

UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA



UNIVERSIDADE
DE LISBOA



NEW APPROACHES TO THE STUDY OF NEUROREHABILITATION PROTOCOLS
IN DOGS AND CATS WITH ACUTE OR CHRONIC SPINAL CORD INJURY WITH
OR WITHOUT DEEP PAIN SENSATION AND POSSIBLE SPINAL SHOCK SIGNS

ÂNGELA PAULA NEVES ROCHA MARTINS

Orientador: Professor Doutor António José Almeida Ferreira

Tese especialmente elaborada para obtenção do grau de Doutor em Ciências Veterinárias
na área de Clínica

2023

UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA



UNIVERSIDADE
DE LISBOA



NEW APPROACHES TO THE STUDY OF NEUROREHABILITATION PROTOCOLS
IN DOGS AND CATS WITH ACUTE OR CHRONIC SPINAL CORD INJURY WITH
OR WITHOUT DEEP PAIN SENSATION AND POSSIBLE SPINAL SHOCK SIGNS

ÂNGELA PAULA NEVES ROCHA MARTINS

Orientador: Professor Doutor António José Almeida Ferreira

Tese especialmente elaborada para obtenção do grau de Doutor em Ciências Veterinárias
na área de Clínica

Júri

Presidente: Professor Doutor Luís Filipe Lopes da Costa

Vogais:

- Professor Doutor Artur Severo Proença Varejão
- Professor Doutora Ana Colette Pereira de Castro Osório Maurício
- Professor Doutora Ana Lúcia Emídio de Jesus Luís
- Professor Doutor António José Almeida Ferreira
- Professor Doutora Graça Maria Alexandre Pires Lopes de Melo

DECLARAÇÃO RELATIVA ÀS CONDIÇÕES DE REPRODUÇÃO DA DISSERTAÇÃO

Nome: Ângela Paula Neves Rocha Martins

Título da Tese ou Dissertação: NEW APPROACHES TO THE STUDY OF NEUROREHABILITATION PROTOCOLS IN DOGS AND CATS WITH ACUTE OR CHRONIC SPINAL CORD INJURY WITH OR WITHOUT DEEP PAIN SENSATION AND POSSIBLE SPINAL SHOCK SIGNS

Ano de conclusão (indicar o da data da realização das provas públicas): 2023

Designação do curso de Mestrado ou de Doutoramento: Ciências Veterinárias

Área científica em que melhor se enquadra (assinale uma):

- ☒ Clínica ☐ Produção Animal e Segurança Alimentar
☐ Morfologia e Função ☐ Sanidade Animal

Declaro sobre compromisso de honra que a tese ou dissertação agora entregue corresponde à que foi aprovada pelo júri constituído pela Faculdade de Medicina Veterinária da ULISBOA.

Declaro que concedo à Faculdade de Medicina Veterinária e aos seus agentes uma licença não-exclusiva para arquivar e tornar acessível, nomeadamente através do seu repositório institucional, nas condições abaixo indicadas, a minha tese ou dissertação, no todo ou em parte, em suporte digital.

Declaro que autorizo a Faculdade de Medicina Veterinária a arquivar mais de uma cópia da tese ou dissertação e a, sem alterar o seu conteúdo, converter o documento entregue, para qualquer formato de ficheiro, meio ou suporte, para efeitos de preservação e acesso.

Retenho todos os direitos de autor relativos à tese ou dissertação, e o direito de a usar em trabalhos futuros (como artigos ou livros).

Concordo que a minha tese ou dissertação seja colocada no repositório da Faculdade de Medicina Veterinária com o seguinte estatuto (assinale um):

- ☒ Disponibilização imediata do conjunto do trabalho para acesso mundial;
- ☐ Disponibilização do conjunto do trabalho para acesso exclusivo na Faculdade de Medicina Veterinária durante o período de ☐ 6 meses, ☐ 12 meses, sendo que após o tempo assinalado autorizo o acesso mundial*;

* Indique o motivo do embargo (OBRIGATÓRIO)

Nos exemplares das dissertações de mestrado ou teses de doutoramento entregues para a prestação de provas na Universidade e dos quais é obrigatoriamente enviado um exemplar para depósito na Biblioteca da Faculdade de Medicina Veterinária da Universidade de Lisboa deve constar uma das seguintes declarações (incluir apenas uma das três):

- É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA TESE/TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.
- É AUTORIZADA A REPRODUÇÃO PARCIAL DESTA TESE/TRABALHO (indicar, caso tal seja necessário, nº máximo de páginas, ilustrações, gráficos, etc.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.
- DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (indicar, caso tal seja necessário, nº máximo de páginas, ilustrações, gráficos, etc.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTA TESE/TRABALHO.

Faculdade de Medicina Veterinária da Universidade de Lisboa, __3__ de ____Fevereiro____ de 2023

(indicar aqui a data da realização das provas públicas)

Assinatura:



Agradecimentos

Agradeço a todos que trabalham por amor incondicional.

Agradeço à imagem importante do Professor Doutor António Ferreira, como homem do saber.

Agradeço por estar viva.

RESUMO

Nova abordagem aos protocolos de neuroreabilitação em cães e gatos com lesão medular aguda ou crônica, com/sem sensibilidade à dor profunda e choque espinal

Os protocolos de neuroreabilitação intensiva (INRP), com as modalidades de reabilitação e o treino locomotor em tapete rolante com suporte de peso (BWSTT), são sugeridos como terapêutica para obter a ambulação em cães e gatos de lesão medular compressiva / não compressiva, completa (DPP⁻), “discompleta” e incompleta (DPP⁺), tal como na medicina humana. Assim, apresenta-se o primeiro artigo, estudo de coorte, prospectivo, controlado e cego, em 22 cães com lesão T11-L3 Hansen tipo I, que demonstrou 100% de ambulação no grupo BWSTT, em média de 4.6 semanas. O segundo artigo refere-se ao estudo controlado e retrospectivo de 367 cães pós-cirúrgicos com lesão aguda T10-L3 de Hansen tipo I. A escala de neuroreabilitação funcional (FNRS-DPP-) foi elaborada e aplicada nestes cães, DPP⁻ ou incompletos, capazes de atingir a locomoção espinal por reflexos (SRL). Verificaram-se diferenças significativas entre grupos, nos DPP⁺ ($p < 0,001$) com 99,4% de ambulação, e nos DPP⁻ ($p = 0,007$) com 58,5%. Em relação à recuperação da sensibilidade profunda ($p = 0,058$), ocorreu 37.3% de SRL, no máximo em 3 meses. O INRP demonstrou-se seguro e a recuperação foi atingida de forma mais precoce. O mesmo foi estudado em 16 cães com recuperação incompleta 3 meses após cirurgia, sendo associada a administração de 4-aminopiridina nos DPP⁻ com 78% de SRL até 45 dias e micção automática em ~62 dias. Obteve-se 100% de ambulação nos cães DPP⁺, em ~47 dias, com evolução positiva nas consultas de seguimento. A ambulação total foi de 88%, estabelecendo este INRP como opção terapêutica, reduzindo o número de eutanásias em âmbito clínico. As mielopatias não compressivas foram, também, estudadas. Assim sendo, estudo prospectivo em 9 gatos revelou 56% de ambulação e 44% de SRL, demonstrando que o INRP poderá ser considerado, no sentido de melhorar a qualidade de vida e bem-estar destes doentes. Após a lesão medular, alguns cães podem desenvolver o choque espinal, principalmente na extrusão aguda não compressiva do núcleo pulposo. Assim, foi desenvolvido estudo prospectivo coorte que elaborou e aplicou escala de choque espinal para monitorização, sugerindo o choque espinal como fator negativo para a rápida recuperação. Este INRP revelou-se seguro, tolerável e viável, com 32% de ambulação em 7 dias e 94% em 60 dias. Consultas de seguimento até 4 anos revelaram evolução positiva. Estes estudos devem ser continuados considerando as suas limitações. **Palavras-passe:** Neuroreabilitação; Lesão medular; Ambulação; Sensibilidade profunda; Choque Espinal

ABSTRACT

New approaches to the study of neurorehabilitation protocols in dogs and cats with acute or chronic spinal cord injury with or without deep pain sensation and possible spinal shock signs

Intensive neurorehabilitation protocols (INRP) with rehabilitation modalities and weight supported treadmill training (BWSTT), are suggested as treatment to obtain ambulation in dogs and cats with complete (DPP-), discomplete and incomplete (DPP+) compressive or non-compressive spinal cord injury (SCI), similarly to what is performed in human medicine. The first study is a cohort, prospective, controlled and blinded study that was performed in 22 dogs with T11-L3 Hansen type I, revealing ambulation in 100% of the BWSTT group, within a mean of 4.6 weeks. One other study, a retrospective controlled clinical study, was developed in 367 acute post-surgical dogs, with T10-L3 Hansen type I. A new functional neurorehabilitation scale (FNRS-DPP-) was performed to evaluate the DPP- or discomplete dogs, that were able to achieve spinal reflex locomotion (SRL). A strong significance between groups was verified in the DPP+ ($p < 0.001$), with 99.4% of ambulation. The same difference was seen in the DPP- ($p = 0.007$) with 58.5% of ambulation and a tendency ($p = 0.058$) was observed in regard to DPP recovery, with 37.2% achieving SRL, within a maximum of 3 months. INRP was demonstrated to be safe and ambulation recovery achieved earlier. The same population was included in another study, on 16 dogs with incomplete recovery 3 months after surgery. DPP- were under INRP associated with 4-aminopyridine administration, achieving 78% of SRL at day 45 and automatic micturition within a mean of 62 days. Also, 100% of ambulation in the DPP+ within a mean of 47 days and positive follow-up evolution. Ambulatory status was achieved in 88%, establishing this INRP as a therapeutic option to reduce euthanasia. Non-compressive myelopathies with contusive patterns were also referred in a prospective study of 9 cats that revealed 56% ($n=5$) of ambulation and 44% ($n=4$) of SRL, showing that INRP should be considered to improve quality of life and the well-being of our patients. Some dogs may develop spinal shock following SCI, including in acute noncompressive nucleus pulposus extrusion. Thus, a cohort prospective study applied a spinal shock scale as a monitoring tool, suggesting spinal shock as a negative factor for a quick recovery. INRP was shown to be safe, tolerable and feasible, allowing 32% of ambulation within 7 days and 94% within 60 days. Follow-ups until 4 years revealed a positive evolution. These studies should be continued, considering each limitation. **Key-words:** Neurorehabilitation; Spinal cord injury; Ambulation; Deep pain perception; Spinal shock.

RESUMO ALARGADO

Nova abordagem aos protocolos de neuorreabilitação em cães e gatos com lesão medular aguda ou crónica, com/sem sensibilidade à dor profunda e choque espinal

A locomoção é um processo sensoriomotor semelhante entre animais bípedes e quadrúpedes. Ambos apresentam a combinação das vias aferentes sensoriais com os circuitos de interneurónios e os geradores de padrão central, permitindo que a informação seja recebida por feixes ascendentes sensoriais. Estes recebem os sinais neurais das vias aferentes da do fuso motor e, ainda, dos mecanorreceptores e proprioceptores das cápsulas articulares. Esta rede neural complexa permite abordar os doentes de lesão medular grave, para os quais a ausência da sensibilidade à dor profunda é sinal de prognóstico negativo.

Assim, acreditamos que após a lesão medular, existe interconexão entre os feixes descendentes motores residuais, os circuitos intrínsecos de interneurónios e os geradores de padrão central, para além dos estímulos por parte dos sinais periféricos sensoriais. Deste modo, estando de acordo com a medicina humana, após lesão grave da medula espinal e na ausência de sensibilidade à dor profunda, podemos obter a ambulação, considerando-se estes doentes “discompletos” em substituição do termo completos, embora seja difícil o entendimento do processo neurológico subjacente.

Para poder classificar os doentes com lesão neurológica definiu-se ambulação como a capacidade destes se levantarem de forma automática/voluntária, perfazerem 10 passos consecutivos em diferentes pisos e curvas, nunca caindo, mas podendo demonstrar sinais de fraqueza muscular. Têm ainda de obter a capacidade de micção automática ou voluntária.

Os protocolos de neuorreabilitação intensiva (INRP), com as modalidades de reabilitação e o treino locomotor em tapete rolante com suporte de peso (BWSTT), são sugeridos como terapêutica para obter a ambulação em cães e gatos de lesão medular compressiva / não compressiva, completa (DPP⁻), “discompleta” e incompleta (DPP⁺), tal como na medicina humana.

Durante o trabalho evidenciamos o papel da estimulação elétrica funcional (FES) e da estimulação elétrica transcutânea da medula espinal (TESCS). A primeira é essencial para manter a monosinapse excitatória e a inibição dissináptica recíproca, permitindo a homeostasia na despolarização e repolarização periféricas. Por outro lado, a

electroestimulação transcutânea da medula espinhal vai permitir ativar os possíveis feixes residuais descendentes motores, sendo a sua amplitude e capacidade de conexões interneurais similares à da estimulação elétrica epidural.

Relativamente ao treino locomotor, este é um método constante em todos os artigos, e deve ser efetuado segundo as regras de Hebb (1949) e da plasticidade equilibrada. Nos estudos em que a população alvo são os doentes DPP-, e que o padrão locomotor flexão-extensão demora mais de 10 dias a ser obtido com modulação, surge o manejo farmacológico com a 4-aminopiridina (4-AP), administrada após 30 dias de treino. Este bloqueador dos canais de potássio permite o influxo de cálcio e a libertação de neurotransmissores serotoninérgicos e noradrenérgicos responsáveis por possíveis novas conexões interneurais.

Todos os doentes, cães e gatos, deste trabalho foram monitorizados e avaliados segundo as escalas OFS para DPP+, FNRS-DPP- para DPP- e a SSS para doentes com sinais de choque espinhal. Sendo as avaliações, normalmente a cada 5 a 7 dias, exceto para os doentes de choque espinhal que no início do estudo foram avaliados diariamente.

Assim, apresenta-se o primeiro artigo, estudo de coorte, prospetivo, controlado e cego, em 22 cães com lesão T11-L3 Hansen tipo I, que demonstrou 100% de ambulação no grupo BWSTT, em média de 4.6 semanas, enquanto o grupo de controlo (alternância de pisos) obteve 78% em 6.1 semanas. Este estudo demonstra a importância de iniciar precocemente o treino locomotor no tapete rolante aquático associado à FES, ao fim de 2 dias, até nos casos de pós-cirúrgico. O treino locomotor aquático vai depender da intensidade, ou seja, número de movimentos repetidos e da duração, respeitando sempre a condição cardiovascular e o alinhamento da coluna vertebral, de modo a evitar alternâncias de postura durante o próprio treino.

O segundo artigo refere-se ao estudo controlado e retrospectivo de 367 cães pós-cirúrgicos com lesão aguda T10-L3 de Hansen tipo I. A escala de neuroreabilitação funcional (FNRS-DPP-) foi elaborada e aplicada nestes cães, DPP- ou incompletos, capazes de atingir a locomoção espinhal por reflexos (SRL). Verificaram-se diferenças significativas entre grupos, nos DPP+ ($p < 0,001$) com 99,4% de ambulação, e nos DPP- ($p = 0,007$) com 58,5%. Em relação à recuperação da sensibilidade profunda ($p = 0,058$), ocorreu 37.3% de SRL, no máximo em 3 meses. Sabendo, ainda, que a locomoção espinhal por reflexos requer o pressuposto de recuperação tardia, e que em relação às médias ajustadas da respetiva escala ao longo dos diversos pontos temporais, obteve-se o pico máximo em T7 (60 dias). O INRP demonstrou-se

seguro e a recuperação foi atingida de forma mais precoce em comparação com estudos prévios.

Em relação aos doentes crónicos de lesão medular grave, após estimulados com protocolos de fisioterapia convencional, foi efetuado estudo de casos clínicos de critério coorte e com recolha de dados durante 10 anos, em 16 cães com recuperação incompleta 3 meses após cirurgia, sendo associada a administração de 4-AP nos DPP- , com 78% de SRL até 45 dias e micção automática em ~62 dias. Obteve-se 100% de ambulação nos cães DPP⁺, em ~47 dias, com evolução positiva nas consultas de seguimento. A ambulação total foi de 88%, estabelecendo este INRP como opção terapêutica, reduzindo o número de eutanásias em âmbito clínico. Os resultados sugerem que a 4-AP, sendo fármaco de ação na membrana celular permite o aumento da amplitude das correntes persistentes ainda existentes após lesão medular grave. Durante a elaboração do treino foi observada sincronização e coordenação do padrão flexor-extensor, com redução dos sinais clínicos de espasticidade, importante pois a neurolocalização da lesão medular é dos segmentos medulares T3-L3, ou seja, lesão de neurónio motor superior.

As mielopatias não compressivas foram, também, estudadas. Assim sendo, a realização de estudo piloto prospetivo em 9 gatos, com recolha de dados durante 7 anos, revelou 56% de ambulação e 44% de locomoção espinhal por reflexos, para além da recuperação de sensibilidade à dor profunda num gato que obteve alta médica atáxico propriocetivo. Sugere-se, ainda, a facilidade de obter reflexos polissinápticos com envolvimento do feixe vestibuloespinhal de forma mais modelada do que em relação ao cão. Desta forma, o INRP demonstrou que poderá ser considerado, no sentido de melhorar a qualidade de vida e bem-estar destes doentes.

Ainda, após a lesão medular, alguns cães podem desenvolver o choque espinhal, principalmente na extrusão aguda não compressiva do núcleo pulposo. Deste modo, foi desenvolvido o estudo prospetivo coorte que elaborou e aplicou a escala de choque espinhal para monitorização, sugerindo o choque espinhal como fator negativo para a rápida recuperação dos doentes. Este INRP revelou-se seguro, tolerável e viável, com 32% de ambulação em 7 dias e 94% em 60 dias (90% em 30 dias). As consultas de seguimento até 4 anos revelaram evolução positiva.

Todos estes estudos devem ser continuados considerando as suas limitações. Assim, destacam-se como principais, a ausência de técnicas de mensuração de elevada

sensibilidade, ausência de avaliação cinética, cinemática e de estudos de eletromiografia, bem como a aplicação de biomarcadores da regeneração, tais como o GFAP, pNFH e S100 β . As presenças destas limitações demonstram a grande dificuldade, durante o percurso dos estudos, de distinguir a evolução clínica pelos INRP e/ou pela neuroplasticidade espontânea.

Como perspectivas futuras, seria fundamental implementar o treino de equipas, em ambiente hospitalar, para o tratamento de animais DPP- e de doentes discompletos. Ainda, a educação dos tutores no manuseio da bexiga neurogénica, de modo a evitar a dissenergia reflexa, e no treino locomotor com suporte do peso, recorrendo ao uso da cadeira de rodas mas sem prender os membros pélvicos, devido à possibilidade de haver recuperação da ambulação e da sensibilidade à dor profunda ao longo do tempo, até dois anos após lesão, podendo desenvolver ataxia propriocetiva.

No futuro, seria importante continuar os estudos com a introdução de observações coorte e grupos de controlo, particularmente na aplicação de células estaminais e com a administração de 4-AP. Por último, a avaliação com ressonância magnética através da técnica de imagem de tensor de difusão e tractografia, quanto à evolução do epicentro da lesão medular.

LIST OF PUBLICATIONS

This dissertation was based on the following publications:

Martins, Â., Gouveia, D., Cardoso, A., Gamboa, Ó., Millis, D., Ferreira, A. 2021. Nervous system modulation through electrical stimulation in companion animals. *Acta Vet. Scand.* 63(22): 1-12.

Martins, Â., Gouveia, D., Cardoso, A., Viegas, I., Gamboa, Ó., Ferreira, A. 2021. A comparison between body weight-supported treadmill training and conventional over-ground training in dogs with incomplete spinal cord injury. *Front. Vet. Sci.* 8(597949):1-14.

Martins, Â., Gouveia, D., Cardoso, A., Carvalho, C., Coelho, T., Silva, C., Viegas, I., Gamboa, Ó., Ferreira, A. 2021. A controlled clinical study of intensive neurorehabilitation in post-surgical dogs with severe acute intervertebral disc extrusion. *Animals.* 11 (3034): 1-22.

Martins, Â., Gouveia, D., Cardoso, A., Carvalho, C., Silva, C., Coelho, T., Gamboa, Ó., Ferreira, A. 2021. Functional neurorehabilitation in dogs with an incomplete recovery 3 months following intervertebral disc surgery: a case series. *Animals.* 11 (2442): 1-21.

Martins, Â., Silva, C.M., Gouveia, D., Cardoso, A., Coelho, T., Gamboa, Ó., Marcelino, E., Ferreira, A. 2021. Spinal locomotion in cats following spinal cord injury: a prospective study. *Animals.* 11 (1994): 1-13.

Gouveia, D., Cardoso, A., Carvalho, C., Gonçalves, A.R., Gamboa, Ó., Canejo-Teixeira, R., Ferreira, A., **Martins, Â.** 2022. Influence of Spinal Shock on the Neurorehabilitation of ANNPE Dogs. *Animals.* 12 (1557): 1-20.

INDEX

<i>Agradecimientos</i>	<i>iii</i>
<i>RESUMO</i>	<i>iv</i>
<i>ABSTRACT</i>	<i>v</i>
<i>RESUMO</i>	<i>vi</i>
<i>LIST OF PUBLICATIONS</i>	<i>x</i>
<i>LIST OF ABBREVIATIONS AND SYMBOLS</i>	<i>xiii</i>
<i>CHAPTER I</i>	<i>1</i>
<i>INTRODUCTION – REVIEW</i>	<i>1</i>
1. <i>The neuroanatomic comparison between the quadrupedal animal and the human biped in locomotion</i>	<i>2</i>
2. <i>Functional Neurorehabilitation Application in Veterinary Medicine</i>	<i>6</i>
3. <i>Spinal Cord Disorders</i>	<i>8</i>
3.1. <i>Intervertebral disc herniation - Hansen type I</i>	<i>8</i>
3.2. <i>Intervertebral disc herniation - Hansen type II</i>	<i>15</i>
3.3. <i>Non-compressive myelopathies</i>	<i>16</i>
3.3.1. <i>Acute non-compressive nucleus pulposus extrusion</i>	<i>16</i>
3.3.2. <i>Fibrocartilaginous embolism of the spinal cord</i>	<i>19</i>
3.3.3. <i>Diagnosis</i>	<i>20</i>
3.3.4. <i>Clinical outcomes</i>	<i>22</i>
4. <i>References</i>	<i>23</i>
<i>CHAPTER II</i>	<i>36</i>
<i>STATE OF ART AND OBJECTIVES – NERVOUS SYSTEM NEUROMODULATION AND NEUROSTIMULATION</i>	<i>36</i>
1. <i>State of Art</i>	<i>37</i>
1.1. <i>Functional Electrical Stimulation</i>	<i>38</i>
1.2. <i>Transcutaneous Electrical Spinal Cord Stimulation</i>	<i>40</i>
1.3. <i>Transcranial Direct Current Stimulation</i>	<i>45</i>
1.4. <i>ARTICLE 1</i>	<i>51</i>
<i>Nervous system modulation through electrical stimulation in companion animals</i>	<i>51</i>
1.5. <i>Locomotor training</i>	<i>76</i>
2. <i>Objectives</i>	<i>81</i>

3. References	83
CHAPTER III	96
RESULTS	96
1. ARTICLE 2	97
<i>A Comparison Between Body Weight-Supported Treadmill Training and Conventional Over-Ground Training in Dogs with Incomplete Spinal Cord Injury</i>	97
2. ARTICLE 3	130
<i>A Controlled Clinical Study of Intensive Neurorehabilitation in Post-Surgical Dogs with Severe Acute Intervertebral Disc Extrusion</i>	130
3. ARTICLE 4	159
<i>Functional Neurorehabilitation in Dogs with an Incomplete Recovery 3 Months following Intervertebral Disc Surgery: A Case Series.</i>	159
4. ARTICLE 5	184
<i>Spinal Locomotion in Cats Following Spinal Cord Injury: A Prospective Study</i>	184
5. ARTICLE 6	201
<i>Influence of Spinal Shock on the Neurorehabilitation of ANNPE Dogs</i>	201
CHAPTER IV	224
DISCUSSION, CONCLUSION AND FUTURE PERSPECTIVES	224
1. Discussion	225
2. Conclusion	229
3. Future Perspectives	230
4. References	232

LIST OF ABBREVIATIONS AND SYMBOLS

4-AP	4-Aminopyridine
AF	Annulus Fibrosus
AIS	American Spinal Injury Association Impairment Scale
ANNPE	Acute Non-compressive Nucleus Pulposus Extrusion
ANOVA	Analyses Of Variance
BDNF	Brain Derived Neurotrophic Factor
BID	Twice a day (from the latin “bis in die”)
BWSTT	Body Weight-Supported Treadmill Training
CG	Control Group
cm ²	Square centimetre
CMAP	Compound Muscle Action Potential
CNS	Central Nervous System
COGI	Conventional Over-Ground Intervention
CPG	Central Pattern Generators
CST	Corticospinal descending motor Tract
CT	Computed Tomography
DEEH	Disc Extrusion with Extensive Epidural Hemorrhage
DPP	Deep Pain Perception
DPP-	Deep Pain Perception Negative
DPP+	Deep Pain Perception Positive
DRG	Dorsal Root Ganglia
EES	Epidural Electrical Stimulation
FEM	Fibrocartilaginous Embolism Myelopathy
FES	Functional Electrical Stimulation
FNR	Functional Neurorehabilitation
FNRIP	Functional Neurorehabilitation Intensive Protocol
FNRS-DPP ⁻	Functional Neurorehabilitation Scale for dogs with Thoracolumbar SCI without Deep Pain Perception
Hz	Hertz
IES	Interferential Electrical Stimulation
INR	Intensive Neurorehabilitation
INRP	Intensive Neurorehabilitation Protocol

IVD	Intervertebral Disc
IVDD	Intervertebral Disc Degeneration
IVDH	Intervertebral Disc Herniation
Km/h	kilometre per hour
LMN	Lower Motor Neuron
mA	Miliampere
mA/cm ²	miliampere per square centimetre
MDPI	Multidisciplinary Digital Publishing Institute
MEP	Motor-Evoked Potential
MFS	Modified Frankel Scale
MMP-2	Matrix Metalloproteinase-2
m/s	metre per second
MS-TESCS	Multisegmental Transcutaneous Electrical Spinal Cord Stimulation
MRI	Magnetic Resonance Image
NGF	Nerve Growth Factor
NMES	Neuromuscular Electrical Stimulation
NP	Nucleus Pulposus
NRMP	Neurorehabilitation Multi-modal Protocol
NSRL	No Spinal Reflex Locomotion
NT-3	Neurotrophin – 3
NT-4	Neurotrophin-4
OFS	Open Field Score
PICs	Persistent Inward Currents
PhD	Doctor of Philosophy
PM	Progressive Myelomalacia
pNF-H	Phosphorylated Neurofilament-Heavy Chain
PROMs	Passive Range of Motion Exercises
QLT	Quadrupedal Locomotor Training
RST	Reticulospinal descending motor Tract
RTM	Rehabilitation Therapeutic Modality
RuST	Rubrospinal descending motor Tract
SBBSL	Spinal cord-Brainstem Brain-Spinal cord Loop

SCI	Spinal Cord Injury
SG	Study Group
SRL	Spinal Reflex Locomotion
SS	Superficial Sensitivity
SSS	Spinal Shock Scale
TDCS	Transcranial Direct Current Stimulation
TESCS	Transcutaneous Electrical Spinal Cord Stimulation
TGF β	Transforming Growth Factor β
TNF- α	Tumor Necrosis Factor- α
TST	Tectospinal descending motor Tract
T1-W	T1-weighted images
T2-W	T2-weighted images
UMN	Upper Motor Neuron
UWTM	Underwater Treadmill
VH/MR	Ventral Horn/Muscle Reflex
VST	Vestibulospinal descending motor Tract

CHAPTER I

INTRODUCTION – REVIEW

1. The neuroanatomic comparison between the quadrupedal animal and the human biped in locomotion

Based on neuroanatomy, in both human biped and animal quadruped there are five major descending motor tracts. The corticospinal descending motor tract (CST), the rubrospinal descending motor tract (RuST), the reticulospinal descending motor tract (RST), the vestibulospinal descending motor tract (VST) and the tectospinal descending motor tract (TST) (Li and Francisco 2015).

CST is the only descending motor tract that originates in the motor cortex, all others have their origin in the brainstem (Petersen 2014). In humans, this is the primary tract responsible for voluntary movement (Hukuda et al. 1973), while in dogs the RuST is the main one, thus classifying the human biped in pyramidal system and the animal quadruped (e.g. dogs and cats) in extrapyramidal system (Barros and Pinheiro, 2009; Thomson and Hahn, 2012; Guertin 2014; Ropper et al. 2014; Solopova et al. 2015). In humans, the RuST is considered to be vestigial, an example of the early descending motor beams in animals (Ropper et al. 2014).

In regard to the RST, there are some neurophysiological differences. In humans, its composed by the dorsal and medial RST, the first controlled by the motor cortex, while the second has no brain involvement (Barros and Pinheiro 2009; Guertin 2014; Ropper et al. 2014). Therefore, dorsal RST has a stretching reflex inhibitory effect and is located, neuroanatomically, along with the lateral CST at the dorsolateral funiculus (Baldwin et al. 2013). On the other hand, medial RST has antagonistic action, which allows the excitatory effect on the spinal stretch reflex (Baldwin et al. 2013).

In quadrupedal animals, reticular formation is under cortical modulation (Uemura 2015a) and has an excitatory/inhibitory action on motor activities through the pontine RST and medullary RST. Pontine RST, that originates from pontine reticular formation, facilitates the excitatory effect of extensor muscles α and γ lower motor neuron (LMN), thus inhibiting α and γ LMN of the flexor muscles. Medullary RST originates from medullary reticular formation and facilitates the excitatory effect of the flexor muscles α and γ LMN, promoting the inhibition of the extensor muscles α and γ LMN. Thus, a balanced between pontine and medullary RST is essential for locomotion, posture and muscle tone (Nathan et al 1996; Edwards 2002; Uemura 2015a).

The VST has an excitatory effect on the α and γ LMN of the ipsilateral anti-gravitational muscles but an inhibitory effect on the contralateral muscle tone, both in humans, dogs and cats (Thomson and Hahn 2012; Martins 2015).

So, according to the past few years studies, locomotion of the human biped and the animal quadruped has similar spinal neural control mechanisms (Dietz 2003). In both, propriospinal tracts are projected from the cervical region to the lumbar region (Nathan et al. 1996; Dietz 2003). Also, co-coordination between limbs during locomotion is similar (Grillner 1981, 1986; Dietz, 1992, 1997; Yang et al. 1998; Pang and Yang 2000), although due to CST primary role, there has been an evolution regarding straight posture and complex and fine hand movements (Dietz 2003; Guertin 2014).

Concerning underlying mechanisms, the locomotion phenomenon doesn't appear to present fundamental differences between the human biped and the animal quadruped (Nilsson et al. 1985; Macpherson et al. 1989; Nicol et al. 1995; Pearson 2000a; Pearson 2000b; Duysens 2002). For Dietz and collaborators (2002), the essential spinal neural mechanism is based on combining the afferent sensory stimulation and the neural intrinsic circuit pattern, which is present in both the biped and quadruped.

Flexion movement, or lower limbs reflex of the human biped (hindlimbs in the animal quadruped), is highly investigated through neurophysiological studies. In these, it was found that in both cases, polysynaptic and multisegmental reflexes are mediated by a complex circuit modulated by the spinal cord and at supraspinal level, (Sandrini et al. 2005), i.e. by the CST (Leblond et al. 2001), RuST (Hongo et al. 1972), VST (Ten Bruggencate and Lundberg, 1974) and medullary RST (Takakusaki et al. 2001).

Once again in both biped and quadruped animals, spinal peripheral nociceptive reflexes are possibly an induced result due to stimulation of A δ nociceptive fibers, with possible contribution of C nociceptive fibers stimulation (Arendt-Nielsen et al. 1995; Herrero and Cervero 1996; Weng and Schouenborg 1998; Clarke et al. 2002; Serrao et al. 2004; Kimura et al. 2005; Sandrini et al. 2005). However, according to Le Bars et al. (1976), Leahy and Durkovic (1991) and Schomburg et al. (2000), in cats, this hypothetic mechanism is already been proven through A β , A δ and C nociceptive fibers activation. Also, the locomotion's flexion phase (a part of the swing phase) may be influenced by the afferent skin receptors (Duysens et al. 1990a, 1990b, 1992; Schomburg et al. 1998; Zehr et al. 1998; Zehr and Stein 1999; Bastiaanse et al. 2000; Spaich et al. 2004).

In the same way, the locomotion phenomenon is presented as a stereotyped rhythmic pattern of motor origin (Calancie et al. 1994; Dimitrijevic et al. 1998; Clarac and Pearlstein 2007; Guertin 2009). Invertebrate investigations, and their contribution to neurobiology in the second half of the 20th century, were based on quadruped neuronal coordination (Dietz 2002; 2012).

Also, it is important to mention that thoracolumbar intervertebral degenerative disk disease in dogs have a histopathological behavior similar to traumatic myelopathies in humans (Levine et al. 2011). Demyelination-associated to axonal injury is common in both (Wright and Palmer 1969; Kakulas 1999; Norenberg et al. 2004; Smith and Jeffery 2006), as is neural necrosis (Kakulas 1999). When there is a spinal cord injury (SCI) in humans, a CST depolarization blockage occurs, resorting to extrapyramidal descending motor tracts (Martins 2015), in order to promote neural reorganization, modulation and memorization, through functional neurorehabilitation (FNR) protocols. FNR protocols activate and stimulate the same descending motor tracts and intrinsic circuits responsible for rhythmic and stereotyped locomotion pattern (Nathan et al. 1996; Martins 2015).

Although the cat belongs to the extrapyramidal system, he depends of CST integrity to achieve locomotion coordination and modulation, thus it is considered to be the most similar animal model for humans (Duysens and Van de Crommert 1998; Knikou 2012; Martins 2015), other than rodent models that appeared not to be the best primate model for SCI. Also, the CST is critically for fine motor control in humans and less critical in rats (Nout et al. 2012). There is some speculation that gradual weakness of the propriospinal neuronal system is accompanied by progressive strenghtning of direct cortical-motoneuron projections, present from cats to primates and stronger in humans, leading to the ability for skilled motor tasks with the forelimbs (Engelhardt and Sobotzik 2017). In addition, the corticospinal fibres account for 20-30% of the total spinal cord white in primates but only nearly 10% in carnivores (Thomson and Hahn 2012), justifying that corticospinal tracts have a bigger importance in ambulation in humans compared to dogs (Hukuda et al. 1973)

Essentially there are clear similarities in motor control between cats and humans, so it is extrapolated that there are no essential differences in regard to the neural networks of both species (Duysens and Van de Crommert 1998). In locomotion it is required to talk about central pattern generators (CPG), which are located in the nervous system and produce an automatic rhythmic output with reciprocity organization between agonists and antagonists, without any signs from descending motor pathways, as well as from movement related to afferent input

(Grillner and Wallen 1985; Grillner 1985). The CPG control and coordinate flexor/extensor motoneurons of the shoulder/forelimbs and pelvis/hindlimbs (Juvin et al. 2005), belonging to vertebrate and invertebrate animals, and not only to the cat (Rossignol and Dubuc 1994). The main difference resides in the fact that in primates, including humans, the CPG circuit is suppressed by cortex inputs through the CST (Vilensky and O'Connor 1997).

In the presence of a lesion that affects the pontomedullary or spinal cord upper motor neuron (UMN) and the central pattern networks, patients that are still able to walk unassisted, may show a delay in the onset of protraction, which is the gait swing phase, and a longer stride caused by a delay on the onset and termination of protraction. Thus, animals (from human primates to dogs and cats) with spinal cord lesions may show ataxia to tetra/paraplegia, depending on injury location (De Lahunta et al. 2021a). Also, dogs with severe T13-L3 injuries may show Schiff-Sherrington posture (Thomas and De Risio 2015), however it has been suggested that only 10-20% of intact descending UMN pathways are needed for recovery of locomotion (Thomson and Hahn 2012).

On the other hand, brainstem lesions may result in alteration of mental status, opisthotonus and decerebrate posture (De Lahunta et al. 2021a). As for forebrain lesions in quadrupeds, they have little effect on basic locomotion, contrary to humans (Thomson and Hahn 2012) that may develop hemiplegia and/or spastic hemiparesis, in which the arm is adducted and internally rotated at the shoulder (Thomson and Nutt 2012).

In both bipeds and quadrupedal animals, SCI has a massive release of glutamate, which blinds the ionotropic receptors that are detected in the surface of astrocytes and oligodendrocytes, causing excitotoxicity (Verkhratsky and Steinhauser 2000; Káradóttir and Attwell 2007; Vanzulli and Butt 2015; Couillard-Despres et al. 2017). This toxicity develops in the grey matter tissue, but also has been shown in the white matter secondary damaged. Furthermore, there is a failure of the Na^+/K^+ ATPase and the collapse of Na^+/K^+ gradient across axonal membranes. In the grey matter, the neuronal imbalanced ionic homeostasis leads to an increase of intracellular Ca^{2+} concentration, leading to apoptosis and cellular necrosis with glutamate releasing by the direct mechanical rupture of cells (Williams et al. 2014). In association to these ionic dysregulations secondary to Na^+ and Ca^{2+} intracellular increase and K^+ decrease, there is a lipid peroxidation that further contributes to the Na^+/K^+ ATPase inhibition. Also, the oligodendrocytes are sensitive to excitotoxicity and submitted to free radical release by the infiltrating neutrophils, activated microglia and immune cells invading the injury

and releasing pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin – 2 (IL-2) (Couillard-Despres et al. 2017).

Neurological recover after SCI is influenced by severity of the injury (Marino 1999) with a steeper curve of recovery in strength and motor scores during the first 3 months. In humans, it is described that the recover of strength is usually steepest in first 100 days (Schubert 2017). This recovery can be attributed to a mechanism of functional compensation, neural plasticity or repair (Curt 2008). The plasticity phenomenon is based on reorganization of neural circuits mechanisms and induced repetitive trained tasks. Repair mechanisms entails remyelination or regeneration and reconnection of damaged spinal tract fibers that reflect changes in the spinal impulse conductivity (Schubert 2017).

2. Functional Neurorehabilitation Application in Veterinary Medicine

FNR is a field of physical medicine and rehabilitation belonging to restorative neurology, based on the evidence of signals transmission through the lesion, both caudal and rostral, as detected by electromiography (Tansey 2010; Dimitrijevic 2012; Kakulas and Kaelan 2015). Therefore, the goal of FNR is to activate by synaptic stimulation the central axons pathways traversing the lesion (Dimitrijevic 2012).

Based on spinal cord neuroanatomy and neurophysiology, Human Medicine has created guidelines for elaborating FNR intensive protocols (FNRIP) (Côté et al. 2018).

One of the FNRIP strategies is locomotor training, which is designed to promote postural control, balance, postural standing, walking and functionality (Smith and Knikou 2016). This will allow the organization of the neuromuscular system below the level of the lesion, due to changes in the spinal cord, generally biochemical (Harkema 2012).

The spinal locomotor CPG circuitry interacts dynamically with afferent inputs from receptors located in muscles, joints and skin (Pearson 2007; Rossignol and Frigon 2011). Thus, FNRIP intends to stimulate these circuits, since the afferent input most relevant to walking arrives primarily from the stretch and sensitive mechanoreceptors (Bouyer and Rossignol 2003). Proprioceptive afferents (groups Ia and Ib) are responsible, in part, for the duration of the step cycle various sub-phases (step frequency and speed) (Rossignol et al 2006; Takeoka et al. 2014), making it very important to apply stretch in order to activate both groups Ia and Ib and, therefore, allowing an exerted force or applied lengthening of the extensor muscle (Van de Crommert et al. 1998). Also, the input from the hip region could be a factor in initiating the swing phase in chronic spinal dogs (Grillner 1986). Hip flexor afferents (group Ia and II), from the iliopsoas and sartorius muscles) regulate the duration of the

stance/swing phases. Thus, hip-joint-related afferent inputs seem to be of crucial importance for the step cycle (Dietz 2012).

FNRIP seeks to recruit and adjust the number of motoneurons that generate the functional rhythmic movements (Côté et al. 2018). Firing of spinal interneurons is continuously adjusted by peripheral receptors that contribute about 30%, affecting motor output with action on segmental and supraspinal neural circuits.

The locomotor training, with or without weight-support, may be a way to perform this same stimulation (Behrman and Harkema 2000; Knikou and Mummidisetty 2014). There are evidences that anatomical and physical changes, which decrease the inflammatory response and increase the neurotrophin levels, may happen, promoting neural regeneration (Côté et al. 2017). These allows FNR, in both animal models (i.e. the cat) and humans, to be able to generate independent steps on the treadmill (Behrman and Harkema 2000). Within the neurotrophins, particularly the brain derived neurotrophic factor (BDNF) plays a critical role in the reorganization of the central nervous system (CNS), as it is a potent neuromodulator and contributes to nervous regeneration and nociceptive regeneration (Knikou 2012; Cassilhas et al. 2015). Also, the nerve growth factor (NGF), neurotrophin – 3 (NT-3) and neurotrophin-4 (NT-4) are shown to be required for axonal growth from sensory neurons. It is proposed that locomotor training can contribute to the disinhibition of the descending motor pathways that are inhibited in humans with SCI, since it stimulates the pre-motor neuronal control and promotes the interlimb and intralimb coordination (Knikou and Mummidisetty 2014).

Several studies have been performed on cats with complete/incomplete SCI, demonstrating step training effectiveness on the treadmill (Dietz et al. 1995; de Leon et al. 1998; van de Crommert et al. 1998) and consequent neurotrophins delivery (Côté et al. 2017). It is essential to understand spinal pathways reorganization after a SCI, that is, to understand anatomical and synaptic plasticity, in order to design and improve the FNRIP (Raineteau and Schwab 2001).

Repeating cyclic movements over a certain period of time, every day, for several and consecutive weeks was shown to promote, after an incomplete/complete SCI, the fictive locomotion, without a pharmacological approach (Knikou 2010). Although with its association this appearance becomes faster and continuous (de Leon et al. 1998), as long as the caudal spinal cord to the lesion has autonomous intrinsic ability to generate locomotor movements, since caudal lesions to L4 compromise the recovery of locomotion (Rossignol et al. 2006; Harkema et al. 2012). This approach allows the plasticity in the reflex pathways (Lavrov et al.

2006; Rossignol et al. 2006), and it is known that limited residual descending inputs may be needed for significant functional improvements (Harkema et al. 2012). Therefore, a motor training with a monotonous repetition of the exact same sensorimotor exercise could result in spinal learning and memorization (Edgerton et al. 2001)

Locomotor training helps to activate the substantial population of long ascending and descending propriospinal interneurons that connects the cervical and lumbar enlargements via the ventrolateral funiculus, contributing significantly to neural coupling between cervical and lumbar spinal segments (Shah et al. 2013; Côté et al. 2018).

3. Spinal Cord Disorders

3.1. Intervertebral disc herniation - Hansen type I

- **Introduction**

Intervertebral disc herniation (IVDH) is one of the most common neurological dysfunctions in dogs (Bos et al. 2012; Muguet-Chanoit et al. 2012; Bergknut et al. 2015), which is highly related to aging (Jeffery et al., 2013) and one of the main causes of spinal pain (Muguet-Chanoit et al. 2012; Roerig et al. 2013; Dewey and Da Costa 2016).

The IVDH is a complex and multifactorial disease, based on multiple changes in the composition of cells and extracellular matrix of Annulus Fibrosus (AF), Nucleus Pulposus (NP), perinuclear zone and endplates. It is described as an altered structural and progressive failure of the cellular mediated response that may be associated with genetic predisposition, chronic mechanisms, trauma, inadequate metabolism and nutritional transport, as well as alterations in enzymatic activity, matrix and water composition of the intervertebral disc (IVD) (Bergknut et al. 2013a; Bergknut et al. 2013b). In the IVDH there is a decrease in glycoaminoglicans and increase in type I and type II collagen level. Thus, the matrix of the disc becomes rigid and with less hydroelastic and hidrostactic properties, essential to his function as an hydraulic protective cushion (Bergknut et al. 2013b). Also, in chondrodystrophic breeds it is common to see dystrophic mineralization of the disc thant can culminate in calcification (Bergknut et al. 2015) and may affect the entire vertebral column. Additionally, the glycoaminoglicans content is lower, when compared to non-chondrodystrophic breeds (Ghosh et al. 1977a; Ghosh et al. 1977b; Bergknut et al. 2015).

This disease is similar in humans (Bergknut et al. 2013b) and may develop initially due to the NP poor nutrition. Although in humans there may be several causes associated with NP

malnutrition, in dogs it is usually related with multigenetic predispositions (Bergknut et al, 2013b; Jeffery et al. 2013; Bacht al. 2014).

Chondroid degeneration is associated with IVD extrusions, also known as Hansen type I hernia. Thus, the nuclear material herniates into the vertebral canal through the ruptured layers of the AF (Fadda et al. 2013; Kranenburg et al. 2013; Smolders and Forterre 2015). Extrusion can occur in any direction, but the most frequent location is at the dorsal level, since the NP has an eccentric position in relation to the AF (Jeffery et al. 2013; Bach et al. 2014; Smolders and Forterre 2015).

Therefore, acute IVDH is characterized by the presence of soft tissue material from the IVD in the spinal canal and by extradural hemorrhage (Tipold et al. 2010). The term “disc extrusion with extensive epidural hemorrhage (DEEH)” has been coined to describe this particular phenomenon (Tartarelli et al. 2005; Fenn and Olby 2020). In addition, spinal cord severe injuries may result in deeper lesions of the white matter that result in decrease/absent deep pain perception (Fingerroth et al. 2015), with possible involvement of grey matter that contains neuronal cell bodies, their dendrites, axons and glia cells, which can compromise prognosis (Uemura 2015b).

Furthermore, in chronic disc extrusion, extradural fibrous adhesions may appear, sometimes as a mineralized mass (Coates 2013), leading to focal compressive myelopathy (Lorenz et al. 2011).

Thus, the extrusion of the degenerated nucleus, in Hansen type I, may result in mixed compressive and contusive injuries, with variable proportions depending on the volume of material and rate of extrusion, possible reproducing the damage of blood vessels in the grey matter of the spinal cord central regions susceptible to the contusion (Jeffery et al 2013). Furthermore, SCI can be described as: $SCI = (\mathcal{X} \times \text{Compression}) + (\mathcal{Y} \times \text{Contusion})$, in which both $\mathcal{X}$ and $\mathcal{Y}$ are the relative contributions to the mechanisms involved ($\mathcal{X} + \mathcal{Y} = 100\%$). However, it has not been identified any correlation between the compressive component and long-term outcomes (Levine et al. 2009).

Herniation of the disc is based on the Newton's laws of physics, translating the impact of a mass (herniated portion of the disc) in the spinal cord with a certain velocity. The kinetic energy delivered can then be defined as $\frac{1}{2} mV^2$ (m = mass; V = velocity) or the force of the herniation as mA (mass times acceleration). So, the greater the kinetic energy or force delivered to the spinal cord, the greater the risk and degree of parenchymal lesion. Again, there

is possibly no connection with the size of the mass (degree of compression) and the severity of clinical signs in dogs (Brasso et al. 1996, Rosenzweig and McDonald 2004).

On the other hand, the compression of the NP results in load transference to the AF and since the dorsal AF is 2-3 times thinner than the ventral, it is usually on the dorsal side that occurs structural failure and disc displacement (Innes and Melrose 2015). Thoracolumbar region is the most affected, although cervical intervertebral disc is also affected (Sanders et al. 2002).

- **Presentation**

It presents a primary SCI given the initial mechanical lesion and the secondary injury due to the development of a complex cascade of biochemical and molecular events, triggered by the primary injury (Brisson 2010). This secondary lesion, consists of vascular dysregulation, neurogenic shock, oxidative stress and erythro-toxicity (Roerig et al. 2013). Also, there is a correlation between the increase in serum matrix metalloproteinase-2 (MMP-2) and the severity of IVDH in dogs (Bergknut et al. 2013b; Bach et al. 2014). Other inflammatory mediators, such as the TNF- α and interleukins -1 β and -6, have also been identified in degenerated IVD (Bergknut et al. 2013b).

This disease is most frequent in chondrodystrophic breeds, such as the Dachshund (Thomas et al. 2015), Pekingese, Welsh Corgi, Beagle, Lhasa Apso and Miniature Poodle (Coates 2013; Dewey and Da Costa 2016). Clinical signs usually appear between two to eight years of age (Kranenburg et al. 2013; Grossbard et al. 2014; Thomas et al. 2015). However, the process has already been described in non-chondrodystrophic breeds such as the Doberman and Labrador Retriever (Olby and Jeffery 2012; Coates 2013; Thomas et al. 2015). In chondrodystrophic breeds, about 75% to 90% manifest the transformation of gelatinous NP into hyaline cartilaginous tissue, around the first year of age. On the other hand, non-chondrodystrophic breeds maintain high levels of non-collagenous proteins until older ages (Brisson 2010).

The thoracolumbar region is the most common in chondrodystrophic breeds diagnosed with IVDH Hansen type I, with the highest incidences between T12-T13 and L1-L2 (Coates 2013; Bach et al. 2014; Smolders and Forterre 2015; Dewey and Da Costa 2016). In regard to the non-chondrodystrophic breeds, the most likely site is between L1-L2 (Coates 2013) and L2-L3 (Bach et al. 2014; Dewey and Da Costa 2016). In addition, the least severe injuries

typically occur in areas where the spinal canal-cord is larger, for example in the cervical region (Lorenz et al. 2011).

Also, there is a correlation between short legged breeds and premature intervertebral disc degeneration (IVDD) (Hansen 1951, 1952). It is well demonstrated that the CFA12 FGF4 retrogene is highly associated to early chondroid metaplasia (Murphy et al. 2019; Dickinson and Bannasch 2020) and consequently the IVDH (Brown et al. 2017; Batchner et al. 2019). This retrogene is commonly associated with mineralization of the IVD, with evidence of calcification (Jensen and Arnbjerg 2001; Rohdin et al. 2010). In regard to the French Bulldog, this retrogene manifests at a significantly younger age (median 3.7 years) compared to the Dachshunds with a median age of 6.5 years (Batchner et al. 2019).

- **Clinical Signs**

As for the clinical signs, the most common is the presence of spinal hyperesthesia that occurs due to the nerve roots and meninges compression (Bos et al. 2012). Hyperesthesia is an exaggerated brain response to a nociceptive stimulus that could result in secondary changes of posture, such as kyphosis (Bos et al. 2012; Dewey and Da Costa 2016). Thus, resulting in exaggerated contraction of the abdominal muscles, with acute abdomen syndrome as one of the main differential diagnoses (Lorenz et al. 2011).

Therefore, the most commonly presented clinical signs are loss of: general proprioception, voluntary motor function, deep pain perception (DPP) and voluntary urination, the latter usually associated with paraplegia (Lorenz et al. 2011; Bos et al. 2012). In Hansen type I hernias, the severity of neurological signs is determined by the neuroanatomical location, speed and amount of compressive material, as well as for the duration of compression (Roerig et al. 2013), although this could be open to discussion debatable. Thus, clinical signs can range from hyperesthesia, ataxia, paresis or paralysis, with or without DPP, urinary and/or fecal incontinence (Fadda et al. 2013) and Schiff-Sherrington postural position (Molina 2015).

Progressive myelomalacia

Progressive myelomalacia (PM) is described as a progressive necrosis of the spinal cord, which can ascend and/or descend along the medullary tissue, potentially affecting several segments or the entire spinal cord (Jeffery et al. 2013; Balducci et al. 2017). In contrast, focal myelomalacia is not characterized by progressive clinical signs and is usually restricted

to the lesion epicenter (Fingerroth and de Lahunta 2015; Gilmour et al. 2016), eventually progressing to a cyst form (Platt et al. 2006).

At the lesion epicenter, hemorrhage, inflammation and medullary necrosis may originate, with possible longitudinal extension throughout the spinal cord from the base of the dorsal funiculus (Griffiths 1972; Lu et al. 2002; Platt et al. 2006). Chondrodystrophic breeds are the ones that show greater susceptibility to the development of PM, specially the Dachshund. The T12-T13 and L3-L6 are the most affected vertebral sites (Balducci et al. 2017; Castel et al. 2017) because, anatomically, there is the dorsolongitudinal ligament over the ventral surface of the spinal canal, and intercapital ligaments that cross the intervertebral disks from T2-T10 (Wisner and Zwingerberger 2015).

Currently, it is believed that nearly 10% of dogs with acute IVDH by extrusion without DPP, may develop PM and meet the criteria for euthanasia (Lu et al. 2002; Olby et al. 2003; Forterre et al. 2010; Muguet-Chanoit et al. 2012; Jeffery et al. 2013; Marquis et al. 2015).

Clinical signs of PM are typically between the first 24 hours and the 5th day after injury, resulting in paraplegia without DPP (Castel et al. 2017). With ascending and/or descending evolution it may be seen the cranial progression of the cutaneous trunci reflex, as well as the shift from upper motor neuron signs to lower motor signs in the hindlimbs (Balducci et al. 2017). The diagnosis could be preventive and the use of the serum phosphorylated neurofilament-heavy chain (pNF-H) is a promising biomarker for ante-mortem diagnosis (Murthy et al. 2021).

PM after SCI may be caused by both contusion and compression events, related to severe changes in the blood supply for the thoracolumbar region by the *arteria radicularis magna*, an important branch of the ventral spinal artery, as well as for the lumbosacral intumescence (Balducci et al. 2017; Castel et al. 2017). The primary injury corresponds to the traumatic event that may cause mechanical destabilization of the neural pathways and microvasculature, resulting in functional and/or morphological changes of the blood supply due to compression, contusion, laceration and/or traction phenomena.

Thus, both compression and contusion play an important role in the compromise of neurological function after IVDH by extrusion, with compression referring to the deforming injury from the severe pressure exerted on the spinal cord parenchyma, and contusion as a parenchyma injury due to the force of the impact (Brisson 2010).

- **Diagnosis**

After neurolocalization by the neurological exam, imaging complementary exams are usually the next step in diagnosis (Jeffery et al. 2013). For dogs with spinal hyperesthesia alone, plain radiographs may be sufficient in diagnosing injuries that may cause bone destruction, such as discospondylitis or neoplasia. Also, x-ray imaging can demonstrate the decreased distance between vertebral endplates, the presence of mineralized disk material on the inner vertebral canal, the narrowing and/or increased opacity of the intervertebral foramen, and vacuum phenomenon (Burk 1989, Lamb et al. 2002), however this is not decisive to give a definitive diagnosis of IVDH (Lamb et al. 2002). Myelography was also used routinely to determine the affected region, but nowadays it is the cross-sectioned imaging with magnetic resonance imaging (MRI) and/or computed tomography (CT) that are considered to be the best techniques in providing accurate information (Shimizu et al. 2009; Robertson and Thrall 2011; Bos et al. 2012). (. Cooper et al. 2014;)

On acute IVDH, the CT may reveal hyperattenuating material within the vertebral canal, loss of epidural fat and distortion of the spinal cord. Whereas in the chronic IVDH, mineralized nucleus pulposus and the extruded material was described as extremely hyperattenuating and homogenous (Olby et al. 2000).

On transverse T1-weighted images (T1-W), MRI it is observed extradural compression caused by extruded nuclear material, typically hypointense, from soft tissue masses and hemorrhage. On T2-weighted images (T2-W), annular and nuclear disc material cannot be distinguished due to the biochemical alterations in the extracellular matrix of the nucleus (Da Costa et al. 2020).

- **Monitoring and Prognosis**

For monitorization of these patients, there was the need to develop a scale to evaluate neurological grade, that could be applied in the daily clinical practice, in an easy and objective way (Levine et al. 2009; Lee et al. 2015 Rousse et al. 2016). Levine et al. (2009) have reported the need to use a repeatable, accurate and easy-to-apply scale, that would allow the evaluation of the neurological degree associated with the neurolocalization of the injury.

Thus, the most common semiquantitative system for gait evaluation in small animals with SCI is the modified Frankel scale (MFS), initially designed for humans with traumatic myelopathies (Levine et al. 2009; Lee et al. 2015). Also, in SCI patients, it is essential a

rigorous clinical examination, whose functional outcome should be punctuated and identified with reliability (Olby et al. 2001).

This injury is usually evaluated on a scale of 0 to 5 (Sharp and Wheeler 2005) with a system that provides useful prognostic information, allowing to make treatment decisions. However, this scale may be less sensitive when evaluating patients admitted in clinical trials, resulting in the development of a movement scale – the Open Field Score (OFS), which is divided into 5 grades and 14 levels. Subsequently, this type of subdivision system allows a more consistent classification of recovery, regarding patients within a specific grade (Olby et al. 2001; Olby et al. 2016)

Injury severity may be evaluated by several scales, but in particular, the evaluation of the DPP, allows the identification of a more severe degree of spinal cord injury (Brisson 2010; Gutierrez-Quintana et al. 2012). Thus, the loss of deep nociception is usually an indicator of bad prognosis (Gutierrez-Quintana et al. 2012; Park et al. 2012; Jeffery et al. 2013; Sims et al. 2015), but, it does not imply a complete section of the spinal cord and that the animal cannot recover, which can happen in nearly 50 % of the cases (Olby et al. 2003; Kerwin et al. 2012; Olby and Jeffery 2012; Jeffery et al. 2013; Jeffery et al. 2016). The functional recovery of grade 0 neurological patients may occur until several months after SCI, even without recovering DPP (Olby et al. 2003).

The Texas Spinal Cord Injury Scale have three major components: gait, proprioceptive positioning (“knuckling”) and nociception (Levine et al. 2009). However, in regard to nociception, it does not consider the neural remodulation of superficial nociception without DPP recovery. Also, in Olby et al. (2014), the stepping score and coordination score, allows the quantification of the number of steps, which provides more detailed information on the reestablishment of communication between the fore and hindlimbs, but not in the DPP negative (DPP⁻) dogs.

Over the years, the absence of DPP has been a major prognostic factor with regard to a patient's ability for obtaining functional locomotion (Ruddle et al. 2006). Nevertheless, it is important to consider that this is a subjective measure with interpretations that may differ between observers (Ruddle et al. 2006) and there is no specific score for monitoring it.

The term of “deep pain perception” has been used historically and refers to the animal’s response to an extremely noxious stimulus applied over the bone of a digit, tested on both medial and lateral digits of each limb (Olby et al. 2020). This assessment, as mentioned before, should be made extremely carefully within a quiet and controlled environment. Thus, DPP

evaluation implies that the animal could feel the stimulus and manifests a repeatable behavioral indication, such as vocalization, looking around, moving away, pupil dilatation due to sympathetic response, which differs from the withdrawal reflex (Fingerroth et al. 2015; Olby et al. 2020). Whenever the patient only demonstrated the withdrawal reflex, with no conscious perception of the noxious stimulus, the reflex wiring is intact, however with possible lesion cranial to the region involving the reflex arc (De Lahunta et al. 2021b).

The pathways that are responsible for DPP are based on reduced diameter axons that make part of multisynaptic tracts - reticulospinal, propriospinal and spinothalamic - located near to the grey matter of the spinal cord, and may be affected more or less severely depending on the injury (Griffin et al. 2009).

Furthermore, in dogs that are DPP⁻ and ambulatory, nearly 40% cannot recover normal fecal continence and 30-53% do not recover normal urinary continence (Olby et al. 2003; Aikawa et al. 2012; Olby et al. 2020). Regarding the DPP⁻ dogs with Hansen type I, 9-33% can develop PM, with most studies reporting a rate between 9 and 17.5%, and one study reporting a rate of 33% in French Bulldogs specifically (Scott and McKee 1999; Aikawa et al. 2014; Balducci et al. 2017; Castel et al. 2019).

There were two studies that reported this specifically and 31% dogs that did not regain DPP, regained the ability to walk within a median time of 9 months (ranging 2-28 months) (Olby et al. 2003; Aikawa et al. 2012). Also, there are reports of nearly 59% of these dogs may regain walking with intensive physical rehabilitation, however this study refers to a mix of etiologies and the DPP assessment is not well described at admission and following outcomes (Gallucci et al. 2017).

For most authors, recovery to a quadrupedal independent ambulation in DPP⁻ dogs, is usually estimated to be around 55%, in contrast to the estimated 90-95% recovery for DPP positive (DPP⁺) dogs (Granger and Carwardine 2014; Freeman and Jeffery 2017; Langerhuus and Miles 2017; Jeffery et al. 2020).

3.2. Intervertebral disc herniation - Hansen type II

The IVDH - Hansen type II can be also called fibroid metaplasia degeneration, due to the progressive thickening of the dorsal AF, which projects dorsally into the vertebral canal. Fibroid metaplasia can lead to gradual disc protrusion. Thus, extrusion of the NP into the extradural space does not occur, but instead a protrusion of the AF (Dewey and Da Costa 2016).

The protrusion of the degenerating annulus into the vertebral canal usually, but not exclusively, occurs within a period of months to years, causing a slow and progressive compression of the spinal cord. This can be accompanied by a dynamic component of compression that may change in severity in each different moment according on the instantaneous position of the vertebrae. Also, the chronic compression leads to axonal loss with evidence of Wallerian degeneration in the distal segments to the injury with prominent damage in the white matter (Jeffery et al. 2013).

Thus, acute clinical signs of IVDH - Hansen type II are less common and compression by protrusion may result in a slow and progressive focal myelopathy (Lorenz et al. 2011), usually manifesting in chronic clinical signs, associated with an insidious onset. Manifestation of pain may occur by the compressive lesion, due to the stimulation of nociceptors in the annulus or nerve compression (Jeffery et al. 2013). Owners usually report a loss of function that may develop from weeks to years, including the damage of both grey and white matter deficits (Fossum et al. 2013; Fenn and Olby 2020). Typically, there is no evidence of hemorrhage and nuclear mineralization, but is possible an aggravation and focal manifestation of acute signs (Brisson 2010).

This disease is mostly frequent in non-chondrodystrophic breeds, such as the German Shepherd and Labrador Retriever (Brisson 2010; Jeffery et al. 2013). Due to its progressive behavior, clinical signs generally appear between five to twelve years of age (Lorenz et al. 2011; Kranenburg et al. 2013).

3.3. Non-compressive myelopathies

3.3.1. Acute non-compressive nucleus pulposus extrusion

- **Introduction**

Acute non-compressive nucleus pulposus extrusion (ANNPE) (Bartner 2020; De Decker and Fenn 2017; Freeman 2020) is described as an extrusion of the non-degenerated or partially degenerated NP that is exposed to excessive force (De Risio et al. 2009). It causes a non-compressive myelopathy due to the explosion of disc material, that is also known as low-volume, high-speed disc extrusion or Hansen type III (Levine and Fingerroth 2015; Coates 2012; Bartner 2020; Freeman 2020; Da Costa et al. 2020).

The ANNPE usually occurs after trauma or intensive exercise, such as running or jumping, without a direct relation to IVDD (De Risio 2015b; Ros et al. 2017) and is mostly described in dogs. After exposure to supra-physiologic forces, the integrity of the IVD may be

compromised (Botsoglou et al. 2021), causing the rupture of the AF (Chang et al. 2007; Bartner 2020). The non-degenerated NP has an osmotic gradient which allows the water transportation and creates a high intradiscal pressure (Bergknut et al. 2013b), leading to the projection of the gelatinous NP into the spinal cord throughout the AF cleft. This can result in focal spinal cord contusion with minimal or no compression (De Risio 2015a, 2015b; Bartner 2020).

After extrusion, the IVD material can lodge in the extradural space (De Lahunta et al. 2021a), as well as in the intramedullary and extramedullary-intradural space (De Risio 2015a).

Thus, the portion of the nucleus that explosively is expelled through the annulus and its displacement, can possibly be observed on MRI and severity varies widely (Jeffery et al. 2013). This sudden extrusion of the nuclear material, and possible penetration into the dura mater (Yarrow and Jeffery 2000; Sanders et al. 2002; Poncelet and Heimann 2011; Kitagawa et al. 2012; Tamura et al. 2015), reflects a contusive injury with minimal compression. Contusion is usually in the ventral and lateral portion of the spinal cord, with disruption of parenchyma (neuronal cell bodies, axons, glial cells, myelin and microvasculature) (Fenn and Olby 2020), that may inclusive even lead to bleeding (Fenn et al. 2016a; Botsoglou et al. 2021).

Contusion is related to axonal and vascular injury, breaking the blood - spinal cord barrier, in association with structural disorder. Also, it promotes the calcium entering into the neuronal cell bodies, axons, astrocytes and oligodendrocytes, which activates a wide range of enzymes (i.e. calpains and caspases), resulting in autodestructive mechanisms that leads to apoptosis or necrosis. Also, free radicals released by enzymatic mitochondrial destruction, activate the microglia (Spitzbarth et al. 2011; Couillard-Despres et al. 2017) and neuronal nitric oxid synthase, causing progressive cycles of cell membranes damage and consequently cells death.

Although, the inflammatory pathways are triggered by microglia activation and secondary cytokine release (i.e. IL-1, TNF- α and nitric oxide), there are also evidences of the neuroprotective effects of the microglia in the SCI due to transforming growth factor β (TGF β) production (David and Kroner 2011; Boekhoff et al. 2012). Furthermore, over longer periods of time, the spinal cord shows the possibility of revascularization (Yasuma et al. 1993) and macrophage invasion (Ito et al. 1996) associated with upregulation of MMP expression (Haro et al. 1999).

In acute cases, the rapid loss of neurologic function can be aggravated by spinal shock appearance, affecting nearly 10% of dogs without deep pain perception, and possibly

worsening with the presence of mechanisms such as vasospasm and thrombosis (Griffiths 1972).

- **Presentation**

It can affect dogs of any breed, but large non-chondrodystrophic are the most common, which usually have slow degenerative changes in the AF (Kim et al. 2020). Most represented breeds are the Border Collie (McKee et al. 2010; Bartner 2020; Freeman 2020), Labrador Retriever and Staffordshire Bull terrier (Fenn et al. 2016a, Fenn et al. 2016b; Mari et al. 2017). Medium breeds can also be affected, such as the Cavalier King Charles Spaniel, Jack Russell Terrier, Miniature Schnauzer, Basenji, Fox terrier, Yorkshire Terrier and Greyhound (De Risio et al. 2009).

Regarding age, most dogs are older than one year old, (Cardy et al. 2015) with median age of diagnostic of 6.7 years, ranging from 2.1 to 10.7 years (De Risio et al. 2009). The association to older animals may be related to AF structure alterations, such as decreased water and proteoglycan content, and also collagen fiber binding changes (Botsoglou et al. 2021). Furthermore, a higher predisposition to males is reported (Fenn et al. 2016a; Mari et al. 2017).

- **Clinical signs**

Clinical presentation of ANNPE is acute and the onset of neurologic deficits may occur in less than 6 hours, given the location and extent of SCI (De Risio 2015a). Affected dogs usually present clinical signs within a maximum of 24 hours, which may be symmetrical or asymmetrical (Bartner 2020).

One of the main reported clinical signs is vocalization (Fenn et al. 2016a; Mari et al. 2017), which seems to be triggered by intensive exercise such as running, jumping, chasing an object, or trauma (De Risio and Platt 2010; Freeman 2020). It has been shown by Ros et al. (2017) that these clinical manifestations occur after running in 66.6% of dogs diagnosed with ANNPE, followed by jumping (4.7%), while traumatic events, such as a car accident was associated with 9.5% and falling from a height with 19% (Ros et al. 2017).

Neurological signs are non-progressive after the first 24 hours and are usually focal. Lateralization of neurological deficits is frequent (De Risio et al. 2009; McKee et al. 2010; Cardy et al. 2015; Fenn et al. 2016a; Fenn et al. 2016b; Behr et al. 2018) with diverse ambulatory

status outcomes, such as possible ambulatory state, non-ambulatory paresis and plegia, with or without DPP (Mari et al. 2017; Ros et al. 2017).

Pain may be present within the first day after the onset of clinical signs, but not if the physical examination is performed long after this period (De Risio et al. 2015; Freeman 2020). Thus, spinal hyperesthesia may be a clinical feature of this disease, but does not usually persist beyond 24 hours (Da Costa et al. 2020). However, studies have reported that clinical signs can also deteriorate (Cardy et al. 2015; Fenn et al. 2016a).

3.3.2. Fibrocartilaginous embolism of the spinal cord

- **Introduction**

The fibrocartilaginous embolism myelopathy (FEM), is the main differential diagnosis of ANNPE with similar clinical presentation (Fenn et al. 2016b; Specchi et al. 2016; Da Costa et al. 2020). Both are common spinal cord diseases in veterinary medicine (De Risio and Platt 2010; Mari et al. 2017). FEM is the most frequent ischemic myelopathy in dogs and occurs when fibrocartilaginous material causes occlusion of the spinal vasculature, followed by ischemic necrosis of dependent regions of the spinal cord parenchyma (Risio and Platt 2010; De Risio 2015b).

Some authors claim that their distinction is not absolutely required, since their treatment and prognosis are very similar (De Risio 2015a; Bartner 2020). However, others report that this differentiation may be crucial in the success of long-term treatment. (Fenn et al. 2016b; Mari et al. 2017). For example, the prevalence of clinical signs, such as fecal incontinence and urinary incontinence can influence the recovery process, making the prognosis guarded (Mari et al. 2017).

Regarding the FEM pathophysiology, several theories have been proposed concerning how a portion of the NP may enter the spinal cord vasculature, although this mechanism is not completely clear (Cauzinille 2000; Moore et al. 2018). These embolisms are more frequent in smaller arteries, but can also be found in veins (De Lahunta and Glass 2009). Also, when the embolus is present in both veins and arteries, it may be due to arteriovenous anastomoses, which are present in the epidural and periradicular space and make this communication possible (Cauzinille 2000; De Risio and Platt 2010).

- **Presentation**

This disease has been mostly reported in middle-aged or older large/giant non-chondrodystrophic breed dogs, however small to medium breeds can also be affected (Freeman 2020). FEM is described in different breeds, such as Miniature Schnauzer, Staffordshire Bull Terrier, Labrador Retriever, German Shepherd, Boxers and crossbreeds (Garosi 2004; De Risio et al. 2007; Fenn et al. 2016b; Mari et al. 2017).

- **Clinical signs**

FEM is usually associated with physical exercise and less frequently related to trauma (Fitzmaurice 2010; Freeman 2020). As mentioned before, clinical presentation of dogs with ANNPE is similar to dogs with FEM (De Risio 2015b). Thus, FEM is described by an acute onset, presenting no pain after the first 24h and clinical signs are typically lateralized (Chang et al. 2007; De Risio et al. 2009). On the other hand, animals with ANNPE may continue to slightly show signs of hyperesthesia more than 24 hours after the clinical onset and this could be the main difference between these two diseases (Chang et al. 2007; De Risio et al. 2009).

On physical examination, it can be shown asymmetry of neurological deficits, as is unlikely the fibrocartilaginous embolism to injure the left and right spinal arteries equally (Freeman 2020). Also, the clinical signs progression is rare and may even show some improvement, depending on the degree of ischemia, presence of other embolisms, or even the secondary spinal cord injury (De Lahunta and Glass 2009; De Risio and Platt 2010).

Treatment of dogs diagnosed with FEM is based on perfusion and neuroprotection in the acute phase, followed by nursing care and physical therapy (De Risio and Platt 2010). Prognosis for recovery may be guarded when the grey matter is injured. However, when this injury affects other regions, such as the white matter, spontaneous recovery is more common (De Lahunta and Glass 2009), with evident improvement two weeks after the onset of clinical signs (Coates 2020).

3.3.3. Diagnosis

The distinction of ANNPE and FEM, given their similar presentation, is only possible through the MRI (Chang et al. 2007; De Risio et al. 2007, 2009; Fenn et al. 2016b; Specchi et al. 2016; Mari et al. 2017; Kim et al. 2020). MRI is the ideal imaging exam that shows images of the intramedullary region and IVDD (De Lahunta and Glass 2009; Fitzmaurice 2010).

However, the definitive diagnosis is only possible through post-mortem histological examination of the injured spinal segments (Mari et al. 2017; De Risio 2015b).

On histological examination it is possible to identify focal contusion of the spinal cord overlying the injured IVD, and occasionally mild epidural hemorrhage (De Risio 2015a) or necrosis (De Decker and Fenn 2017). Also, the AF may possibly show some fissures and material from the non-degenerated extradural NP can be visible within the spinal canal (De Decker and Fenn 2017). Microscopic changes are consistent with grey and white matter malacia and loss of normal architecture (De Risio 2015a). Sometimes, it is possible to note edematous spinal cord and little evidence of IVD material in the epidural space (De Lahunta et al. 2021a).

CT is a diagnostic imaging method that is used in neurological patients (Da Costa et al. 2020). Their advantages, when compared to MRI, are the lower cost, greater availability and possibility to perform in less time. Definitive diagnostic of ANNPE or FEM may not be possible, but it is considered an ideal modality for cases of vertebral fracture and/or luxation (Da Costa et al. 2020).

The CT with myelography may be more sensitive in the diagnosis of extra/intramedullary and intradural NP extrusion, with accumulation of iodinated contrast in the subarachnoid space and/or in the spinal cord parenchyma (Botsoglou et al. 2021). The dispersion of iodinated contrast into the extradural space suggests a dural fissure (De Risio 2015a). Myelography can show a small focal extradural compressive lesion with an intramedullary pattern caused by spinal cord swelling, suggesting that myelography could be used to differentiate between ANNPE and IVDH (Ros et al. 2017).

In regard to the MRI, its non-invasive nature and excellent resolution for soft tissues, makes it a better diagnostic imaging for ANNPE and FEM, and has made the description of intervertebral disc extrusions more accurate (Henke et al. 2013), given their ability to show the spinal cord parenchyma in detail (Jeffery et al. 2013, Maddox 2018).

The T1-W images have excellent resolution to identify anatomical structures, while T2-W are used to identify the presence of disease (Lorenz et al. 2011). On T1-W, the image is most commonly isointense, with no intensification after contrast administration (De Risio et al. 2009). In T2-W images, it can be observed an intramedullary hyperintensity, that is occasionally accompanied by a hypointense center within the linear tracts (Kim et al. 2020).

Studies carried out on the diagnosis by MRI, have shown that the reduction in the volume of the NP was the most observed feature in animals with suspected ANNPE (Fenn et

al. 2016b; Specchi et al. 2016; Mari et al. 2017). The ANNPE presents as a focal lesion due to its potential for contusion, while FEM is an ischemic lesion, with a tendency towards a wider and longer lesion (Fenn et al. 2016b), primarily affecting the grey matter (Abramson et al. 2005). An AF cleft is also a main feature of ANNPE (Specchi et al. 2016; Mari et al. 2017), due to the forces applied during exercise or trauma, that may cause a slight tear in the AF (Bartner 2020).

The adipose tissue is hyper-intensive on T1-W images, so the presence of fat can make it difficult to distinguish between epidural, meningeal and intramedullary lesions (Specchi et al. 2016). Dogs with a presumptive diagnosis of ANNPE have greater evidence of fat in the epidural and meningeal space compared to animals with a presumptive diagnosis of FEM, since adipose tissue may be associated with inflammation secondary to high-velocity extrusion of hydrated NP and revascularization of disc fragments in the epidural space (Specchi et al. 2016).

In regard to neurolocalization, animals with ANNPE are often diagnosed in the T3-L3 spinal cord segment (De Risio et al. 2009; Cardy et al. 2015; Fenn et al. 2016a; Ros et al. 2017) and there are studies with MRI that revealed the most commonly affected site as the T12-T13 (De Risio et al. 2009; McKee et al. 2010; Mari et al. 2017, 2019), following by the T13-L1 spinal cord segment (De Risio et al. 2009; McKee et al. 2010; Mari et al. 2017, 2019; Ros et al. 2017).

3.3.4. Clinical outcomes

The clinical outcomes of dogs diagnosed with presumptive ANNPE or FEM have been previously explored. Ambulation and complete urinary/fecal normal continence were shown in 66.6% of the ANNPE cases and in 84% of the FEM cases (De Risio et al. 2009; Mari et al. 2017). Dogs diagnosed with ANNPE had significantly lower success in the clinical outcome and were found to have a higher risk of developing fecal incontinence (23%), when compared to dogs with FEM (7.5%) (Mari et al. 2017). The inability to control defecation was reported in dogs with fecal incontinence, and the urinary incontinence was frequently described as intermittent rather than persistent (Olby et al. 2003; McKee et al. 2010; Aikawa et al. 2012; Fenn et al. 2016a). Also, the longitudinal extension of the injury does not seem to be associated with the risk of incontinence (Mari et al. 2019).

It is important to remember that during compression and contusion, a series of secondary biochemical alterations occurs, which include inflammation, oxidative stress, vascular events and neurohormonal changes.

This compression may result in slight ischemia that could aggravate demyelination process (Kinns et al. 2006; Carrera et al. 2011). Although a pure compression can be tolerated by the spinal cord to substantial degree, internal disruption of the parenchyma with compromise of the spinal cord structural integrity implies more severe neurological deficits, with less probability to complete recover (or even no recovery). Thus, in compression of SCI, the most important factor is the kinetic energy delivered to the spinal cord, in other words the force of acceleration that is defined by mass times acceleration. It is not the size of the mass that is crucial, both the kinetic force delivered, which correlates to the risk and severity of parenchymal injury (Brasso et al. 1996; Rosenzweig and McDonald 2004). In some extreme situations, mostly in ANNPE, there can be a rupture of the internal vertebral venous plexus causing an haematoma formation (Mckee et al. 2010).

4. References

- Abramson CJ, Garosi L, Platt SR, Dennis R, McConnell JF. 2005. Magnetic resonance imaging appearance of suspected ischemic myelopathy in dogs. *Vet Radiol Ultrasound*. 46:225-229.
- Aikawa T, Fujita H, Kanazono S, Shibata M, Yoshigae Y. 2012. Long-term neurologic outcome of hemilaminectomy and disk fenestration for treatment of dogs with thoracolumbar intervertebral disk herniation: 831 cases (2000-2007). *J Am Vet Med Assoc*. 241:1617-26.
- Aikawa T, Shibata M, Asano M, Hara Y, Tagawa M, Orima H. 2014. A comparison of thoracolumbar intervertebral disc extrusion in French bulldogs and dachshunds and association with congenital vertebral anomalies. *Vet Surg*. 43: 301-7.
- Arendt-Nielsen L, Petersen-Felix S, Fischer M, Bak P, Bjerring P, Zbinden AM. 1995. The effect of N-methyl-D-aspartate antagonist (ketamine) on single and repeated nociceptive stimuli: a placebo-controlled experimental human study. *Anesth Analg*. 81 (1): 63-68.
- Bach FC, Willems N, Penning LC, Ito K, Meij BP, Tryfonidou MA. 2014. Potential regenerative treatment strategies for intervertebral disc degeneration in dogs. *BMC Vet Res*. 10 (3): 1-9.
- Balducci F, Canal S, Contiero B, Bernardini M. 2017. Prevalence and risk factors for presumptive ascending/descending myelomalacia in dogs after thoracolumbar intervertebral disk herniation. *J Vet Intern Med*. 31(2):498-504.
- Baldwin KM, Haddad F, Pandorf CE, Roy RR, Edgerton RV. 2013. Alterations in muscle mass and contractile phenotype in response to unloading models: role of transcriptional/pretranslational mechanisms. *Front Physiol*. 4:284.
- Barros P, Pinheiro J. 2009. In MJ Sá. *Neurologia clínica: compreender as doenças neurológicas*. 1st Ed. Brasil: Edições Universidade Fernando Pessoa.
- Bartner L. 2020. Neurological Disease of the Thoracic Limb. In: Duerr F, ed. *Canine Lameness*, 1st ed. Hoboken, NJ: Wiley-Blackwell. p. 255-269.

Bastiaanse CM, Duysens J, Dietz V. 2000. Modulation of cutaneous reflexes by load receptor input during human walking. *Exp Brain Res*. 135 (2): 189-198.

Batcher K, Dickinson P, Giuffrida M, Sturges B, Vernau K, Knipe M, Rasouliha SH, Drogemuller C, Leeb T, Maciejczyk K. et al. 2019. Phenotypic effects of FGF4 retrogenes on intervertebral disc disease in dogs. *Genes*. 10(6) :435.

Behr S, Radaelli, Tiziana. 2018. Neurological causes of lameness. In: Arthurs G, Brown G, Pettitt R, eds. *Bsava Manual of Canine and Feline Musculoskeletal Disorders*, 2nd ed. Quedgeley, UK: British Small Animal Veterinary Association. p. 167-186.

Behrman AL, Harkema SJ. 2000. Locomotor Training After Human Spinal Cord Injury: A Series of Case Studies. *Phys. Ther*. 80: 688–700.

Bergknut N, Meij BP, Hagman R, De Nies KS, Rutges JP, Smolders LA, Creemers LB, Lagerstedt AS, Hazewinkel HAW, Grinwis GCM. 2013a. Intervertebral disc disease in dogs – part 1: a new histological grading scheme for classification of intervertebral disc degeneration in dogs. *Vet J*. 195: 156-163.

Bergknut N, Smolders LA, Grinwis GCM, Hagman R, Lagerstedt AS, Hazewinkel HAW, Tryfonidou MA, Meij BP. 2013b. Intervertebral disc degeneration in the dog - part 1: anatomy and physiology of the intervertebral disc and characteristics of intervertebral disc degeneration. *Vet J*. 195: 282-291.

Bergknut N, Forterre F, Levine JM, Lasser SD, Fingerroth JM. 2015. Comparisons between biped (human) and quadruped (canine/feline) intervertebral disc disease. In: JM Fingerroth and WB Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*, 1st ed. Iowa: American College of Veterinary Surgeons Foundation (ACVS Foundation) & Wiley-Blackwell. p. 14-19.

Boekhoff TM, Ensinger EM, Carlson R, Bock P, Baumgartner W, Rohn K, Tipold A, Stein VM. 2012. Microglial contribution to secondary injury evaluated in a large animal model of human spinal cord trauma. *J Neurotrauma*. 29:1000–1011.

Bos AS, Brisson BA, Nykamp SG, Poma R, Foster RA. 2012. Accuracy, intermethod agreement, and inter-reviewer agreement for use of magnetic resonance imaging and myelography in small-breed dogs with naturally occurring first-time intervertebral disk extrusion. *J Am Vet Med Assoc*. 240 (8): 969-977.

Botsoglou R, Sarpekidou E, Patsikas M, Kazakos G. 2021. Acute non-compressive nucleus pulposus extrusion in dogs and cats: An overview. *J Hellenic Vet Med Soc*. 72(2): 2809-2816.

Burk RL. 1989. Problems in the radiographic interpretation of intervertebral disc disease in the dog. *Probl Vet Med*. 1:381–401.

Bouyer LJG, Rossignol S. 2003. Contribution of cutaneous inputs from the hindpaw to the control of locomotion in intact cats. *J Neurophysiol*. 90(6): 3625–3639.

Brasso DM, Beattie MS, Bresnahan JC. 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol*. 139:244-56.

Brisson BA. 2010. Intervertebral disc disease in dogs. *Vet Clin North Am Small Anim Pract*. 40 (5): 829-841.

Brown EA, Dickinson PJ, Mansour T, Sturges BK, Aguilar M, Young AE, Korff C, Lind J, Ettinger CL, Varon S, et al. 2017. FGF4 retrogene on CFA12 is responsible for chondrodystrophy and intervertebral disc disease in dogs. *Proc Natl Acad Sci USA*. 114:11476– 81.

Calancie B, Needham-Shropshire B, Jacobs P, Willer K, Zych G, Green BA. 1994. Involuntary stepping after chronic spinal cord injury: evidence for a central rhythm generator for locomotion in man. *Brain*. 117 (5): 1143-1159.

- Cardy TJA, De Decker S, Kenny PJ, Volk HA. 2015. Clinical reasoning in canine spinal disease: what combination of clinical information is useful?. *Vet Rec.* 177(7): 171.
- Carrera I, Sullivan M, McConnell F, Goncalves R. 2011. Magnetic resonance imaging features of discospondylitis in dogs. *Vet Radiol Ultrasound.* 52(2):125-31.
- Cassilhas RC, Tufik S, Mello MT. 2015. Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol Life Sci.* 73(5):975-83.
- Castel A, Olby N, Mariani CL, Muñana KR, Early PJ. 2017. Clinical characteristics of dogs with progressive myelomalacia following acute intervertebral disc extrusion. *J Vet Intern Med.* 31(6):1782–1789.
- Castel A, Olby NJ, Ru H, Mariani CL, Muñana KR, Early PJ. 2019. Risk factors associated with progressive myelomalacia in dogs with complete sensorimotor loss following intervertebral disc extrusion: a retrospective case-control study. *BMC Vet Res.* 15:1–9.
- Cauzinille L. 2000. Fibrocartilaginous embolism in dogs. *Vet Clin North Am Small Anim Pract.* 30(1): 155-167.
- Chang Y, Dennis R, Platt SR, Penderis J. 2007. Magnetic resonance imaging of traumatic intervertebral disc extension in dogs. *Vet Rec.* 160: 795-799.
- Clarac F, Pearlstein E. 2007. Invertebrate preparations and their contribution to neurobiology in the second half of the 20th century. *Brain Res Rev.* 54: 113-161.
- Clarke RW, Eves S, Harris J, Peachey JE, Stuart E. 2002. Interactions between cutaneous afferent inputs to a withdrawal reflex in the decerebrated rabbit and their control by descending and segmental systems. *Neurosci.* 112 (3): 555-571.
- Coates J. 2012. Acute Disc Disease. In: S Platt, L Garosi, eds. *Small Animal Neurological Emergencies*, 1st ed. London: Manson Publishing Ltd. p. 399-416.
- Coates JR. 2013. Paraparesis. In: SR Platt & NJ Olby, eds. *BSAVA Manual of Canine and Feline Neurology*, 4th ed. Gloucester: British Small Animal Veterinary Association. p. 297-327.
- Coates JR. 2020. Myelopathy. In: DS Bruyette, N Bexfield, JD Chretien, L Kidd, S Kube, C Langston, TJ Owen, MA Oyama, N Peterson, LV Reiter, EA Rozanski, C Ruaux, SMF Torres, eds. *Clinical Small Animal Internal Medicine*, volume 1, 1st ed. California: John Wiley & Sons, Inc. p.815- 822.
- Cooper JJ, Young BD, Griffin JF, Fosgate GT, Levine JM. 2014. Comparison between noncontrast computed tomography and magnetic resonance for detection and characterization of thoracolumbar myelopathy caused by intervertebral disk herniation in dogs. *Vet Radiol Ultrasound.* 55:182-189.
- Couillard-Despres S, Bieler L, Vogl M. 2017. Pathophysiology of traumatic spinal cord injury. In: N. Weidner, R. Rupp, K. Tansey, eds. *Neurological aspects of spinal cord injury*. Switzerland: Springer International Publishing. p. 503- 528.
- Côté MP, Murray M, Lemay MA. 2017. Rehabilitation strategies after spinal cord injury: inquiry into the mechanisms of success and failure. *J Neurotrauma.* 34: 1841-1857.
- Côté MP, Murray LM, Knikou M. 2018. Spinal Control of locomotion: Individual neurons, their circuits and functions. *Front. Physiol.* 9: 784.
- Curt A, Van Hedel HJA, Klaus D, Dietz V, EM-SCI Study Group. 2008. Recovery from spinal cord injury: significance of compensation, neural plasticity and repair. *J Neurotrauma.* 25(6): 677-685
- Da Costa RC, De Decker S, Lewis MJ, Volk H, Cansort SCI. 2020. Diagnostic imaging in intervertebral disc disease. *Front Vet Sci.* 7: 782.
- David S, Kroner A. 2011. Repertoire of microglial and macro- phage responses after spinal cord injury. *Nat Rev Neurosci.* 12:388–399.

- De Decker S, Fenn J. 2017. Acute Herniation of Nondegenerate Nucleus Pulposus: Acute Noncompressive Nucleus Pulposus Extrusion and Compressive Hydrated Nucleus Pulposus Extrusion. *Veterinary Clinics of North America: Small Animal Practice*. 48(1): 95-109.
- De Lahunta A, Glass E. 2009. Small animal spinal cord disease. In: A De Lahunta, E Glass, eds. *Veterinary Neuroanatomy and Clinical Neurology*, 3rd ed. Missouri: Elsevier Saunders. p. 243-284.
- De Lahunta A, Glass E, Kent M. 2021a. Small animal spinal cord disease. In: A de Lahunta, EN Glass, M Kent, eds. *Veterinary Neuroanatomy Clinical Neurology*, 5th ed. Philadelphia: Elsevier Saunders. p. 267-311.
- De Lahunta A, Glass E, Kent M. 2021b. General sensory systems: General proprioception and general somatic afferent. In: A de Lahunta, EN Glass, M Kent, eds. *Veterinary Neuroanatomy Clinical Neurology*, 5th ed. Philadelphia: Elsevier Saunders. p. 246-266.
- De Leon RD, Hodgson JA, Roy RR, Edgerton VR. 1998. Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J Neurophysiol*. 80:83–91.
- De Risio L, Adams V, Dennis R, McConnell FJ, Platt SR. 2007. Magnetic resonance imaging findings and clinical associations in 52 dogs with suspected ischemic myelopathy. *J Vet Intern Med*. 21: 1290-1298.
- De Risio L, Adams V, Dennis R, McConell F. 2009. Association of clinical and magnetic resonance imaging findings with outcome in dogs with presumptive acute noncompressive nucleus pulposus extrusion: 42 cases (2000-2007). *J Am Vet Med Assoc*. 234(4): 495-504.
- De Risio L, Platt SR. 2010. Fibrocartilagenous embolic myelopathy in small animals. *Vet Clin North Am Small Anim Pract*. 40: 859-869.
- De Risio L. 2015a. A review of fibrocartilagenous embolic myelopathy and different types of peracute non-compressive intervertebral disk extrusions in dogs and cats. *Front Vet Sci*. 2(24): 1-9.
- De Risio L. 2015b. What is fibrocartilagenous embolism and is it related to IVDD? In: J Fingerroth, W Thomas, eds. *Advances in Intervertebral Disc Diseases in Dogs and Cats*. Iowa: Wiley-Blackwell. p.75-87.
- De Risio L, Thomas W, Fingerroth J. 2015. Traumatic Disc Extrusions. In: J Fingerroth, W Thomas, eds. *Advances in Intervertebral Disc Diseases in Dogs and Cats*. Iowa: Wiley-Blackwell. p.121-126.
- Dewey CW, Da Costa RC. 2016. Myelopathies: Disorders of the spinal cord. In: CW Dewey, RC Da Costa, eds. *Practical Guide to Canine and Feline Neurology*, 3rd ed. Iowa: Wiley-Blackwell. p. 331-337.
- Dickinson PJ, Bannasch DL. 2020. Current Understanding of the Genetics of Intervertebral Disc Degeneration. *Front. Vet. Sci*. 7: 431.
- Dietz V. 1992. Human neuronal control of automatic functional movements: interaction between central programs and afferent input. *Physiol Rev*. 72 (1): 33-69.
- Dietz V, Colombo G, Jensen L, Baumgartner L. 1995. Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol*. 37: 574–582.
- Dietz V. 1997. Neurophysiology of gait disorders: present and future applications. *Electroencephalogr Clin Neurophysiol*. 103 (3): 333-355.
- Dietz V. 2002. Proprioception and locomotor disorders. *Nat Rev Neurosci*. 3:781–90.
- Dietz V, Müller R, Colombo G. 2002. Locomotor activity in spinal man: significance of afferent input from joint and load receptors. *Brain*. 125 (12): 2626-2634.
- Dietz V. 2003. Spinal cord pattern generators for locomotion. *Clin Neurophysiol*. 114:1379–89.
- Dietz V. 2012. Neuronal plasticity after a human spinal cord injury: positive and negative effects. *Exp Neurol*. 235 (1): 110-115.

- Dimitrijevic MR, Gerasimenko Y, Pinter MM. 1998. Evidence for a spinal central pattern generator in humans. *Ann N Y Acad Sci.* 860 (1): 360-376.
- Dimitrijevic M. 2012. Residual motor function after spinal cord injury. In: Dimitrijevic M, Kakulas B, McKay W, Vrbová G, eds. *Restorative neurology of spinal cord injury*. New York: Oxford University Press. p. 1–9.
- Duysens J, Trippel M, Horstmann GA, Dietz V. 1990a. Gating and reversal of reflexes in ankle muscles during human walking. *Exp Brain Res.* 82 (2): 351-358.
- Duysens J, Tax AA, Trippel M, Dietz V. 1990b. Ipsilateral reflex reversal during human locomotion suggest that the biceps femoris reflex is not a simple flexion reflex. In T. Brandt, W. Paulus, W Bles, M Dieterich, S Krafczyk, A Straube (Eds.). *Disorders of Posture and Gait*. Stuttgart: Georg Thieme Verlag. p. 124-127.
- Duysens J, Tax AM, Trippel M, Dietz V. 1992. Phase-dependent reversal of reflexly induced movements during human gait. *Exp Brain Res.* 90 (2): 404-414.
- Duysens J, Van de Crommert HW. 1998. Neural control of locomotion; the central pattern generator from cats to humans. *Gait Posture.* 7:131–41.
- Duysens J. 2002. Human gait as a step in evolution. *Brain*, 125: 2589-90.
- Edgerton VR, De Leon RD, Harkema SJ, Hodgson JA, London N, Reinkensmeyer DJ, Roy RR, Talmadge RJ, Tillakaratne NJ, Timoszyk W, et al. 2001. Retraining the injured spinal cord. *J. Physiol.* 533: 15–22.
- Edwards S. 2002. Abnormal tone and movement as a result of neurological impairment: considerations for treatment. In S. Edwards (Ed.), *Neurological Physiotherapy: A problem-solving approach*. 2nd Ed., Churchill Livingstone: Elsevier Health Sciences. p. 97-132.
- Engelhardt M, Sobotzik JM. 2017. Functional neuroanatomy of the spinal cord. In: N. Weidner, R. Rupp, K. Tansey, eds. *Neurological aspects of spinal cord injury*. Switzerland: Springer International Publishing. p. 19-60.
- Fadda A, Oevermann A, Vandevelde M, Doherr MG, Forterre F, Henke D. 2013. Clinical and pathological analysis of epidural inflammation in intervertebral disk extrusion in dogs. *J Vet Intern Med.* 27: 924-934.
- Fenn J, Drees R, Volk HA, De Decker S. 2016a. Comparison of clinical signs and outcomes between dogs with presumptive ischemic myelopathy and dogs with acute noncompressive nucleus pulposus extrusion. *J Am Vet Med Assoc.* 249(7): 767-775.
- Fenn J, Drees R, Volk HA, Decker S. 2016b. Inter and intraobserver agreement for diagnosing presumptive ischemic myelopathy and a cute noncompressive nucleus pulposus extrusion in dogs using magnetic resonance imaging. *Vet Radiol Ultrasound.* 57(1): 33-40.
- Fenn J, Olby N. 2020. Classification of intervertebral disc disease. *Front Vet Sci.* 7:707.
- Fingerroth JM, de Lahunta A. 2015. Ascending/Descending Myelomalacia Secondary to Intervertebral Disc Herniation Traumatic Disc Extrusions. In: J Fingerroth, W Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*, 1st ed. Ames (IA): CVS Foundation. p.115-120.
- Fitzmaurice S. 2010. *Saunders solutions in veterinary medicine: small animal neurology*, 1st ed. Oxford: Saunders-Elsevier.
- Freeman P, Jeffery ND. 2017. Re-opening the window on fenestration as a treatment for acute thoracolumbar intervertebral disc herniation in dogs. *J Small Anim Pract.* 58:199–204.
- Freeman PM. 2020. Paresis, Paralysis, and Proprioceptive Ataxia. In: PM Freeman, E Ives, eds. *A Practical Approach to Neurology for the Small Animal Practitioner*, 1st ed. Hoboken, NJ: Wiley-Blackwell. p. 275-296.
- Forterre F, Gorgas D, Dickomeit M, Jaggy A, Lang J, Spreng D. 2010. Incidence of spinal compressive lesions in chondrodystrophic dogs with abnormal recovery after

hemilaminectomy for treatment of thoracolumbar disc disease: A prospective magnetic resonance imaging study. *Vet Surg.* 39(2):165–172.

Fossum TW, Dewey CW, Horn CV, Johnson AL, MacPhail CM, Radlinsky MG, Schulz KS, Willard MD. 2013. *Surgery of the Cervical Spine*. In: *Small Animal Surgery*. 4th ed. St. Louis, Missouri: Elsevier.

Gallucci A, Dragone L, Menchetti M, Gagliardo T, Pietra M, Cardinali M, Gandini G. 2017. Acquisition of involuntary spinal locomotion (spinal walking) in dogs with irreversible thoracolumbar spinal cord lesion: 81 dogs. *J Vet Intern Med.* 222:762–6.

Garosi L. 2004. Lesion localization and differential diagnosis. In: SR. Platt, NJ Olby, eds. *Bsava Manual of Canine and Feline Neurology*, 3rd ed. Quedgeley, Gloucs, UK: British Small Animal Veterinary Association. p. 24-34.

Ghosh P, Taylor TK, Braund KG. 1977a. Variation of the glycosaminoglycans of the intervertebral disc with ageing. I. Non-chondrodystrophoid breed. *Gerontology.* 23(2):87-98.

Ghosh P, Taylor TK, Braund KG. 1977b. Variation of the glycosaminoglycans of the intervertebral disc with ageing. II. Non-chondrodystrophoid breed. *Gerontology.* 23(2):99-109.

Gilmour LJ, Jeffery ND, Miles K, Riedesel E. 2016. Single-shot turbo spin echo pulse sequence findings in dogs with and without progressive myelomalacia. *Vet Radiol Ultrasound.* 58(2):197–205.

Granger N, Carwardine D. 2014. Acute spinal cord injury: tetraplegia and paraplegia in small animals. *Vet Clin North Am Small Anim Pract.* 44:1131– 56.

Griffin JF, Levine JM, Kerwin SC, Cole RC. 2009. Canine thoracolumbar intervertebral disk disease: diagnosis, prognosis, and treatment. *Comp. Cont. Educ. Pract. Vet.* 31: E1-E14.

Griffiths IR. 1972. The extensive myelopathy of intervertebral disc protrusions in dogs ('the ascending syndrome'). *J Small Anim Pract.* 13(8):425–437.

Grillner S. 1981. Control of locomotion in bipeds, tetrapods, and fish. *Handbook of Physiology – The Nervous System. Supplement 2*. Washington DC: American Physiological Society. p. 1179-1236

Grillner S, Wallen P. 1985. Central pattern generators for locomotion, with special reference to vertebrates. *Ann Rev Neurosci.* 8:233–61.

Grillner S. 1985. Neurobiological bases on rhythmic motor acts in vertebrates. *Science.* 228:143– 9.

Grillner S. 1986. Interaction between sensory signals and the central networks controlling locomotion in lamprey, dogfish and cat. In S Grillner, P Stein, D Stuart, H Forssberg, R Hermann (Eds) *Neurobiology of vertebrate locomotion*. London: Macmillan. p. 505-512.

Grossbard BP, Loughin CA, Marino DJ, Marino LJ, Sackman J, Umbaugh SE, Solt PS, Afruz J, Leando P, Lesser ML, et al. 2014. Medical infrared imaging (thermography) of type I thoracolumbar disk disease in chondrodystrophic dogs. *Vet Surg.* 43 (7): 869-876.

Guertin PA. 2009. The mammalian central pattern generator for locomotion. *Brain Res Rev.* 62 (1): 45-56.

Guertin PA. 2014. Preclinical evidence supporting the clinical development of central pattern generator-modulating therapies for chronic spinal cord-injured patients. *Front Hum Neurosci.* 8: 272.

Gutierrez-Quintana R, Edgar J, Wessmann A, Cherubini GB, Penderis J. 2012. The cutaneous trunci reflex for localising and grading thoracolumbar spinal cord injuries in dogs. *J Small Anim Pract.* 53: 470-475.

Hansen HJ. 1951. A pathologic-anatomical interpretation of disc degeneration in dogs. *Acta Orthop Scand.* 20:280–93.

Hansen HJ. 1952. A pathologic-anatomical study on disc degeneration in dog, with special reference to the so-called enchondrosis intervertebralis. *Acta Orthop Scand Suppl.* 11:1–117.

- Haro H, Shinomiya K, Murakami S, Spengler DM. 1999. Up-regulated expression of matrilysin and neutrophil collagenase in human herniated discs. *J Spinal Disord.* 12:245–249.
- Harkema SJ, Schmidt-Read M, Lorenz DJ, Edgerton VR, Behrman AL. 2012. Balance and ambulation improvements in individuals with chronic incomplete spinal cord injury using locomotor training-based rehabilitation. *Arch Phys Med Rehabil.* 93 (9): 1508-1517.
- Henke D, Gorgas D, Flegel T, Vandevelde M, Lang J, Doherr MG, Forterre F. 2013. Magnetic resonance imaging findings in dogs with traumatic intervertebral disk extrusion with or without spinal cord compression: 31 cases (2006-2010). *J Am Vet Med Assoc.* 242(2): 217-222.
- Herrero JF, Cervero F. 1996. Supraspinal influences on the facilitation of rat nociceptive reflexes induced by carrageenan monoarthritis. *Neurosci Lett.* 209 (1); 21-24.
- Hongo T, Jankowska E, Lundberg A. 1972. The rubrospinal tract. IV. Effects on interneurons. *Exp Brain Res.* 15 (1): 54-78.
- Hukuda S, Jameson HD, Wilson CB. 1973. Experimental cervical myelopathy. 3. The canine corticospinal tract. Anatomy and function. *Surg Neurol.* 1(2):107-114.
- Innes JF, Melrose J. 2015. Embryology, innervation, morphology, structure and function of the canine intervertebral disc. In: JM Fingerroth, WB Thomas, eds. *Advances in intervertebral disc disease in dogs and cats.* Iowa: Wiley-Blackwell. p. 3-7.
- Ito T, Yamada M, Ikuta F, Fukuda T, Hoshi SI, Kawaji Y, Uchiyama S, Homma T, Takahashi HE. 1996. Histologic evidence of absorption of sequestration-type herniated disc. *Spine (Phila Pa 1976).* 21:230–234.
- Jeffery ND, Levine JM, Olby NJ, Stein VM. 2013. Intervertebral disk degeneration in dogs: consequences, diagnosis, treatment, and future directions. *J Vet Intern Med.* 27 (6): 1318-1333.
- Jeffery ND, Barker AK, Hu HZ, Alcott CJ, Kraus KH, Scanlin EM, & Levine JM. 2016. Factors associated with recovery from paraplegia in dogs with loss of pain perception in the pelvic limbs following intervertebral disk herniation. *J Am Vet Med Assoc.* 248: 386-394.
- Jeffery ND, Olby NJ, Moore SA, CANSORT-SCI. 2020. Clinical Trial Design—A Review-With Emphasis on Acute Intervertebral Disc Herniation. *Front Vet Sci.* 7: 583
- Jensen VF, Arnbjerg J. 2001. Development of intervertebral disk calcification in the dachshund: a prospective longitudinal radiographic study. *J Am Anim Hosp Assoc.* 37:274–82.
- Juvin L, Simmers J, Morin D. 2005. Propriospinal circuitry underlying interlimb coordination in mammalian quadrupedal locomotion. *J Neurosci.* 25:6025-6035.
- Kakulas AB. 1999. A review of the neuropathology of human spinal cord injury with emphasis on special features. *J Spinal Cord Med.* 22 (2), 119-124.
- Kakulas BA, Kaelan C. 2015. The neuropathological foundations for the restorative neurology of spinal cord injury. *Clin. Neurol. Neurosurg.* 129: 51–57.
- Káradóttir R, Attwell D. 2007. Neurotransmitter receptors in the life and death of oligodendrocytes. *NSC.* 145: 1426-1438.
- Kerwin SC, Levine JM, Hicks DG. 2012. Thoracolumbar spine. In: KM Tobias & SA Johnston, eds. *Veterinary Surgery: Small Animal*, 1st volume. St. Louis, Missouri: Elsevier Saunders.
- Kim J, Kim H, Hwang J, Eom K. 2020. Preliminary study of presumptive intradural-intradural intervertebral disc extrusion in 20 dogs. *J Vet Sci.* 21(4): e52.
- Kimura S, Tanabe M, Honda M, Ono H. 2005. Enhanced wind-up of the C-fiber-mediated nociceptive flexor reflex movement following painful diabetic neuropathy in mice. *J Pharmacol Sci.* 97 (2): 195-202.

- Kinns J, Mai W, Seiler G, Zwingenberger A, Johnson V, Caceres A, Valdés-Martínez A, Schwarz T. 2006. Radiographic sensitivity and negative predictive value for acute canine spinal trauma. *Vet Radiol Ultrasound*. 47(6):563-70.
- Kitagawa M, Okada M, Kanayama K, Sakai T. 2012. Identification of ventrolateral intramedullary intervertebral disc herniation in a dog. *J S Afr Vet Assoc*. 83:103.
- Knikou M. 2010. Neural control of locomotion and training-induced plasticity after spinal and cerebral lesions. *Clin. Neurophysiol*. 121: 1655–1668.
- Knikou M. 2012. Plasticity of corticospinal neural control after locomotor training in human spinal cord injury. *Neural Plast*. 2012:254948.
- Knikou M, Mummidisetty CK. 2014. Locomotor training improves premotoneuronal control after chronic spinal cord injury. *J Neurophysiol*. 111:2264–75.
- Kranenbrug HJC, Grinwis GCM, Bergknut N, Gahrman N, Voorhout G, Hazewinkel HAW, Meij BP. 2013. Intervertebral disc disease in dogs – Part 2: comparison of clinical, magnetic resonance imaging, and histological findings in 74 surgically treated dogs. *Vet J*. 195: 164-171
- Lamb CR, Nichols A, Targett M, Mannion P. 2002. Accuracy of survey radiographic diagnosis of intervertebral disc protrusion in dogs. *Vet Radiol Ultrasound*. 43:222–228.
- Langerhuus L, Miles J. 2017. Proportion recovery and times to ambulation for non-ambulatory dogs with thoracolumbar disc extrusions treated with hemilaminectomy or conservative treatment: a systematic review and meta-analysis of case-series studies. *Vet J*. 220:7–16.
- Lavrov I, Gerasimenko YP, Ichiyama RM, Courtine G, Zhong H, Roy RR, Edgerton VR. 2006. Plasticity of spinal cord reflexes after a complete transection in adult rats: relationship to stepping ability. *J Neurophysiol*. 96:1699–710.
- Le Bars D, Guilbaud G, Jurna I, Besson JM. 1976. Differential effects of morphine on responses of dorsal horn lamina V type cells elicited by A and C fibre stimulation in the spinal cat. *Brain Res*. 115 (3): 518-524.
- Leahy JC, Durkovic RG. 1991. Differential synaptic effects on physiological flexor hindlimb motoneurons from cutaneous nerve inputs in spinal cat. *J Neurophysiol*. 66 (2): 460-472.
- Leblond H, Ménard A, Gossard JP. 2001. Corticospinal control of locomotor pathways generating extensor activities in the cat. *Exp Brain Res*. 138 (2): 173-184.
- Lee CS, Bentley RT, Weng HY, Breur GJ. 2015. A preliminary evaluation of the reliability of a modified functional scoring system for assessing neurologic function in ambulatory thoracolumbar myelopathy dogs. *BMC veterinary research*. 11: 241247.
- Levine JM, Fosgate GT, Chen AV, Rushing R, Nghiem PP, Platt SR, Bagley RS, Kent M, Hicks DG, Young BD, et al. 2009. Magnetic resonance imaging in dogs with neurologic impairment due to acute thoracic and lumbar intervertebral disk herniation. *J Vet Intern Med*. 23(6): 1220-6.
- Levine GJ, Levine JM, Budke CM, Kerwin SC, Au J, Vinayak A, Hettlich BF, Slater MR. 2009. Description and repeatability of a newly developed spinal cord injury scale for dogs. *Prev Vet Med*. 89: 121-127.
- Levine J, Levine G, Porter B, Topp K, Noble-Haesslein L. 2011. Naturally occurring disk herniation in dogs: an opportunity for pre-clinical spinal cord injury research. *J Neurotrauma*. 28:675–88.
- Levine JM, Fingerroth JM. 2015. Historical and Current Nomenclature Associated with Intervertebral Disc Pathology. In: J Fingerroth, W Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*. Iowa: Wiley-Blackwell. p. 25-31.
- Li S, Francisco GE. 2015. New insights into the pathophysiology of post-stroke spasticity. *Front Hum Neurosci*. 9:192.

- Lorenz MD, Coates JR, Kent M. 2011. Pelvic Limb Paresis, Paralysis, or Ataxia. In: Handbook of Veterinary Neurology, 5th ed. St Louis: Elsevier Saunders. p. 109-118.
- Lu D, Lamb CR, Targett MP. 2002. Results of myelography in seven dogs with myelomalacia. *Vet Radiol Ultrasound*. 43(4):326–330.
- Macpherson JM, Horak FB, Dunbar DC, Dow RS. 1989. Stance dependence of automatic postural adjustments in humans. *Exp Brain Res*. 78 (3): 557-566.
- Maddox TW. 2018. Imaging Techniques. In: G Arthurs, G Brown, R Pettitt. *Bsava Canine and Feline Musculoskeletal Disorders*, 2nd ed. Quedgeley, Gloucs, United Kingdom: British Small Animal Veterinary Association. p. 359-361.
- Mari L, Behr S, Shea A, Dominguez E, Johnson PJ, Ekiri A, De Risio L. 2017. Outcome comparison in dogs with presumptive diagnosis of thoracolumbar fibrocartilaginous embolic myelopathy and acute non-compressive nucleus pulposus extrusion. *Vet Rec*. 181(11): 293.
- Mari L, Behr S, Shea A, Dominguez E, Ricco C, Alcoverro E, Ekiri A, Sanchez-Masian D, De Risio L. 2019. Predictors of urinary or fecal incontinence in dogs with thoracolumbar acute non-compressive nucleus pulposus extrusion. *J Vet Internal Med*. 33 (66): 2693-2700.
- Marino RJ. 1999. Neurologic recovery after traumatic spinal cord injury: data from the model spinal cord injury systems. *Arch Phys Med Rehabil*. 80 (11): 1391-1396.
- Marquis A, Packer RA, Borgens RB, Duerstock BS. 2015. Increase in oxidative stress biomarkers in dogs with ascending-descending myelomalacia following spinal cord injury. *J Neurol Sci*. 353(1–2):63–69.
- Martins A. 2015. The importance of the quadruped animal model in functional neurorehabilitation for human biped. *International Archives of Medicine: Physical Medicine and Rehabilitation*. 8 (178): 1-9.
- McKee WM, Downes CL, Pink JJ, Gemmill TJ. 2010. Presumptive exercise- associated peracute thoracolumbar disc extrusion in 48 dogs. *Vet Rec*. 166(17): 523-528.
- Molina JJ. 2015. Síndromes neurológicos frecuentes. In: *Manual Práctico de Neurología Veterinaria*. Zaragoza: Servet. p. 62-70.
- Moore BJ, Batterson AM, Luetmer MT, Reeves RK. 2018. Fibrocartilaginous embolic myelopathy: demographics, clinical presentation, and functional outcomes. *Spinal Cord*. 56(12): 1144-1150.
- Muguet-Chanoit AC, Olby NJ, Lim JH, Gallagher R, Niman Z, Dillard S, Campbell J, Early P, Mariani CL, Munana KR, et al. 2012. The cutaneous trunci muscle reflex: a predictor of recovery in dogs with acute thoracolumbar myelopathies caused by intervertebral disc extrusions. *Vet Surg*. 41: 200-206 .
- Murphy BG, Dickinson P, Marcellin-Little DJ, Batcher K, Raverty S, Bannasch D. 2019. Pathologic features of the intervertebral disc in young nova scotia duck tolling retrievers confirms chondrodystrophy degenerative phenotype associated with genotype. *Vet Pathol*. 56:895–902.
- Murthy VD, Li C, Hicks J, Kroll J, Giuffrida M, Dickinson P, Toedebusch CM. 2021. Serum phosphorylated neurofilament heavy chain as a diagnostic biomarker for progressive myelomalacia in dogs with thoracolumbar intervertebral disc herniation. *J Vet Intern Med*. 35(5): 2366-2373.
- Nathan PW, Smith M, Deacon P. 1996. Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. *Brain*. 119:1809–33.
- Nicol DJ, Granat MH, Baxendale RH, Tuson SJ. 1995. Evidence for a human spinal stepping generator. *Brain Res*. 684 (2): 230-232.
- Nilsson J, Thorstensson A, Halbertsma J. 1985. Changes in leg movements and muscle activity with speed of locomotion and mode of progression in humans. *Acta Physiol*. 123 (4): 457-475.

- Norenberg MD, Smith J, Marcillo A. 2004. The pathology of human spinal cord injury: defining the problems. *J Neurotrauma*. 21 (4): 429-440.
- Nout YS, Rosenzweig ES, Brock JH, Strand SC, Moseanko R, Hawbecker S, Zdunowski S, Nielson JL, Roy RR, Courtine G, et al. 2012. Animal models of neurologic disorders: a nonhuman primate model of spinal cord injury. 9: 380-392.
- Olby NJ, Muñana KR, Sharp NJ, Thrall DE. 2000. The computed tomographic appearance of acute thoracolumbar intervertebral disc herniations in dogs. *Vet Radiol Ultrasound*. 41:396-402.
- Olby NJ, De Risio L, Muñana KR, et al. Development of a functional scoring system in dogs with acute spinal cord injuries. *Am J Vet Res* 2001. 62:1624-1628.
- Olby N, Levine J, Harris T, Muñana K, Skeen T, Sharp N. 2003. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord: 87 cases (1996-2001). *JAVMA*. 222 (6): 762-9.
- Olby NJ, Jeffery ND. 2012. Pathogenesis and physiology of central nervous system disease and injury. In: KM Tobias, SA Johnston, eds. *Veterinary Surgery Small Animal*. St Louis: Elsevier Saunders. p. 374-387.
- Olby NJ, Lim JH, Babb K, Bach K, Domaracki C, Williams K, Griffith E, Harris T, Muguet-Chanoit A. 2014. Gait scoring in dogs with thoracolumbar spinal cord injuries when walking on a treadmill. *BMC Veterinary Research*. 10: 58-65.
- Olby NJ, Muguet-Chanoit AC, Lim JH, Davidian M, Mariani CL, Freeman AC, Platt SR, Humphrey J, Kent M, Giovanella C, et al. 2016. A placebo-controlled, prospective, randomized clinical trial of polyethylene glycol and methylprednisolone sodium succinate in dogs with intervertebral disk herniation. *J Vet Intern Med*. 30: 206-214.
- Olby NJ, Da Costa RC, Levine JM, Stein VM. 2020. The Canine Spinal Cord Injury Consortium (CANSORT SCI). Prognostic Factors in Canine Acute Intervertebral Disc Disease. *Front Vet Sci*. 7 (596059): 144-157.
- Pang MY, Yang JF. 2000. The initiation of the swing phase in human infant stepping: importance of hip position and leg loading. *J Physiol* 528 (2): 389-404.
- Park EH, White GA, Tieber LM. 2012. Mechanisms of injury and emergency care of acute spinal cord injury in dogs and cats. *J Vet Emerg Crit Care*. 22: 160-178.
- Pearson K. 2000a. Motor systems. *Curr Opin in Neurobiology*. 10 (5): 649-654.
- Pearson K. 2000b. Neural adaptation in the generation of rhythmic behavior. *Annu Rev Physiol*. 62 (1): 723-753.
- Pearson KG. 2007. Role of sensory feedback in the control of stance duration in walking cats. *Brain Res Rev*. 57: 222-227.
- Petersen TH. 2014. Technical device for measurement of spasticity, developed for bedside use. In W. Jensen, OK. Andersen & M. Akay (Eds.), *Proceedings of the 2nd International Conference of Neurorehabilitation (ICNR): Replace, Repair, Restore, Relieve - Bridging Clinical and Engineering Solutions in Neurorehabilitation*; 24-26 June; Aalborg: Springer. p. 127-128.
- Platt SR, McConnell J, Bestbier M. 2006. Magnetic resonance imaging characteristics of ascending hemorrhagic myelomalacia in a dog. *Vet Radiol Ultrasound*. 47(1):78-82.
- Poncelet L, Heimann M. 2011. Intradural vertebral disc herniation in a dog. *Vet Rec*. 168:486.
- Raineteau O, Schwab ME. 2001. Plasticity of motor systems after incomplete spinal cord injury. *Nat. Rev. Neurosci*. 2 (4): 263-73.
- Robertson I, Thrall DE. 2011. Imaging dogs with suspected disc herniation: Pros and cons of myelography, computed tomography, and magnetic resonance. *Vet Radiol Ultrasound*. 52(1 Suppl 1):S81-S84.

Roerig A, Carlson R, Tipold A, Stein VM. 2013. Cerebrospinal fluid tau protein as a biomarker for severity of spinal cord injury in dogs with intervertebral disc herniation. *Vet J.* 197 (2): 253-258.

Rohdin C, Jeserevic J, Viitmaa R, Cizinauskas S. 2010. Prevalence of radiographic detectable intervertebral disc calcifications in Dachshunds surgically treated for disc extrusion. *Acta Vet Scand.* 52:24.

Ropper A, Samuels M, Klein J. 2014. Motor paralysis. In: Victor M, Ropper AH, Adams RD, editors. *Adams and Victor's principles of neurology*. 1st Ed. China: McGraw-Hill Education. p. 45–63.

Ros C, de la Fuente R, Rodenas S, Novellas R, Viu J, Añor S. 2017. Myelographic and low-field magnetic resonance imaging findings in dogs with presumptive acute hydrated non-compressive nucleus pulposus extrusion. *Vet Rec.* 181(22): 594-599.

Rosenzweig ES, McDonald JW. 2004. Rodent models for treatment of spinal cord injury: research trends and progress toward useful repair. *Curr Opin Neurol.* 17(2):121-31.

Rossignol S, Dubuc R. 1994. Spinal pattern generation. *Curr Opin Neurobiol.* 894 – 902.

Rossignol S, Dubuc R, Gossard JP. 2006. Dynamic Sensorimotor Interactions in Locomotion. *Physiol. Rev.* 86: 89–154.

Rossignol S, Frigon A. 2011. Recovery of Locomotion After Spinal Cord Injury: Some Facts and Mechanisms. *Annu. Rev. Neurosci.* 34: 413–440.

Rousse CA, Olby NJ, Williams K, Harris TL, Griffith E, Mariani CL, Muñana KR, Early P. 2016. Recovery of stepping and coordination in dogs following acute thoracolumbar intervertebral disc herniations. *Vet J.* 213: 59-63.

Ruddle TL, Allen DA, Schertel ER, Barnhart MD, Wilson ER, Lineberger JA, Klocke NW, Lehenbauer TW. 2006. Outcome and prognostic factors in nonambulatory Hansen type I intervertebral disc extrusions: 308 cases. *Vet Comp Orthop Traumatol.* 19(01): 29-34.

Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC. 2005. The lower limb flexion reflex in humans. *Prog Neurobiol.* 77 (6):353-395.

Sanders SG, Bagley RS, Gavin PR. 2002. Intramedullary spinal cord damage associated with intervertebral disk material in a dog. *J Am Vet Med Assoc.* 221:1594-6.

Schomburg ED, Petersen N, Barajon I, Hultborn H. 1998. Flexor reflex afferents reset the step cycle during fictive locomotion in the cat. *Exp Brain Res.* 122 (3): 339-350.

Schomburg ED, Jankowska E, Fernstrom KW. 2000. Nociceptive input to ascending tract neurones forwarding information from low threshold cutaneous and muscle afferents in cats. *Neurosci res.* 38 (1): 117-120.

Schubert M. 2017. Natural course of disease of spinal cord injury. In: N Weidner, R Rupp, K Tansey, eds. *Neurological aspects of spinal cord injury*. Switzerland:Springer International Publishing.p. 77- 105.

Scott HW, McKee WM. 1999. Laminectomy for 34 dogs with thoracolumbar intervertebral disc disease and loss of deep pain perception. *J Small Anim Pract.* 40:417–22.

Serrao M, Rossi P, Sandrini G, Parisi L, Amabile GA, Nappi G, Pierelli F. 2004. Effects of diffuse noxious inhibitory controls on temporal summation of the RIII reflex in humans. *Pain.* 112 (3): 353-360.

Shah PK, Garcia-Alias G, Choe J, Gad P, Gerasimenko Y, Tillakaratne N, Zhong H, Roy RR, Edgerton VR. 2013. Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain.* 136:3362–77.

Sharp NJH, Wheeler SJ. 2005. Thoracolumbar disc disease. In: *Small animal spinal disorders: diagnosis and surgery*, 2nd ed. Oxford, England: Elsevier Mosby.

Shimizu J, Yamada K, Mochida K, Kako T, Muroya N, Teratani Y, Kishimoto M, Lee K, Iwasaki T, Miyake Y. 2009. Comparison of the diagnosis of intervertebral disc herniation in dogs by CT before and after contrast enhancement of the subarachnoid space. *Vet Rec.* 165:200-202.

Sims C, Waldron R, Marcellin-Little DJ. 2015. Rehabilitation and Physical Therapy for the Neurologic Veterinary Patient. *Vet Clin North Am Small Anim Pract.* 45(1): 123-143.

Smith PM, Jeffery ND. 2006. Histological and Ultrastructural Analysis of White Matter Damage after Naturally-occurring Spinal Cord Injury. *Brain Pathol.* 16 (2): 99-109.

Smith AC, Knikou M. 2016. A review on locomotor training after spinal cord injury: reorganization of spinal neuronal circuits and recovery of motor function. *Neural Plast.* 1216258:1-20.

Smolders LA, Forterre F. 2015. Biomechanics of the intervertebral disc and why do discs displace?. In: JM Fingerroth and WB Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*, 1st ed. Iowa: American College of Veterinary Surgeons Foundation (ACVS Foundation) & Wiley Blackwell. p. 14-19.

Solopova IA, Selionov VA, Sylos-Labini F, Gurfinkel VS, Lacquaniti F, Ivanenko YP. 2015. Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Front Syst Neurosci.* 9:14.

Spaich EG, Arendt-Nielsen L, Andersen OK. 2004. Modulation of lower limb withdrawal reflexes during gait: a topographical study. *J Neurophysiol.* 91 (1): 258-266.

Specchi S, Johnson P, Beauchamp G, Masseau I, Pey P. 2016. Assessment of interobserver agreement and use of selected magnetic resonance imaging variables for differentiation of acute noncompressive nucleus pulposus extrusion and ischemic myelopathy in dogs. *J Am Vet Med Assoc.* 248(9): 1013-1021.

Spitzbarth I, Bock P, Haist V, Stein VM, Tipold A, Wewetzer K, Baumgartner W, Beineke A. 2011. Prominent microglial activation in the early proinflammatory immune response in naturally occurring canine spinal cord injury. *J Neuropathol Exp Neurol.* 70:703–714.

Takakusaki K, Kohyama J, Matsuyama K, Mori S. 2001. Medullary reticulospinal tract mediating the generalized motor inhibition in cats: parallel inhibitory mechanisms acting on motoneurons and on interneuronal transmission in reflex pathways. *Neurosci.* 103(2):511-27.

Takeoka A, Vollenweider I, Courtine G, Arber S. 2014. Muscle Spindle Feedback Directs Locomotor Recovery and Circuit Reorganization after Spinal Cord Injury. *Cell.* 159: 1626–1639.

Tamura S, Doi S, Tamura Y, Takahashi K, Enomoto H, Ozawa T, Uchida, K. 2015. Thoracolumbar intradural disc herniation in eight dogs: clinical, low-field magnetic resonance imaging, and computed tomographic myelography findings. *Vet Radiol Ultrasound.* 56:160-7.

Tansey KE. 2010. Neural plasticity and locomotor recovery after spinal cord injury. *PM R.* 2: S220-6.

Tartarelli CL, Baroni M, Borghi M. 2005. Thoracolumbar disc extrusion associated with extensive epidural haemorrhage: a retrospective study of 23 dogs. *J Small Anim Pract.* 46:485–90.

Ten Bruggencate G, Lundberg A. 1974. Facilitatory interaction in transmission to motoneurons from vestibulospinal fibres and contralateral primary afferents. *Exp Brain Res.* 19 (3): 248-270.

Thomas WB, De Risio L. 2015. History, Neurologic Examination, and Neuroanatomic Localization for Spinal Cord and Nerve Root Disease. In: JM Fingerroth and WB Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*. Iowa: John Wiley & Sons, Inc. p. 92-106.

Thomas WB, Fingerroth JM, Hagman R. 2015. What do we know about the incidence of disc disease in chondrodystrophoid dogs?. In: JM Fingerroth and WB Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*, 1st ed. Iowa: John Wiley & Sons, Inc. p. 32-34.

Thomson C, Hahn C. 2012. Reflexes and motor systems. In: Thomson C, Hahn C, editors. *Veterinary neuroanatomy: a clinical approach*. 1st Ed. Edinburgh: Elsevier. p. 47–57.

Thompson PD, Nutt JG. 2012. Gait disorders. In: RB Daroff, J Jankovic, Mazziotta JC. Pomeroy SL, eds. *Bradley's Neurology in Clinical Practice* 6th ed. China: Elsevier Inc. p.250-261.

Tipold A, Bernardini M, Kornberg M. 2010. Spinal cord. In: A Jaggy, SR Platt, eds. *Small Animal Neurology: An Illustrated Text*. Germany: Schlütersche. p. 333-362.

Uemura E. Motor system. 2015a. In: Uemura E, editor. *Fundamentals of canine neuroanatomy and neurophysiology*. 1st Ed. Iowa: Wiley. p. 257–85.

Uemura E. Spinal Cord. 2015b. In: Uemura E, editor. *Fundamentals of canine neuroanatomy and neurophysiology*. 1st Ed. Iowa: Wiley. p. 99-119.

Van de Crommert HW, Mulder T, Duysens J. 1998. Neural control of locomotion: Sensory control of the central pattern generator and its relation to treadmill training. *Gait Post.* 7: 251–263.

Vanzulli I, Butt AM. 2015. mGluR5 protect astrocytes from ischemic damage in postnatal CNS white matter. *Cell Calcium*. 58:423-430.

Verkhatsky A, Steinhäuser C. 2000. Ion channels in glial cells. *Brain Res Rev.* 32: 380-412.

Vilensky JA, O'Connor BL. 1997. Stepping in humans with complete spinal cord transection: a phylogenetic evaluation. *Mot Cont.* 1:284 – 92.

Weng HR, Schouenborg J. 1998. On the cutaneous receptors contributing to withdrawal reflex pathways in the decerebrate spinal rat. *Exp Brain Res.* 118 (1):71-77.

Williams PR, Marincu BN, Sorbara CD, Mahler CF, Schumacher AM, Griesbeck O, Kerschensteiner M, Mischak H. 2014. A recoverable state of axon injury persists for hours after spinal cord contusion in vivo. *Nat Commun.* 5: 5683.

Wisner E, Zwingenberger A. 2015. Vertebral Column & Spinal Cord. In: E Wisner and AL Zwingenberger. *Atlas of Small Animal CT and MRI*, 1st ed. Iowa: John Wiley & Sons, Inc. p. 355-375.

Wright F, Palmer AC. 1969. Morphological changes caused by pressure on the spinal cord. *Pathol Vet.* 6 (4): 355-368.

Yang JF, Stephens MJ, Vishram R. 1998. Transient disturbances to one limb produce coordinated, bilateral responses during infant stepping. *J Neurophysiol.* 79 (5): 2329-2337.

Yarrow TG, Jeffery ND. 2000. Dura mater laceration associated with acute paraplegia in three dogs. *Vet Rec.* 146:138-9.

Yasuma T, Arai K, Yamauchi Y. 1993. The histology of lumbar intervertebral disc herniation. The significance of small blood vessels in the extruded tissue. *Spine (Phila Pa 1976).* 18:1761– 1765.

Zehr EP, Stein RB, Komiyama T. 1998. Function of sural nerve reflexes during human walking. *J Physiol.* 507 (1): 305-314.

Zehr EP, Stein RB. 1999. What functions do reflexes serve during human locomotion? *Prog Neurobiol.* 58 (2): 185-205.

CHAPTER II

STATE OF ART AND OBJECTIVES – NERVOUS SYSTEM NEUROMODULATION AND NEUROSTIMULATION

1. State of Art

Severe spinal cord injuries after a surgical approach, require neural modulation and reorganization protocols based on reflexes and their automatization. Thus, the coordination and stimulation of the intramuscular spindle, mechanoreceptors, medullary segments, ascending sensory and descending motor tracts, is essential.

Emerging in the field of human medicine, electrical stimulation modalities associated with programs of movement repetition, allow a positive evolution and clinical success with this new approach to neurorehabilitation, based on therapeutic modalities and locomotor training.

This approach may be translated to the small animals, in situations of compressive or non-compressive myelopathies, such as spinal contusions.

In veterinary clinical practice, one of the most frequent neurological problems are severe spinal cord injuries, since they limit the individual's functionality due to total loss of sensory and motor function (Min et al. 2014).

The most common etiologies of SCI in dogs are IVDD, ischemic myelopathy due to fibrocartilaginous embolism and trauma (Olby 2010). IVDD is the most common cause in dogs, which corresponds to 1% of all cases observed in animal clinical practice, being this an important cause of disability or euthanasia (Olby et al. 2001; Olby et al. 2003; Olby et al. 2016).

Severe SCI are associated to all patients classified as grade 0 or 1, according to the MFS, having as a prognostic indicator the absence or presence DPP. DPP is transmitted by small diameter axons encompassed in the multisynaptic bundles, that is, on the spinoreticular tracts, on the propriospinal tracts and, possibly, on the spinothalamic tracts, located near the spinal cord grey matter (Olby et al. 2003; De Lahunta and Glass 2014).

In these severe SCI, there is a massive destruction of the medullar parenchyma, although there may be some maintained axonal connections, that are targeted for measures with therapeutic potential (Lim et al. 2014).

Lewis et al. (2018), suggested that there is an interconnection between residual motor descending tracts and motor interneurons intrinsic circuits, responsible for flexion-extension pattern of locomotion, which can allow functional fictive locomotion. That is, a considered reflex-based spinal movement, but in which the grade 0 patient can get up and walk independently.

Therefore, FNR approaches emerge in order to promote voluntary locomotion or even functional fictive locomotion, based on neural modulated reorganization, that can be also called, in a clinical setting, spinal reflex locomotion (SRL).

FNR explores the intrinsic spinal cord properties, such as synaptic and anatomical neuroplasticity (Martins 2015; Solopova et al. 2015). The synaptic neuroplasticity obtains the modification of pre-existent neural circuits, while anatomic neuroplasticity develops new circuits and connections, allowing growth of new axonal branches and dendrites (Grasso et al. 2004). Neural plasticity stimulation, is not enough to obtain the patient's functionality, but it is needed to perform neural plasticity modulation, that is, neuromodulation (Raineteau and Schwab 2001).

Neuromodulation is a field of the neurosciences, medicine and bioengineering that aims to inhibit, stimulate, modify and regulate, or even to obtain therapeutic changes in electrical and chemical activity of the peripheral and CNS, thus modulating its function, in order to promote an increase in quality of life and functionality in humans (Krames et al. 2009).

Towards these FNR guidelines, and according to neural modulation and neural reorganization, in the last decades of human medicine, there has been many studies in regard to the application of three electrostimulation techniques: functional electrical stimulation (FES), transcutaneous electrical spinal cord stimulation (TESCS) and transcranial direct current stimulation (TDCS).

1.1. Functional Electrical Stimulation

FES is a neuromodulation modality that is based on the use of short bursts of electrical pulses sequences, aiming to stimulate the LMN near the motor endplate region, or through peripheral afferent stimulation, resulting in peripheral spinal reflex (Kralj et al. 1983; Holsheimer 1998b).

It may be used to initiate a motor response in patients with SCI, with the intent of restoring functionality (Granat et al. 1993). FES was applied for the first time in 1961, by Liberson and collaborators, in bipedal hemiplegic patients, in which the stimulation of the peroneal nerve was able to correct proprioceptive positioning deficits that occurred during the swing phase of locomotion (Liberson et al. 1961; Granat et al. 1993).

This modality is based on the use of surface electrodes to stimulate the quadriceps femoris muscle group, therefore restoring and promoting the postural and swing phase of gait (Kralj and Grobelnik 1973). FES is a rehabilitation therapeutic modality (RTM) that applies the basic principle of adaptation of an electric current to a nerve, in order to make an increase in neuron action potentials, that may be decreased and not able to generate a physiological muscle contraction (Pilkar et al. 2012).

Also, FES is considered to be a coordination modality of the neuromuscular system, promoting functional movement. Its mechanism of action was based on nerve stimulation (nerve fibers) through low intensity currents, but enough to allow the association of an action potential, necessary for the depolarization of the same and future muscle contraction (Richard et al. 2015).

As in all RTM, accurate electrical current parameters are paramount to the success of the excitability of a given tissue. Therefore, all FES protocols should be programmed according to current magnitude/amplitude, duration, frequency, waveform, and the cycle between the start and the end of the current emission, which is also known as the duty cycle (Hamid and Hayek 2011).

FES is the result of an unnatural recruitment of muscle fibers, beginning by large diameter motor neurons, which are considered functionally quicker, instead of smaller diameter motor neurons, which are considered slower and more resistant (Fouad and Tetzlaff 2012). Therefore, with a knowledge of the recruitment physiology action behind FES, it is of great importance that its protocols are properly programmed, regarding both frequency and pulse, so that it is possible to avoid muscle fatigue with lactic acid accumulation, while simultaneously promoting neural excitability in a non-invasive way (Fouad and Tetzlaff 2012).

The neuromuscular or neurophysiological effect of electrical stimulation can be registered through surface electromyography, though there are some difficulties in the interpretation of this exam, due to the presence of electrical artifacts (Pilkar et al. 2012).

Johnston et al. (2005) supported the viability of FES as a therapeutic modality for patients without LMN lesions and stated that it should be applied using surface electrodes in order to promote muscle strengthening and neurological recovery, avoiding progression of muscle atrophy.

When performed as an RTM, FES may contribute to a functional ambulatory state through the development of spinal polysynaptic reflexes, which are used to identify the spinal interneuronal intrinsic circuits, related to both voluntary and fictive locomotion (spinal reflex locomotion) (Hubli et al. 2012).

Nowadays, human medicine considers the electrical stimulation of the skeletal muscle to be an important tool for excitability modulation of spinal reflexes, particularly in patients with complete spinal injury, allowing development of long-lasting peripheral spinal reflexes, similar to those of cats suffering from total spinal cord section. These reflexes are considered to be

the result of the interneurons activity in spinal locomotor centers, such as the intrinsic spinal circuits of motor interneurons and CPG (Serrao 2014).

The combination of FES with locomotor training is highly reported amongst both quadrupedal animals and humans (Hesse et al. 1995; Daly et al. 2004; Lindquist et al. 2007; Lee et al. 2013; Thompson 2014), with evidences of promoting neuroplasticity and allowing functional development of gait, mainly in regard to speed, step length and to the cadence itself (Hesse et al. 1995).

Thus, FES should be incorporated in veterinary neurorehabilitation protocols, as a RTM that promotes functional neural reorganization, especially in the initial phase of gait, similarly to what currently happens in human medicine for the management of UMN syndromes (Daly et al. 2004; Serrao 2014).

1.2. Transcutaneous Electrical Spinal Cord Stimulation

TESCS is a therapeutic method based on electrical activation of spinal cord circuits, by means of surface electrodes placed on the skin in the lower thoracic region or in the lumbosacral area (Gerasimenko et al. 2015).

It is a non-invasive and non-painful RTM with the ability to neuro-modulate the physiological state of the spinal cord (Gerasimenko et al. 2015).

TESCS has synergists and interactive multi-segmental effects, which converge with the motor descending tracts and with the ascending sensorial tracts, projecting into the neural intrinsic circuits during spinal cord electrical stimulation (Sayenko et al. 2015).

This type of multifocal TESCS has a key role when it is necessary to activate and stimulate the spinal neurons intrinsic circuits, in order to achieve a position of postural standing and locomotor activity (Courtine et al. 2007; Minassian et al. 2007a; Sayenko et al. 2015).

In 1942, Lloyd investigated the first electrical stimulation, through the dorsal and ventral horn of the same spinal segment, obtaining a peak of spinal peripheral reflex, in the transmission between the two neurons, designated by ventral horn/muscle reflex (VH/MR) (Lloyd 1942; Minassian et al. 2007).

Afterwards in human medicine, this reflex was considered important for achieving results during epidural electrical stimulation (EES) of the lumbar intumescence, a neuroanatomical region of the motor interneurons intrinsic circuit, associated to the CPG (Shah et al. 2013; Lavrov et al. 2015; Martins 2015). In addition, the current flow and distribution of

the electric potential generated by TESCS and EES are very similar (Holsheimer 1998b; Rattay et al. 2000; Holsheimer 2002; Hofstoetter et al. 2014)

In 2007, Minassian and collaborators performed a study to demonstrate the action of TESCS placed over the T11-T12 vertebrae, with the purpose of stimulating the VH/MR of the quadriceps femoris and flexor muscles. Furthermore, it was performed a TESCS control, with two electrodes placed on L4 to L5 region.

Minassian et al. (2007a) reported that to obtain a depolarized current, cathodes should be placed bilaterally on T11- T12 and the anode at the abdominal area, specifically in the umbilical region, also bilaterally. This stimulation of surface electrodes allows a perpendicular current penetration, migrating by the ligaments between the spinous processes in the vertebral lamina, and so penetrating on the vertebral canal, allowing a current conduction into the cerebrospinal fluid (Holsheimer 1998a; Minassian et al. 2007a).

In 2009, Kitano and Kocaja, conducted a study that aimed to compare the effect of the TESCS and of the traditional peripheral electrical stimulation, that is, through the H-reflex technique, which is an electrophysiological response record. H-reflex is a reflex produced by afferent conduction through Ia afferent fibers, and by efferent conduction through α -motor neurons. Thus, it is a spinal monosynaptic reflex produced after an electrical stimulus. It reflects muscle spindle action, as well as the stretch reflex after the Achilles tendon mechanical stimulation (Fisher 2012; Kimura 2013).

Kitano and Kocaja (2009) study, intended to observe what should be the maximum H value obtained between the two techniques, using three muscle groups, one group with about 80% of slow nervous fibers and two groups with about 50% of the same fibers. In the study it was verified that the TESCS and the traditional peripheral electrical stimulation techniques showed no significant difference in the maximum H value, although in the groups with higher number of high-speed nervous fibers, TESCS technique had a greater H-maximum value. Therefore, TESCS showed superior power in the monosynaptic multisegmental response (Kitano and Kocaja 2009), allowing a selective excitation of the sensorial fibers, producing a direct motor response and an antidromic current on the motor fibers (Courtine et al. 2007).

The progressive study of TESCS, has demonstrated that the best-chosen anatomical region until 2011, was the previously referred (Minassian et al. 2007a; Hofstoetter et al. 2008; Kitano and Kocaja, 2009; Danner et al. 2011) with the multisegmental monosynaptic as a response (Courtine et al. 2007; Minassian et al. 2007a; Kitano and Kocaja 2009). Although for Akaza et al. (2010) and Troni et al. (2011), the supra-stimulation of TESCS is performed with

electrodes placing on the region of T12-L1 and for the last author the best placement of the electrodes should be always tested, considering an individual basis.

Roy et al. (2012), intended to study the several possible positions of the electrodes in the spinal cord, so that it could stimulate a higher number of connections in the lumbosacral intumescence. According to these authors, the best location was considered to be at the beginning of the enlargement of the lumbosacral intumescence, thus T11-T12 or T12-L1. The sensorial and motor nervous fibers stimulation depends on the cathode position along the vertebral column, noting that the sensorial nervous fibers stimulation is supported by the L1-L3 region, but for the recruitment of motor nervous fibers the best location should be caudally, near the anatomical region L5-S1.

Also, the geometric changings of the thoracolumbar vertebral column, as well as the position of the adjacent vertebra, could influence the progression of current depolarization and the stimulation of possible intra-spinal interconnections at the lumbosacral intumescence (Roy et al. 2012).

Hofstoetter et al. (2014), with electrodes in the standard location at T11/T12 and a continuous current of 50 Hz, intended to depolarize the large afferent diameter fibers of L2-S2 region and its posterior roots on the human biped (Dimitrijevic et al. 2004; Minassian et al. 2011). In establishing this current, the segmental neural circuit of the lumbar intumescence is activated, triggering neural connections in a higher way when comparing with the epidural technique (Minassian et al. 2011; Minassian et al. 2012), as referred previously.

With the application of a 50 Hz continuous current, it would be expected to observe a contraction of the paraspinal and abdominal muscles, in addition to paresthesia at the lower limbs in the biped patient (Hofstoetter et al. 2014; Hofstoetter et al. 2018). The duration of this current was nearly 30 minutes, and the evaluation of spasticity, as a clinical sign, was done immediately at the end of each session, through the correlation between the Wartenburg pendulum test and the Ashworth scale, in order to validate results. The final result showed a decrease in muscle contraction co-activation, leading to a faster gait. On three patients of this study there was evidence of decrease on the tonic and clonic phases during the examination of withdraw reflex (Minassian et al. 2012).

For Hofstoetter et al. (2014), the progressive current is due to the depolarization of afferent large diameter fibers on L2-S2 segments, passing through ligaments and discs, which have the role to enable a faster path, allowing the current to flow by the medullar canal (Ladenbauer et al. 2010; Danner et al. 2011; Minassian et al. 2011).

When the current enters the medullar canal, only about 8% (Ladenbauer et al. 2010) depolarizes the nervous root, given the progression of the current through the descending tracts until the neural intrinsic circuit (Hofstoetter et al. 2014). This reflects in the expression of active vibrations on the Achilles tendon (Minassian et al. 2007a) and by the presence of passive or active movements at the lower limbs in humans (Minassian et al. 2007a; Hofstoetter et al. 2008; Dy et al. 2010; Hofstoetter et al. 2018).

Also, the Ia afferent fibers perform synaptic connections with Ia inhibitor interneurons, which allows the reduction of the clinical sign of spasticity (Hofstoetter et al. 2014). Thus, it is the author belief that the depolarization of motor descending tracts doesn't occur directly, but instead by the phenomenon of spinal cord-brainstem brain-spinal cord loop (SBBSL) (Hofstoetter et al. 2014).

In 2013, Shah et al. described the importance of the activation of this SBBSL, for the stimulation of motor neurons and interneurons, that interconnect to the proprioceptive neurons. For the authors, the afferent stimuli of the forelimbs on the quadrupedal animal, influences directly the activity of the hindlimbs, by activation of proprioceptive descending tracts. Therefore, the afferent stimuli are transmitted by the spinothalamic and spinocerebellar tracts, culminating in the brainstem locomotor region of quadrupeds, and in the motor cortex of humans.

Thus, by the spinal cord descending bulbo-spinal tracts, the motor interneurons intrinsic circuits and the CPG of the lumbar-sacral intumescence are activated (Shah et al. 2013; Martins 2015).

Also, SBBSL allows the inhibition of the neural excitability to increase, secondarily to uncontrolled activation of descending motor tracts, responsible for the α and γ LMN contraction of anti-gravitational muscles (Li and Francisco 2015; Martins 2015), in a clinical situation of incomplete SCI.

Thus, the SBBSL, by activating the afferent receptors, that is, A β afferent fibers from cutaneous mechanoreceptors (Hunter and Ashby 1994; Linderroth and Foreman 1999; Holsheimer 2002), and secondarily the spinothalamic tract, may justify the appearance of paresthesia as a clinical sign. When there is paresthesia on human patients, it may indicate SBBSL activation and subsequently motor descending tracts inhibition, which are possibly involved in the neurophysiological explanation of spasticity. Therefore, in humans, the TESCS modality is used to activate the SBBSL and control this imbalance, which may be secondary to pontine reticulospinal lesions in quadrupeds and dorsal reticulospinal lesions in humans

(Richardson et al. 1979; Gybels and Van Roost 1985; Dimitrijevic et al. 1986;; Pinter et al. 2000).

Therefore, through this investigation regarding the SBBSL mechanism, it is suggested that 50 Hz TESCOs during 30 minutes allowed the decrease of spasticity, promoting a physiological motor control for a balanced α - γ co-contraction, allowing functional recovery. TESCOs is a RTM, which promotes the ability to restore the neurologic normal pattern (Hofstoetter et al. 2014).

In 2013, Hofstoetter et al. adapted the TESCOs standard protocol (T11-T12 with 30 Hz), simultaneously with the locomotor training on a land treadmill with current intensity implementation until paresthesia of the lower limbs, at the level of their dermatomes.

The goal of this association was to increase sensorial information, with cutaneous receptors stimulation and even mechanoreceptors, relating to interneurons and CPG of the lumbosacral intumescence, by the locomotor training (Dietz 2012; Harkema et al. 2012a; Harkema et al. 2012b; Hofstoetter et al. 2013; Knikou 2012; Martins 2015).

In 2015, an identical study was performed by the same team (Hofstoetter et al. 2013; Hofstoetter et al. 2015), concluding that the lumbar afferent depolarization and the sacral nervous roots, including the Ia afferent fibers from the muscle spindle, could produce a reflex response, while the 50 hz current promoted lower limbs paresthesia, although below the threshold of muscle contraction. For these authors, the possible TESCOs stimulation was identical to EES (Rattay et al. 2000; Huang et al. 2006; Ladenbauer et al. 2010). Synchronized action potential of the afferent axons, through its terminal branches communicate with the spinal cord, by trans-synaptic modulator pathways, organizing the neural intrinsic circuit (Hofstoetter *et al.* 2015).

Scientific studies demonstrated that the most used frequency, in order to allow the neural stimulation, was about 100 Hz, or even a lower value (Minassian et al. 2004; Minassian et al. 2005; Krames et al. 2009; Angeli et al. 2014; Hofsoetter et al. 2014; Hofsoetter et al. 2015; Rejc et al. 2015; Minassian et al. 2016; Gad et al. 2017).

Nowadays, the Hz values indicated to control spasticity should be around 50 to 100 Hz (Pinter et al. 2000; Hofsoetter et al. 2014; Estes et al. 2017), although for locomotor function in SCI patients, 20 to 50 Hz are needed (Minassian et al. 2005; Harkema et al. 2011; Angeli et al. 2014; Hofsoetter et al., 2015).

In addition to TESCOs (T11-T12), there were studies with other location options, which allowed stimulation based on neuromodulation, namely the multisegmental TESCOs (MS-

TESCS). The MS-TESCS performs the stimulation of interconnections between the forelimbs and the hindlimbs motor interneurons, preserving these connections, which are essential in quadrupedal locomotion (Juvin et al. 2005; Juvin et al. 2012; Gerasimenko et al. 2012; Gerasimenko et al. 2014).

MS-TESCS applied a 5 Hz current with electrodes placed on C5 to C6, T11 to T12, and L1-L2. During the investigation, current intensity was increased according to patient's welfare, avoiding pain. Maximum values could reach 180 mA, without any undesirable effects, due to the biphasic current and values never higher than 10 Hz (Ward 2009).

According to Yamaguchi (1986), long propriospinal neurons play a key role in functional connections between the cervical and lumbosacral regions, allowing coordination between limbs. The stimulation of locomotion coordination depends on the propriospinal system, which can be activated by the stimulation of the forelimbs peripheral nerves (Lloyd 1942; Menetrey et al. 1985) and the activation of cervical stimulation (Miller et al. 1998; Cowley et al. 2008). Therefore, MS-TESCS allows neuromodulation of the propriospinal system, promoting coordination of the gait cycle.

MS-TESCS is a convergent multisegmental stimulation of ascending and descending spinal cord tracts, which are associated to propriospinal system neuromodulation that will influence positively the spinal intrinsic circuits and locomotion. As a non-invasive RTM, it became interesting to the clinical practice (Gerasimenko et al. 2014).

Gerasimenko et al. (2014), also presented a new MS-TESCS technique, applying a 5 to 40 Hz current, and likewise absent from pain manifestation. Thereby, a biphasic current, that could reach 30 to 200 mA, and exactly with the same neuroanatomical location for electrodes placing.

1.3. Transcranial Direct Current Stimulation

TDCS is based on the non-invasive transcranial stimulation through a weak direct current (< 1,5 mA). This stimulation is non-painful, easy to apply, and does not need expensive logistics (Nitsche et al. 2003; Fregni et al. 2006; Ferruci et al. 2008a; Ferruci et al. 2008b; Antal et al. 2011).

Many studies have demonstrated that TDCS may be responsible for the modulated excitability of the motor cortex on humans (Nitsche et al. 2003; Nitsche et al. 2004; Nitsche et al. 2005).

Motor cortex excitability is maintained for a few minutes after TDCS, allowing the ability to create interconnections next to the electrodes placement, but also in the structural

neuronal areas further away (Lang et al. 2005; Ferruci et al. 2008a; Ferruci et al. 2008b; Groppa et al. 2010).

This RTM has been applied in stroke patients since 2005, in an attempt to promote recovery of motor deficits (Hummel et al. 2005; Fregni et al. 2006), as well as in neuropsychiatric diseases and in chronic neuropathic pain (Liebetanz et al. 2006; Fregni and Pascual-Leone 2007; Nitsche et al. 2008; Liebetanz et al. 2009; Antal et al. 2011).

In TDCS, the stimulation parameters are of great importance, depending on the number of sessions, frequency, intensity and stimulation areas (Nitsche et al. 2006). Repeated sessions have greater efficacy due to their cumulative effect when compared to isolated sessions. It is considered that the TDCS effects should last longer when it is applied for a period of two weeks, though more studies are required to create a successful protocol (Fregni and Pascual-Leone 2007) regarding to its electrophysiological effects (Nitsche et al. 2008).

Bindman et al. (1962), Purpura and McMurtry (1965), Nitsche and Paulus (2001), verified that neural action potential, depends on cathode and anode activity. Thus, the cathode contributes to neuronal hyperpolarization, and consequently to the reduction of neuronal excitability, and the anode is responsible for the desired neuronal depolarization, allowing neuronal excitability. TDCS success depends on the interaction between electrical flux and neuronal geometry. Its effects are described to persist for up to five hours, or even more, whenever TDCS is applied for a stimulation period of 10 to 30 minutes (Artola et al. 1990; Nitsche and Paulus 2000; Nitsche and Paulus 2001).

Even though TDCS is based on the use of a weak current, it aims to induce motor-evoked potentials (MEP), demonstrated in the right abductor digital minimal muscle of humans. This was proved through transcranial magnetic stimulation, using contralateral motor cortex tracts, with the observation that MEPs would always increase during anodal current stimulation, while the opposite was observed for the cathode current (Nitsche and Paulus 2000; Fregni and Pascual-Leone 2007; Groppa et al. 2010; Antal et al. 2011).

Therefore, Nitsche and Paulus (2000), in agreement with Bindman et al. (1964) and Purpura and McMurtry (1965), performed the same technique of anode and cathode stimulation in animals, namely in rats. These authors confirmed that, according to the neurophysiology, anode stimulation promoted motor cortex excitability, leading to neuronal depolarization. On the other hand, cathode stimulation reduced motor cortex excitability (Bindman et al. 1964; Nitsche and Paulus 2000; Bikson et al. 2010; Antal et al. 2011). Although in the same study by Nitsche and Paulus (2000), there was not the same neurophysiological

results, in regard to inhibitory interneurons hyperpolarization after anode stimulation, when compared to cathode stimulation of superficial membranes (Bindman *et al.* 1964; Purpura and McMurtry 1965; Nitsche and Paulus, 2000).

Therefore, the interaction between electric fields and neuronal geometry is paramount for the success of TDCS electrical flux and in neuronal excitability modification (Nitsche and Paulus 2000; Ferruci *et al.* 2008a; Ferruci *et al.* 2008b).

In 2000, the cellular and molecular neurophysiological mechanisms behind the neuronal excitability at both cellular and molecular levels was not yet understood. It was assumed that motor cortex excitability could induce changes in the spinal cord excitability, whenever the anode stimulation would stimulate the spontaneous excitability of medullary pyramid cells (Nitsche and Paulus 2000). In conclusion, TDCS is a non-invasive, non-painful, focal and selective modality that allows motor cortex modulated excitability (Hummel *et al.* 2005; Ferruci *et al.* 2008a; Ferruci *et al.* 2008b).

TDCS was considered a RTM from 2000 to 2003, due to the possibility of neuronal neuroplasticity modulation as an approach to motor memorization and learning (Rosenkranz *et al.*, 2000; Nitsche *et al.*, 2003; Fregni *et al.*, 2005; Hummel *et al.*, 2005).

Nitsche *et al.* (2003) and Islam *et al.* (1995), supported that TDCS neurophysiology was due to the existence of intracellular Ca^{2+} ion concentration increase, in addition to ionic alterations.

Ca^{2+} ion transportation and its intracellular levels are very essential since they are deeply related with neuronal neuroplasticity (Bennett 2000). Therefore, Ca^{2+} channels modulation might alter the percentage of neurotransmitter concentration and consequently the neuronal excitability (Nitsche *et al.* 2003). Beyond this, there is also N-metil-D-Aspartate receptors modulation, essential for the initial phase of neuronal neuroplasticity (Bennett 2000; Nitsche *et al.* 2003).

In 2004, a new experimental approach was presented with electrodes placement based on the anode as being the excitatory electrode, and the cathode as the inhibitory electrode, and a week continuous current. An example of this would be the emission of 1 mA current for 10 minutes (Siebner *et al.* 2004). On this study, 10 minutes of TDCS increased MEPs amplitude, which was the opposite to what occurred with TDCS cathode current that reduced this amplitude. Thus, the author considered that TDCS has the ability to improve excitability-related alterations in plasticity (Nitsche *et al.* 2005), which are known as homeostatic plasticity (Siebner *et al.* 2004).

Homeostatic plasticity is paramount to the control of synaptic plasticity *in vivo*, observed in rats, which allows motor, visual and somatosensorial synaptic changes (Rioul-Pedotti et al. 2000; Desai et al. 2002; Allen et al. 2003). Therefore, it was suggested that homeostatic plasticity have an interesting therapeutic ability when related to brain diseases, such as neuropsychiatric diseases in humans (Klein et al. 1999; Siebner et al. 1999; Hoffman et al. 2003; Liebetanz et al. 2006).

In 2005 Stecker, confirmed that cranial stimulation is a complex phenomenon, which depended on cerebral geometry and on the different conductivities of intracranial membranes, as well as other factors (i.e. the complex skin geometry, cranium, cerebrospinal fluid, brain), associated to the anisotropic conductivity of the different structures, and the variability amongst patients.

This neurophysiological explanation is important to understand why electrodes should be placed next to the anatomical region that we need to stimulate, knowing that the anatomical region next to the anode electrode will be the one stimulated (Stecker 2005).

Lang et al. (2005) have shown that TDCS leads to changes in excitability of the corticospinal tract in humans, when two electrodes were placed next to the motor cortex and one other electrode was placed at the frontal contralateral pole, knowing that the excitability direction can change depending on the polarity of the electrodes.

It is of main importance that the electrodes are correctly placed, since corticospinal tract excitability increases when the anode is placed next to the motor cortex and the cathode is placed next to the frontal pole. This stimulation is called anode TDCS, and it can suffer a reduction whenever the current flux is inverted, turning it into cathode TDCS (Liebetanz et al. 2009).

Also, whenever TDCS lasts longer than 10 minutes, with a 1 mA current, its effects may persist for at least one hour (Nitsche and Paulus 2001; Nitsche et al. 2003; Hummel et al. 2005; Lang et al. 2005; Liebetanz et al. 2009).

In 2005, Lang and collaborators created the first neuroimaging study that explored the effects of TDCS in the human brain, both at rest and during tasks, with electrodes placed as previously mentioned. This study described a bi-directional and diffuse modification in the regional neuronal activity, associated with diffuse cerebral blood flow alterations, which gradually normalized after 50 minutes.

In 2006, Fregni and collaborators, performed a double-blind randomized study with a control group (placebo group) to evaluate the efficacy of five TDCS sessions in patients with

chronic central neuropathic pain following spinal cord trauma. In this study, the current was applied for 20 minutes, with 2 mA, in a group of healthy people and one single session. In the group of the chronic neuropathic pain patients, the protocol was similar, with the difference of a current intensity of 1 mA. The electrodes were placed as suggested by Nitsche and Paulus (2001), for maximum motor cortex excitability.

In this study, alterations in the activity of the cortical areas and motor cortex contralateral activity, were observed whenever the electrodes were placed at the M1 region, as already proven by Lang et al. (2005). This observation allowed the same electrode placement to be used in patients with both symmetric and asymmetric chronic neuropathic pain, which suggests that unilateral TDCS is enough for treatment of patients with bilateral chronic neuropathic pain (Fregni et al. 2006).

In this same study, participants also described the existence of a tingling sensation in the anatomical region of both electrodes during the emission of the current, particularly during the initial phase of stimulation. This study also mentioned that no other secondary effects were found (Fregni et al. 2006).

The same tingling sensations are described to be minimal and patients should be able to adapt within seconds (Fregni and Pascual-Leone 2007; Antal et al. 2011).

A study by Fregni et al. (2006) suggested that the excitability of the motor cortex, modulated pain perception through indirect effects in the thalamic and sub-thalamic neuroanatomical areas (Lang et al. 2005; Fregni and Pascual-Leone 2007), which may be responsible for the inhibition of nociceptive impulses in the spinal cord (García-Larrea et al. 1997; García-Larrea et al. 1999).

Hummel et al. (2005) and Gandiga et al. (2006) performed a randomized study in clinical neurorehabilitation, with a control group, which associated this RTM with motor training. In this study, it was concluded that TDCS was a RTM safe and successful in the neurorehabilitation of voluntary healthy people and stroke patients, when used with the correct parameters.

Nitsche et al. (2008) refers that TDCS should only be applied in patients that do not show any predisposition for cutaneous eczema, to avoid burns where the electrodes are placed. The author also mentioned the need for a responsible approach with proper monitorization in cases where TDCS was applied to neurological patients, such as epileptic patients.

TDCS protocols applied to humans are based on the use of 1 mA currents and electrodes with either 35 cm² (Nitsche and Paulus 2000; Fregni et al. 2006; Liebetanz et al. 2009) or 25 cm² (Hummel et al. 2005; Gandiga et al. 2006), for 20 min. Liebetanz et al. (2009), performed a study in rats, where they applied up to 10 mA with the goal of promoting a greater increase in cerebral cortex modulated excitability, without having any thermal secondary effects or neurotoxicity (Liebetanz et al. 2009).

In veterinary clinical practice, severe SCI after surgical or conservative management, according to its etiology, are becoming more frequent. Dogs at grade 0, according to the MFS may present a severe prognosis, with functional limitations. Therefore, it is essential to introduce RTMs, in order to promote spinal cord neuroplasticity and neuromodulation, reducing the number of non-functional animals and euthanasia.

In humans, as we can see from this review, the three RTMs are essential for central and peripheral nervous system neural modulation and reorganization. Following the biophysical and neurophysiological explanation of the three RTMs, there is a relevant integration in human medicine multidisciplinary programs.

Therefore, it is possible to apply RTMs to patients with severe SCI, in order to obtain locomotor independence and functionality. With the clinical application of RTMs, future clinical studies may be carried out in order to observe the evolution of these patients.

1.4. ARTICLE 1

Acta Veterinaria Scandinavica (2021), 63(22):1-12

Nervous system modulation through electrical stimulation in companion animals

Nervous system modulation through electrical stimulation in companion animals

Ângela Martins^{1,2*}, Débora Gouveia¹, Ana Cardoso¹, Óscar Gamboa³, Darryl Millis⁴ and António Ferreira³

¹Arrábida Veterinary Hospital – Animal Rehabilitation Center, Azeitão, Setúbal, Portugal

²Faculty of Veterinary Medicine, Lusófona University, Campo Grande, Lisboa, Portugal

³Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal

⁴Department of Small Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN USA

*Corresponding author

Ângela Martins

E-mail: vetarabida.lida@gmail.com

Abstract

Domestic animals with severe spontaneous spinal cord injury (SCI), including dogs and cats that are deep pain perception negative (DPP-), can benefit from specific evaluations involving neurorehabilitation integrative protocols.

In human medicine, patients without deep pain sensation, classified as grade A on the American Spinal Injury Association (ASIA) impairment scale, can recover after multidisciplinary approaches that include rehabilitation modalities, such as functional electrical stimulation (FES), transcutaneous electrical spinal cord stimulation (TESCS) and transcranial direct current stimulation (TDCS).

This review intends to explore the history, biophysics, neurophysiology, neuroanatomy and the parameters of FES, TESCS, and TDCS, as safe and noninvasive rehabilitation modalities applied in the veterinary field.

Additional studies need to be conducted in clinical settings to successfully implement these guidelines in dogs and cats.

Key words: Cat, Dog, Electrostimulation, Functionality, Neurorehabilitation modalities, Spinal cord

Background

Locomotion requires the coordination of movement and extensor/flexor muscles in all limbs. This coordination is due to the partial influence of all descending motor tracts in humans as well as dogs and cats [1].

In humans, the influence of the corticospinal tract is greater than in dogs and cats. The corticospinal tract is less developed in domestic animals, especially dogs, resulting in difficulties to perform complex and precise movements [2, 3].

Bipeds, including humans, and quadrupeds share many similarities. They have the same major descending motor tracts, however with the pyramidal system predominating in humans as opposed to dogs and cats, where the extrapyramidal system predominates [4-6]. In humans, the rubrospinal descending motor tract is considered to be vestigial in humans [7, 8], but this is of major importance in domestic animals. In dogs, this tract is considered the main tract that controls voluntary movement, as it can facilitate action of the lower motor neurons (LMNs) in flexor muscles [4]. Moreover, in cats, apart from rubrospinal tract influence, the corticospinal tract has a participative role in precise and complex movements [9].

The reticulospinal tract (RST) plays a prominent role in the motor and postural control, which has been well demonstrated in cats. Additionally, a balance between pontine and medullary RST is essential for locomotion, posture and muscle tone [9, 10]. The pontine RST facilitates spinal motoneurons (α and γ) for the extensor muscles, and the medullary RST inhibits spinal motoneurons (α and γ) for the extensor muscles and simultaneously facilitates motoneurons for the flexor muscles [3, 4, 9] in both species.

For neurorehabilitation, the reorganization of these tracts derived from reticular formation is essential to promote spinal reflexes, motor activity and postural standing, as these tracts play an important role in muscle tone [9].

There are some neurophysiological differences between dogs and humans; for example, in humans, the RST is composed of the dorsal and medial RSTs [11]. However, in the two species, the propriospinal tracts share similar spinal neural control mechanisms [12] and exhibit similar coordination patterns between limbs during locomotion [13].

Neurorehabilitation training for dogs and cats that are deep pain perception positive (DPP+), as well as for human patients who have American Spinal Injury Association (ASIA) impairment grades of B, C, or D [14], can improve functional gait after injury by improving coordination, balance, and muscle strength and shortening the recovery time [15, 16].

Thus, functionality can be defined in a similar way as 'ambulatory', describing the ability to stand up, maintain active postural balance during standing and take at least ten consecutive active, weight-bearing steps [3] without falling. Impairments in functionality may be related to proprioceptive deficits.

Dogs and cats classified as DPP+, as well as human patients with ASIA B/C/D spinal cord injuries (SCIs), are considered to have an incomplete SCI. Therefore, it can be beneficial to stimulate descending motor spinal cord pathways and afferent inputs that have branches connecting to central pattern generators (CPGs). CPGs are connected to propriospinal neurons, the majority of which are interneurons connecting multiple segments that can control LMNs, and re-establish basic gait motor rhythmicity [17, 18].

In humans with subacute and chronic motor incomplete SCI, different approaches can be considered to provide assistance during stepping [19], such as manual locomotor training (body weight-supported treadmill training or overground training) and robotic devices [20]. Additionally, in different studies, locomotor training has been performed with functional electrical stimulation (FES) [21-24].

In both human and veterinary medicine, permanent deep pain perception negative (DPP-) can be an indication of a complete spinal cord injury [25, 26].

In cats classified as DPP-, repetitions of the same movement and the associated sensory feedback that occurs from that movement may induce the essential plastic adaptations of network neurons. Thus, rehabilitation modalities can be useful tools and require the involvement of not only network neurons but also some residual supraspinal pathways [27].

In dogs with severe SCI, DPP should be evaluated for 24 hours in the event that the clinical presentation changes [28-30]. The most severe injuries are related to paraplegic DPP. A previous study, in DPP- dogs, showed a recovery rate of 61% after surgical decompression and 10% after conservative treatment [31]. Thus, DPP assessments are performed to determine whether there is a conscious response to a mechanical stimulus in the medial and lateral digits of both forelimbs and hindlimbs, the tip/base of the tail and the perineal region (scrotum/vulva and anus). This assessment is essential as a prognostic indicator.

Incomplete to complete SCIs involve primary and secondary injuries. The primary injury is usually caused by a relative contribution of both compressive and contusive forces caused by structures anatomically located ventral to the spinal cord [32]. Concussion manifests in a more severe form, and dogs are usually paralyzed with absent DPP [13, 33], which indicates an extremely poor prognosis for functional recovery [34].

The secondary SCI are caused by biochemical and metabolic damage 2 to 48 hours postinjury. At this time, there is a massive release of glutamate and other central excitatory neurotransmitters, which promote excitotoxicity [35], as well as oxidative damage and inflammation [36].

If DPP is lost, the spinoreticular tracts, propriospinal tracts, and possibly even the spinothalamic tracts near the spinal cord gray matter can be affected [37, 38].

In clinical settings, a relation between the presence of DPP and ambulation has been examined. Thus, paraplegic dogs with intact DPP are more likely to regain the ability to ambulate when compared to DPP- dogs [39]. In these cases, the ability to locomote is dependent on the neural reorganization of motor descending tracts and sensory inputs [3].

Deep pain perception negative dogs and cats, as well as human patients, can exhibit functional recovery through neurorehabilitation, given the similarities between the three species [40-42]. These multidisciplinary treatment protocols are based on a group of neurorehabilitation modalities, such as FES, transcutaneous electrical spinal cord stimulation (TESCS) and transcranial direct current stimulation (TDCS).

Neurorehabilitation modalities can be useful for stimulating neurogenesis and strengthening the pre-existing neural tracts [43] that promote anatomic and synaptic neuroplasticity [5], both cranial and caudal to the injury site and possibly through it. Spontaneous reorganization of the motor system leads to the sprouting of spared fibers of the RST [44, 45].

Previous researchers have focused on neural modulation and have studied reflexes and their roles in improving functionality and generating flexion-extension locomotion patterns [46]. The therapeutic potential of neural modulation can be illustrated by TESCS, which promotes the maintenance of axonal connections in severe SCI when massive destruction of the spinal parenchyma occurs, especially in trauma patients [47].

This review article intends to explore the history, biophysics, neurophysiology and parameters of the following neuromodulation modalities: FES, TESCS, and TDCS.

Review

Functional electrical stimulation as a neurorehabilitation therapeutic modality

Functional electrical stimulation is a general tool used for all applications targeting the activation or restoration of function by electrical stimulation, including efferent and afferent nerve stimulation and neuromuscular and muscle stimulation [48].

This neuromodality uses sequences of short bursts of electrical pulses to stimulate the LMNs near the motor endplate region or through peripheral afferent nerves, resulting in the activation of a peripheral spinal reflex [49, 50].

FES was applied for the first time by Liberson et al. [51], who used two superficial electrodes to stimulate the peroneal branch of the sciatic nerve of human patients who exhibited unilateral weakness following a stroke.

Furthermore, FES was used in human patients with SCI to initiate a motor response and restore lost function by the stimulation of the quadriceps group during the stance phase and the end of the swing phase. Additionally, with the stimulation of the peroneal nerve in the popliteal fossa region and the stimulation of the saphenous nerve, the results showed a flexion withdrawal response producing movement in the swing phase [52].

To obtain that response, the current administered by FES is a low intensity current but sufficient to trigger an action potential to induce a visible muscle contraction [53]. In some cases, this stimulation can be performed by placing the electrodes near the muscle mechanoreceptors [34, 54].

To prevent excessive neuromuscular fatigue, it is important to select the correct electrical parameters, including the current magnitude, pulse amplitude, total treatment time, current frequency, current waveform and duty cycle [55].

FES induces the unnatural recruitment of muscle fibers, mostly by recruiting large-diameter motor neurons, which are considered to have fast conduction velocity fibers, instead of recruiting small-diameter motor neurons, which are slower and fatigue resistant [56].

On this basis, using superficial electrodes, FES is used to strengthen muscles, improve blood flow and avoid progression of muscle atrophy (Figures 1 and 2). Additionally, FES can electrically stimulate noninnervated tissue and paralyzed and spastic muscles [57, 58]. To decrease discomfort, in dogs, a short pulse duration as low as 20 Hz is usually recommended for a tetanic muscle contraction. However, the maximal force of contraction generally occurs between 60 and 100 Hz (more commonly in noninnervated tissue). Thus, a lower tetanic frequency within the range of 25 to 50 Hz and a lower intensity, but over 2.5 mA, will minimize fatigue (more commonly in innervated tissue) [59].

Patients with severe atrophy (neurogenic atrophy) may need longer off-time to recover, starting with duty cycle ratios of 1:5, and these patients should be monitored for signs of fatigue. Additionally, a ramp-up time of 2-4 seconds and a ramp-down time of 1-2 seconds should be considered [59].

Since 2012, in the field of restorative neurology, FES has been considered a possible tool for achieving a functional ambulatory state by activating spinal interneuron intrinsic circuits and CPGs that are involved in both voluntary and spinal reflex locomotion [60, 61].

FES allows long-latency flexor reflex development in humans with complete SCI, which has been compared to the late flexor reflex observed in animals with the same injury. This reflex in cats is considered to utilize interneurons of spinal locomotor centers that are associated with afferent stimulation [62], generating the flexor reflex and the swing phase of stepping [63].

Thus, FES protocols can promote the excitability of new connections, which can be created during the process of neural reorganization as a result of rehabilitative programs. Moreover, it is known that these programs are more effective when they are implemented only a few days after spinal injury [20].

FES is considered a noninvasive modality that is easy to perform in a clinical setting and can be used for neuromodulation.

The combination of FES and locomotor training protocols has been shown to have therapeutic benefits among both animals and human patients [64, 65], which indicates that these protocols affect neuroplasticity and functional locomotion, particularly in regard to speed, step length and cadence [64]. It has also been proposed that FES can facilitate or enhance reciprocal inhibition, which is mainly mediated by interneuron activation through Ia fibers on the contralateral side [66].

Transcutaneous electrical spinal cord stimulation as a neurorehabilitation therapeutic modality

Transcutaneous electrical spinal cord stimulation is a therapeutic method of electrically activating spinal cord circuits by means of surface electrodes placed on the skin in the cervical, thoracic and lumbosacral regions [67].

TESCS is a noninvasive and nonpainful neuromodulation modality that induces stimulation throughout synergistic and interactive multisegmental paths, combining the central components of motor descending tracts and ascending sensorial tracts. Thus, it may recruit a different population of motor neurons by projecting sensory and intraspinal connections, directly resulting in the amplification of spinal evoked motor potentials [68]. Multifocal TESCS may have a role in human locomotor activities that involve the monosynaptic neural circuitry of the extensors

and flexors of the knee and ankle and in achieving unassisted standing with correct weight distributions [69].

Lloyd [70] was the first to investigate electrical stimulation through the dorsal and ventral horn of the same spinal segment, maximally inducing the spinal peripheral reflex in the transmission between the two neurons, which was named the ventral horn/muscle reflex (RCV/M).

Minassian et al. [71] performed a study to demonstrate the action of TESCS with electrodes (cathodes) placed over the T11-T12 vertebral region and the anode on the umbilical region to create two channels. This stimulation through surface electrodes allowed perpendicular current penetration, migrating the ligaments between the spinous processes in the vertebral lamina, penetrating the vertebral canal and promoting a current in the cerebrospinal fluid, with the purpose of stimulating the RCV/M from the quadriceps femoris, hamstrings, tibial anterior and triceps surae. The results provided evidence that TESCS can depolarize the lumbosacral posterior roots.

Kitano and Koceja [72] showed that the monosynaptic multisegmental response of TESCS is superior to that of traditional peripheral electrical stimulation, with a maximum RCV/M or H-reflex, because TESCS allows selective excitation of the sensorial fibers, thereby producing a direct motor response and an antidromic current on the motor fibers.

Many authors studied the T11-T12 anatomical region, as previously described. However, for other authors, maximal stimulation with TESCS was best performed with the electrodes placed in the T12-L1 intervertebral region [71-76]. However, Akaza et al. (2010) concluded that the placement of the electrodes should be determined on an individual basis [76].

According to Roy et al. [77] the best location to stimulate a large number of connections in the lumbosacral intumescence with biphasic current was considered to be T11-T12 or T12-L1. However, for the same authors, the position of the cathode along the vertebral column was of greater importance, and the authors noted that sensory neural fiber stimulation is supported by the L1-L3 region; however, for the recruitment of motor neuron fibers, the best neuroanatomical location was caudal to the L5-S1 anatomical region.

Regarding the anatomical region of electrode placement in humans, geometric changes in the thoracolumbar vertebral column, as well as the position of adjacent vertebra, can influence the progression of current depolarization. Therefore, the stimulation of interconnections at the level of the lumbosacral intumescence may be affected [77].

Hofstoetter et al. [79] placed electrodes at the standard location of T11/T12 and applied a symmetric biphasic continuous current of 50 Hz, intending to depolarize the large-diameter afferent fibers of the L2-S2 neuroanatomical region and its posterior roots in humans, and the authors concluded that the activated connections were superior to those of the epidural technique [78]. In 2012 the same authors used a biphasic continuous current with a range ≤ 100 Hz and observed contractions of the paraspinal and abdominal muscles, in addition to paraesthesia in the lower limbs in bipedal patients, which was expected [61].

Currently, in human medicine, the application of this modality leads to a decrease in muscle contraction coactivation, leading to a faster gait in bipedal patients. This outcome was observed in three patients; decreases in coactivation during the tonic and clonic phases in peripheral flexor reflex examinations and thus a decrease in muscle spasms were observed [61, 78, 79].

As mentioned before and confirmed by different authors, the current enters the spinal canal, but only a small portion, approximately 8%, of the current enters the canal [80]. The current depolarizes the nerve root because it progresses through the motor descending spinal cord tracts until it reaches the neural intrinsic circuitry of the lumbosacral region, normally preserved caudal to the injury [78]. This event can be assessed by visual vibrations on the Achilles tendon and the presence of passive or active contractions at the level of the lower limbs in humans [79]. By our own experience, this event also occurs in dogs and cats.

Hofstoetter et al. [82] showed that motor descending tracts can be depolarized by the spinal cord-brainstem-brain-spinal cord loop (SBBSL) phenomenon [81]. The stimulation of afferent nerves of the forelimbs in quadrupedal animals directly influences pelvic limb activity by activating proprioceptive descending tracts. Therefore, afferent stimuli cross the spinothalamic and spinocerebellar tracts to the locomotor region of the brainstem or the motor cortex in humans. Moreover, through descending bulbospinal tracts, it is possible to activate the spinal network of the lumbosacral intumescence.

In human patients with spasticity, when electrodes are placed below the level of the lesion, TESCS can promote temporary anti-spastic effects through nonspecific inhibitory mechanisms [83]. The most commonly used frequencies for neural stimulation are approximately 100 Hz and lower [84-86]. However, to control spasticity, the frequency should range between 50 and 100 Hz [87] because stimulation at higher frequencies can not only electrically activate the same neural input structures but also lead to maximum action potentials.

For locomotor function, approximately stimulation at frequencies of 20 to 50 Hz should be used in patients with SCI [88, 89].

Simulation at frequencies within this range can evoke action potentials of the descending motor tracts and stimulate Ia afferent fibers that have a strong synaptic connection to Ia inhibitory interneurons (reciprocal inhibition mechanism) while avoiding paresthesia, allowing muscle contractions, and reducing the risk of spasticity [81].

According to different authors, long propriospinal neurons play a key role in the functional connection between the cervical and lumbosacral regions and allow coordination between limbs. Locomotor coordination depends on the propriospinal system, which can be activated by the stimulation of limb peripheral nerves in the thoracic and cervical regions [90, 91].

Therefore, multisegmental TESCOs (MS-TESCOs) with electrodes placed at C5-C6, T11-T12, and L1-L2 promotes propriospinal system neuromodulation and thus gait cycle coordination [92, 93]. Some studies used the same modality with a 5-40 Hz biphasic current that could reach 30 to 200 mA while maintaining the severity of pain below a predetermined threshold [94, 95].

MS-TESCOs enables the convergent multisegmental stimulation of ascending and descending spinal cord tracts, which is associated with propriospinal system neuromodulation and positively influences spinal intrinsic circuits and triggers locomotion. MS-TESCOs is a noninvasive neuromodulation modality that is used in human patients, and it can be considered a potential treatment option for DPP+ or DPP- paraplegic dogs [94].

TESCOs, with a 50 Hz biphasic current in a 30-minute session, has been proposed to be a viable nonpharmacological treatment [96]. This type of current has a rectangular ramp that helps control and improve patient comfort, with a ramp-up time of 4 seconds, ramp-down of 2 seconds and *plateau* close to 10 seconds [59]. In the study, the amplitude was increased until lower limb paresthesia was present, ranging from 15 to 90 mA. These stimulations can induce spinal reflexes in the lower limbs. Within two days, consecutive TESCOs demonstrated moderate-to-high reproducibility of the spinal reflex recruitment properties [97].

It is important to mention the human randomized, sham-controlled, double-blinded, parallel design study published by Awosika et al. [98] as the results indicated that anodal TESCOs may be related to the neurorehabilitation of locomotor function after neurological injury. Anodal TESCOs affected speed and symmetry, as well as flexion/extension locomotor pattern modulation, and increased blood flow to the spinal cord, especially when TESCOs was repeated daily.

Ammendolia et al. [99] showed an increase in blood flow to the spinal cord and cauda equina with TESCS, and the magnitude of effect was dependent on the intensity of the electrical stimulus.

Cervical TESCS has also been shown to improve the voluntary control of hand function in tetraplegic patients [100], although the neurophysiological mechanisms are still poorly understood. The amplitude of the current used ranged from 50 to 90 mA, and there were evident similarities with lumbosacral TESCS [101].

The spinal neural mechanism is mostly based on both afferent sensory stimuli and the neural intrinsic circuitry pattern, which are present in both humans and dogs [102]. Although there is no literature regarding TESCS applied in small animals, it is possible to translate the therapeutic potential of this modality to veterinary patients, considering all neuroanatomic differences.

The intrinsic circuitry pattern and CPGs constitute the neural network located in the intumescence in both the brachial and lumbosacral plexuses. However, in dogs, the lumbosacral plexus is located in L4-S3 (spinal cord segments corresponding to L3-L7, S1-S3, Co1 to Co 3 or more) [4, 103], and in humans, it is located in L2-L3, with the spinal cord ending at the inferior side of the L1 vertebrae [104].

Therefore, for translational medicine, in dogs, the cathode should be placed in the L2-L3 region, that is, at the beginning of the pelvic intumescence. The anode should be placed on the dorsal edge of the iliac crest, corresponding to the spinous process of L7 (Figures 3 and 4).

Transcranial direct current stimulation as a neurorehabilitation therapeutic modality

Transcranial direct current stimulation is a method of noninvasive transcranial stimulation that uses a weak direct current (< 1.5 milliamperes (mA)), which is not painful and easy to apply [95, 105].

Studies have demonstrated that TDCS is responsible for motor cortex-modulated excitability in humans [106-108]. This excitability is maintained for a few minutes after TDCS application, which allows interconnections to be created not only at anatomical region at which the electrodes are placed but also in other regions [109-111].

This neuromodality has been used in stroke patients since 2005 to promote motor deficit recovery [112] and in patients with neuropsychiatric diseases and chronic neuropathic pain [113-115].

Previous studies have demonstrated the use of TDCS in neuropathic pain management through A delta fiber activation (as well as A beta fibers and sometimes C fibers) and through the spinothalamic pathway [116] with the use of 2 and 2.5 mA currents for 15-20 minutes, respectively [117].

The number of stimulation sessions, frequency, intensity and stimulation region are important factors of treatment to consider [118]. Sessions repeated every 24 hours have higher efficacy than do single sessions due to the cumulative effect, and the effects are expected to last longer when TDCS is applied over longer periods of time, including a period of two weeks [119].

The characteristics of a neural action potential depend on the cathode and anode placement and the current of the electrodes. The cathode contributes to neuronal hyperpolarization and consequently to a reduction in neuronal excitability (Cathode TDCS), and the anode is responsible for the desired neuronal depolarization, allowing neuronal excitability to take place (Anode TDCS) [114, 115] (Figure 5).

Thus, TDCS success depends on the interaction between the electrical current and geometrical parameters [120]. Stecker [8] found that cranial stimulation is a complex phenomenon that depends not only on cerebral geometry and the conductivity of intracranial membranes but also on the complex skin and skull geometry, the cerebrospinal fluid and the brain, which are associated with the anisotropic conductivity of the different structures and their variability among patients [110].

TDCS effects can persist for five hours or sometimes more when the stimulation is applied for 10 to 30 minutes [7]. According to different authors, whenever TDCS is applied for longer than 10 minutes, with a current close to 1 mA, the effects persist for at least one hour [112, 114].

The standard range of the current is usually between 1 and 2 mA, and currents within this range lead to smaller peripheral side effects than do larger currents [121, 122] due to the electrochemical production of toxic substances at the electrode interface, which has been observed both in rats and in humans [114]. Additionally, this type of electrical current does not intend to achieve muscle contraction however can have some cumulative effects. However, the existing information on TDCS current intensification is limited [114, 121]. Thus, standard TDCS protocols performed in humans usually use 1 mA pulsed currents ramped up and then down over 30 second windows and 35 cm² and 25 cm² electrodes [105, 112, 123], which are chosen to minimize the risk of unintended effects [124]. This stimulation can be applied for a duration of 20 minutes for 5 consecutive days [124] (Figure 6).

A study in rats used currents of up to 10 mA to increase cerebral cortex-modulated excitability, and there was no evidence of thermal secondary effects or neurotoxicity. However, additional studies need to be conducted to determine the morphological long-term changes and the therapeutic effects of intensifying protocols without causing harmful effects [114].

In 2000, the neurophysiological mechanism of TDCS was presumed to be associated with motor cortex excitability, which can induce changes in spinal cord excitability [109]. TDCS induces changes in cerebral excitability by up to 40%, likely due to functional synaptic mechanisms that are dependent on current intensity and stimulation duration [125].

In 2003, research suggested that the neurophysiological effects of TDCS are caused by ionic alterations at the cellular level, which lead to increased intracellular Ca^{2+} concentrations. Additionally, in 2005, the same author suggested that TDCS can improve excitability-related changes in plasticity [95, 108, 126]. Thus, recent results from animal studies show that intracellular calcium Ca^{2+} concentrations determine the magnitude of increase/decrease in synaptic connections and that the strength of plasticity depends on the amount of calcium influx. Long and periods of Ca^{2+} influx cause the depression of axonal synapses, and moderate periods of calcium influx do not induce synaptic modulation, whereas a large Ca^{2+} influx increases the potentiation of synaptic modulation [127]. Additionally, glutamatergic activity with N-methyl-D-aspartate (NMDA) receptors, which have Ca^{2+} channel properties, has been proposed to be a possible mechanism of induced plasticity [128]. In vivo animal studies have revealed that the TDCS current impacts the neuronal firing rate, modifying synaptic efficacy at both the single-cell and network levels. Furthermore, TDCS generates static electric fields in the brain, whose cellular mechanisms are focused on the neuronal elements activated, such as glial and endothelial cells, as well as Ca^{2+} influx-induced astrocytes [128].

Therefore, it is suggested that this type of synaptic modulation can be used to treat related brain diseases, such as neuropsychiatric diseases and chronic neuropathic pain, in humans [105].

Human participants subjected to TDCS treatment described a tingling sensation in the anatomical regions of the two electrodes during current emission, particularly in the initial stimulation phase. The same tingling sensations have been observed at the cranial level and are considered minimal, and patients should be able to adapt to the sensations within seconds [115, 119].

It seems that motor cortex excitability can modulate pain perception through indirect effects on the thalamic and subthalamic neuroanatomical regions. These factors may be responsible for the inhibition of nociceptive impulses in the spinal cord [119].

In human patients, there is recent evidence suggesting that TDCS combined with TESCS may enhance the effects of robotic-assisted gait training [129]. The use of TDCS with FES should also be considered [130], and the combination of TDCS with conventional locomotor training appears to promote balance recovery. However, there is a need for additional studies on this topic [124].

In our daily veterinary practice, we use a protocol with similar parameters: a current intensity of 1-2 mA; a monophasic/biphasic pulsed rectangular current with ramp-up and ramp-down periods of 30 seconds; electrode sizes close to 25-35 cm²; a session duration of 20 minutes; and a treatment duration of 5 consecutive days (Figures 5 and 6).

Conclusions

Both human and small animal patients with SCI can benefit from neurorehabilitation treatment. Dogs and cats that are DPP+, as well as human patients with ASIA B/C/D impairment, may regain the ability to ambulate with only mild residual proprioceptive deficits with neurorehabilitation treatment.

On the other hand, neurorehabilitation with multidisciplinary protocols is important for dogs and cats that are DPP-, and different approaches, including some electrical stimulation modalities, may help improve flexor-extensor patterns by stimulating a peripheral component (FES) or central component (TDCS and TESCS).

This review was essential for understanding the potential of these modalities in veterinary medicine, addressing three major questions in regard to safety, clinical applications, and the parameters to be used.

Multidisciplinary protocols can benefit from these three electrical stimulation modalities, and the appropriate method can be selected according to the neurologic deficits on an individual basis. Additionally, to increase repetitive training, these protocols are commonly combined with locomotor exercises with an underwater treadmill, land treadmill and/or overground training. However, to determine the effects of these neurorehabilitation modalities in small animals, additional studies are needed.

List of abbreviations

ASIA: American Spinal Injury Association

CPG: Central pattern generator

DPP: Deep pain perception

DPP+: Deep pain perception positive
 DPP-: Deep pain perception negative
 FES: Functional electrostimulation
 FNR: Functional neurorehabilitation
 LMN: Lower motor neuron
 mA: Milliampere
 MS - TESCS: Multisegmental TESCS
 RCV/M: Ventral horn/muscle reflex
 RST: Reticulospinal descending motor tract
 SBBSL: Spinal cord-brainstem-brain-spinal cord loop
 SCI: Spinal cord injury
 TDCS: Transcranial direct current stimulation
 TESCS: Transcutaneous electrical spinal cord stimulation

References

1. Dobkin BH, Apple D, Barbeau H, Basso M, Behrman A, Deforge D, et al. Methods for a randomized trial of weight-supported treadmill training versus conventional training for walking during inpatient rehabilitation after incomplete traumatic spinal cord injury. *Neurorehabil Neural Repair*. 2003;17:153-67.
2. García-Álías G, Truong K, Shah PK, Roy RR, Edgerton VR. Plasticity of subcortical pathways promote recovery of skilled hand function in rats after corticospinal and rubrospinal tract injuries. *Exp Neurol*. 2015;266:112-9.
3. Lewis MJ, Jeffery ND, Olby NJ. Ambulation in dogs with absent pain perception after acute thoracolumbar spinal cord injury. *Front Vet Sci*. 2020;7:560.
4. Thomson C, Hahn C. Reflexes and motor systems. In: Thomson C, Hahn C, editors. *Veterinary neuroanatomy: a clinical approach*. Edinburgh: Saunders Elsevier; 2012. p. 47-57.
5. Solopova IA, Selionov VA, Sylos-Labini F, Gurfinkel VS, Lacquaniti F, Ivanenko YP. Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Front Syst Neurosci*. 2015;9:14.
6. Li S, Francisco GE. New insights into the pathophysiology of post-stroke spasticity. *Front Hum Neurosci*. 2015;9:192.
7. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*. 2001;57:1899-901.
8. Stecker MM. Transcranial electric stimulation of motor pathways: a theoretical analysis. *Comput Biol Med*. 2005;35:133-55.
9. Uemura E. Motor system. In: Uemura E, editor. *Fundamentals of canine neuroanatomy and neurophysiology*. Iowa: Wiley Blackwell; 2015. p. 257-85.
10. Nathan PW, Smith M, Deacon P. Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. *Brain*. 1996;119:1809-33.
11. Ropper A, Samuels M, Klein J. Motor paralysis. In: Victor M, Ropper AH, Adams RD, editors. *Adams and Victor's principles of neurology*. China: McGraw-Hill Education; 2014. p. 45-63.

12. Dietz V. Spinal cord pattern generators for locomotion. *Clin Neurophysiol.* 2003;114:1379-89.
13. Pang MY, Yang JF. The initiation of the swing phase in human infant stepping: importance of hip position and leg loading. *J Physiol.* 2000;528:389-404.
14. Roberts TT, Leonard GR, Cepela DJ. Classifications in brief: American Spinal Injury Association (ASIA) impairment scale. *Clin Orthop Relat Res.* 2017;475:1499-504.
15. Alexeeva N, Sames C, Jacobs PL, Hobday L, Distasio MM, Mitchell SA, et al. Comparison of training methods to improve walking in persons with chronic spinal cord injury: a randomized clinical trial. *J Spinal Cord Med.* 2011;34:362-79.
16. Dobkin BH, Duncan PW. Should body weight-supported treadmill training and robotic-assistive steppers for locomotor training trot back to the starting gate? *Neurorehabil Neural Repair.* 2012;26:308-17.
17. Duysens J, Van de Crommert HW. Neural control of locomotion; the central pattern generator from cats to humans. *Gait Posture.* 1998;7:131-41.
18. Meyns P, Van de Crommert HW, Rijken H, Van Kuppevelt DH, Duysens J. Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed. *Spinal Cord.* 2014;52:887-93.
19. Sandler EB, Roach KE, Field-Fote EC. Dose-Response outcomes associated with different forms of locomotor training in persons with chronic motor-incomplete spinal cord injury. *J Neurotrauma.* 2017;34:1903-8.
20. Winchester P, McColl R, Querry R, Foreman N, Mosby J, Tansey K, et al. Changes in supraspinal activation patterns following robotic locomotor therapy in motor-incomplete spinal cord injury. *Neurorehabil Neural Repair.* 2005;19:313-24.
21. Field-Fote EC. Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. *Arch Phys Med Rehabil.* 2001;82:818-24.
22. Popovic DB, Popovic MB, Sinkjaer T. Neurorehabilitation of upper extremities in humans with sensory-motor impairment. *Neuromodulation.* 2002;5:54-66.
23. Thrasher T, Popovic M. FES-assisted walking for rehabilitation of incomplete spinal cord injury. In: *Proceedings of the International functional electrical stimulation society conference.* Queensland: Australia; 2003. p. 131-4.
24. Popovic MR, Keller T. Modular transcutaneous functional electrical stimulation system. *Med Eng Phys.* 2005;27:81-92.
25. Amsellem P, Toombs JP, Lavery PH, Breur GJ. Loss of deep pain sensation following thoracolumbar intervertebral disk herniation in dogs: treatment and prognosis. *Compend Contin Educ Pract Vet.* 2003;25:266-74.
26. Fingerroth J, Forterre F, Levine J. Compressive and contusive spinal cord injury secondary to intervertebral disc displacement: a clinical perspective. In: Fingerroth J, Thomas W, editors. *Advances in intervertebral disc disease in dogs and cats.* Oxford: Wiley Blackwell; 2015. p. 131-4.
27. Escalona M, Delivet-Mongrain H, Kundu A, Gossard JP, Rossignol S. Ladder treadmill: a method to assess locomotion in cats with an intact or lesioned spinal cord. *J Neurosci.* 2017;37:5429-46.
28. Scott HW, McKee WM. Laminectomy for 34 dogs with thoracolumbar intervertebral disc disease and loss of deep pain perception. *J Small Anim Pract.* 1999;40:417-22.
29. Jeffery ND, Levine JM, Olby NJ, Stein VM. Intervertebral disk degeneration in dogs: consequences, diagnosis, treatment, and future directions. *J Vet Intern Med.* 2013;27:1318-33.
30. Jeffery ND, Barker AK, Hu HZ, Alcott CJ, Kraus KH, Scanlin EM, et al. Factors associated with recovery from paraplegia in dogs with loss of pain perception in the pelvic limbs following intervertebral disk herniation. *J Am Vet Med Assoc.* 2016;248:386-94.

31. Moore SA, Tipold A, Olby NJ, Stein V, Granger N. Current approaches to the management of acute thoracolumbar disc extrusion in dogs. *Front Vet Sci.* 2020;7:610.
32. Fenn J, Olby NJ. Classification of intervertebral disc disease. *Front Vet Sci.* 2020;7:579025.
33. Fletcher D, Dewey C, Da Costa R. Spinal trauma management. In: Dewey C, Da Costa R, editors. *Practical guide to canine and feline neurology*. London: Wiley Blackwell; 2016. p. 423-5.
34. Zidan N, Sims C, Fenn J, Williams K, Griffith E, Early PJ, et al. A randomized, blinded, prospective clinical trial of postoperative rehabilitation in dogs after surgical decompression of acute thoracolumbar intervertebral disc herniation. *J Vet Intern Med.* 2018;32:1133-44.
35. Couillard-Despres S, Bieler L, Vogl M. Pathophysiology of traumatic spinal cord injury. In: Weidner N, Rupp R, Tansey K, editors. *Neurological aspects of spinal cord injury*. Switzerland: Springer Nature; 2017. p. 503-6.
36. Olby N. Spinal trauma. In: Platt S, Garosi L, editors. *Small animal neurological emergencies*. London: CRC Press; 2011. p. 383-5.
37. Olby N, Levine J, Harris T, Muñana K, Skeen T, Sharp N. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord: 87 cases (1996-2001). *J Am Vet Med Assoc.* 2003;222:762-9.
38. De Lahunta A, Glass E. General sensory systems: general proprioception and general somatic afferent. In: De Lahunta A, Glass E, Kent M, editors. *Veterinary neuroanatomy and clinical neurology*. Philadelphia, PA: Saunders Elsevier; 2014. p. 221-42.
39. Muguet-Chanoit AC, Olby NJ, Lim JH, Gallagher R, Niman Z, Dillard S, et al. The cutaneous trunci muscle reflex: a predictor of recovery in dogs with acute thoracolumbar myelopathies caused by intervertebral disc extrusions. *Vet Surg.* 2012;41:200-6.
40. Dietz V, Fouad K. Restoration of sensorimotor functions after spinal cord injury. *Brain.* 2014;137:654-67.
41. Takeoka A, Vollenweider I, Courtine G, Arber S. Muscle spindle feedback directs locomotor recovery and circuit reorganization after spinal cord injury. *Cell.* 2014;159:1626-39.
42. Knikou M, Mummidisetty CK. Locomotor training improves premotoneuronal control after chronic spinal cord injury. *J Neurophysiol.* 2014;111:2264-75.
43. Grasso R, Ivanenko YP, Zago M, Molinari M, Scivoletto G, Castellano V, et al. Distributed plasticity of locomotor pattern generators in spinal cord injured patients. *Brain.* 2004;127:1019-34.
44. Lewis MJ, Yap PT, McCullough S, Olby NJ. The relationship between lesion severity characterized by diffusion tensor imaging and motor function in chronic canine spinal cord injury. *J Neurotrauma.* 2018;35:500-7.
45. Ballermann M, Fouad K. Spontaneous locomotor recovery in spinal cord injured rats is accompanied by anatomical plasticity of reticulospinal fibers. *Eur J Neurosci.* 2006;23:1988-96.
46. Raineteau O. Plastic responses to spinal cord injury. *Behav Brain Res.* 2008;192:114-23.
47. Dimitrijevic M. Residual motor function after spinal cord injury. In: Dimitrijevic M, Kakulas B, McKay W, Vrbová G, editors. *Restorative neurology of spinal cord injury*. New York, NY: Oxford University Press; 2012. p. 1-9.
48. Tansey KE. Neural plasticity and locomotor recovery after spinal cord injury. *PM R.* 2010;2:S220-6.
49. Kralj A, Bajd T, Turk R, Krajnik J, Benko H. Gait restoration in paraplegic patients: a feasibility demonstration using multichannel surface electrode FES. *J Rehabil R D.* 1983;20:3-20.

50. Holsheimer J. Computer modelling of spinal cord stimulation and its contribution to therapeutic efficacy. *Spinal Cord*. 1998;36:531-40.
51. Liberson WT, Holmquest HJ, Scot D, Dow M. Functional electrotherapy: stimulation of the peroneal nerve synchronized with the swing phase of the gait of hemiplegic patients. *Arch Phys Med Rehabil*. 1961;42:101-5.
52. Granat MH, Heller BW, Nicol DJ, Baxendale RH, Andrews BJ. Improving limb flexion in FES gait using the flexion withdrawal response for the spinal cord injured person. *J Biomed Eng*. 1993;15:51-6.
53. Pilkar RB, Yarossi M, Forrest G. Empirical mode decomposition as a tool to remove the function electrical stimulation artifact from surface electromyograms: preliminary investigation. In: 2012 annual international conference of the IEEE engineering in medicine and biology society. San Diego, CA: IEEE; 2012. p. 1847-50.
54. Gallucci A, Dragone L, Menchetti M, Gagliardo T, Pietra M, Cardinali M, et al. Acquisition of involuntary spinal locomotion (spinal walking) in dogs with irreversible thoracolumbar spinal cord lesion: 81 dogs. *JVIM*. 2017;31:492-497.
55. Hamid S, Hayek R. Role of electrical stimulation for rehabilitation and regeneration after spinal cord injury: an overview. *Eur Spine J*. 2008;17:1256-69.
56. Fouad K, Tetzlaff W. Rehabilitative training and plasticity following spinal cord injury. *Exp Neurol*. 2012;235:91-9.
57. McDonald JW, Becker D, Sadowsky CL, Jane JA, Sr., Conturo TE, Schultz LM. Late recovery following spinal cord injury. Case report and review of the literature. *J Neurosurg*. 2002;97:252-65.
58. Johnston TE, Greco MN, Gaughan JP, Smith BT, Betz RR. Patterns of lower extremity innervation in pediatric spinal cord injury. *Spinal Cord*. 2005;43:476-82.
59. Levine D, Bockstahler B. Electrical stimulation. In: Millis D, Levine D, editors. *Canine rehabilitation and physical therapy*. Philadelphia, PA: Saunders Elsevier; 2014. p. 342-56.
60. Hubli M, Dietz V, Bolliger M. Spinal reflex activity: a marker for neuronal functionality after spinal cord injury. *Neurorehabil Neural Repair*. 2012;26:188-96.
61. Minassian K, Hofstoetter U, Tansey K, Mayr W. Neuromodulation of lower limb motor control in restorative neurology. *Clin Neurol Neurosurg*. 2012;114:489-97.
62. Spaich E, Andersen O. Using painful sensory stimulation to improve the hemiparetic gait. In: Jensen W, Andersen O, Akay M, editors. In: *Proceedings of the 2nd international conference of neurorehabilitation (ICNR): replace, repair, restore, relieve - bridging clinical and engineering solutions in neurorehabilitation*. Aalborg: Springer; 2014. p. 165-67.
63. Lavrov I, Gerasimenko YP, Ichiyama RM, Courtine G, Zhong H, Roy RR, et al. Plasticity of spinal cord reflexes after a complete transection in adult rats: relationship to stepping ability. *J Neurophysiol*. 2006;96:1699-710.
64. Lee HJ, Cho KH, Lee WH. The effects of body weight support treadmill training with power-assisted functional electrical stimulation on functional movement and gait in stroke patients. *Am J Phys Med Rehabil*. 2013;92:1051-9.
65. Thompson A. Learning to change a reflex to improve locomotion. In: Jensen W, Andersen O, Akay M, editors. In: *Proceedings of the 2nd international conference of neurorehabilitation (ICNR): replace, repair, restore, relieve - bridging clinical and engineering solutions in neurorehabilitation*. Aalborg: Springer; 2014. p. 179-85.
66. Hamilton LD, Mani D, Almuklass AM, Davis LA, Vieira T, Botter A, et al. Electrical nerve stimulation modulates motor unit activity in contralateral biceps brachii during steady isometric contractions. *J Neurophysiol*. 2018;120:2603-13.
67. Gerasimenko Y, Gorodnichev R, Moshonkina T, Sayenko D, Gad P, Reggie Edgerton V. Transcutaneous electrical spinal-cord stimulation in humans. *Ann Phys Rehabil Med*. 2015;58:225-31.

68. Sayenko DG, Atkinson DA, Floyd TC, Gorodnichev RM, Moshonkina TR, Harkema SJ, et al. Effects of paired transcutaneous electrical stimulation delivered at single and dual sites over lumbosacral spinal cord. *Neurosci Lett*. 2015;609:229-34.
69. Courtine G, Harkema SJ, Dy CJ, Gerasimenko YP, Dyhre-Poulsen P. Modulation of multisegmental monosynaptic responses in a variety of leg muscles during walking and running in humans. *J Physiol*. 2007;582:1125-39.
70. Lloyd DP. Mediation of descending long spinal reflex activity. *J Neurophysiol*. 1942;5:435-58.
71. Minassian K, Persy I, Rattay F, Dimitrijevic MR, Hofer C, Kern H. Posterior root-muscle reflexes elicited by transcutaneous stimulation of the human lumbosacral cord. *Muscle Nerve*. 2007;35:327-36.
72. Kitano K, Koceja DM. Spinal reflex in human lower leg muscles evoked by transcutaneous spinal cord stimulation. *J Neurosci Methods*. 2009;180:111-5.
73. Hofstoetter US, Minassian K, Hofer C, Mayr W, Rattay F, Dimitrijevic MR. Modification of reflex responses to lumbar posterior root stimulation by motor tasks in healthy subjects. *Artif Organs*. 2008;32:644-8.
74. Danner SM, Hofstoetter US, Ladenbauer J, Rattay F, Minassian K. Can the human lumbar posterior columns be stimulated by transcutaneous spinal cord stimulation? A modeling study. *Artif Organs*. 2011;35:257-62.
75. Troni W, Di Sapio A, Berra E, Duca S, Merola A, Sperli F, et al. A methodological reappraisal of non invasive high voltage electrical stimulation of lumbosacral nerve roots. *Clin Neurophysiol*. 2011;122:2071-80.
76. Akaza M, Kanouchi T, Inaba A, Numasawa Y, Irioka T, Mizusawa H, et al. Motor nerve conduction study in cauda equina with high-voltage electrical stimulation in multifocal motor neuropathy and amyotrophic lateral sclerosis. *Muscle Nerve*. 2010;43:274-82.
77. Roy FD, Gibson G, Stein RB. Effect of percutaneous stimulation at different spinal levels on the activation of sensory and motor roots. *Exp Brain Res*. 2012;223:281-9.
78. Hofstoetter U, Danner S, Minassian K. Paraspinal magnetic and transcutaneous electrical stimulation. In: Jaeger D, Jung R, editors. *Encyclopedia of computational neuroscience*. New York, NY: Springer; 2014. p. 1-21.
79. Hofstoetter US, Freundl B, Binder H, Minassian K. Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: elicitation of posterior root-muscle reflexes. *PLoS One*. 2018;13:e0192013.
80. Ladenbauer J, Minassian K, Hofstoetter US, Dimitrijevic MR, Rattay F. Stimulation of the human lumbar spinal cord with implanted and surface electrodes: a computer simulation study. *IEEE Trans Neural Syst Rehabil Eng*. 2010;18:637-45.
81. Hofstoetter US, McKay WB, Tansey KE, Mayr W, Kern H, Minassian K. Modification of spasticity by transcutaneous spinal cord stimulation in individuals with incomplete spinal cord injury. *J Spinal Cord Med*. 2014;37:202-11.
82. Shah PK, Garcia-Alias G, Choe J, Gad P, Gerasimenko Y, Tillakaratne N, et al. Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain*. 2013;136:3362-77.
83. Pinter MM, Gerstenbrand F, Dimitrijevic MR. Epidural electrical stimulation of posterior structures of the human lumbosacral cord: 3. Control Of spasticity. *Spinal Cord*. 2000;38:524-31.
84. Minassian K, Hofstoetter US, Danner SM, Mayr W, Bruce JA, McKay WB, et al. Spinal rhythm generation by step-induced feedback and transcutaneous posterior root stimulation in complete spinal cord-injured individuals. *Neurorehabil Neural Repair*. 2016;30:233-43.
85. Rejc E, Angeli C, Harkema S. Effects of lumbosacral spinal cord epidural stimulation for standing after chronic complete paralysis in humans. *PLoS One*. 2015;10:e0133998.

86. Gad P, Gerasimenko Y, Zdunowski S, Turner A, Sayenko D, Lu DC, et al. Weight bearing over-ground stepping in an exoskeleton with non-invasive spinal cord neuromodulation after motor complete paraplegia. *Front Neurosci.* 2017;11:333.
87. Estes SP, Iddings JA, Field-Fote EC. Priming neural circuits to modulate spinal reflex excitability. *Front Neurol.* 2017;8:17.
88. Angeli CA, Edgerton VR, Gerasimenko YP, Harkema SJ. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain.* 2014;137:1394-409.
89. Hofstoetter US, Krenn M, Danner SM, Hofer C, Kern H, McKay WB, et al. Augmentation of voluntary locomotor activity by transcutaneous spinal cord stimulation in motor-incomplete spinal cord-injured individuals. *Artif Organs.* 2015;39:E176-86.
90. Yamaguchi T. Descending pathways eliciting forelimb stepping in the lateral funiculus: experimental studies with stimulation and lesion of the cervical cord in decerebrate cats. *Brain Res.* 1986;379:125-36.
91. Cowley KC, Zaporozhets E, Schmidt BJ. Propriospinal neurons are sufficient for bulbospinal transmission of the locomotor command signal in the neonatal rat spinal cord. *J Physiol.* 2008;586:1623-35.
92. Juvin L, Le Gal JP, Simmers J, Morin D. Cervicolumbar coordination in mammalian quadrupedal locomotion: role of spinal thoracic circuitry and limb sensory inputs. *J Neurosci.* 2012;32:953-65.
93. Gerasimenko Y, Edgerton VR, Roy RR, Lu DC. Multi-site transcutaneous electrical stimulation of the spinal cord for facilitation of locomotion. U.S. Patent No. 9,993,642; 2018.
94. Gerasimenko Y, Gorodnichev R, Puhov A, Moshonkina T, Savochin A, Selionov V, et al. Initiation and modulation of locomotor circuitry output with multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. *J Neurophysiol.* 2014;113:834-42.
95. Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, et al. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *J Cogn Neurosci.* 2003;15:619-26.
96. Hofstoetter US, Freundl B, Danner SM, Krenn MJ, Mayr W, Binder H, et al. Transcutaneous spinal cord stimulation induces temporary attenuation of spasticity in individuals with spinal cord injury. *J Neurotrauma.* 2020;37:481-93.
97. Saito A, Masugi Y, Nakagawa K, Obata H, Nakazawa K. Repeatability of spinal reflexes of lower limb muscles evoked by transcutaneous spinal cord stimulation. *PLoS One.* 2019;14:e0214818.
98. Awosika OO, Sandrini M, Volochayev R, Thompson RM, Fishman N, Wu T, et al. Transcutaneous spinal direct current stimulation improves locomotor learning in healthy humans. *Brain Stimul.* 2019;12:628-34.
99. Ammendolia C, Côté P, Rampersaud YR, Southerst D, Schneider M, Ahmed A, et al. Effect of active TENS versus de-tuned TENS on walking capacity in patients with lumbar spinal stenosis: a randomized controlled trial. *Chiropr Man Therap.* 2019;27:24.
100. Inanici F, Samejima S, Gad P, Edgerton VR, Hofstetter CP, Moritz CT. Transcutaneous electrical spinal stimulation promotes long-term recovery of upper extremity function in chronic tetraplegia. *IEEE Trans Neural Syst Rehabil Eng.* 2018;26:1272-8.
101. Milosevic M, Masugi Y, Sasaki A, Sayenko DG, Nakazawa K. On the reflex mechanisms of cervical transcutaneous spinal cord stimulation in human subjects. *J Neurophysiol.* 2019;121:1672-9.
102. Dietz V. Proprioception and locomotor disorders. *Nat Rev Neurosci.* 2002;3:781-90.
103. Prada I. Neuroanatomia funcional em medicina veterinária com correções clínicas. São Paulo: Editora Terra Molhada; 2014.

104. Schenkman M, Bowman J, Gisber R, Butler R, Giddings D, Sawyer S. Anatomia regional e suprimento sanguíneo. In: Schenkman ML, Bowman JP, Gisbert RL, Butler RB, editors. *Neurociência clínica e reabilitação*. Barueri: Editora Manole; 2016. p. 27-9.
105. Fregni F, Boggio PS, Lima MC, Ferreira MJ, Wagner T, Rigonatti SP, et al. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. *Pain*. 2006;122:197-209.
106. Nitsche MA, Liebetanz D, Antal A, Lang N, Tergau F, Paulus W. Modulation of cortical excitability by weak direct current stimulation--technical, safety and functional aspects. *Suppl Clin Neurophysiol*. 2003;56:255-76.
107. Nitsche MA, Niehaus L, Hoffmann KT, Hengst S, Liebetanz D, Paulus W, et al. MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clin Neurophysiol*. 2004;115:2419-23.
108. Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, et al. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *J Physiol*. 2005;568:291-303.
109. Ferrucci R, Mameli F, Guidi I, Mrakic-Spota S, Vergari M, Marceglia S, et al. Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology*. 2008;71:493-8.
110. Ferrucci R, Marceglia S, Vergari M, Cogiamanian F, Mrakic-Spota S, Mameli F, et al. Cerebellar transcranial direct current stimulation impairs the practice-dependent proficiency increase in working memory. *J Cogn Neurosci*. 2008;20:1687-97.
111. Groppa S, Bergmann TO, Siems C, Mölle M, Marshall L, Siebner HR. Slow-oscillatory transcranial direct current stimulation can induce bidirectional shifts in motor cortical excitability in awake humans. *Neuroscience*. 2010;166:1219-25.
112. Hummel F, Celnik P, Giraux P, Floel A, Wu WH, Gerloff C, et al. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain*. 2005;128:490-9.
113. Liebetanz D, Klinker F, Hering D, Koch R, Nitsche MA, Potschka H, et al. Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia*. 2006;47:1216-24.
114. Liebetanz D, Koch R, Mayenfels S, König F, Paulus W, Nitsche MA. Safety limits of cathodal transcranial direct current stimulation in rats. *Clin Neurophysiol*. 2009;120:1161-7.
115. Antal A, Polania R, Schmidt-Samoa C, Dechent P, Paulus W. Transcranial direct current stimulation over the primary motor cortex during fMRI. *Neuroimage*. 2011;55:590-6.
116. Sun Y, Zehr EP. Sensory enhancement amplifies interlimb cutaneous reflexes in wrist extensor muscles. *J Neurophysiol*. 2019;122:2085-94.
117. Choi YA, Kim Y, Shin HI. Pilot study of feasibility and effect of anodal transcutaneous spinal direct current stimulation on chronic neuropathic pain after spinal cord injury. *Spinal Cord*. 2019;57:461-70.
118. Nitsche MA, Lampe C, Antal A, Liebetanz D, Lang N, Tergau F, et al. Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. *Eur J Neurosci*. 2006;23:1651-7.
119. Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. *Nat Clin Pract Neurol*. 2007;3:383-93.
120. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol*. 2000;527:633-9.
121. Liu A, Vöröslakos M, Kronberg G, Henin S, Krause MR, Huang Y, et al. Immediate neurophysiological effects of transcranial electrical stimulation. *Nat Commun*. 2018;9:5092.
122. Bestmann S, Walsh V. Transcranial electrical stimulation. *Curr Biol*. 2017;27:R1258-62.

123. Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin Neurophysiol.* 2006;117:845-50.
124. Raithatha R, Carrico C, Powell ES, Westgate PM, Chelette li KC, Lee K, et al. Non-invasive brain stimulation and robot-assisted gait training after incomplete spinal cord injury: a randomized pilot study. *NeuroRehabilitation.* 2016;38:15-25.
125. Rosenkranz K, Nitsche MA, Tergau F, Paulus W. Diminution of training-induced transient motor cortex plasticity by weak transcranial direct current stimulation in the human. *Neurosci Lett.* 2000;296:61-3.
126. Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci.* 2004;24:3379-85.
127. Fertonani A, Miniussi C. Transcranial electrical stimulation: what we know and do not know about mechanisms. *Neuroscientist.* 2017;23:109-23.
128. Samani MM, Agboada D, Kuo MF, Nitsche MA. Probing the relevance of repeated cathodal transcranial direct current stimulation over the primary motor cortex for prolongation of after-effects. *J Physiol.* 2020;598:805-16.
129. Picelli A, Brugnera A, Filippetti M, Mattiuz N, Chemello E, Modenese A, et al. Effects of two different protocols of cerebellar transcranial direct current stimulation combined with transcutaneous spinal direct current stimulation on robot-assisted gait training in patients with chronic supratentorial stroke: a single blind, randomized controlled trial. *Restor Neurol Neurosci.* 2019;37:97-107.
130. Seo HG, Lee WH, Lee SH, Yi Y, Kim KD, Oh BM. Robotic-assisted gait training combined with transcranial direct current stimulation in chronic stroke patients: a pilot double-blind, randomized controlled trial. *Restor Neurol Neurosci.* 2017.

FIGURES



Figure 1. Functional electrical study - Application of segmental FES modality on a dog.



Figure 2. FES protocol to promote new connections – Application of the anode near the motor point region.



Figure 3. Transcutaneous electrical spinal cord stimulation - Application of L2-L3 TESCS modality on a dog.



Figure 4. Transcutaneous electrical spinal cord stimulation - Application of L2-L3 TESCS, according to the vertebral column geometric anatomy.

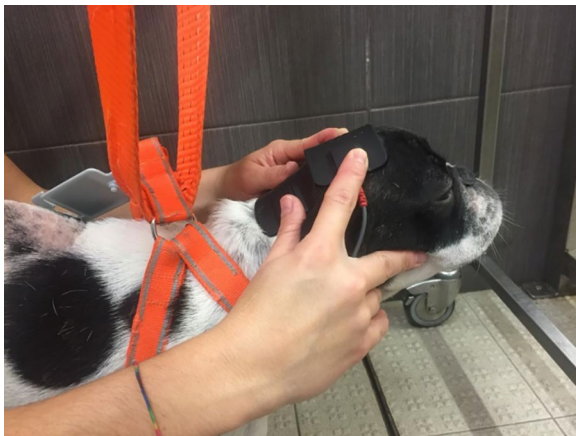


Figure 5. Application of TDCS modality on a dog - Anode localization near the brainstem region.



Figure 6. Transcranial direct current stimulation - TDCS protocol based on a 1 mA current for 20 minutes on a dog.

1.5. Locomotor training

Neurorehabilitation therapies are based on task-specific repetitive training, sensorimotor stimulation and intensive exercises (Cecatto and Chadi 2007) that promotes functional reorganization of the neuromuscular system (Harkema et al. 2012a)

Treadmill training provides a tool for relearning stepping but it depends of much practice is done regarding the exercises, even for professional human athletes. Thus, success of the performance is highly variable, suggesting the fundamental feature of the neural control of movement (Edgerton et al. 2008). However, always remembering that plasticity improvement on spinal neuronal circuits has already been proved in experiments with “complete” SCI cats (Lovely et al. 1986; Barbeau and Rossignol 1987; De Leon et al. 1998; Cai et al. 2006; Edgerton et al. 2008; Hubli and Dietz 2013).

Battistuzzo et al. 2012 selected a systematic review about exercise training intervention in quadrupedal animals (23% of the studies in cats, 65% in rats and 12% in mice) with thoracic SCI. These exercise protocols were based on body weight-supported treadmill training (BWSTT) (41% of the studies) or robotic devices to assist stepping (35%).

Thus, outcome of a neurorehabilitation approach depends on the type, number of repetitions and the quality of the motor function. These allows free synaptic territories to become occupied by sprouting of intraspinal fibres (Hou et al. 2008).

One of the first attempt to translate the neuroanatomical principles from the laboratory into the clinical setting was based on the evidence of CPG. The CPG of the spinal cord promote oscillating activity of the flexor/extensor muscles groups in all limbs, regardless of any supraspinal or afferent input (Grillner and Zangger 1975, 1979; Grillner and Wallen 1985; Grillner and Dubuc 1988; Basso 2000; Yakovenko et al 2004).

The CPG are located in the lumbar segments of the spinal cord in rats, cats and dogs (Brown 1914; Grillner and Zangger 1979; Grillner and Wallen 1985; Grillner and Dubuc 1988) and can be triggered by external stimuli, such as the treadmill belt moving, that is a typical exercise in complete SCI dogs and cats to achieve normal stepping (Robinson and Goldberger 1986; Barbeau and Rossignol 1987; Howland et al. 1995).

Edgerton and colleagues (2008) confirmed that an experimental group of animals that were trained to learn how to performed a specify task had a higher competency than animals relying on spontaneous recovery, reflecting the spinal cord ability to “Learn” and execute motor tasks in quadrupeds.

Therefore, quadruped animals exhibit a direct evidence that spinal networks (CPG and motor interneurons) are the basis of generating locomotor rhythm (Barthélemy et al. 2015). The BWSTT may be a possible method to enhance the activity-dependent plasticity, as well as the residual pathways and networks (Dobkin 2000; Barbeau and Fung 2001; Edgerton et al. 2001, 2003).

The flexion/extension locomotor pattern may be induced by the spinal cord EES in adult cats, which is reported to occur in the L5-S1 segments (Iwahara et al. 1991) and L4-L5 (Gerasimenko et al. 2002, 2003). This is also reported in rats (Lavrov et al 2006) and in humans (Dimitrijevic et al. 1998; Gerasimenko et al. 2001; Minassian et al. 2007b).

Rossignol et al. (2006) have shown that L4 segment is essential for initiating stepping in cats, which is supported by other authors (Langlet et al. 2005; Barthélemy et al. 2007). Thus, until now there are three main procedures that have proven to be successful to postural and locomotion activity: locomotor training, which involves modulation of the spinal network based on learning ability; combination of pharmacological agonists and antagonists to neurotransmitter systems, promoting disinhibition of some inhibitory systems, such as the glycinergic and GABAergic (Tillakaratne et al. 2002); and the modulation by electrical stimulation (Gerasimenko et al. 2008).

In rats, Lavrov and colleagues (2008a), proved a higher independent positive effect of electrical stimulation to the spinal afferents that projects to the neural network, when associated to the proprioceptive information from the hindlimbs placed on a moving treadmill belt.

In human medicine, active assisted movements are studied in regard to the potential benefit on motor control and spasticity (Harel and Tansey 2017). Particularly, the BWSTT directed to the lumbar locomotor CPG circuits with the main aim of improving coordination and ambulation (Colombo et al 2000; Swinnen et al. 2010; Adams and Hicks 2011; Alexeeva et al. 2011; Tefertiller et al. 2011; Mehrholz et al. 2012; Morawietz and Moffat 2013; Smith and Knikou 2016). It has been shown, in human patients, that these exercises may reduce spasticity by replacing abnormal hyper-excitable sensory firing with functional afferent signaling that leads to less muscle spasm and co-contraction (Thomas and Gorassini 2005; Norton and Gorassini 2006).

Thus, depending on injury severity, to perform locomotor training in human patients, it may be needed 1 to 2 therapists that have to perform supported coordinated leg movements during the swing and stance, aiming to increase walking speed, reduce weight support and the

amount of manual support. The treadmill type of training is an effective restorative therapy option in the acute phase following SCI, in particular in cases with severe sensorimotor impairment, because it allows the repetition of exercises at a higher frequency and speed (Morawietz and Moffat 2013).

In regard to the overground training, it can be combined with body-weight support such as the LiteGait system (Mobility Research Inc., Tempe, USA), which is a mobile device that allows weight bearing through a strap system attached to two overhead supports. This training device can be used over a treadmill or over ground (Hidler et al. 2011).

Other type of system is the Zero G system (Aretex, Ashburn, Virginia 20147, USA) that also provides body weight support through a harness attached to a ceiling track, which can be equipped with a static or dynamic support system while walking overground (Hidler et al. 2011).

These robotic devices promote neuroplasticity induced by sensorimotor training during the rehabilitation sessions (Mehrholz et al. 2008), while reducing staff requirements and physical demands upon the therapists (Wu et al. 2012).

Repetitive movements during locomotor training potentially enhances glutamatergic dorsal root ganglia (DRG) sensory feedback, promoting excitation of the primary extrinsic source and entering the spinal cord below the injury to engage the local circuits, which already been evidenced in animal models (Lavrov et al. 2008b).

The muscle spindle is innervated by proprioceptive sensory neurons that conduct the information about muscle contraction to the spinal cord. They constitute the proprioceptors that exhibit the most widespread central projection of all DRG sensory neurons and establish synaptic connections with motor neurons and some interneurons of the intrinsic circuits (Rossignol et al. 2006). Thus, muscle spindle afferent is probably the key to direct excitation of spinal circuits relevant to motor control regulation, mainly under conditions of disconnected descending input (Takeoka et al. 2014).

Also, locomotor training may stimulate cholinergic propriospinal cells essential for the control of CPG and the coordinated locomotor output, the basis for the recovery of locomotor ability in the absence of descending locomotor control (Jordan et al. 2014).

Therefore, descending propriospinal neurons excite neural networks regardless of supraspinal control and activation of interneural networks to enhance locomotor coordination has a major importance for designing strategies to stepping recovery (Behrman and Harkema 2000; Shah et al. 2013).

The early treadmill training initiated soon after severe SCI, can induce leg muscle electromyography and activity modulated over the step cycle in a similar way to as observed in healthy human patients. In these types of situations there is a diminished noradrenergic input to neural networks and the signal amplitude is considered to be smaller (Barbeau and Rossignol 1994). Also, dendritic persistent inward currents (PICs) don't make motoneuron dendrites highly active (Powers et al. 2002; Binder 2003; Heckman et al. 2003, 2005), which could be restored with monoamines that have several effects on motoneurons, including their main effect on the PICs (Powers and Binder 2001; Alaburda et al. 2002).

Principles of motor learning have to be implemented based on the number of repetitions and the functional consequences of the locomotor training on mediating neuronal plasticity, increasing performance (Smith and Knikou 2016). In an early phase after SCI, automated locomotor training is an important therapy, especially in human patients with severe motor impairment. The choice of the type of assisted training with different devices, depends on the extent of sensorimotor deficits in the lower limbs and trunk, as well as the cardiopulmonary restrictions (Hensel et al. 2017).

Most protocols consisted on one daily training session (82%) (Stevens et al. 2006; Carvalho et al. 2008; Heng and de Leon 2009; Ichiyama et al. 2009; Oh et al. 2009; Robert et al. 2010; Singh et al. 2011). Only three studies (18%) were based on two to three daily sessions (Moshonkina et al. 2002; Nothias et al. 2005; Foret et al. 2010). Also, duration of each session was diverse, from 5-15 min (53%) to 20-30 min (47%). Period of time, regarding therapy, also varied widely from 1-36 weeks (12% with < 4 weeks; 53% with 4-9 weeks; 35 % with > 10 weeks), and 15 studies reported training 5 days/week.

In regard to the type of training implemented, the most frequent was treadmill training (33%), followed by the BWSTT (17%) and results were assessed, regarding quality, with randomization into groups (Engesser-Cesar et al 2005; Carvalho et al. 2008; Kuerzi et al. 2010; Lee et al 2010; Tillakaratne et al 2010; Singh et al. 2011).

Locomotor training in addition to electrical or magnetic stimulation therapies and pharmacological drugs, such as serotonergic and noradrenergic agonists, can activate spinal locomotor circuitries in cats, rats and mice (Hoyer et al. 1994; Hsiao et al. 1998; Collins et al. 2002). Thus, designing effective neurorehabilitation protocols depends on task-specific sensory exercises, supporting the recruitment of both neural networks and spared supraspinal connections (Jacobs et al. 2002).

In addition, complete SCI studies with positive outcomes were registered in 55% of studies in rats, 50 % in mice and 100% in cats. Only 40% of the studies were considered successful with less than 6 weeks of training, but 100% were successful with 6 to 9 weeks.

In human patients, intensive therapy was performed 5 times a week for 90 min sessions, with step training for a minimum of 20 min and long-term follow up evaluations for 6-12 months (Harkema et al 2012b). Motor recovery in these type of patients with incomplete motor SCI after intensive training, was registered within the first 2 months of rehabilitation (Mehrholtz et al. 2008).

Specific amount of time needed for standing or stepping also depends on each subject level of exercise induced fatigue (Dobkin et al. 2006).

Hensel et al. (2017) has said that the treadmill training should be mixed with overground exercises and replaced by these activities as soon as possible. Treadmill training with body weight support has become essential for gait rehabilitation in patients with incomplete SCI, allowing an almost physiological joint movement of the hip, knee and ankle, with a main emphasis on the hip joint (Behrman and Harkema 2000; Dietz et al. 2002).

Locomotor training has been correlated to an increase in bone and muscle mass along with positive cardiovascular effects (Behrman and Harkema 2000; Wirz et al. 2001; Hicks et al 2005; Dobkin et al. 2006; Harkema et al. 2012c) and intralimb hip-knee coordination (Field-Fote and Tepavac 2002).

2. Objectives

The field of neurorehabilitation has grown in human medicine in the last decades, but it is still a challenge in patients with complete sensorimotor injury with absence of voluntary movement and/or deep pain perception below the injury, classified with the American Spinal Injury Association Impairment Scale (AIS) as grade A. The translation for veterinary medicine takes place through the MFS in grade 0 patients, who are a subset severely injured dogs and cats that fail to regain pain perception caudally to the injury, referring to DPP⁻ patients classified as having an unsuccessful outcome (Scott and McKee 1999; Granger and Carwardine 2014; Lewis et al. 2020).

Therefore, neurorehabilitation based on therapeutic modalities and repetitive intensive training may help to regain ambulation, which is define as the ability to rise and take at least 10 consecutive weight-bearing steps, unassisted and without falling (Moore et al. 2016, 2020). This ambulation, when based on reflex stimulation is called spinal reflex locomotion (SRL), meaning coordinated and balanced locomotion and not the traditional “Spinal Walking” gait. Gallucci and colleagues (2017) believe that this reflexive stepping is generated by the spinal cord caudal to the injury site, as described by experimental studies performed in cats and other species (Sherington 1910; Brown 1911; Forssberg and Grillner 1973; Blaich 1977; Rossignol 1986; Lovely et al. 1986, 1990; Barbeau and Rossignol 1987, 1991; Naito et al. 1990; Edgerton et al. 1991; Belanger et al. 1996; De Leon et al. 1999).

Furthermore, Barrett et al. (2013) proposed neurorehabilitation as a field that involves a wide range of treatments, which include neural regeneration, repair and dynamic reorganization of functional neural systems (Grau et al. 2020).

Thus, the first and main objective was to improve ambulation faster than spontaneous recovery or with standard physiotherapy in DPP⁻ dogs, but also in DPP⁺ and MFS grade 1, with compressive or non-compressive myelopathies, always in the acute phase (at least up to 7 days from injury to rehabilitation).

We hypothesize that multidisciplinary protocols with locomotor training, FES and, in some cases, pharmacological management, improve the integration between sensory input and CPG activity to coordinate motor output due to limited or residual lack of supraspinal tracts, contributing to the motor and sensory control. This reorganization potentially demonstrates intact brain and spinal cord communication with new connections traversing the epicenter of the injury, which in human medicine refers to the “discomplete” patients (Militskova et al. 2020). In this PhD program, the pharmacological approach was made with 4-aminopyridine (4-AP), a

potassium channel antagonist that has been shown to restore pelvic limbs motor function, already proven in dogs with chronic thoracolumbar SCI (Nishida et al. 2014; Wang-Leandro et al. 2017; Lewis et al. 2020).

The second objective was to prove that the same neurorehabilitation protocols that aim to improve anatomical and synaptic changes in the SCI (Van de Crommert et al. 1998; Côté and Gossard 2004; Takeoka et al. 2014; Rossignol et al. 2015), might result in recovery of motor/sensory function in chronic DPP⁻ dogs and cats, but also in DPP⁺ dogs, with more than 3 months of SCI, with or without decompressive surgery, most of them already stimulated by home exercises or standard physical therapy.

The third objective was to prove that electrostimulation programs, such as FES, interferential electrical stimulation (IES) and TESCOs, associated with locomotor training, are safe, tolerable, repeatable, feasible and a professional therapeutic plan to treat all dogs and cats that present severe SCI (with or without spinal shock) in acute, subacute or chronic stage, increasing ambulation and decreasing unsuccessful cases that end up on euthanasia.

3. References

- Adams MM, Hicks AL. 2011. Comparison of the effects of body-weight-supported treadmill training and tilt-table standing on spasticity in individuals with chronic spinal cord injury. *J Spinal Cord Med.* 34: 488-494.
- Akaza M, Kanouchi T, Inaba A, Numasawa Y, Irioka T, Mizusawa H, Yokota T. 2010. Motor nerve conduction study in cauda equina with high-voltage electrical stimulation in multifocal motor neuropathy and amyotrophic lateral sclerosis. *Muscle & Nerve.* 43 (2): 274-282.
- Alaburda A, Perrier JF, Hounsgaard J. 2002. Mechanisms causing plateau potentials in spinal motoneurons. *Adv Exp Med Biol.* 508:219-226.
- Alexeeva N, Sames C, Jacobs PL, Hobday L, Distasio MM, Mitchell SA, Calancie B. 2011. Comparison of training methods to improve walking in persons with chronic spinal cord injury: a randomized clinical trial. *J Spinal Cord Med.* 34: 362-379.
- Allen CB, Celikel T, Feldman DE. 2003. Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. *Nature Neurosci.* 6 (3): 291-299.
- Angeli CA, Edgerton VR, Gerasimenko YP, Harkema SJ. 2014. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain.* 137 (5): 1394-1409.
- Antal A, Polania R, Schmidt-Samoa C, Dechent P, Paulus W. 2011. Transcranial direct current stimulation over the primary motor cortex during fMRI. *Neuroimage.* 55 (2): 590-596.
- Artola A, Bröcher S, Singer W. 1990. Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. *Nature.* 347 (6288): 69-72.
- Barbeau H, Rossignol S. 1987. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* 412: 84–95.
- Barbeau H, Rossignol S. 1991. The effects of serotonergic drugs on the locomotor pattern and on cutaneous reflexes of the adult chronic spinal cat. *Brain Res.* 514: 55–67.
- Barbeau H, Rossignol S. 1994. Enhancement of locomotor recovery following spinal cord injury. *Curr. Opin. Neurol.* 7: 517–524.
- Barbeau H, Fung, J. 2001. The role of rehabilitation in the recovery of walking in the neurological population. *Curr Opin Neurol.* 14:735-40.
- Barrett AM, Oh-Park M, Chen P, Ifejika NL. 2013. Neurorehabilitation: five new things. *Neurol Clin Pract.* 3:484-492.
- Barthelemy D, Willerslev-Olsen M, Lundell H, Biering-Sorensen F, Bo Nielsen J. 2015. Assessment of transmission in specific descending pathways in relation to gait and balance following spinal cord injury. *Prog Brain Res.* 218:79-101.
- Basso DM. 2000. Neuroanatomical substrates of functional recovery after experimental spinal cord injury: Implications of basic science research for human spinal cord injury. *Phys Ther.* 80 (8):808-817.
- Battistuzzo CR, Callister RJ, Callister R, Galea MP. 2012. A systematic review of exercise training to promote locomotor recovery in animal models of spinal cord injury. *J Neurotrauma.* 29:1600-1613.
- Behrman AL, Harkema SJ. 2000. Locomotor Training After Human Spinal Cord Injury: A Series of Case Studies. *Phys. Ther.* 80: 688–700.
- Belanger M, Drew T, Provencher J, Rossignol S. 1996. A comparison of treadmill locomotion in adult cats before and after spinal transection. *J Neurophysiol.* 76:471–91.
- Bennett MR. 2000. The concept of long-term potentiation of transmission at synapses. *Prog Neurobiol.* 60 (2): 109-137.

Bikson M, Datta A, Rahman A, Scaturro J. 2010. Electrode montages for tDCS and weak transcranial electrical stimulation: role of “return” electrode’s position and size. *Clin Neurophysiol.* 121 (12): 1976-1978

Bindman LJ, Lippold OCJ, Redfearn JWT. 1962. Long-lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. *Nature.* 196 (4854): 584-585.

Bindman LJ, Lippold OCJ, Redfearn JWT. 1964. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J Physiol.* 172 (3): 369-382.

Binder MD. 2003. Intrinsic dendritic currents make a major contribution to the control of motoneurone discharge. *J Physiol.* 552:665.

Blauch B. Spinal reflex walking in the dog. 1977. *Vet Med Sm Anim Clin.* 169:173.

Brown TG. 1911. The intrinsic factors in the act of progression in the mammal. *Proc Royal Soc.* 84:308–19.

Brown TG. 1914. On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of evolution of function in the nervous system. *J Physiol.* 48:18-46.

Cai LL, Courtine G, Fong AJ, Burdick JW, Roy RR, Edgerton VR. 2006. Plasticity of functional connectivity in the adult spinal cord. *Philos Trans R Soc Land B Biol Sci.* 361(1473)

Carvalho KAT, Cunha RC, Vialle EM, Osiecki R, Moreira GHG, Simeoni RB, Francisco JC, Guarita-Souza LC, Oliveira L, Zocche L, et al. 2008. Functional outcome of bone marrow stem cells (CD45(+)/CD34(-)) after cell therapy in acute spinal cord injury: in exercise training and in sedentary rats. *Transplant. Proc.* 40: 847-849.

Cecatto RB, Chadi G. 2007. The importance of neuronal stimulation in central nervous system plasticity and neurorehabilitation strategies. *Functional Neurology.* 22(3): 137-143.

Collins DF, Gorassini M, Bennett D, Burke D, Gandevia SC. 2002. Recent evidence for plateau potentials in human motoneurons. *Adv Ex Med Biol.* 508:227-235.

Colombo G, Joerg M, Schreider R, Dietz V. 2000. Treadmill training of paraplegic patients using a robotic orthosis. *J Rehabil Res Dev.* 37: 693-700.

Côté MP, Gossard JP. Step training-dependent plasticity in spinal cutaneous pathways. 2004. *J Neurosci.* 24:11317-27.

Courtine G, Harkema SJ, Dy CJ, Gerasimenko YP, Dyhre-Poulsen P. 2007. Modulation of multisegmental monosynaptic responses in a variety of leg muscles during walking and running in humans. *J Physiol.* 582 (3): 1125-1139.

Cowley KC, Zaporozhets E, Schmidt BJ. 2008. Propriospinal neurons are sufficient for bulbospinal transmission of the locomotor command signal in the neonatal rat spinal cord. *J Physiol.* 586 (6): 1623-1635.

Daly JJ, Roenigk KL, Butler KM, Gansen JL, Fredrickson E, Marsolais EB, Rogers J, Ruff RL. 2004. Response of sagittal plane gait kinematics to weight-supported treadmill training and functional neuromuscular stimulation following stroke. *J Rehabil Res Dev.* 41 (6A): 807-820.

Danner SM, Hofstoetter US, Ladenbauer J, Rattay F, Minassian K. 2011. Can the human posterior columns be stimulated by transcutaneous spinal cord stimulation? A modeling study. *Artif Organs.* 35 (3): 257-262.

De Lahunta A, Glass E. 2014. General Sensory Systems: General Proprioception and General Somatic Afferent. In: De Lahunta A, Glass E, Kent M, editors. *Veterinary Neuroanatomy and Clinical Neurology.* 4th Ed. Philadelphia: Saunders Elsevier. p. 221-242.

De Leon RD, Hodgson JA, Roy RR, Edgerton VR. 1998. Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J Neurophysiol.* 80 (1): 83-91.

- Desai NS, Cudmore RH, Nelson SB, Turrigiano GG. 2002. Critical periods for experience-dependent synaptic scaling in visual cortex. *Nature neurosci.* 5 (8): 783-790.
- Dietz V, Müller R, Colombo G. 2002. Locomotor activity in spinal man: significance of afferent input from joint and load receptors. *Brain.* 125 (12): 2626-2634.
- Dietz V. 2012. Neuronal plasticity after a human spinal cord injury: positive and negative effects. *Exp Neurol.* 235 (1): 110-115.
- Dimitrijevic MR, Illis LS, Nakajima K, Sharkey PC, Sherwood AM. 1986. Spinal cord stimulation for the control of spasticity in patients with chronic spinal cord injury: II. Neurophysiological Observations. *Cent Nerv Syst Trauma.* 3 (2): 145-152
- Dimitrijevic M, Gerasimenko Y, Pinter M. 1998. Evidence for a spinal central pattern generator in humans. *Ann NY Acad Sci.* 860: 360-376.
- Dimitrijevic MR, Dimitrijevic MM, Kern H, Minassian K, Rattay F. 2004. Electrophysiological characteristics of H-reflexes elicited by percutaneous stimulation of the cauda equina. Program No. 417.11. Abstract presentation. Washington, DC: Society for Neuroscience.
- Dobkin B. 2000. Functional rewiring of brain and spinal cord after injury: the three R's of neural repair and neurological rehabilitation. *Curr Opin Neurol.* 13:655-9.
- Dobkin B, Apple D, Barbeau H, Basso M, Behrman A, Deforge D, Ditunno J, Dudley G, Elashoff R, Fugate L, et al. 2006. Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI. *Neurology.* 66: 484–493.
- Dy CJ, Gerasimenko YP, Edgerton VR, Dyhre-Poulsen P, Courtine G, Harkema SJ. 2010. Phase-dependent modulation of percutaneously elicited multisegmental muscle responses after spinal cord injury. *J Neurophysiol.* 103 (5): 2808-2820.
- Edgerton VR, Roy RR, Hodgson JA, Prober RJ, de Guzman CP, de Leon R. 1991. A physiological basis for the development of rehabilitative strategies for spinally injured patients. *J Am Paraplegia Soc.* 14: 150–157.
- Edgerton V, de Leon R, Harkema S, Hodgson JA, London N, Reinkensmeyer DJ, Roy RR, Talmadge RJ, Tillakaratne NJ, Timoszyk W, et al. 2001. Retraining the injured spinal cord. *J Physiol.* 533:15-22.
- Edgerton V, Harkema S, Dobkin B. 2003. Retraining the human spinal cord. In: Lin V, editor. *Spinal cord medicine: principles and practice.* New York: Demos Medical. p. 817-28.
- Edgerton VR, Courtine G, Gerasimenko YP, Lavrov I, Ichiyama RM, Fong AJ, Cai LL, Otsoshi CK, Tillakaratne NJK, et al. 2008. Training locomotor networks. *Brain Res Rev.* 57(1):241-254.
- Engesser-Cesar C, Anderson AJ, Basso DM, Edgerton VR, Cotman CW. 2005. Voluntary wheel running improves recovery from a moderate spinal cord injury. *J Neurotrauma.* 22: 157-171.
- Estes SP, Iddings JA, Field-Fote EC. 2017. Priming neural circuits to modulate spinal reflex excitability. *Front Neurol* 3 (8): 17.
- Ferrucci R, Mameli F, Guidi I, Mrakic-Sposta S, Vergari M, Marceglia S, Cogiamanian F, Barbieri S, Scarpini E, Priori A. 2008a. Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology.* 71 (7): 493-498.
- Ferruci R, Marceglia S, Vergari M, Cogiamanian F, Mrakic-Sposta S, Mameli F, Zago S, Barbieri S, Priori A. 2008b. Cerebellar transcranial direct current stimulation impairs the practice-dependent proficiency increase in working memory. *J Cogn Neuroscience.* 20 (9): 1687-1697.
- Field-Fote EC, Tepavac D. 2002. Improved intralimb coordination in people with incomplete spinal cord injury following training with body weight support and electrical stimulation. *Phys Ther.* 82: 707-715.

Fisher MA. 2012. H-reflex and F-response studies. In: Aminoff MJ. Editor. *Aminoff's Electrodiagnosis in Clinical Neurology*, 6th Ed. China: Elsevier Saunders. p. 407-420.

Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, Marcolin MA, Rigonatti SP, Silva MTA, Paulus W, Pascual-Leone A. 2005. Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp Brain Res*. 166 (1): 23-30.

Fregni F, Boggio PS, Lima MC, Ferreira MJL, Wagner T, Rigonatti SP, Castro AW, Souza DR, Riberto M, Freedman SD, Nitsche MA, Pascual-Leone A. 2006. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. *Pain*. 122: 197-209.

Fregni F, Pascual-Leone A. 2007. Technology insight: noninvasive brain stimulation in neurology - perspectives on the therapeutic potential of rTMS and tDCS. *Nat Clin Pract Neurol*. 3 (7): 383-93.

Foret A, Quertainmont R, Botman O, Bouhy D, Amabili P, Brook G, Schoenen J, Frazen R. 2010. Stem cells in the adult rat spinal cord: plasticity after injury and treadmill training exercise. *J Neurochem*. 112: 762-772.

Forssberg H, Grillner S. 1973. The locomotion of the acute spinal cat injected with clonidine iv. *Brain Res*. 50: 184-186.

Fouad K, Tetzlaff W. 2012. Rehabilitative training and plasticity following spinal cord injury. *Exp Neurol*. 235: 91-99.

Gad P, Gerasimenko Y, Zdunowski S, Turner A, Sayenko D, Lu DC, Edgerton VR. 2017. Weight bearing over-ground stepping in an exoskeleton with non-invasive spinal cord neuromodulation after motor complete paraplegia. *Front Neurosci*. 11: 333.

Gallucci A, Dragone L, Menchetti M, Gagliardo T, Pietra M, Cardinali M, Gandini G. 2017. Acquisition of involuntary spinal locomotion (spinal walking) in dogs with irreversible thoracolumbar spinal cord lesion: 81 dogs. *J Vet Intern Med*. 31: 492-7.

Gandiga PC, Hummel FC, Cohen LG. 2006. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin Neurophysiol*. 117 (4): 845-850.

García-Larrea LG, Peyron R, Mertens P, Gregoire MC, Lavenne F, Bonnefoi F, Mauguière F, Laurent B, Sindou M. 1997. Positron emission tomography during motor cortex stimulation for pain control. *Stereotact Funct Neurosurg*. 68: 141-148.

García-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Le Bars D, Convers P, Mauguière F, Sindou M, Laurent B. 1999. Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. *Pain*. 83 (2): 259-273.

Gerasimenko Y, Daniel O, Regnaud J, Combeaud M, Bussel B. 2001. Mechanisms of locomotor activity generation under epidural spinal cord stimulation. In: R Dengler, A Kossev, editors. *Sensorimotor Control*. Washington DC: IOS Press. p. 64-171.

Gerasimenko Y, Makarovskii A, Nikitin O. 2002. Control of locomotor activity in humans and animals in the absence of supraspinal influences. *Neurosci Behav Physiol*. 32: 417-423.

Gerasimenko YP, Avelev VD, Nikitin OA, Lavrov IA. 2003. Initiation of locomotor activity in spinal cats by epidural stimulation of the spinal cord. *Neurosci Behav Physiol*. 33: 247-254.

Gerasimenko Y, Roy RR, Edgerton R. 2008. Epidural stimulation: comparasion of the spinal circuits that generate and control locomotion in rats, cats and humans. *Exp Neurol*. 209 (2): 417-425.

Gerasimenko Y, Gorodnichev R, Puchov A, Moshonkina T, Savochin A, Roy RR, Edgerton VR. 2012. Multi-site transcutaneous electrical stimulation of the spinal cord effectively facilitates the locomotor circuitry in non-injured humans. U.S. Patent Application No. 15/975,678.

Gerasimenko Y, Gorodnichev R, Puhov A, Moshonkina T, Savochin A, Selionov V, Roy RR, Lu DC, Edgerton VR. 2014. Initiation and modulation of locomotor circuitry output with

multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. *J Neurophysiol.* 113 (3): 834-842.

Gerasimenko Y, Gorodnichev R, Moshonkina T, Sayenko D, Gad P, Edgerton VR. 2015. Transcutaneous electrical spinal-cord stimulation in humans. *Ann Phys Rehabil Med.* 58 (4): 225-231.

Granat MH, Heller BW, Nicol DJ, Baxendale RH, Andrews BJ. 1993. Improving limb flexion in FES gait using the flexion withdrawal response for the spinal cord injured person. *J Biomed Eng.* 15 (1): 51-56.

Granger N, Carwardine D. 2014. Acute spinal cord injury: tetraplegia and paraplegia in small animals. *Vet Clin North Am Small Anim Pract.* 44:1131– 56.

Grasso R, Yvanenko YP, Zago M, Molinari M, Scivoletto G, Castellano V, Macellari V, Lacquaniti F. 2004. Distributed plasticity of locomotor pattern generators in spinal cord injured patients. *Brain.* 127: 1019-1034.

Grau JW, Baine RE, Bean PA, Davis JA, Fauss GN, Henwood MK, Hudson KE, Johnson DT, Tarbet MM, Strain MM. 2020. Learning to promote recovery after spinal cord injury. *Exp Neurol.* 330: 113334.

Grillner S, Zangger P. 1975. How detailed is the central pattern generation for locomotion? *Brain Res.* 88: 367-371.

Grillner S, Zangger P. 1979. On the central generation of locomotion in the low spinal cat. *Exp Brain Res.* 34:241-261.

Grillner S, Wallen P. 1985. Central pattern generators for locomotion, with special reference to vertebrates. *Ann Rev Neurosci.* 8:233-261.

Grillner S, Dubuc R. 1988. Control of locomotion in vertebrates: spinal and supraspinal mechanisms. *Adv Neurol.* 47:425-453.

Groppa S, Bergmann TO, Siems C, Mölle M, Marshall L, Siebner HR. 2010. Slow-oscillatory transcranial direct current stimulation can induce bidirectional shifts in motor cortical excitability in awake humans. *Neurosci.* 166 (4): 1219-1225.

Gybels J, Van Roost D. 1985. Spinal cord stimulation for the modification of dystonic and hyperkinetic conditions: a critical review. In: Eccles JC, Dimitrijevic MR, editors. *Recent achievements in restorative neurology.* Karger: Basel. p. 56-70.

Hamid S, Hayek R. 2008. Role of electrical stimulation for rehabilitation and regeneration after spinal cord injury: an overview. *Eur Spine J.* 17 (9): 1256- 1269.

Harkema SJ, Gerasimenko Y, Hodes J, Burdick J, Angeli C, Chen Y, Ferreira C, Willhite A, Rejc E, Grossman RG, Edgerton VR. 2011. Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *Lancet.* 377 (9781): 1938-1947.

Harkema SJ, Schmidt-Read M, Lorenz DJ, Edgerton VR, Behrman AL. 2012b. Balance and ambulation improvements in individuals with chronic incomplete spinal cord injury using locomotor training-based rehabilitation. *Arch Phys Med Rehabil.* 93 (9): 1508-1517.

Harkema SJ, Schmidt-Read M, Behrman AL, Bratta A, Sisto SA, Edgerton VR. 2012a. Establishing the neuro recovery network: multisite rehabilitation centers that provide activity-based therapies and assessments for neurologic disorders. *Arch Phys Med Rehabil.* 93 (9): 1498-1507.

Harkema SJ, Hillyer J, Schmidt-Read M, Ardolino E, Sisto SA, Behrman AL. 2012c. Locomotor training: as a treatment of spinal cord injury and in the progression of neurologic rehabilitation. *Arch Phys Med Rehabil.* 93(9): 1588-1597.

Harel NY, Tansey KE. 2017. Spasticity. In: N Weidner et al., editors. *Neurological aspects of spinal cord injury.* Switzerland: Springer International. p. 303-324.

Heckman CJ, Lee RH, Brownstone RM. 2003. Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. *Trends Neurosci.* 26:688-695.

- Heckman CJ, Gorassini MA, Bennett DJ. 2005. Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle & Nerve*. 31: 135-156.
- Heng C, de Leon RD. 2009. Treadmill training enhances the recovery of normal stepping patterns in spinal cord contused rats. *Exp Neurol*. 216: 139-147.
- Hensel C, Kaschuba R, Schmidt E-M. 2017. In: N Weidner et al. editors. *Neurological aspects of spinal cord injury*. Switzerland: Springer International. p. 649-688.
- Hesse S, Malezic M, Schaffrin A, Mauritz KH. 1995. Restoration of gait by combined treadmill training and multichannel electrical stimulation in non-ambulatory hemiparetic patients. *Scand J Rehabil Med*. 24 (4):199-204.
- Hicks AL, Adams MM; Martin GK, Giangregorio L, Latimer A, Phillips SM, McCartney N. 2005. Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being. *Spinal Cord*. 43 (5):291-298.
- Hidler J, Brennan D, Black I, Nichols D, Brady K, Nef T. 2011. ZeroG: overground gait and balance training system. *J Rehabil Res Dev*. 48 (4): 287-298.
- Hoffman RE, Hawkins KA, Gueorguieva R, Boutros NN, Rachid F, Carroll K, Krystal JH. 2003. Transcranial magnetic stimulation of left temporoparietal cortex and medication-resistant auditory hallucinations. *Arch Gen Psychiatry*. 60 (1): 49-56.
- Hofstoetter US, Minassian K, Hofer C, Mayr W, Rattay F, Dimitrijevic MR. 2008. Modification of reflex responses to lumbar posterior root stimulation by motor tasks in healthy subjects. *Artif Organs*. 32 (8): 644-648.
- Hofstoetter US, Hofer C, Kern H, Danner SM, Mayr W, Dimitrijevic MR, Minassian K. 2013. Effects of transcutaneous spinal cord stimulation on voluntary locomotor activity in an incomplete spinal cord injured individual. *Biomed Tech (Berl)*.58, Suppl. 1.
- Hofstoetter US, Danner SM, Minassian K. 2014. Paraspinal magnetic and transcutaneous electrical stimulation. In: Jaeger D, Jung R, editors. *Encyclopedia of Computational Neuroscience*. New York: Springer, p. 1-21.
- Hofstoetter US, Krenn M, Danner SM, Hofer C, Kern H, McKay WB, Mayr W, Minassian K. 2015. Augmentation of voluntary locomotor activity by transcutaneous spinal cord stimulation in motor-incomplete spinal cord-injured individuals. *Artif Organs*. 39 (10): E176-E186.
- Hofstoetter US, Freundl B, Binder H, Minassian K. 2018. Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: elicitation of posterior root-muscle reflexes. *PloS One*. 13 (1): 1-22.
- Holsheimer J. 1998a. Computer modelling of spinal cord stimulation and its contribution to therapeutic efficacy. *Spinal Cord*. 36: 531-540.
- Holsheimer J. 1998b. Concepts and methods in neuromodulation and functional electrical stimulation: an introduction. *Neuromodulation*. 1 (2): 57-61.
- Holsheimer J. 2002. Which neuronal elements are activated directly by spinal cord stimulation. *Neuromodulation*. 5 (1): 25-31.
- Hongo T, Jankowska E, Lundberg A. 1972. The rubrospinal tract. IV. Effects on interneurons. *Exp Brain Res*. 15 (1): 54-78.
- Hou S, Duale H, Cameron AA, Abshire SM, Lyttle TS, Rabchevsky AG. 2008. Plasticity of lumbosacral propriospinal neurons is associated with the development of autonomic dysreflexia after thoracic spinal cord transection. *J Comp Neurol*. 509 (4): 382-399.
- Howland DR, Bregman BS, Tessler A,Goldberger ME. 1995. Development of locomotor behavior in the spinal kitten. *Exp Neurol*.135:108-122.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA. 1994. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev*. 46: 157-293.

- Hsiao CF, Del Negro CA, Trueblood PR, Chandler SH. 1998. Ionic basis for serotonin-induced bistable membrane properties in guinea pig trigeminal motoneurons. *J Neurophysiol.* 79: 2847-2856.
- Huang H, He J, Herman R, Carhart MR. 2006. Modulation effects of epidural spinal cord stimulation on muscle activities during walking. *IEEE Trans Neural Syst Rehabil Eng.* 14 (1): 14-23.
- Hubli M, Dietz V, Bolliger M. 2012. Spinal reflex activity: a marker for neuronal functionality after spinal cord injury. *Neurorehabil Neural Repair.* 26 (2): 188-196.
- Hubli M, Dietz V. 2013. The physiological basis of neurorehabilitation – locomotor training after spinal cord injury. *J Neuroeng Rehabil.* 10:1–8.
- Hummel F, Celnik P, Giraux P, FLoel A, Wu WH, Gerloff C, Cohen LG. 2005. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain.* 128 (3): 490-499.
- Hunter JP, Ashby P. 1994. Segmental effects of epidural spinal cord stimulation in humans. *J Physiol.* 474 (3): 407-419.
- Ichiyama R, Potuzak M, Balak M, Kalderon N, Edgerton VR. 2009. Enhanced motor function by training in spinal cord contused rats following radiation therapy. *PLoS One.* 4(8):e6862
- Islam N, Aftabuddin M, Moriwaki A, Hattori Y, Hori Y. 1995. Increase in the calcium level following anodal polarization in the rat brain. *Brain Res.* 684 (2): 206-208.
- Iwahara T, Atsuta Y, Garsia-Rill E, Skinner R. 1991. Spinal cord stimulation-induced locomotion in the adult cat. *Brain Res Bull.* 28: 99-105.
- Jacobs BL, Martin-Cora FJ, Fornal CA. 2002. Activity of medullary serotonergic neurons in freely moving animal. *Brain Res Rev.* 40:45-52.
- Johnston TE, Greco MN, Gaughan JP, Smith BT, Betz RR. 2005. Patterns of lower extremity innervation in pediatric spinal cord injury. *Spinal Cord.* 43: 476-482.
- Jordan LM, McVagh JR, Noga BR, Cabaj AM, Majczynski H, Slawinska U, Provencher J, Leblond H, Rossignol S. 2014. Cholinergic mechanisms in spinal locomotion – potential target for rehabilitation approaches. *Front Neural Circ.* 8 (132):1-25.
- Juvin L, Simmers J, Morin D. 2005. Propriospinal circuitry underlying interlimb coordination in mammalian quadrupedal locomotion. *J Neurosci.* 25 (25): 6025-6035.
- Juvin L, Le Gal JP, Simmers J, Morin D. 2012. Cervicolumbar coordination in mammalian quadrupedal locomotion: role of spinal thoracic circuitry and limb sensory inputs. *J Neurosci.* 32 (3): 953-965.
- Kimura J. 2013. H, T, and masseter reflexes and the silent period. In: *Electrodiagnosis in diseases of nerve and muscle: principles and practice.* 4th Ed. Oxford: Oxford University Press. p. 208-222.
- Kitano K, Koceja DM. 2009. Spinal reflex in human lower leg muscles evoked by transcutaneous spinal cord stimulation. *J Neurosci Methods.* 180 (1):111-5.
- Klein E, Kreinin I, Chistyakov A, Koren D, Mecz L, Marmur S, Bem-Shachar D, Feinsod M. 1999. Therapeutic efficacy of right prefrontal slow repetitive transcranial magnetic stimulation in major depression: a double-blind controlled study. *Arch Gen Psychiatry.* 56 (4): 315-320.
- Kralj A, Grobelnik S. 1973. Functional electrical stimulation - a new hope for paraplegic patients? *Buletin of Prosthetics Research.* 20: 75-102.
- Kralj A, Bajd T, Turk R, Krajnik J, Benko H. 1983. Gait restoration in paraplegic patients: a feasibility demonstration using multichannel surface electrode FES. *J Rehabil R D.* 20 (1): 3-20.

- Krames E, Rezai A, Peckham P, Aboelsaad F. 2009. What is neuromodulation? In: Krames E, Peckham P, Rezai A, editors. *Neuromodulation*. London: Elsevier-Academic Press. P. 3-8.
- Knikou M. 2012. Plasticity of corticospinal neural control after locomotor training in human spinal cord injury. *Neural Plast*. 2012:245948.
- Kuerzi J, Brown EH, Shum-Siu A, Siu A, Burke D, Morehouse J, Smith RR, Magnuson DSK. 2010. Task-specificity vs ceiling effect: step-training in shallow water after spinal cord injury. *Exp Neurol*. 224: 178-187.
- Ladenbauer J, Minassian K, Hofstoetter US, Dimitrijevic MR, Rattay F. 2010. Stimulation of the human lumbar spinal cord with implanted and surface electrodes: a computer stimulation study. *IEEE Trans Neural Syst Rehabil Eng*. 18 (6): 637-645.
- Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, Rothwell JC, Lemon RM, Frackowiak RS. 2005. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain?. *Eur J Neurosci*. 22 (2): 495-504.
- Langlet C, Leblond H, Rossignol S. 2005. The mid-lumbar segments are needed for the expression of locomotion in chronic spinal cats. *J Neurophysiol*. 93: 2474-2488.
- Lavrov I, Gerasimenko YP, Ichiyama RM, Courtine G, Zhong H, Roy RR, Edgerton VR. 2006. Plasticity of spinal cord reflexes after a complete transection in adult rats: relationship to stepping ability. *J Neurophysiol*. 96:1699-1710.
- Lavrov I, Dy CJ, Fong AJ, Gerasimenko Y, Courtine G, Zhong H, Roy RR, Edgerton VR. 2008a. Epidural stimulation induced modulation of spinal locomotor networks in adult spinal rats. *J Neurosci*. 28 (23): 6022-6029.
- Lavrov I, Courtine G, Dy CJ, Brand R, Fong AJ, Gerasimenko Y, Zhong H, Roy RR, Edgerton VR. 2008b. Facilitation of stepping with epidural stimulation in spinal rats : role of sensory input. *J Neurosci*. 28 (31): 7774 – 7780.
- Lavrov I, Musienko PE, Selionov VA, Zdunowski S, Roy RR, Edgerton VR, Gerasimenko Y. 2015. Activation of spinal locomotor circuits in the decerebrated cat by spinal epidural and/or intraspinal electrical stimulation. *Brain Res*. 1600: 84-92.
- Lee YS, Zdunowski S, Edgerton VR, Roy RR, Zhong H, Hsiao I, Lin VW. 2010. Improvement of gait patterns in step-trained, complete spinal cord-transected rats treated with a peripheral nerve graft and acidic fibroblast growth factor. *Exp Neurol*. 224: 429-437.
- Lee HJ, Cho KH, Lee WH. 2013. The effects of body weight support treadmill training with power-assisted functional electrical stimulation on functional movement and gait in stroke patients. *Am J Phys Med Rehabil*. 92 (12): 1051-1059.
- Lewis MJ, Yap PT, McCullough S, Olby NJ. 2018. The relationship between lesion severity characterized by diffusion tensor imaging and motor function in chronic canine spinal cord injury. *J Neurotrauma*. 35 (3): 500-507.
- Lewis MJ, Jeffery ND, Olby NJ, CANSORT-SCI. 2020. Ambulation in dogs with absent pain perception after acute thoracolumbar spinal cord injury. *Front Vet Sci*. 7 (560): 19-28.
- Li S, Francisco GE. 2015. New insights into the pathophysiology of post-stroke spasticity. *Front Hum Neurosci*. 9 (192): 1-9.
- Liberson WT, Holmquest HT, Scot D, Dow M. 1961. Functional electrotherapy: stimulation of the peroneal nerve synchronized with the swing phase of the gait of hemiplegic patients. *Arch Phys Med Rehabil*. 42: 101-105.
- Liebetanz D, Klinker F, Hering D, Koch R, Nitsche MA, Potschka H, Loscher W, Paulus W, Tergau F. 2006. Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia*. 47 (7): 1216-1224.
- Liebetanz D, Koch R, Mayenfels S, König F, Paulus W, Nitsche MA. 2009. Safety limits of cathodal transcranial direct current stimulation in rats. *Clin Neurophysiol*. 120: 1161-1167.

- Lim JH, Muguet-Chanoit AC, Smith DT, Laber E, Olby NJ. 2014. Potassium channel antagonists 4-aminopyridine and the T-butyl carbamate derivative of 4-aminopyridine improve hind limb function in chronically non-ambulatory dogs; a blinded, placebo-controlled trial. *PLoS One*. 9 (12): 1-19.
- Lindquist AR, Prado CL, Barros RM, Mattioli R, da Costa PH, Salvini TF. 2007. Gait training combining partial body-weight support, a treadmill, and functional electrical stimulation: effects on poststroke gait. *Phys Ther*. 87 (9): 1144-1154.
- Linderer B, Foreman RD. 1999. Physiology of spinal cord stimulation: review and update. *Neuromodulation*. 2 (3): 150-164.
- Lloyd DP. 1942. Mediation of descending long spinal reflex activity. *J Neurophysiol*. 5 (6): 435-458.
- Lovely RG, Gregor RJ, Roy RR, Edgerton VR. 1986. Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. *Exp Neurol*. 92 (2): 421-435.
- Lovely RG, Gregor RJ, Roy RR, Edgerton VR. 1990. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats. *Brain Res*. 514: 206-218.
- Martins A. 2015. Functional neurorehabilitation - the locomotor quadrupedal animal training adapted to the bipedal human. *International Archives of Medicine: Physical Medicine and Rehabilitation*. 8 (179): 1-11.
- Mehrholz J, Kugler J, Pohl M. 2008. Locomotor training for walking after spinal cord injury. *Cochr. Datab. Syst. Rev*. 11: CD006676.
- Menetrey D, De Pommery J, Roudier F. 1985. Propriospinal fibers reaching the lumbar enlargement in the rat. *Neurosci Lett*. 58 (2): 257-261.
- Militskova A, Mukhametova E, Fatykhova E, Sharifullin S, Cuellar CA, Calvert JS, Grahn PJ, Baltina T, Lavrov I. 2020. Supraspinal and Afferent Signaling Facilitate Spinal Sensorimotor Network Excitability After Discomplete Spinal Cord Injury: A Case Report. *Front. Neurosci*. 14: 552.
- Miller KE, Douglas VD, Richards AB, Chandler MJ, Foreman RD. 1998. Propriospinal neurons in the C1-C2 spinal segments project to the L5-S1 segments of the rat spinal cord. *Brain Res Bull*. 47 (1): 43-47.
- Min J, Kim JY, Seo CH, Jeon SR, Choi KH, Jeong JH. 2014. Changes of the electrophysiological study in dogs with acute spinal cord injury. *Korean J Neurotrauma*. 10 (1): 1-5.
- Minassian K, Jilge B, Rattay F, Pinter MM, Binder H, Gerstenbrand F, Dimitrijevic MR. 2004. Stepping-like movements in humans with complete spinal cord injury induced by epidural stimulation of the lumbar cord: electromyographic study of compound muscle action potentials. *Spinal Cord*. 42 (7): 401-416.
- Minassian K, Persy I, Rattay F, Dimitrijevic M. 2005. Peripheral afferent and central afferent input to the human lumbar spinal cord isolated from brain control. *Biocybernetics and Biomedical Engineering*. 25: 11-29.
- Minassian K, Persy I, Rattay F, Dimitrijevic MR, Hofer C, Kern H. 2007a. Posterior root-muscle reflexes elicited by transcutaneous stimulation of the human lumbosacral cord. *Muscle & Nerve*. 35: 327-336.
- Minassian K, Persy I, Rattay F, Pinter MM, Kern H, Dimitrijevic MR. 2007b. Human lumbar cord circuitries can be activated by extrinsic tonic input to generate locomotor-like activity. *Hum Mov Sci*. 26: 275-295.
- Minassian K, Hofstoetter US, Rattay F. 2011. Transcutaneous lumbar posterior root stimulation for motor control studies and modification of motor activity after spinal cord injury. In: Dimitrijevic MR, Kakulas BA, McKay WB, Vrbova G, editors. *Restorative Neurology of Spinal Cord Injury*. USA: Oxford University Press. p. 226-255.

- Minassian K, Hofstoetter US, Tansey K, Mayr W. 2012. Neuromodulation of lower limb motor control in restorative neurology. *Clin Neurol Neurosurg.* 114: 489-497.
- Minassian K, Hofstoetter US, Danner SM, Mayr W, Bruce JA, McKay WB, Tansey KE. 2016. Spinal rhythm generation by step-induced feedback and transcutaneous posterior root stimulation in complete spinal cord-injured individuals. *Neurorehabil Neural Repair* 30 (3): 233-243.
- Moore SA, Early PJ Hettlich BF. 2016. Practice patterns in the management of acute intervertebral disc herniation in dogs. *J Small Anim Pract.* 57:409-15.
- Moore SA, Tipold A, Olby NJ, Stein V, Granger N, CANSORT SCI. 2020. Current approaches to the management of acute thoracolumbar disc extrusion in dogs. *Front Vet Sci.* 7 (610): 41-55.
- Morawietz C, Moffat F. 2013. Effects of locomotor training after incomplete spinal cord injury: a systematic review. *Arch Phys Med Rehabil.* 94:2297-2308.
- Moshonkina T, Avelev V, Gerasimenko Y, Mathur R, Bijlani RL. 2002. Treadmill training accelerates restoration of locomotion after complete spinal cord transection in the rat. *Indian J Physiol Pharmacol.* 46: 499-503.
- Naito A, Shimizu Y, Handa Y. Analyses of treadmill locomotion in adult spinal dogs. 1990. *Neurosci Res.* 8:281-90.
- Nishida H, Nakayama M, Tanaka H, Kamishina H, Izawa T, Hatoya S. Sugiura K, Suzuki Y, Ide C, Inaba T. 2014. Evaluation of serum phosphorylated neurofilament subunit NF-H as a prognostic biomarker in dogs with thoracolumbar intervertebral disc herniation. *Vet Surgery.* 43:289-93.
- Nitsche MA, Paulus W. 2000. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol.* 527 (3): 633-639.
- Nitsche MA, Paulus W. 2001. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology.* 57 (10): 1899-1901.
- Nitsche MA, Liebetanz D, Antal A, Lang N, Tergau F, Paulus W. 2003. Modulation of cortical excitability by weak direct current stimulation-technical, safety and functional aspects. *Suppl Clin Neurophysiol.* 56: 255-276.
- Nitsche MA, Niehaus L, Hoffmann KT, Hengst S, Liebetanz D, Paulus W, Meyer BU. 2004. MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clin Neurophysiol.* 115 (10): 2419-2423.
- Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, Fricke K, Liebetanz D, Lang N, Antal A, Paulus W, Tergau F. 2005. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *J of Physiol.* 568 (1): 291-303.
- Nitsche MA, Lampe C, Antal A, Liebetanz D, Lang N, Tergau F, Paulus W. 2006. Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. *Eur J Neurosci.* 23 (6): 1651-1657.
- Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A. 2008. Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* (3): 206-223.
- Norton JA, Gorassini MA. 2006. Changes in cortically related intermuscular coherence accompanying improvements in locomotor skills in incomplete spinal cord injury. *J Neurophysiol.* 95: 2580-2589.
- Nothias JM, Mitsui T, Shumsky JS, Fischer I, Antonacci MD, Murray M. 2005. Combined effects of neurotrophin secreting transplants, exercise, and serotonergic drug challenge improve function in spinal rats. *Neurorehabil Neural Repair.* 19: 296-312.

Oh MJ, Seo TB, Kwon KB, Yoon SJ, Elzi DJ, Kim BG, Namgung U. 2009. Axonal outgrowth and Erk1/2 activation by training after spinal cord injury in rats. *J Neurotrauma*. 26: 2071-2082.

Olby NJ, Risio LD, Muñana KR, Wosar MA, Skeen TM, Sharp NJ, Keene BW. 2001. Development of a functional scoring system in dogs with acute spinal cord injuries. *Am J Vet Res*. 62 (10): 1624-1628.

Olby NJ, Levine J, Harris T, Muñana K, Skeen T, Sharp N. 2003. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord: 87 cases (1996–2001). *J Am Vet Med Assoc*. 222 (6): 762-769.

Olby NJ. 2010. The pathogenesis and treatment of acute spinal cord injuries in dogs. *Vet Clin North Am Small Anim Pract*. 40: 791-807.

Olby NJ, Muguet-Chanoit AC, Lim JH, Davidian M, Mariani CL, Freeman AC, Platt SR, Humphrey J, Kent M, Giovanella C, et al. 2016. A placebo-controlled, prospective, randomized clinical trial of polyethylene glycol and methylprednisolone sodium succinate in dogs with intervertebral disk herniation. *J Vet Intern Med*. 30 (1): 206-214.

Pilkar RB, Yarossi M, Forrest G. 2012. Empirical mode decomposition as tool to remove the function electrical stimulation artifact from surface electromyograms: preliminary investigation. In: *Proceedings of the 34th Annual International Conference of the IEEE EMBS*, San Diego, California, 28 August to 1 September 2012, IEEE, p. 1847-1850.

Pinter MM, Gerstenbrand F, Dimitrijevic MR. 2000. Epidural electrical stimulation of posterior structures of the human lumbosacral cord: 3. Control of spasticity. *Spinal Cord*. 38: 524-531.

Powers RK, Binder MD. 2001. Input-output functions of mammalian motoneurons. *Rev Physiol Biochem Pharmacol*. 143: 137-263.

Powers Rk, Turker KS, Binder MD. 2002. What can be learned about motoneurone properties from studying firing patterns? *Adv Exp Med Biol*. 508:199-205.

Purpura DP, McMurtry JG. 1965. Intracellular activities and evoked potential changes during polarization of motor cortex. *J Neurophysiol*. 28 (1): 166-185.

Raineteau O, Schwab ME. 2001. Plasticity of motor systems after incomplete spinal cord injury. *Nat Rev Neurosci*. 2 (4): 263-273.

Rattay F, Minassian K, Dimitrijevic MR. 2000. Epidural electrical stimulation of posterior structures of the human lumbosacral cord: 2. quantitative analysis by computer modeling. *Spinal Cord*. 38 (8): 473-489.

Rejc E, Angeli C, Harkema S. 2015. Effects of lumbosacral spinal cord epidural stimulation for standing after chronic complete paralysis in humans. *PloS One*. 10 (7): e0133998.

Richard MA, Spaich EG, Serrao M, Andersen OK. 2015. Stimulation site and phase modulation of the withdrawal reflex during gait initiation. *Clin Neurophysiol*. 126 (12): 2282-2289.

Richardson RR, Cerullo LJ, McLone DG, Gutierrez FA, Lewis V. 1979. Percutaneous epidural neurostimulation in modulation of paraplegic spasticity. Six case reports. *Acta Neurochir (Wien)* 49(3-4): 235-243.

Riout-Pedotti MS, Friedman D, Donoghue JP. 2000. Learning-induced LTP in neocortex. *Science*. 290 (5491): 533-536.

Robert AA, Ibrahim AL, Jadid MS, Bin Afif S, Abdulmohsen AL, Sowyed A, Al-Mubarak S. 2010. The effects of different rehabilitation strategies on the functional recovery of spinal cord injured rats: An experimental study. *Spine*. 35: E1273-E1277.

Robinson GA, Goldberger ME. 1986. The development and recovery of motor function in spinal cats, I: the infant lesion effect. *Exp Brain Res*. 62: 373-386.

Rosenkranz K, Nitsche MA, Tergau F, Paulus W. 2000. Diminution of training-induced transient motor cortex plasticity by weak transcranial direct current stimulation in the human. *Neurosci Lett*. 296 (1): 61-63.

Rossignol S, Barbeau H, Julien C. 1986. Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In: M Goldberger, A Gorio, M Murray, eds. *Development and Plasticity of the Mammalian Spinal Cord*. Padova, Italy: Liviana Press Padova. p. 323–345.

Rossignol S, Dubuc R, Gossard JP. 2006. Dynamic sensorimotor interactions in locomotion. *Physiol Rev*. 86:89-154.

Rossignol S, Martinez M, Escalona M, Kundu A, Delivet-Mongrain H, Alluin O, Gossard JP. 2015. The “beneficial” effects of locomotor training after various types of spinal lesions in cats and rats. *Prog Brain Res*. 218:173-98.

Roy FD, Gibson G, Stein RB. 2012. Effect of percutaneous stimulation at different spinal levels on the activation of sensory and motor roots. *Exp Brain Res*. 223: 281-289.

Sayenko DG, Atkinson DA, Floyd TC, Gorodnichev RM, Moshonkina TR, Harkema SJ, Edgerton VR, Gerasimenko YP. 2015. Effects of paired transcutaneous electrical stimulation delivered at single and dual sites over lumbosacral spinal cord. *Neurosci Lett*. 609: 229-234.

Scott HW, McKee WM. 1999. Laminectomy for 34 dogs with thoracolumbar intervertebral disc disease and loss of deep pain perception. *J Small Anim Pract*. 40:417–22.

Serrao M. 2014. Adaptive behavior of the spinal cord in the transition from quiet stance to walking: the use of withdrawal reflexes to support gait initiation. In: Jensen W, Andersen OK, Akay M, editors. *Proceedings of the 2nd International Conference of Neurorehabilitation (ICNR): Replace, Repair, Restore, Relieve - Bridging Clinical and Engineering Solutions in Neurorehabilitation*, 24-26 June 2014, Aalborg : Springer. p. 161-163.

Shah PK, Garcia-Alias G, Choe J, Gad P, Gerasimenko Y, Tillakaratne N, Zhong H, Roy RR, Edgerton VR. 2013. Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain*. 136 (11): 3362-3377.

Sherrington CS. 1910. Flexion-reflex of the limb, crossed extension-reflex and reflex stepping and standing. *J Physiol*. 40:28–121.

Siebner HR, Tormos JM, Ceballos-Baumann AO, Auer C, Catala MD, Conrad B, Pascual-Leone A. 1999. Low-frequency repetitive transcranial magnetic stimulation of the motor cortex in writer’s cramp. *Neurology*. 52 (3): 529-529.

Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, Rothwell JC. 2004. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci*. 24 (13): 3379-3385.

Singh A, Murray M, Houle JD. 2011. A training paradigm to enhance motor recovery in contused rats: effects of staircase training. *Neurorehabil Neural Repair*. 25: 24-34.

Smith AC, Knikou M. 2016. A review on locomotor training after spinal cord injury: reorganization of spinal neuronal circuits and recovery of motor function. *Neural Plast*. 2016: 1216258.

Solopova IA, Selionov VA, Sylos-Labini F, Gurfinkel VS, Lacquaniti F, Ivanenko YP. 2015. Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Front Syst Neurosci*. 9: 14.

Stecker MM. 2005. Transcranial electric stimulation of motor pathways: a theoretical analysis. *Comput Biol Med*. 35 (2): 133-155.

Stevens JE, Liu M, Bose P, O’Steen WA, Thompson FJ, Anderson DK, Vandenborne K. 2006. Changes in soleus muscle function and fiber morphology with one week of locomotor training in spinal cord contusion injured rats. *J Neurotrauma*. 23: 1671-1681.

- Swinnen E, Duerinck S, Baeyens J-P, et al. 2010. Effectiveness of robot-assisted gait training in persons with spinal cord injury: a systematic review. *J Rehabil Med.* 42:520-526.
- Takeoka A, Vollenweider I, Courtine G, Arber S. 2014. Muscle Spindle Feedback Directs Locomotor Recovery and Circuit Reorganization after Spinal Cord Injury. *Cell.* 159: 1626–1639.
- Tefertiller C, Pharo B, Evans N, Winchester P. 2011. Efficacy of rehabilitation robotics for walking training in neurological disorders: a review. *J Rehabil Res Dev.* 48: 387-416.
- Thomas SL, Gorassini MA. 2005. Increases in corticospinal tract function by treadmill by treadmill training after incomplete spinal cord injury. *J Neurophysiol.* 94: 2844-2855
- Thompson AK. 2014. Learning to change a reflex to improve locomotion. In: Jensen W, Andersen OK, Akay M, editors. *Proceedings of the 2nd International Conference of Neurorehabilitation (ICNR): Replace, Repair, Restore, Relieve - Bridging Clinical and Engineering Solutions in Neurorehabilitation*, 26 June 2014. Aalborg: Springer. p. 179-185.
- Tillakaratne NJ, de Leon RD, Hoang TX, Roy RR, Edgerton VR, Tobin AJ. 2002. Use-dependent modulation of inhibitory capacity in the feline lumbar spinal cord. *J Neurosci.* 22: 3130-3143.
- Tillakaratne NJK, Guu JJ, de Leon RD, Bigbee AJ, London NJ, Zhong H, Ziegler MD, Joynes RL, Roy RR, Edgerton VR. 2010. Functional recovery of stepping in rats after a complete neonatal spinal cord transection is not due to regrowth across the lesion site. *Neuroscience.* 166: 23-33.
- Troni W, Sapien AD, Berra E, Duca S, Merola A, Sperli F, Bertolotto A. 2011. A methodological reappraisal of non-invasive high voltage electrical stimulation of lumbosacral nerve roots. *Clin Neurophysiol.* 122: 2071-2080.
- Van de Crommert HW, Mulder T, Duysens J. 1998. Neural control of locomotion: Sensory control of the central pattern generator and its relation to treadmill training. *Gait. Post.* 7: 251–263.
- Ward AR. 2009. Electrical stimulation using kilohertz-frequency alternating current. *Phys Ther.* 89 (2): 181-190.
- Wang-Leandro A, Siedenburg JS, Hobert MK, Dziallas P, Rohn K, Stein VM, Tipold A. 2017. Comparison of preoperative quantitative magnetic resonance imaging and clinical assessment of deep pain perception as prognostic tools for early recovery of motor function in paraplegic dogs with intervertebral disk herniations. *J Vet Intern Med.* 31:842-8.
- Wirz M, Colombo G, Dietz V. 2001. Long term effects of locomotor training in spinal humans. *J Neurol Neurosurg Psychiatry.* 71(1): 93-96.
- Wu M, Landry JM, Schmit BD, Hornby G, Yen SC. 2012. Robotic resistance treadmill training improves locomotor function in human spinal cord injury: a pilot study. *Arch Phys Med Rehabil.* 93:782-789.
- Yakovenko S, Gritsenko V, Prochazka A. 2004. Contribution of stretch reflexes to locomotor control: a modeling study. *Biol Cybern.* 90: 146-155.
- Yamaguchi T. 1986. Descending pathways eliciting forelimb stepping in the lateral funiculus: experimental studies with stimulation and lesion of the cervical cord in decerebrate cats. *Brain Res.* 379 (1): 125-136.

CHAPTER III

RESULTS

1. ARTICLE 2

Frontiers of Veterinary Science (2021), 8(597949):1-14

A Comparison Between Body Weight-Supported Treadmill Training and Conventional Over-Ground Training in Dogs with Incomplete Spinal Cord Injury

A Comparison Between Body Weight-supported Treadmill Training and Conventional Over-ground Training in Dogs with Incomplete Spinal Cord Injury

Ângela Martins^{1,2*}, Débora Gouveia¹, Ana Cardoso¹, Inês Viegas¹, Óscar Gamboa³, António Ferreira³

¹ Arrábida Veterinary Hospital – Animal Rehabilitation Center, Azeitão, Setúbal, Portugal

² Lusófona University, Faculty of Veterinary Medicine, Campo Grande, Lisboa, Portugal

³ University of Lisbon, Faculty of Veterinary Medicine, Lisboa, Portugal

***Correspondence:** Ângela Martins, vetarrabida.lda@gmail.com

Keywords: Spinal cord injury, dogs, rehabilitation, treadmill, over-ground training.

Abstract

In human medicine there was no evidence registered of a significant difference in recovery between body weight-supported treadmill training (BWSTT) and conventional over-ground (COGI). There isn't any similar study in veterinary medicine. Thus, this study aimed to compare the locomotor recovery obtained in incomplete SCI (T11–L3 Hansen type I) post-surgical dogs following BWSTT or COGI protocols, describing their evolution during 7 weeks in regard to OFS classifications.

At admission, dogs were blindly randomized in two groups but all were subjected to the same protocol (underwater treadmill training) for the first two weeks. After, they were divided in the BWSTT group (n=10) and the COGI group (n=10) for the next two weeks, where they performed different training. In both groups locomotor training was accompanied by functional electrical stimulation (FES) protocols.

Results reported statistically significant differences between all OFS evaluations time-points ($p < 0.001$) and between the two groups ($p < 0.001$). In particular with focus on T1 to T3 a two-way repeated measures ANOVA was performed and similar results were obtained ($p = 0.007$). Functional recovery was achieved in 90% (17/19) of all dogs and 100% recovered bladder function. The BWSTT group showed 100% (10/10) recovery within a mean time of 4.6 weeks, while the COGI group had 78% (7/9) within 6.1 weeks.

Therefore, BWSTT leads to a faster recovery with a better outcome in general.

Introduction

Intervertebral degenerative hernia (IVDH) is the most common cause of acute spinal cord injury (SCI) in dogs, with a prevalence as high as 19% in some breeds (1-3). The most prevalent type of IVDH is extrusion thoracolumbar IVDH Hansen type I (4). IVDH can occur in any breed of dogs (5), but it typically affects chondrodystrophic dogs at an age ranging from 3 to 6 years. However, it also occurs in non-chondrodystrophic dogs (6,7).

The clinical signs of IVDH vary, ranging from spinal hyperesthesia to hindlimb paralysis (8, 9). Severe clinical signs that imply the loss of voluntary motor function after acute SCI are mostly caused by contusion (7). IVDH Hansen type I results in mixed contusive and compressive injury (10-12) and causes both primary and secondary lesions (13). This condition can benefit from decompressive surgery (14), particularly in the case of dogs with rapidly deteriorating neurological status (15).

Moore and colleagues (2016) reported that 43% of veterinarians routinely recommend a postsurgical rehabilitation program, with the treatment beginning immediately after surgery. It has been reported that nursing care of these dogs benefits their recovery of ambulation. In addition, there are many diverse recommendations, ranging from no restriction to strict cage rest up to 8 weeks post-surgery (3).

In human medicine, rehabilitation strategies aim to improve stepping recovery after SCI (16). Locomotor training exercises with step repetitions for several minutes each day for several consecutive weeks improve muscle force output and endurance (17) and also promote functional plasticity in locomotor central pattern generators (CPGs) present in the spinal network (18).

Locomotor training based on repetitions also affects the characterization and quality of gait through more complex mechanisms, such as the stimulation of residual descending/ascending pathways. Thus, locomotor training facilitates the recovery of ambulation after incomplete SCI (17).

The spinal cord depends on sensory feedback to generate force during stepping. In cats with spinal injury, a direct access between the cutaneous inputs and spinal rhythm-generating circuitry is suggested and treadmill training can provide 50% of the force generated during the stance phase (19).

In humans, high-intensity locomotor training promotes the release of various trophic factors, such as brain-derived neurotrophic factor (BDNF), which may facilitate angiogenesis, neurogenesis, neuro-reorganization changes, and muscle metabolism (20,21). This type of

training in humans with SCI includes body weight-supported treadmill training (BWSTT), which plays an important role in posture and gait (22). In addition, BWSTT can increase the amplitude and coordination of firing of muscle motor units (23).

Dobkin and collaborators in 2007, compared gait evolution by BWSTT and by conventional over-ground intervention (COGI) and found that there was no significant difference between the two treatments up to 3 months (24). Thus, BWSTT did not differ significantly from COGI in terms of kinematic and kinetic parameters (25-27). A study by Field-Fote and Roach (2010) indicated that gains in walking speed obtained with COGI were equivalent to gains achieved with BWSTT and that regarding walking distance, COGI was superior (28).

An association between functional electrostimulation (FES) and locomotor training that improves normality in speed, distance, and limb coordination has been reported (29). In addition, FES improves stride, length, cadence, and gait quality, as well as muscle strength and activation (29,30). FES with locomotor training is implemented in individuals with complete or incomplete SCI (31). This electrostimulation modality, targets restorative neurology by modifying residuals and remaining nervous system circuits and pathways (32).

Given that rehabilitation protocols are already successfully used in humans, it is considered to be a therapy with reasonable translational potential for veterinary medicine (33). Thus, this study aimed to compare the application of the BWSTT protocol versus the COGI protocol in dogs with incomplete sub-acute SCI (T11–L3 IVDH Hansen type I) after surgery.

Materials and Methods

Participants

This was a controlled, blinded, prospective clinical study using a cohort criterion in dogs. Dogs presenting with an acute history of thoracolumbar SCI with IVDH Hansen type I after hemilaminectomy at one or two sites were included. The time between onset of disease and surgery was approximately 2–4 days, during which they were treated with a pharmacological approach with non-steroidal anti-inflammatories or with low doses of corticosteroids. All dogs were diagnosed using computerized tomography (CT) or magnetic resonance imaging (MRI) with T11–L3 neuro-localization.

All modified Frankel Scale (MFS) grade I dogs, regardless of sex and breed, were included in this study and were admitted to the rehabilitation center up to 7 days postoperatively. Other inclusion criteria were weight ≤ 20 kg and age from 2 to 8 years.

At admission, all participants underwent a neurorehabilitation functional examination and a standard upper motor neuron examination and showed decreased or absent withdrawal reflexes in one or both pelvic limbs. This status, in the absence of an SCI in the lumbosacral intumescence, was compatible with spinal shock diagnosis, which can be associated with disk herniation (34).

All subjects demonstrated a neurogenic bladder observed at admission, which was compatible with SCI neuro-localization: cranial to the spinal cord segment S1 or cranial to the L4 vertebra, resulting in increased urethral resistance and difficulties in vesical expression (35).

This study was performed between November 2017 and January 2020 at the Arrábida Veterinary Hospital Rehabilitation Center after approval by the Lisbon Veterinary Medicine Faculty ethics committee.

Dogs were excluded from this study if they had suspected or confirmed spinal cord neoplasia, myelomalacia phenomenon, meningomyelitis, IVDH Hansen type II, thoracolumbar fibrocartilaginous embolic myelopathy, acute non-compressive nucleus pulposus extrusion, or thoracolumbar myelitis. They were also excluded if they were diagnosed with IVDH Hansen type I but had different neuro-localization and/or had normal/increased withdrawal reflexes. Dogs with T11–L3 IVDH Hansen type I were excluded if they underwent conservative management and/or if they were admitted to the center more than 7 days postoperatively.

Study Design

Twenty-two subjects were admitted to the study and underwent a neurorehabilitation examination according to clinical history and physical and neurologic examination after the owners provided consent.

All subjects had an alert mental status, passive standing posture, diverse postures (kyphosis and scoliosis), upper motor neuron peripheral reflexes with the exception of the withdrawal reflex (absent/decreased in one or both limbs), and cutaneous trunci reflexes present at or below the injury site. Overall, superficial and deep pain perception was present.

Deep pain perception was evaluated in a controlled environment, with respect to movement and noise, with 12-cm Halsted mosquito forceps, in both medial/lateral digits of both

pelvic limbs. For a more accurate pain perception examination, this was also tested in the perineal region and at the tip and base of the tail.

Spinal hyperesthesia was also assessed with the correct technique of vertebral column palpation. This technique is performed by placing slight pressure on the vertebral column, placing one hand on the abdominal muscles, palpating throughout the dorsal longitudinal ligament between each spinous process, and between each intervertebral space, in order to activate the nociceptors located in the peripheral fibrous annulus (36).

With the dogs in a standing posture, both pelvic limbs were palpated to compare symmetry and tonus. All dogs showed hypotonicity in the flexor muscle group. Proprioceptive positioning was evaluated in dogs while supported by the examiner.

This study used the Open Field Score (OFS) (37) to quantify recovery of stepping ability and to observe coordination between the thoracic and pelvic limbs.

Videotaping was performed on admission (T0) and 2 weeks later (T1). Dogs were randomized into two groups. Dogs were randomly assigned to groups according to the order of admission in the center, and the first selected dog was designated by a blinded procedure.

Evaluations were performed and recorded each week from T1 (T2 to T6) and at follow-ups (F1, F2, and F3). Videotapes were analyzed and scored by two blinded observers, board-certified canine rehabilitation veterinarians, and if interobserver disagreement exceeded 20%, a third blinded observer, working at a different institution, performed the same analysis.

Twenty-two dogs were admitted in the study and were subjected to the same protocol of functional neuro-rehabilitation (FNR) during the first 2 weeks. The protocol of FNR included: Underwater treadmill (FNR-UWTM); IES and FES.

After the first 2 weeks, they were allocated to different groups, but two dogs left the study before randomization due to the owners' decision. The remaining twenty dogs (n=20) were separated into the FNR-BWSTT protocol group (n=10) and the FNR-COGI protocol group (n=10). Both groups performed these protocols during the next 2 weeks and had outcomes observed at T2 and T3, based on neurological examination and OFS interobserver observations.

The group with the lower success rate after these first 4 weeks of FNR, was transferred to the protocol with higher results, and evaluations were performed at T4, T5 and T6. As can be seen in figure 1 the diagram of this study within a 7-week period is described and explains the two-week interval between T0 and T1, and also the evaluations at T2 (3rd week), T3 (4th week), T4 (5th week), T5 (6th week) and T6 (7th week).

Interventions

The 22 dogs that were included in the study started the FNR-UWTM protocol within 24 hours after admission. The first day was considered an adaptation to all neurorehabilitation interventions.

FNR-UWTM Protocol

In the first two weeks (T0-T1), all dogs received interferential electrical stimulation (IES) for pain management throughout a 4-channel electrotherapy device (BTL-5645 PULS, BTL, USA). IES is a premodulated interferential current commonly used in canine rehabilitation.

This technique used two different channels with four rubber and carbon electrodes (7 x 5 cm) (BTL, USA) placed crossing each other at a 90° angle and located proximally two spaces cranial and caudal to the spinal hyperesthesia site, after clipping the hair and application of gel (Fig 2). The current applied was biphasic, symmetric and continuous and was performed two times/day, five days/week in the first week. On the second week this was applied once a day, each 48 hours. Pain level was assessed daily by a correct technique of vertebral column palpation and recorded to better evaluation in slow motion mode.

Current parameters were applied as followed:

- Channel 1 (Acute pain) – Frequency was 80–150 Hz; Intensity was 0.5-1 mA; Pulse duration was 2–50 µs; Time of treatment of 10 minutes (38,39);
- Channel 2 (Chronic pain) – Frequency was 1–10 Hz; Intensity was 0.5-1 mA; Pulse duration was 100–400 µs; Time of treatment of 10 minutes (38,39).

Participants were also subjected to locomotor training based on repetitive water training, with established pelvic limb bicycle movements, stretching of pelvic limb muscles, and stimulation of cutaneous receptors on the treadmill surface (Fig. 3). This exercise was alternated with perineal stimulation to obtain the flexion and extension cycles of small amplitude used for paralysis of the hindlimbs (40). All exercises were performed with weight support according to the patient's capacity at the time.

In the first days of FNR-UWTM training, each patient was allowed to adapt, with speeds beginning at ≤0.8 km/h and progressively increasing to 1.2 km/h, 1.9 km/h and 2.5 km/h (41,42), first for 3–10 minutes and extending to 20 minutes once a day within the first 2 weeks.

Water temperature was maintained between 24°C and 26°C, and the water level was positioned between the lateral malleolus of the tibia and the lateral condyle of the femur (43). Dogs were monitored by assessing the mucous membranes, capillary refill time, and pulse.

The use of combined approaches in rehabilitation may be more effective than a single intervention, simultaneously targeting different injury mechanisms (44). Therefore, all participants underwent FES within this period with a 2-channel electrotherapy device (BTL-5645 PULS, BTL, USA).

FES is described to use low pulse frequency electrical currents to restore motor functions after SCI, promoting control standing, balance, posture and gait training. Also, increase tonus in the hamstring muscle and polysynaptic reflex (45), essential in this population of dogs that showed decreased or absent withdrawal reflexes.

Superficial rubber and carbon electrodes (7 x 5 cm) (BTL, USA) were applied after trichotomy in a segmental technique and were placed over the peripheral nerve trunk and motor point (46) with patients in the standing position (Fig. 4). Thus, the main hamstring muscles were stimulated (biceps femoris, semitendinosus and semimembranosus), both cranial and caudal groups, allowing the stimulation of the stance and swing phase, respectively.

This was a symmetric, biphasic and pulsed alternating current with the following parameters: Frequency of 30 Hz (47); intensity of 6–16 mA (48); pulse duration 100–150 μ s (49); duty cycle 1:4 ratio, 2–4 seconds ramp-up, 8 seconds on-time, 1–2 seconds ramp-down (38), and time of treatment of 10 minutes. FES was performed 2–3 times/day for 5 days/week.

During treatment, parameters were adjusted according to the monitoring of muscle fatigue signs, such as muscle tremors, increased respiratory rate and/or pain. In case any of these signs appeared, parameters were adjusted: intensity (mA) was reduced until they disappear and if they continued, frequency (Hz) was also reduced. Also, if they persist in time, duty cycle was increased (i.e., until 1:6).

FNR-BWSTT Protocol

In human medicine, BWSTT can be manually or robotically assisted, such as via the Lokomat system, which supports 60% of an individual's weight at the beginning, decreasing in accordance with load tolerance to no less than 25% support (50). A similar manual support approach was implemented in our dogs.

As all dogs were acclimated to the underwater treadmill, very few dogs resisted treadmill training. However, in dogs that offered resistance, bipedal step training was required, wherein the forelimbs remained stationary on a fixed platform above the belt (51) (Fig. 5).

The aim of the BWSTT protocol was to achieve quadrupedal step training, starting with:

- Speeds of 1 km/h - 2.5 km/h, during 20–30 minutes, 2–3 times/day, 6 days/week according to patients' capabilities (Fig. 6) (52-54);
- The treadmill slope was elevated from 10° to 25°, with thoracic and pelvic limb coordination (55,56).

All 10 dogs in this group underwent BWSTT within a therapeutic window of 40 minutes after application of FES, using the same technique as described earlier.

During these two weeks (T1-T3) it was performed the FNR-BWSTT protocol without alteration regarding time, frequency during the day and frequency during the week. Thus, the locomotor training had a total range from 40-90 min plus 20-30 min of FES in a daily basis.

FNR-COGI Protocol

At the same time (T1-T3), COGI was applied in 10 dogs, by one or two veterinarians' technicians, with the help of a harness system that allowed the dogs to perform step movements with similar weight support as described before, decreasing the load tolerance according to dogs' abilities.

During gait stimulation, all dogs had to touch the floor surface, beginning with a knuckling position and progressing to cutaneous receptor stimulation (placed in the interdigital space).

Dogs were stimulated to walk for 20–30 minutes, 2–3 times/day, 6 days/week on different floor surfaces, such as sand, stones, and grass (Fig. 7).

After the dogs achieved a flexion–extension locomotor pattern, it was associated with gait stimulation on an uneven floor as well as up and down ramps (Fig. 8), for the same amount of time.

All 10 dogs were subjected to the same FES protocol (Table 1) before they were stimulated to walk. Dogs performed a daily locomotor training from 40-90 min total and 20-30 min of FES during these two weeks.

Thus, in both protocols (COGI and BWSTT), exactly the same time of CNS stimulation was promoted.

Supportive Care

Dogs were hospitalized full-time, resting in soft beds to avoid pressure sores or decubital ulcers, and doughnut bandages were placed under bone prominences. If they were recumbent, they were turned every 4–6 hours from left to right lateral recumbency to prevent atelectasis or accumulation of lung secretions (57). They were always encouraged to maintain sternal recumbency.

Dogs were fed three times per day with an intake of 30% and hydric support of 100–120 ml/kg. All had neurogenic bladders, which were expressed every 4–6 hours as a daily rule (57,58).

Outcomes/Follow-ups

All dogs underwent an FNR examination and an OFS evaluation every 7 days. The first evaluation was performed at admission (T0), followed by an evaluation at 2 weeks after initiating FNR-UWTM (T1). At this stage, the presence of the withdrawal reflex and crossed extensor reflex was evaluated, as the main outcome.

Blinded randomization was implemented at T1, when the dogs were divided into two groups: the BWSTT group and the COGI group. Evaluations were performed weekly over the subsequent 5 weeks (T2–T6), finishing the study within a 7-week period.

If neurological evolution was not evident in one of these groups until the finish of T3, the protocol was switched to the one that yielded the best apparent results. Thus, from T4 to T6, all dogs were treated with the same FNR protocol.

Follow-up was advised after the first week of release, also after 1 and 2 months. All were evaluated (FNR exam and OFS score) and videotaped.

In this study, functional outcome was considered when dogs achieved an OFS score classification of ≥ 11 . An OFS of 11 is equivalent to a locomotor state in ambulatory paraparesis and OFS > 11 is equivalent to that in proprioceptive ataxia. A functional state can be defined as the possibility of a dog standing up, maintaining an active standing posture, giving five or more steps, and having the ability to urinate and defecate voluntarily.

Statistical Analysis

Quantitative, qualitative, and categorical data were analyzed using IBM SPSS Statistics software, Version 22 (International Business Machines Corporation, Armonk, NY, USA), and the results were interpreted at a level of significance of $p < 0.05$.

For inter-observer validation regarding OFS scores, normality tests were performed, and normality was not verified. Thus, independent-samples Mann–Whitney U tests were performed.

For comparison between groups and between evaluation time-points, a univariate analysis of variance was performed. The statistical model was considered significant ($p < 0.001$). Estimated marginal means with 95% confidence interval and an interaction plot for the comparison of mean OFS at each time-point between groups were derived.

Also, for better evaluation between T1 and T3, a two-way repeated measures ANOVA with time as a repeated measure, was performed. Thus, a measured dependent variable (OFS) was evaluated regarding two independent variables (time points and different protocols), with the primary purpose of understanding if there is an interaction between these two factors on the dependent variable.

Results

This study described the evolution of progress in 22 dogs throughout their FNR protocol over a 7-week period, according to the diagram in figure 9 and 10, and their evaluation using the OFS score.

In the total population ($n = 22$), the average age was 5.3 years and average weight was 9.65. There were 10 males and 12 females; 63.6% (14/22) were chondrodystrophic dogs and 36.4% (8/22) of non-chondrodystrophic dogs. The following was the breed distribution: six Dachshunds, four French Bulldogs, four Mixed breeds, two Beagles, two Basset Hounds, one Labrador Retriever; one Pincher; one Yorkshire Terrier; and one German Shepherd. Before randomization, two dogs left the study, of which one was Dachshund and one was a German Shepherd.

All dogs had spinal hyperesthesia at admission and were under the IES protocol. At T1 no dog had pain in both groups (COGI and BWSTT), which was assessed by palpation and recorded in slow motion mode.

After randomization (T1), dogs were divided into two groups ($n = 10$ each), with similar chondrodystrophy, age, and average weight.

For inter-observer validation, independent-samples Mann–Whitney U tests were performed, revealing the absence of a statistical difference between observers in every moment of evaluation ($p = 0.799$).

In the total of 156 observations, that were performed and recorded between the two observers, there were only 21 inconsistencies, resulting in 13% interobserver disagreement.

For comparison between groups and between evaluation time-points, a univariate analysis of variance was performed. The statistical model was considered significant ($p < 0.001$), with statistically significant differences between all OFS evaluation time-points ($p < 0.001$) and between groups (COGI $\times$ BWSTT) ($p < 0.001$). Both variables (group and time-point) also demonstrated a positive interaction ($p = 0.019$).

A higher mean OFS was observed in the BWSTT group (10.132) than in the COGI group (8.001) (Table 2).

In particular in T2 and T3, at each evaluation point there was a higher mean of OFS in the BWSTT group (Table 3). Additionally, it can be observed that, at 4 weeks after admission (T3), FNR-BWSTT dogs showed a borderline functional outcome ($\text{OFS} \geq 11$), which was only demonstrated in the COGI group at T6 (after 7 weeks).

At T3, the functional outcome ($\text{OFS} \geq 11$) was achieved within the BWSTT group in six dogs and in the COGI group in only one dog. The functional outcomes at the subsequent time-points (T4, T5, and T6) are described in figure 11. In addition, at T3, the best result was seen in the BWSTT group, with four dogs classified as OFS 13, while one dog in the COGI group achieved only OFS 11.

Before T4 evaluation, one dog left the study by the owners' decision, thus the number of dogs decreased ($n=19$), as shown in the flow diagram (Fig. 10).

A two-way repeated measures ANOVA that compared the OFS mean differences between groups from T1 to T3 was done and the interaction plot obtained is shown in figure 12. There was an interaction effect between time and treatment, thus OFS may vary based on time differently between the two treatments. Thus, there was a clear difference between the estimated mean of OFS after T1, with the highest difference observed at T3. According to the multivariate tests there was clear statistical significance between groups regarding treatment ($p = 0.006$) and during each evaluation time point ($p=0.000$).

Upon medical discharge at the 7th week, a total functionality of 90% was achieved (17/19). Within the groups, functionality was 78% (7/9) in the COGI group by the 7th week, and 100 % (10/10) in the BWSTT group but already demonstrating these results in the 6th week (Fig.11).

Thus, for the COGI group, functional outcomes were achieved within a mean time of 6.1 weeks (range, 4–8 weeks) and for the BWSTT group within a mean time of 4.6 weeks (range, 3–6 weeks).

Discussion

Our study population characterization agrees with that of most documented studies on IVDH Hansen type I, which report that the Dachshund is the breed most frequently diagnosed with this disease (59-63), followed by the French bulldog (64,65). The main reason for this was explained by Hansen (1951,1959) (66,67) as involving transformation of the gelatinous nucleus pulposus into hyaline cartilage, which can begin as early as 2 months of age in Dachshunds and French Bulldogs.

In addition, chondrodystrophic breeds were the majority with 64% (14/22), in agreement with Kinzel et al. (2005) (68), and Aikawa et al. (2012) (5). However, 36.4% (8/22) were non-chondrodystrophic dogs, such as the German Shepherd and Labrador Retrievers, as described by Jeffery et al. (2013) (11) and Dewey & da Costa (2016) (36). The most frequent localization was the thoracolumbar region (69).

The average age of the affected dogs was 5.3 years, similar to that reported in previous studies (8, 62,70), and the average weight was 9.65 kg, which was similar to that reported in earlier studies (65, 71, 72).

Chondroid metaplasia was an inclusion criterion. According to Ball and collaborators (1982)(73) and Thomas and colleagues (2015) (74), this should have demonstrated skewing of the sample population to males, but in our study, 55% (12/22) were females.

In T0, the 22 dogs started the FNR-UWTM protocol based on the IES technique to manage spinal hyperesthesia and locomotor training during two weeks. Spinal hyperesthesia manifestation decreased during this time (T0-T1), in particular in the 2nd week, and IES application was performed according to the pain level assessed by a correct technique of vertebral column palpation.

The repetitive locomotor training allowed stimulation of the muscle spindle and the cutaneous receptors during the bicycle movements performed by the technician, under water, in direct contact with the treadmill surface (22).

The treadmill locomotor training in incomplete SCI patients can induce plasticity in reflex pathways (75-77), as proven by Gossard and collaborators (2015) in incomplete SCI cats, who obtained functional plasticity of the locomotor CPGs (16).

The main goal of the FNR-UWTM training was to acquire the flexion–extension pattern before randomization (T1), equivalent to a minimum classification of OFS 3. In our study, this was not achieved in all dogs. Two dogs left the study before achieving this pattern (one with

OFS 1; and one with OFS 2) while the remainder were randomly divided into the BWSTT and COGI groups (Fig. 1).

At T1, the population was assigned to two groups (FNR-BWSTT × FNR-COGI) and dogs were evaluated, by two blinded observers using the OFS, resulting in a total of 156 observations with only 13% interobserver disagreement, confirming the reliability of the results obtained in the evaluations.

By a univariate analysis of variance, it was observed that after T1, both the BWSTT group and the COGI group showed a positive evolution over time in terms of an increase in OFS mean values ($p < 0.001$). Also, a two-way repeated measures ANOVA was performed to carefully evaluate from T1 to T3 and a similar significance was obtained ($p=0.007$).

It is the authors opinion that just like in human medicine, repetitive step training for several consecutive weeks (by BWSTT or underwater treadmill) promotes an increase in muscle force output and endurance as well as an increase in rhythmic activity by the spinal locomotor circuitry and stimulation of remnant descending/ascending pathways (15). This selects the activation of motor neuron populations to obtain muscle synergy by descending motor pathway control and afferent input modulation, proposing rehabilitation strategies as a toll to improve stepping recovery after SCI (78).

Therefore, the limb displacement compensation mechanism, which involves increasing the hip angle, can cause activation of adductor muscles, and decreasing the angle can promote activation of the abductor muscles (79). In addition, the limb load compensation mechanism, which can stimulate stretch receptors and the Golgi tendon organs of extensor muscles, allows for faster rehabilitation in animal models (40,80) and in humans (81, 82).

The interaction plot documented the biggest difference in the estimated means of OFS between groups in T3 (Fig. 11), with an average of 10.7 in the BWSTT and 6.3 in the COGI groups (table 2). In addition, the first time-point for assessing the difference between groups was at T2 (at 1 week after initiating differentiated training) with a clear distinction between them, this difference was maintained until T5, after 2 weeks of the same BWSTT training was implemented for ethical reasons.

At T5, the estimated OFS confidence interval showed an overlap of the upper limit of the COGI dogs and the lower limit of the BWSTT dogs, and functionality at this stage was considered to be 79% (15/19).

At the time of clinical discharge (T6), functional recovery was achieved in 90% (17/19) of all dogs and 100% had recovered bladder function. Within the BWSTT group, 100% (10/10)

of the dogs recovered, while only 78% (7/9) in the COGI group recovered. The functional outcomes at the time of clinical discharge (90% functional recovery) agreed with the results of previous studies (1,4,8,14,8,73). However, in the BWSTT group, there was 100% functional recovery within an average time of 4.6 weeks. Considering that these dogs had severe grade I MFS, this protocol is a viable tool for implementation in sub-acute postsurgical IVDH dogs.

The results obtained in this population of subacute postsurgical IVDH dogs that had absent/decrease withdrawal reflex at admission, and a consequential descending pathway blockage compatible with spinal shock, showed that the underwater treadmill training could have promoted the appearance of the flexion/extension pattern. Also, the allocation of dogs in different groups demonstrated that in the BWSTT a functionality of 100% (10/10) was achieved within a mean time of 4.6 weeks, while in the COGI group 78% (7/9) were functional within 6.1 weeks, suggesting that even spontaneous recovery could occur in both groups, the BWSTT had the major success in recovery and in less time.

Recovery of function due to neurological injuries can be variable and difficult to predict. The most important challenge for the recovery of function is axonal regeneration and the formation of new functional synapses across the injury site (62,83), mainly between the axons of damaged tracts and the new collateral axons (84). Thus, locomotor training is a strategy that promotes cutaneous reflex excitability (85). These can result from different mechanisms, involving the sprouting of undamaged fibers, regeneration of damaged fibers, and/or changes in synaptic efficiency (86,87).

In humans BWSTT allows for greater trunk stabilization, a rhythmic timing exercise, and also assists with retropulsion of the stance phase, which promotes the hip extension critical to the initiation of the swing phase (88). Moreover, the combination of BWSTT and FES clearly improved walking speed, distance, limb coordination, strength, and muscle activation patterns (29). This combination showed significant improvements in cadence and step length and had a synergistic effect on muscle mass, balance, coordination, and rhythm. Complementary FES with low pulse frequency has been recommended for humans with incomplete SCI, probably because it facilitates training in standing (31,46).

FES may also have been essential for its action in muscle atrophy and fatigue (89). Following SCI there are proportionally more fibers type II (fast-fatigable, anaerobic) than type I (slow, aerobic), which are also atrophied, leading to weakness and fatigue. This atrophy is also accompanied by a conversion of type I to type II muscle fibers. Thus, FES technique can increase contraction time and resistance to fatigue by reversing the muscle mass loss and the

conversion of muscle fibers to a different type, probably resulting in more muscle fibers that become type I (90, 91). It also increases capillary density and blood flow, decreases spasticity and pain, increases the range of motion, and decreases contractures (92-95).

Research indicates that BWSTT promotes greater control of body posture and balance, both of which depend on organization of the multisensory system (96) and recruitment of muscles in the categories of the flexors/extensors and abductors/adductors (97). This may be among the main reasons for the faster recovery of dogs in the BWSTT group and also for the positive recovery of dogs who had been treated with the COGI protocol initially, but were switched to the BWSTT protocol. This last protocol leads to an increase in the amplitude and coordination of motor unit firing in leg muscles (23).

Our results indicated that COGI training could not promote as many repetitive exercises as BWSTT. For improved walking function, motor learning requires more than a simple repetition (20) and benefits from the opportunity to make and correct errors. This facilitates recovery of locomotion after incomplete SCI (17,98).

Nevertheless, COGI dogs showed slow recovery, with positive evolution, probably due to the effects of walking training on the cutaneous muscular reflex, which comprises part of the flexor afferent pathway. This pathway is considered to be activated by excitatory and inhibitory interneurons within the CPGs (99).

In humans with incomplete SCI, it was reported no difference between BWSTT and COGI interventions. Likewise, no distinctions between the two approaches were seen from the kinematic and kinetic aspects (24, 28). This was not the case in our study, where BWSTT showed a markedly better performance. In human medicine, the combination of these types of training is considered to be beneficial (100). This is likely due to the ability of BWSTT to stimulate the stance phase and promote hip, knee, and ankle control, while COGI training allows integrated volitional control of all components of gait (101).

The biggest limitation of this study was the absence of a kinematic and kinetic evaluation, as well as electromyographic studies useful for neuromuscular monitoring, which could promote understanding of the differences between the groups. In addition, the decrease in the number of dogs that attended the first follow-up ($n = 10$) did not allow reliable assessment of the efficacy of these protocols in the long term. Finally, this study was also limited by the fact that dogs only differed in treatment for 2 weeks, as all dogs were transferred to the protocol with best results in T3 (BWSTT), which could be the cause of the overall differences between groups to be relatively small. Given these, further studies are warranted.

The difficulty in comparing differences between both groups is probably due to the fact that the dogs underwent different treatment for only 2 weeks.

Conclusion

The differences in successful recovery between the BWSTT group and the COGI group were clearly demonstrated in this study. After 4 weeks of the FNR implementation 60 % (6/10) had recovered in the BWSTT group, while 10% (1/10) had recovered in the COGI group.

Despite these differences, dogs in the COGI group managed to evolve positively, primarily after switching to the BWSTT protocol. This may suggest that conventional protocols can be applied to dogs with incomplete SCI, but can delay functional recovery, while treating dogs with SCI by BWSTT can yield better functional outcomes in less time.

Both protocols, which included FES and locomotor training, were considered to be safe and not harmful to any of the dogs, and promoted a positive evolution in dogs with grade I MFS incomplete SCI, diagnosed with T11–L3 IVDH Hansen type I, after surgery. Future studies should be continued with a focus on longer period times in a single treatment.

References

1. Olby N, Levine J, Harris T, et al. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord:87 cases (1996-2001). J Am Vet Med Assoc (2003) 222(6): 762-9
2. Levine J, Levine G, Porter B et al. Naturally occurring disk herniation in dogs: an opportunity for pre-clinical spinal cord injury research. J Neurotrauma (2011) 28: 675-688
3. Moore SA, Early PJ, Hettlich BF. Practice patterns in the management of acute intervertebral disc herniation in dogs. J Small Anim Pract (2016) 57(8): 409-15
4. Langerhuus L, Miles J. Proportion recovery and times to ambulation for non-ambulatory dogs with thoracolumbar disc extrusions treated with hemilaminectomy or conservative treatment: a systematic review and meta-analysis of case-series studies. Vet J (2017) 220: 2-34
5. Aikawa T, Fujita H, Kanazono S, et al. Long-term neurologic outcome of hemilaminectomy and disk fenestration for treatment of dogs with thoracolumbar intervertebral disk herniation:831 cases (2000-2007). J Am Vet Med Assoc (2012) 241(12):1617-26.
6. Brisson BA. Intervertebral disc disease in dogs. Vet Clin Small Anim (2010) 40(5): 829-858

7. Freeman P, Jeffery ND. Re-opening the window on fenestration as a treatment for acute thoracolumbar intervertebral disc herniation in dogs. *J Small Anim Pract* (2017) 58:199-204
8. Loughin CA, Dewey CW, Ringwood PB, et al. Effect of durotomy on functional outcome of dogs with type I thoracolumbar disc extrusion and absent deep pain perception. *Vet Comp Orthop Traumatol* (2005) 3: 141-146
9. Sterna J, Burzykowski T. Assessment of the usefulness of the fenestration method in cases of disc extrusion in the cervical and thoraco-lumbar spine in chondrodystrophic dogs. *Pol J Vet Sci* (2008) 11(1): 55-62
10. Kazakos G, Polizopoulou ZS, Patsikas MN, et al. Duration and severity of clinical signs as prognostic indicators in 30 dogs with thoracolumbar disk disease after surgical decompression. *J Vet Med* (2005) 52:147-152
11. Jeffery ND, Levine JM, Olby NJ, et al. Intervertebral disk degeneration in dogs: consequences, diagnosis, treatment, and future directions. *J Vet Intern Med* (2013) 27(6): 1318-33
12. Sims C, Waldron R, Marcellin-Little DJ. Rehabilitation and physical therapy for the neurologic veterinary patient. *Vet Clin North Am Small Anim Pract* (2015) 45: 123-43
13. Bush WW, Tiches DM, Kamprad C, et al. Functional outcome following hemilaminectomy without methylprednisolone sodium succinate for acute thoracolumbar disk disease in 51 non-ambulatory dogs. *J Vet Emerg Crit Care* (2007) 17(1):72-76
14. Olby NJ, Harris T, Burr J, et al. Recovery of pelvic limb function in dogs following acute intervertebral disc herniations. *J Neurotrauma* (2004) 21(1): 49-59
15. Mann FA, Wagner-Mann CC, Dunphy ED, et al. Recurrence rate of presumed thoracolumbar intervertebral disc disease in ambulatory dogs with spinal hyperpathia treated with anti-inflammatory drugs:78 cases (1997-2000). *J Vet Emerg Crit Care* (2007) 17(1): 53-60
16. Gossard J, Delivet-Mongrain H, Martinez M, et al. Plastic Changes in lumbar locomotor networks after a partial spinal cord injury in cats. *J Neurosci* (2015) 35(25): 9446-9455
17. Escalona M, Delivet-Mongrain H, Kundu A, et al. Ladder Treadmill: a method to assess locomotion in cats with an intact or lesioned spinal cord. *J Neurosci* (2017) 37(22):5429-5446

18. Martinez M, Delivet-Mongrain H, Leblond H, et al. Effect of locomotor training in completely spinalized cats previously submitted to a spinal hemisection. *J Neurosci* (2012) 32:10961-10970
19. Hurteau M, Thibaudier Y, Dambreville C, et al. Nonlinear modulation of cutaneous reflexes with increasing speed of locomotion in spinal cats. *J Neurosci* (2017) 37:3896-3912
20. Brazg G, Fahey M, Holleran CL, et al. Effects of training intensity on locomotor performance in individuals with chronic spinal cord injury: a randomized crossover study. *Neurorehabil Neural Repair* (2017) 31: 944-954
21. Engelhardt M, Sobotzik J. "Functional neuroanatomy of the spinal cord". In: Weidner N, Rupp R, Tansey K, editors. *Neurological Aspects of Spinal Cord Injury*. Switzerland: Springer Nature (2017). p. 3-15
22. Dobkin BH, Apple D, Barbeau H, et al. Methods for a randomized trial of weight-supported treadmill training versus conventional training for walking during inpatient rehabilitation after incomplete traumatic spinal cord injury. *Neurorehabil Neural Repair* (2003) 17:153-167
23. Dobkin BH, Duncan PW. Should body weight-supported treadmill training and robotic-assistive steppers for locomotor training trot back to the starting gate? *Neurorehabil Neural Repair* (2012) 26: 308-317
24. Dobkin B, Barbeau H, Deforge D, et al. The evolution of walking-related outcomes over the first 12 weeks of rehabilitation for incomplete traumatic spinal cord injury: The multicenter randomized spinal cord injury locomotor trial. *Neurorehabil Neural Repair* (2007) 21: 25-35
25. Labruyère R, van Hedel HJ. Strength training versus robot-assisted gait training after incomplete spinal cord injury: a randomized pilot study in patients depending on walking assistance. *J Neuroeng Rehabil* (2014) 11: 1-12
26. Riley PO, Paolini G, Croce U, et al. A kinematic and kinetic comparison of overground and treadmill walking in healthy subjects. *Gait Posture* (2007) 26:17-24
27. Lee SJ, Hidler J. Biomechanics of overground vs treadmill walking in healthy individuals. *J Appl Physiol* (2008) 104: 747-755
28. Field-Fote EC, Roach KE. Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: A randomized clinical trial. *J Am Phys Ther Assoc* (2010) 91:48-60

29. Field-Fote EC, Lindley SD, Sherman AL. Locomotor training approaches for individuals with spinal cord injury: a preliminar report of walking-related outcomes. *J Neurol Phys Ther* (2005) 29(3):127-37
30. Postans NJ, Hasler JP, Granat MH, et al. Functional electric stimulation to augment partial weight-bearing supported treadmill training for patients with acute incomplete spinal cord injury: a pilot study. *Arch Phys Med Rehabil* (2004) 85: 604-10
31. Momeni K, Ramanujam A, Garbarini EL, et al. Multi-muscle electrical stimulation and stand training: effects on standing. *J Spinal Cord Med* (2019) 42(3):378-386
32. Minassian K, Hofstoetter U, Tansey K, et al. Neuromodulation of lower limb motor control in restorative neurology. *Clin Neurol Neurosurg* (2012) 114: 489-497
33. Kwon BK, Okon E, Hillyer J, et al. A systematic review of non-invasive pharmacologic neuroprotective treatments for acute spinal cord injury. *J Neurotrauma* (2011) 28:1545-1588
34. Levine JM, Levine GJ, Johnson SI, et al. Evaluation of the success of medical management for presumptive thoracolumbar intervertebral disk herniation in dogs. *Vet Surg* (2007) 36: 482-491
35. Amsellem P, Toombs J, Laverty P, et al. Loss of deep pain sensation following thoracolumbar intervertebral disk herniation in dogs: Treatment and prognosis. *Comp Cont Edu Pract* (2003) 25(4): 266-74.
36. Dewey CW, da Costa RC, Thomas WB. "Performing the neurologic examination". In: Dewey CW, da Costa RC, editors. *Practical guide to canine and feline neurology*. USA: Wiley Blackweel (2016). p. 9-28
37. Olby NJ, De Risio L, Muñana KR, et al. Development of a functional scoring system in dogs with acute spinal cord injuries. *Am J Vet Res* (2001) 62(10): 1624-8
38. Levine D, Bockstahler B. "Electrical Stimulation". In: DL Millis & D Levine, editors. *Canine Rehabilitation and Physical Therapy 2nd*. Philadelphia: Elsevier Saunders (2014). p. 342–358
39. Platt S, Olby NJ. "Nursing and Rehabilitation of the neurological patient". In: SR Platt; NJ Olby, editors. *Canine and Feline Neurology 3rd Ed*. England: British Small Animal Veterinary Association (2014). p. 394- 407
40. Alluin O, Delivet-Mongrain H, Rossignol S. Inducing hindlimb locomotor recovery in adult rat after complete thoracic spinal cord section using repeated treadmill training with perineal stimulation only. *J Neurophysiol* (2015) 114(3):1931-46

41. Chau C, Barbeau H, Rossignol S. Early locomotor training with clonidine in spinal cats. *The American Physiological Society* (1998) 79:392-409
42. Meyns P, Van de Crommert H, Rijken H, et al. Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed. *Spinal Cord* (2014) 52: 887-893
43. Levine D, Millis DL, Flocker J, et al. "Aquatic therapy". In: DL Millis & D Levine, editors. *Canine Rehabilitation and Physical Therapy*, 2nd. Philadelphia: Saunders Elsevier (2014). p.526-542
44. Gomes-Osman J, Cortes M, Guest J, et al. A systematic review of experimental strategies aimed at improving motor function after acute and chronic spinal cord injury. *J Neurotrauma* (2016) 33:425-438
45. Mushahwar VK, Jacobs PL, Normann RA, et al. New functional electrical stimulation approaches to standing and walking. *J Neural Eng* (2007) 4: 181-197
46. Malesevic NM, Popovic LZ, Schwirtlich L, et al. Distributed low-frequency functional electrical stimulation delays muscle fatigue compared to conventional stimulation. *Muscle & Nerve* (2010) 42:556-562
47. Bochezanian V, Newton RU, Trajano GS, et al. Effect of tendon vibration during wide-pulse neuromuscular electrical stimulation (NMES) on muscle force production in people with spinal cord injury (SCI). *BMC Neurol* (2018) 18(1):17
48. Kapadia N, Masani K, Craven BC, et al. A randomized trial of functional electrical stimulation for walking in incomplete spinal cord injury: effects on walking competency. *J Spinal Cord Med* (2014) 37: 511-524
49. Barbeau H, Fung J, Leroux A, et al. A review of the adaptability and recovery of locomotion after spinal cord injury. *Prog Brain Res* (2002) 137: 9-25.
50. Alcobendas-Maestro M, Esclarín-Ruz A, Casado-López RM, et al. Lokomat robotic-assisted versus overground training within 3 to 6 months of incomplete spinal cord lesion: randomized controlled trial. *Neurorehabil Neural Repair* (2012) 26(9): 1058-63
51. de Leon RD, Hodgson JA, Roy RR, et al. Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J Neurophysiol* (1998) 80: 83-91
52. Engesser-Cesar C, Ichiyama RM, Nefas AL, et al. Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. *Eur J Neurosci* (2007) 25(7):1931-9

53. Leech KA, Kinnaird CR, Holleran CL, et al. Effects of locomotor exercise intensity on gait performance in individuals with incomplete spinal cord injury. *Phys Ther* (2016) 96(12): 1919-1929
54. Cassilhas RC, Tufik S, Mello MT. Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol Life Sci* (2016) 73(5): 975-83
55. Maier IC, Ichiyama RM, Courtine G, et al. Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain* (2009) 132: 1426-1440
56. Shah PK, Garcia-Alias G, Choe J, et al. Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain* (2013) 136: 3362-77
57. Campbell MT, Huntingford JL. "Nursing care and rehabilitation therapy for patients with neurologic disease". In: Dewey CW, da Costa RC, editors. *Practical guide to canine and feline neurology*. USA: Wiley Blackweel (2016). p. 559-583
58. Martins Â, Ferreira A. "Neuroreabilitação funcional em lesões medulares". In: Lopes RS, Diniz R, editors. *Fisiatria em pequenos animais* 1st Ed. São Paulo: Editora Inteligente (2018). p. 287–298
59. Ingram EA, Kale DC, Balfour RJ. Hemilaminectomy for thoracolumbar Hansen type I Intervertebral disk disease in ambulatory dogs with or without neurologic deficits: 39 cases (2008-2010). *Vet Surg* (2013), 42(8):924-31
60. Castel A, Olby NJ, Mariani CL, et al. Clinical characteristics of dogs with progressive myelomalacia following acute intervertebral disc extrusion. *J Vet Intern Med* (2017) 31(6): 1782-1789
61. Fenn J, Laber E, Williams K, et al. Associations between anesthetic variables and functional outcome in dogs with thoracolumbar intervertebral disk extrusion undergoing decompressive hemilaminectomy. *J Vet Intern Med* (2017) 31(3): 814-824
62. Lewis MJ, Cohen EB, Olby NJ. Magnetic resonance imaging features of dogs with incomplete recovery after acute, severe spinal cord injury. *Spinal Cord* (2018) 56 (2):133-141.
63. Olby NJ, Lim J, Wagner N, et al. Time course and prognostic value of serum GFAP, pNFH, and S100 β concentrations in dogs with complete spinal cord injury because of intervertebral disc extrusion. *J Vet Intern Med* (2019) 33:726-734

64. Aikawa T, Shibata M, Hara Y. et al. A comparison of thoracolumbar intervertebral disc extrusion in french bulldogs and dachshunds and association with congenital vertebral anomalies. *Vet Surg* (2014) 43: 301-3017
65. Bennaim M, Porato M, Jarleton A, et al Preliminary evaluation of the effects of photobiomodulation therapy and physical rehabilitation on early postoperative recovery of dogs undergoing hemilaminectomy for treatment of thoracolumbar intervertebral disk disease. *AJVR* (2017) 78(2): 195- 2005
66. Hansen HJ. A pathologic-anatomical interpretation of disc degeneration in dogs. *Acta Orthop Scand Suppl* (1951) 20:280-293
67. Hansen HJ. Comparative views of the pathology of disk degeneration in animals. *Lab Invest* (1959) 8:1242-1265.
68. Kinzel S, Wolff M, Buecker A, et al. Partial percutaneous discectomy for treatment of thoracolumbar disc protrusion: retrospective study of 331 dogs. *J Small Anim Practice* (2005) 46:479- 484
69. Bergknut N, Auriemma E, Wijsman S, et al. Evaluation of intervertebral disk degeneration in condrodystrophic and noncondrodystrophic dogs by use of Pfirrmann grading of images obtained with low-field magnetic resonance imaging. *Am J Vet Res* (2011) 72(7): 893-898
70. Lainiten OM, Puerto DA. Surgical decompression in dogs with thoracolumbar intervertebral disc disease and loss of deep pain perception: A retrospective study of 46 cases. *Acta Vet Scand* (2005) 46:79-85
71. Ruddle TL, Allen DA, Schertel ER, et al. Outcome and prognostic factors in non-ambulatory Hansen type I intervertebral disk extrusions: 308 caes. *Vet Comp Orthop Traumatol* (2006) 19:29-34
72. Zidan N, Sims C, Fenn J, et al. A randomized, blinded, prospective clinical trial of postoperative rehabilitation in dogs after surgical decompression of acute thoracolumbar intervertebral disc herniation. *J Vet Intern Med* (2018) 32:1133-1144.
73. Ball MU, McGuire JA, Swaim SF, et al. Patterns of occurrence of disk disease among registered dachshunds. *J Am Vet Med Assoc* (1982) 180(5): 519-22
74. Thomas WB, Fingerroth JM, Hagman R. "What Do We Know about the Incidence of Disc Disease in Chondrodystrophoid Dogs?" In: Fingerroth JM & Thomas WB, editors. *Advances in Intervertebral Disc Disease in Dogs and Cats*. (1st Ed.). Iowa: American College of Veterinary Surgeons Foundation & Wiley Blackwell (2015)

75. Côté MP, Ménard A, Gossard JP. Spinal cats on the treadmill: changes in load pathways. *J Neurosci* (2003) 23:2789-2796
76. Côté MP, Gossard JP. Step training-dependent plasticity in spinal cutaneous pathways. *J Neurosci* (2004) 24:11317-11327
77. Lavrov I, Gerasimenko YP, Ichiyama RM, et al. Plasticity of spinal cord reflexes after a complete transection in adult rats: relationship to stepping ability. *J Neurophysiol* (2006) 96:1699-1710
78. Brand RVD, Heutschi J, Barraud Q, et al. Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science* (2012) 336:1182-1185
79. Musienko PE, Deluagina TG, Gerasimenko YP, et al. Limb and trunk mechanisms for balance control during locomotion in quadrupeds. *J Neurosci* (2014) 34(16):5704-5716
80. Rossignol S, Martinez M, Escalona M, et al. "The "beneficial" effects of locomotor training after various types of spinal lesions in cats and rats". In: Dancause N, Nadeau S, Rossignol S, editors. *Sensorimotor rehabilitation: at the crossroad of basic and clinical sciences*. Oxford: Elsevier (2015). p-173-198
81. Harkema SJ, Behrman AL, Barbeau H. *Locomotor training: principles and practice*. New York:Oxford UP (2011)
82. Smith AC, Mummidisetty CK, Rymer WZ, et al. Locomotor training alters the behavior of flexor reflexes during walking in human spinal cord injury. *J Neurophysiol* (2014) 112: 2164-2175
83. Leech KA, Hyosub EK, Hornby G. Strategies to augment volitional and reflex function may improve locomotor capacity following incomplete spinal cord injury. *J Neurophysiol* (2017) 119(3):894-903
84. Lang C, Bradley PM, Jacobi A, et al. STAT3 promotes corticospinal remodelling and functional recovery after spinal cord injury. *EMBO Reports* (2013) 14(10): 931-937.
85. Martinez M, Brezun JM, ZEnnou-Azogui Y, et al. Sensorimotor training promotes functional recovery and somatosensory cortical map reactivation following cervical spinal cord injury. *Eur J Neurosci* (2009) 30:2356-2367
86. Bregman BS. Regeneration in the spinal cord. *Curr Opin neurobiol* (1998) 8:800-807
87. Thomas SL, Gorassini MA. Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury. *J Neurophysiol* (2005) 94: 2844-2855

88. Field-Fote E, Tepavac D. Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. *Arch Phys Med Rehabil* (2001) 82:818-824
89. Ibitoye MO, Hamzaid NA, Hasnan N, et al. Strategies for rapid muscle fatigue reduction during FES exercise in individuals with spinal cord injury: A systematic review. *PLoS One*. (2016) 11(2):1-28
90. Ragnarsson KT. Functional electrical stimulation after spinal cord injury: current use, therapeutic effects and future directions. *Spinal cord* (2008) 46:255-274
91. Côté MP, Murray M, Lemay MA. Rehabilitation strategies after spinal cord injury: Inquiry into the mechanisms of success and failure. *J Neurotrauma* (2017) 34(10): 1841-1857
92. Pette D, Vrbová G. The contribution of neuromuscular stimulation in elucidating muscle plasticity revisited. *Eur J Transl Myol* (2017) 27(1):33-39
93. Sivaramakrishnan A, Solomon JM, Manikandan N. Comparasion of transcutaneous electrical nerve stimulation (TENS) and functional electrical stimulation (FES) for spasticity in spinal cord injury – a pilot randomized cross-over trial. *J Spinal Cord Med* (2018) 41(4):397-406
94. Doucet BM, Lam A, Griffin L. Neuromuscular electrical stimulation for skeletal muscle function. *Yale J Biol Med* (2012) 85:201-215
95. Glinsky J, Harvey L, Van Es P. Efficacy of electrical stimulation to increase muscle strength in people with neurological conditions: a systematic review. *Physiother Res Int* (2007) 12(3): 175-194
96. Hubli M, Dietz V. The physiological basis of neurorehabilitation – locomotor training after spinal cord injury. *J Neuroeng Rehabil* (2013) 10(5): 1-8
97. Honeycutt CF, Gottschall JS, Nichils TR. Electromyographic responses from the hindlimb muscles of the decerebrate cat to horizontal support surface perturbations. *J Neurophysiol* (2009) 101:2751-2761
98. Musselman KE, Fouad K, Misiaszek JE, et al. Training of walking skills overground and on the treadmill: case series on individuals with incomplete spinal cord injury. *J Am Phys Ther Ass* (2009) 89(6):601-611
99. Zewdie E, Roy FD, Yang JF, et al. Facilitation of descending excitatotory and spinal inhibitory networks from training of endurance and precision walking in participants with incomplete spinal cord injury. *Prog Brain Res* (2015) 218: 127-155

100. Wessels M, Lucas C, Eriks I, et al. Body weight-supported gait training for restoracion of walking in people with an incomplete spinal cord injury: a systematic review. J Rehabil Med (2010) 42:513-519

101. Daly JJ, Zimbelman J, Roenigk KL, et al. Recovery of coordinated gait: randomized controlled stroke trial of functional electrical stimulation (FES) versus no FES, with weight-supported treadmill and Over-Ground training. Neurorehabil Neural Repair (2011) 25(7):588-596

FIGURES AND TABLES

Table 1. Functional Electrical Stimulation (FES) Protocol

Current	Symmetric Biphasic Pulsed
Frequency	30 Hz
Intensity	6-16 mA
Pulse Duration	100–150 μ s
Duty Cycle	1:4
Shape	2–4 seconds ramp-up, 8 seconds on-time, 1–2 seconds ramp-down
Time of treatment	10 min; 2–3 times/day for 5 days/week

Table 2. OFS total mean between BWSTT and COGI groups

Group	OFS Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
BWSTT	10,132	0,249	9,640	10,623
COGI	8,001	0,247	7,512	8,490

OFS: Open Field Score; BWSTT: Body weight-supported treadmill training; COGI: Conventional overground intervention

Table 3. T2 and T3 OFS evaluation in both groups.

OFS Moment		Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
T2	BWSTT	8,300	0,589	7,136	9,464
	COGI	5,500	0,589	4,336	6,664
T3	BWSTT	10,700	0,589	9,536	11,864
	COGI	6,300	0,589	5,136	7,464

OFS: Open Field Score; BWSTT: Body weight-supported treadmill training; COGI: Conventional overground intervention

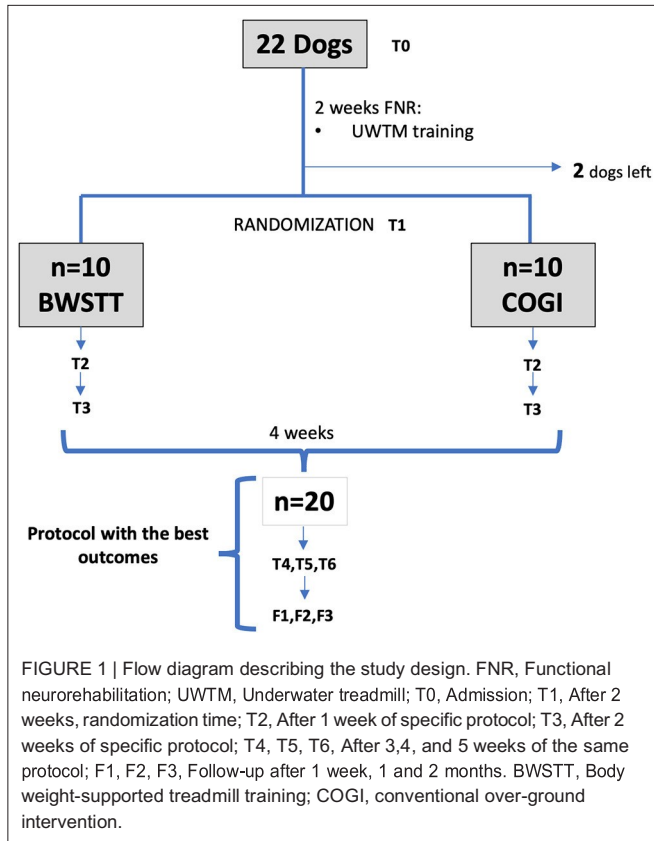




FIGURE 3 | Underwater treadmill locomotor training, with pelvic limb bicycle movements performed by the technician.



FIGURE 4 | Functional electrical stimulation (FES) through the sciatic nerve. Superficial electrodes applied in a segmental technique, placed on the sciatic nerve root (L7-S1 region) and near the flexor muscle motor point.



FIGURE 5 | Body weight-supported treadmill training with bipedal step training. Forelimbs stationary on a fixed platform above the belt.



FIGURE 6 | Body weight-supported treadmill training with quadrupedal step training. Technician performing bicycle movements.



FIGURE 7 | Conventional over ground intervention. Gait Stimulation in different floors surfaces, such as sand, stones, and grass.



FIGURE 8 | Gait Stimulation on uneven floor, such as ramp up and down

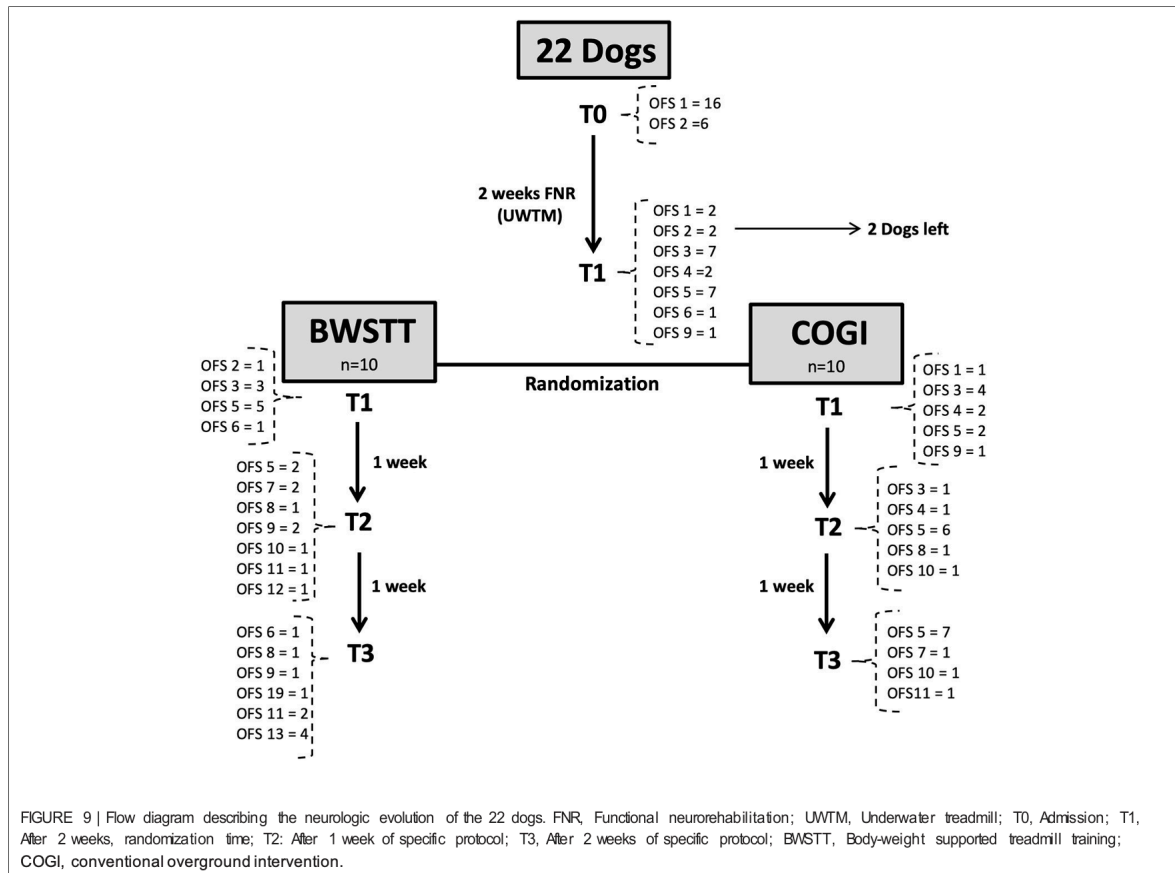


FIGURE 9 | Flow diagram describing the neurologic evolution of the 22 dogs. FNR, Functional neurorehabilitation; UWTM, Underwater treadmill; T0, Admission; T1, After 2 weeks, randomization time; T2, After 1 week of specific protocol; T3, After 2 weeks of specific protocol; BWSTT, Body-weight supported treadmill training; COGI, conventional overground intervention.

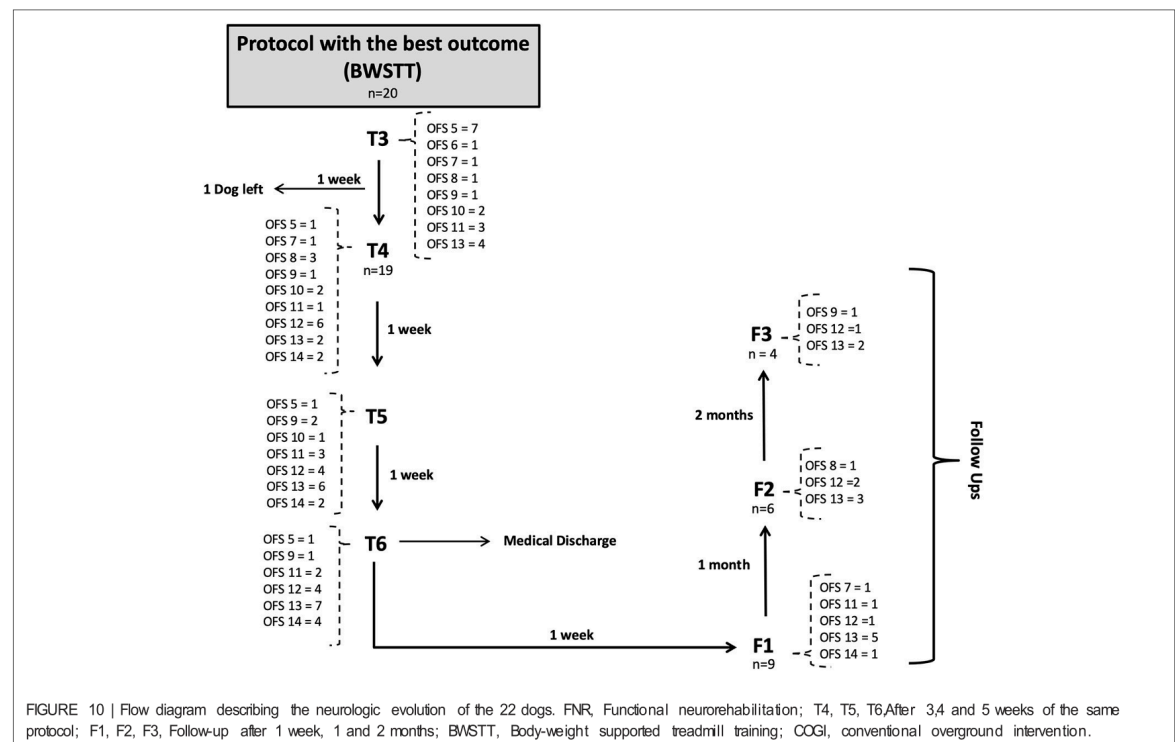


FIGURE 10 | Flow diagram describing the neurologic evolution of the 22 dogs. FNR, Functional neurorehabilitation; T4, T5, T6, After 3, 4 and 5 weeks of the same protocol; F1, F2, F3, Follow-up after 1 week, 1 and 2 months; BWSTT, Body-weight supported treadmill training; COGI, conventional overground intervention.

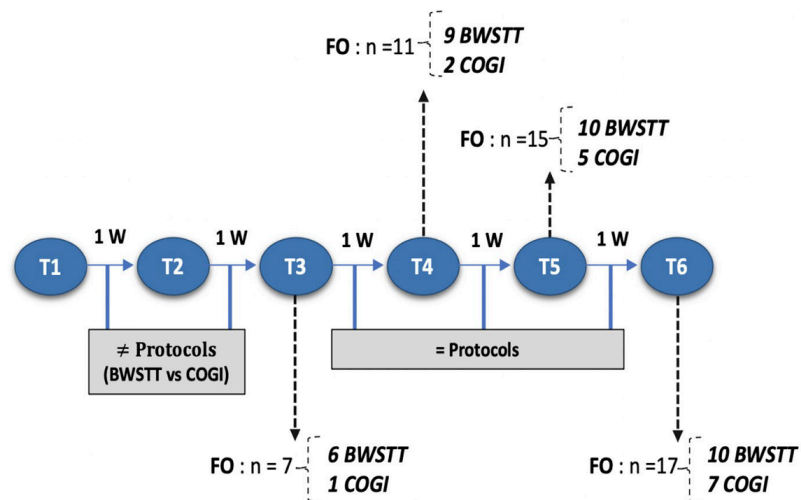


FIGURE 11 | Flow diagram describing each moment according to number of weeks and functional outcome recovery. FNR, Functional neurorehabilitation; UWTM, Underwater treadmill; T1, After 2 weeks, randomization time; T2, After 1 week of specific protocol; T3, After 2 weeks of specific protocol; T4, T5, T6, After 3, 4, and 5 weeks of the same protocol; BWSTT, Body-weight supported treadmill training; COGI, conventional overground intervention; FO, Functional outcome.

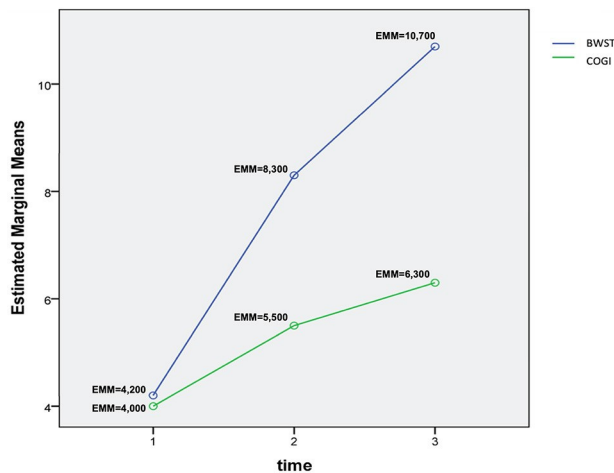


FIGURE 12 | Interaction plot describing the evolution of OFS means in T1, T2, and T3 between groups. OFS, Open Field Score; BWSTT, Body weight-supported treadmill training; COGI, Conventional overground intervention; EMM, Estimated marginal means.

2. ARTICLE 3

Animals (2021), 11(3034):1-22

A Controlled Clinical Study of Intensive Neurorehabilitation in Post-Surgical Dogs with Severe Acute Intervertebral Disc Extrusion

A Controlled Clinical Study of Intensive Neurorehabilitation in Post-Surgical Dogs with Severe Acute Intervertebral Disc Extrusion

Ângela Martins ^{1,2,3,4*}, Débora Gouveia ^{2,4}, Ana Cardoso ², Carla Carvalho ², Tiago Coelho ², Cátia Silva ², Inês Viegas², Óscar Gamboa ⁵ and António Ferreira ^{3,5}

¹ Faculty of Veterinary Medicine, Lusófona University, Campo Grande, 1300-477 Lisboa, Portugal;

² Animal Rehabilitation Center, Arrábida Veterinary Hospital, Azeitão, 2925-583 Setúbal, Portugal;

³ CIISA-Centro Interdisciplinar-Investigação em Saúde Animal—Faculdade de Medicina Veterinária, Av. Universidade Técnica de Lisboa, 1300-477 Lisboa, Portugal

⁴ Superior School of Health, Protection and Animal Welfare, Polytechnic Institute of Lusophony, Campo Grande, 1300-477 Lisboa, Portugal

⁵ Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisboa, Portugal;

* Correspondence: vetarrabida.lda@gmail.com

Simple Summary: This study explores the potential intensive neurorehabilitation plasticity effects in post-surgical paraplegic dogs with severe acute intervertebral disc extrusion, aiming to achieve ambulatory status. The intensive neurorehabilitation protocol translated in 99.4% (167/168) of recovery in deep pain perception positive dogs and 58.5% (55/94) in deep pain perception negative dogs. There was 37.3% (22/59) of spinal reflex locomotion, obtained within a maximum period of 3 months. Thus, intensive neurorehabilitation may be a useful approach for this population of dogs, avoiding future euthanasia and promoting an estimated time window of 3 months to recover.

Abstract: This retrospective controlled clinical study aimed to verify if intensive neurorehabilitation (INR) could improve ambulation, faster than spontaneous recovery or conventional physiotherapy, and provide a possible therapeutic approach in post-surgical paraplegic deep pain perception positive (DPP⁺) (with absent/decreased flexor reflex) and DPP negative (DDP⁻) dogs, with acute intervertebral disc extrusion. A large cohort of T10-L3 Spinal Cord Injury (SCI) dogs (n=367) were divided into a study group (SG) (n=262) and a control group (CG) (n=105). SG was based on prospective clinical cases and CG was created by retrospective medical records. All SG dogs performed an INR protocol by hospitalization regime, based on locomotor training, electrical stimulation, and, for DPP⁻, the combination with pharmacological management. All were monitored throughout the process and measured outcome for DPP⁺ was performed by OFS, and for the DPP⁻, by the new Functional Neurorehabilitation Scale (FNRS-DPP). In the SG, DPP⁺ dogs had an ambulation rate of 99.4% (n=167) and in DPP⁻ of 58.5% (n=55). Moreover, in DPP⁺, there was a strong statistically significant difference between groups regarding ambulation ($p < 0.001$). The same significant difference was verified in the DPP⁻ dogs ($p = 0.007$). Furthermore, it was demonstrated a tendency to a significant statistical difference ($p = 0.058$) regarding DPP recovery, between groups. Of the 59 dogs that did not recover DPP, 22 dogs achieved spinal reflex locomotion (SRL), 37.2% within a maximum of 3 months. Progressive myelomalacia cases were 14.9% (14/94). Therefore, although it is difficult to assess the contribution of INR for recovery, the results suggested that ambulation success may be improved, mainly regarding time.

Keywords: spinal cord injury; locomotor training; functional electrical stimulation; transcutaneous electrical SC stimulation; 4-aminopyridine; neurorehabilitation; acute dogs

1. Introduction

Spinal Cord Injury (SCI) leads to temporary or permanent changes in the motor, sensory, and automatic functions [1]. Following SCI, it is possible to activate and strengthen connections until spontaneous recovery [2], although it is not clear how intensive neurorehabilitation (INR) training can induce plasticity.

INR is a field of physical medicine and rehabilitation that is based on the evidence of signal transmission throughout the lesion, caudally and rostrally, which can be detected by electromyography [3–5]. Thus, the main goal of INR is to trigger the central axon pathways, traversing the lesion, by synaptic stimulation [4,6] and to stimulate propriospinal connections that may bypass the injury site and possibly mediate recovery [6,7].

The INR intends to facilitate central nervous system (CNS) reorganization at multiple levels. Mostly, it intends to achieve a balance between the central pattern generators (CPG), spinal rhythm-generating circuitry plasticity, persistent descending pathways [8,9], and the sensory feedback, which stimulates the CPG [10–13]. While descending pathways can produce start–stop signals, which are essential for coordination and posture [12], propriospinal interneurons may promote new intraspinal circuits after severe SCI [2]. Therefore, INR, in addition to spontaneous recovery, could induce recovery based on similar mechanisms.

INR protocols are based on a multimodal approach, which includes locomotor training [10,14–16], electrostimulation protocols [2,17–22], and, in circumstances of absent deep pain perception (DPP⁻), pharmacological management [23–29].

Locomotor training promotes the activation of the spinal locomotor circuitry, which interacts dynamically with afferent inputs from the receptors of muscles, joints, and skin [16,30,31].

Functional electrical stimulation (FES) promotes an unnatural recruitment pattern of muscle fibers, starting with large-diameter ones (fast neurons) instead of fatigue-resistant slow motoneurons. In addition, FES may stimulate new intraspinal circuits and persistent residual spinal circuits [3,32–35].

Furthermore, transcutaneous electrical stimulation can promote neuromodulation effects in the spinal cord locomotor network of the lumbar region [36–38] and plays a role in the spinal rhythm-generating circuitry [39,40].

The use of multidisciplinary approaches in human patients and in animal models may improve ambulation [41,42]. A similar approach could be applied in DPP⁻ and DPP⁺ dogs, in a clinical setting.

The main aim of this study was to verify if INR could improve ambulatory status faster than spontaneous recovery or conventional physiotherapy, with a minimum sensory deficit, and, also, to provide a possible therapeutic approach in post-surgical dogs with acute intervertebral disc extrusion (IVDE).

This study hypothesized that a multimodal training protocol may improve ambulation in paraplegic dogs DPP⁻ and DPP⁺.

2. Materials and Methods

This study was conducted between May 2011 and May 2020 at the Arrábida Veterinary Hospital (Arrábida Animal Rehabilitation Center), after approval by the Lisbon Veterinary Medicine Faculty Ethics Committee and after the owners' consent.

2.1. Participants

This was a retrospective controlled clinical study using a large cohort of dogs (n=367). The Study Group (SG) was composed of 262 dogs and the Control Group (CG) consisted of 105 dogs.

Dogs from the SG were prospective clinical cases that were monitored and all data was collected and registered during the rehabilitation process.

The 105 dogs of the CG were selected from the Arrábida Veterinary Hospital data management system and the medical records were retrospectively investigated. These medical records focus on breed, age, weight, etiology, surgical treatment, neurological examination at admission and at medical discharge.

All the dogs (n=367) had compressive myelopathy by extruded material (Hansen Type I IVDE) with T10-L3 segment injury diagnosed by computed tomography (CT) with/without myelogram or magnetic resonance imaging (MRI). Standard CT signs observed were hyperattenuating material inside the vertebral canal, a loss of epidural fat, and deformity of the spinal cord. Dogs were included in two different imaging patterns: acute extruded mineralized nucleus pulposus or acute extrusion of nucleus pulposus with hemorrhage. The myelogram findings reported lateralized focal spinal cord compression or herniation into the ventral subdural space. Regarding MRI, only some dogs of both groups needed this type of exam, showing hyperintensity on T2W images, which could correlate with the severity and presentation of the clinical neurological findings, such as necrosis, myelomalacia, inflammation, edema, and intramedullary hemorrhage.

Dogs were treated by hemilaminectomy 3 to 5 days after injury (first clinical sign appearance) and classified with the Frankel Modified Scale (FMS) as grade 0 (DPP⁻) or 1 (DPP⁺), before and after surgery.

Regarding the SG, all dogs were admitted to the rehabilitation center less than 7 days after surgery. One hundred and sixty-eight dogs were paraplegic DPP⁺ with absent/decreased flexor peripheral reflex and classified as grade 1 according to the Open Field Score (OFS) [43].

The other 94 dogs were paraplegic DPP⁻, evaluated according to a new score scale—the Functional Neurorehabilitation Scale for dogs with Thoracolumbar SCI without Deep Pain Perception (FNRS-DPP) [44] and classified as grade 0 or 1 (Fig.1). Dogs were classified with an FNRS-DPP grade 1 only if the patellar reflex was present.

Concerning the CG, 62 dogs were paraplegic DPP⁺ with absent/decreased flexor peripheral reflex and classified with OFS 1, and 43 dogs were paraplegic DPP⁻.

This group was subjected to cage rest, passive range of motion exercises (PROMS), massage, supported postural standing, gait stimulations, and neuromuscular electrical stimulation (NMES).

All animals in the study were less than 7 years old and weighed less than 25 kg. Most were of chondrodystrophic breeds and lacked other

concomitant diseases. Dogs were excluded if they presented other SCI, outside T10–L3 or if they had a surgical approach before 3 days or more than 5 days after injury. Moreover, animals were excluded if they had higher OFS scores (> 1) or higher grades of FNRS-DPP (> 1). Regarding the SG, dogs that were admitted to the rehabilitation center more than 7 days post-surgery were also excluded.

FUNCTIONAL NEUROREHABILITATION SCALE (FNS) FOR DOGS WITH TORACOLUMBAR INJURY, WITHOUT DEEP PAIN SENSATION		
Nociception Evaluation	Deep pain sensation present in the digits	(+1)
	Deep pain sensation present in the tail	(+1)
	Deep pain sensation present in the perineum (dermatomes S3)	(+1)
	Deep pain sensation present in the vulva (dermatomes S2)	(+1)
Spinal Reflexes Evaluation	Patellar reflex	Absent 0
	Cranial tibial reflex	Decreased 1
	Withdrawal reflex	Normal 2
		Increased 3
	Crossed extensor reflex	Absent 0
		Present 1
Muscle Tone Evaluation	Hypotonic extensors muscles and hypotonic flexors muscles	0
	Hypertonic extensors muscles and hypotonic flexors muscles	1
	Spasticity of the extensors muscles and hypotonic flexors muscles, with passive range of motion difficult or absent ROM	2
	Hypertonic extensors muscles and hypotonic flexors muscles, with decreased ROM	3
	Normal muscle tone or slightly hypotonic flexors muscles	4
Gait Evaluation	Paraplegia	0
	Presence of movement without deep pain sensation, non-functional	1
	Presence of movement with deep pain sensation, non-functional	3
	Presence of movement without deep pain sensation, functional	2
	Presence of movement with deep pain sensation, functional	4
Proprioception and Locomotor #Coordination Evaluation	Coordination between PL and TL < 10% of the time*; +/- knuckling	0
	Coordination between PL and TL between 10-25% of the time*; +/- knuckling	1
	Coordination between PL and TL between 25-50% of the time*; without knuckling	2
	Coordination between PL and TL between 50-75% of the time*; without knuckling	3
	Coordination between PL and TL > 75% of the time*; without knuckling	4

Figure 1. Functional neurorehabilitation scale for dogs with thoracolumbar spinal cord injury without deep pain perception; 31st Annual Symposium of the ESVN-ECVN [44].

2.2. Study Design

The 367 dogs were subjected to a neurorehabilitation consultation and evaluated according to their history and physical and neurorehabilitation examinations. In the neurorehabilitation examination, the following were examined: mental status; posture; postural reactions; spinal reflexes; cutaneous trunci muscle reflex; spinal palpation; pain perception and gait. For the SG (n=262), gait for DPP⁺ dogs was evaluated using the OFS and for DPP⁻ dogs using the FNRS-DPP⁻.

Regarding gait, SG dogs were evaluated during the same time (3 to 7 PM), on the same 4 m ceramic surface floor, by the same observer always in the same position. The neurorehabilitation examination was performed in a controlled environment, without external noise and a restricted number of people. Study participants underwent an accurate evaluation regarding DPP, tested on the medial and lateral digits of the hindlimb bilaterally and on the tip and base of the tail. Superficial sensitivity was assessed on the S1–S2 dermatomes with 12 cm Halsted mosquito forceps.

The perineal region, including the bulbocavernosus reflex, was also assessed.

INR protocol started 24 h after admission, for all dogs. Within this period, dogs were prepared for training and rehabilitation modalities (e.g., trichotomy), adapted to the treadmill, and presented to the rehabilitation team, who attended to their tender, love, and care needs.

All SG dogs were subjected to weekly evaluations by a certified canine rehabilitation professional (CCRP) examiner/instructor at the University of Tennessee. Data were recorded (Canon EOS Rebel T6 1300 D camera) and all images were then revised by another CCRP instructor and a non-CCRP neurologist at the Lisbon Veterinary Medicine Faculty. SG dogs were admitted to an INR through a hospitalization regimen for a maximum period of 3 months, as described in the Figure 2.

As acute IVDE dogs, all SG and CG dogs were under a nonsteroidal anti-inflammatory treatment with carprofen (2.2 mg/kg BID) or meloxicam (0.1mg/kg SID) for 5 days or corticosteroids with prednisolone (0.5 mg/kg per os BID/SID) for 3–5 days. After 7 days post-surgery, this treatment was totally discontinued in both the SG and CG. The SG dogs remained only with the INR protocol, and CG remained with cage rest, PROMS, massage, postural standing, gate stimulation, and NMES.

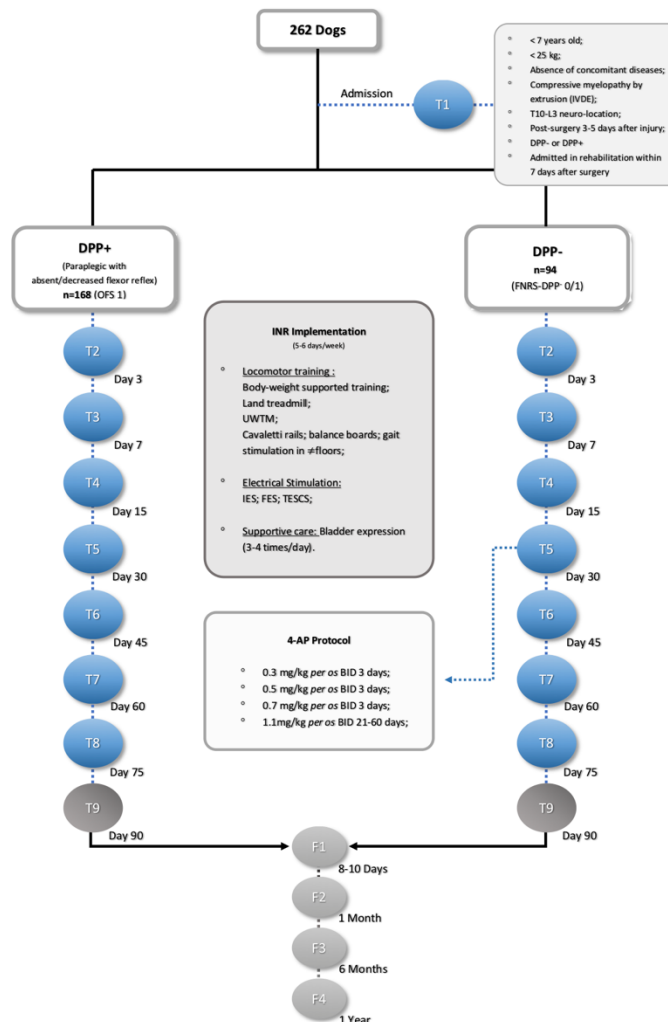


Figure 2. Flow diagram (as recommended by Strengthening the Reporting of Observational Studies in Epidemiology STROBE guidelines) illustrating the study design. IVDE: intervertebral disc extrusion; DPP: deep pain perception; INR:

intensive neurorehabilitation; OFS: open field score; FNRS-DPP: functional neurorehabilitation scale for thoracolumbar SCI dogs without DPP; UWTM: underwater treadmill; IES: interferential electrical stimulation; FES: functional electrical stimulation; TESCS: transcutaneous electrical spinal cord stimulation; 4-AP: 4-aminopyridine.

2.3. Interventions

Dogs started INR training with an association of locomotor training and electrical stimulation, 24 hours after admission. Pharmacological management was only applied in DPP- dogs, who did not recover DPP until day 30 but demonstrated a flexion-extension locomotor pattern.

2.3.1. Locomotor training

Dogs were accustomed to the land treadmill and started with a higher body weight support (BWS) (60–80% body weight) [45], which was decreased with the load tolerance [46], always supervised by a rehabilitator.

BWS was achieved with a harness, allowing quadrupedal step training as part of their daily protocol. However, when some resistance was offered, a change to bipedal step training was required [47,48]. During bipedal training, the forelimbs rested on a platform raised above the treadmill belt [49] while the perineal area was stimulated, by suspending and crimping the tail [50] or with assisted bicycle hindlimb movements [51].

For each training session, variables such as the walking speed and duration were increased and recorded, starting from 0.8 km/h (0.5 mph) to a maximum of 1.9 km/h (1.2 mph) [52–54] over 5 min (4–6 times/day, 6 days/week), with the aim of reaching 20 min (2 times/day, 6 days/week) [55].

Clinical study participants in quadruped training received similar stimulation with the same speed and frequency variables. The goal in this group was to reach 30–40 min (2–3 times/day, 6 days/week) [56]. The treadmill slope was then elevated from 10° [47] to 25° [57], to encourage forelimb–hindlimb coordination [58].

All patients began underwater treadmill training 2–7 days after admission. All started with a water temperature of 26°C [59,60], 5 min walk until reaching 1 h of training once a day (5 days/week), from 1–3.5 km/h (2.2 mph) [61, 62] while overtraining signs were monitored.

Furthermore, kinesiotherapy exercises were performed, such as: cavaletti rails (3 rounds, 3 times/day), balance boards (1–2 min increase to 5 min, 3 times/day—with or without BWS), and gait stimulation on different surfaces (1–5 min, 3 times/day).

2.3.2. Electrical stimulation

Electrical stimulation protocols were used to manage pain through interferential electrical stimulation (IES): increase muscle strength and neural connections with FES and increase potential descending pathway depolarization with transcutaneous electrical spinal cord stimulation (TESCS).

- Interferential electrical stimulation

This technique is a form of stimulation that has two separate channels and uses alternating currents [63] through four electrodes placed on the skin near the region of spinal hyperesthesia and crossed at a 90° angle with the following parameters: acute pain, 80–150 Hz and 2–50 ms; chronic pain, 1–10 Hz, 100–400 ms [64,65], once a day (Figure 3).



Figure 3. Interferential electrical stimulation. Four electrodes crossed at a 90° angle near the spinal hyperesthesia region.

- Functional electrical stimulation

This neuromodulation modality uses a short electrical pulse sequence resulting in spinal reflexes. It aims to stimulate the lower motoneuron near the motor region or through peripheral afferent stimulation [66–68]. This modality was performed in all patients with superficial electrodes, using a segmental technique. One electrode was placed on the skin region corresponding to L7–S1 and the other electrode was placed near the ventromedial motor region of the hindlimb flexor muscle group, using a pulsated and biphasic current.

The parameters were 60 Hz, 6–24 mA [69,70]; 1:4 duty cycle; 2–4 s ramp up, 8 s plateau, and 1–2 s ramp down [64], during 10 min. This routine was performed 2–3 times/day (5 days/week) and was discontinued according to each patient's neurological improvement. After the dogs were subjected to the FES modality, they had a therapeutic window of 40 min, during which the locomotor training was conducted [71–73].

- Transcutaneous electrical spinal cord stimulation

All the patients underwent TESCOs 3 times/day (5 days/week), which was gradually discontinued when the flexion–extension locomotor pattern appeared. The surface electrodes were placed on the paravertebral muscles (one electrode at T11–T12 and the other at L7–S1) [74–77] with a continuous current of 50 Hz, 2 mA for 10 min [36,37,78–80] (Fig.4).



Figure 4. Transcutaneous electrical spinal cord stimulation. One electrode of each canal at the T11–T12 region and the others at L7–S1 region.

2.3.3. Pharmacological management

During the 3rd to 4th week (T4–T5) of the training, if the flexion–extension locomotor pattern was present with a DPP⁺ result (tested on the medial and lateral digits of the hindlimb bilaterally, on the tip and base of the tail), it was added with the owner’s consent—the pharmacological management.

It was administered 4-aminopyridine (4-AP), a K⁺ channel-blocking compound [25,26,29,81,82–84], under the following regime: 0.3 mg/kg per os BID for 3 days; 0.5 mg/kg BID for 3 days; 0.7 mg/kg BID for 3 days; and 1.1 mg/kg BID for 21 days. The 4-AP protocol was implemented for a maximum of 2 months.

If any side effects (seizures, diarrhea, and vomiting) occurred, dogs were immediately treated and withdrawn from the clinical study.

INR protocol application was consistently performed within the patient’s cardiorespiratory capacity and according to the evolution observed on the functional neurorehabilitation examination and OFS (DPP⁺ dogs) or FNRS-DPP[−] (DPP[−] dogs) assessment, over a maximum of 3 months.

2.3.4. Supportive measures

Most of the dogs in the clinical study had neurogenic bladders. Thus, bladder expression was performed 3–4 times/day [85] and the urine was monitored daily for odor and color changes. If there was a suspected urinary tract infection, urine culture (by cystocentesis) and specific antibiotic treatment were administered.

The dogs were maintained under a full-time hospitalization regime. They were able to rest on soft beds with multiple disposable absorbent pads and were encouraged to maintain sternal recumbency. Dogs were fed three times per day with an intake increase of 30% and oral hydric support of 100–120 ml/kg, after resistance training alternated with strength training, according to the patient’s needs. At the end of the day, class IV laser therapy was administered to reduce pain at trigger points [86].

All dogs started training at 9:00 a.m. and finishing at 7:00 p.m. They were assisted only by veterinarians and veterinary nurses, who had taken the CCRP course.

2.4. Outcome measures

SG dogs were assessed by neurological examination every 5–7 days by the same certified CCRP examiner/instructor. The measured outcomes, including the OFS or FNRS-DPP⁻, were evaluated at different time points: admission (T1), day 3 (T2), day 7 (T3), day 15 (T4), day 30 (T5), day 45 (T6), day 60 (T7), day 75 (T8), and day 90 (T9) after starting the INR. Follow-ups were performed after 8–10 days (F1), 1 month (F2), 6 months (F3), and one year (F4) (Figure 2).

The presence of DPP, the flexor reflex, flexion–extension locomotor pattern, and postural standing ability were monitored in these evaluations, which facilitated the establishment of an accurate and systematic evaluation of ambulation recovery among dogs.

Ambulation was defined as the patient's ability to stand up, maintain postural standing, take at least ten steps without assistance or weight support on any walking surface, and obtain voluntary or automatic micturition and defecation.

Autonomous ability in movement control suggests that parts of the brain, and also the spinal cord may presumably activate movements with some conscious control [87].

"Spinal Reflex" Locomotion (SRL) can promote autonomous ability to stand up and walk in "DPP⁻ dogs", maintaining some coordination between the forelimbs and hindlimbs, perhaps by propriospinal system reorganization, promoting the dog's ability to not fall when changing directions on a non-slippery floor.

DPP⁻ SG dogs were considered ambulatory if they showed an SRL score of ≥ 14 on FNRS-DPP⁻. For DPP⁺ SG dogs, ambulation was considered when the OFS was ≥ 11 .

At the end of the study, dogs that become DPP⁺ or DPP⁻ but with functional SRL or non-functional SRL were discharged and released to the owner's guardianship.

DPP⁻ dogs, who showed signs compatible with progressive myelomalacia (PM), and upon the owner's request, were euthanized by induction with Propofol IV after they fell asleep, followed by Pentobarbital IV, within a quiet room.

CG dogs were evaluated at admission and at discharge, regarding the neurological examination and FMS. For the CG, ambulation was considered within the same parameters as described for the SG.

2.5. Statistical analysis

Database and statistical analyses were performed through the Microsoft Office Excel 2016 software (Microsoft, USA). Quantitative, qualitative, and categorical data were analyzed using the IBM SPSS Statistics software, version 22 (International Business Machines Corporation, Armonk, NY, USA), and the results were interpreted at the $p \leq 0.05$ level of significance. The categorical data were presented as frequencies and proportions (95% confidence interval).

Chi-square tests were performed to demonstrate the presence of statistically significant differences between the SG and the CG. Independent *t*-tests of samples were also performed to compare groups regarding population characterization.

In the SG, estimated marginal means and interaction plots for comparison at each time-point regarding the OFS scores or FNRS-DPP⁻ scores were performed.

3. Results

The total sample of this study was 367 participants, distributed by an SG (n=262) and a CG (n=105). Acknowledging that the size of the Sg and the CG could impair the analysis, Cohen's d tests were performed, when applicable, and the effect size was always small.

In each group, dogs were admitted in both grade 0 (DPP-) and 1 (DPP+), according to the FMS. Thus, the results are divided as follows.

3.1. DPP+ Dogs

Grade 1 (DPP+) was represented by 62.7% of the total population (n=230). Of these, 168 dogs belong to the SG (73%) and 62 (27%) were controls.

Regarding population characterization, the most common breed among the DPP+ dogs was the Mixed breed (18.7%; n=43), followed by the French Bulldog (17.4%; n=40) and the Dachshund 11.3%; n=26). However, 68.3% (157/230) were of a chondrodystrophic breed.

In the total DPP+ population (SG+CG), there were 59.6% males and 40.4% females and, regarding the neuro-location, the main region was T12–T13 (30.4%), followed by T13–L1 (26.1%). The mean age of the population was 4.07 years (median of 4.00) and the mean weight was 8.79 kg (median of 8.00). Individual means and medians of age and weight for both groups are represented in Table 1.

Table 1. DPP+ Population characterization at admission (n=230)

	DPP+ (n=230)			SG (n=168)			CG (n=62)		
	Mean (SD)	95% CI	Median	Mean (SD)	95% CI	Median	Mean (SD)	95% CI	Median
Age (years)	4.07 (1.574)	3.86–4.27	4.00	4.07 (1.642)	3.86–4.27	4.00	3.76 (1.339)	3.42–4.10	4.00
Bodyweight (kg)	8.79 (4.032)	8.27–9.32	8.00	8.79 (4.484)	8.27–9.32	8.00	7.79 (2.159)	7.24–8.34	7.00

Abbreviations: DPP: deep pain perception; SG: study group; CG: control group; CI: confidence interval; SD: standard deviation.

Concerning ambulation, 99.4% (n=167) of dogs became ambulatory in the SG, whereas only 75.8% (n=47) achieved ambulation in the CG. Furthermore, while only one dog did not achieve ambulation in the SG, 24.2% (n=15) were considered non-ambulatory in the CG. Comparing both groups, there was a strong difference regarding statistical significance (χ^2 (1, N = 230) = 38.963, $p < 0.001$).

Dogs of the SG had clinical discharge as followed: 23.2% (n=39) in T3; 44.6% (n=75) in T4; 25% (n=42) in T5; 4.8 % (n=8) in T6; 0.6% (n=1) in T7 and 1.8% (n=3) in T8 (Figure 5 and 6).

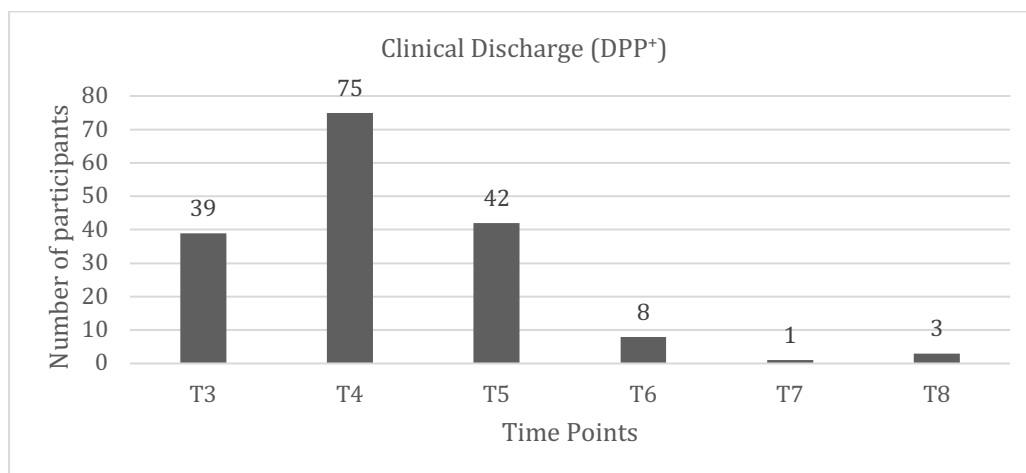


Figure 5. Graphic describing the number of DPP+ dogs in the study group (SG) with clinical discharge in each time point. DPP+: deep pain perception positive; T3: day 7; T4: day 15; T5: day 30; T6: day 45; T7: day 60; T8: day 75.

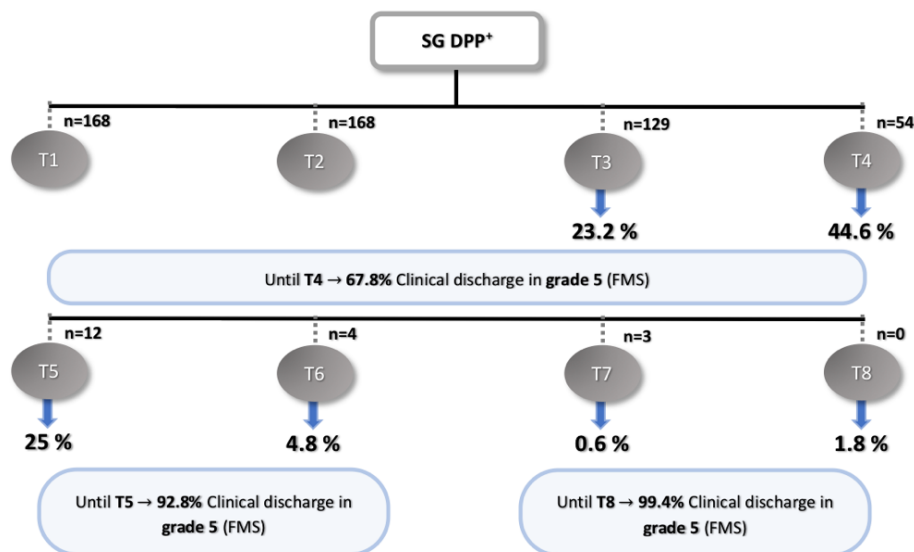


Figure 6. Flow diagram describing the results of the DPP+ dogs in the study group. SG: study group; DPP+: deep pain perception positive; T1: admission; T2: day 3; T3: day 7; T4: day 15; T5: day 30; T6: day 45; T7: day 60; T8: day 75; FMS: Frankel modified scale.

Moreover, in each time point and follow-up consultations, OFS values of each dog in the SG were registered and their estimated mean values revealed a progressive evolution in time (Figure 7), achieving a maximum value in time-point day 30 with an OFS mean of 11.8 (n=54). The graph obtained showed a slight decrease in time-point day 45 with an OFS mean of 10.7 (n=12).

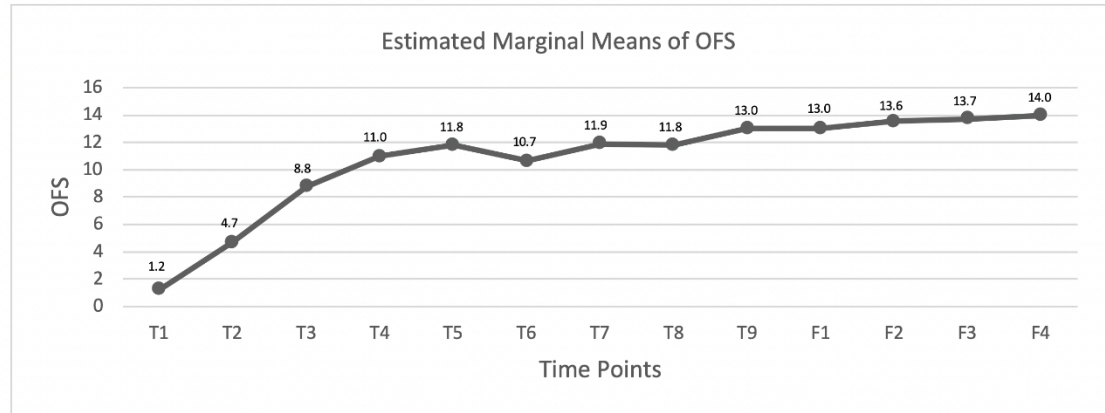


Figure 7. Graphic describing the OFS mean evolution within the DPP+ dogs in the study group (SG). T1: admission; T2: day 3; T3: day 7; T4: day 15; T5: day 30; T6: day 45; T7: day 60; T8: day 75; T9: day 90; F1: 8–10 days follow-up; F2: 1-month follow-up; F3: 6-months follow-up; F4: one year follow-up.

3.2. DPP- Dogs

Grade 0 (DPP-) was represented by 37.3% of the total population (n=137), with 94 dogs belonging to the SG and 43 dogs to the CG.

From these 137 dogs, the French Bulldog was the most common with 27% (n=37), followed by the Mixed breed with 25.5% (n=35) and the Dachshund with 15.3% (n=20). Most dogs were chondrodystrophic, representing 72.3% of the DPP- population (n=99), with 39.4% females and 60.6% males.

For the total DPP- population (SG + CG), regarding neuro-location, T12–T13 had 27% of representation, followed by T13–L1 (19.7%) and L1–L2 (18.2%). Thus, most lesions occurred caudally to T12. The mean age of the population was 4.03 years (median of 4.00) and the mean weight was 8.14 kg (median of 8) (Table 2).

Table 2. DPP- population characterization at admission (n=137)

DPP - (n=137)				SG (n=94)				CG (n=43)	
Mean	95%	Median		Mean	9	Median	M 9	Median	
(SD)	CI			(SD)	5		e 5		
					%		a %		
					C		n C		
					I		(I		
							S		
							D		
							)		
Age (years)	4.03	3.76 -	4.00	3.90	3	4.00	4 3	4.00	
	(1.576)	4.30		(1.566)	.		. .		
					5		3 8		
					8		0 2		
					–		(-		

					4		1 4	
					.		.	.
					2		5 7	
					3		8 9	
							2	
							)	
Body	8.14	7.59 –	8.00	8.51	7	8.00	7 6	7.00
weight (kg)	(3.218)	8.68		(3.466)	.		.	.
					8		3 5	
					0		3 8	
					–		(–	
					9		2 8	
					.		.	.
					2		4 0	
					2		3 8	
							7	
							)	

Abbreviations: DPP: Deep pain perception; SG: study group; CG: control group; CI: confidence interval; SD standard deviation

Having tested the equality of variances (non-significant), the t-test for independent samples in the categories age and weight, revealed no difference with statistical significance between the SG and CG ($p = 0.864$; $p = 0.112$), thus making them comparable. The means and medians of age and weight for both groups are represented in Table 2.

The DPP recovery in the SG was observed in 37.2% (35/94) and 21% (9/43) in the CG. Participants that did not recover DPP were 62.8% (59/94) in the SG and 79% (34/43) in the CG. Thus, there was a tendency towards a significant statistical difference between groups ($X^2 (1, N = 137) = 3.597$; $p = 0.058$).

Of the 59 dogs that did not recover DPP, 22 dogs regained ambulation through SRL, 37.3% (22/59) of them within a maximum of 3 months.

In regard to ambulation, 58.5% ($n=55$) recovered ambulatory status in the SG, while there were only 32.6% ($n=14$) in the CG. On the other hand, 41.5% ($n=39$) did not achieve ambulation in the SG and 67.4% ($n=29$) in the CG. Therefore, comparison between groups showed a strong difference with statistical significance, regarding ambulation recovery ($X^2 (1, N = 137) = 7.311$; $p = 0.007$).

As mentioned earlier, from the total DPP population, 35 dogs recovered DPP: two dogs in T4, although they were withdrawn from the study by owner's decision; seven dogs in T5, achieving ambulation with grade 5 (FMS); five dogs in T7 (grade 5 FMS); 21 dogs in T9 (grade 5 FMS) (Figure 8).

Regarding the 39 dogs that did not achieve ambulation, two left the study in T4 as mentioned above and 37 were discharged in grade 0 (FMS). From these, in T3 ($n=3$), T4 ($n=9$), and T5 ($n=2$), dogs presented

signs of PM. Thus, this study presented 14.9% (14/94) of participants with myelomalacia. Furthermore, in T7 and T9, the remaining 23 dogs were discharged in grade 0 (FMS).

For these dogs in the SG, after 30 days of INR, it was associated the pharmacological management with 4-AP. Thus, in the group of dogs that had DPP recovery and regained ambulation, 26 of them were after 4-AP. SRL was also obtained in T7 (n=10) and T9 (n=12), always after 4-AP administration.

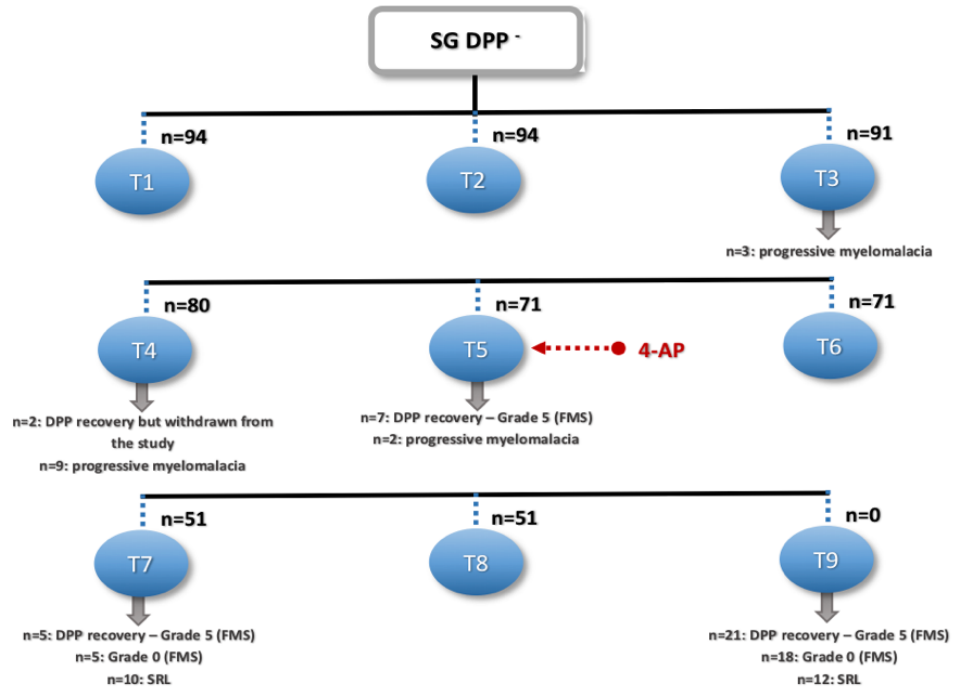


Figure 8. Flow diagram describing the results of the DPP- dogs in the study group. SG: study group; DPP-: deep pain perception negative; T1: admission; T2: day 3; T3: day 7; T4: day 15; T5: day 30; T6: day 45; T7: day 60; T8: day 75; T9: day 90; FMS: Frankel modified scale; SRL: Spinal reflex locomotion.

Comparing each time-points and follow-ups, dogs that did not recover DPP (n=59), were monitored with the FNRS-DPP- scale. The estimated mean values were recorded and are shown in Figure 9, demonstrating an ascending curve throughout the 90 days of INR, with the first maximum value achieved in the time-point—day 60. Moreover, in the 6 months follow-up, the mean score value was 11.3, which increased to 17.3 in the one-year follow-up (n=11).

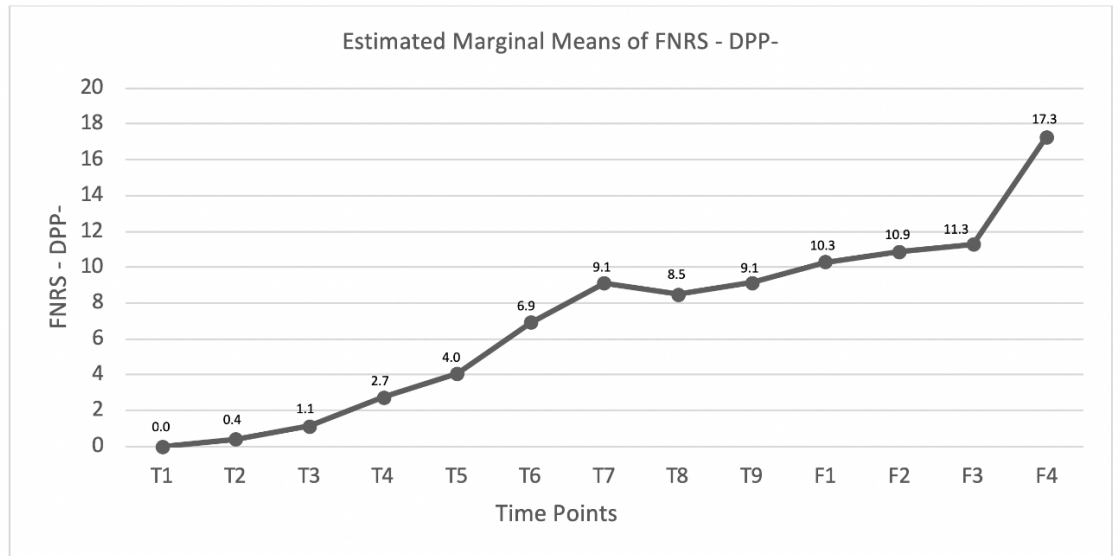


Figure 9. Graphic describing the FNRS-DPP- mean evolution within the DPP- dogs in the study group (SG). T1: admission; T2: day 3; T3: day 7; T4: day 15; T5: day 30; T6: day 45; T7: day 60; T8: day 75; T9: day 90; F1: 8–10 days follow-up; F2: 1-month follow-up; F3: 6 months follow-up; F4: one-year follow-up.

The 35 dogs that recovered DPP were monitored according to the OFS, and estimated means were registered (Figure 10), revealing an OFS of 0 until time-point day 7, ascending from on that day with a maximum value in time-point day 90 (OFS mean 12.3), and achieving a mean score of 13 in the 6 month follow-up that was maintained until one year.

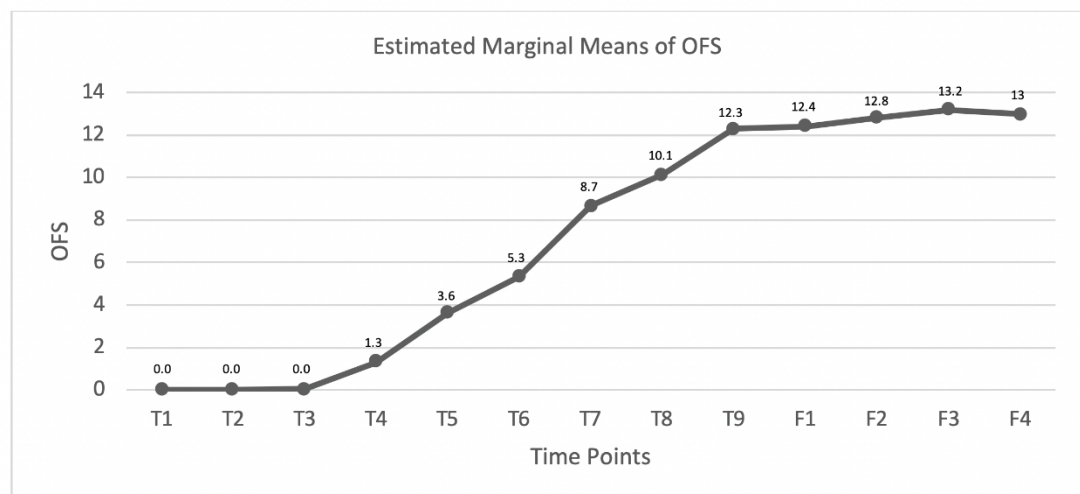


Figure 10. Graphic describing the OFS mean evolution within the DPP⁻ dogs that recovered DPP in the study group (SG). T1: admission; T2: day 3; T3: day 7; T4: day 15; T5: day 30; T6: day 45; T7: day 60; T8: day 75; T9: day 90; F1: 8–10 days follow-up; F2: 1-month follow-up; F3: 6 months follow-up; F4: one-year follow-up.

4. Discussion

This retrospective-controlled clinical study intended to evaluate the effect of INR in the SG versus the CG in both DPP⁺ and DPP⁻ dogs.

The total population was 367 dogs, allowing an approximate level of power ($1-\beta$) of 0.90 and an α (type I error) of 0.01. For the SG ($n=262$), an approximate level of power ($1-\beta$) of 0.90 and an α (type I error) of 0.05 [88] was possible.

The population sample of both the SG and CG was not easy to obtain, needing a total of nine years, given the strict and specific inclusion criteria. A pilot study was previously carried out with 84 dogs subjected to similar INR protocol guidelines as this study. Results were accepted for publication in the ISCOS meeting and were continued in this clinical study [89].

In acute IVDE dogs, it is challenging to differ recovery due to spontaneous plasticity or by possible induced plasticity due to rehabilitation techniques. Thus, this study explored the possible INR plasticity effects, considering that spontaneous neurological recovery usually reaches a plateau within the first few weeks after surgery [90–92].

Furthermore, selecting the participants—DPP⁻ dogs or DPP⁺, but with absent/decreased flexor peripheral reflex, similar to Jeffery et al. (2020) [93]—makes recovery harder to achieve. Participants were classified at admission using the FNRS-DPP⁻ scale with grade 0/1 or OFS 1, respectively, in order to fully understand the possible effects of INR.

The absence/decreased flexor reflex may be attributed to the incidence of spinal shock, which can lead to a secondary lesion within the spinal cord segments containing the hind limbs reflex circuitry; when this clinical sign persists over time, it may be consistent with descending myelomalacia.

At admission, the FNRS-DPP⁻ grade 0 is attributed to the absence of any peripheral reflex in the hind-limbs, whereas dogs in grade 1 have a positive patellar reflex, making the possibility of PM less probable. In the future, it would be of interest to relate the presence of this reflex with the ability to achieve SRL.

All the DPP⁻ dogs underwent a strict evaluation regarding DPP—decreasing variability and increasing the degree of accuracy in testing—which is essential for predicting outcomes [94–96].

The SG and CG could be considered analogous and a homogenous sample population, making comparison possible. Both had similar breed prevalence, with the majority of French Bulldogs and Dachshunds, justifying the chondrodystrophic high incidence. In addition, the *t*-test revealed no statistically significant difference between groups in the DPP⁻ dogs.

4.1. DPP⁺ Dogs

The ambulation rate obtained in the SG was 99.4% ($n=167$), with one dog achieving ambulatory paraparesis (but without the ability to perform 10 consecutive steps without falling). Between the SG and CG,

there was a strong statistical significance regarding ambulation recovery ($p < 0.001$).

Within the SG, 68% of ambulation was achieved from T3–T4 (Figure 6)—a similar result found in previous studies [65,97,98]. An ambulatory state was considered when OFS ≥ 11 , and at T4 the OFS mean obtained in the DPP+ dogs was 11.0, higher than that previously reported by Zidan et al (2018) [96] at the same time, which obtained an OFS mean of 7.87 in the basic group and 7.73 in the intensive group.

Furthermore, at time-point T5 (day 30), a maximum result in the graphic curve was shown: 93.4% (156/167) of ambulation achieving an OFS mean of 11.8. The following decline in the graphic curve may be explained by the remaining small number of dogs in the next time points, not allowing results to be discussed.

In this study, all dogs from the SG underwent the same INR protocol, which differs from most protocols, given the early start of the underwater treadmill (UWTM) training (2–7 days after admission), in contrast to the 7–14 days reported by Zidan et al (2018) [96]. Moreover, a major difference an association of electrical stimulation with FES and not NMES, complementing with TESCOs. Early training has been previously reported as having major importance to recovery [6,99,100].

The BWS training was based on repetitive movements, depending on intensity, volume, and duration [6], with some clinical evidence suggesting that this training may improve the excitatory influence of descending pathways. In this study, all SG dogs performed locomotor training, in which parameters were according to different authors [45,53–56]. Slope training was also performed (inclination 10–25°) to promote standing up ability [47,57].

This INR applied to DPP+ dogs was shown to be a feasible, safe, and repeatable protocol—able to be performed with some knowledge in the field of neuro-rehabilitation and acquiring a land treadmill and a UWTM.

Furthermore, electrical stimulation by FES may potentiate large diameter motor neurons recruitment, capable of fast conduction velocity fibers, instead of recruiting small diameter motor neurons, which are slower and more susceptible to fatigue [101,102]. FES may also increase muscle tonus in the hindlimbs and enhance polysynaptic reflex, essential in this population of dogs that showed absent or decreased withdrawal reflex [66,67,103,104].

This multidisciplinary treatment also included TESCOs, which is considered a non-invasive and non-painful neuro-modulation modality. TESCOs has been proposed to induce stimulation through multi-segmental interactive and synergistic pathways, which combine the central components of motor descending paths and ascending sensorial paths [102], recruiting a diverse population of motor neurons by projecting sensory and intraspinal connections [75].

4.2. DPP- Dogs

In regard to the assessment of DPP- dogs, the OFS has limited value, given the fact that it is unable to evaluate the neurological signs based on peripheral reflexes—mainly the evaluation of the flexion/extension locomotor pattern. Thus, there is a need to implement a precise and restricted outcome with the FNRS-DPP- scale (published at the 31st ESVN-ECVN Symposium in Copenhagen 2018 [44]).

Total DPP⁺ dogs were 137, and 94 dogs were within the SG. The ambulation rate within this group was 58.5% (55/94) and when comparing DPP recovery between the SG and CG, there was 37.2% (35/94) of recovery in the SG, higher than the CG, with a tendency to a significant statistical difference (X^2 (1, $N = 137$) = 3.597, $p = 0.058$); although a lower number, when compared to results published by the CANSORT-SCI (2021) [105], which refer to nearly 60% of DPP recovery [105,106].

DPP recovery assessment in the CG was very limited because these were retrospective data with most dogs euthanized by the owner's decision after 3 weeks. A decision that did not allow for investigating the theory that permanent DPP⁺ dogs may demonstrate spontaneous motor recovery over time [2,107-109].

This reflects the major importance of 22 dogs in the SG that regained ambulation by SRL—37.3% in a maximum period of 3 months. This percentage was higher than the one reported by Olby et al. (2003) [107], with 32% (7/18), that regained ambulation within 9 months on average (range 4–18 months). Furthermore, among a cohort of 94 dogs examined in a chronic setting, nine became ambulatory within a median time of 12 months (range 3–89 months) [109].

On the other hand, Gallucci et al (2017) [108] found, via a retrospective review, a median time of only 75 days to regain ambulation by SRL (range, 16–350 days), with 59% (48/81) of the DPP⁺ dogs recovering and 81% of a full-time hospitalization regime implemented, however, with mixed etiology including acute IVDE or trauma (e.g., vertebral luxation/fracture).

Most studies reported that a shorter average time to achieve ambulation may be related to an early post-injury intensive rehabilitation, which could have a positive influence on recovery [2,108]. In these studies [108–110], participants had T3–L3 as the major neurolocation, which is similar to the present study.

In this type of population, bodyweight distribution allows body support in the forelimbs, with the ability of postural standing but not regaining ambulation, which could be explained due to the larger demand on the supraspinal postural control that is needed to maintain balance, that may be missing after severe injury [2].

Several investigations have reported that multimodal approaches may facilitate motor recovery and are useful in improving outcome, in combination with additional approaches directed to the lesion epicenter [2,111–115].

To Gallucci et al. (2017) [108], spinal walking is a “reflex gait with complex dynamic interactions between CPG of pelvic limbs and proprioceptive feedback from the body in the absence of superior control by the brain after complete spinal cord damage”. For the present study, it is the author's belief that the future aim for INR techniques is to develop strategies that directly target the spinal cord injury and limit the secondary injury, enhancing axons regeneration and/or increasing compensatory plasticity of the persisting tissue. Thus, it is essential to understand broad knowledge regarding injury and recovery mechanisms in order to develop new strategies.

The INR protocol focused on the ability to perform the flexion/extension locomotor pattern, settled on the specific needs of each dog, and promoting exercises based on the flexor reflex and crossed extensor reflex stimulation. The UWTM locomotor training was critical for the DPP⁺ dogs, facilitating the possibility to regain the

flexion/extension pattern [108]. In Martins, 2021 [104], randomization in different locomotor training groups was only possible after 15 days of UWTM training, warranted that possible spinal chock dogs were stimulated at a maximum level with a multidisciplinary treatment.

As a part of the treatment protocol, some authors recommend cage rest for 6–8 weeks [106,116], preventing future traumatic injury and decreasing pain and inflammation [117,118]. It is known that INR may be beneficial to locomotor recovery in a post-surgical patient, mainly in populations similar to our SG, which are severely affected dogs. Studies have shown that intensive locomotor training can promote anatomic and physiologic changes, allowing an improved motor function [119,120].

This study is in accordance with Zidan et al (2018) and Jeong et al (2019) [96,121], however, the latter received decompressive surgery alone, reporting only 17%, which is much lower than the typical results reported in the literature, which usually range from 50 to 60%.

Moore et al. (2020) [106] refer to a similar training protocol when compared to our own, stating that locomotor training (land or UWTM) may help to regain movement. Thus, these types of rehabilitation strategies should be rationally implemented and initially encouraged in the recovery process for IVDE [122], as already shown in human medicine [123].

SG ambulation was achieved in 58.5% (55/94) of patients, in contrast to the 32.6% of the CG, probably because there is no case of SRL in the CG group. Thus, comparison between groups, regarding ambulation, has shown a strong statistically significant difference ($X^2(1, N = 137) = 7.311; p = 0.007$).

In the SG, until T5 there was clinical evidence of PM, with dogs being euthanized in T3 and T4. PM cases were 14.9% in total, a result that agrees with previously reported studies [97,107,124,127]. However, the two dogs that remained until T5 had only signs of descending myelomalacia.

Jeffery et al. (2020) [93] has suggested that an increase in ambulation rate and decrease in cases of PM could be associated with the durotomy surgical technique, as a means to examine the spinal cord and potentiate tissue perfusion, with an approximately 4% reduction.

In the present study, regarding DPP recovery, the greatest percentage occurred after T5, with 74.3% (26/35), as well as the appearance of the SRL, which was only present in T7 and T9. Both situations occurred after the pharmacological support with 4-AP. Thus, it is the author's belief that the combination of these neurorehabilitation modalities, locomotor training, and the 4-AP administration, may help in recovery within a maximum of 3 months, including the possibility of the spontaneous plasticity contribution.

The 4-AP is a potassium channel antagonist that may improve hindlimb motor function in chronic thoracolumbar spinal cord dogs [81,115,128]. This beneficial effect could be achieved through the enhancement of central conduction by anatomically intact axons traversing the injury site, as well as direct synaptic effects [29,129,130].

Dogs that did not regain DPP throughout the 3 months of hospitalization were monitored by the FNSR-DPP scale in each time point outcome. The appearance of the flexion/extension locomotor pattern was a critical outcome, which was the moment of starting the 4-AP administration. In Figure 9, the graphic represents a gradual increase throughout the time points with an ascending curve, although not constant between each time point. Substantial evidence at the beginning

of the curve may be explained by the unlocked inhibition of the nervous system pathways, followed by a fast excitation.

In T7 and T8, there is a slight decrease in the graphic curve, given the fact that 15 dogs were clinically discharged within this period. In T9, the mean scale punctuation was 9.1, although 22 dogs achieved ambulation by SRL. All 22 dogs reached a minimum FNRS-DPP score 14 and the mean of FNRS-DPP ≥ 14 was only achieved in the follow-ups F3 and F4.

This mean scale punctuation may be justified by each variability (ranging from dogs with PM with lower scores to dogs with higher scores) and by the fact that dogs had clinical discharge throughout the process, decreasing the number of dogs that remained and with a worst neurological state.

For Olby et al (2020) [131] and the CANSORT-SCI (2021) [105], approximately 60% of dogs with IVDE recovered DPP and ambulation by 6 months after SCI. In the present study, we had 57.4% of ambulation by 3 months, which might have been influenced by the INR that helped do decrease the time of recovery. Moreover, in the same study, 31% of dogs did not achieve DPP but regained the ability to walk within a mean time of 9 months (range 2–28 months), similar to other authors [115,131]. In our study, we achieved a higher prevalence with 37.3% (22/59), but at a period of 3 months, possibly leading to a better understanding from the owners when discussing prognosis and decreasing future euthanasia. In regard to dogs that regained DPP (n=35), all were monitored with the OFS scale at each time point. Maximum OFS mean was in T0 with 12.3, always increasing and maintained in the 6 months and one-year follow-ups, proving the spinal cord memorization property in the neural regeneration process.

Study limitations included the inability to achieve a level of power ($1-\beta$) of 0.99 and an α (type I error) of 0.05 for each group, a lack of a prospective control group with the same conditions and with the same study group size, the need to statistically relate the presence of peripheral reflexes with ambulation recovery and a non-existent biomarker of regeneration, usually associated to structural proteins (e.g., glial fibrillary acidic protein—GFAP), although this would imply highly sensitive measuring techniques.

5. Conclusions

In acute IVDE dogs classified with OFS1 or FNRS-DPP grade 0/1 and after surgical management, the outcomes obtained were difficult to attribute to spontaneous recovery or with intensive rehabilitation. However, the results suggested that with INR ambulation success could be improved, mainly regarding time, within an estimated period of 3 months. Thus, its implementation may be useful in acute post-surgical IVDE dogs.

Author Contributions: Conceptualization, Â.M.; methodology, Â.M.; validation, Â.M., I.V., A.F.; formal analysis, I.V., A.F.; investigation, Â.M., D.G., A.C., C.S., C.C., T.C. and Ó.G.; writing—original draft preparation, Â.M., C.S., D.G., C.C., and A.C.; writing—review and editing A.F.; supervision, A.F.; project administration, Â.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study received the approval of the Ethics and Animal Welfare Commission of the Veterinary Medicine Faculty of the Lisbon University (CEBEA) (N/Refª001/2019).

Informed Consent Statement: Not applicable

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Fakhoury, M. Spinal cord injury: overview of experimental approaches used to restore locomotor activity. *Rev Neurosci.* **2015**, 26(4), 397–405; doi:10.1515/revneuro-2015-0001.
2. Lewis, M.J.; Jeffery, N.D.; Olby, N.J.; The Canine Spinal Cord Injury Consortium (CANSORT-SCI). Ambulation in Dogs With Absent Pain Perception After Acute Thoracolumbar Spinal Cord Injury. *Front. Vet. Sci.* **2020**, 7, 560; doi: [10.3389/fvets.2020.00560](https://doi.org/10.3389/fvets.2020.00560).
3. Tansey K. E. Neural plasticity and locomotor recovery after spinal cord injury. *PM & R : the journal of injury, function, and rehabilitation* **2010**. 2(12 Suppl 2), S220–S226; doi: [10.1016/j.pmrj.2010.10.007](https://doi.org/10.1016/j.pmrj.2010.10.007).
4. Dimitrijevic M.R. Residual motor function after spinal cord injury. In: *Restorative Neurology of Spinal Cord Injury*, Dimitrijevic M.R., Kakulas B.A., McKay W.B., Vrbová G., Eds.; 1st ed. New York: Oxford University Press; **2012**. p.1–9.
5. Kakulas B.A.; Kaelan C. The neuropathological foundations for the restorative neurology of spinal cord injury. *Clin Neurol Neurosurg.* **2015**, 129, 51–57; doi: [10.1016/j.clineuro.2015.01.012](https://doi.org/10.1016/j.clineuro.2015.01.012).
6. Côté M.P.; Murray M.; Lemay M.A. Rehabilitation strategies after spinal cord injury: inquiry into the mechanisms of success and failure. *J Neurotrauma.* **2017**, 34, 1841–1857; doi: [10.1089/neu.2016.4577](https://doi.org/10.1089/neu.2016.4577).
7. Courtine G.; Song B.; Roy R.R.; Zhong H.; Herrmann J.E.; Ao Y. *et al.* Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. *Nat Med.* **2008**, 14,1, 69–74; doi:[10.1038/nm1682](https://doi.org/10.1038/nm1682).
8. Raineteau O., Schwab M.E.; Plasticity of motor systems after incomplete spinal cord injury. *Nat Rev Neurosci.* **2001**, 2,4, 263–73; doi: [10.1038/35067570](https://doi.org/10.1038/35067570).
9. Dimitrijevic M.R.; Danner S.M.; Mayr W. Neurocontrol of movement in humans with spinal cord injury. *Artif Organs.* **2015**, 39,10, 823–33; doi: [10.1111/aor.12614](https://doi.org/10.1111/aor.12614).
10. van de Crommert H.W.; Mulder T.; Duysens J. Neural control of locomotion: sensory control of the central pattern generator and its relation to treadmill training. *Gait Posture.* 1998; 7: 251–263; doi: [10.1016/s0966-6362\(98\)00010-1](https://doi.org/10.1016/s0966-6362(98)00010-1).
11. Rossignol, S.; Dubuc, R.; Gossard, J.P. Dynamic sensorimotor interactions in locomotion. *Physiol. Rev.* **2006**, 86, 89–154; doi: [10.1152/physrev.00028.2005](https://doi.org/10.1152/physrev.00028.2005).
12. Barrière, G.; Leblond, H.; Provencher, J.; Rossignol, S. Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries. *J. Neurosci.* **2008**, 28, 3976–3987. doi: 10.1523/JNEUROSCI.5692-07.2008.
13. Chen Y.; Chen L.; Wang Y.; Chen X.Y.; Wolpaw J.R. Why new spinal cord plasticity does not disrupt old motor behaviors. *J Neurosci.* **2017**, 37,34, 8198–8206; doi: 10.1523/JNEUROSCI.0767-17.2017.
14. Edgerton, R.; de Leon, R.; Harkema, S.; Hodgson, J.; London, N.; Reinkensmeyer, D.; Roy, R.; Talmadge, R.; Tillakaratne, N.; Timoszyk, W.; *et al.* Retraining the injured spinal cord. *J. Physiol.* **2001**, 533, 15–22; doi: 10.1111/j.1469-7793.2001.0015b.x.
15. Wirz, M.; Zemon, D.H.; Rupp, R.; Scheel, A.; Colombo, G.; Dietz, V.; *et al.* Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: a multicenter trial. *Arch Phys Med Rehabil.* **2005**, 86, 672–80; doi: 10.1016/j.apmr.2004.08.004.

16. Knikou, M. Neural control of locomotion and training-induced plasticity after spinal and cerebral lesions. *Clin Neurophysiol.* **2010**, 121,10, 1655-1668; doi: 10.1016/j.clinph.2010.01.039.
17. Behrman, A.; Harkema, S. Locomotor training after human spinal cord injury: a series of case studies. *Phys Ther.* **2000**, 80,7, 688-700; PMID: 10869131.
18. Barbeau, H.; Fung, J.; Leroux, A.; Ladouceur, M. A review of the adaptability and recovery of locomotion after spinal cord injury. *Prog Brain Res.* **2002**, 137, 9-25; doi: 10.1016/s0079-6123(02)37004-3.
19. Lavrov, I.; Gerasimenko, Y.P.; Ichiyama, R.M.; Courtine, G.; Zhong, H.; Roy, R.R.; *et al.* Plasticity of spinal cord reflexes after a complete transection in adult rats: relationship to stepping ability. *J Neurophysiol.* **2006**, 96: 1699-1710; doi: 10.1152/jn.00325.2006.
20. Possover M. Recovery of sensory and supraspinal control of leg movement in people with chronic paraplegia: a case series. *Arch Phys Med Rehabil.* **2014**, 95: 610-614; doi: 10.1016/j.apmr.2013.10.030.
21. Mehrholz, J.; Kugler, J.; Pohl, M. Locomotor training for walking after spinal cord injury. *Cochrane Database Syst Rev.* **2012**, 11; doi: 10.1002/14651858.CD006676.pub2.
22. Mehrholz, J.; Harvey, L.A.; Thomas, S.; Elsner, B. Is body-weight-supported treadmill training or robotic-assisted gait training superior to overground gait training and other forms of physiotherapy in people with spinal cord injury? A systematic review. *Spinal Cord.* **2017**, 55: 722-729; doi: 10.1038/s41393-017-0059-6.
23. Barbeau, H.; Norman, K.; Fung, J.; Visintin, M.; Ladouceur, M. Does neurorehabilitation play a role in the recovery of walking in neurological populations?. *Ann N Y Acad Sci.* **1998**, 860:377-92.; doi: 10.1111/j.1749-6632.1998.tb09063.x.
24. Wolpaw, J.R.; Tennissen, A.M.; Activity-dependent spinal cord plasticity in health and disease. *Annu Rev Neurosci.* **2001**, 24: 807-43; doi: [10.1146/annurev.neuro.24.1.807](https://doi.org/10.1146/annurev.neuro.24.1.807).
25. Hayes, K.C. The use of 4-aminopyridine (fampridine) in demyelinating disorders. *CNS Drug Rev.* **2004**, 10:4: 295–316; doi: [10.1111/j.1527-3458.2004.tb00029.x](https://doi.org/10.1111/j.1527-3458.2004.tb00029.x).
26. Hayes, K.C. Fampridine-SR for multiple sclerosis and spinal cord injury. *Expert Rev Neurother.* **2007**, 7:5: 453-61; doi: 10.1586/14737175.7.5.453.
27. Fong, A.J.; Roy, R.R.; Ichiyama, R.M.; Lavrov, I.; Courtine, G.; Gerasimenko, Y.; *et al.* Recovery of control of posture and locomotion after a spinal cord injury: solutions staring us in the face. *Prog Brain Res.* **2009**, 175: 393-418; doi: 10.1016/S0079-6123(09)17526-X.
28. Guertin, P.A. Central pattern generator for locomotion: anatomical, physiological, and pathophysiological considerations. *Front Neurol.* **2013**, 3:183; doi: 10.3389/fneur.2012.00183.
29. Lewis, M.J.; Laber, E.B.; Olby, N.J. Predictors of response to 4-aminopyridine in chronic canine spinal cord injury. *J Neurotrauma.* **2019**, 36:9: 1428-1434; doi: 10.1089/neu.2018.5975.
30. Pearson, K.G. Role of sensory feedback in the control of stance duration in walking cats. *Brain Res Rev.* **2008**, 57: 222-7; doi: 10.1016/j.brainresrev.2007.06.014.
31. Rossignol, S.; Frigon, A. Recovery of locomotion after spinal cord injury: Some facts and mechanisms. *Annu. Rev. Neurosci.* **2011**, 34, 413–440; doi: 10.1146/annurev-neuro-061010-113746.
32. Edgerton, V.R.; Harkema, S. Epidural stimulation of the spinal cord in spinal cord injury: current status and future challenges. *Expert Rev Neurother.* **2011**, 11:10: 1351-3; doi: 10.1586/ern.11.129.
33. Harkema SJ, Gerasimenko Y, Hodes J, Burdick J, Angeli C, Chen Y, *et al.* Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *Lancet.* **2011**, 377, 9781, 1938–47; doi: 10.1016/S0140-6736(11)60547-3.
34. Minassian, K.; Hostoetter, U.S.; Danner, S.M.; Mayr, W.; Bruce, J.A.; McKay, W.B.; *et al.* Spinal Rhythm Generation by Step-Induced Feedback and Transcutaneous Posterior Root Stimulation in Complete Spinal Cord-Injured Individuals. *Neurorehabil Neural Repair.* **2016**, 30,3, 233-243; doi: 10.1177/1545968315591706.

35. Minassian, K.; Hofstoetter, U.S. Spinal Cord Stimulation and Augmentative Control Strategies for Leg Movement after Spinal Paralysis in Humans. *CNS Neurosci Ther.* **2016**, 22,4, 262-270; doi: 10.1111/cns.12530.
36. Hofstoetter, U.S.; Hofer, C.; Kern, H.; Danner, S.M.; Mayr, W.; Dimitrijevic, M.R.; *et al.* Effects of transcutaneous spinal cord stimulation on voluntary locomotor activity in an incomplete spinal cord injured individual. *Biomed Tech (Berl).* **2013**, 58,1; doi: 10.1515/bmt-2013-4014.
37. Hofstoetter, U.S.; McKay, W.B.; Tansey, K.E.; Mayr, W.; Kern.; Minassian, K. Modification of spasticity by transcutaneous spinal cord stimulation in individuals with incomplete spinal cord injury. *J Spinal Cord Med.* **2014**, 37,2, 202-11; doi: 10.1179/2045772313Y.0000000149.
38. Estes, S.P.; Iddings, J.A.; Field-Fote, E.C. Priming neural circuits to modulate spinal reflex excitability. *Front Neurol.* **2017**, 3, 8-17; doi: 10.3389/fneur.2017.00017.
39. Knikou, M.; Mummidisetty, C.K. Locomotor training improves premotoneuronal control after chronic spinal cord injury. *J Neurophysiol.* **2014**, 111, 2264-2275; doi: 10.1152/jn.00871.2013.
40. Hofstoetter, U.S.; Knikou, M.; Guertin, P.A.; Minassian, K. Probing the Human Spinal Locomotor Circuits by Phasic Step-Induced Feedback and by Tonic Electrical and Pharmacological Neuromodulation. *Curr Pharm Des.* **2017**, 23,12, 1805-1820; doi: 10.2174/1381612822666161214144655.
41. Gomes-Osman, J.; Cortes, M.; Guest, J.; Pascual-Leone, A. A systematic review of experimental strategies aimed at improving motor function after acute and chronic spinal cord injury. *J Neurotrauma.* **2016**, 33,425-38; doi: 10.1089/neu.2014.3812.
42. Gant, K.L.; Nagle, K.G.; Cowan, R.E.; Field-Fote, E.C.; Nash, M.S.; Kressler, J.; *et al.* Body system effects of a multi-modal training program targeting chronic, motor complete thoracic spinal cord injury. *J Neurotrauma.* **2018**, 35,3, 411-423; doi: 10.1089/neu.2017.5105.
43. Olby NJ, De Risio L, Muñana KR, Wosar MA, Skeen, TM, Sharp, NJ, *et al.* Development of a functional scoring system in dogs with acute spinal cord injuries. *Am J Vet Res.* **2001**, 62,10, 1624-8; doi: 10.2460/ajvr.2001.62.1624.
44. Martins, Â.; Cardoso, A.; Cruz, R.; Gouveia, D.; Pina, R.; Moisés, M. *et al.* Functional neurorehabilitation scale for dogs with thoracolumbar spinal cord injury without deep pain sensation. **2018**. Presented in 31st Annual Symposium of the ESVN-ECVN.
45. Dietz, V.; Muller, R. Degradation of neuronal function following a spinal cord injury: mechanisms and countermeasures. *Brain.* **2004**, 127,10, 2221-31; doi: 10.1093/brain/awh255.
46. Millis, D.L.; Ciuperca, I.A. Evidence for canine rehabilitation and physical therapy. *Vet Clin North Am Small Anim Pract.* **2015**, 45, 1-27; doi: 10.1016/j.cvsm.2014.09.001.
47. Maier, I.C.; Ichiyama, R.M.; Courtine, G.; Schnell, L.; Lavrov, I.; Edgerton, V.R.; *et al.* Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain.* **2009**, 132, 1426-1440; doi: 10.1093/brain/awp085.
48. Shah, P.K.; Sureddi, S.; Alam, M.; Zhong, H.; Roy, R.R.; Edgerton, V.R.; *et al.* Unique spatiotemporal neuromodulation of the lumbosacral circuitry shapes locomotor success after spinal cord injury. *J Neurotrauma.* **2016**, 33,1709-23; doi: 10.1089/neu.2015.4256.
49. de Leon, R.D.; Hodgson, J.A.; Roy, R.R.; Edgerton, V.R. Full weight-bearing hindlimb standing following stand training in the adult spinal cat. *J Neurophysiol.* **1998**, 80, 83-91; doi: 10.1152/jn.1998.80.1.83.
50. Zhang, S. X., Huang, F., Gates, M., White, J., & Holmberg, E. G. Tail nerve electrical stimulation induces body weight-supported stepping in rats with spinal cord injury. *J neurosci met.* **2010**, 187,183-189; doi:10.1016/j.jneumeth.2010.01.008.
51. Alluin, O.; Delivet-Mongrain, H.; Rossignol, S. Inducing hindlimb locomotor recovery in adult rat after complete thoracic spinal cord section using repeated treadmill training with perineal stimulation only. *J Neurophysiol.* **2015**, 114,3,1931-46; doi: 10.1152/jn.00416.2015.

52. Dietz, V.; Colombo, G.; Jensen, L.; Baumgartner, L. Locomotor capacity of spinal cord in paraplegic patients. *Ann Neurol.* 1995; 37,5, 574-82; doi: [10.1002/ana.410370506](https://doi.org/10.1002/ana.410370506).
53. Benito-Penalva, J.; Edwards, D.; Opisso, E.; Cortes, M.; Lopez-Blazquez, R.; Murillo, N.; *et al.* Gait training in human spinal cord injury using electromechanical systems: effect of device type and patient characteristic. *Arch Phys Med Rehabil.* **2012**, 93,3, 404-12; doi: [10.1016/j.apmr.2011.08.028](https://doi.org/10.1016/j.apmr.2011.08.028).
54. Meyns, P.; van de Crommert, H.W.; Rijken, H.; van, Kuppevelt, D.H.; Duysens, J. Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed. *Spinal Cord.* **2014**, 52, 887-893; doi: [10.1038/sc.2014.172](https://doi.org/10.1038/sc.2014.172).
55. Battistuzzo, C.; Callister, R.J.; Callister, R.; Galea, M. A systematic review of exercise training to promote locomotor recovery in animal models of spinal cord injury. *J Neurotrauma.* **2012**, 29,8, 1600-13; doi: [10.1089/neu.2011.2199](https://doi.org/10.1089/neu.2011.2199).
56. Cassilhas, R.C.; Tufik, S.; Mello, M.T. Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol Life Sci.* **2016**, 73,5, 975-83; doi: [10.1007/s00018-015-2102-0](https://doi.org/10.1007/s00018-015-2102-0).
57. Tillakaratne, N.J.; Duru, P.; Fujino, H.; Zhong, H.; Xiao, M.S.; Edgerton, V.R.; *et al.* Identification of interneurons activated at different inclines during treadmill locomotion in adult rats. *J Neurosci Res.* **2014**, 92, 1714-22; doi: [10.1002/jnr.23437](https://doi.org/10.1002/jnr.23437).
58. Shah, P.K.; Garcia-Alias, G.; Choe, J.; Gad, P.; Gerasimenko, Y.; Tillakaratne, N.; *et al.* Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain.* **2013**, 136, 3362-77; doi: [10.1093/brain/awt265](https://doi.org/10.1093/brain/awt265).
59. Levine, D.; Millis, D.L.; Flocker, J.; Macguire, L. Aquatic therapy. In: DL Millis & D Levine, Eds. *Canine Rehabilitation and Physical Therapy*, 2nd. Philadelphia: Saunders Elsevier. **2014**, p.526-542
60. Sims, C.; Waldron, R.; Marcellin-Little, D.J. Rehabilitation and physical therapy for the neurologic veterinary patient. *Vet Clin North Am Small Anim Pract.* **2015**; 45, 123-43; doi: [10.1016/j.cvsm.2014.09.007](https://doi.org/10.1016/j.cvsm.2014.09.007).
61. Engesser-Cesar, C.; Ichiyama, R.M.; Nefas, A.L.; Hill, M.A.; Edgerton, V.R.; Cotman, C.W.; *et al.* Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. *Eur J Neurosci.* **2007**, 25,7, 1931-9; doi: [10.1111/j.1460-9568.2007.05469.x](https://doi.org/10.1111/j.1460-9568.2007.05469.x).
62. Leech, K.A.; Kinnaird, C.R.; Holleran, C.L.; Kahn, J.; Hornby, T.G. Effects of locomotor exercise intensity on gait performance in individuals with incomplete spinal cord injury. *Phys Ther.* **2016**, 96,12, 1919-1929; doi: [10.2522/ptj.20150646](https://doi.org/10.2522/ptj.20150646).
63. Sherman & Olby. Nursing and Rehabilitation of the neurological patient. In: SR Platt; NJ Olby, editors. *Canine and Feline Neurology* 3rd Ed. England: British Small Animal Veterinary Association. **2014**. p. 394- 407
64. Levine, D.; Bockstahler, B. Electrical Stimulation. In: DL Millis & D Levine, editors. *Canine Rehabilitation and Physical Therapy* 2nd. Philadelphia: Elsevier Saunders. **2014**. p. 342-358
65. Hady, L.L.; Schwarz, P.D.; Recovery times for dogs undergoing thoracolumbar hemilaminectomy with fenestration and physical rehabilitation: a review of 113 cases. *JVMAH.* **2015**; 7:8: 278-289
66. Kralj, A.; Bajd, T.; Turk, R.; Krajnik, J.; Benko, H. Gait restoration in paraplegic patients: a feasibility demonstration using multichannel surface electrode FES. *J Rehabil R D.* **1983**; 20,1, 3-20; PMID: 6887064
67. Holsheimer, J. Computer modelling of spinal cord stimulation and its contribution to therapeutic efficacy. *Spinal Cord.* **1998**; 36,8, 531-40; doi: [10.1038/sj.sc.3100717](https://doi.org/10.1038/sj.sc.3100717).
68. Hamid, S.; Hayek, R. Role of electrical stimulation for rehabilitation and regeneration after spinal cord injury: an overview. *Eur Spine J.* **2008**, 17, 1256-69; doi: [10.1007/s00586-008-0729-3](https://doi.org/10.1007/s00586-008-0729-3).
69. Field-Fote, E.C.; Lindley, S.D.; Sherman, A.L. Locomotor training approaches for individuals with spinal cord injury: a preliminar report of walking-related outcomes. *J Neurol Phys Ther.* **2005**, 29,3, 127-37; doi: [10.1097/01.npt.0000282245.31158.09](https://doi.org/10.1097/01.npt.0000282245.31158.09).
70. Kapadia, N.; Masani, K.; Craven, B.C.; Giangregorio, L.M.; Hitzig, S.L.; Richards, K.; *et al.* A randomized trial of functional electrical stimulation for walking in incomplete spinal cord injury:

- effects on walking competency. *J Spinal Cord Med.* **2014**, 37, 511-524; doi: 10.1179/2045772314Y.0000000263.
71. Postans, N.J.; Hasler, J.P.; Granat, M.H.; Maxwell, D.J. Functional electric stimulation to augment partial weight-bearing supported treadmill training for patients with acute incomplete spinal cord injury: a pilot study. *Arch Phys Med Rehabil.* **2004**, 85, 604-10; doi: 10.1016/j.apmr.2003.08.083.
 72. Nooijen, C.F.; Hoeve, N, Field-Fote EC. Gait quality is improved by locomotor training in individuals with SCI regardless of training approach. *J Neuroeng Rehabil.* **2009**, 6,36: 1-11; doi: 10.1186/1743-0003-6-36.
 73. van Hedel, H.J.; Dietz, V. Rehabilitation of locomotion after spinal cord injury. *Restor Neurol Neurosci.* **2010**, 28, 123-34; doi: 10.3233/RNN-2010-0508.
 74. Rath, M.; Vette, A.H.; Ramasubramaniam, S.; Kun, L.; Burdick, J.; Edgerton, V.R.; *et al.* Trunk stability enabled by non-invasive spinal electrical stimulation after spinal cord injury. *J Neurotrauma.* **2018**, 35,1-44; doi: 10.1089/neu.2017.5584.
 75. Sayenko, D.G.; Atkinson, D.A.; Floyd, T.C.; Gorodnichev, R.M.; Moshonkina, T.R.; Harkema, S.J.; *et al.* Effects of paired transcutaneous electrical stimulation delivered at single and dual sites over lumbosacral spinal cord. *Neurosci Lett.* **2015**, 609, 229-234; doi: 10.1016/j.neulet.2015.10.005.
 76. Sayenko, D. G., Atkinson, D. A., Mink, A. M., Gurley, K. M., Edgerton, V. R., Harkema, S. J., & Gerasimenko, Y. P. Vestibulospinal and Corticospinal Modulation of Lumbosacral Network Excitability in Human Subjects. *Frontiers in physiology.* **2018**, 9, 1746; doi: 10.3389/fphys.2018.01746.
 77. Gerasimenko, Y.; Sayenko, D.; Gad, P.; Kozesnik, J.; Moshonkina, T.; Grishin.; *et al.* Electrical spinal stimulation, and imaging of lower limb movements to modulate brain-spinal connectomes that control locomotor-like behavior. *Front Physiol.* **2018**, 9,1196; doi: 10.3389/fphys.2018.01196.
 78. Ladenbauer, J.; Minassian, K.; Hofstoetter, U.S.; Dimitrijevic, M.R.; Rattay, F. Stimulation of the human lumbar spinal cord with implanted and surface electrodes: a computer stimulation study. *IEEE Trans Neural Syst Rehabil Eng.* **2010**, 18,6, 637-645; doi: 10.1109/TNSRE.2010.2054112.
 79. Angeli, C.; Edgerton, V.; Gerasimenko, Y.; Harkema, S. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain.* **2014**, 137,5, 1394-409; doi: 10.1093/brain/awu038.
 80. Hofstoetter, U.S.; Krenn, M.; Danner, S.M.; Hofer, C.; Kern, H.; McKay, W.B.; *et al.* Augmentation of voluntary locomotor activity by transcutaneous spinal cord stimulation in motor-incomplete spinal cord-injured individuals. *Artif Organs.* **2015**, 39,10, 176-86; doi: 10.1111/aor.12615.
 81. Lim, J.H.; Muguet-Chanoit, A.C.; Smith, D.T.; Laber, E.; Olby, N.J. Potassium channel antagonists 4-aminopyridine and the t-butyl carbamate derivative of 4-aminopyridine improve hind limb function in chronically non-ambulatory dogs; a blinded, placebo-controlled trial. *PLoS One.* **2014**, 9,12, 1-19; doi: 10.1371/journal.pone.0116139.
 82. Savin, Z.; Lejbkiewicz, I.; Glass-Marmor, L.; Lavi, I.; Rosenblum, S.; Miller, A. Effect of Fampridine-PR (prolonged released 4-aminopyridine) on the manual functions of patients with Multiple Sclerosis. *J Neurol Sci.* **2016**, 360, 102–109; doi: 10.1016/j.jns.2015.11.035.
 83. Tseng, K.C.; Li, H.; Clark, A.; Sundem, L.; Zuscik, M.; Noble, M.; *et al.* 4- Aminopyridine promotes functional recovery and remyelination in acute peripheral nerve injury. *EMBO Mol Med.* **2016**, 8,12,1409–1420; doi: 10.15252/emmm.201506035.
 84. Zörner, B.; Filli, L.; Reuter, K.; Kapitza, S.; Lorincz, L.; Sutter, T.; *et al.* Prolonged-release fampridine in multiple sclerosis: Improved ambulation effected by changes in walking pattern. *Mult Scler.* **2016**, 22,11, 1463–1475; doi: 10.1177/1352458515622695.
 85. Martins, Â.; Ferreira, A. Neuroreabilitação funcional em lesões medulares. In: RS Lopes & R Diniz, Editors. *Fisiatria em pequenos animais* 1st Ed. São Paulo: Editora Inteligente; **2018**. p. 287–298.
 86. Bennaim, M.; Porato, M.; Jarleton, A.; Hamon, M.; Carroll, J.; Gommeren, K.; *et al.* Preliminary evaluation of the effects of photobiomodulation therapy and physical rehabilitation on early

- postoperative recovery of dogs undergoing hemilaminectomy for treatment of thoracolumbar intervertebral disk disease. *Am J Vet Res.* **2017**, 78,2, 195-206; doi: 10.2460/ajvr.78.2.195.
87. Shik, M.L.; Orlovsky, G.N.; Neurophysiology of locomotor automatism. *Physiol Rev.* **1976**, 56,3, 465-501; doi: 10.1152/physrev.1976.56.3.465.
 88. Schulz, K.F.; Grimes, D.A. Sample size calculations in randomized trials: mandatory and mystical. *Lancet.* **2005**, 365,1348,53; doi: 10.1016/S0140-6736(05)61034-3.
 89. Martins, A.; Gouveia, D.; Cardoso, A.; Viegas, I. Ferreira, A. Intensive Neurorehabilitation of 84 paraplegic dogs affected by spinal cord injury. **2021**. Poster presentation in 60st International Spinal Cord Society Annual Scientific Meeting (ISCOS).
 90. Burns, A.S.; Marino, R.J.; Flanders, A.E.; Flett, H. Clinical diagnosis and prognosis following spinal cord injury. *Handb. Clin. Neurol.* **2012**, 109, 47-62; doi: 10.1016/B978-0-444-52137-8.00003-6.
 91. Burns, A.S.; Marino, R.J.; Kalsi-Ryan, S.; Middleton, J.W.; Tetreault, L.A.; Dettori, J.R.; Mihalovich, K.E.; Fehlings, M.G.; Type and timing of rehabilitation following acute and subacute spinal cord injury: a systemic review. *Glob. Spine J.* 2017, 7,175-194; doi: 10.1177/2192568217703084.
 92. Martins, A.; Silva, C.M.; Gouveia, D.; Cardoso, A.; Coelho, T.; Gamboa, O.; Marcelino, E.; Ferreira, A. Spinal locomotion in cats following spinal cord injury: a prospective study. *Animals.* **2021a**, 11, 1-13; doi:10.3390/ani11071994.
 93. Jeffery, N.D.; Mankin, J.M.; Ito, D.; Boudreau, C.E.; Kerwin, S.C.; Levine, J.M.; Krasnow, M.S.; Andruzzi, M.N.; Alcott, C.J.; Granger, N.; Extended durotomy to treat severe spinal cord injury after acute thoracolumbar disc herniation in dogs. *Vet Surg.* **2020**, 1-10; doi: 10.1111/vsu.13423.
 94. Whiteneck, G.; Gassaway, J.; Dijkers, M.P.; Heinemann, A.W.; Kreider, S.E. Relationship of patient characteristics and rehabilitation services to outcomes following spinal cord injury: the SCIREhab Project. *J Spinal Cord Med.* **2012**, 35, 484-502; doi: 10.1179/2045772312Y.0000000057.
 95. Kornhaber, R.; Mclean, L.; Betihavas, V.; Cleary, M. Resilience and the rehabilitation of adult spinal cord injury survivors: a qualitative systematic review. *J Adv Nurs.* **2017**, 72, 980-911; doi: 10.1111/jan.13396.
 96. Zidan, N.; Sims, C.; Fenn, J.; Williams, K.; Griffith, E.; Early, P.J.; Mariani, C.L.; Munana, K.R.; Guevar, J.; Olby, N.J. A randomized, blinded, prospective clinical trial of postoperative rehabilitation in dogs after surgical decompression of acute thoracolumbar intervertebral disc herniation. *J Vet Intern Med*, **2018**, 32, 1133-1144; doi: 10.1111/jvim.15086.
 97. Aikawa T, Fujita H, Kanazono S, Shibata M, Yoshigae Y. Long-term neurologic outcome of hemilaminectomy and disk fenestration for treatment of dogs with thoracolumbar intervertebral disk herniation:831 cases (2000-2007). *J Am Vet Med Assoc.* **2012**, 241,12,1617-26; doi:
 98. Davis, G.J.; Brown, D.C. Prognostic indicators for time to ambulation after surgical decompression in nonambulatory dogs with acute thoracolumbar disk extrusions: 112 cases. *Vet Surg.* **2002**, 31, 513-518; doi: 10.1053/jvet.2002.36015.
 99. Smith, R.R.; Brown, E.H.; Shum-Siu, A.; Whelan, A.; Burke, D.A.; Benton, R.L.; Magnuson, D.S. Swim training initiated acutely after spinal cord injury is ineffective and induces extravasation in and around the epicenter. *J Neurotrauma.* **2009**, 26, 1017-1027; doi: 10.1089/neu.2008-0829.
 100. Detloff, M.R.; Smith, E.J.; Molina, D.Q.; Ganzer, P.D.; Houle, J.D. Acute exercise prevents the development of neuropathic pain and the sprouting of non-peptidergic (GDNF-and-artemin-responsive) c-fibers after spinal cord injury. *Exp Neurol.* **2014**, 255, 38-48; doi: 10.1016/j.expneurol.2014.02.013.
 101. Fouad, K.; Tetzlaff, W. Rehabilitative training and plasticity following spinal cord injury. *Exp Neurol.* **2012**, 235, 91-99; doi: 10.1016/j.expneurol.2011.02.009.
 102. Martins, A.; Gouveia, D.; Cardoso, A.; Gamboa, Ó.; Millis, D.; Ferreira, A. Nervous system modulation through electrical stimulation in companion animals. *Acta Vet Scand.* **2021b**, 63,22, 1-12; doi: 10.1186/s13028-021-00585-z.

103. Mushahwar, V.K.; Jacobs, P.L.; Normann, R.A.; Triolo, R.J.; Kleitman, N. New functional electrical stimulation approaches to standing and walking. *J Neural Eng.* **2007**, *4*, 181-197; doi: 10.1088/1741-2560/4/3/S05.
104. Martins, A.; Gouveia, D.; Cardoso, A.; Viegas, I.; Gamboa, Ó.; Ferreira, A. A comparison between body weight-supported treadmill training and conventional over-ground training in dogs with incomplete spinal cord injury. *Front Vet Sci.* **2021c**, *8*, 1-14; doi: 10.3389/fvets.2021.597949.
105. Olby, N.J.; Tipold, A., eds. Canine intervertebral disc disease: The current state of Knowledge. Lausanne: Frontiers Media SA. *Front Vet Sci.* **2021**. doi: 10.3389/fvets.2021.656764.
106. Moore, S.A.; Tipold, A.; Olby, N.J.; Stein, V.; Granger, N.; and CANSORT-SCI. Current approaches to the management of acute thoracolumbar disc extrusion in dogs. *Front Vet Sci.* **2020**, *7*, 41-55; doi: 10.3389/fvets.2020.00610.
107. Olby, N.J.; Levine, J.; Harris, T.; Munana, K.; Skeen, T.; Sharp, N. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord: 87 cases (1996-2001). *J Am Vet Med Assoc.* **2003**, *222*, 762-769; doi: 10.2460/javma.2003.222.762.
108. Gallucci, A.; Dragone, L.; Menchetti, M.; Gagliardo, T.; Pietra, M.; Cardinali, M., et al. Acquisition of involuntary spinal locomotion (spinal walking) in dogs with irreversible thoracolumbar spinal cord lesion: 81 dogs. *J Vet Intern Med.* **2017**, *31*, 492-497; doi: 10.1111/jvim.14651.
109. Hu, H.Z.; Jeffery, N.D.; Granger, N. Somatosensory and motor evoked potentials in dogs with chronic severe thoracolumbar spinal cord injury. *Vet J.* **2018**, *237*, 49-54; doi: 10.1016/j.tvjl.2018.05.007.
110. Lewis, M.J.; Cohen, E.B.; Olby, N.J. Magnetic resonance imaging features of dogs with incomplete recovery after acute, severe spinal cord injury. *Spinal Cord.* **2018**, *56*, 133-141; doi: 10.1038/s41393-017-0004-8.
111. Edgerton, V.R.; Tillakaratne, N.; Bigbee, A.J.; de Leon, R.D.; Roy, R.R.; Plasticity of the spinal neural circuitry after injury. *Ann Rev Neurosci.* **2004**, *27*, 145-167; doi: 10.1002/cphy.c130056.
112. AuYong, N.; Lu, D.C. Neuromodulation of the lumbar spinal locomotor circuit. *Neurosurg Clin N Am.* **2014**, *25*, 15-23; doi: 10.1016/j.nec.2013.08.007.
113. Courtine, G.; Sofroniew, V.R. Spinal cord repair: advances in biology and technology. *Nat Med.* **2019**, *25*, 898-908; doi: 10.1038/s41393-017-0004-8.
114. Branco, É.; Alves, J.G.R.; Pinheiro, L.L. Coutinho, L.N.; Gomes, C.R.M.; Galvão, G.R., et al. Can paraplegia by disruption of the spinal cord tissue be reversed? The signs of a new perspective. *Anat Rec.* **2019**, *303*, 1812-1820; doi: 10.1002/ar.24262.
115. Lewis, M.J.; Granger, N.; Jeffery, N.D.; and CANSORT-SCI. Emerging and adjunctive therapies for spinal cord injury following acute canine intervertebral disc herniation. *Front Vet Sci.* **2020**, *7*, 73-85; doi: 10.3389/fvets.2020.579933.
116. Coates, J.R. Intervertebral disk disease. *Vet Clin North Am Small Anim Pract.* **2000**, *30*, 77-110; doi: 10.1016/s0195-5616(00)50004-7.
117. Moore, S.A.; Early, P.J.; Hettlich, B.F. Practice patterns in the management of acute intervertebral disc herniation in dogs. *J Small anim Pract.* **2016**, *57*, 409-415; doi: 10.1111/jsap.12496.
118. Dewey, C.W.; Costa, R.C. In Practical guide to canine and feline neurology. **2015**. Ames, IA: John Wiley & Sons.
119. Takeoka, A.; Vollenweider, I.; Courtine G.; Arber, S.; Muscle spindle feedback directs locomotor recovery and circuit reorganization after spinal cord injury. *Cell.* **2014**, *159*, 1626-1639; doi: 10.1016/j.cell.2014.11.019.
120. Rossignol, S.; Martinez, M.; Escalona, M.; Kundu, A.; Delivet-Mongrain, H.; Alluin O. et al. The beneficial effects of locomotor training after various types of spinal lesions in cats and rats. *Prog Brain Res.* **2015**, *218*, 173-198; doi: 10.1016/bs.pbr.2014.12.009.

121. Jeong, I.S.; Piao, Z.; Rahman, M.M.; Kim, S.; Kim, N.S. Canine thoracolumbar intervertebral disk herniation and rehabilitation therapy after surgical decompression: a retrospective study. *J Adv Vet Anim Res.* **2019**, *6*, 394-402; doi: 10.5455/javar.2019.f359.
122. Yu, P.; Zhang, W.; Liu, Y.; Sheng, C.; So, K.F.; Zhou, L. et al. The effects and potential mechanisms of locomotor training on improvements of functional recovery after spinal cord injury. *Int Rev Neurobiol.* **2019**, *147*, 199-217; doi: 10.1016/bs.irn.2019.08.003.
123. Aravind, N.; Harvey, L.A.; Glinsky, J.V. Physiotherapy interventions for increasing muscke strength in people with spinal cord injuries: a systematic review. *Spinal Cord.* **2019**, *57*, 449-460; doi: 10.1038/s41393-019-0242-z.
124. Griffiths, I.R.; The extensive myelopathy of intervertebral disc protrusions in dogs ("the ascending syndrome"). *J Small Anim Pract.* **1972**, *13*, 425-438; doi: 10.1111/j.1748-5827.1972.tb06870.x.
125. Jeffery, N.D.; Barker, A.K.; Hu, H.Z. et al. Factors associated with recovery from psaraplegia in dogs with loss of pain perception in the pelvic limbs following intervertebral disk herniation. *J Am Vet Med Assoc.* **2016**, *248*, 386-394; doi: 10.2460/javma.248.4.386.
126. Olby, N.J.; Muguet-Chanoit, A.C.; Lim, J.H., et al. A placebo-controlled, prospective, randomized clinical trial of polyethylene glycol and methylprednisolone sodium succinate in dogs with intervertebral disk herniation. *J Vet Intern Med.* **2016**, *30*, 206-214; doi: 10.1111/jvim.13657.
127. Olby, N.J.; Lim, J.H.; Wagner, N. et al. Time course and prognostic value of serum GFAP, pNFH, and S100 β concentrations in dogs with complete spinal cord injury because of intervertebral disc extrusion. *J Vet Intern Med.* **2019**, *33*, 726-734; doi: 10.1111/jvim.15439.
128. Blight, A.R.; Toombs, J.P.; Bauer, M.S.; Widmer, W.R. the effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: a phase i clinical trial. *J Neurotrauma.* **1991**, *8*, 103-19; doi:10.1089/neu.1991.8.103.
129. Blight, A.R. Effect of 4-aminopyridine on axonal conduction-block in chronic spinal cord injury. *Brain Res Bull.* **1989**, *22*, 46-52; doi: 10.1016/0361-9230(89)90126-3.
130. Smith, K.J.; Felts, P.A.; John G.R. Effects of 4-aminopyridine on demyelinated axons, synapses and muscle tension. *Brain.* **2000**, *123*, 171-84; doi: 10.1093/brain/123.1.171.
131. Olby, N.J.; da Costa, R.C.; Levine, J.M.; Stein, V.M. and CANSORT-SCI. Prognostic factors in canine acute intervertebral disc disease. *Front Vet Sci.* **2020**, *7*, 144-157; doi: 10.3389/fvets.2020.596059.

3. ARTICLE 4

Animals (2021), 11(2442):1-21

**Functional Neurorehabilitation in Dogs with an Incomplete Recovery 3
Months following Intervertebral Disc Surgery: A Case Series.**

Functional Neurorehabilitation in Dogs with an Incomplete Recovery 3 Months Following Intervertebral Disc Surgery: A Case Series

Ângela Martins ^{1,2,3,4,*}, Débora Gouveia ^{2,4}, Ana Cardoso ², Carla Carvalho ², Cátia Silva ², Tiago Coelho ², Óscar Gamboa ⁵ and António Ferreira ^{3,5}

¹ Faculty of Veterinary Medicine, Lusófona University, Campo Grande, 1300-477 Lisboa, Portugal

² Animal Rehabilitation Center, Arrábida Veterinary Hospital, Azeitão, 2925-583 Setúbal, Portugal; deborahisabel@msn.com (D.G.); anacardosocatarina@gmail.com (A.C.); mv.carla.c@gmail.com (C.C.); Catiasilva@outlook.com (C.S.); tiagoccoelho@netcabo.pt (T.C.)

³ CIISA – Centro Interdisciplinar-Investigação em Saúde Animal, Faculdade de Medicina Veterinária, Av. Universidade Técnica de Lisboa, 1300-477 Lisboa, Portugal; aferreira@fmv.ulisboa.pt

⁴ Superior School of Health, Protection and Animal Welfare, Polytechnic Institute of Lusophony, Campo Grande, 1300-477 Lisboa, Portugal

⁵ Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisboa, Portugal; ogamboa@fmv.ulisboa.pt

* Correspondence: vetarrabida.lda@gmail.com

Simple Summary: A non-invasive neurorehabilitation multimodal protocol (NRMP) may be applicable to chronic T3-L3 dogs 3 months after undergoing surgery for acute Intervertebral Disc Disease (IVDD) Hansen type I; this protocol has been shown to be safe, feasible, and potentially effective at improving ambulation in both open field score (OFS) 0 and OFS 1 dogs. The specific sample population criteria limit the number of dogs included, mainly due to owners withdrawing over time. Thus, the present case series study aimed to demonstrate that an NRMP could contribute to a functional treatment possibly based on synaptic and anatomic reorganization of the spinal cord.

Abstract: This case series study aimed to evaluate the safety, feasibility, and positive outcome of the neurorehabilitation multimodal protocol (NRMP) in 16 chronic post-surgical IVDD Hansen type I dogs, with OFS 0/DPP– (n = 9) and OFS 1/DPP+ (n = 7). All were enrolled in the NRMP for a maximum of 90 days and were clinically discharged after achieving ambulation. The NRMP was based on locomotor training, functional electrical stimulation, transcutaneous electrical spinal cord stimulation, and 4-aminopyridine (4-AP) pharmacological management. In the Deep Pain Perception (DPP)+ dogs, 100% recovered ambulation within a mean period of 47 days, reaching OFS ≥ 11, which suggests that a longer period of time is needed for recovery. At follow-up, all dogs presented a positive evolution with voluntary micturition. Of the DPP– dogs admitted, all achieved a flexion/extension locomotor pattern within 30 days, and after starting the 4-AP, two dogs were discharged at outcome day 45, with 78% obtaining Spinal Reflex Locomotion (SRL) and automatic micturition within a mean period of 62 days. At follow-up, all dogs maintained their neurological status. After the NRMP, ambulatory status was achieved in 88% (14/16) of dogs, without concurrent events. Thus, an NRMP may be an important therapeutic option to reduce the need for euthanasia in the clinical setting.

Keywords: spinal cord injury; locomotor training; functional electrical stimulation; transcutaneous electrical SC stimulation; chronic dogs; 4-aminopyridine; neurorehabilitation

1. Introduction

Spinal cord injury (SCI) is a commonly observed event in dogs following spinal trauma and Intervertebral Disc Disease (IVDD). Clinically, the SCI can be considered complete/incomplete if Deep Pain Perception (DPP) is absent (DPP-) or incomplete if DPP is present (DPP+) [1,2].

After IVDD Hansen type I, the spinal cord is able to sustain variable degrees of contusion and compression, depending on the extrusion velocity and amount of extruded disc material [3]. DPP- dogs can regain DPP after surgical decompression and thus return to a clinically ambulatory status [1,4,5]. Dogs without DPP recovery after surgery can become ambulatory through the reorganization of the spinal cord's intrinsic circuit and central pattern generators (CPG) and with weight-bearing ability due to recovery of the residually intact descending axons traversing the lesion epicenter [6–8]. The absence of DPP is considered a negative prognostic factor for ambulation recovery [9]. However, if 10% of the axons in the malacic zone are preserved, there is a possibility of recovery [10].

More severely affected dogs can benefit from controlled activity programs in the field of neurorehabilitation. Functional neurorehabilitation belongs to physical medicine and rehabilitation in the area of restorative neurology and can be implemented in veterinary medicine [11], based on the evidence of signal transmission through the lesion, both caudal and rostral, as detected by electromyography for the motor tracts and evoked potentials for the sensory pathways [12–14].

Functional neurorehabilitation (FNR) protocols are based on repetitive task-specific therapy to optimize the spinal locomotor performance [15–17] and can stimulate and promote new connection formation, proving the spinal cord's ability to interpret complex sensory inputs for posture and locomotion [8,18]. Therefore, FNR provides certain advantages in the recovery of muscle and motor functions in chronic dogs [19].

Dogs with chronic neurologic diseases can benefit from physical and rehabilitation support, which is common in human medicine, as well as in dogs and cats [11]. After SCI, both humans and cats can demonstrate spontaneous recovery, which can be explained by the similar cellular mechanisms responsible for spontaneous remodeling. Therefore, regarding the neurological clinical signs' evolution, it is difficult to determine whether recovery is due to rehabilitation treatment or spontaneous recovery. Over time, in chronic dogs, it is possible that the neurological evolution could be mainly due to the neurorehabilitation protocol implemented [20–22].

Intensive FNR protocols modify motor interneuron intrinsic circuitry excitability and CPG through the stimulation of the cutaneous afferent pathway [23,24] and the amplitude of the motoneuron synaptic inputs, essentially from the axons descending in the ventrolateral funiculus (VLF) from the reticular formation [25], which involves the activation of the reticulospinal pathways [26–29]. Thus, increasing locomotor exercise intensity with repeated training can lead to significant changes in peak gait speed, stride length, and cadence [30]. Changes that promote the

functionality of the neural network in animals with complete or incomplete SCI are entirely related to sensorimotor rehabilitation [7,31–35].

Quadrupedal step locomotor training could promote the activation of some long ascending and descending propriospinal interneurons [24,25] and improve the recruitment of muscles, mainly the flexors/extensors and adductors/abductors, which are very important for coordination and sensorimotor rehabilitation.

Pelvic limb muscle strength in SCI individuals can be improved by functional electrical stimulation (FES). FES includes efferent and afferent nerve stimulation, neuromuscular and muscle stimulation [12,36], and, depending on the cathode and anode orientation, has the potential to lead to regeneration and the activation of new connections [37,38].

Intralimb hip–knee coordination may be promoted by the combination of FES and locomotor training [37–39]. In chronic patients, electrical stimulation protocols with FES and Transcutaneous Electrical Spinal Cord Stimulation (TESCS) could be applied as a possible therapeutic neurorehabilitation tool for IVDD [40–43]. TESCS can lead to synergistic and interactive multi-segmental effects, converging with the ascending sensorial pathways and the descending motor pathways, that might be important in activating the spinal network [44]. Moreover, it is implied that this modality may stimulate new intraspinal circuits and persistent residual spinal circuits [12,45–50].

There have been investigations regarding pharmacological management with 4-AP in thoracolumbar chronic SCI dogs to improve neurological deficits [51–53]. The 4-AP is a potassium channel antagonist that could be used to block exposed fast-gated potassium channels in demyelinated axons and increase synaptic transmission at the pre-synaptic level [53]. Thus, it has been proposed as a possible intervention given its effect on the neural synaptic reorganization of the motor interneuron circuit and CPG—the spinal network.

Chronic dogs are usually not modulated considering peripheral spinal reflexes, manifesting a non-coordinated and non-synchronized flexion/extension locomotor pattern with clonic reflexes. It is believed that 4-AP administration may enhance motor neuron pool excitability in dogs with depressed activity; however, studies with larger cohorts are needed in order to determine how this drug could be useful [54].

Today, it is well known that the Central Nervous System (CNS) contains a complex network of neural connections, which are supported and modified by a population of glial cells, including astrocytes, oligodendrocytes, and microglia, which play an important role in neural plasticity. The astrocytes are the major components of the fibroglial scar that develops in the injury site after SCI [55]. This glial scar attempts to stabilize the injured parenchyma by reestablishing its physical and chemical integrity through different mechanisms, such as closing the blood–brain barrier, decreasing the infiltration of non-CNS cells, limiting possible infection [56], and playing an important pro-regenerative role [57].

Furthermore, the fibroglial scar, which contains a rich mixture of cells and extracellular matrix [58], also inhibits axonal growth and remyelination.

Despite this disparity regarding the glial scar, there is increasing evidence that suggests that it can support CNS repair, essentially via astrocytes, NG2 glia, and microglia.

Historically, synaptic plasticity in pre-existent pathways and the formation of new circuits—in other words, synaptic and anatomical neuroplasticity, respectively—can promote the sprouting of lesioned fibers, contributing to regeneration [59]. Thus, the induction of an activity-dependent form of spinal cord plasticity may play an important role in the development of effective new rehabilitation modalities to be applied after SCI [60].

This case series clinical study intends to verify whether a neurorehabilitation multimodal protocol (NRMP) is a safe and feasible therapeutic protocol to be applied in SCI chronic dogs and to assess whether this NRMP allows a positive neurological evolution until ambulation and within a time period that is clinically acceptable.

2. Materials and Methods

This study was conducted between March 2011 and January 2021 at Arrábida Veterinary Hospital and Rehabilitation Centre, (HVA/CRAA) after obtaining approval from the Lisbon Veterinary Medicine Faculty Ethics Committee and after obtaining the owners' consent.

2.1. Participants

Participants were included if they were chronic post-surgical IVDD Hansen type I dogs and more than 3 months had passed since their surgery. All had to be of chondrodystrophic breed, aged less than 7 years old, with a weight of less than 15 kg, with neuro-location at T10-L3, and classified at admission as paraplegic with DPP (OFS 1/ DPP+) or without DPP (OFS 0/DPP-). All DPP- dogs with voluntary movement of the tail, pain perception on the perineal region, scrotum, or vulva, and pain perception at the tip and base of the tail were excluded.

The dogs were evaluated and the findings were documented (Canon EOS Rebel T6 1300D camera) by two Certified Canine Rehabilitation Practitioners (CCRPs) from the University of Tennessee and a non-CCRP neurologist from Lisbon University.

The breed distribution was diverse: five (31%) Dachshunds, five (31%) French bulldogs, two (13%) Shi Tzus, two (13%) Pugs, one (6%) Pinscher, and one (6%) Pekingese. They had a mean age of 3.63 years (median of 4) and a mean weight of 7 kg (median of 7), and 56% (9/16) were males and 44% (7/16) were females. The most frequent neuro-location was L1–L2, for 37% (6/16) of dogs. Nine dogs were DPP- (56%) and seven dogs were DPP+ (44%).

2.2. Interventions

The dogs underwent a Home Work Plan (HWP) after the hemilaminectomy for Hansen type I IVDD, for 3 months, and performed three different types of protocols as described in Table 1, with no positive evolution for at least 6–8 weeks.

Table 1. Home Work Plan: Different types of protocols.

Protocol	Description	Frequency
A	30 min land treadmill	3 days/week
B	1 h leash ground walk	4 days/week
C	30 min underwater treadmill	1–2 days/week

All were enrolled in the NRMP for a maximum of 90 days and were clinically discharged after achieving ambulation. Ambulation was

considered when dogs showed “the ability to rise and take at least 10 consecutive weight-bearing steps unassisted without falling” [6]. Regarding DPP- dogs, they had to be able to perform curves without falling, with a wider support base and automatic micturition.

After admission, the dogs were redirected to a neurorehabilitation consultation and evaluated based on their history and neurorehabilitation examination (mental status, posture, OFS gait, postural reactions, peripheral spinal reflexes, cutaneous trunci muscle reflex, spinal cord palpation, and pain perception).

For DPP evaluation, 12 cm Halsted forceps were used to test the response to a noxious stimulus applied to the bone of both medial/lateral digits of the pelvic limbs, always in a controlled environment, regarding movement and noise. Moreover, to perform an accurate pain examination, we also tested the perineal region and the tip and base of the tail.

The flexion/extension locomotor pattern that was also monitored comprises interneurons interposed between the input and output. Thus, these interneurons allow divergence and promote input distribution to a wide population of output neurons. One type of input can then stimulate both agonist muscle contraction and antagonist muscle relaxation [61]. In SCI, a disruption in descending motor pathways, particularly in the lateral funiculus and the opposing pathways in the ventral funiculus, exerts a powerful excitatory influence on the alpha and gamma motoneurons of the extensor muscles [62]. A local spinal reflex and the upper motor neurons influence the activity of the alpha and gamma motoneurons, which allows the appearance of the crossed extensor reflex. Therefore, a normal dog, in the lateral recumbency position, should not exhibit the crossed extensor reflex, which is manifested as an ipsilateral flexion and contralateral extension reflex that helps to maintain posture when the dog withdraws one of its limbs, such as in stepping.

2.2.1. Neurorehabilitation Multimodal Protocol

The dogs were then subjected to an NRMP. For DPP+ dogs, the protocol comprised a combination of locomotor training and electrical stimulation. Electrical stimulation was based on Functional Electrical Stimulation (FES) and Transcutaneous Electrical Spinal Cord Stimulation (TESCS). For DPP- dogs, the protocol implemented was the same as described, with the inclusion of pharmacological management with 4-aminopyridine (4-AP).

This pharmacological management was applied in DPP- dogs because they were considered to have a complete SCI with a poor prognosis.

- Locomotor training

The locomotor training began on the second day of admission after the dogs had become accustomed to the land treadmill. Dogs were adapted with the help of technicians in a calm environment.

The primary type of exercise was quadrupedal step training. For each training session, variables such as the walking speed and duration were increased and recorded, starting with 0.8 km/h (0.22 m/s) with a maximum of 1.9 km/h (0.53 m/s) [63–65] for 5 min (4–6 times/day, 6 days/week), to achieve 20 min (2–3 times/day, 6 days/week) [66]. The goal was to achieve 30–40 min (2–3 times/day, 5–6 days/week) [67], and the

land treadmill slope was elevated to 10° [68] to a maximum of 25° [69], with forelimb–hindlimb coordination (Table 2).

The underwater treadmill (UWTM) training was introduced in all dogs from the second day of admission, with a water temperature of 26 °C [70] for 5 min to a maximum of 1 h training per day (5 days/week), monitoring for signs of overtraining. The speed ranged from 1 km/h (0.28 m/s) to 3.5 km/h (0.97 m/s) [26,71]. The training session consisted of increasing speed and duration under the same rule: four sessions with good performance indicated a 10% improvement in both variables [72–74] (Table 3).

Table 2. Land treadmill locomotor training.

Week	Time Duration	Walking Speed	Frequency	Slope
1st–2nd	Up to 5–10 min	0.8–1.9 km/h	4–6 times/day 6 days/week	-
3rd–4th	Up to 20 min	2 km/h	2–4 times/day 6 days/week	-
5th–6th	Up to 30 min	2.2 km/h	2–3 times/day 6 days/week	-
7th–8th	Up to 40 min	2.5 km/h	2 times/day 6 days/week	5°
9th–10th	Up to 40 min	2.5 km/h	2 times/day 5 days/week	10°
11th–12th	Up to 40 min	2.5 km/h	1 time/day 5 days/week	25°

Table 3. Underwater treadmill locomotor training.

Week	Time Duration	Walking Speed	Slope
1st–2nd	Up to 5–10 min	1–1.2 km/h	-
3rd–4th	Up to 10–20 min	1.8–2 km/h	-
5th–6th	Up to 30 min	2–2.5 km/h	5°
7th–8th	Up to 40 min	2.8–3 km/h	5°
9th–10th	Up to 40 min	3–3.5 km/h	5°
11th–12th	Up to 60 min	3.5 km/h	10°

Frequency of underwater treadmill training: 5 days a week, once a day, always in the morning.

Locomotor training was always associated with active and assisted kinesiotherapy exercises, such as postural standing, flexor movements, bicycle movements, balance board, and different floor surfaces [75] (Table 4).

Table 4. Kinesiotherapy exercise training.

Week	Exercise	Time Duration	Frequency
1st–4th	Postural standing	Until 10 min	2–4 times/day
			6 days/week
	Flexor movements	10–20 repetitions	2–3 times/day
	Bicycle movements	5–10 repetitions	5 days/week
5th–8th	Dog with active postural standing	Different floors gait stimulation	3–5 min 2–3 times/day

Dog without active postural standing	Postural standing	10 min	5 days/week
	Different floors gait stimulation	3–5 min	
9th–12th	Different floors gait stimulation	5–10 min	2 times/day 5
	Balance board	5 min	days/week

All exercises were performed accordingly to dog's cardiorespiratory ability.

- Electrical Stimulation

The electrical stimulation protocol applied in all dogs was a combination of FES, aiming to achieve muscle contraction and neural connection, and TESCS to increase the descending pathway depolarization.

Functional Electrical Stimulation

A neuromodulation modality uses a short electrical pulse sequence to stimulate the lower motoneurons near the motor point region or by peripheral afferent stimulation, resulting in peripheral spinal reflexes [76,77]. This modality was performed in all dogs with superficial electrodes. One electrode was placed on the skin region corresponding to L7-S1 and the other electrode was close to the motor point of the hamstring muscles (biceps femoris, semitendinosus, and semimembranosus).

The current parameters were as follows: 60 Hz and 6–24 mA [78–81]; duty cycle: 1:4 ratio; ramp-up/down: between 2 and 4 s for ramp-up, 8 s on time, and 1–2 s for ramp down [82]. This was performed 2 to 3 times a day (5 days/week) and was discontinued as per neurological evolution (Figure 1).



Figure 1. Functional electrical stimulation. One electrode was placed at L7-S1 region and one close to the motor point of the hamstring muscles (biceps femoris, semitendinosus, and semimembranosus).

Transcutaneous electrical spinal cord stimulation

This is a non-invasive and non-painful rehabilitation therapeutic modality with the ability to neuromodulate the spinal cord's physiological status [83].

All dogs underwent TESCS three times a day (5 days/week), which was gradually discontinued when the flexion/extension locomotor pattern appeared; thus, the surface electrodes were placed in the paravertebral muscles (one electrode on L1-L2 and the other on L7-S1)

and a continuous current of 50 Hz and 2 mA was applied for 10 min (Figure 2A,B).



Figure 2. (A,B) Transcutaneous electrical spinal cord stimulation. One electrode placed on L1-L2 and the other one on L7-S1.

- Pharmacological management

After 4 weeks of the NRMP, dogs who displayed a flexion/extension locomotor pattern but were DPP- started pharmacological management in combination with the training protocol, after the owners' consent was obtained.

This pharmacological treatment was based on a potassium channel blocker, 4-AP [53,84–86], according to the following protocol: 0.3 mg/kg per os BID 3 days; 0.5 mg/kg BID 3 days; 0.7 mg/kg BID 3 days; 1.1 mg/kg BID for 21 days. In the event of side effects (e.g., vomiting, diarrhea, and seizures), dogs would have been immediately treated and withdrawn from the study; however, none of the dogs showed adverse effects in response to this medication that warranted withdrawal.

- Supportive care

All the dogs had neurogenic bladders; therefore, their bladders were manually expressed 3–4 times/day. Urine was monitored daily for odor and color changes, and in the case of suspected urinary tract infection (UTI), urine culture (by cystocentesis) and specific antibiotic treatment were performed. Only two dogs were UTI-positive and required antibiotic therapy with water consumption control. The dogs were maintained on a full-time hospitalization regimen and rested in soft beds. Feeding was performed three times/day (intake increase of 30%) and a hydric support of 100–120 mL/kg/day was provided after resistance training (training intended not to reach the maximum activity within an extended time period) that was alternated with fortification training (training intended to achieve the maximum activity within a short time period) according to the dog's needs. All were trained during the day, starting at 9:00 am and finishing at 7:00 pm, assisted only by veterinarians and veterinary nurses that were CCRPs.

2.2.2. Outcomes/Follow-Up

During full-time NRMP, the dogs were evaluated neurologically every 3 to 7 days in order to make necessary changes concerning exercise duration and intensity. This evaluation included the assessment of the flexor reflex, flexion/extension locomotor pattern, postural standing, and the OFS [87], including deep pain assessment. Thus, pain perception assessment was specifically evaluated in all dogs as described above.

Outcomes were performed at 15, 30, 45, 60, 75, and 90 days, and they were followed up after 8–10 days, 1 month, and 6 months by the same CCRP instructor/examiner, based on the neurorehabilitation examination criteria and OFS.

The primary outcome parameters of this study were the ambulatory status with an OFS ≥ 11 , which indicates ambulation for DPP+ dogs. Regarding DPP-, ambulatory dogs will show spinal reflex locomotion (SRL). DPP- dogs without ambulatory status will show no spinal reflex locomotion (NSRL). Dogs with NSRL demonstrated the flexion/extension pattern but without the ability to rise.

A secondary outcome was the pain perception assessment, evaluated on the tip and base of the tail and the perineum region (S1 and S2 dermatomes). Deep Pain Perception was assessed with 12 cm Halsted forceps in the lateral and medial digits of both pelvic limbs in a controlled environment regarding noise and the circulation of people.

The evolution of the 16 chronic dogs was recorded after HWP and NRMP implementation, as illustrated in the flow diagram, as well as at follow-up. All the dogs were medically discharged when they achieved ambulation, with subsequent assessment by long-term follow-up after 8–10 days, 1 month, and 6 months (Figure 3).

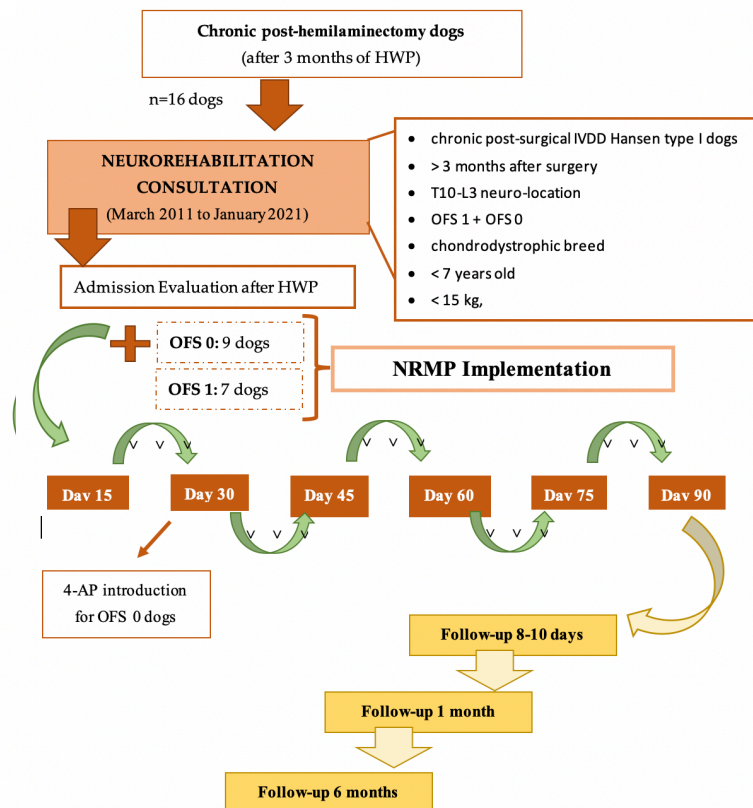


Figure 3. Flow diagram illustrating the study design of chronic paraplegic post-hemilaminectomy dogs. Abbreviations: OFS—open field score [64]; NRMP—neurorehabilitation multimodal protocol; HWP—Home Work Plan; SRL—Spinal Reflex Locomotion; NSRL—Non-Spinal Reflex Locomotion.

2.2.3. Statistical Analysis

Database and statistical analysis were performed using Microsoft Office Excel 2016® (Microsoft, Redmond, WA, USA). Categorical data were described as frequencies and proportions (with 95% confidence intervals). The collected data included breed, sex, age, weight, lesion neuro-location, DPP presence, OFS at admission and at each time point (15, 30, 45, 60, 75, and 90 days), ambulatory status, time when ambulation was achieved, time of medical discharge, follow-ups (8–10 days, 1 month, and 6 months), and the presence of concurrent events. Concurrent events during the NRMP included: pressure ulcers, alveolo-interstitial pulmonary disease, gastrointestinal disease, and bacteria/fungal dermatitis.

3. Results

The NRMP was applied to nine DPP– dogs (OFS 0) and seven DPP+ dogs (OFS 1) for a maximum period of 90 days. All the dogs had been paraplegic for more than 3 months after IVDD surgery and HWP. Dogs who achieved ambulation were discharged, as demonstrated in Figure 4.

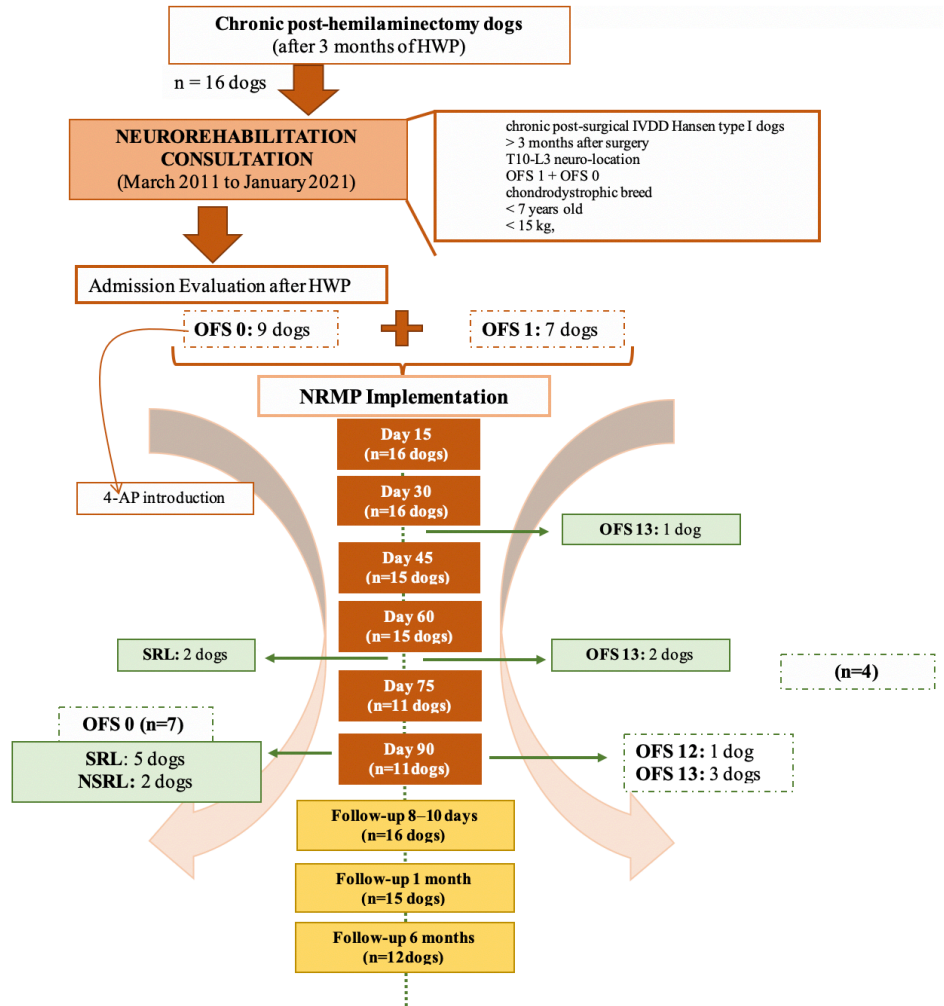


Figure 4. Flow diagram illustrating chronic paraplegic post-hemilaminectomy dogs' evolution and medical discharge. Abbreviations: OFS—open field score [64]; NRMP—neurorehabilitation multimodal protocol; HWP—Home Work Plan; SRL—Spinal Reflex Locomotion; NSRL—Non-Spinal Reflex Locomotion.

As mentioned above, data were collected regarding breed, age, gender, weight, neuro-location, and the presence of DPP. Moreover, this study included 100% (n = 16) paraplegic dogs, of which 56% (9/16) were DPP– and 44% (7/16) were DPP+.

At medical discharge and after the NRMP, ambulatory status was achieved in 88% (14/16) of dogs. No concurrent events were observed during the NRMP.

3.1. DPP– Dogs' Results

Among the nine DPP– dogs that were admitted with an OFS of 0, 78% (7/9) were discharged with SRL and 22% (2/9) remained with NSRL.

On day 15 and day 30, the dogs showed no significant evolution. Adverse events, such as vomiting, diarrhea, and seizures, were not seen in any of the dogs receiving 4-AP medication.

At outcome day 45, two dogs (22% (2/9)) obtained SRL with medical discharge at day 60. On day 60, two more dogs (22% (2/9)) obtained SRL with medical discharge at day 90. Three more dogs (33% (3/9)) achieved SRL at outcome day 75, remaining until day 90. Only two dogs (22% (2/9)) remained with NSRL on day 90. The appearance of SLR occurred only after day 30, which may have been coincidental or may have been due to the contribution of 4-AP to their recovery.

At the 8–10-day follow-up, all nine dogs maintained similar neurological status, as well as at the 1-month follow-up, although one dog missed their follow-up appointment. At the 6-month follow-up, only six dogs attended, and they all maintained the same neurological status. The evolution of the ambulatory status and medical discharge statistics are presented in Figure 5, with the OFS 0 dogs represented in blue.

The mean time in which ambulation was achieved, in DPP– dogs, was 62 days (median 60 days) and all regained automatic micturition.

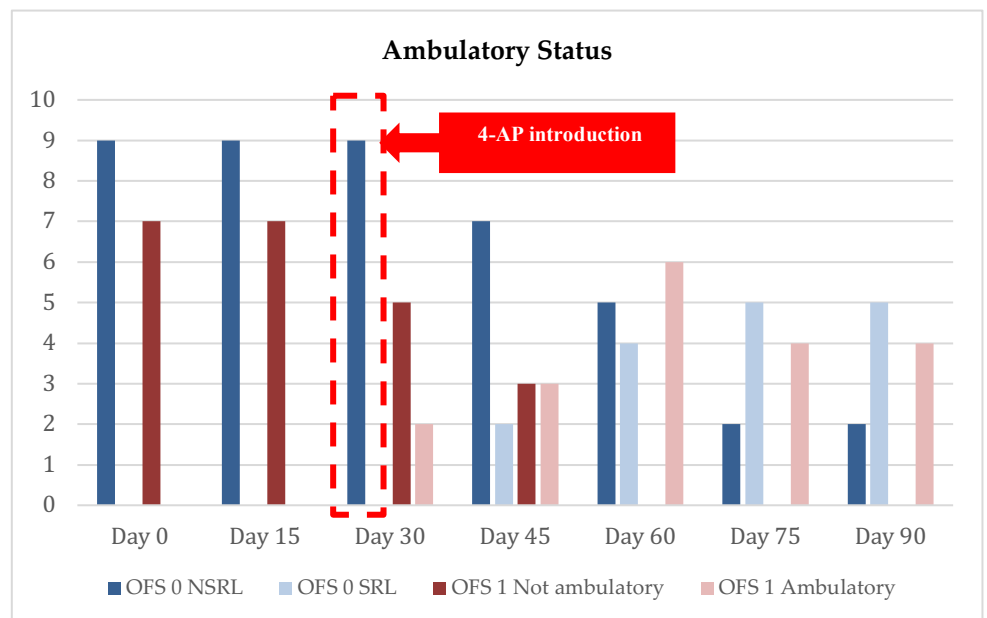


Figure 5. Evolution of ambulatory status and medical discharge from admission (day 0) until day 90. OFS—open field score [64]; SRL—Spinal Reflex Locomotion; NSRL—Non-Spinal Reflex Locomotion; OFS 0: blue color; OFS 1: brown color.

3.2. DPP+ Dogs' Results

Seven DPP+ dogs were admitted with an OFS of 1, and 100% were discharged with ambulatory status.

On outcome day 15, all dogs showed an improvement in the OFS (range 3 to 9) and two dogs achieved ambulatory status at outcome day 30: one with OFS 13 and the other with OFS 11. The first one was medically discharged on day 30, and the second only on day 60.

On outcome day 45 (n = 6), two more dogs achieved ambulatory status with an OFS of 11, and on outcome day 60 (n = 6), all the dogs were ambulatory, with two medical discharges with an OFS of 13.

On outcome day 75 (n = 4), three dogs had OFS 12 and one had OFS 11. On day 90, the former improved to OFS 13 and the latter to OFS 12. The OFS results were registered during the outcomes, as shown in Table 5, and their evolution is displayed in Figure 6.

The mean time to achieve ambulation in DPP+ dogs was 47 days (median 45 days) and all achieved voluntary micturition.

Table 5. OFS gait data records and mean time since admission and during outcomes (day 15, 30, 45, 60, 75, and 90), OFS at medical discharge and during follow-up (8–10-day, 1-month, and 6-month).

DPP+ Dogs ID	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90	OFS Medical Discharge	8–10-Day Follow-Up	1-Month Follow-Up	6-Month Follow-Up
1	1	3	7	10	11	12	13	13	13	13	14
2	1	4	9	11	13	12		13	13	14	14
3	1	3	7	10	11	12	13	13	13	13	14
4	1	7	13			12		13	13	13	13
5	1	7	9	11	12	12	13	13	13	13	13
6	1	9	11	12	13			13	13	14	
7	1	7	9	10	11	11	12	12	12	12	13
OFS Mean	1	5.7	9.3	10.7	11.8	11.8	12.8				

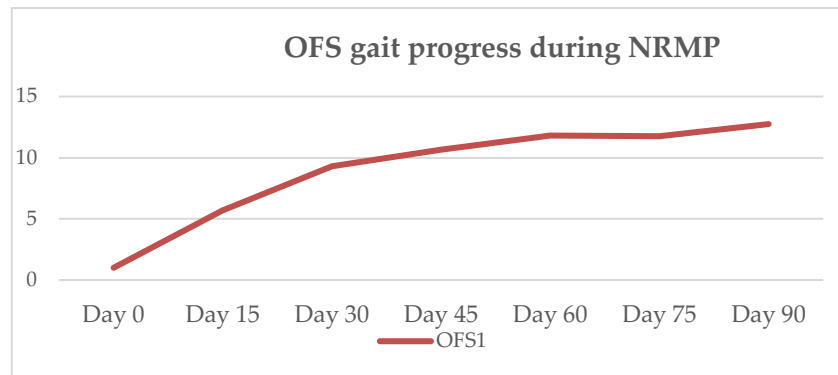


Figure 6. Mean OFS gait progression of OFS 1 dogs during NRMP. OFS—open field score [64]; NRMP—neurorehabilitation multimodal protocol.

The evolution of the ambulatory status and medical discharges are presented in Figure 5, with the OFS 1 dogs represented in brown.

At the 8–10-day follow-up, all seven dogs maintained similar OFS results, and at the 1-month follow-up, two dogs improved to an OFS of 14. At the 6-month follow-up, one dog did not attend, two improved to an OFS of 14, and one improved to an OFS of 13. The evolution of mean

OFS results since medical discharge and during follow-up is represented in Figure 7.

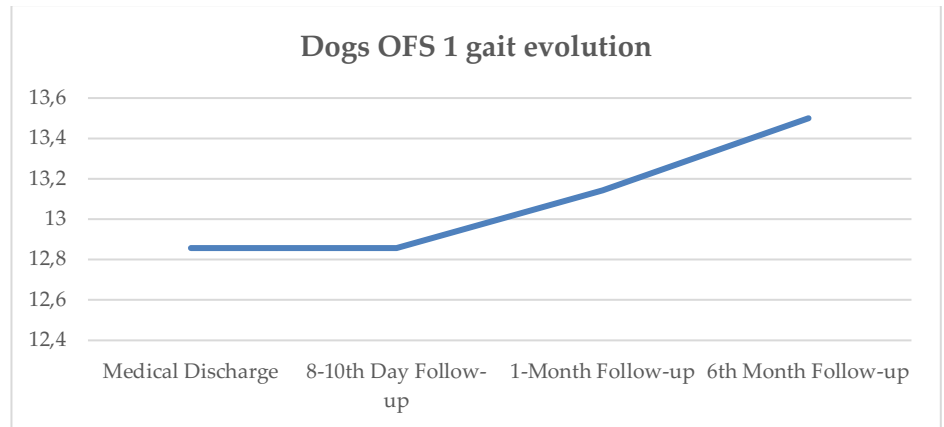


Figure 7. Dogs' OFS gait evolution since medical discharge and follow-up (8–10day, 1-month, and 6-month); OFS—open field score described by the y-axis [64].

4. Discussion

In this study's population, the median age was 4 years old and 31% (5/16) of the dogs were French Bulldogs, which can be explained by the fact that this breed is fixed for the CFA12 FGF4 retrogene at a significantly younger age, as reported by Dickinson et al. (2020) [88], with a median age of 3.7 years. Dachshunds displayed the same prevalence (5/16); this breed is homozygous for the same retrogene but with a median age of 6.5 years [89], also according to our population inclusion criteria. The presence of the CFA12 FGF4 retrogene seems to be sufficient to cause the premature degeneration of the intervertebral disc [89], associated with early chondroid metaplasia in chondrodystrophic breeds [90], which includes all our dogs.

Regarding this study's population, the mean weight was 7 kg, in line with most recent studies [91–93]. The same comparison could be made regarding the lesion site, focusing on the thoracolumbar region, which can be justified by anatomical reasons [2,92,93].

In the present study, these dogs had a chronic spinal injury that was present more than 3 months after surgery. Thus, the positive evolution in the locomotor pattern to achieve ambulation may indicate the benefits of the NRMP [16]. This type of neurorehabilitation protocol originated in human medicine; however, there are also several recent studies that use a population of chronically injured dogs that simulate a situation in humans [94–98].

The NRMP implemented was based on locomotor training, which included land treadmill by body weight supported treadmill training (BWSTT), UWTM, and kinesiotherapy exercises [99], as recently reinforced by the Canine Spinal Cord Injury Consortium (CANSORT SCI) [6].

The land BWSTT enhances muscle endurance, promotes the rhythmic activity of the spinal network, and increases the stimulation of the remaining descending/ascending pathways [17,100]. Locomotor training is essential as a strong neuromodulator that may contribute to neuroregeneration and to the disinhibition of the descending motor pathways that are inhibited in SCI dogs, stimulating pre-motor neuronal control and promoting intra-limb and inter-limb coordination [101–103].

In this case series study, the results showed that in DPP– dogs, the neurological deficits improved after implementation of the pharmacological approach, with one dog achieving SRL 15 days after starting 4-AP, i.e., at the 45-day outcome. Moreover, 78% of ambulatory DPP– dogs achieved SRL by the 75-day outcome, i.e., within two and a half months. Thus, the mean time to achieve ambulation in DPP– dogs was 62 days and all achieved automatic micturition. At follow-up, all dogs maintained similar neurological status, due to the spinal cord's memorization of the locomotor pattern. These results suggest that more studies are needed in this field.

Furthermore, it is important to mention that, to the best of our knowledge, there is no study that unites the effects of intensive locomotor training, electrical stimulation, and 4-AP administration. Therefore, this is the first case series study that may prove the relevance of a multimodal approach.

Lewis and colleagues (2019) [54] administered a mean drug dosage of 0.78 mg/kg, with all the dogs receiving at least three 4-AP doses, separated by at least 8 h. In the present study, the dosage was gradually administered from 0.3mg/kg until 1.1 mg/kg per os BID, to avoid increased drug-related side effects, such as seizure activity, as reported in the aforementioned study. There were no adverse effects reported in our results, which suggests that this was a safe and non-harmful protocol.

In order to be able to repeat this NRMP, it is necessary to use an electrical stimulation device, a land treadmill, and, ideally, a UWTM (given the possibility to stimulate afferent pathways, cutaneous receptors, and proprioceptive receptors) [104–106]. Moreover, given the benefits of water, such as floatability, viscosity, and resistance, superficial tension and vasodilation/vasoconstriction are promoted by the water temperature [107–110].

In our dogs, pharmacological management was only applied within a period of two months. The possible lack of response may suggest that there is a limited functional effect of 4-AP in chronic SCI dogs when the neuronal matter is already in a state of hyperexcitability [111,112]. However, it is also suggested that 4-AP can potentiate the spinal network in chronic dogs, with the observation of low spasticity signs [54], important given the fact that severe chronic thoracolumbar SCI dogs usually develop signs of spasticity in weeks to months after the initial injury [113,114]. Thus, 4-AP could possibly decrease clonic reflexes and increase a synchronized and coordinated flexion/extension locomotor pattern.

In chronic SCI, the axons fail to regrow, and this axonal regrowth could be inhibited by scar tissue formation [115]. The glial scar and cavity formation are highlighted as the main pathophysiological feature in chronic SCI, characterized by reactive astrocytes in the chronic phase after the injury [116]. When mainly accentuated within the grey matter, it could be related to severe necrosis, with the destruction of the tissue structure

[117]. At the same time, extensive oligodendrocyte remyelination of the remaining axons could also occur [116].

A recent analysis that assessed transcranial magnetic motor evoked potential suggested that spinal locomotion could be associated with remaining intact conduction through the descending motor tracts [5].

Granger et al. (2012) [94] and Hu et al. (2018) [118] studied the hypotheses regarding the relation between spinal walking and spinal “long tract” conduction. It was demonstrated that in some dogs, even those with complete SCI, a persistent passage of electrophysiological stimuli across the lesion with recorded spinal somatosensory evoked potentials above the injury [118], which could also be seen in humans [119]. In the aforementioned study, it was suggested that this remaining passage is a possibility, or not, in spinal walking dogs; however, Lewis et al. (2017) [5] support this positive relationship and mention a non-relation between the lesion site and recovery of spinal walking.

Hu et al. (2018) [118] concluded that, in chronic dogs, this remaining electrophysiological conduction throughout the long tracts is extremely variable and there is still very little evidence regarding the benefits in improving functional outcomes. Other studies in humans have suggested that propriospinal connections could bypass the injury site, improving functional recovery [120–126].

Locomotor training activity after SCI is a well-established modality to be applied after SCI, promoting the recovery of stepping in cats [124,125]. There is also evidence that the same could be seen in clinically spinal-cord-injured dogs, as shown by Gallucci et al. (2017) [92], but also in IVDD and traumatic SCI dogs.

All DPP– dogs in this study manifested some spinal network plasticity, allowing a flexion/extension locomotor pattern with a fast but modulated flexor reflex and crossed extensor reflex, characteristic of the NSRL [126,127], which was obtained in 22% (2/9) through the quadrupedal training with sensory feedback, which sends signals to the CPG [28,83,123,128]. The remaining seven dogs achieved ambulation by SRL, through the possibility of SCI regeneration and/or the activation of residual descending pathways [5].

In the chronic phase, mainly in DPP– dogs, there is no standard treatment to regain ambulation, aiming to promote synapse plasticity, increase axonal sprouting, and lead to regeneration [129]. The existing gap in chronic post-surgical IVDD dogs has led to recent research in regenerative medicine that has investigated Schwann cells [130], embryonic stem cells [131], macrophages [132], neural stem cells [133], and pluripotent stem cells [115,134]. All these studies aimed to demonstrate the effects of regenerative medicine on axonal regeneration with possible benefits in recovery.

It was shown that neurogenic induction, in both canines and humans, with dental pulp stem cells (neuroectodermal stem progenitor cells—NSPCs) could cause morphological changes [135].

In rats with spinal cord transection, the combination of NSPCs with electroacupuncture has been shown to improve neurological evolution and regeneration [136,137]. Similar studies with the association between locomotor training and stem cells were performed [97].

In Prado et al. (2019) [138], in 16 dogs ranging from 5 to 11 years old, with acute IVDD, who remained paralyzed for at least 3 months and were subjected to dorsal hemilaminectomy, stem cells were injected intramedullary into the spinal cord parenchyma—in the middle, cranial, and

caudal to the lesion. A second transplantation was performed after seven days, followed by three percutaneous intraspinal injections. Locomotor training 3 times a week was performed after 8–11 days, for the initial 7 weeks, followed by twice a week for a further 5 weeks, in association with UWTM twice a week, for 3–15 min, for a total of 12 weeks. The results demonstrated that no beneficial effects could be associated with the cells alone or with the combined treatment.

Compared to the present study, the results described above may be related to the fact that locomotor training should be performed every day in order to induce plasticity in the reflex pathways [17,126,139,140]. In Martins et al. (2020) [17], the authors reported a statistically significant positive evolution over time, with an increase in mean OFS related to the locomotor training implemented. Thus, repetitive locomotor training tasks could promote cutaneous reflex excitability [141], by different mechanisms, such as the sprouting and regeneration of damaged fibers and changes in synapses' efficiency [142,143].

One other study that used adipose tissue mesenchyme stem cells (AT-MSCs) demonstrated a regenerative function, either by mediating replaced lost cells, such as oligodendrocytes, which would facilitate the injured axons' remyelination, or by mediating astrocytes. Therefore, some degree of neuroplasticity was evolved in axonal sprouting and the formation of new circuits. Moreover, they reported that the locomotor recovery was obtained within 8 weeks, with a tendency to stabilize between 8 to 16 weeks [129].

In the present study, 100% of the DPP+ dogs achieved ambulatory status at clinical discharge. On day 15, all dogs had improved but only two achieved ambulation by day 30, the time point at which an OFS peak is achieved, as seen in Figure 6. At outcome day 45, the mean OFS was 10.7, and at day 60, it increased to 11.8, as shown in Table 5. Such results may suggest that chronic post-surgical dogs need more time to achieve ambulation, within a mean time of 47 days (median 45 days). This is in contrast to acute post-surgical IVDD dogs, who usually demonstrate 90% to 100% of recovery, with 70.8% recovering within the first 15 days [144]. All DPP+ dogs achieved voluntary micturition. At follow-up, the average evolution was positive regarding the neurological status (Figure 7).

Regarding DPP– dogs, there was no DPP recovery, in contrast to the acute dogs, who usually regain 41–62% of ambulation [1,145–149]. According to the Canine Spinal Cord Injury Consortium (CANSORT SCI), DPP– dogs recover DPP to ambulation ~60% within 6 months after injury. In our results, DPP– chronic dogs achieved 78% of ambulation within 2 and a half months; however, all had achieved the flexion/extension locomotor pattern within the first two weeks, which allowed the introduction of the pharmacological management protocol with 4-AP.

Since this was a case series study, we suggest that more studies are needed in this area, given the clinical interest and the need to be open to multidisciplinary approaches, mainly on post-surgical DPP– chronic dogs. This study's population was difficult to obtain due to the strict inclusion criteria, because chronic post-surgical IVDD Hansen type I paraplegic dogs do not typically survive. We propose a multimodal approach in order to achieve ambulation, decrease the need to perform euthanasia, and promote the quality of life of these dogs.

Thus, limitations included the small number of dogs, due to the criteria mentioned above, and the lack of control cases, which is a significant limitation of this study. The appearance of the SRL may have

been due to spontaneous recovery, coincidental, or due to the contribution of 4-AP, and a control group would be required in order to assess this.

Future controlled studies with a larger number of dogs with chronic DPP+ or DPP- incomplete recovery are needed. It would also be interesting to continue this study considering the association between the NRMP, stem cell administration, and/or a pharmacological management protocol with 4-AP.

5. Conclusions

This prospective case series clinical study proposes a neurorehabilitation multimodal protocol (NRMP) as a safe and feasible therapeutic approach that was applied in all 16 chronic post-surgical SCI dogs without any side effects.

Chronic dogs with OFS 0/DPP- (n = 9) could not recover DPP within the 90 days of the NRMP, but 78% (7/9) were able to achieve ambulation by SRL, with a mean time of 62 days. Thus, it is suggested that this type of ambulation may be possible with a multidisciplinary protocol with the influence or not of spontaneous recovery. Moreover, it is possible that dogs that were admitted in a more severe state (DPP-) may simply take longer to respond to the non-pharmacological rehabilitation modalities when compared to DPP+ dogs.

On the other hand, dogs with OFS 1/DPP+ (n = 7) were able to achieve 100% of ambulation within a mean period of 47 days, suggesting the need for a longer period of time when compared to the average acute dogs who are submitted to similar rehabilitation approaches.

The type of population considered in this study, which achieved 88% (14/16) of ambulation, usually could not persevere. Thus, an NRMP may be an important therapeutic strategy to reduce the need for euthanasia in the clinical setting. This study should be continued, given all the limitations mentioned.

Author Contributions: Conceptualization Â.M.; methodology Â.M.; validation Â.M. and A.F.; formal analysis, A.F.; investigation, Â.M., D.G., A.C., C.S., C.C., T.C. and Ó.G.; writing—original draft preparation, Â.M., C.S., D.G., C.C. and A.C.; writing—review and editing A.F.; supervision A.F.; project administration Â.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study received the approval of the Ethics and Animal Welfare Commission of the Veterinary Medicine Faculty of the Lisbon University (CEBEA) (N/Ref 001/2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Olby, N.; Levine, J.; Harris, T.; Muñana, K.; Skeen, T.; Sharp, N. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord: 87 cases (1996–2001). *J. Am. Vet. Med. Assoc.* **2003**, *222*, 762–769, doi:10.2460/javma.2003.222.762.
2. Jeffery, N.D.; Barker, A.K.; Hu, H.Z.; Alcott, C.J.; Kraus, K.H.; Scanlin, E.M.; Granger, N.; Levine, J.M. Factors associated with recovery from paraplegia in dogs with loss of pain perception in the pelvic limbs following intervertebral disk herniation. *J. Am. Vet. Med. Assoc.* **2016**, *248*, 386–394, doi:10.2460/javma.248.4.386.
3. Fingerroth, J.M.; Forterre, F.; Levine, J.M. Compressive and contusive spinal cord injury secondary to intervertebral disc displacement: A clinical perspective. In *Advances in Intervertebral Disc Disease in Dogs and Cats*; Fingerroth, J.M., Thomas, W.B., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2015; pp. 131–134.
4. Ingram, E.A.; Kale, D.C.; Balfour, R.J. Hemilaminectomy for thoracolumbar hansen type I intervertebral disk disease in ambulatory dogs with or without neurologic deficits: 39 cases (2008–2010). *Vet. Surg.* **2013**, *42*, 924–931, doi:10.1111/j.1532-950X.2013.12061.x.
5. Lewis, M.J.; Howard, J.F.; Olby, N.J. The relationship between trans-lesional conduction, motor neuron pool excitability, and motor function in dogs with incomplete recovery from severe spinal cord injury. *J. Neurotrauma* **2017**, *34*, 2994–3002, doi:10.1089/neu.2017.5012.
6. Lewis, M.J.; Jeffery, N.D.; Olby, N.J. Ambulation in dogs with absent pain perception after acute thoracolumbar Spinal Cord Injury. *Front. Vet. Sci.* **2020**, *7*, 560–572, doi:10.3389/fvets.2020.00560.
7. Alluin, O.; Delivet-Mongrain, H.; Rossignol, S. Inducing hindlimb locomotor recovery in adult rat after complete thoracic spinal cord section using repeated treadmill training with perineal stimulation only. *J. Neurophysiol.* **2015**, *114*, 1031–1046, doi:10.1152/jn.00416.2015.
8. Militskova, A.; Mukhametova, E.; Fatykhova, E.; Sharifullin, S.; Cuellar, C.A.; Calvert, J.S.; Grahm, P.J.; Baltina, T.; Lavrov, I. Supraspinal and Afferent Signaling Facilitate Spinal Sensorimotor Network Excitability After Discomplete Spinal Cord Injury: A Case Report. *Front. Neurosci.* **2020**, *14*, 552, doi:10.3389/fnins.2020.00552.
9. Olby, N.J.; Lim, J.H.; Wagner, N.; Zidan, N.; Early, P.J.; Mariani, C.L.; Muñana, K.R.; Laber, E. Time course and prognostic value of serum GFAP, pNFH, and S100 β concentrations in dogs with complete spinal cord injury because of intervertebral disc extrusion. *J. Vet. Intern. Med.* **2019**, *33*, 726–734, doi:10.1111/jvim.15439. Epub 2019 Feb 13.
10. Fingerroth, J.M.; De Lahunta, A. Ascending/Descending myelomalacia secondary to Intervertebral Disc Herniation. In *Advances in Intervertebral Disc Disease in Dogs and Cats*; Fingerroth, J.M., Thomas, W.B., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2015; pp. 115–120.
11. Moore, S.A.; Tipold, A.; Olby, N.J.; Stein, V.; Granger, N. Current approaches to the management of acute thoracolumbar disc extrusion in dogs. *Front. Vet. Sci.* **2020**, *7*, 1–15, doi:10.3389/fvets.2020.00610.
12. Tansey, K.E. Neural plasticity and locomotor recovery after spinal cord injury. *PM R* **2010**, *2*, 220–226, doi:10.1016/j.pmrj.2010.10.007.
13. Dimitrijevic, M.R. Residual motor function after spinal cord injury. In *Restorative Neurology of Spinal Cord Injury*; Dimitrijevic, M.R., Ed.; Oxford University Press: New York, NY, USA, 2012; pp. 1–9.
14. Kakulas, B.A.; Kaelan, C. The neuropathological foundations for the restorative neurology of spinal cord injury. *Clin. Neurol. Neurosurg.* **2015**, *129*, 51–57, doi:10.1016/j.clineuro.2015.01.012.
15. Alisauskaite, N.; Spitzbarth, I.; Baumgartner, W.; Dziallas, P.; Kramer, S.; Denning, R.; Stein, V.M.; Tipold, A. Chronic post-traumatic intramedullary lesion in dogs, a translational model. *PLoS ONE* **2017**, *12*, e0187746, doi:10.1371/journal.pone.0187746.
16. Martins, Â.; Silva, C.M.; Gouveia, D.; Cardoso, A.; Coelho, T.; Gamboa, Ó.; Marcelino, E.; Ferreira, A. Spinal Locomotion in Cats Following Spinal Cord Injury: A Prospective Study. *Animals* **2021**, *11*, 1994, doi.org/10.3390/ani11071994.
17. Martins, Â.; Gouveia, D.; Cardoso, A.; Viegas, I.; Gamboa, Ó.; Ferreira, A. A Comparison Between Body Weight-Supported Treadmill Training and Conventional Over-Ground Training in Dogs with Incomplete Spinal Cord Injury. *Front. Vet. Sci.* **2021**, *8*, 597949, doi.org/10.3389/fvets.2021.597949.
18. Solopova, I.A.; Selionov, V.A.; Sylos-Labini, F.; Gurfinkel, V.S.; Lacquaniti, F.; Ivanenko, Y.P. Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Front. Syst. Neurosci.* **2015**, *18*, 9–14, doi:10.3389/fnsys.2015.00014.
19. Harness, E.T.; Yozbatiran, N.; Cramer, S.C. Effects of intense exercise in chronic spinal cord injury. *Spinal Cord* **2008**, *46*, 733–737, doi:10.1038/sc.2008.56.
20. Rossignol, S.; Bouyer, L. Adaptive Mechanisms of Spinal Locomotion in Cats. *Integr. Comp. Biol.* **2004**, *44*, 71–79, doi:10.1093/icb/44.1.71.
21. De Leon, R.D.; Hodgson, J.A.; Roy, R.R.; Edgerton, V.R. Retention of hindlimb stepping ability in adult spinal cats after the cessation of step training. *J. Neurophysiol.* **1999**, *81*, 85–94, doi:10.1152/jn.1999.81.1.85.
22. Fouad, K.; Tetzlaff, W. Rehabilitative training and plasticity following spinal cord injury. *Exp. Neurol.* **2012**, *235*, 91–99, doi:10.1016/j.expneurol.2011.02.009.

23. Kiehn, O. Locomotor circuits in the mammalian spinal cord. *Annu. Rev. Neurosci.* **2006**, *29*, 279–306, doi:10.1146/annurev.neuro.29.051605.112910.
24. Côté, M.P.; Murray, L.M.; Knikou, M. Spinal Control of locomotion: Individual neurons, their circuits and functions. *Front. Physiol.* **2018**, *9*, 1–27, doi:10.3389/fphys.2018.00784.
25. Guertin, P.A. Preclinical evidence supporting the clinical development of central pattern generator-modulating therapies for chronic spinal cord-injured patients. *Front. Hum. Neurosci.* **2014**, *8*, 1–17, doi:10.3389/fnhum.2014.00272.
26. Barbeau, H.; Rossignol, S. Enhancement of locomotor recovery following spinal cord injury. *Curr. Opin. Neurol.* **1994**, *7*, 517–524, doi:10.1097/00019052-199412000-00008.
27. Wirz, M.; Zemon, D.H.; Rupp, R.; Anke, S.; Colombo, G.; Dietz, V.; Hornby, T.G. Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: A multicenter trial. *Arch. Phys. Med. Rehabil.* **2005**, *86*, 672–680, doi:10.1016/j.apmr.2004.08.004.
28. Barrière, G.; Leblond, H.; Provencher, J.; Rossignol, S. Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries. *J. Neurosci.* **2008**, *28*, 3976–3987; doi:10.1523/JNEUROSCI.5692-07.2008.
29. Gossard, J.P.; Delivet-Mongrain, H.; Martinez, M.; Kundu, A.; Escalona, M.; Rossignol, S. Plastic changes in lumbar locomotor networks after a partial spinal cord injury in Cats. *J. Neurosci.* **2015**, *35*, 9446–9455, doi:10.1523/jneurosci.4502-14.2015.
30. Leech, K.A.; Kinnaird, C.R.; Holleran, C.L.; Kahn, J.; Hornby, T.G. Effects of locomotor exercise intensity on gait performance in individuals with incomplete spinal cord injury. *Phys. Ther.* **2016**, *96*, 1919–1929, doi:10.2522/ptj.20150646.
31. Rossignol, S. Neural control of stereotypic limb movements. In: Rowell LB, Sheperd JT, editors. *Handbook of Physiology. Sect. 12. Exercise: Regulation and Integration of Multiple Systems*. American Physiological Society: Oxford, UK, 1996, pp. 173–216.
32. Edgerton, V.R.; Leon, R.D.; Harkema, S.J.; Hodgson, J.A.; London, N.; Reinkensmeyer, D.J.; Roy, R.R.; Talmadge, R.J.; Tillakaratne, N.J.; Timoszyk, W.; et al. Retraining the injured spinal cord. *J. Physiol.* **2001**, *533*, 15–22, doi:10.1111/j.1469-7793.2001.0015b.x.
33. de Leon, R.D.; Kubasak, M.D.; Phelps, P.E.; Timoszyk, W.K.; Reinkensmeyer, D.J.; Roy, R.R.; Edgerton, V.R. Using robotics to teach the spinal cord to walk. *Brain Res. Rev.* **2002**, *40*, 267–273, doi:10.1016/s0165-0173(02)00209-6.
34. Ichiyama, R.M.; Broman, J.; Roy, R.R.; Zhong, H.; Edgerton, V.R.; Havton, L.A. Locomotor training maintains normal inhibitory influence on both alpha- and gamma-motoneurons after neonatal spinal cord transection. *J. Neurosci.* **2011**, *31*, 26–33, doi:10.1523/jneurosci.6433-09.2011.
35. Martinez, M.; Delivet-Mongrain, H.; Leblond, H.; Rossignol, S. Effect of locomotor training in completely spinalized cats previously submitted to a spinal hemisection. *J. Neurosci.* **2012**, *32*, 10961–10970, doi:10.1523/JNEUROSCI.1578-12.2012.
36. Martins, Â.; Gouveia, D.; Cardoso, A.; Gamboa, Ó.; Millis, D.; Ferreira, A. Nervous system modulation through electrical stimulation in companion animals. *Acta Vet. Scand.* **2021**, *63*, 1–22, doi:10.1186/s13028-021-00585-z.
37. Thompson, A.K.; Wolpaw, J.R. The simplest motor skill: Mechanisms and applications of reflex operant conditioning. *Exerc. Sport Sci. Rev.* **2014**, *42*, 82–90, doi:10.1249/JES.0000000000000010.
38. Thompson, A.K.; Wolpaw, J.R. Operant conditioning of spinal reflexes: From basic science to clinical therapy. *Front. Integr. Neurosci.* **2014**, *18*, 8–25, doi:10.3389/fnint.2014.00025.
39. Richard, M.A.; Spaich, E.G.; Serrao, M.; Andersen, O.K. Stimulation site and phase modulation of the withdrawal reflex during gait initiation. *Clin. Neurophysiol.* **2015**, *126*, 2282–2289, doi:10.1016/j.clinph.2015.01.019.
40. Krueger, E.; Magri, L.M.S.; Botelho, A.S.; Bach, F.S.; Rebellato, C.L.K.; Fracaro, L.; Fragoso, F.Y.I.; Villanova, J.A.; Brofman, P.R.S.; Popović-Maneski, L. Effects of low-intensity electrical stimulation and adipose derived stem cells transplantation on the time-domain analysis-based electromyographic signals in dogs with SCI. *Neurosci. Lett.* **2019**, *696*, 38–45, doi:10.1016/j.neulet.2018.12.004.
41. Noonan, V.K.; Kopec, J.A.; Zhang, H.; Dvorak, M.F. Impact of associated conditions resulting from spinal cord injury on health status and quality of life in people with traumatic central cord syndrome. *Arch. Phys. Med. Rehabil.* **2008**, *89*, 1074–1082, doi:10.1016/j.apmr.2007.10.041.
42. Taghavi, N.; Luecke, G.R.; Jeffery, N.D. A wearable body controlling device for application of functional electrical stimulation. *Sensors* **2018**, *18*, 1251, doi:10.3390/s18041251.
43. Milinis, K.; Young, C.A.; On behalf of the Trajectories of Outcome in Neurological Conditions (TONiC) Study. Systematic review of the influence of spasticity on quality of life in adults with chronic neurological conditions. *Disabil. Rehabil.* **2015**, *29*, 1–11, doi:10.3109/09638288.2015.1106592.

44. Sayenko, D.G.; Atkinson, D.A.; Floyd, T.C.; Gorodnichev, R.M.; Moshonkina, T.R.; Harkema, S.J.; Edgerton, V.R.; Gerasimenko, Y.P. Effects of paired transcutaneous electrical stimulation delivered at single and dual sites over lumbosacral spinal cord. *Neurosci. Lett.* **2015**, *609*, 229–234, doi:10.1016/j.neulet.2015.10.005.
45. Ladenbauer, J.; Minassian, K.; Hofstoetter, U.S.; Dimitrijevic, M.R.; Rattay, F. Stimulation of the human lumbar spinal cord with implanted and surface electrodes: A computer simulation study. *IEEE Trans. Neural. Syst. Rehabil. Eng.* **2010**, *18*, 637–645, doi:10.1109/TNSRE.2010.2054112.
46. Edgerton, V.R.; Harkema, S. Epidural stimulation of the spinal cord in spinal cord injury: Current status and future challenges. *Expert Rev. Neurother.* **2011**, *11*, 1351–1353, doi:10.1586/ern.11.129.
47. Harkema, S.; Gerasimenko, Y.; Hodes, J.; Burdick, J.; Angeli, C.; Chen, Y.; Ferreira, C.; Willhite, A.; Rejc, E.; Grossman, R.G.; et al. Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: A case study. *Lancet* **2011**, *377*, 1938–1947, doi:10.1016/S0140-6736(11)60547-3.
48. Hofstoetter, U.S.; Krenn, M.; Danner, S.M.; Hofer, C.; Kern, H.; McKay, W.B.; Mayr, W.; Minassian, K. Augmentation of voluntary locomotor activity by transcutaneous spinal cord stimulation in motor-incomplete spinal cord-injured individuals. *Artif. Organs* **2015**, *39*, 176–186, doi:10.1111/aor.12615.
49. Minassian, K.; Hofstoetter, U.S.; Danner, S.M.; Mayr, W.; Bruce, J.A.; McKay, W.B.; Tansey, K.E. Spinal Rhythm Generation by Step-Induced Feedback and Transcutaneous Posterior Root Stimulation in Complete Spinal Cord-Injured Individuals. *Neurorehabil. Neural. Repair.* **2016**, *30*, 233–243, doi:10.1177/1545968315591706.
50. Hofstoetter, U.S.; Knikou, M.; Guertin, P.A.; Minassian, K. Probing the Human Spinal Locomotor Circuits by Phasic Step-Induced Feedback and by Tonic Electrical and Pharmacological Neuromodulation. *Curr. Pharm. Des.* **2017**, *23*, 1805–1820, doi:10.2174/1381612822666161214144655.
51. Lewis, M.J.; Granger, N.; Jeffery, N.D.; Canine Spinal Cord Injury Consortium (CANSORT-SCI). Emerging and Adjunctive Therapies for Spinal Cord Injury Following Acute Canine Intervertebral Disc Herniation. *Front. Vet. Sci.* **2020**, *7*, 579933, doi:10.3389/fvets.2020.579933.
52. Blight, A.R.; Toombs, J.P.; Bauer, M.S.; Widmer, W.R. The effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: A phase i clinical trial. *J. Neurotrauma* **1991**, *8*, 103–119, doi:10.1089/neu.1991.8.103.
53. Lim, J.H.; Muguet-Chanoit, A.C.; Smith, D.T.; Laber, E.; Olby, N.J. Potassium channel antagonists 4-aminopyridine and the T-butyl carbamate derivative of 4-aminopyridine improve hind limb function in chronically nonambulatory dogs; a blinded, placebo-controlled trial. *PLoS ONE* **2014**, *9*, e116139, doi:10.1371/journal.pone.0116139.
54. Lewis, M.J.; Laber, E.; Olby, N.J. Predictors of response to 4-aminopyridine in chronic canine spinal cord injury. *J. Neurotrauma* **2019**, *36*, 1428–1434, doi:10.1089/neu.2018.5975.
55. Karimi-Abdolrezaee, S.; Billakanti, R. Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. *Mol. Neurobiol.* **2012**, *46*, 251–264, doi:10.1007/s12035-012-8287-4.
56. Couillard-Despres, S.; Bieler, L.; Vogl, M. Pathophysiology of traumatic Spinal Cord Injury, In *Neurological Aspects of Spinal Cord Injury*; Weider, N., Rupp, R., Tansey, K.E., Eds.; Springer: Cham, Switzerland, 2017; pp. 503–528.
57. Rolls, A.; Shechter, R.; Schwartz, M. The bright side of the glial scar in CNS repair. *Nat. Rev. Neurosci.* **2009**, *10*, 235–241, doi:10.1038/nrn2591.
58. Adams, K.L.; Gallo, V. The diversity and disparity of the glial scar. *Nat. Neurosci.* **2018**, *21*, 9–15, doi:10.1038/s41593-017-0033-9.
59. Raineteau, O.; Schwab, M.E. Plasticity of motor systems after incomplete spinal cord injury. *Nat. Rev. Neurosci.* **2001**, *2*, 263–273, doi:10.1038/35067570.
60. Wolpaw, J.R. Spinal cord plasticity in acquisition and maintenance of motor skills. *Acta Physiol.* **2007**, *189*, 155–169, doi:10.1111/j.1748-1716.2006.01656.x.
61. Thomson, C.; Hahn, C. *Veterinary Neuroanatomy, A Clinical Approach*, 1st ed.; Edwards, R., Leng, H., Eds.; Elsevier Saunders: Philadelphia, PA, USA, 2012; pp. 47–57.
62. Uemura, E.E. *Fundamentals of Canine Neuroanatomy and Neurophysiology*, 1st ed.; Uemura, E.E., Ed.; Wiley Blackwell: Oxford, UK, 2015; pp. 128–155.
63. Dietz, V.; Colombo, G.; Jensen, L.; Baumgartner, L. Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol.* **1995**, *37*, 574–582, doi:10.1002/ana.410370506.
64. Benito-Penalva, J.; Edwards, D.J.; Opisso, E.; Cortes, M.; Lopez-Blazquez, R.; Murillo, N.; Costa, U.; Tormos, J.M.; Vidal-Samsó, J.; Valls-Solé, J.; et al. Gait training in human spinal cord injury using electromechanical systems: Effect of device type and patient characteristic. *Arch. Phys. Med. Rehabil.* **2012**, *93*, 404–412, doi:10.1016/j.apmr.2011.08.028.
65. Meyns, P.; Van de Crommert, H.W.A.A.; Rijken, H.; Van Kuppevelt, D.H.J.M.; Duysens, J. Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed. *Spinal Cord* **2014**, *52*, 887–893, doi:10.1038/sc.2014.172.

66. Battistuzzo, C.R.; Callister, R.J.; Callister, R.; Galea, M.P. A systematic review of exercise training to promote locomotor recovery in animal models of spinal cord injury. *J. Neurotrauma* **2012**, *29*, 1600–1613, doi:10.1089/neu.2011.2199.
67. Cassilhas, R.C.; Tufik, S.; Mello, M.T. Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol. Life Sci.* **2016**, *73*, 975–983, doi:10.1007/s00018-015-2102-0.
68. Maier, I.C.; Ichiyama, R.M.; Courtine, G.; Schnell, L.; Lavrov, I.; Edgerton, V.R.; Schwab, M.E. Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain* **2009**, *132*, 1426–1440, doi:10.1093/brain/awp085.
69. Tillakaratne, N.; Duru, P.; Fujino, H.; Zhong, H.; Xiao, M.S.; Edgerton, V.R.; Roy, R.R. Identification of interneurons activated at different inclines during treadmill locomotion in adult rats. *J. Neurosci. Res.* **2014**, *92*, 1714–1722, doi:10.1002/jnr.23437.
70. Muir, G.D.; Steeves, J.D. Sensorimotor stimulation to improve locomotor recovery after spinal cord injury. *Trends Neurosci.* **1997**, *20*, 72–77, doi:10.1016/s0166-2236(96)10068-0.
71. Engesser-Cesar, C.; Ichiyama, R.M.; Nefas, A.L.; Hill, M.A.; Edgerton, V.R.; Cotman, C.W.; Anderson, A.J. Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. *Eur. J. Neurosci.* **2007**, *25*, 1931–1939, doi:10.1111/j.1460-9568.2007.05469.x.
72. Lang, C.E.; MacDonald, J.R.; Gnip, C. Counting repetitions: An observational study of outpatient therapy for people with hemiparesis post-stroke. *J. Neurol. Phys. Ther.* **2007**, *31*, 3–10, doi:10.1097/01.npt.0000260568.31746.3.
73. Dobkin, B.; Apple, D.; Barbeau, H.; Basso, M.; Behrman, A.; Deforge, D.; Ditunno, J.; Dudley, G.; Elashoff, R.; Fugate, L.; et al. Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI. *Neurology* **2006**, *66*, 484–493, doi:10.1212/01.wnl.0000202600.72018.39.
74. Lang, C.E.; Lohse, K.R.; Birkenmeier, R.L. Dose and timing in neurorehabilitation: Prescribing motor therapy after stroke. *Curr. Opin. Neurol.* **2015**, *28*, 549–555, doi:10.1097/wco.0000000000000256.
75. Platt, S.R.; Olby, N.J. Nursing and Rehabilitation of the neurological patient. In *Canine and Feline Neurology*, 3rd ed.; Platt, S.R., Olby, N.J., Eds.; British Small Animal Veterinary Association: Quedgeley, Gloucester, UK, 2004; p. 542.
76. Kralj, A.; Bajd, T.; Turk, R.; Krajnik, J.; Benko, H. Gait restoration in paraplegic patients: A feasibility demonstration using multichannel surface electrode FES. *J. Rehabil. R D* **1983**, *20*, 3–20. Available online: <https://pubmed.ncbi.nlm.nih.gov/6887064/> (accessed on 11 June 2021).
77. Holsheimer, J. Computer modelling of spinal cord stimulation and its contribution to therapeutic efficacy. *Spinal Cord* **1998**, *36*, 531–540, doi:10.1038/sj.sc.3100717.
78. Field-Fote, E.C. Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. *Arch. Phys. Med. Rehabil.* **2001**, *82*, 818–824, doi:10.1053/apmr.2001.23752.
79. Field-Fote, E.C.; Lindley, S.D.; Sherman, A.L. Locomotor training approaches for individuals with spinal cord injury: A preliminary report of walking-related outcomes. *J. Neurol. Phys. Ther.* **2005**, *29*, 127–137, doi:10.1097/01.npt.0000282245.31158.09.
80. Field-Fote, E.C.; Roach, K.E. Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: A randomized clinical trial. *Phys. Ther.* **2010**, *91*, 48–60, doi:10.2522/ptj.20090359.
81. Kapadia, N.; Masani, K.; Craven, B.C.; Giangregorio, L.M.; Hitzing, S.L.; Richards, K.; Popovic, M.R. A randomized trial of functional electrical stimulation for walking in incomplete spinal cord injury: Effects on walking competency. *J. Spinal Cord Med.* **2014**, *37*, 511–524, doi:10.1179/2045772314y.00000000263.
82. Levine, D.; Bockstahler, B. Electrical Stimulation. In *Canine Rehabilitation and Physical Therapy*, 2nd ed.; Millis, D.L., Levine, D., Eds.; Elsevier Saunders: Philadelphia, PA, USA, 2014; pp. 342–358.
83. Gerasimenko, Y.; Sayenko, D.; Gad, P.; Kozesnik, J.; Moshonkina, T.; Grishin, A.; Pukhoy, A.; Moiseyev, S.; Gorodnichev, R.; Selionov, V.; et al. Electrical spinal stimulation, and imaging of lower limb movements to modulate brain-spinal connectomes that control locomotor-like behavior. *Front. Physiol.* **2018**, *9*, 1–13, doi:10.3389/fphys.2018.01196.
84. Savin, Z.; Lejbkiewicz, I.; Glass-Marmor, L.; Lavi, I.; Rosenblum, S.; Miller, A. Effect of Fampridine-PR prolonged released 4-aminopyridine on the manual functions of patients with Multiple Sclerosis. *J. Neurol. Sci.* **2016**, *360*, 102–109, doi:10.1016/j.jns.2015.11.035.
85. Tseng, K.C.; Li, H.; Clark, A.; Sundem, L.; Zuscik, M.; Noble, M.; Elfar, J. 4-Aminopyridine promotes functional recovery and remyelination in acute peripheral nerve injury. *EMBO Mol. Med.* **2016**, *8*, 1409–1420, doi:10.15252/emmm.201506035.
86. Zörner, B.; Filli, L.; Reuter, K.; Kapitza, S.; Lörincz, L.; Sutter, T.; Weller, D.; Farkas, M.; Easthope, C.S.; Czaplinski, A.; et al. Prolonged-release fampridine in multiple sclerosis: Improved ambulation effected by changes in walking pattern. *Mult. Scler. J.* **2016**, *22*, 1463–1475, doi:10.1177/1352458515622695.

87. Olby, N.J.; De Risio, L.; Muñana, K.R.; Wosar, M.A.; Skeen, T.M.; Sharp, N.J.; Keene, B.W. Development of a functional scoring system in dogs with acute spinal cord injuries. *Am. J. Vet. Res.* **2001**, *62*, 1624–1628, doi:10.2460/ajvr.2001.62.1624.
88. Dickinson, P.J.; Bannasch, D.L. Current Understanding of the Genetics of Intervertebral Disc Degeneration. *Front. Vet. Sci.* **2020**, *7*, 431, doi:10.3389/fvets.2020.00431.
89. Murphy, B.G.; Dickinson, P.; Marcellin-Little, D.J.; Batcher, K.; Raverty, S.; Bannasch, D. Pathologic Features of the Intervertebral Disc in Young NovaScotia Duck Tolling Retrievers Confirms Chondrodystrophy Degenerative Phenotype Associated with Genotype. *Vet. Pathol.* **2019**, *566*, 895–902, doi:10.1177/0300985819868731.
90. Fenn, J.; Olby, N.J.; Canine Spinal Cord Injury Consortium (CANSORT-SCI). Classification of Intervertebral Disc Disease. *Front. Vet. Sci.* **2020**, *7*, 579025, doi:10.3389/fvets.2020.579025.
91. Aikawa, T.; Fujita, H.; Shibata, M.; Takahashi, T. Recurrent Thoracolumbar Intervertebral Disc Extrusion after Hemilaminectomy and Concomitant Prophylactic Fenestration in 662 Chondrodystrophic Dogs. *Vet. Surg.* **2012**, *41*, 381–390, doi:10.1111/j.1532-950X.2012.00970.x.
92. Gallucci, A.; Dragone, L.; Menchetti, M.; Gagliardo, T.; Pietra, M.; Cardinali, M.; Gandini, G. Acquisition of Involuntary Spinal Locomotion (Spinal Walking) in Dogs with Irreversible Thoracolumbar Spinal Cord Lesion: 81 Dogs. *J. Vet. Intern. Med.* **2017**, *31*, 492–497, doi:10.1111/jvim.14651.
93. Zidan, N.; Fenn, J.; Griffith, E.; Early, P.J.; Mariani, C.L.; Muñana, K.R.; Guevar, J.; Olby, N.J. The Effect of Electromagnetic Fields on Post-Operative Pain and Locomotor Recovery in Dogs with Acute, Severe Thoracolumbar Intervertebral Disc Extrusion: A Randomized Placebo-Controlled, Prospective Clinical Trial. *J. Neurotrauma* **2018**, *35*, 1726–1736, doi:10.1089/neu.2017.5485.
94. Granger, N.; Blamires, H.; Franklin, R.J.; Jeffery, N.D. Autologous olfactory mucosal cell transplants in clinical spinal cord injury: A randomized double-blinded trial in a canine translational model. *Brain* **2012**, *135*, 3227–3237, doi:10.1093/brain/aww268.
95. Granger, N.; Chew, D.; Fairhurst, P.; Fawcett, J.W.; Lacour, S.P.; Craggs, M.; Mosse, C.A.; Donaldson, N.; Jeffery, N.D. Use of an implanted sacral nerve stimulator to restore urine voiding in chronically paraplegic dogs. *J. Vet. Intern. Med.* **2013**, *27*, 99–105, doi:10.1111/jvim.12011.
96. Nishida, H.; Nakayama, M.; Tanaka, H.; Kitamura, M.; Hatoya, S.; Sugiura, K.; Suzuki, Y.; Ide, C.; Inaba, T. Evaluation of transplantation of autologous bone marrow stromal cells into the cerebrospinal fluid for treatment of chronic spinal cord injury in dogs. *Am. J. Vet. Res.* **2011**, *72*, 1118–1123, doi:10.2460/ajvr.72.8.1118.
97. Cizkova, D.; Murgoci, A.N.; Cubinkova, V.; Humenik, F.; Mojzisova, Z.; Maloveska, M.; Cizek, M.; Fournier, I.; Salzet, M. Spinal Cord Injury: Animal Models, Imaging Tools and the Treatment Strategies. *Neurochem. Res.* **2020**, *45*, 134–143, doi:10.1007/s11064-019-02800-w.
98. Jeffery, N.; Smith, P.M.; Lakatos, A.; Ibanez, C.; Ito, D.; Franklin, R. Clinical canine spinal cord injury provides an opportunity to examine the issues in translating laboratory techniques into practical therapy. *Spinal Cord* **2006**, *44*, 584–593, doi:10.1038/sj.sc.3101912.
99. Thomas, W.B.; Olby, N.; Sharon, L. Neurologic Conditions and Physical Rehabilitation of the Neurologic Patient. In *Canine Rehabilitation and Physical Therapy*, 2nd ed.; Millis, D.L., Levine, D., Eds.; Elsevier Saunders: Philadelphia, PA, USA, 2014; pp. 609–627.
100. Mann, F.; Wagner-Mann, C.; Dunphy, E.; Ruben, D.; Rochat, M.; Bartels, K. Recurrence rate of presumed thoracolumbar intervertebral disc disease in ambulatory dogs with spinal hyperpathia treated with anti-inflammatory drugs: 78 cases (1997–2000). *J. Vet. Emerg. Crit. Care* **2007**, *17*, 53–60, doi:10.1111/j.1476-4431.2006.00195.x.
101. English, A.W.; Wilhelm, J.C.; Ward, P.J. Exercise, neurotrophins, and axon regeneration in the PNS. *Physiology* **2014**, *29*, 437–445, doi:10.1152/physiol.00028.2014.
102. López-Álvarez, V.M.; Modol, L.; Navarro, X.; Cobianchi, S. Early increasing-intensity treadmill exercise reduces neuropathic pain by preventing nociceptor collateral sprouting and disruption of chloride cotransporters homeostasis after peripheral nerve injury. *Pain* **2015**, *156*, 1812–1825, doi:10.1097/j.pain.0000000000000268.
103. Knikou, M.; Mummidisetty, C.K. Locomotor training improves premotoneuronal control after chronic spinal cord injury. *J. Neurophysiol.* **2014**, *111*, 2264–2275, doi:10.1152/jn.00871.2013.
104. Rossignol, S.; Frigon, A. Recovery of locomotion after spinal cord injury: Some facts and mechanisms. *Annu. Rev. Neurosci.* **2011**, *34*, 413–440, doi:10.1146/annurev-neuro-061010-113746.
105. MacKay-Lyons, M. Central pattern generation of locomotion: A review of the evidence. *Phys. Ther.* **2002**, *82*, 69–83, doi:10.1093/ptj/82.1.69.
106. Roy, R.R.; Harkema, S.J.; Edgerton, V.R. Basic concepts of activity-based interventions for improved recovery of motor function after spinal cord injury. *Arch. Phys. Med. Rehabil.* **2012**, *93*, 1487–1497, doi:10.1016/j.apmr.2012.04.034.
107. Chiquoine, J.; McCauley, L.; Van Dyke, J.B. Aquatic Therapy. In *Canine Sports Medicine and Rehabilitation*, 1st ed.; Zink, M.C., Van Dyke, J.B., Eds.; Wiley-Blackwell: Ames, IA, USA, 2013; pp. 158–175.

108. Dragone, L. *Idroterapia. Fisioterapia Riabilitativa del Cane e del Gatto*, 1st ed.; Mutignani, A., Leschiera, P., Eds.; Elsevier Srl: Milano, Italy, 2011; pp. 57–75.
109. Hernández, M.P. Hidrocinestoterapia. In *Manual de Fisioterapia en Pequeños Animales*, 1st ed.; Martin, F.M., Ed.; Multimédica Ediciones Veterinarias: Barcelona, España, 2014; pp. 394–424.
110. Diniz, R. Hidroterapia. In *Fisiatria em Pequenos Animais*, 1st ed.; Lopes, R.S., Diniz, R., Eds.; Editora Inteligente: São Paulo, Brasil, 2018; pp. 156–162.
111. Languth, H.W.; Teasdall, R.D.; Magladery, J.W. Electrophysiological studies of reflex activity in patients with lesions of the nervous system. III. Motoneuron excitability following afferent nerve volleys in patients with rostrally adjacent spinal cord damage. *Bull. Johns Hopkins Hosp.* **1952**, *9*, 1257–1266.
112. Magladery, J.W.; Teasdall, R.D. Stretch reflexes in patients with spinal cord lesions. *Bull. Johns Hopkins Hosp.* **1958**, *103*, 236–241.
113. Blauch, B. Spinal reflex walking in the dog. *Vet. Med. Small Anim. Clin.* **1977**, *72*, 169–173.
114. McBride, W.J.; Hall, P.V.; Chernet, E.; Patrick, J.T.; Shapiro, S. Alterations of Amino Acid Transmitter Systems in Spinal Cords of Chronic Paraplegic Dogs. *J. Neurochem.* **1984**, *42*, 1625–1631, doi:10.1111/j.1471-4159.1984.tb12752.x.
115. Tamura, K.; Maeta, N. Efficacy of autologous bone marrow mononuclear cell transplantation in dogs with chronic spinal cord injury. *Open Vet. J.* **2020**, *10*, 206–215, doi:10.4314/ovj.v10i2.10.
116. Hu, R.; Zhou, J.; Luo, C.; Lin, J.; Wang, X.; Li, X.; Bian, X.; Li, Y.; Wan, Q.; Yu, Y.; et al. Glial scar and neuroregeneration: Histological, functional, and magnetic resonance imaging analysis in chronic spinal cord injury. *J. Neurosurg. Spine* **2010**, *13*, 169–180, doi:10.3171/2010.3.SPINE09190.
117. Moore, S.A.; Zidan, N.; Spitzbarth, I.; Nout-Lomas, Y.S.; Granger, N.; da Costa, R.C.; Levine, J.M.; Jeffery, N.D.; Stein, V.M.; Tipold, A.; et al. Development of an International Canine Spinal Cord Injury observational registry: A collaborative data-sharing network to optimize translational studies of SCI. *Spinal Cord* **2018**, *56*, 656–665, doi:10.1038/s41393-018-0145-4.
118. Hu, H.Z.; Jeffery, N.D.; Granger, N. Somatosensory and motor evoked potentials in dogs with chronic severe thoracolumbar spinal cord injury. *Vet. J.* **2018**, *237*, 49–54, doi:10.1016/j.tvjl.2018.05.007.
119. Dimitrijevic, M.R.; Hsu, C.Y.; McKay, W.B. Neurophysiological assessment of spinal cord and head injury. *J. Neurotrauma* **1992**, *9*, S293–S300.
120. Courtine, G.; Song, B.; Roy, R.R.; Zhong, H.; Herrmann, J.E.; Ao, Y.; Qi, J.; Edgerton, V.R.; Sofroniew, M.V. Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. *Nat. Med.* **2008**, *14*, 69–74, doi:10.1038/nm1682.
121. Van Den Brand, R.; Heutschi, J.; Barraud, Q.; DiGiovanna, J.; Bartholdi, K.; Huerlimann, M.; Friedli, L.; Vollenweider, I.; Moraud, E.M.; Duis, S. Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science* **2012**, *336*, 1182–1185, doi:10.1126/science.1217416.
122. Filli, L.; Engmann, A.K.; Zorner, B.; Weinmann, O.; Moraitis, T.; Gullo, M.; Kasper, H.; Schenider, R.; Schwab, M.E. Bridging the gap: A reticulo-propriospinal detour bypassing an incomplete spinal cord injury. *J. Neurosci.* **2014**, *34*, 13399–13410, doi:10.1523/JNEUROSCI.0701-14.2014.
123. Hollis, E.R.; Ishiko, N.; Pessian, M.; Tolentino, K.; Lee-Kubli, C.A.; Calcutt, N.A.; Zou, Y. Remodelling of spared proprioceptive circuit involving a small number of neurons supports functional recovery. *Nat. Commun.* **2015**, *6*, 6076–6079, doi:10.1038/ncomms7079.
124. Lovely, R.G.; Gregor, R.J.; Roy, R.R.; Edgerton, V.R. Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. *Exp. Neurol.* **1986**, *92*, 421–435, doi:10.1016/0014-4886(86)90094-4.
125. Barbeau, H.; Rossignol, S. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* **1987**, *412*, 84–95, doi:10.1016/0006-8993(87)91442-9.
126. Lavrov, I.; Gerasimenko, Y.P.; Ichiyama, R.M.; Courtine, G.; Zhong, H.; Roy, R.R.; Edgerton, V.R. Plasticity of spinal cord reflexes after a complete transection in adult rats: Relationship to stepping ability. *J. Neurophysiol.* **2006**, *96*, 1699–1710, doi:10.1152/jn.00325.2006.
127. Gerasimenko, Y.; Gorodnichev, R.; Machueva, E.; Pivovarova, E.; Semyenov, D.; Savochin, A.; Roy, R.R.; Edgerton, V.R. Novel and direct access to the human locomotor spinal circuitry. *J. Neurosci.* **2010**, *30*, 3700–3708, doi:10.1523/JNEUROSCI.4751-09.2010.
128. Rossignol, S.; Dubuc, R.; Gossard, J.P. Dynamic sensorimotor interactions in locomotion. *Physiol. Rev.* **2006**, *86*, 89–154, doi:10.1152/physrev.00028.2005.
129. Escalhão, C.C.M.; Ramos, I.P.R.; Hochman-Mendez, C.; Kasai-Brunswick, T.H.; Souza, S.A.L.; Gutfilen, B.; Goldenberg, R.C.D.S.; Coelho-Sampaio, T. Safety of Allogeneic Canine Adipose Tissue-Derived Mesenchymal Stem Cell Intraspinal Transplantation in Dogs with Chronic Spinal Cord Injury. *Stem Cells Int.* **2017**, *2017*, 1–11, doi:10.1155/2017/3053759.
130. Martin, D.; Robe, P.; Franzen, R.; Delree, P.; Schoenen, J.; Stevenaert, A.; Moonen, G. Effects of Schwann cell transplantation in a contusion model of rat spinal cord injury. *J. Neurosci. Res.* **1996**, *45*, 588–597, doi:10.1002/(SICI)1097-4547(19960901)45:5<588::AID-JNR8>3.0.CO;2-8.

131. McDonald, J.W.; Liu, X.Z.; Qu, Y.; Liu, S.; Mickey, S.K.; Turetsky, D.; Gottlieb, D.I.; Choi, D.W. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat. Med.* **1999**, *5*, 1410–1412, doi:10.1038/70986.
132. Knoller, N.; Auerbach, G.; Fulga, V.; Zelig, G.; Attias, J.; Bakimer, R.; Marder, J.B.; Yoles, E.; Belkin, M.; Schwartz, M.; et al. Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: Phase I study results. *Clin. Trial* **2005**, *3*, 173–181, doi:10.3171/spi.2005.3.3.0173.
133. Ogawa, M.; Uchida, K.; Yamato, O.; Mizukami, K.; Chambers, J.K.; Nakayama, H. Expression of Autophagy-Related Proteins in the Spinal Cord of Pembroke Welsh Corgi Dogs With Canine Degenerative Myelopathy. *Vet. Pathol.* **2015**, *52*, 1099–1107, doi:10.1177/0300985815570070.
134. Tsuji, O.; Miura, K.; Okada, Y.; Fujiyoshi, K.; Mukaino, M.; Nagoshi, N.; Kitamura, K.; Kumagai, G.; Nishino, M.; Tomisato, S.; et al. Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12704–12709, doi:10.1073/pnas.0910106107.
135. Dissanayaka, W.L.; Zhu, X.; Zhang, C.; Jin, L. Characterization of dental pulp stem cells isolated from canine premolars. *J. Endod.* **2011**, *37*, 1074–1080, doi:10.1016/j.joen.2011.04.004.
136. Ding, Y.; Yan, Q.; Ruan, J.W.; Zhang, Y.Q.; Li, W.J.; Zhang, Y.J.; Li, Y.; Dong, H.; Zeng, Y.S. Electroacupuncture promotes survival, differentiation of the bone marrow mesenchymal stem cells as well as functional recovery in the spinal cord-transected rats. *BMC Neurosci.* **2009**, *10*, 35, doi:10.1186/1471-2202-10-35.
137. Yan, Q.; Ruan, J.W.; Ding, Y.; Li, W.J.; Li, Y.; Zeng, Y.S. Electro-acupuncture promotes differentiation of mesenchymal stem cells, regeneration of nerve fibers and partial functional recovery after spinal cord injury. *Exp. Toxicol. Pathol.* **2011**, *63*, 151–156, doi:10.1016/j.etp.2009.11.002.
138. Prado, C.; Fratini, P.; Matias, G.S.; Bocabello, R.Z.; Monteiro, J.; Santos, C.J.; Joaquim, J.G.; Giglio, R.F.; Possebon, F.S.; Sakata, S.H.; et al. Combination of stem cells from deciduous teeth and electroacupuncture for therapy in dogs with chronic spinal cord injury: A pilot study. *Res. Vet. Sci.* **2019**, *123*, 247–251, doi:10.1016/j.rvsc.2019.01.011.
139. Côté, M.P.; Ménard, A.; Gossard, J.P. Spinal cats on the treadmill: Changes in load pathways. *J. Neurosci.* **2003**, *23*, 2789–2796, doi:10.1523/JNEUROSCI.23-07-02789.2003.
140. Côté, M.P.; Gossard, J.P. Step training-dependent plasticity in spinal cutaneous pathways. *J. Neurosci.* **2004**, *24*, 11317–11327, doi:10.1523/JNEUROSCI.1486-04.2004.
141. Martinez, M.; Brezun, J.M.; Zennou-Azogui, Y.; Baril, N.; Xerri, C. Sensorimotor training promotes functional recovery and somatosensory cortical map reactivation following cervical spinal cord injury. *Eur. J. Neurosci.* **2009**, *30*, 2356–2367, doi:10.1111/j.1460-9568.2009.07019.x.
142. Bregman, B.S. Regeneration in the spinal cord. *Curr. Opin. Neurobiol.* **1998**, *8*, 800–807, doi:10.1016/S0959-4388(98)80124.
143. Thomas, S.L.; Gorassini, M.A. Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury. *J. Neurophysiol.* **2005**, *94*, 2844–2855, doi:10.1152/jn.00532.2005.
144. Olby, N.J.; da Costa, R.C.; Levine, J.M.; Stein, V.M.; The Canine Spinal Cord Injury Consortium (CANSORT SCI). Prognostic Factors in Canine Acute Intervertebral Disc Disease. *Front. Vet. Sci.* **2020**, *7*, 596059, doi:10.3389/fvets.2020.596059.
145. Duval, J.; Dewey, C.; Roberts, R.; Aron, D. Spinal cord swelling as a myelographic indicator of prognosis: A retrospective study in dogs with intervertebral disc disease and loss of deep pain perception. *Vet. Surg.* **1996**, *25*, 6–12, doi:10.1111/j.1532-950x.1996.tb01371.x.
146. Scott, H.W. Hemilaminectomy for the treatment of thoracolumbar disc disease in the dog: A follow-up study of 40 cases. *J. Small Anim. Pract.* **1997**, *38*, 488–494, doi:10.1111/j.1748-5827.1997.tb03303.x.
147. Scott, H.W.; McKee, W.M. Laminectomy for 34 dogs with thoracolumbar intervertebral disc disease and loss of deep pain perception. *J. Small Anim. Pract.* **1999**, *40*, 417–422, doi:10.1111/j.1748-5827.1999.tb03114.x.
148. Ito, D.; Matsunaga, S.; Jeffery, N.D.; Sasaki, N.; Nishimura, R.; Mochizuki, M.; Kasahara, M.; Fujiwara, R.; Ogawa, H. Prognostic value of magnetic resonance imaging in dogs with paraplegia caused by thoracolumbar intervertebral disk extrusion: 77 cases (2000–2003). *J. Am. Vet. Med. Assoc.* **2005**, *227*, 1454–1460, doi:10.2460/javma.2005.227.1454.
149. Tamura, K.; Harada, Y.; Nagashima, N.; Itoi, T.; Ishino, H.; Yogo, T.; Nezu, Y.; Hara, Y.; Suzuki, Y.; Ide, C.; et al. Autotransplanting of Bone Marrow-Derived Mononuclear Cells for Complete Cases of Canine Paraplegia and Loss of Pain Perception, Secondary to Intervertebral Disc Herniation. *Exp. Clin. Transplant* **2012**, *10*, 263–272, doi:10.6002/ect.2011.0151.

4. ARTICLE 5

Animals (2021), 11(1994):1-13.

Spinal Locomotion in Cats Following Spinal Cord Injury: A Prospective Study

Spinal locomotion in cats following spinal cord injury: A prospective study

Ângela Martins ^{1,2,5*}, Cátia Silva^{1,2}, Débora Gouveia^{2,3}, Ana Cardoso ², Tiago Coelho ², Óscar Gamboa ⁴, Eduardo Marcelino ^{1,5} and António Ferreira ^{4,5}

¹Faculty of Veterinary Medicine, Lusófona University, Campo Grande, Lisboa, Portugal;

²Animal Rehabilitation Center, Arrábida Veterinary Hospital, Azeitão, Setúbal, Portugal;

³Superior School of Health, Protection and Animal Welfare, Polytechnic Institute of Lusophony, Campo Grande, Lisboa, Portugal;

⁴Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal;

⁵CIISA-Centro Interdisciplinar -investigação em Saúde Animal – Faculdade de Medicina Veterinária, Av. Universidade Técnica de Lisboa, 1300-477 Lisboa, Portugal.

* Correspondence: vetarrabida.lda@gmail.com

Simple Summary: Functional neurorehabilitation promotes neural reorganization by stimulating subjects without deep pain perception, leading to a faster recovery when compared to spontaneous recovery and achieving fewer compensatory errors, or even deviations to neuropathic or adaptive pain pathways, such as spasticity. The present study demonstrates the importance of intensive and repetition-based functional neurorehabilitation, essential for subjects classified as Grade 0 according to the modified Frankel scale.

Abstract: This article aimed to evaluate the safety and efficacy of intensive neurorehabilitation in paraplegic cats, with no deep pain perception (Grade 0 on the modified Frankel scale), with more than three months of injury. Nine cats admitted to the Arrábida Veterinary Hospital/Arrábida Animal Rehabilitation Center (CRAA) were subjected to a 12-week intensive functional neurorehabilitation protocol based on ground and underwater treadmill locomotor training, electrostimulation, and kinesiotherapy exercises, aiming to obtain a faster recovery to ambulation and a modulated locomotor pattern of flexion/extension. Of the nine cats that were admitted in this study, 56% (n=5) recovered ambulation, 44% of which (4/9) did so through functional spinal locomotion by reflexes, while one achieved this through the recovery of deep pain perception. These results suggest that intensive neurorehabilitation can play an important role in ambulation recovery, allowing for a better quality of life and well-being, which may lead to a reduction in the number of euthanasia procedures performed on paraplegic animals.

Keywords: spinal cord injury; treadmill training; central pattern generator; spinal locomotion; reflexes; cats; neurorehabilitation

1. Introduction

Locomotion is based on the synchronization between flexion and extension of the limbs and reflex circuits of the spinal cord. However, the spinal cord alone is not sufficient to enable walking, and the input of the brain centers is also necessary [1]. The alternation between extension and flexion of the pelvic limbs involves the central pattern generators (CPGs). The CPGs are spinal locomotor circuits with pacemaker properties,

located in the thoracolumbar spinal cord, which generates bilateral rhythmic and repetitive contractions and relaxation of the flexors and extensor muscles in the absence of descending motor tracts and supraspinal inputs [1,2]. The supraspinal control of the CPGs originates in the nuclei located in the midbrain ("mesencephalic locomotor region"). These nuclei initiate locomotion by activating the reticulospinal neurons in the brain stem. In cats, there are two major descending motor tracts: the medial longitudinal fascicle, with cells originating in the medial pontomedullary reticular formation, and the lateral vestibulospinal tract [3].

Locomotor training after spinal cord injury is based on the principle that sensory stimuli reactivate and reorganize the spinal locomotor circuit [4]. Over the last decade, the expression of the hind limb locomotion after SCI at T13 in kittens [5,6] and adult cats has been investigated [7,8]. After 2–3 weeks of land treadmill training, both were able to walk and recover full weight bearing. To modulate the flexor-extensor pattern, some researchers suggest the association of pharmacologic management [9,10]. Thus, the adult cat spinal cord, *in vivo*, was proposed as a classical model in studies of mammalian hindlimb CPGs [11-13], allowing the translation of these findings to a clinical setting.

In this study, intensive neurorehabilitation is used to promote neural locomotion control. This control is based on spinal cord autonomy, the influence of sensory stimuli on the spinal cord autonomy, neuromodulation, and learning capacity of the spinal cord locomotor circuit and, finally, the importance of the descending pathways in the control of locomotion [14]. This paper aimed to evaluate the safety and efficacy of intensive neurorehabilitation in Grade 0 paraplegic cats, with more than three months of injury, in a clinical environment.

2. Materials and Methods

This prospective clinical study was conducted in HVA/CRAA, from 15 September 2014 to 15 March 2021, according to the Faculty of Veterinary Medicine, ULHT ethics committee, after the owner's consent.

2.1 Characterization of the population

For the present study, only cats with a severe contusion of traumatic origin, hit by a car or injured from a fall, were included. The diagnosis was obtained by radiology and myelotomography. At 24/48 h after SCI, imaging exams showed edema (a presence of hypoattenuating fluid/tissue adjacent to the lesion site) and an absence of vertebral spine damage (fracture and/or luxation). The lesions were localized between T3 and L3 and had to be more than 3 months old. In addition, cats had to exhibit Grade 0 in the modified Frankel scale (MFS), i.e., be paraplegic with an absence of deep pain perception (DPP) and a lack of superficial sensitivity (SS), at the level of the tail (base and tip) and perineum. The upper motor neuron (UMN) injury led to a neurogenic bladder.

Cats with compressive myelopathies, intervertebral disc disease (IVDD), mainly spinal cord extrusion, neoplastic disease, and others were excluded from the study. Cats presenting severe contusions with neurolocalization beyond T3-L3 as well as cats with contusions at T3-L3, but with a degree higher than 0, according to MFS, were also excluded.

2.2 Plan of the study

Upon admission to the study, the neurorehabilitation consultation was performed, and the subjects were redirected to CRAA. The Intensive Neurorehabilitation Protocol (INRP) was initiated 24 h after admission, allowing for accommodation to the new environment. The INRP was performed 6 days a week, from 10 a.m. to 6 p.m. In addition to the re-evaluations of the INRP in accordance to the neurological development, cats were evaluated over a period of 2 to 3 months, always by the same person, in the same environment, and at the same time, with a video recording of steps taken on alternate floor types, using a Canon EOS Rebel T6 1300D camera. The reevaluation was only recorded at specific time points, as described later. The cats that reached ambulation before 3 months (time limit for INRP) were discharged from the hospital and redirected to the neurorehabilitation follow-up appointment.

2.3 Neurorehabilitation consultation at study admission

Cats were classified as Grade 0 in the MFS and as paraplegic. Peripheral spinal reflexes were evaluated through percussion with Taylor's hammer. In the evaluation of peripheral spinal reflexes for the pelvic limb, the patellar reflex (PR), the tibial cranial reflex (TCR), the crossed extensor reflex (CER), and the flexor reflex (FR) were assessed. With cats in sternal recumbency, the cutaneous trunci reflex (CTR) was evaluated. Contracture or shortening of the femoral quadriceps muscle, associated with hypotonicity of the flexor muscle group, was observed in all subjects. The muscle tone of the rectus abdominis (RA) was also evaluated. Finally, sensitivity to superficial pain was assessed, i.e., the dermatomes of the pelvic limb, followed by an evaluation of deep pain perception in the medial and lateral digit of each limb. Twelve-centimeter straight-ended Halstead mosquito forceps were used for this procedure.

2.4 Intensive Neurorehabilitation Protocol

The INRP consisted mainly of locomotor training and electrostimulation. Regarding locomotor training, there were two modalities: bipedal locomotor training (BLT) and quadrupedal locomotor training (QLT) (Table 1).

In BLT, the subject was placed on the land treadmill with the thoracic limbs on a smooth surface platform, and gait movement was assisted only in the pelvic limbs. The therapist sat behind the cat and performed cycling movements at a constant pace. Initially, the BLT was executed 3–6 times a day for 2–5 min, increasing progressively to 3 times a day, for about 20 min, 6 days a week. Regarding the velocity of the treadmill, in the initial stage, we started at 0.8 Km/h (0.22 m/s) and increased to 1.2 Km/h (0.33 m/s), without slope.

In the first stage, QLT (Figure 1) was performed 4–8 times a day for 2–5 min, aiming at sessions longer than 30 min. In the final stage, the training advanced to 3 times a day, 6 days a week. The velocity of the treadmill was between 1 Km/h (0.27 m/s) and 1.8 Km/h (0.5 m/s), with a 10–25% slope.



Figure 1. Quadrupedal Locomotor Training (QLT) performed on a land treadmill.

In both modalities, the therapist stimulated the perineal region and base of the tail to induce stepping and progressively supported the weight by holding the tail. In the initial stage, the therapist performed cycling movements in alternation with the perineal and base of the tail stimulation.

Table 1. Locomotor training performed by the cats.

Locomotor Training		
Animal	Treadmill	Underwater Treadmill
I	Bipedal first 15 days and then quadrupedal	Bipedal first 15 days and then quadrupedal
II	Bipedal	Not tolerated
III	Bipedal first 15 days and then quadrupedal	Bipedal first 15 days and then quadrupedal
IV	Bipedal first 15 days and then quadrupedal	Bipedal first 7 days and then quadrupedal
V	Quadrupedal	Not tolerated
VI	Quadrupedal	Quadrupedal
VII	Bipedal first 15 days and then quadrupedal	Not tolerated
VIII	Bipedal first 15 days and then quadrupedal	Not tolerated
IX	Bipedal	Bipedal

In the underwater treadmill locomotor training (UWT), cats were fitted on a harness in such a way that a guidance rope system was provided by the rehabilitation technician outside the underwater treadmill. This type of training was conducted once a day, 5 days a week, from 5 min up to 40 min, according to subject compliance, with a 10% slope. The temperature of the water was 24–26 °C, and after UWT, cats were dried with an absorbent towel in a calm environment.

To complement the locomotor training, passive, passive-assisted, and active kinesiotherapy exercises were administered, such as postural standing stimulation (2–5 min, 4–6 times a day), gradually introducing difficulties with postural standing on the trampoline or on balance boards (3–5 times a day, 5 days a week) (Figure 2), and on cavaletis rails, for 2 min, 2–4 times a day, 5 days a week.



Figure 2. Kinesiotherapy exercises—postural standing stimulation on a balance board.

The locomotor training was associated with electrical stimulation. Segmental electrical stimulation was prescribed according to the sciatic nerve path, with one electrode in the anatomical region L7- S1/S2 and the other in the ventral-medial region of the thigh, close to the motor point of the hamstring muscles (biceps femoris, semitendinosus, and semimembranosus) (Figure 3). The parameters of the pulsed biphasic electric current were 40-60 Hz, up to 24 mA, with a working cycle from 1 shot to 4 at rest (1:4) and a ramp 4 s upward, 8 s at the plateau, and 2 s downward. This was executed twice a day, for 10 min, 5 days a week. Milliamperage was applied with a constant observation of the muscle so that the limit was a maximum involuntary contraction but still comfortable for the subject.



Figure 3. Functional electrical segmental stimulation of the sciatic nerve.

2.4 Monitoring of the study population

All cats were evaluated at the time of inclusion in the study (M0). The neurological evolution up to the ambulatory state was evaluated after 15 days of hospitalization (M1) and then, reevaluated after 4 weeks (M2), 8 weeks (M3), and 12 weeks (M4) of hospitalization. Follow-up appointments were made 15 days (R1), 1 month (R2), 3 months (R3), 6 months (R4), 1 year (R5), 2 years (R6), and 3 years (R7) after medical discharge. The temporal space between neurorehabilitation appointments is schematically shown in Figure 4.

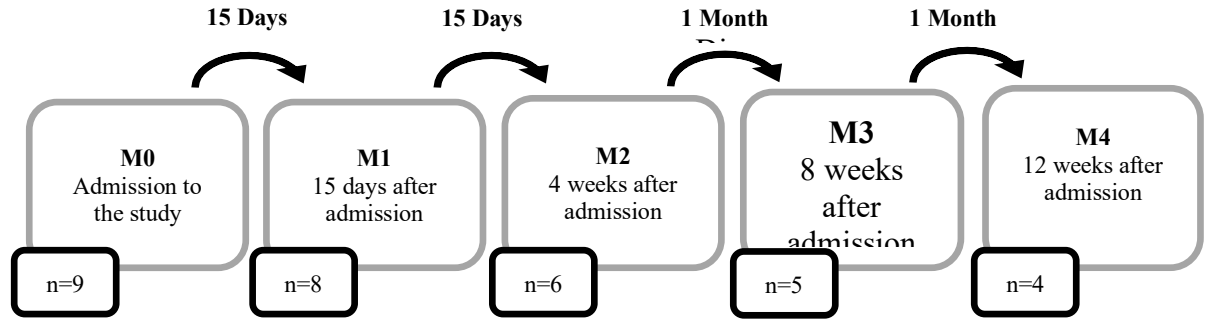


Figure 4. The schematization of the time interval between neurorehabilitation appointments.

At every appointment (from M0 to M4 and from R1 to R7), cats were evaluated according to the following: active postural standing capacity, the presence of a flexor reflex, a crossed extensor reflex, CTR, rectus abdominis muscle tone, superficial sensitivity in the perineum and tail (base and tip), evaluations of deep pain perception in the medial and lateral digits, and gait stimulation on different floors.

Ambulation is related to motor capacity whereby one can perform more than 10 steps independently of the floor and perform curves with a greater support base, demonstrating slight squat secondary to muscle weakening, without ever falling, to perform, for example, micturition, automatic or voluntary urination.

3. Results

The database and statistical analyses were performed with Excel 2013 ® (Microsoft, USA).

In this study (n=9), cats had a mean age of 3.9 years, 67% (n=6) female and 33% (n=3) male, with a mean body weight of 4.4 kg.

Four cats (44%) presented the flexor reflex bilaterally, four (44%) presented a diminished flexor reflex, and one (11%) presented an exaggerated flexor reflex. After the INRP, 89% (n=8) of the cats presented a flexor reflex and only 1 (11%) presented a clonic flexor reflex, as seen in Table 2.

Table 2. Classification of peripheral spinal reflexes and muscle tone of the rectus abdominis, before and after the application of INRP (medical discharge).

Animal	FR		CTR		CER		RA	
	Before INRP	After INRP	Before INRP	After INRP	Before INRP	After INRP	Before INRP	After INRP
I	Present bilateral	Normal	Present at the site of lesion *	Recovery (since the lumbosacral region)	Present bilateral	Normal/ Modeled with flexor reflex	Normal	Normal
II	Present bilateral exaggerated	Clonic	Present at the site of lesion *	Present at the site of lesion *	Present bilateral	Normal/ Modeled with flexor reflex	Decreased	Normal

III	Present bilateral	Normal	Present at the site of lesion *	Recovery (since the lumbosacral region)	Present bilateral	Normal/ Modeled with flexor reflex	Decreased	Normal
IV	Present bilateral decreased	Normal	Present since the lumbosacral region	Recovery (since the lumbosacral region)	Present bilateral decreased	Normal/ Modeled with flexor reflex	Decreased	Normal
V	Present bilateral decreased	Normal	Present at the site of lesion *	Recovery (since the lumbosacral region)	Present bilateral	Normal/ Modeled with flexor reflex	Decreased	Normal
VI	Present bilateral	Normal	Present at the site of lesion *	Recovery (since the lumbosacral region)	Present bilateral	Normal/ Modeled with flexor reflex	Normal	Normal
VII	Present bilateral	Normal	Absent at the site of lesion	Present at the site of lesion *	Present bilateral decreased	Decreased	Decreased	Normal
VIII	Present bilateral decreased	Normal	Absent at the site of lesion	Present at the site of lesion *	Absent	Decreased	Decreased	Decreased
IX	Present bilateral decreased	Normal	Absent at the site of lesion	Absent at the site of lesion	Present bilateral decreased	Decreased	Decreased	Decreased

FR: flexor reflex; CTR: cutaneous trunci reflex; CER: crossed extensor reflex; RA: Muscle tone of the rectus abdominis; * lesion is approximately two vertebral bodies cranial to the cutoff point.

Regarding the CTR, at admission, 56% (n=5) had a CTR at the lesion site (note that lesion is approximately two vertebral bodies cranial to the cutoff point), 33% (n=3) exhibited an absence of a CTR at the lesion site, and one showed a CTR in the lumbosacral region. Following the INRP, 56% (n=5) demonstrated a recovery with the CTR in the lumbosacral region, 33% (n=3) presented a CTR at the lesion site, and one showed no signs of neurological improvement, the CTR remaining absent at the site of the lesion.

Regarding the neurological grade (Table 3), all cats (n=9) were paraplegic, without DPP, at admission, whereas, after undergoing INRP, 44% (n=4) of the cats presented functional spinal locomotion by reflexes, i.e., ambulation, 22% (n=2) had non-functional spinal locomotion by reflexes, 22% remained paraplegic without DPP, and 11% (n=1) recovered DPP and stayed paraparesic ambulatory with proprioceptive ataxia.

In the study, non-functional spinal locomotion was considered when the cats exhibited a flexor-extensor pattern, moving on the floor or with weight support by holding the tail, without being able to stand up.

In 44% (n=4) of the cats, the remodeling regarding superficial sensitivity was evident because three cats had superficial pain at the perineum, although two of them also presented this at the tip and base of the tail. Only one cat regained DPP, although when comparing the deep

pain of the thoracic limbs, a slower reaction was found. The results of the present study are summarized in Figure 5.

Neurological Grade before INRP		Neurological Grade after INRP
I	Paraplegic	Functional spinal locomotion by reflexes
II	Paraplegic	Non-functional spinal locomotion by reflexes
III	Paraplegic	Functional spinal locomotion by reflexes
IV	Paraplegic	Paraparesis ambulatory with proprioceptive ataxia
V	Paraplegic	Functional spinal locomotion by reflexes
VI	Paraplegic	Functional spinal locomotion by reflexes
VII	Paraplegic	Non-functional spinal locomotion by reflexes
VIII	Paraplegic	Paraplegic
IX	Paraplegic	Paraplegic

Table 3. Ambulation capacity at the end of the study.

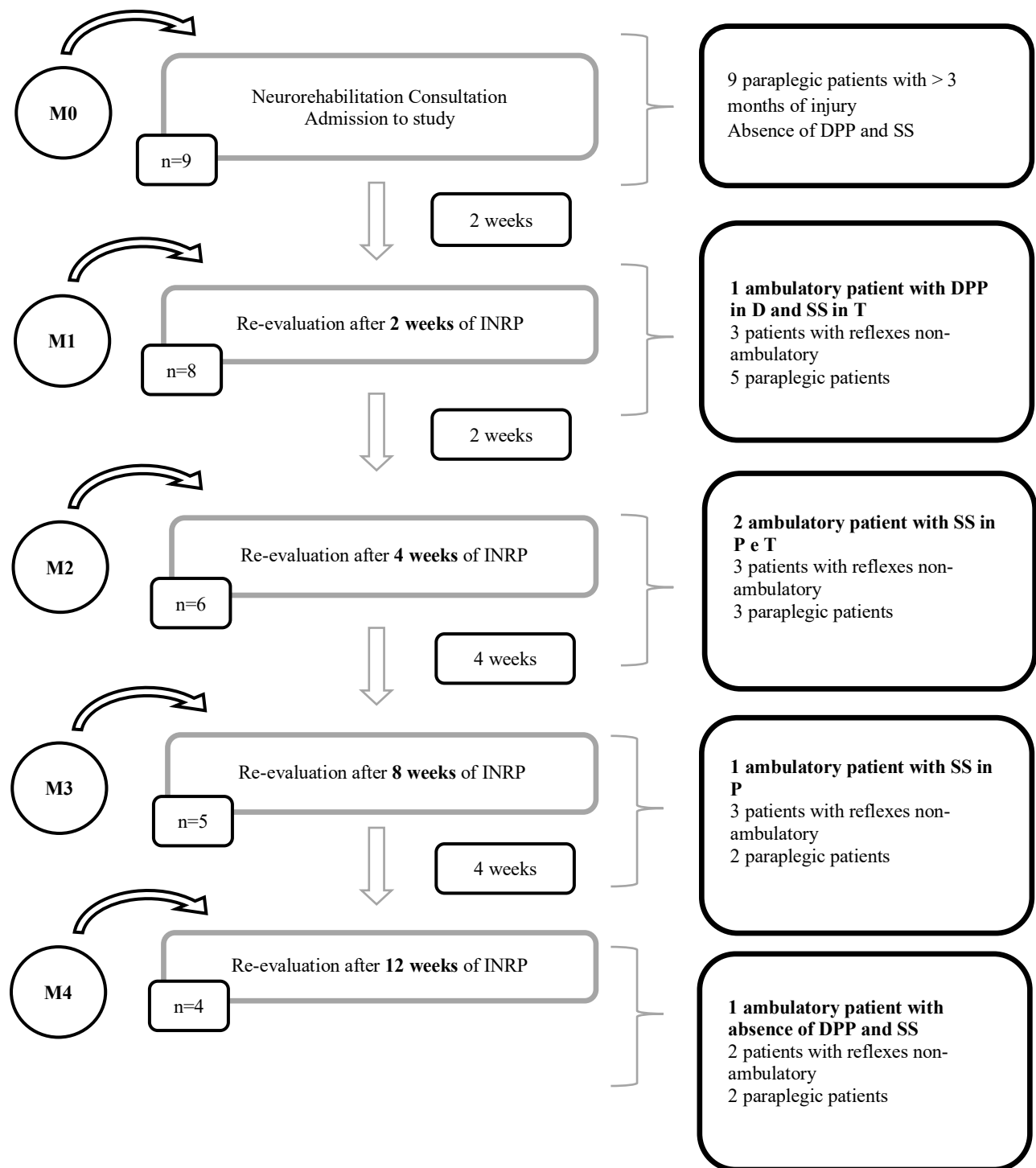


Figure 5. Patient progression algorithm in the study. DPP: Deep Pain Perception; SS: Superficial Sensitivity; P: Perineum; T: Tail; D: Digits.

4. Discussion

This study used nine cats diagnosed with a severe contusion of traumatic origin, in a chronic state, i.e., more than 3 months after injury.

Since spontaneous neurological recovery after injury reaches a plateau usually within the first few months, the neurological evolution observed is expected to be the consequence of the INRP, rather than the continuation of spontaneous recovery [15-16]. Furthermore, the fact that stimulation through INRP was extended for 3 months, and that the plateau indicative of motor recovery occurs between 2 to 3 months [7,17,18], also accounts for INRP for the neurologic improvement of cats.

After spinal cord injury, both humans and cats can experience spontaneous recovery that is observed even without functional rehabilitation and can be explained by the same cellular mechanisms that can occur in spontaneous remodeling [19-21]. This makes it difficult to separate, concerning the clinical signs indicative of neurological recovery over time, the evolution that is mainly due to the action of rehabilitation training from that of spontaneous recovery [22].

Early and important studies in neuroscience were performed in acute and chronic adult spinal cats and were based on a complete section of the spinal cord in a laboratory setting, which resulted in the paralysis of the pelvic limbs (Grade 0 in MFS) [4,7,8,23,24]. The breakthrough of intensive rehabilitation in SCI was based on the studies mentioned above [22]. Furthermore, training procedures that showed small stepping movements on the land treadmill led to a marked improvement in the locomotor performance [25], although it appears that the type of locomotor training was the most important factor in the recovery of locomotion [26].

The main difference between those studies, when compared to ours, is the velocity applied on the treadmill. In our study, locomotor training was performed, in some cases, bipedal training that progressed to quadrupedal training, which allowed the spinal cats to adapt to the treadmill and made it possible to reach higher treadmill speeds. Lovely et al., 1990 [26], achieved stepping on the land treadmill with velocities of 0.05-0.15 m/s-1, which was a slow speed when compared to our study (0.22 m/s increasing to 0.5 m/s). The maximum land treadmill speed at which spinal locomotion could be obtained was reported from 0.5 to 0.6 m/s, only after 3 weeks of training [27]. Our study accords with these training procedures.

Lovely et al., 1990 [26], also performed daily locomotor training, similar to our protocol, but with an early training approach in the acute phase, justifying their results. The same study showed that a period of 4–6 months was needed to achieve ambulation, while Barbeau and Rossignol (1987) proved that acute spinalized adult cats (T13) were capable of demonstrating a gait with the weight support of the hindlimbs without showing knuckling, from 3 weeks up to 1 year [7]. On the other hand, Edgerton et al., 1991 [28], performed 30 min of daily training for 6 months reaching a performance plateau at 2–4 months. Regarding time, this training was similar to the one implemented in our protocol, although 33% (n=3) reached a plateau performance in 1 month.

Barbeau and Rossignol, 1990 [29], introduced pharmacological management in cats between 1 and 3 months after spinalization. Cats reached a treadmill velocity of 1.0 m/s before and after an injection of a serotonergic monoamine drug (5-HT). They concluded that the injections of 5-HT increased the cycle duration by about 80% and that noradrenergic, dopaminergic, and GABAergic drugs could improve the initiation of the

locomotion (in an early stage) and modulate the well-established locomotor pattern (in a chronic stage). In addition, studies were performed on the association between pharmacologic management and FES [30]. In this regard, some results showed that electrical stimulation could initiate or modify locomotor activity in a similar way to pharmacological neural modulation [25]. These findings show that the use of electrostimulation can be a neurorehabilitative modality used to enhance the restoration of full weight-bearing locomotor function [31].

Smith et al., 1982 [32], demonstrated that spinal cats could exhibit excellent weight support during locomotion, applying treadmill speeds of ≤ 0.8 m/s, which suggested velocity as a major factor in the recovery of ambulation. In addition, results have shown that, at the first 7–10 days post section, cats only executed small hindlimb movements with little to no weight support, which could be enhanced with pharmacological treatment, stimulating the central plasticity that could be reflected by the evolution of the locomotor pattern over time [10].

When compared to our study, on both modalities of the locomotor training (BLT and QLT), combined with FES, ambulation was obtained in 56% (n=5). Thus, only 10% of the white matter of the spinal cord is required to initiate and maintain the locomotor pattern, in a voluntary way, on the land treadmill [33], which can probably be justified by the residual descending motor tracts post-injury [34]. In our study, one cat recovered DPP, obtaining medical discharge after 2 weeks of INRP. As for the other three cats exhibiting SS, two had medical discharge 1 month after INRP and the other one did 2 months afterwards. It is suggested that severe injury contains sub-functional connections that are capable of transmitting a supraspinal influence on the neural circuits of CPG, below the injury [35,36]. Dimitrijevic et al., 1987, refers to this type of lesion as “discomplete SCI”, in agreement with Gerasimenko et al., 2017 [37], which suggests that locomotor training can significantly improve sensorimotor and autonomic function after SCI [38].

In the neurophysiological field, it has been proposed that the use of an $\alpha 2$ -noradrenergic agonist, such as clonidine, could initiate treadmill locomotion faster in cats with acute and chronic spinal injury [39] and modulate the spinal locomotor pattern [10], allowing fictive locomotion, which may be recovered spontaneously in several weeks. Though in chronic spinal cats, the activation of the receptors does not express the spontaneous spinal locomotion [40] excitatory amino acid receptor agonist NMDA, injected intrathecally, resulted in a dramatic improvement in the locomotor pattern.

In some studies, fictive locomotion has been achieved. This fictive locomotion could be defined as a rhythmic pattern that occurs in the absence of any movement [2, 41], and in chronic spinal cats, it can occur spontaneously, which indicates functional changes in the interneurons and CPGs [4,42].

Our strict INRP suggested that BLT could activate proprioceptive afferents (Groups Ia, Ib, and II) [42, 43] throughout stretch bicycle movements, performed by the therapist, in alternation with the stimulation of the perineal region [44] and the base of the tail [19], which regulated, in some part, the duration of various sub-phases of the step cycle (frequency and speed). Moreover, the hip joint influences the

locomotor rhythmic generation and has a potent effect on the intrinsic neural circuits because, when the hip is flexed, the spinal cat stops the stepping movement [45]. Furthermore, the QLT may activate the CPG that regulates the fore and hindlimb locomotion, and this can be blocked when the propriospinal pathways that connect the cervical and lumbar enlargements are interrupted [46, 47]. Thus, the QLT stimulates the coordinating propriospinal system, providing forelimb–hindlimb coordination [48]. In our study, the cat that showed the recovery of DPP could perform QLT in the first instance.

The QLT presumably increases the synaptic activity by interneuron stimulation, responsible for locomotor patterns and intrinsic electrophysiological proprieties [49,50], vital to neural control [51]. Locomotor training can have an impact on spinal cord autonomy, influence sensory inputs, induce neuromodulation and learning abilities of the spinal network, promote depolarization of the descending motor pathways [51], and upregulate the neurotrophins, particularly the neurotrophic factor derived from the brain (BDNF) that plays an important role in central nervous system (CNS) neuroplasticity, contributing to the restoration of function [25]. The intense repetitive training may have driven neuroplasticity, which eventually results in the ability of voluntary movement, by reorganizing its structure, function, and connections [52-56].

The QLT promotes stimulation of the afferent pathways by receptors, located in the muscles (intrafusal fibers), joints (nerve endings), and skin. This type of stimulation allows a dynamic interaction with the CPG circuit [3,14,57], and cutaneous neural stimuli in the digit region that stimulates the expression of reflex locomotion, allowing the observation of neural reorganization after four weeks of locomotor training on the treadmill [7,19,58]. During the exercises several interconnected components are activated, necessary to obtain correct locomotion [4].

The vestibulospinal and reticulospinal tracts are responsible for posture, meaning that they have a greater positive effect on the extensor muscles [59], so the introduction of the slope during QLT will stimulate these muscles and therefore the neuroplasticity of the tracts mentioned above. Thus, 10° of a slope are suggested by Maier et al., 2009 [60], and 25° are suggested by Tillakaratne et al., 2014 [61].

Locomotor training that allows neural plasticity should be performed for 30–60 min [14,20], in agreement with the INRP presented in this study. The INRP is associated with kinesiotherapy exercises that play a role in synaptic neuroplasticity in the sense of neuroremodeling [62,63]. To achieve a balance between excitation and inhibition, in the passage of an obstacle, there is a stimulation of receptors located in the distal dorsal region of the limb that will allow excitation of the flexor muscle group of the ipsilateral limb, when performing the protraction phase, and that, with the same stimulus, can excite the extensor muscles necessary to obtain the postural phase of the step cycle [64]. An example of the above is active-assisted and/or active kinesiotherapy exercises, which are included in locomotor training [65]. Included in the INRP are the cavaletis rails for stimulating the passage of obstacles.

The cat that recovered DPP showed fast progression when performing these exercises, supporting the notion that DPP is considered

a favorable prognostic factor [66-68,34]. Therefore, the ability to execute early locomotor training, reaching the mentioned performance guidelines, may suggest a higher probability of recovery ambulation in a shorter amount of time.

The limitations of our study are the small population sample and the fact that the cats had greater difficulties adapting to the INRP requiring a longer period.

5. Conclusions

The present prospective study demonstrates the importance of intensive and repetition-based functional neurorehabilitation in spinal cord injuries since INRP allowed 56% of chronic paraplegic cats with no DPP to recover ambulation.

The protocols of intensive functional neurorehabilitation are safe and can allow a significant improvement of the neurological state of spinal cats.

Thus, in cats with Grade 0 in the MFS, it is suggested that early locomotor training for at least 30-60 min, attaining speeds of 0.5-0.6 m/s, associated with FES, is needed, allowing for the stimulation of the descending motor tracts and the afferent inputs, inducing neuromodulation, and promoting the learning ability of the spinal network. Given its limitations, this study should be continued in a clinical setting.

Author Contributions: Conceptualization A.M.; methodology A.M.; validation A.M. and E.M.; formal analysis, A.M., E.M., and A.F.; investigation, A.M., D.G., A.C., T.C., O.G., writing—original draft preparation, A.M., C.S., D.G., and A.C.; writing—review and editing E.M.; supervision A.M.; project administration A.M.; all authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study received the approval of the Ethics and Animal Welfare Commission of the Lusófona University of Humanities and Technology (CEBEA- ULHT) (process nº 106-2021).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Thomson, C., & Hahn, C. (2012). *Veterinary Neuroanatomy A Clinical Approach*. Elsevier.
2. Uemura, E. (2017b). *Sistema Motor*. In H. H. Dukes & W. O. Reece, Dukes: *Fisiologia Dos Animais Domésticos*. 13th ed. Guanabara Koogan.
3. Mackay-Lyons, M. (2002). Central Pattern Generation of Locomotion: A Review of the Evidence. In *Physical Therapy* (Vol. 82). <https://academic.oup.com/ptj/article-abstract/82/1/69/2837028>
4. Rossignol, Serge, & Frigon, A. (2011). Recovery of locomotion after spinal cord injury: Some facts and mechanisms. *Annual Review of Neuroscience*, 34, 413–440. <https://doi.org/10.1146/annurev-neuro-061010-113746>
5. Forssberg, H., Grillner, S., & Halbertsma, J. (1980a). The locomotion of the low spinal cat I. Coordination within a hindlimb. *Acta Physiologica Scandinavica*, 108(3), 269–281. <https://doi.org/10.1111/j.1748-1716.1980.tb06533.x>

6. Forssberg, H., Grillner, S., Halbertsma, J., & Rossignol, S. (1980b). The locomotion of the low spinal cat. II. Interlimb coordination. *Acta Physiologica Scandinavica*, 108(3), 283–295. <https://doi.org/10.1111/j.1748-1716.1980.tb06534.x>
7. Barbeau, H., & Rossignol, S. (1987). Recovery of locomotion after chronic spinalization in the adult cat. In *Brain Research* (Vol. 412).
8. Belanger, M., Drew, T., Provencher, J., & Rossignol, S. (1996). A Comparison of Treadmill Locomotion in Adult Cats Before and After Spinal Transection. In *JOURNAL OF NEUROPHYSIOLOGY* (Vol. 76, Issue 1). www.physiology.org/journal/jn
9. Barthélemy, D., Leblond, H., Provencher, J., & Rossignol, S. (2006). Nonlocomotor and locomotor hindlimb responses evoked by electrical microstimulation of the lumbar cord in spinalized cats. *Journal of Neurophysiology*, 96(6), 3273–3292. <https://doi.org/10.1152/jn.00203.2006>
10. Chau, C., Barbeau, H., & Rossignol, S. (1998). Early Locomotor Training With Clonidine in Spinal Cats.
11. Graham Brown, T. (1914). On The Nature of The Fundamental Activity of The Nervous Centers; Together With an Analysis of The Conditioning of Rhythmic Activity in Progression, and a Theory of The Evolution of Functional in The Nervous System.
12. Grillner, S., & Zangger, P. (1979). On the Central Generation of Locomotion in the Low Spinal Cat. In *Brain Res* (Vol. 34).
13. Jankowska, E., Jukes, M.G.M., Lund, S. and Lundberg, A. (1967), The Effect of DOPA on the Spinal Cord 6. Half-centre organization of interneurons transmitting effects from the flexor reflex afferents. *Acta Physiologica Scandinavica*, 70: 389-402. <https://doi.org/10.1111/j.1748-1716.1967.tb03637.x>
14. Roy, R., Harkema, S., & Edgerton, R. (2012). Basic concepts of activity-based interventions for improved recovery of motor function after spinal cord injury. In *Archives of Physical Medicine and Rehabilitation* (Vol. 93, Issue 9, pp. 1487–1497). W.B. Saunders. <https://doi.org/10.1016/j.apmr.2012.04.034>
15. Burns, A. S., Marino, R. J., Flanders, A. E., & Flett, H. (2012). Clinical diagnosis and prognosis following spinal cord injury. In *Handbook of Clinical Neurology* (Vol. 109, pp. 47–62). Elsevier B.V. <https://doi.org/10.1016/B978-0-444-52137-8.00003-6>
16. Burns, A. S., Marino, R. J., Kalsi-Ryan, S., Middleton, J. W., Tetreault, L. A., Dettori, J. R., Mihalovich, K. E., & Fehlings, M. G. (2017). Type and Timing of Rehabilitation Following Acute and Subacute Spinal Cord Injury: A Systematic Review. *Global spine journal*, 7(3 Suppl), 175S–194S. <https://doi.org/10.1177/2192568217703084>
17. Edgerton, V. R., de Leon, R. D., Tillakaratne, N., Recktenwald, M. R., Hodgson, J. A., & Roy, R. R. (1997). Use-dependent plasticity in spinal stepping and standing. *Advances in neurology*, 72, 233–247.
18. Lovely, R. G., Gregor, R. J., Roy, R. R., & Edgerton, V. R. (1986). Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. *Experimental neurology*, 92(2), 421–435. [https://doi.org/10.1016/0014-4886\(86\)90094-4](https://doi.org/10.1016/0014-4886(86)90094-4)
19. Rossignol, Serge, & Bouyer, L. (2004). Adaptive Mechanisms of Spinal Locomotion in Cats 1 (Vol. 44). Delcomyn. <https://academic.oup.com/icb/article/44/1/71/599761>
20. Côté, M. P., Ménard, A., & Gossard, J. P. (2003). Spinal cats on the treadmill: changes in load pathways. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 23(7), 2789–2796. <https://doi.org/10.1523/JNEUROSCI.23-07-02789.2003>
21. De Leon, R. D., Hodgson, J. A., Roy, R. R., & Edgerton, V. R. (1999b). Retention of hindlimb stepping ability in adult spinal cats after the cessation of step training. *Journal of neurophysiology*, 81(1), 85–94. <https://doi.org/10.1152/jn.1999.81.1.85>
22. Fouad, K., & Tetzlaff, W. (2012). Rehabilitative training and plasticity following spinal cord injury. *Experimental neurology*, 235(1), 91–99.
23. Barbeau, I., Chau, C., Rossignol, S., & ROSSIGNOL Noradrenergic, S. (1993). Noradrenergic Agonists and Locomotor Training Affect Locomotor Recovery After Cord Transection in Adult Cats. In *Brain Research Bulletin* (Vol. 30).
24. Rossignol, S., Barbeau, H., and Julien, C. (1986). Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In: *Development and Plasticity of the Mammalian Spinal Cord*, edited by M. Goldberger, A. Gorio, and M. Murray. Padova: Liviana, p. 323–345.
25. Frigon, A. (2012). Central pattern generators of the mammalian spinal cord. In *Neuroscientist* (Vol. 18, Issue 1, pp. 56–69). <https://doi.org/10.1177/1073858410396101>
26. Lovely, R. G., Gregor, R. J., Roy, R. R., & Edgerton, V. R. (1990). Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats. In *Brain Research* (Vol. 514).
27. Barrière, G., Leblond, H., Provencher, J., & Rossignol, S. (2008). Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries. *Journal of Neuroscience*, 28(15), 3976–3987. <https://doi.org/10.1523/JNEUROSCI.5692-07.2008>
28. Edgerton, V. R., Roy, R. R., Hodgson, J. A., Prober, R. J., de Guzman, C. P., & de Leon, R. (1991). A physiological basis for the development of rehabilitative strategies for spinally injured patients. *The Journal of the American Paraplegia Society*, 14(4), 150–157. <https://doi.org/10.1080/01952307.1991.11735848>

29. Barbeau, H., & Rossignol, S. (1990). The effects of serotonergic drugs on the locomotor pattern and on cutaneous reflexes of the adult chronic spinal cat. In *Brain Research* (Vol. 514).
30. Barbeau, H., & Rossignol, S. (1994). Enhancement of locomotor recovery following spinal cord injury. *Current opinion in neurology*, 7(6), 517–524. <https://doi.org/10.1097/00019052-199412000-00008>
31. Gerasimenko, Y., Gad, P., Sayenko, D., McKinney, Z., Gorodnichev, R., Puhov, A., Moshonkina, T., Savochin, A., Selionov, V., Shigueva, T., Tomilovskaya, E., Kozlovskaya, I., & Reggie Edgerton, V. (2016). Rapid Report Integration of sensory, spinal, and volitional descending inputs in regulation of human locomotion. *J Neurophysiol*, 116, 98–105. <https://doi.org/10.1152/jn.00146.2016>
32. Smith, J. L., Smith, L. A., Zernicke, R. F., & Hoy, M. (1982). Locomotion in Exercised and Nonexercised Cats Cordotomized at Two or Twelve Weeks of Age. In *EXPERIMENTAL NEUROLOGY* (Vol. 76).
33. Delivet-Mongrain, H., Dea, M., Gossard, J.-P., & Rossignol, S. (2020). Recovery of locomotion in cats after severe contusion of the low thoracic spinal cord. *J Neurophysiol*, 123, 1504–1525. <https://doi.org/10.1152/jn.00498.2019>-Large
34. Lewis, M. J., Jeffery, N. D., Olby, N. J., & Canine Spinal Cord Injury Consortium (CANSORT-SCI) (2020). Ambulation in Dogs With Absent Pain Perception After Acute Thoracolumbar Spinal Cord Injury. *Frontiers in veterinary science*, 7, 560. <https://doi.org/10.3389/fvets.2020.00560>
35. Dimitrijevic, M. R., Dimitrijevic, M. M., Faganel, J., & Sherwood, A. M. (1984). Suprasegmentally Induced Motor Unit Activity in Paralyzed Muscles of Patients with Established Spinal Cord Injury.
36. Dimitrijevic, M. R., Haller, J. A., Sharkey, P. C., & Sherwood, A. M. (1987). Epidural Spinal Cord Stimulation and Carry-Over Effect in Chronic Spinal Cord Injury Patients. In *Appl. Neurophysiol* (Vol. 50).
37. Gerasimenko, Y., Sayenko, D., Gad, P., Liu, C. T., Tillakaratne, N. J. K., Roy, R. R., Kozlovskaya, I., & Edgerton, V. R. (2017). Feed-Forwardness of Spinal Networks in Posture and Locomotion. In *Neuroscientist* (Vol. 23, Issue 5, pp. 441–453). SAGE Publications Inc. <https://doi.org/10.1177/1073858416683681>
38. Militskova, A., Mukhametova, E., Fatykhova, E., Sharifullin, S., Cuellar, C. A., Calvert, J. S., Grah, P. J., Baltina, T., & Lavrov, I. (2020). Supraspinal and Afferent Signaling Facilitate Spinal Sensorimotor Network Excitability After Discomplete Spinal Cord Injury: A Case Report. *Frontiers in Neuroscience*, 14. <https://doi.org/10.3389/fnins.2020.00552>
39. Forssberg, H., & Grillner, S. (1973). The locomotion of the acute spinal cat injected with clonidine i.v.
40. Giroux, N., Chau, C., Barbeau, H., Reader, T. A., & Rossignol, S. (2003). Effects of intrathecal glutamatergic drugs on locomotion. II. NMDA and AP-5 in intact and late spinal cats. *Journal of Neurophysiology*, 90(2), 1027–1045. <https://doi.org/10.1152/jn.00758.2002>
41. Delcomyn F. (1980). Neural basis of rhythmic behavior in animals. *Sci Wash DC*, 210:492–498.
42. Pearson, K. G., & Rossignol, S. (1991). Fictive Motor Patterns in Chronic Spinal Cats (Vol. 66, Issue 6). www.physiology.org/journal/jn
43. Saltiel, P., & Rossignol, S. (2004). Critical points in the forelimb fictive locomotor cycle and motor coordination: Effects of phasic retractions and protractions of the shoulder in the cat. *Journal of Neurophysiology*, 92(3), 1342–1356. <https://doi.org/10.1152/jn.00564.2003>
44. Côté, M. P., Azzam, G. A., Lemay, M. A., Zhukareva, V., & Houlié, J. D. (2011). Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after spinal cord injury. *Journal of Neurotrauma*, 28(2), 299–309. <https://doi.org/10.1089/neu.2010.1594>
45. Rossignol, Serge, Barrière, G., Frigon, A., Barthélemy, D., Bouyer, L., Provencher, J., Leblond, H., & Bernard, G. (2008). Plasticity of locomotor sensorimotor interactions after peripheral and/or spinal lesions. In *Brain Research Reviews* (Vol. 57, Issue 1, pp. 228–240). <https://doi.org/10.1016/j.brainresrev.2007.06.019>
46. Viala, D., & Vidal, C. (1978). Evidence for distinct spinal locomotion generators supplying respectively fore- and hindlimbs in the rabbit. In *Brain Research* (Vol. 155).
47. Juvin, L., Simmers, J., & Morin, D. (2005). Propriospinal circuitry underlying interlimb coordination in mammalian quadrupedal locomotion. *Journal of Neuroscience*, 25(25), 6025–6035. <https://doi.org/10.1523/JNEUROSCI.0696-05.2005>
48. Gerasimenko, Y., Musienko, P., Bogacheva, I., Moshonkina, T., Savochin, A., Lavrov, I., Roy, R. R., & Edgerton, V. R. (2009). Propriospinal bypass of the serotonergic system that can facilitate stepping. *Journal of Neuroscience*, 29(17), 5681–5689. <https://doi.org/10.1523/JNEUROSCI.6058-08.2009>
49. Gosgnach, S. (2011). The role of genetically-defined interneurons in generating the mammalian locomotor rhythm. *Integrative and Comparative Biology*, 51(6), 903–912. <https://doi.org/10.1093/icb/acr022>
50. Rybak, I. A., Dougherty, K. J., & Shevtsova, N. A. (2015). Organization of the mammalian locomotor CPG: Review of computational model and circuit architectures based on genetically identified spinal interneurons. In *eNeuro* (Vol. 2, Issue 5). Society for Neuroscience. <https://doi.org/10.1523/ENEURO.0069-15.2015>
51. Fawcett, J. W., Curt, A., Steeves, J. D., Coleman, W. P., Tuszynski, M. H., Lammertse, D., Bartlett, P. F., Blight, A. R., Dietz, V., Ditunno, J., Dobkin, B. H., Havton, L. A., Ellaway, P. H., Fehlings, M. G., Privat, A., Grossman, R., Guest, J. D., Kleitman, N., Nakamura, M., ... Short, D. (2007). Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: Spontaneous recovery after spinal cord injury and

- statistical power needed for therapeutic clinical trials. In *Spinal Cord* (Vol. 45, Issue 3, pp. 190–205). <https://doi.org/10.1038/sj.sc.3102007>
52. Angeli, C. A., Edgerton, V. R., Gerasimenko, Y. P., & Harkema, S. J. (2014). Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain*, 137(5), 1394–1409. <https://doi.org/10.1093/brain/awu038>
 53. Escalona, M., Delivet-Mongrain, H., Kundu, A., Gossard, J. P., & Rossignol, S. (2017). Ladder treadmill: A method to assess locomotion in cats with an intact or lesioned spinal cord. *Journal of Neuroscience*, 37(22), 5429–5446. <https://doi.org/10.1523/JNEUROSCI.0038-17.2017>
 54. Harkema, S., Gerasimenko, Y., Hodes, J., Burdick, J., Angeli, C., Chen, Y., Ferreira, C., Willhite, A., Rejc, E., Grossman, R. G., & Edgerton, V. R. (2011). Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: A case study. *The Lancet*, 377(9781), 1938–1947. [https://doi.org/10.1016/S0140-6736\(11\)60547-3](https://doi.org/10.1016/S0140-6736(11)60547-3)
 55. Cramer, S. C., Sur, M., Dobkin, B. H., O'Brien, C., Sanger, T. D., Trojanowski, J. Q., Rumsey, J. M., Hicks, R., Cameron, J., Chen, D., Chen, W. G., Cohen, L. G., Decharms, C., Duffy, C. J., Eden, G. F., Fetz, E. E., Filart, R., Freund, M., Grant, S. J., ... Vinogradov, S. (2011). Harnessing neuroplasticity for clinical applications. In *Brain* (Vol. 134, Issue 6, pp. 1591–1609). <https://doi.org/10.1093/brain/awr039>
 56. Khan, F., Amatyia, B., Galea, M. P., Gonzenbach, R., & Kesselring, J. (2017). Neurorehabilitation: applied neuroplasticity. *Journal of Neurology*, 264(3), 603–615. <https://doi.org/10.1007/s00415-016-8307-9>
 57. Rossignol, S., Dubuc, R., & Gossard, J. P. (2006). Dynamic sensorimotor interactions in locomotion. *Physiological reviews*, 86(1), 89–154. <https://doi.org/10.1152/physrev.00028.2005>
 58. Rossignol, S., Chau, C., Giroux, N., Brustein, E., Bouyer, L., Marcoux, J., Langlet, C., Barthelmy, D., Provencher, J., Leblond, H., Barbeau, H., & Reader, T. A. (2002). The cat model of spinal injury. In *Progress in Brain Research* (Vol. 137).
 59. Jaggy, A., & Platt, S. (2010). *Small Animal Neurology An Illustrated Text*. Schlütersche .
 60. Maier, I. C., Ichiyama, R. M., Courtine, G., Schnell, L., Lavrov, I., Edgerton, V. R., & Schwab, M. E. (2009). Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain*, 132(6), 1426–1440. <https://doi.org/10.1093/brain/awp085>
 61. Tillakaratne, N. J. K., Duru, P., Fujino, H., Zhong, H., Xiao, M. S., Edgerton, V. R., & Roy, R. R. (2014). Identification of interneurons activated at different inclines during treadmill locomotion in adult rats. *Journal of Neuroscience Research*, 92(12), 1714–1722. <https://doi.org/10.1002/jnr.23437>
 62. Edgerton, R., de Leon, R., Harkema, S., Hodgson, J., London, N., Reinkensmeyer, D., Roy, R., Talmadge, R., Tillakaratne, N., Timoszyk, W., & Tobin, A. (2001). Retraining the injured spinal cord. *Journal of Physiology*, 533(1), 15–22.
 63. Martinez, M., Delivet-Mongrain, H., Leblond, H., & Rossignol, S. (2012). Effect of locomotor training in completely spinalized cats previously submitted to a spinal hemisection. *Journal of Neuroscience*, 32(32), 10961–10970. <https://doi.org/10.1523/JNEUROSCI.1578-12.2012>
 64. Forssberg H. (1979). Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. *Journal of neurophysiology*, 42(4), 936–953.
 65. Mccrea, D. A. (2001). Topical Review Spinal circuitry of sensorimotor control of locomotion. In *The Journal of Physiology Symposium on Spinal Cord Function and Rehabilitation* (Issue 1).
 66. Aikawa, T., Shibata, M., Asano, M., Hara, Y., Tagawa, M., & Orima, H. (2014). A comparison of thoracolumbar intervertebral disc extrusion in French Bulldogs and Dachshunds and association with congenital vertebral anomalies. *Veterinary surgery: VS*, 43(3), 301–307. <https://doi.org/10.1111/j.1532-950X.2014.12102.x>
 67. Olby, N., Harris, T., Burr, J., Muñana, K., Sharp, N., & Keene, B. (2004). Recovery of pelvic limb function in dogs following acute intervertebral disc herniations. *Journal of neurotrauma*, 21(1), 49–59. <https://doi.org/10.1089/089771504772695940>
 68. Olby, N. J., da Costa, R. C., Levine, J. M., Stein, V. M., & Canine Spinal Cord Injury Consortium (CANSORT SCI) (2020). Prognostic Factors in Canine Acute Intervertebral Disc Disease. *Frontiers in veterinary science*, 7, 596059. <https://doi.org/10.3389/fvets.2020.596059>.

5. ARTICLE 6

Animals (2022), 12(1557):1-20.

Influence of Spinal Shock on the Neurorehabilitation of ANNPE Dogs

Influence of Spinal Shock on the Neurorehabilitation of ANNPE Dogs

Débora Gouveia ^{1,2*}, Ana Cardoso ¹, Carla Carvalho ¹, Ana Rita Gonçalves ³, Óscar Gamboa ⁴, Rute Canejo-Teixeira ³, António Ferreira ^{4,5} and Ângela Martins ^{1,2,3,5}

¹ Arrábida Veterinary Hospital—Arrábida Animal Rehabilitation Center, Azeitão, 2925-538 Setúbal, Portugal; anacardosocatarina@gmail.com (A.C.); mv.carla.c@gmail.com (C.C.); vetarrabida.lda@gmail.com (Â.M.)

² Superior School of Health, Protection and Animal Welfare, Polytechnic Institute of Lusophony, Campo Grande, 1950-396 Lisboa, Portugal

³ Faculty of Veterinary Medicine, Lusófona University, Campo Grande 376, 1749-024 Lisboa, Portugal; ana.rita.silva.goncalves@hotmail.com (A.R.G.); p5637@ulusofona.pt (R.C.-T.)

⁴ Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisboa, Portugal; ogamboa@fmv.ulisboa.pt (Ó.G.); aferreira@fmv.ulisboa.pt (A.F.)

⁵ CIISA—Centro Interdisciplinar-Investigação em Saúde Animal, Faculdade de Medicina Veterinária, Av. Universidade Técnica de Lisboa, 1300-477 Lisboa, Portugal

* Correspondence: deborahisabel@msn.com

Simple Summary: Acute noncompressive nucleus pulposus extrusion (ANNPE) is characterized by a peracute onset of neurological clinical signs. It is based on the spinal cord contusion with possible manifestation of signs compatible with spinal shock. Treatment is conservative and may involve the implementation of physical rehabilitation protocols. Thus, this study intends to contribute to the knowledge surrounding the ANNPE research and to verify if the presence of spinal shock can affect the outcome, as well as recovery time after neurorehabilitation.

Abstract: Acute noncompressive nucleus pulposus extrusion (ANNPE) is related to contusive spinal cord injuries, and dogs usually appear to be exercising vigorously at the time of onset. ANNPE has a characteristic peracute onset of clinical signs during exercise or following trauma, with non-progressive signs during the first 24 h and possibly signs of spinal shock. The main aim was to assess if the presence of spinal shock affects the neurorehabilitation outcomes of ANNPE dogs. This prospective controlled cohort clinical study was conducted at the Arrábida Rehabilitation Center. All of the dogs had T3–L3 injuries and were paraplegic/monoplegic with/without nociception, the study group (n = 14) included dogs with ANNPE spinal shock dogs, and the control group (n = 19) included ANNPE dogs without spinal shock. The study group was also evaluated using a new scale—the Spinal Shock Scale (SSS)—and both groups were under the same intensive neurorehabilitation protocol. Spinal shock was a negative factor for a successful outcome within less time. SSS scores > 4 required additional hospitalization days. The protocol was safe, tolerable, and feasible and accomplished 32% ambulation within 7 days, 29% in 14 days, and 29% in 30 days. The results were better than those obtained in previous studies—94% at 60 days—and 75% of the dogs without nociception recovered ambulation. Long-term follow-ups carried out 4 years later revealed a positive evolution.

Keywords: ANNPE; neurorehabilitation; dogs; spinal shock; nociception; spinal shock scale

In the scientific literature, acute noncompressive nucleus pulposus extrusion (ANNPE) was previously known as high velocity–low volume disk extrusion, type III disk extrusion, traumatic intervertebral disk extrusion, or intervertebral disk explosion [1–6].

The ANNPE is predominantly related to contusive spinal cord injury (SCI). It usually presents in association with an external trauma, such as a car crash or a fall from a height [5], although reports have shown that at onset, most dogs are exercising vigorously. A tearing of the complex lamellar structure of the annulus fibrosus may occur along with a sudden material extrusion of the non-degenerated nucleus pulposus into the vertebral canal, suggesting that in dogs of increasing age, the annular lamellar is vulnerable [7].

Contusions may cause the release of local inflammatory mediators, cytotoxins, and excitatory neurotransmitters that promote a chain of secondary events and that lead to ischemia and the depletion of cellular energy [8,9]. In humans, it has been shown that elevated cytokine levels, such as the tumor necrosis factor alpha (TNF- α) and interleukin 1-beta (IL-1 β) appear within minutes after injury and can last up to 2 h. Additionally, increased levels of glutamate, a neurotransmitter of the central nervous system (CNS), can be present, leading to astrocytic dysfunction [10].

Thus, ANNPE is characterized by a peracute onset of the non-progressive, often lateralized paresis/paralysis of one to four limbs depending on the neurolocalization of the injury [11]. The affected dogs often show vocalization at the onset of clinical signs and moderate spinal hyperesthesia noted on initial examination [12,13]. Additionally, the non-progression of clinical signs has been observed 24 h after injury [14,15]. This usually manifests as a highly asymmetrical T3–L3 myelopathy that affects medium and large dog breeds [9,16,17]. The prognosis for recovery in dogs with ANNPE that present with paraplegia without nociception is considered poor, and the majority of these dogs are euthanized within a week of injury [18].

The main differential diagnosis of ANNPE is the fibrocartilaginous embolic myelopathy (FEM), which has a similar clinical presentation [1,2,19]. MRI is the ideal imaging exam [3,16,19–22]; however, a definitive diagnosis is only possible through post-mortem histological examinations of the injured spinal cord segments [22,23]. The MRI findings can help to differentiate the ANNPE from FEM based on reductions in the volume and signal of the nucleus pulposus on T2-weighted (T2W) images, a focal hyperintense lesion within the spinal cord dorsally to the affected disc on T2W, an abnormal signal intensity in the epidural space, and a narrow intervertebral disc space with spinal cord compression that is absent or less than 10% [20,24].

On occasion, clinical signs may be considered compatible with spinal shock when there are temporary reduced spinal reflexes, including the withdrawal reflex in one or both hindlimbs. This is observed in the absence of any type of injury in the L4–S3 spinal cord segments or nerves during magnetic resonance imaging [16,18], which is a relevant clinical feature that may lead to an incorrect neuroanatomic localization [25].

Spinal shock syndrome has been described as a cause of the loss of motor and sensory function in both humans [26,27] and dogs [21,28,29] with severe SCI or in spinal cord transection. Spinal shock syndrome is a profound depression in the segmental spinal reflexes and a loss of muscle tone caudally to the lesion, even in physically intact reflex arcs (Jeffery

and Smith 2005). It has also been described as involving the development of fecal and urinary incontinence [2,30,31].

Spinal shock syndrome is complex, and in dogs, the anal sphincter reflex typically recovers within 15 min of spinal cord transection; however, the withdrawal reflex may need from 2 days to 6 weeks to recover [32,33]. Furthermore, it manifests with a sudden interruption to the descending supraspinal input on motor neurons and interneurons as well as fusimotor inhibition and increased segmental inhibition [29]. For some authors, the end of spinal shock is considered to be the return of certain reflexes, and in humans, there is evidence of a slow transition from spinal shock to signs of spasticity [34].

ANNPE treatment is conservative and involves the implementation of physical rehabilitation [11,16,25,35] that does not require surgery [36] and that ideally includes an initial period of rest [17,37]. Some rehabilitation strategies may include kinesiotherapy exercises [37], electrical stimulation, and laser therapy [38–41]. Pharmacological management is based on opioids as well as NSAIDs such as paracetamol and gabapentin, which may be useful as additional analgesics in the early stages. Supportive care is essential, especially in the management of the urinary bladder, with possible need for manual expression [8].

Thus, the aim of this study was to contribute to the knowledge gaps in the field of ANNPE research and to verify if the presence of spinal shock syndrome may affect the neurorehabilitation outcomes of ANNPE dogs as well as time to recovery. It was hypothesized that this syndrome may delay the recovery of these patients. A second aim was to prove if intensive neurorehabilitation is safe, tolerable, and feasible in a clinical setting.

2. Materials and Methods

This prospective controlled cohort clinical study was conducted at the Arrábida Animal Rehabilitation Center (Setúbal, Portugal) between 1 February 2017 and 1 February 2022 after receiving approval from the Lusófona Veterinary Medicine Faculty ((Lisbon, Portugal) ethics committee (No. 114-2021) and consent from the owners.

All of the dogs had to meet the following criteria for inclusion in the study with the onset of neurological dysfunction associated with vigorous exercise and T3–L3 lesions. All of the included dogs were paraplegic/monoplegic with or without nociception and therefore grade 1 or 0 according to the Modified Frankel Scale (MFS) [42], and characterized by peracute clinical signs, such as an asymmetric presentation, spinal discomfort, and evidence of spinal shock (reduced segmental spinal reflexes and muscle tone caudally to the injury) without L4–S3 lesions or peripheral nerve dysfunction. All of the dogs were diagnosed after computed tomography (CT), CT–myelography (Figure 1), or MRI and admitted to the rehabilitation center within the first 48 h after injury. Dogs were excluded if they presented with vertebral fractures, luxation, and/or compressive myelopathies. Additionally, the dogs diagnosed with presumptive FEM by a veterinary neurology specialist were immediately excluded from the study.

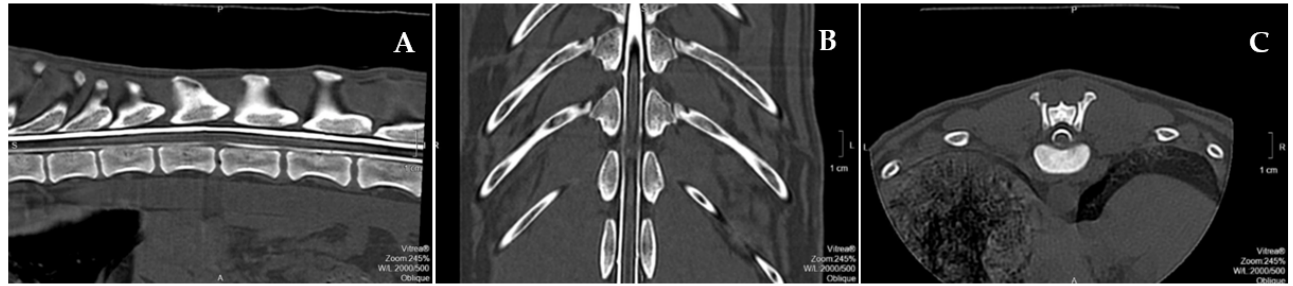


Figure 1. Computed Tomography–Myelogram image from a 6-year-old Labrador with T12–T13 acute non-compressive nucleus pulposus extrusion. (A) Vacuum phenomenon and light attenuation of the ventral contrast line diameter (sagittal view); (B) T12–T13 hyperintensity sign and bilateral light attenuation of the diameter of the contrast lines (dorsal view); (C) normal T12–T13 disk space with residual mineralized disk material ventrally (transverse view).

2.1. Study Design

In this present prospective controlled cohort clinical study, 33 dogs with thoracolumbar neuroanatomical localization received a neurorehabilitation consultation and were randomized by means of aleatory stratification according to the clinical signs that are consistent with spinal shock syndrome. They were divided into the study group (SG) ($n = 14$), which comprised ANNPE dogs manifesting signs of spinal shock, and a control group (CG) ($n = 19$) comprising ANNPE dogs without spinal shock. The total population characterization is described in Table 1 along with the registration of concomitant occurrences. The main occurrence was hip dysplasia associated with painful osteoarthritis of the coxofemoral joint, which was mainly observed in the SG (71.4%).

Table 1. Sample characterization of both the control and study groups.

	SG ($n = 14$)	CG ($n = 19$)	Total ($n = 33$)
Breed	Breed: 64.3% (9/14) Mixed Breed: 35.7% (5/14)	Breed: 89.5% (17/19) Mixed Breed: 10.5% (2/19)	Breed: 78.8% (26/33) Mixed Breed: 21.2% (7/33)
Sex	Male: 50% (7/14) Female: 50% (7/14)	Male: 57.9% (11/19) Female: 42.1% (8/19)	Male: 54.5% (18/33) Female: 45.5% (15/33)
Age	<7 years old: 71.4% (10/14) ≥7 years old: 28.6% (4/14) Mean: 5.64 years	<7 years old: 57.9% (11/19) ≥7 years old: 42.1% (8/19) Mean: 6.42 years	<7 years old: 63.6% (21/33) ≥7 years old: 36.4% (12/33) Mean: 6 years
Weight	≤15 kg: 35.7% (5/14) >15 kg: 64.3% (9/14) Mean: 26.86 kg	≤15 kg: 36.8% (7/19) >15 kg: 63.2% (12/19) Mean: 21.89 kg	≤15 kg: 36.4% (12/33) >15 kg: 63.6% (21/33) Mean: 24 kg.
Complementary Exams	MRI: 35.7% (5/14) CT: 64.3% (9/14)	MRI: 42.1% (8/19) CT: 57.9% (11/19)	MRI: 39.4% (13/33) CT: 60.6% (20/33)
Neuroanatomical Localization	T12–T13: 14.3% (2/14) T13–L1: 35.7% (5/14) L1–L2: 21.4% (3/14)	T12–T13: 21.1% (4/19) T13–L1: 36.8% (7/19) L1–L2: 31.6% (6/19)	T12–T13: 18.2% (6/33) T13–L1: 36.4% (12/33) L1–L2: 27.3% (9/33)

	L2–L3: 28.6% (4/14)	L2–L3: 10.5% (2/19)	L2–L3: 18.2% (6/33)
Lateralization signs	Absent: 14.3% (2/14) Present: 85.7% (12/14)	Absent: 21.1% (4/19) Present: 78.9% (15/19)	Absent: 18.2% (6/33) Present: 81.8% (27/33)
Spinal discomfort	Absent: 78.6% (11/14) Present: 21.4% (3/14)	Absent: 84.2% (16/19) Present: 15.8% (3/19)	Absent: 81.8% (27/33) Present: 18.2% (6/33)
Nociception	Absent: 28.6% (4/14) Present: 71.4% (10/14)	Absent: 21.1% (4/19) Present: 78.9% (15/19)	Absent: 24.2% (8/33) Present: 75.8% (25/33)
Modified Frankel Scale	Grade 0: 28.6% (4/14) Grade 1: 71.4% (10/14)	Grade 0: 21.1% (4/19) Grade 1: 78.9% (15/19)	Grade 0: 24.2% (8/33) Grade 1: 75.8% (25/33)
Fecal and Urinary Incontinence	Present: 14% (2/14) (progressive myelomalacia) Absent: 86% (12/14)	Absent: 100% (19/19)	Present: 6% (2/33) (progressive myelomalacia) Absent: 94% (31/33)
Occurrences	Absent: 28.6% (4/14) Present: 71.4% (10/14)	Absent: 57.9% (11/19) Present: 42.1% (8/19)	Absent: 45.5% (15/33) Present: 54.5% (18/33) (2 progressive myelomalacia)

Legend: SG (study group); CG (control group).

Dogs from both groups were subjected to an evaluation of their mental status, passive standing posture, spinal reflexes (patellar reflex, cranial tibial reflex, withdrawal reflex, crossed-extensor reflex, and perineal reflex), cutaneous trunci reflex, superficial pain, and deep pain perception (DPP). The DPP was evaluated in a controlled environment using 12 cm Halsted mosquito forceps in both the medial/lateral digits of both hindlimbs. This was also tested in the perineal region and at the tip and base of the tail. Spinal hyperesthesia was assessed with the vertebral palpation technique, which is performed by placing slight pressure on the vertebral column and one hand on the abdominal muscles, palpating throughout the ligamentum supraspinale between each spinous process (*processus spinosus*) and between each intervertebral space to activate the nociceptors located in the outer annulus fibrosus or of the dorsal longitudinal ligament.

An orthopedic examination including joint palpation distally to proximally was executed. Any signs of inflammation, edema, or crepitation and the presence of Ortolani in the hip that were observed were registered. Additionally, muscle tone was evaluated via the palpation of the quadriceps femoris, biceps femoris, semitendinosus and semimembranosus muscles, and the abdominal region (*rectus abdominis* and *obliquus externus abdominis*).

All of dogs were classified according to the Open Field Score (OFS) [43] at admission, and in the following time points, they were always in the same controlled 6 m floor, even if the dogs presented monoplegia without DPP. The SG was also evaluated using a pioneer scale that is currently in development to assess spinal shock—the Spinal Shock Scale (SSS) (Table 2).

The SSS is a functional punctuation scale that is based on the evaluation of three main spinal reflexes of the hindlimbs, the perineal reflex, the cutaneous trunci reflex, perineal tone, and hindlimbs tone. Thus, a dog with a classification score of 7 is considered to have severe signs of spinal shock.

Table 2. Spinal Shock Scale (SSS).

Reduced hindlimb reflexes	Withdrawal reflex	(+1)
	Patellar reflex	(+1)
	Cranial tibial reflex	(+1)
	Reduced hindlimb tone	(+1)
	Reduced perineal reflex	(+1)
	Reduced perineal tone	(+1)
	Absent/abnormal cutaneous trunci cut off	(+1)
TOTAL SCORE		

Neurorehabilitation consultations and the following evaluations were always performed by a certified canine rehabilitation professional (CCRP) examiner/instructor. Data were recorded (Canon EOS Rebel T6 1300 D camera), and all videos were revised by two CCRP instructors.

The ambulatory status was considered according to OFS scores ≥ 11 , although the dogs remained at the rehabilitation center for a maximum of 2 months to observe OFS improvements over time.

2.2. Procedures

Both groups were subjected to the same intensive neurorehabilitation protocol (INRP), which was based on electrical stimulation, laser therapy, and locomotor training.

2.2.1. Electrical Stimulation

The electrical stimulation (BTL® 4000 Smart, Famões, Portugal) programs included functional electrical stimulation (FES), a neuromodulation modality based on the application of superficial electrodes after trichotomy, according to the segmental technique [44–46]. All of the patients who showed an evident decrease in muscle tone were subjected to the co-contraction protocol, which consisted of the simultaneous contraction of both the quadriceps femoris group and the hamstring muscles group (Figure 2A). If the patients showed good muscle tone in the quadriceps femoris muscle tone associated with patellar hyperreflexia, then the electrodes were only placed following the sciatic nerve trajectory, stimulating the contraction of the hamstring muscles (Figure 2B). The current parameters were as follows: 40–60 Hz; 10–48 mA; 150 μ s duty cycle 1:4; and trapezoid current [44,45]. Electrical stimulation was carried out for 10 min and was conducted first three times/day at first before decreasing to once a day (Table 3).

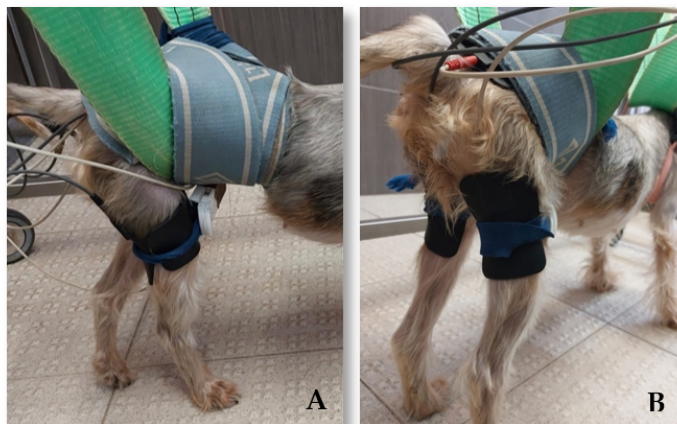


Figure 2. Electrical stimulation protocols on a dog in a postural standing position. (A) Co-contraction protocol with the stimulation of both the quadriceps femoris group and the hamstring muscles group. (B) Segmental technique of the sciatic nerve by means of functional electrical stimulation.

2.2.2. Class IV Laser Therapy

Photostimulation programs using class IV laser therapy (Companion Therapy Laser—LiteCure®, New Castle, USA) were performed with a 5 cm probe and transcutaneous application after trichotomy according to the neurolocalization of each dog. The protocol was the same as the one described by Bennaim et al. (2017) [47] and used 12 J/cm² of energy, a wavelength of 810–987 nm, and the pulsed mode. The probe was applied to the injury site with slight pressure, and circulatory movements were always carried out in the cranial and caudal segments for one minute each (Figure 3). This protocol was performed daily for the first 15 days (Table 3).



Figure 3. Laser therapy class IV program applied on the spinal cord injury.

Dogs with signs of hip osteoarthritis were treated with an 8–10 J/cm² emission protocol and a four-point technique, which was also applied in continuous circulatory movements and 2 to 3 times a week [48,49]. Figure 4 depicts all of the points: the first point was located at the proximo cranial region of the hip joint; the second point was located at the proximo caudal; the third point was located at the disto caudal; and the fourth point was located at the disto cranial region.

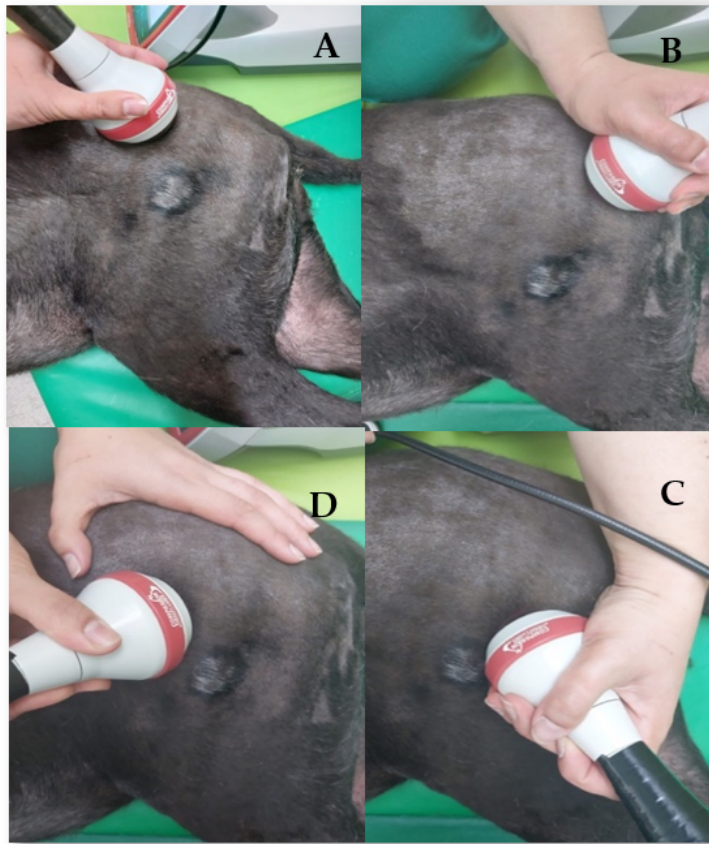


Figure 4. Laser therapy class IV program applied to the coxofemoral joint using the four-point technique. (A) Proximo cranial region; (B) proximo caudal region; (C) disto caudal region; (D) disto cranial region.

2.2.3. Kinesiotherapy Protocol

- Locomotor training

Locomotor exercise was initiated on the second day after admission, and all of the dogs performed land treadmill (Superior Fit Fur Life Treadmill®, Fernhurst, Surrey, UK) and underwater treadmill (Hidro Pysio®, Broseley, UK) exercises (Figure 5). However, in some dogs with poor adaptation, bipedal training was implemented until the dogs were able to complete the exercises. Because all of the dogs were paraplegic/monoplegic, in the few first days of exercise, three rehabilitators helped to maintain postural standing and performed bicycle movements with the plegic limb(s) on the treadmill with the help of a body-weighted harness device.

The first sessions were performed on the land treadmill over a short period of time and several times a day (i.e., 3–5 min, 6–8 times/day) and gradually increased to longer training, i.e., 40 min, 2–3 times/day. The speed was also adjustable, from 1.8 km to 4.5 km/h, and the inclination was 2–10%. Land treadmill exercise was performed 6 times/week, and the underwater treadmill exercise was performed 5 times/week, according to the specifications described in Table 3.



Figure 5. Locomotor exercise in two dogs. (A) Land treadmill; (B) underwater treadmill.

- Kinesiotherapy exercises

On the second day after admission, the kinesiotherapy exercise circuit, the one described in Table 3, was initiated. First, passive, and assisted exercises were carried out, always with the help of a rehabilitator.

All of the dogs performed a set of obstacle rails (5–6 times/day) via the stimulation of the monoplegic limb by the dorsal region of the paw touching the obstacle (Figure 6A). Additionally, gait stimulation using alternating floors, namely sand, grass, gravel, and soft ground (1–2 min; 3–6 times/day) (Figure 6B), and postural standing on a balance board (1–2 min; 3–6 times/day) (Figure 7A) were also implemented. These were followed by assisted bicycling while standing on a central stimulation pad with a rough surface (15 bicycle movements, 2–3 times/day) (Figure 7B). All of the exercises were carried out 5 days a week and, according to the frequency of the exercises, were decreased until they were only being performed 2–3 times a day, which was determined according to the dogs' neurological evolution.



Figure 6. Kinesiotherapy exercises. (A) Obstacle rail; (B) gait stimulation on alternating floors; ● knuckling position.



Figure 7. Kinesiotherapy exercises. (A) Postural standing on a balance board; (B) bicycle movements on a central stimulation pad.

Table 3. Intensive neurorehabilitation protocol.

Admission to Ambulation	Study Group	DPP Positive	Electrical Stimulation	Laser Therapy Class IV	Kinesiotherapy Exercises	
					Land Treadmill	Underwater Treadmill
			50 Hz, 10 mA, 150 μ s, duty cycle 1:4; 10 min; TID; SSS score < 4; BID.	After trichotomy, 12 J/cm ² , 2.5 Hz, duty cycle 88%, 3 min, pulsed mode, SID, 15 days.	3–10 min; no slope; 1.8 km/h 4–6 times/day; 6 days/week	5–10 min; no slope; 1.2–2 km/h SID; 5 days/week.
						Obstacle rails, postural standing: 1–2 min, 3–6 times/day; bicycles: 2–3 times/day alternating floors: 1 min, 3–6 times/day, 6 days/week.

Ambulation to Clinical Discharge	Control Group	DPP Negative	40 Hz; 10–48 mA; 150 μ s, duty cycle 1:4. 10 min; TID SSS score < 4; BID.	3–10 min; 2–5% slope; 1.5 km/h 6–8 times/day; 6 days/week	10–20 min; no slope; 1.2–2 km/h SID; 5 days/week.	Obstacle rails, postural standing: 1–2 min, 3–6 times/day; bicycles: 2–3 times/day; alternating floors: 1 min, 3–6 times/day, 6 days/week
		DPP Positive	50 Hz; 10 mA; 150 μ s, duty cycle 1:4; 10 min; BID.	3–10 min; no slope; 1.8 km/h 4–6 times/day; 6 days/week	5–10 min; no slope; 1.2–2 km/h SID; 5 days/week.	Obstacle rails, postural standing: 1–2 min, 3–6 times/day; bicycles: 2–3 times/day; alternating floors: 1 min, 3–6 times/day, 6 days/week.
		DPP Negative	40 Hz; 10–48 mA; 150 μ s, duty cycle 1:4; 10 min; BID.	3–10 min; 2–5% slope; 1.5 km/h 6–8 times/day; 6 days/week	10–20 min; no slope; 1.2–2 km/h SID; 5 days/week.	Obstacles rails, postural standing: 1–2 min, 3–6 times/day; bicycles: 2–3 times/day; alternating floors: 1 min, 3–6 times/day, 6 days/week.
	Study Group	DPP Positive	50 Hz; 10 mA; 150 μ s, duty cycle 1:4; 10 min; SID.	10–40 min; 2–5% slope; 2– 2.5 km/h 2–3 times/day; 3 days/week	30 min; 2–5% slope; 2–2.5 km/h SID; 3 days/week.	Obstacle rails, postural standing: 1–2 min, 2–3 times/day; alternating floors: 2 min, 2–3 times/day, 3 days/week.
		DPP Negative	40 Hz; 10–48 mA; 150 μ s, duty cycle 1:4; 10 min; SID.	10–40 min; 2–5% slope; 1.8– 2.5 km/h 2–3 times/day; 5 days/week	40 min; 5–10% slope; 2.8–4.5 km/h SID; 5 days/week.	Obstacle rails, postural standing: 1–2 min, 3 times/day; alternating floors: 2 min, 3 times/day, 5 days/week.
	Control Group	DPP Positive	50 Hz; 10 mA; 150 μ s, duty cycle 1:4; 10 min; SID.	10–40 min; 2–5% slope; 2– 2.5 km/h 2–3 times/day; 3 days/week	30 min; 2–5% slope; 2–2.5 km/h SID; 3 days/week.	Obstacle rails, postural standing: 1–2 min, 2–3 times/day; alternating floors: 2 min, 2–3 times/day, 3 days/week.

DPP Negative	40 Hz; 10–48 mA; 150 μs, duty cycle 1:4; 10 min; SID.	10–40 min; 2–5% slope; 1.8– 2.5 km/h 2–3 times/day; 5 days/week	40 min; 5–10% slope; 2.8–4.5 km/h SID; 5 days/week.	Obstacle rails, postural standing: 1–2 min, 3 times/day; alternating floors: 2 min, 3 times/day, 5 days/week.
		.	.	.

Legend: DPP (deep pain perception); TID (three times a day); BID (twice a day); SID (once daily).

2.2.4. Supportive Care

Hydric support was maintained for each dog at a rate of 100–150 mL/kg, and nutritional care was supported with an increased intake of 30%, according to the needs of each patient. Manual expression of the bladder was completed 3–4 times a day, and urine was monitored with regard to color, smell, and quantity. All of the dogs performed passive range of motion (PROM) exercises, completing 15–30 repetitions per session three times a day (Figure 8A), followed by massage (stroking, effleurage, wringing-up, kneading, friction, wringing-up, effleurage, and stroking) for a total of 5 min two times a day (Figure 8B).

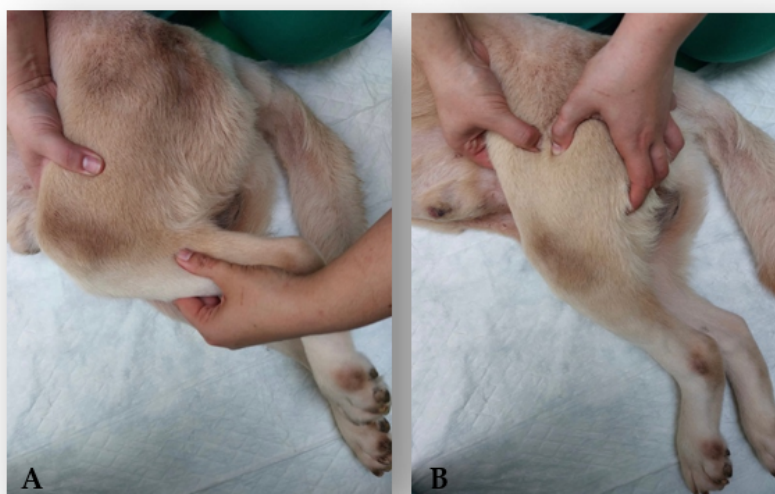


Figure 8. Supportive treatment. (A) Passive range of motion exercises for the knee joint; (B) deep kneading massage technique for direct muscular treatment.

2.3. Outcomes and Follow-Ups

The SG and CG dogs were assessed by neurological examination, as described in the flow cohort diagram (Figure 9). Assessments were always carried out by the same certified CCRP examiner/instructor. The measured outcomes for the SG were the OFS and the SSS scales, and for the CG, the only measured outcome was the OFS. The time points of evaluation were admission (T0), day 1 (T1), day 2 (T2), day 3(T3), day 5 (T4), day 7 (T5), day 14 (T6), day 21 (T7), day 30 (T8), and day 60 (T9).

Follow-ups were performed after clinical discharge at 7 days (F1), 15 days (F2), one month (F3), three months (F4), six months (F5), one year (F6), two years (F7), three years (F8), and four years (F9).

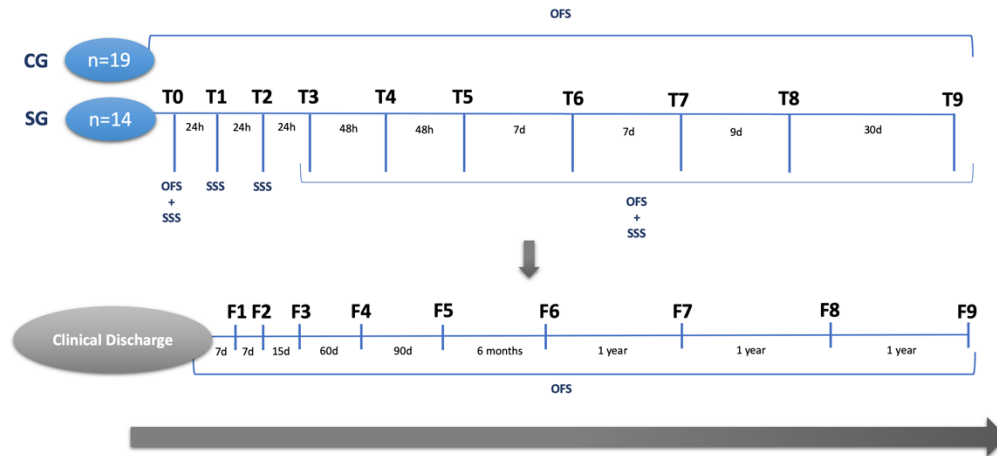


Figure 9. Flow cohort diagram describing the outcome measures of both the control and study group throughout the rehabilitation period and after clinical discharge. OFS: Open Field Score; SSS: Spinal Shock Scale; CG: control group; SG: study group; T0 (day 0); T1 (day 1); T2 (day 2); T3 (day 3); T4 (day 5); T5 (day 7); T6 (day 14); T7 (day 21); T8 (day 30); T9 (day 60); F1 (7 days); F2 (15 days); F3 (one month); F4 (three months); F5 (six months); F6 (one year); F7 (two years); F8 (three years); F9 (four years).

2.4. Data Collection

For all of the dogs ($n = 33$), data collection included the following categorical binominal variables: breed, sex, type of complementary exams carried out, neuroanatomical localization, presence of lateralization signs, spinal discomfort, nociception, fecal and urinary incontinence, occurrences, and flexion/extension pattern. The continuous quantitative variables included age, weight, number of days to achieve ambulation (categorized in ≤ 14 days, >14 to ≤ 30 days and >30 days), number of days in hospital (categorized as ≤ 14 days, 30 days, and 60 days), and number of days to recover DPP. Data collection also included categorical ordinal variables, such as OFS evaluation at admission, time points, and follow-ups, and ambulatory status with OFS scores ≥ 11 . Ambulation was defined as the patient's ability to stand up, maintain postural standing, take at least ten steps without assistance or weight support on any walking surface, and obtain voluntary or automatic micturition and defecation [44].

The SSS evaluation of the study group was carried out at admission and at the remaining time points, with scores ≤ 4 being considered a possible better prognosis and >4 being considered a possible worst one.

The cut-off of 4 was established due to clinical evidence regarding spinal shock severity. Thus, until a score of 4 points, reduced hindlimb reflexes and muscle tone may be considered the standard for spinal shock, whereas a score of more than 4 points may be associated with difficulties and an increased time to achieve a positive outcome.

2.5. Statistical Analysis

Quantitative and categorical data were recorded in Microsoft Office Excel 365® (Microsoft Corporation, Redmond, Washington, USA) and processed using IBM SPSS Statistics 25® (International Business Machines Corporation, Armonk, NY, USA) software. Continuous variables (age and

weight) were examined for normal distribution using the Shapiro–Wilk test ($n < 50$). As the data were normally distributed, continuous variables were reported as the arithmetic means, minimum, maximum, standard deviation (SD), and standard error of mean (SEM) with a 95% confidence interval. Descriptive statistics were performed by comparing the clinical and outcome variables between dogs with ANNPE by considering the presence or absence of spinal shock and allowing study population characterization.

The two groups were compared using a one-way repeated measures variance analysis (ANOVA) that considered the “number of hospitalization days” and “number of days until ambulation”. Chi-square tests were also performed to identify the differences between the SG and the CG regarding “the presence of spinal shock” and “the number of hospitalization days”. Univariate analysis of variance was carried out by considering the OFS scores with time as a repeated measure.

3. Results

In this prospective controlled cohort clinical study, as mentioned above, of the 33 dogs with a presumptive diagnosis of ANNPE, 21.2% were mixed-breed dogs, and 78.8% were pure-breed dogs, including Labrador retrievers ($n = 7$), Portuguese water dogs ($n = 3$), bullmastiffs ($n = 2$), Yorkshire terriers ($n = 2$), Spitz ($n = 2$), golden retrievers ($n = 1$), dachshunds ($n = 1$), grand danois ($n = 1$), French mastiffs ($n = 1$), poodles ($n = 1$), whippets ($n = 1$), Jack Russell terriers ($n = 1$), Bouvier bernois ($n = 1$), German shepherds ($n = 1$), and pitbulls ($n = 1$).

Age and weight had a normal distribution ($p = 0.088$ and $p = 0.127$, respectively), according to the Shapiro–Wilk Normality Test ($n < 50$). In each group, data normality was also achieved with a mean age of 5.6 years SD (5.94) in the study group and 6.4 years (SD) 9.48 in the CG, whereas a mean weight of 26.9 kg SD (15.57) was achieved in the SG and a mean weight of 21.9 kg was achieved in the SD (14.38) in the CG. Table 4 reports the results of the descriptive analyses in both the SG and CG.

Ambulatory status was achieved in 94% (31/33) of the total population, with two dogs (**one from the SG**) being euthanatized due to signs of progressive myelomalacia, one after T4 (day 5) and one after T6 (day 14). In the total sample, of the six dogs that did not have DPP, 67% (4/6) recovered: three dogs in the first 3 days after INRP (**one from the SG; two from the CG**) and one dog after 30 days (**one from the CG**). The two dogs that did not recover from DPP achieved the flexion/extension locomotor pattern within ~7 days in the monoplegic limb, leading to spinal reflex locomotion (**two from the CG**).

Regarding time until ambulation of the total population, 32% (10/31) of the dogs became ambulatory within 7 days after starting the INRP (**one from the SG; nine from the CG**), 29% (9/31) became ambulatory within 14 days (**two from the SG; seven from the CG**), and the same number of dogs became ambulatory in 30 days (**eight from the SG; one from the CG**). Only three of the dogs remained non-ambulatory after 30 days (**one from the SG; two from the CG**).

Table 4. Descriptive analysis of age and weight in both the study and control group.

		SG (n = 14)	CG (n = 19)	Total (n = 33)
Age (years)	Mean	5.64	6.42	6.09
	Median	5	6	6
	Variance	5.94	9.48	7.898
	SD	2.437	3.079	2.81
	Minimum	2	1	1
	Maximum	11	12	12
	SEM	0.654	0.706	0.489
	Shapiro–Wilk Normality Test	0.55	0.326	0.088
Weight (kg)	Mean	26.86	21.89	24
	Median	24	21	21
	Variance	242.44	206.655	220.938
	SD	15.57	14.375	14.864
	Minimum	8	4	4
	Maximum	60	57	60
	SEM	4.161	3.298	2.587
	Shapiro–Wilk Normality Test	0.31	0.149	0.127

Legend: SG (study group); CG (control group); SD (standard deviation); SEM (standard error of mean).

In the study group, as for the relation between the presence of spinal shock syndrome and days until ambulation, there was an evident difference between groups, which was demonstrated by one-way ANOVA ((F (1, 29) = [6.109], $p = 0.02$). Furthermore, a similar difference was observed when comparing the SG and CG with regard to the number of hospitalization days and the presence of spinal shock syndrome (X^2 (2, $n = 33$) = 8.806, $p = 0.012$).

The SSS scores of the SG dogs decreased over time (Figure 10 and Table 5). In addition, a strong significance was observed between higher SSS scores during the first 48 h and the number of hospitalization days (X^2 (2, $n = 14$) = 7.000, $p = 0.03$)

**Spinal Shock Scale Estimated Marginal Means
- Time Points -**

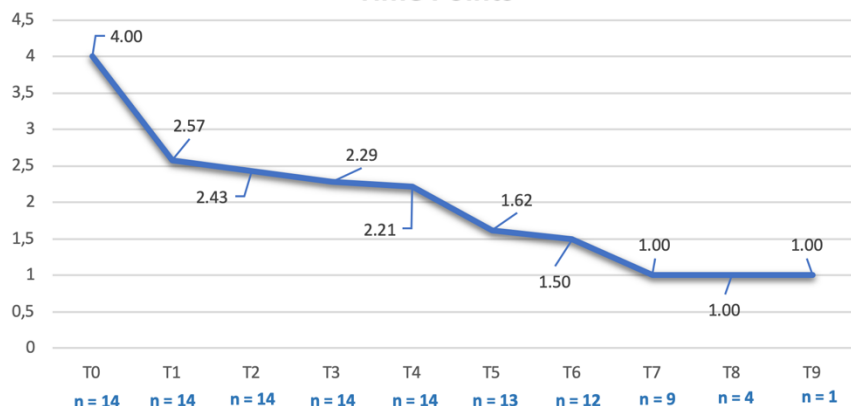


Figure 10. Evolution of the spinal shock scale-estimated marginal means in the study group throughout the intensive neurorehabilitation process. Y-axis: Spinal shock scale score; T0 (day 0); T1 (day 1); T2 (day 2); T3 (day 3); T4 (day 5); T5 (day 7); T6 (day 14); T7 (day 21); T8 (day 30); T9 (day 60).

Table 5. Evolution of the SSS score in the study group over time.

Time points ID	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	6	2	2	1	1	1	.	.	.	.
2	6	2	1	1	1	1	1	1	.	.
3	3	2	2	2	1	1	1	1	1	.
4	3	2	2	2	1	1	1	1	.	.
5	3	2	2	2	2	1	1	1	1	.
6	3	2	2	2	2	1	1	1	.	.
7	3	2	2	2	2	2	1	1	1	.
8	3	2	1	1	1	1	1	.	.	.
9	3	2	2	2	2	1	1	1	.	.
10	3	2	2	2	2	2	1	1	1	1
11	3	2	2	1	1	1	1	1	.	.
12	3	2	1	1	1	1	1	.	.	.
13	7	6	6	6	7	7	7	.	.	.
14	7	6	7	7	7	.	.	.	.	.

Legend: SSS (Spinal Shock Scale); ID (identification); T0 (day 0); T1 (day 1); T2 (day 2); T3 (day 3); T4 (day 5); T5 (day 14); T6 (day 14); T7 (day 21); T8 (day 30); T9 (day 60).

According to the univariate analysis of variance, a significant difference was observed between the groups at each time point ($p < 0.001$; *Adjusted R*²= 0.865), a finding that could be also verified in the OFS-estimated marginal means evolution chart (Figure 11).

The long-term follow ups, which were carried out for a maximum of 4 years, implied a general positive exponential evolution regarding the OFS-estimated marginal means over the follow-up period (Figure 12).

There were no registered adverse effects to the INRP, such as hemodynamic alterations, which were only observed in two dogs who showed signs of progressive myelomalacia.

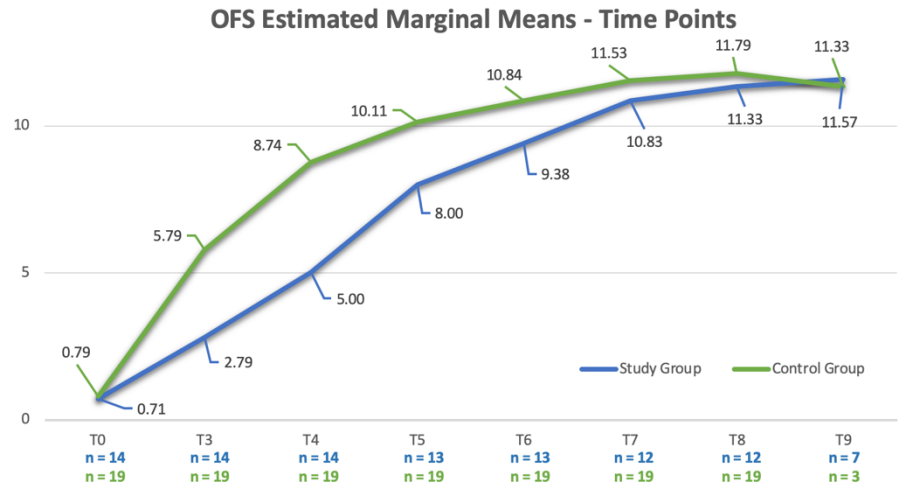


Figure 11. Evolution of the Open Field Score (OFS)-estimated marginal means in both the study and control groups throughout intensive neurorehabilitation. Y-axis: OFS; T0 (day 0); T1 (day 1); T2 (day 2); T3 (day 3); T4 (day 5); T5 (day 7); T6 (day 14); T7 (day 21); T8 (day 30); T9 (day 60).

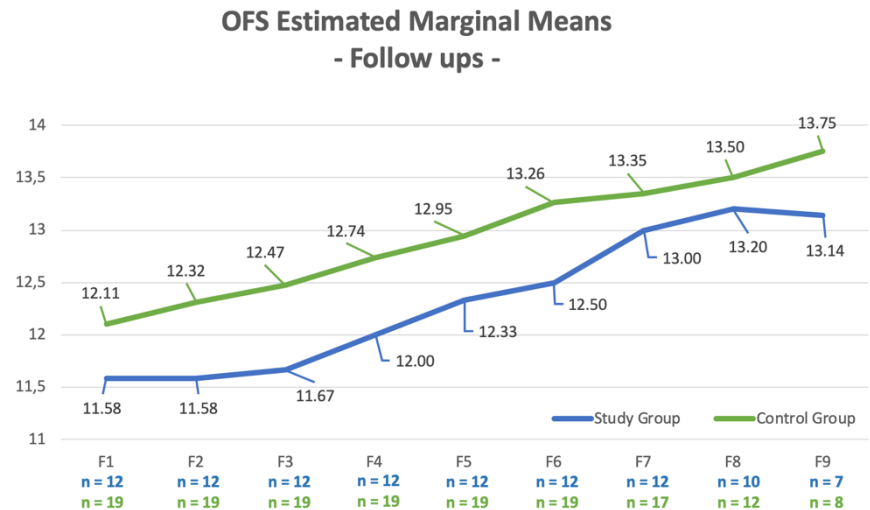


Figure 12. Evolution of the OFS-estimated marginal means in both the study and control groups during the follow-up periods. Y-axis: OFS (Open Field Score); F1 (7 days); F2 (15 days), F3 (one month), F4 (three months), F5 (six months), F6 (one year), F7 (two years), F8 (three years); F9 (four years).

4. Discussion

In the present study, variability was observed in the breed distribution, with the Labrador retriever showing 21% prevalence (7/33), which is in agreement with Freeman (2020) [4]. This could be because it is a large non-chondrodystrophic breed, which is an important feature that has been referred to by McKee et al. (2010), Henke et al. (2013), and Mari et al. (2017) [22,35,37]. However, there was also an evident presence of small breeds, such as the Jack Russell terrier and Yorkshire terrier, as described by De Risio et al. (2009) [16]. The Portuguese water dog showed 9% (3/33) prevalence, as it is an autochthonous breed with a sample expression. Additionally, regarding sex, males were dominant in both groups, a finding that has been observed by other authors [2,16,22,24,50].

For the total sample population, the mean age was 6 years, and the mean weight was 24 kg, also similar to previous studies [11,16,22].

As for the SG and CG, the normality of the data for both age and weight were verified according to the Shapiro–Wilk Test, making the groups comparable.

Between groups, DPP recovery was mostly seen in the CG. For Dewey and Da Costa (2016) [51], ANNPE prognosis is favorable in dogs with DPP. Thus, from the eight dogs admitted to the study who did not have DPP, six of them became ambulatory (75%), with four recovering from DPP (50%) and two achieving spinal reflex locomotion (25%). These two dogs without DPP until clinical discharge showed signs of nociception recovery during the long-term follow-ups, a finding that is in contrast to many authors [16,18,24,37], and this can possibly be justified by the presence of intact trans-lesion connections [29,52,53]. Thus, ANNPE dogs without DPP at admission should be treated carefully according to their prognosis, but it may be possible to achieve a successful outcome. It is essential to identify the appropriate treatment for these dogs who show severe neurological signs [13].

The efficacy of INRP has already been proven in other situations, but the question remains as to how long dogs need to recover. Even with spinal shock syndrome, the total population of dogs revealed 32% (10/31) ambulation within the first 7 days of admission, 29% (9/31) ambulation in 14 days, and 29% ambulation in 30 days. As for the comparison between groups, according to the descriptive analysis, there is a clear delay in recovery in the SG. Previous studies showed successful outcomes of 67%, which were usually obtained within 1 month after injury [5,16], which can be compared to our results achieving 90 % (28/31) recovery within 30 days (T8) and 94% (31/33) recovery in 60 days (T9).

Thus, the differences between groups were evident, especially in regard to time until ambulatory status ($p = 0.02$), the number of hospitalization days ($p = 0.012$), and the OFS-estimated means at each time point registered by the univariate analysis of variance ($p < 0.001$; *Adjusted* $R^2 = 0.9$) (Figure 1), demonstrating the negative relevance of spinal shock syndrome.

Considering the presence of spinal shock syndrome as a negative factor for a quick and successful clinical outcome, it is important for the neuro-rehabilitator to rely on a scale that is able to indicate the possible prognosis and time needed for recovery, i.e., the SSS. This evaluation is mainly essential in the 48 h after injury because within this timeframe, the first signs appear and may shift, with the possible quick recovery of the anal sphincter reflex after 15 min but with the withdrawal reflex only recovering any time from 2 days to 6 weeks [31,32,54]. In our study, only two dogs were euthanized after 5 and 14 days. These dogs had fecal and urinary incontinence at admission, signs that may be present as reported by other authors [50].

Therefore, the INRP may have a role in decreasing the time until recovery, and although the association between the presence of the withdraw reflex and the number of hospitalization days was not studied, the SSS score demonstrated a marked significance in relation to time ($p = 0.03$) in the SG, with dogs having an SSS > 4 requiring more days of hospitalization. On the CG, there was no need to apply this scale, as the two groups were separated after randomized stratification in regard to the presence of spinal shock.

Additionally, spinal shock areflexia may be present for a variable length of time and is followed by a gradual return of reflexes, possibly shifting to a state of hyperreflexia [29,55]. This state can be treated by resorting to electrical stimulation by means of FES [56] to avoid secondary spasticity signs, something that has already been suggested in human patients as a result of the involvement of residual descending connections [57] and a strong contribution from the reticulospinal tract [58,59], possibly delaying recovery, which were signs that were not observed in our patients.

Thus, our results suggest that spinal shock syndrome may not interfere in achieving ambulatory status, which is supported by other studies [18,22,31], although it may delay the time to complete recovery and require more hospitalization days, affecting the neurorehabilitation outcome and agreeing with our hypothesis.

Different authors have recommended restricted activity and cage rest for 4–6 weeks after injury [11,13,37,60], which is the opposite of our study, although the implementation of our INRP was carried out gradually regarding the type, frequency, and intensity of the exercises. This study has established a safe, tolerable, and a feasible protocol that can be applied in ANNPE dogs in a clinical setting. No side effects were observed, except in two dogs, who were determined to be showing myelomalacia progression.

The long-term follow-ups, which were carried out for maximum of 4 years, also revealed the positive evolution of the OFS-estimated marginal means in both groups, again with slightly lower values in the SG (Figure 12), which is indicative of the importance of spinal shock and SSS classification at admission, even for later recovery. The interpretation of the follow-up results should be conducted carefully due to the loss of the sample population after F6 (one year), decreasing the number of dogs who attended these consultations.

The limitations of this study included the reduced sample size, a lack of intra and inter-observers, no SSS validation, and the absence of CNS biomarkers. Additionally, it would be interesting to study the role of neurorehabilitation protocols in terms of hemodynamic alterations as well as fecal and urinary incontinence and their consequences when more dogs without DPP are included. Further studies should be continued with validation of the SSS.

5. Conclusions

This prospective controlled cohort clinical study showed a total of 94% recovery up to a maximum of 60 days, and these results are higher than those obtained in previous studies. Additionally, 75% of the dogs without DPP showed signs of recovery during the long-term follow-up period up to 4 years after intervention, revealing a positive evolution, results that can be accomplished in a clinical setting. However, the SG revealed a clinical and statistical delay compared to the CG regarding time until ambulation and the number of hospitalization days. Thus, it is suggested that the presence of spinal shock had a negative influence on the neurorehabilitation of ANNPE dogs.

Author Contributions: Conceptualization, Â.M. and D.G.; methodology, D.G., A.C. and C.C.; validation, A.F., R.C.-T. and Â.M.; formal analysis, C.C.; investigation, Â.M., D.G., A.C., C.C., A.R.G. and Ó.G.; data curation, C.C. and A.R.G.; writing—original draft preparation, D.G.; writing—review and editing, Â.M., A.C., R.C.-T. and A.F.; visualization, D.G.; supervision, Â.M. and A.F.;

project administration, Â.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted and approved by the ethics committee Comissão de Ética e Bem Estar Animal (CEBEA) of the Faculty of Veterinary Medicine, Lusófona University.

Informed Consent Statement: Informed consent was obtained from all of the owners of the dogs involved in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Da Costa, R.C.; De Decker, S.; Lewis, M.J.; Volk, H.; Consort, C. Diagnostic imaging in intervertebral disc disease. *Front. Vet. Sci.* **2020**, *7*, 782.
2. Fenn, J.; Drees, R.; Volk, H.A.; De Decker, S. Comparison of clinical signs and outcomes between dogs with presumptive ischemic myelopathy and dogs with acute noncompressive nucleus pulposus extrusion. *J. Am. Vet. Med. Assoc.* **2016**, *249*, 767–775.
3. Bartner, L. Neurological Disease of the Thoracic Limb. In *Canine Lameness*, 1st ed.; Duerr, F.M., Ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2020; pp. 255–269.
4. Freeman, P.M. Paresis, Paralysis, and Proprioceptive Ataxia. In *A Practical Approach to Neurology for the Small Animal Practitioner*, 1st ed.; Freeman, P.M., Ives, E., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2020; pp. 275–296.
5. Kim, J.; Kim, H.; Hwang, J.; Eom, K. Preliminary study of presumptive intradural-intramedullary intervertebral disc extrusion in 20 dogs. *J. Vet. Sci.* **2020**, *21*, e52.
6. Levine, J.M.; Fingerroth, J.M. Historical and current nomenclature associated with intervertebral disc pathology. In *Advances in Intervertebral Disc Diseases in Dogs and Cats*, 1st ed.; Fingerroth, J.M., Thomas, W.B., Eds.; Wiley-Blackwell: Ames, IA, USA, 2015; pp. 26–31.
7. Schollum, M.L.; Robertson, P.A.; Broom, N.D. How age influences unravelling morphology of annular lamellae—A study of interfibre cohesivity in the lumbar disc. *J. Anat.* **2010**, *216*, 310–319.
8. Ives, E.; Freeman, P.M. Neurological Emergencies. In *A Practical Approach to Neurology for the Small Animal Practitioner*, 1st ed.; Freeman, P.M., Ives, E., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2020; pp. 329–370.
9. Gandini, G.; Cizinauskas, S.; Lang, J.; Fatzer, R.; Jaggy, A. Fibrocartilaginous embolism in 75 dogs: Clinical findings and factors influencing the recovery rate. *J. Small Anim. Pract.* **2003**, *44*, 76–80.
10. Snyder, L.A.; Tan, L.; Gerard, C.; Fessler, R.G. Spinal cord trauma. In *Bradley's Neurology in Clinical Practice*, 7th ed.; Daroff, R.B., Jankovic, J., Mazziotta, J.C., Pomeroy, S.L., Eds.; Elsevier: St. Louis, MO, USA, 2016; Volume 2, pp. 881–902.
11. Cardy, T.J.A.; De Decker, S.; Kenny, P.J.; Volk, H.A. Clinical reasoning in canine spinal disease: What combination of clinical information is useful? *Vet. Rec.* **2015**, *177*, 171.
12. Cauzinille, L.; Kornegay, J.N. Fibrocartilaginous embolism of the spinal cord in dogs: Review of 36 histologically confirmed cases and retrospective study of 26 suspected cases. *J. Vet. Intern. Med.* **1996**, *10*, 241–245.
13. De Decker, S.; Fenn, J. Acute Herniation of Nondegenerate Nucleus Pulposus, Acute Noncompressive Nucleus Pulposus Extrusion and Compressive Hydrated Nucleus Pulposus Extrusion. *Vet. Clin. N. Am. Small Anim. Pract.* **2018**, *48*, 95–109.
14. Griffiths, I.R. A syndrome produced by dorso-lateral “explosions” of the cervical intervertebral discs. *Vet. Rec.* **1970**, *87*, 737–741.
15. Bagley, R.S. Clinical features of diseases—Spinal cord. In *Fundamentals of Veterinary Clinical Neurology*, 1st ed.; Bagley, R.S., Ed.; Wiley-Blackwell Publishing: Hoboken, NJ, USA, 2005; pp. 174–175.
16. De Risio, L.; Adams, V.; Dennis, R.; McConnell, F.J. Association of clinical and magnetic resonance imaging findings with outcome in dogs with presumptive acute noncompressive nucleus pulposus extrusion: 42 cases (2000–2007). *J. Am. Vet. Med. Assoc.* **2009**, *234*, 495–504.
17. De Risio, L.; Platt, S.R. Fibrocartilaginous embolic myelopathy in small animals. *Vet. Clin. N. Am. Small Anim. Pract.* **2010**, *40*, 859–869.
18. Olby, N.J.; da Costa, R.C.; Levine, J.M.; Stein, V.M. Canine Spinal Cord Injury Consortium (CANSORT SCI). Prognostic Factors in Canine Acute Intervertebral Disc Disease. *Front. Vet. Sci.* **2020**, *7*, 596059.

19. Specchi, S.; Johnson, P.; Beauchamp, G.; Masseau, I.; Pey, P. Assessment of interobserver agreement and use of selected magnetic resonance imaging variables for differentiation of acute noncompressive nucleus pulposus extrusion and ischemic myelopathy in dogs. *J. Am. Vet. Med. Assoc.* **2016**, *248*, 1013–1021.
20. Chang, Y.; Dennis, R.; Platt, S.R.; Penderis, J. Magnetic resonance imaging of traumatic intervertebral disc extension in dogs. *Vet. Rec.* **2007**, *160*, 795–799.
21. De Risio, L.; Adams, V.; Dennis, R.; McConnell, F.J.; Platt, S.R. Magnetic resonance imaging findings and clinical associations in 52 dogs with suspected ischemic myelopathy. *J. Vet. Intern. Med.* **2007**, *21*, 1290–1298.
22. Mari, L.; Behr, S.; Shea, A.; Dominguez, E.; Johnson, P.J.; Ekiri, A.; De Risio, L. Outcome comparison in dogs with presumptive diagnosis of thoracolumbar fibrocartilaginous embolic myelopathy and acute non-compressive nucleus pulposus extrusion. *Vet. Rec.* **2017**, *181*, 293.
23. De Risio, L. What is fibrocartilaginous embolism and is it related to IVDD? In *Advances in Intervertebral Disc Diseases in Dogs and Cats*, 1st ed.; Fingerroth, J.M., Thomas, W.B., Eds.; Wiley-Blackwell: Ames, IA, USA, 2015; pp. 75–87.
24. Ros, C.; de la Fuente, R.; Rodenas, S.; Novellas, R.; Viu, J.; Añor, S. Myelographic and low-field magnetic resonance imaging findings in dogs with presumptive acute hydrated non-compressive nucleous pulposus extrusion. *Vet. Rec.* **2017**, *181*, 594–599.
25. De Risio, L. A review of fibrocartilaginous embolic myelopathy and different types of peracute non-compressive intervertebral disk extrusions in dogs and cats. *Front. Vet. Sci.* **2015**, *2*, 24.
26. Ko, H.-Y.; Ditunno, J.F., Jr.; Graziani, V.; Little, J.W. The pattern of reflex recovery during spinal shock. *Spinal Cord* **1999**, *37*, 402–409.
27. Santiago, P.; Fessler, R.G. Spinal cord trauma. In *Neurology in Clinical Practice: The Neurological Disorders*, 4th ed.; Bradley, W.G., Daroff, R.B., Fenichel, G.M., Jankovic, J., Eds.; Elsevier: Philadelphia, PA, USA, 2004; pp. 1149–1178.
28. De Lahunta, A.; Glass, E. Small animal spinal cord disease. In *Veterinary Neuroanatomy and Clinical Neurology*, 3rd ed.; de Lahunta, A., Glass, E.; Saunders: St. Louis, MO, USA, 2009; pp. 248–250.
29. Smith, P.M.; Jeffery, N.D. Spinal shock—Comparative aspects and clinical relevance. *J. Vet. Intern. Med.* **2005**, *19*, 788–793.
30. Mari, L.; Behr, S.; Shea, A.; Dominguez, E.; Ricco, C.; Alcoverro, E.; Ekiri, A.; Sanchez-Masian, D.; De Risio, L. Predictors of urinary or fecal incontinence in dogs with thoracolumbar acute noncompressive nucleus pulposus extrusion. *J. Vet. Intern. Med.* **2019**, *33*, 2693–2700.
31. Full, A.M.; Heller, H.L.B.; Mercier, M. Prevalence, clinical presentation, prognosis, and outcome of 17 dogs with spinal shock and acute thoracolumbar spinal cord disease. *J. Vet. Emerg. Crit. Care* **2016**, *26*, 412–418.
32. Blauch, B. Spinal reflex walking. *Vet. Med. Small Anim. Clin.* **1977**, *72*, 169–173.
33. Little, J.W. Serial recording of reflexes after feline spinal cord transection. *Exp. Neurol.* **1986**, *93*, 510–521.
34. Ditunno, J.F.; Little, J.W.; Tessler, A.; Burns, A.S. Spinal shock revisited: A four-phase model. *Spinal Cord* **2004**, *42*, 383–395.
35. Henke, D.; Gorgas, D.; Flegel, T.; Vandeveld, M.; Lang, J.; Doherr, M.G.; Forterre, F. Magnetic resonance imaging findings in dogs with traumatic intervertebral disk extrusion with or without spinal cord compression: 31 cases (2006–2010). *J. Am. Vet. Med. Assoc.* **2013**, *242*, 217–222.
36. Wessmann, A.; Posporis, C. Compressive hydrated nucleus pulposus extrusion: Is surgery necessary? *Vet. Rec.* **2017**, *181*, 622–662.
37. McKee, W.M.; Downes, C.J.; Pink, J.J.; Gemmill, T.J. Presumptive exercise associated peracute thoracolumbar disc extrusion in 48 dogs. *Vet. Rec.* **2010**, *166*, 523–528.
38. Yang, X.; Liu, B.; Ouyang, B. Effect of acupuncture combined with rehabilitative training on neural functional recovery of stroke patients during recovery phase: A randomised controlled trial. *World J. Acupunct. Moxibustion* **2014**, *24*, 17–23.
39. Draper, W.E.; Schubert, T.A.; Clemmons, R.M.; Miles, S.A.; Low-level laser therapy reduces time to ambulation in dogs after hemilaminectomy : A preliminary study. *J. Small Anim. Pract.* **2012**, *53*, 465–469.
40. De Freitas, G.R.; Santo, C.C.; De Machado-Pereira, N.A.; Bobinski, F.; Dos Santos, A.R.; Ilha, J. Early cyclical neuromuscular electrical stimulation improves strenght and trophism by Akt pathway signaling in partially paralyzed biceps muscle after spinal cord injury in rats. *Phys. Ther.* **2018**, *93*, 172–181.
41. Canto, S.; Momeni, K.; Ramanujam, A.; Garbarini, E.; Forrest, G.F. Neuromotor response of the leg muscles following a supine, stand retraining with/without neuromuscular electrical stimulation training intervention for individuals with SCI: A case series. In Proceedings of the 2016 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Orlando, FL, USA, 16–20 August **2016**; pp. 3143–3146.
42. Frankel, H.L.; Hancock, D.O.; Hyslop, G.; Melzak, J.; Michaelis, L.S.; Ungar, G.H.; Vernon, J.D.S.; Walsh, J.J. The value of postural reduction in the initial management of closed injuries of the spine with paraplegia and tetraplegia. *Spinal Cord* **1969**, *7*, 179–192.

43. Olby, N.J.; De Risio, L.; Munana, K.R.; Wosar, M.A.; Skeen, T.M.; Sharp, N.; and Keene, B.W. Development of a functional scoring system in dogs with acute spinal cord injuries. *Am. J. Vet. Res.* **2001**, *62*, 1624–1628.
44. Martins, Â.; Gouveia, D.; Cardoso, A.; Carvalho, C.; Coelho, T.; Silva, C.; Viegas, I.; Gamboa, Ó.; Ferreira, A. A controlled clinical study of intensive neurorehabilitation in post-surgical dogs with severe acute intervertebral disc extrusion. *Animals* **2021**, *11*, 3034.
45. Martins, Â.; Gouveia, D.; Cardoso, A.; Carvalho, C.; Silva, C.; Coelho, T.; Gamboa, Ó.; Ferreira, A. Functional neurorehabilitation in dogs with an incomplete recovery 3 months following intervertebral disc surgery: A case series. *Animals* **2021**, *11*, 2442.
46. Liberson, W.T.; Holmquest, H.J.; Scot, D.; Dow, M. Functional electrotherapy stimulation of the peroneal nerve synchronized with the swing phase of the gait of hemiplegic patients. *Arch. Phys. Med. Rehabil.* **1961**, *42*, 101–105.
47. Bennaim, M.; Porrato, M.; Jarleton, A.; Hamon, M.; Carroll, J.D.; Gommeren, K.; Balligand, M. Preliminary evaluation of the effects of photobiomodulation therapy and physical rehabilitation on early postoperative recovery of dogs undergoing hemilaminectomy for treatment of thoracolumbar intervertebral disk disease. *Am. J. Vet. Res.* **2017**, *78*, 195–206.
48. Shaw, K.K.; Brown, L. Modalities Part 2: Laser Therapy. In *Physical Rehabilitation for Veterinary Technicians and Nurses*, 1st ed.; Goldberg, M.E., Tomlinson, J.E., Eds.; Wiley Blackwell: Hoboken, NJ, USA, **2018**; pp. 231–240.
49. Monici, M.; Millis, D.; Ciuperca, I.; McCarthy, D. Laser Therapy. In *Essential Facts of Physical Medicine, Rehabilitation and Sports Medicine in Companion Animals*, 1st ed.; Bockstahler, B., Wittek, K., Levine, D., Maierl, J., Millis, D., Eds.; VBS GmbH: Babenhausen, Germany, **2019**; pp. 245–254.
50. Fenn, J.; Drees, R.; Volk, H.A.; Decker, S. Inter and intraobserver agreement for diagnosing presumptive ischemic myelopathy and acute noncompressive nucleus pulposus extrusion in dogs using magnetic resonance imaging. *Vet. Radiol. Ultrasound* **2016**, *57*, 33–40.
51. Dewey, C.W.; da Costa, R.C. Spinal Trauma Management. In *Practical Guide to Canine and Feline Neurology*, 3rd ed.; Dewey, C.W., da Costa, R.C., Eds.; Wiley Blackwell: Ames, IA, USA, **2016**; pp. 423–433.
52. Fenn, J.; Ru, H.; Jeffery, N.D.; Moore, S.; Tipold, A.; Soebbele, F.J.; Wang-Leandro, A.; Mariani, C.L.; Early, P.J.; Muñana, K.R.; et al. Association between anesthesia duration and outcome in dogs with surgically treated acute severe spinal cord injury caused by thoracolumbar intervertebral disk herniation. *J. Vet. Intern. Med.* **2020**, *34*, 1507–1513.
53. Lewis, M.J.; Howard, J.F.; Olby, N.J. The relationship between trans-lesional conduction, motor neuron pool excitability and motor function in dogs with incomplete recovery from severe spinal cord injury. *J. Neurotrauma* **2017**, *34*, 2994–3002.
54. Thomas, W.B.; Dewey, C.W. Performing the neurologic examination. In *A Practical Guide to Canine and Feline Neurology*, 2nd ed.; Dewey, C.W., Ed.; Wiley-Blackwell: Ames, IA, USA, **2008**; p. 64.
55. Sehl, J.; Gruber, A.D.; Lazzerini, K.; Loderstedt, S. Ischaemic paraspinal myopathy along with myelopathy due to putative fibrocartilaginous embolism in a dog. *Vet. Rec. Case Rep.* **2017**, *5*, e000400.
56. Sivaramakrishnan, A.; Solomon, J.M.; Manikandan, N. Comparison of transcutaneous electrical nerve stimulation(TENS) and functional electrical stimulation(FES) for spasticity in spinal cord injury—A pilot randomized cross-over trial. *J. Spinal Cord Med.* **2018**, *41*, 397–406.
57. Sangari, S.; Lundell, H.; Kirshblum, S.; Perez, M.A. Residual descending motor pathways influence spasticity after spinal cord injury. *Ann. Neurol.* **2019**, *86*, 28–41.
58. Sangari, S.; Perez, M.A. Imbalanced Corticospinal and Reticulospinal Contributions to Spasticity in Spinal Cord Injury. *J. Neurosci.* **2019**, *39*, 7872–7881.
59. Baker, S.N.; Perez, M.A. Reticulospinal contributions to gross hand function after human spinal cord injury. *J. Neurosci.* **2017**, *37*, 9778–9784.
60. Botsoglou, R.; Sarpekidou, E.; Patsikas, M.; Kazakos, G. Acute non-compressive nucleus pulposus extrusion in dogs and cats: An overview. *J. Hell. Vet. Med. Soc.* **2021**, *75*, 2809–2816.

CHAPTER IV

DISCUSSION, CONCLUSION AND FUTURE PERSPECTIVES

1. Discussion

Human, dogs and cats share similar neural control mechanisms (Dietz 2003). The RST and the VST play an essential role for locomotion, posture and muscle, mainly when the CST (in humans) or the RuST (in dogs) are affected (Nathan et al. 1996; Martins 2015a; Uemura 2015). The propriospinal tract is the key for co-ordination during locomotion (Pang and Yang 2000). Thus, combination between afferent sensory stimulation and the neural intrinsic circuit pattern is fundamental to recover ambulation after SCI (Dietz et al. 2002), in both dogs and cats DPP⁻ or DPP⁺ in grade 1 (MFS).

After SCI, with or without surgical approach, it is required to improve postural control, balance, standing ability and walking, achieving functionality based on a consistent ambulation. The guidelines of FNR may be the therapeutic approach to these dogs and cats, which are established on the principles of neuroanatomy and neurophysiology (Côté et al. 2018).

This has been suggested in the past and confirmed by the work of Lewis et al. (2018), with reports of an inter-connection and possible depolarization between residual motor descending tracts, motor interneurons and CPG. Thus, the modulation of a flexion-extension locomotor pattern promotes the locomotion by reflexes in individuals who chronically didn't recover DPP.

In the first 24 hours after a compressive or non-compressive T3-L3 SCI, it is possible the appearance of clinical signs compatible with spinal shock syndrome. This syndrome is complex and has been described as a cause for loss of motor and sensory functions in humans (Ko et al. 1999; Santiago and Fessler 2004)) and in dogs (Smith and Jeffery 2005; De Risio et al. 2007; De Lahunta and Glass 2009). It is shown as a profound depression of segmental spinal reflexes and loss of muscle tone caudally to the injury site, revealing a decreased/absent flexion-extension locomotor pattern, which is essential in DPP- dogs and cats, possibly causing a delay or inability to achieve locomotion by reflexes. It is important to break this phenomenon and promote stimulation of neural pathways throughout neurorehabilitation (Knikou 2012).

The FNR approach explores this new concept of neural reorganization (Martins 2015b; Solopova et al. 2015), possible through multidisciplinary protocols with electrical stimulation modalities and repetitive intensive training (Martins et al. 2021a, 2021b).

Furthermore, it is important to understand that repetitive intensive locomotor training based on the BWSTT may have a superior value in comparison with the conventional over-ground intervention (COGI), a regular practice in the home exercises plan. Although a univariate analysis of variance allowed to observe that both groups (BWSTT vs COGI) improved in regard to the OFS mean values ($p < 0.001$), suggesting the efficacy of combining IES for pain management (with a 4-channel electrotherapy device), the locomotor training in the UWMT (for 3-10 min until 20 min) and FES (with symmetric, biphasic and pulsed current),

adjusted to the muscle fatigue signs. This can also contribute to the maintenance and strengthening of the muscle mass that is lost in the first 6 weeks after the injury (Zidan et al. 2018; Marrero et al. 2021).

After randomization in different groups, a two-way repeated measures analyses of variance (ANOVA) showed a strong significant difference ($p=0.007$) that, in the authors opinion, demonstrated the benefits of BWSTT and repetitive step training on increasing muscle force output, endurance and rhythmic activity by the spinal locomotor circuitry and residual descending/ascending pathways stimulation, similar to other results in human medicine (Mann et al. 2007; Martins et al. 2021b).

At clinical discharge, ambulation was seen in 100% (10/10) of the dogs in the BWSTT group at 4.6 weeks, and only 78% (7/9) in the COGI group at 6.1 weeks. In total, 90% of the population recovered, in agreement with previous studies (Ball et al. 1982; Olby et al. 2003, 2004; Loughin et al. 2005; Langerhuus and Miles 2017).

Thus, the first protocol (FNR-UWTM) promoted the appearance of the flexion-extension locomotor pattern in the first weeks, in subacute post-surgical IVDH dogs with signs of spinal shock. Also, the FNR-BWSTT protocol had higher success in recovery with less time, showing evident better performance and not agreeing with a few studies of human patients with incomplete SCI (Dobkin et al. 2007; Field-Fote and Roach 2010; Daly et al. 2011).

The fact that IVDH is one of the most common neurological dysfunctions in dogs (Muguet-Chanoit et al. 2012; Bos et al. 2012; Bergknut et al. 2015), explains the importance of studying and proving the effect of INR on this population of acute dogs. In the manuscript “A Controlled Clinical Study of Intensive Neurorehabilitation in Post-Surgical Dogs with Severe Acute Intervertebral Disc Extrusion”, a population of 367 acute dogs after IVDH by extrusion, allowed an approximate level of power ($1 - \beta$) of 0.90 and an α (type I error) of 0.01.

Also, this study revealed that in the SG ($n=262$), 68% of ambulation was achieved from day 7 to day 15, similarly to previous studies (Davis and Brown 2002; Aikawa et al. 2012; Hady and Schwarz 2015), but in the DPP⁺, the OFS mean at day 15 was 11.0, higher than the 7.87 previously reported by Zidan et al. (2018), which is indicative of non-ambulation.

The early introduction of FES and UWTM (2-7 days) may justify the success of recovery (Smith et al. 2009; Detloff et al. 2014; Côté et al. 2017). The UWTM, depending on the intensity (number of repetitive movements), volume and duration (Côté et al. 2017), was applied in an early phase, agreeing with different authors, who stated that a premature training has a major importance in recovery (Smith et al. 2009; Detloff et al. 2014; Côté et al. 2017). The locomotor training implemented agreed with different authors (Dietz 2004; Battistuzzo et al. 2012; Benito-Penalva et al. 2012; Meyns et al. 2014; Cassilhas et al. 2015) and inclination of the treadmill was from 10-25° to promote postural standing ability (Maier et al. 2009; Tillakaratne et al. 2014).

The INRP was shown to be feasible, safe and repeatable, proving one of the main goals of this study. Particularly, to evaluate DPP⁻ dogs the FNRS-DPP⁻ scale was developed, allowing the monitorization of dogs under 4-AP administration.

Recently, Immekeppel and colleagues (2021) also assumed that dogs with severe SCI may indeed benefit from neurorehabilitation, although with no expression of statistical significance by a multivariable regression model. This was probably due to the multivariate neurorehabilitation protocols implemented between different clinics and practitioners and the absence of tool to assess gait, such as the OFS. Still, current results showed that these protocols implemented in DPP⁻ dogs have a lower percentage of euthanasia, so owners should be encouraged to do so.

For Olby et al. (2020) and the CANSORT-SCI (2021), nearly 60% of dogs recover DPP/ambulation by 6 months after SCI, which can be compared to our 58,5% of ambulation by 3 months, revealing the possibilities of INR and proving the first aim of this PhD. The positive outcome is difficult to attribute to spontaneous recovery or only to the INRP, but with these results it is proposed that INR contributed to the success regarding time of recovery.

In order to complete a second aim, a case series clinical study was performed with 16 chronic dogs diagnosed with IVDD by extrusion after 3 months, and also, a prospective pilot study with 9 chronic DPP⁻ cats after 3 months of SCI. Thus, the goal was to verify the role of INR in chronic SCI dogs and cats, with or without compressive myelopathies, therefore with or without decompressive surgery.

In the DPP⁻ chronic dogs, the INR was implemented with pharmacological management during two months. The 4-AP is administered to increase PICs amplitude and to modulate a synchronized and coordinated flexion-extension locomotor pattern, with low signs of spasticity (Blauch 1977; McBride et al 1984; Lewis et al. 2019).

In dogs with an incomplete recovery by 3 months following IVD surgery, 100% of the DPP⁺ achieved ambulatory status within a mean time of 47 days (median 45 days), and during follow up the average evolution was positive.

In DPP⁻ dogs, there was no recovery of DPP, in contrast to what observed in acute dogs with the same disease and approach (Martins et al. 2021c). These acute dogs usually regain ambulation in 41 to 62% (Duval et al. 1996; Scott 1997; Scott and McKee 1999; Ito et al. 2005; Tamura et al. 2012; Olby et al. 2003, 2020), in some cases by 6 months (Olby et al. 2020), but there are no similar studies in chronically injured dogs. Thus, in this case series study, it was shown 78% of ambulation within two and a half months by SRL.

On the other hand, the prospective study with 9 cats diagnosed with severe contusion of traumatic origin, 3 months after injury, allowed a comparison with important studies in neuroscience, especially in regard to locomotor training, which is a crucial factor on ambulation

recovery (Forssberg 1980a, 1980b; Barbeau and Rossignol 1987; Lovely et al. 1990; Belanger et al. 1996; Barrière et al. 2008; Frigon 2012).

Therefore, it was considered and suggested that, in cats, bipedal locomotor training may be the first to be implemented, helping the adaptation. These repetitive exercises could activate proprioceptive afferents receptors (groups Ia, Ib and II) (Pearson and Rossignol 1991; Saltiel and Rossignol 2004) throughout stretch bicycle movements performed by the technician in alternation with perineal and base of the tail stimulation (Rossignol and Bouyer 2004; Côté et al. 2011), which partially regulate the sub-phases of step cycle, frequency and speed. Also, the movement of the hip and the potent effect on the intrinsic neural circuits (Rossignol et al. 2008) may be obtained with QLT, activating the CPG and regulating fore/hindlimbs locomotion by the influence of propriospinal pathways and the connection of cervical and lumbar enlargements (Viala and Vidal 1978; Juvin et al. 2005).

Thus, in this study, there was one cat that recovered DPP, which was the one that achieved an early QLT with a good performance since the first instance, suggesting that this recovery could have the influence of a higher quadrupedal stimulation. The QLT presumably increases synaptic activity by interneurons stimulation and neural control reorganization (Fawcett et al. 2007; Gosgnach 2011; Harkema et al. 2011; Angeli et al. 2014; Rybak et al. 2015; Escalona et al. 2017; Khan et al. 2017), eventually resulting in “voluntary movement”. Once again, the 10-25° treadmill inclination, promoted the neuroplasticity of VST and RST, responsible for posture (Jaggy and Platt 2010) that is essential to neurorehabilitation. In this study, the INRP allowed 56% (5/9) of chronic paraplegic cats without DPP to recover ambulation, 4 cats with SRL and 1 cat that regained DPP.

In order to complete the third aim, the influence of spinal shock on the neurorehabilitation of ANNPE dogs was study. A population of 33 dogs classified as paraplegic/monoplegic, with T3-L3 lesions, with or without nociception, was evaluated and divided into a SG (n=14) and a CG (n=19).

The SG, with dogs that presented signs of spinal shock, was evaluated by a new scale – The Spinal Shock Scale (SSS). Results showed that SSS scores > 4 required a longer period time until ambulation, but the intensive neurorehabilitation protocol applied was safe, tolerable and feasible for all dogs, with a total of 32% of ambulation within 7 days.

These results, suggested that the presence of spinal shock syndrome may be a negative factor for a faster successful outcome. Also, the areflexia state that is usually present for a variable length of time is followed by a gradual reflexes' recovery (Fenn et al. 2016; Nacimiento and Noth 1999; Ditunno et al. 2004; Full et al. 2016) and in this study, that was shown in our dogs, with 6 DPP⁻ dogs becoming ambulatory (4 that recovered DPP and 2 that achieved spinal reflex locomotion). However, along the long-term follow-ups, all dogs

recovered DPP, not agreeing with other authors (De Risio et al. 2009; McKee et al. 2010; Ros et al. 2017; Olby et al. 2020).

In regard to ANNPE dogs, previous studies reported success in 67% within 1 month after injury (De Risio et al. 2009; Kim et al. 2020), whereas in this study there was 90% (28/31) within 1 month. The differences between groups were clear regarding time until ambulation and the number of days of hospitalization, proving the negative relevance of spinal shock syndrome.

These PhD published works should be continued, taking in consideration particular limitations. As major limitations, all studies were performed in a clinical setting and without highly sensitive measuring techniques to monitor outcomes and minor limitations of each study were discussed at the end of each discussion section, respectively.

2. Conclusion

Neurorehabilitation with multidisciplinary protocols could be applied in the veterinary field to SCI dogs and cats, in the acute, sub-acute and chronic stages.

Electrical stimulation modalities may help to improve the flexion-extension locomotor pattern in paraplegic dogs and cats, with or without DPP, by stimulation of the peripheral component of the peripheral nervous system with FES, and by stimulation of the central components with TESCS. Along this PhD, it was proved that these modalities were safe, tolerable, repeatable and with practical application in a clinical setting.

Also, the locomotor training based on BWSTT applied on paraplegic DPP⁺ dogs with spinal shock signs, secondary to acute history of thoraco-lumbar SCI with IVDH- Hansen type I after surgery, was shown to be safe, repeatable and promoted better functional outcome in less time, when compared to conventional over ground stimulation.

After INR, the same type of DPP⁺ population achieved an OFS mean of 11, higher than previously described by Zidan et al. (2018). The evolution of the DPP⁻ was positive in 58.5% of dogs that regained ambulation by 3 months, 37.3% without recovering DPP. Thus, a higher prevalence in less time, comparing to the results reported by others (Olby et al. 2020; Olby and Tipold 2021). Therefore, the implementation of an INR program showed better outcomes in a short period of time.

Considering the possible link between these results and spontaneous recovery, a similar population was considered but with chronic presentation. In chronic dogs it was observed 100% of ambulation recovery in DPP⁺ and 78% in DPP⁻ with SRL within 2 and a half months. All achieved the flexion-extension locomotor pattern within the first two weeks, allowing the 4-AP administration protocol and all dogs showed positive neurological evolution in the follow-up consultation.

Regarding DPP⁻ cats with chronic severe thoracolumbar contusion after trauma, the prospective pilot study showed a 56% rate of ambulation after the implementation of an intensive protocol without similar studies to compare.

In ANNPE paraplegic/monoplegic dogs, the prospective controlled cohort clinical study, revealed 75% of ambulation in the DPP⁻ dogs and a total of 94% of ambulation (DPP⁻ and DPP⁺) within a maximum of 60 days.

All PhD studies resulted in successful ambulation rates, proving that neurorehabilitation should be considered and advised in order to achieve ambulation/functionality. Studies should be continued in SCI, as well as for other neurological disorders.

3. Future Perspectives

Dogs presenting ANNPE or other SCI, that may develop clinical signs compatible with spinal shock syndrome, should continue to be studied. A higher number of dogs under the evaluation with the SSS, could help in the validation and confirmation of this scale utility for neurorehabilitation.

Also, dogs and cats that are DPP⁻ have worst prognosis, and clinical management with substantial nursing care may be needed for weeks to months, especially in patients with severe conditions, decreasing the possibility for long-term complications (Ducoté 2019).

This type of situations requires trained veterinary nursing staff, not only for usual treatment but to avoid and prevent future complications (Sharp and Wheeler 2005), such as pressure ulcers and urinary tract infections. Also, it is of main importance to teach owners to adjust their home environment, such as adding non-slippery surfaces that provide traction, and to make them aware of the need to a good neurogenic bladder approach in these T3-L3 patients. An accurate urinary management will prevent from permanent damage to the detrusor muscle and decrease the presence of reflex dyssynergia.

The use of a wheelchair not in a typical way, but as a locomotor training device similar to the BWSTT, may help to recover DPP in long term (one to two years after injury), and to improve spinal reflex locomotion, intensifying stepping repetition. Future studies should be done to assess this possibility.

Other conditioning factors, are reported to be associated with increasing age, body condition (obesity) and a sedentary lifestyle, which have been negatively correlated with activity (Robertson 2003; Chan et al. 2005; Morrison et al. 2014; Frye et al. 2022)

Thus, in the future, as observed in human patients with SCI (AIS A and AIS B), there are several aspects of the locomotor electromyographic pattern (Côté et al. 2017) that should improve the ability to shape the neurorehabilitation program to each individual specific needs. This could be integrated with the OFS, specifying the protocols and reducing the possibility of

overtraining that was not clear in our studies when we talked about intensity, volume, duration, timing and number of repetitions.

The compound muscle action potential (CMAP) that is recorded with surface electrodes, indirectly assess the conduction of motor fibers axons. The CMAP amplitude also depends on the relative conduction speed of the axons, the integrity of the neuromuscular functions and the number of muscle fibers that are able to generate action potentials (Katirji 2012). This may improve the programming of FES parameters, given electrical stimulation intensity is measured by current density (mA/cm^2), and explain the pharmacological management with blocked potassium drugs, such as the 4-AP. The length of stimulus intervention is also a key parameter to evoke an effective neuromuscular effect (Garcia et al. 2020).

It would be of great interest to perform a deeper study about 4-AP applicability, both in DPP^- and DPP^+ patients, proving its safety, tolerability and evaluation of possible side effects, and in prospective cohort-controlled studies to verify the success of INRP with 4-AP.

In addition, kinematic and kinetic gait analysis could be used in future clinical practice as an outcome measure, eventually in association with OFS. Thus, computer-assisted gait analysis of this type may be an important tool to avoid observation errors of the human eye, as demonstrated by other researchers (van Klaveren et al. 2005; Bockstahler et al. 2012; Druen et al. 2012; Deisenroth et al. 2013; Souza et al. 2019).

More investigations are needed in the future, i.e. specialized applications of MRI, which may include diffusion tensor imaging, beneficial to the evaluation of DPP^- dogs and cats. The eminent need to study axonal damage, demyelination, Wallerian degeneration and loss of tissue architecture (Vedantam et al. 2014), leads to the tractography (Basser et al. 2000; Ducreux et al. 2007).

The tractography, already used in human medicine, could be a long-term functional outcome in this population of DPP^- dogs and cats after SCI (Facon et al. 2005; Schwartz et al. 2005; Endo et al. 2011; Peterson et al. 2012; Koskinen et al. 2013; Martin et al. 2016), such as functional MRI, myelin water fraction imaging, magnetization transfer imaging and magnetic resonance spectroscopy (Filippi and Rocca 2007; Martin et al. 2016; Cohen-Adad 2017; Wyss et al. 2017; Laule and Moore 2018), that have been reported in dogs with spondylomyelopathy and in experimental canine IVDD models. However, this has not been translated to the clinical setting as a potential outcome to be applied in the monitoring of neural modulation, allowing the design and modification of specific and individual INR protocols.

4. References

- Aikawa T, Fujita H, Kanazono S, Shibata M, Yoshigae Y. 2012. Long-term neurologic outcome of hemilaminectomy and disk fenestration for treatment of dogs with thoracolumbar intervertebral disk herniation: 831 cases (2000–2007). *J. Am Veter-Med Assoc.* 241: 1617–1626.
- Angeli CA, Edgerton VR, Gerasimenko YP, Harkema SJ. 2014. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain.* 137: 1394–1409.
- Ball MU, McGuire JA, Swaim SF, Hoerlein BF. 1982. Patterns of occurrence of disk disease among registered dachshunds. *J Am Vet Med Assoc.* 180: 519–22.
- Barbeau H, Rossignol S. 1987. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* 412: 84–95.
- Barrière G, Leblond H, Provencher J, Rossignol S. 2008. Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries. *J Neurosci.* 28: 3976–3987.
- Basser PJ, Pajevic S, Pierpaoli C, Duda J, Aldroubi A. 2000. In vivo fiber tractography using DT-MRI data. *Magn Reson Med.* 44:625–32.
- Battistuzzo CR, Callister RJ, Callister R, Galea MP. 2012. A systematic review of exercise training to promote locomotor recovery in animal models of spinal cord injury. *J Neurotrauma.* 29:1600–1613.
- Belanger M, Drew T, Provencher J, Rossignol S. 1996. A Comparison of Treadmill Locomotion in Adult Cats Before and After Spinal Transection. *J Neurophysiol.* 76: 471–491.
- Benito-Penalva J, Edwards DJ, Opisso E, Cortes M, Lopez-Blazquez R, Murillo N, Costa U, Tormos JM, Vidal-Samsó J, Valls-Solé J, Medina J. 2012. Gait training in human spinal cord injury using electromechanical systems: Effect of device type and patient characteristic. *Arch Phys Med Rehabil.* 93: 404–412.
- Bergknut N, Forterre F, Levine JM, Lasser SD, Fingerroth JM. 2015. Comparisons between biped (human) and quadruped (canine/feline) intervertebral disc disease. In: JM Fingerroth and WB Thomas, eds. *Advances in Intervertebral Disc Disease in Dogs and Cats*, 1st ed. Iowa: American College of Veterinary Surgeons Foundation (ACVS Foundation) & Wiley-Blackwell. p. 14–19.
- Bockstahler BA, Prickler B, Lewy E, Holler PJ, Voborni A, Peham C. 2012. Hind limb kinematics during therapeutic exercises in dogs with osteoarthritis of the hip joints. *Am J Vet Res.* 73: 1371–1376.
- Blauch B. Spinal reflex walking in the dog. 1977. *Vet Med Sm Anim Clin.* 169:173.
- Bos AS, Brisson BA, Nykamp SG, Poma R, Foster RA. 2012. Accuracy, intermethod agreement, and inter-reviewer agreement for use of magnetic resonance imaging and myelography in small-breed dogs with naturally occurring first-time intervertebral disk extrusion. *J Am Vet Med Assoc.* 240 (8): 969–977.
- Cassilhas RC, Tufik S, Mello MT. 2015 Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol Life Sci.* 73(5):975–83.
- Chan CB, Spierenburg M, Ihle SL, Tudor-Locke C. 2005. Use of pedometers to measure physical activity in dogs. *J Am Vet Med Assoc.* 226:2010– 5.
- Cohen-Adad J. 2017. Functional magnetic resonance imaging of the spinal cord: current status and future developments. *Semin Ultrasound CT MRI.* 38:176–86.
- Côté MP, Azzam GA, Lemay MA, Zhukareva V, Houlié JD. 2011. Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after spinal cord injury. *J Neurotrauma.* 28: 299–309.
- Côté MP, Murray M, Lemay MA. 2017. Rehabilitation strategies after spinal cord injury: inquiry into the mechanisms of success and failure. *J Neurotrauma.* 34: 1841–1857.
- Côté MP, Murray LM, Knikou M. 2018. Spinal Control of locomotion: Individual neurons, their circuits and functions. *Front. Physiol.* 9: 784
- Daly JJ, Zimbelman J, Roenigk KL, McCabe JP, Rogers JM, Butler K, Burdsall R, Holcomb JP, Marsolais EB, Ruff RL. 2011. Recovery of coordinated gait: randomized

controlled stroke trial of functional electrical stimulation (FES) versus no FES, with weight-supported treadmill and Over-Ground training. *Neurorehabil Neural Repair*. 25: 588-96.

Davis GJ, Brown DC. 2002. Prognostic indicators for time to ambulation after surgical decompression in nonambulatory dogs with acute thoracolumbar disk extrusions: 112 cases. *Veter Surg*. 31: 513–518.

De Lahunta A, Glass E. 2009. Small animal spinal cord disease. In: De Lahunta A, Glass E, Eds. *Veterinary Neuroanatomy and Clinical Neurology*. 3rd ed. St. Louis, USA: Saunders. p. 248–250.

De Risio L, Adams V, Dennis R, McConnell FJ, Platt SR. 2007. Magnetic resonance imaging findings and clinical associations in 52 dogs with suspected ischemic myelopathy. *J Vet Intern Med*. 21: 1290–1298.

De Risio L, Adams V, Dennis R, McConnell FJ. 2009. Association of clinical and magnetic resonance imaging findings with outcome in dogs with presumptive acute noncompressive nucleus pulposus extrusion: 42 cases (2000–2007). *J Am Vet Med Assoc*. 234: 495–504.

Deisenroth A, Nolte I, Wefstaedt P. 2013. Use of gold implants as a treatment of pain related to canine hip dysplasia – a review. Part 2: Clinical trials and case reports. *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 41: 244–254.

Detloff MR, Smith EJ, Molina DQ, Ganzer PD, Houlié JD. 2014. Acute exercise prevents the development of neuropathic pain and the sprouting of non-peptidergic (GDNF- and artemin-responsive) c-fibers after spinal cord injury. *Exp Neurol*. 255: 38–48.

Dietz V, Müller R, Colombo G. 2002. Locomotor activity in spinal man: significance of afferent input from joint and load receptors. *Brain*. 125 (12): 2626–2634.

Dietz V. 2003. Spinal cord pattern generators for locomotion. *Clin Neurophysiol*. 14:1379–89.

Dietz V. 2004. Degradation of neuronal function following a spinal cord injury: mechanisms and counter measures. *Brain*. 127:2221–2231.

Ditunno JF, Little JW, Tessler A, Burns AS. 2004. Spinal shock revisited: a four- phase model. *Spinal Cord*. 42:383–395.

Dobkin B, Barbeau H, Deforge D, Ditunno J, Elashoff R, Apple D, Basso M, Behrman A, Harkema S, Saulino M, et al. 2007. The evolution of walking-related outcomes over the first 12 weeks of rehabilitation for incomplete traumatic spinal cord injury: the multicenter randomized spinal cord injury locomotor trial. *Neurorehabil Neural Repair*. 21: 25–35.

Druen S, Boddeker J, Meyer-Lindenberg A, Fehr M, Nolte I, Wefstaedt P. 2012. Computer-based gait analysis of dogs: evaluation of kinetic and kinematic parameters after cemented and cementless total hip replacement. *Vet Comp Orthop Traumatol*. 25: 375–384.

Ducoté JM. 2019. Common neurologic problems: impact on patient welfare, caregiver burden and veterinarian wellbeing. *Vet Clin North Am Small Anim Pract*. 49(3): 463–476.

Ducreux D, Fillard P, Facon D, Ozanne A, Lepeintre J, Renoux J, Tadié M, Lasjaunias P. 2007. Diffusion tensor magnetic resonance imaging and fiber tracking in spinal cord lesions: current and future indications. *Neuroimaging Clin N Am*. 17:137–47

Duval J, Dewey C, Roberts R, Aron D. 1996. Spinal cord swelling as a myelographic indicator of prognosis: A retrospective study in dogs with intervertebral disc disease and loss of deep pain perception. *Vet. Surg*. 25: 6–12

Endo T, Suzuki S, Utsunomiya A, Uenohara H, Tominaga T. 2011. Prediction of neurological recovery using apparent diffusion coefficient in cases of incomplete spinal cord injury. *Neurosurgery*. 68:329–36.

Escalona M, Delivet-Mongrain H, Kundu A, Gossard JP, Rossignol S. 2017. Ladder treadmill: A method to assess locomotion in cats with an intact or lesioned spinal cord. *J Neurosci*. 37: 5429–5446.

Facon D, Ozanne A, Fillard P, Lepeintre JF, Tournoux-Facon C, Ducreux D. 2005. MR diffusion tensor imaging and fiber tracking in spinal cord compression. *Am J Neuroradiol*. 26:1587–94.

Fawcett JW, Curt A, Steeves JD, Coleman WP, Tuszynski MH, Lammertse D, Bartlett PF, Blight AR, Dietz V, Ditunno J, et al. 2007. Guidelines for the conduct of clinical trials for

spinal cord injury as developed by the ICCP panel: Spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. *Spinal Cord*. 45: 190–205.

Fenn J, Drees R, Volk HA, Decker S. 2016. Inter and intraobserver agreement for diagnosing presumptive ischemic myelopathy and acute noncompressive nucleus pulposus extrusion in dogs using magnetic resonance imaging. *Vet Radiol Ultrasound*. 57: 33–40.

Field-Fote EC, Roach KE. 2010. Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: A randomized clinical trial. *J Am Phys Ther Assoc*. 91: 48-60.

Filippi M, Rocca MA. 2007. Magnetization transfer magnetic resonance imaging of the brain, spinal cord, and optic nerve. *J Am Soc Exper Neurother*. 4:401–13.

Forssberg H, Grillner S, Halbertsma J. 1980a. The locomotion of the low spinal cat I. Coordination within a hindlimb. *Acta Physiol Scand*. 108: 269–281.

Forssberg H, Grillner S, Halbertsma J, Rossignol S. 1980b. The locomotion of the low spinal cat. II. Interlimb coordination. *Acta Physiol Scand*. 108: 283–295.

Frigon A. 2012. Central pattern generators of the mammalian spinal cord. *Neuroscientist*. 18: 56–69.

Full AM, Heller HLB, Mercier M. 2016. Prevalence, clinical presentation, prognosis, and outcome of 17 dogs with spinal shock and acute thoracolumbar spinal cord disease. *J Vet Emerg Crit*. 26: 412–8.

García AM, Serrano-Munoz D, Taylor J, Avendano-Coy J, Gómez-Soriano J. 2020. Transcutaneous spinal cord stimulation and motor rehabilitation in spinal cord injury: a systematic review. *Neurorehabil Neural Repair*. 34(1): 3-12.

Gosgnach S. 2011. The role of genetically-defined interneurons in generating the mammalian locomotor rhythm. *Integr Comp Biol*. 51: 903–912.

Hady LL, Schwarz PD. 2015. Recovery times for dogs undergoing thoracolumbar hemilaminectomy with fenestration and physical rehabilitation: A review of 113 cases. *JVMAH*. 7: 278–289.

Harkema S, Gerasimenko Y, Hodes J, Burdick J, Angeli C, Chen Y, Ferreira C, Willhite A, Rejc E, Grossman RG, et al. 2011. Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: A case study. *Lancet* 377: 1938–1947.

Immekeppel A, Rupp S, Demierre S, Rentmeister K, Meyer-Lindenberg A, Goessmann J, Bali MS, Schmidli-Davies F, Forterre F. 2021. Investigation of timing of surgery and other factors possibly influencing outcome in dogs with acute thoracolumbar disc extrusion: a retrospective study of 1501 cases. *Acta Vet Scand*. 63:30.

Ito D, Matsunaga S, Jeffery ND, Sasaki N, Nishimura R, Mochizuki M, Kasahara M, Fujiwara R, Ogawa H. 2005. Prognostic value of magnetic resonance imaging in dogs with paraplegia caused by thoracolumbar intervertebral disk extrusion: 77 cases (2000–2003). *J Am Vet Med Assoc*. 227: 1454–1460.

Jaggy A, Platt S. 2010. *Small Animal Neurology An Illustrated Text*; Hannover, Germany: Schlütersche Verlagsgesellschaft mbH & Co. KG. p. 171-181.

Juvin L, Simmers J, Morin D. 2005. Propriospinal circuitry underlying interlimb coordination in mammalian quadrupedal locomotion. *J Neurosci*. 25: 6025–6035.

Katirji B. 2016. Clinical Electromyography. In: RB Daroff, J Jankovic, JC Mazziotta, SL Pomeroy, eds. *Bradley's Neurology in clinical practice*, 7th ed. London: Elsevier. p.366-390.

Khan F, Amatya B, Galea MP, Gonzenbach R, Kesselring J. 2017. Neurorehabilitation: Applied neuroplasticity. *J Neurol*. 264: 603–615.

Kim J, Kim H, Hwang J, Eom K. 2020. Preliminary study of presumptive intradural-intramedullary intervertebral disc extrusion in 20 dogs. *J Vet Sci*. 21: e52.

Knikou M. 2012. Plasticity of corticospinal neural control after locomotor training in human spinal cord injury. *Neural Plast*. 2012:254948.

Ko HY, Ditunno JF, Graziani V, Little JW. 1999. The pattern of reflex recovery during spinal shock. *Spinal Cord*. 37: 402–409.

Koskinen E, Brander A, Hakulinen U, Luoto T, Helminen M, Ylinen A, Ohman J. 2013. Assessing the state of chronic spinal cord injury using diffusion tensor imaging. *J Neurotrauma*. 30:1587–95.

Langerhuus L, Miles J. 2017. Proportion recovery and times to ambulation for non-ambulatory dogs with thoracolumbar disc extrusions treated with hemilaminectomy or conservative treatment: a systematic review and meta-analysis of case-series studies. *Vet J*. 220:7–16.

Laule C, Moore GRW. 2018. Myelin water imaging to detect demyelination and remyelination and its validation in pathology. *Brain Pathol*. 28:750–64.

Lewis MJ, Yap PT, McCullough S, Olby NJ. 2018. The relationship between lesion severity characterized by diffusion tensor imaging and motor function in chronic canine spinal cord injury. *J Neurotrauma*. 35 (3): 500-507.

Lewis MJ, Laber E, Olby NJ. 2019. Predictors of response to 4-aminopyridine in chronic canine spinal cord injury. *J Neurotrauma*. 36: 1428–1434.

Loughin CA, Dewey CW, Ringwood PB, Pettigrew RW, Kent M, Budsberg SC. 2005. Effect of durotomy on functional outcome of dogs with type I thoracolumbar disc extrusion and absent deep pain perception. *Vet Comp Orthop Traumatol*. 3:141-6.

Lovely RG, Gregor RJ, Roy RR, Edgerton VR. 1990. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats. *Brain Res*. 514: 206–218.

Maier IC, Ichiyama RM, Courtine G, Schnell L, Lavrov I, Edgerton VR, Schwab ME. 2009. Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. *Brain*. 132: 1426–1440.

Mann F, Wagner-Mann C, Dunphy E, Ruben D, Rochat M, Bartels K. 2007. Recurrence rate of presumed thoracolumbar intervertebral disc disease in ambulatory dogs with spinal hyperpathia treated with anti-inflammatory drugs: 78 cases (1997-2000). *J Vet Emerg Crit Care*. 17: 53-60.

Marrero NPA, Thomovsky SA, Linder JE, Bowditch J, Lind M, Kazmierczak KA, Moore GE, Lewis MJ. 2022. Static body weight distribution and girth measurements over time in dogs after acute thoracolumbar intervertebral disc extrusion. *Front Vet Sci*. 9:877402.

Martin AR, Alexsanderek I, Cohen-Adad J, Tarmohamed Z, Tetreault L, Smith N, Cadotte DW, Crawley A, Ginsberg H, Mikulis DJ, et al. 2016. Translating state-of-the-art spinal cord MRI techniques to clinical use: a systematic review of clinical studies utilizing DTI, MR, MWF, MRS, and fMRI. *Neuroimage*. 10:192–238.

Martins A. 2015a. The importance of the quadruped animal model in functional neurorehabilitation for human biped. *International Archives of Medicine: Physical Medicine and Rehabilitation*. 8 (178): 1-9.

Martins A. 2015b. Functional neurorehabilitation - the locomotor quadrupedal animal training adapted to the bipedal human. *International Archives of Medicine: Physical Medicine and Rehabilitation*. 8 (179): 1-11.

Martins A, Gouveia D, Cardoso A, Gamboa O, Millis D, Ferreira A. 2021a. Nervous system modulation through electrical stimulation in companion animals. *Acta Vet. Scand*. 63(22): 1-12.

Martins A, Gouveia D, Cardoso A, Viegas I, Gamboa O, Ferreira A. 2021b. A comparison between body weight-supported treadmill training and conventional over-ground training in dogs with incomplete spinal cord injury. *Front. Vet. Sci*. 8(597949):1-14.

Mcbride WJ, Hall PV, Chernet E, Patrick JT, Shapiro S. 1984. Alterations of amino acid transmitter systems in spinal cords of chronic paraplegic dogs. *J Neurochem*. 42: 1625-1631.

McKee WM, Downes CJ, Pink JJ, Gemmill TJ. 2010. Presumptive exercise associated peracute thoracolumbar disc extrusion in 48 dogs. *Vet. Rec*. 166: 523–528.

Meyns P, Van de Crommert HWAA, Rijken H, Van Kuppevelt DHJM, Duysens J. 2014. Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed. *Spinal Cord*. 52: 887–893.

Morrison R, Reilly JJ, Penpraze V, Pendlebury E, Yam PS. 2014. A 6- month observational study of changes in objectively measured physical activity during weight loss in dogs. *J Small Anim Pract*. 55:566– 70.

Muguet-Chanoit AC, Olby NJ, Lim JH, Gallagher R, Niman Z, Dillard S, Campbell J, Early P, Mariani CL, Munana KR, et al. 2012. The cutaneous trunci muscle reflex: a predictor of recovery in dogs with acute thoracolumbar myelopathies caused by intervertebral disc extrusions. *Vet Surg.* 41: 200-206

Nacimiento W, Noth J. 1999. What, if anything is spinal shock? *Arch Neurol.* 56:1033–1035.

Nathan PW, Smith M, Deacon P. 1996. Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. *Brain.* 119:1809–33.

Olby NJ, Levine J, Harris T, Muñana K, Skeen T, Sharp N. 2003. Long-term functional outcome of dogs with severe injuries of the thoracolumbar spinal cord: 87 cases (1996–2001). *J Am Vet Med Assoc.* 222 (6): 762-769.

Olby N, Harris T, Burr J, Muñana K, Sharp N, Keene B. 2004. Recovery of pelvic limb function in dogs following acute intervertebral disc herniations. *J Neurotrauma.* 21: 49–59.

Olby NJ, Da Costa RC, Levine JM, Stein VM. 2020. The Canine Spinal Cord Injury Consortium (CANSORT SCI) Prognostic Factors in Canine Acute Intervertebral Disc Disease. *Front Vet Sci.* 7 (596059): 144–157.

Olby NJ, Tipold A. 2021. Editorial: Canine Intervertebral Disc Disease: The Current State of Knowledge. *Front Veter Sci.* 8.

Pang MY, Yang JF. 2000. The initiation of the swing phase in human infant stepping: importance of hip position and leg loading. *J Physiol* 528 (2): 389-404.

Pearson KG, Rossignol S. 1991. Fictive Motor Patterns in Chronic Spinal Cats. *J Neurophysiol.* 66: 1874–1887.

Peterson JA, Wilm BJ, von Meyenburg J, Schubert M, Seifert B, Najafi Y, Dietz V, Kollias S. 2012. Chronic cervical spinal cord injury: DTI correlates with clinical and electrophysiological measures. *J Neurotrauma.* 29:1556–66.

Robertson ID. 2003. The association of exercise, diet and other factors with owner-perceived obesity in privately owned dogs from metropolitan Perth, WA. *Prev Vet Med.* 58:75–83.

Ros C, de la Fuente R, Rodenas S, Novellas R, Viu J, Añor S. 2017. Myelographic and low-field magnetic resonance imaging findings in dogs with presumptive acute hydrated non-compressive nucleus pulposus extrusion. *Vet. Rec.* 181: 594–599.

Rossignol S, Bouyer L. 2004. Adaptive Mechanisms of Spinal Locomotion in Cats. *Integr Comp Biol.* 44: 71–79.

Rossignol S, Barrière G, Frigon A, Barthélemy D, Bouyer L, Provencher J, Leblond H, Bernard G. 2008. Plasticity of locomotor sensorimotor interactions after peripheral and/or spinal lesions. *Brain Res Rev.* 57: 228–240.

Rybak IA, Dougherty KJ, Shevtsova NA. 2015. Organization of the mammalian locomotor CPG: Review of computational model and circuit architectures based on genetically identified spinal interneurons. *eNeuro.* 2.

Saltiel P, Rossignol S. 2004. Critical points in the forelimb fictive locomotor cycle and motor coordination: Effects of phasic retractions and protractions of the shoulder in the cat. *J Neurophysiol.* 92: 1342–1356.

Santiago P, Fessler RG. 2004. Spinal cord trauma. In: Bradley WG, Daroff RB, Fenichel GM, Jankovic J, Eds. *Neurology in Clinical Practice: The Neurological Disorders*, 4th ed. Philadelphia, USA: Elsevier. p. 1149–1178.

Schwartz ED, Duda J, Shumsky JS, Cooper ET, Gee J. 2005. Spinal cord diffusion tensor imaging and fiber tracking can identify white matter tract disruption and glial scar orientation following lateral funiculotomy. *J Neurotrauma.* 22:1388–98.

Sharp NJH, Wheeler SJ. 2005. Postoperative care. In: *Small animal spinal disorders: diagnosis and surgery*. 2nd edition. London: Elsevier Limited; 2005. p. 339–62.

Scott HW. 1997. Hemilaminectomy for the treatment of thoracolumbar disc disease in the dog: A follow-up study of 40 cases. *J Small Anim Pract.* 38: 488–494.

Scott HW, McKee WM. 1999. Laminectomy for 34 dogs with thoracolumbar intervertebral disc disease and loss of deep pain perception. *J Small Anim Pract.* 40: 417–422.

Smith PM, Jeffery ND. 2005. Spinal shock—Comparative aspects and clinical relevance. *J Vet Intern Med.* 19: 788–793.

Smith RR, Brown EH, Shum-Siu A, Whelan A, Burke DA, Benton RL, Magnuson D. 2009. Swim Training Initiated Acutely after Spinal Cord Injury Is Ineffective and Induces Extravasation in and around the Epicenter. *J Neurotr.* 26: 1017–1027.

Solopova IA, Selionov VA, Sylos-Labini F, Gurfinkel VS, Lacquaniti F, Ivanenko YP. 2015. Tapping into rhythm generation circuitry in humans during simulated weightlessness conditions. *Front Syst Neurosci.* 9: 14.

Souza ANA, Escobar ASA, Germano B, Farias CLF, Gomes LFF, Matera JM. 2019. Kinetic and Kinematic analysis of dogs suffering from hip osteoarthritis and healthy dogs across different physical activities. *Vet Comp Orthop Traumatol.* 32:104-111.

Tamura K, Harada Y, Nagashima N, Itoi T, Ishino H, Yogo T, Nezu Y, Hara Y, Suzuki Y, Ide C, et al. 2012. Autotransplanting of Bone Marrow-Derived Mononuclear Cells for Complete Cases of Canine Paraplegia and Loss of Pain Perception, Secondary to Intervertebral Disc Herniation. *Exp Clin Transpl.* 10: 263–272.

Tillakaratne NJ, Duru P, Fujino H, Zhong H, Xiao MS, Edgerton VR, Roy RR. 2014. Identification of interneurons activated at different inclines during treadmill locomotion in adult rats. *J Neurosci Res.* 92: 1714-1722.

Uemura E. Motor system. 2015. In: Uemura E, editor. *Fundamentals of canine neuroanatomy and neurophysiology.* 1st Ed. Iowa: Wiley. p. 257–85.

Van Klaveren NJ, Suwankong N, De Boer S, van den Brom WE, Voorhout G, Hazewinkel HA, Meij BP. 2005. Force plate analysis before and after dorsal decompression for treatment of degenerative lumbosacral stenosis in dogs. *Vet Surg.* 34:450-456.

Vedantam A, Jirjis MB, Schmit BD, Wang MC, Ulmer JL, Kurpad SN. 2014. Diffusion tensor imaging of the spinal cord: insights from animal and human studies. *Neurosurgery.* 74:1–8.

Viala D, Vidal C. 1978. Evidence for distinct spinal locomotion generators supplying respectively fore-and hindlimbs in the rabbit. *Brain Res.* 155: 182–186.

Wyss PO, Hock A, Kollias S. 2017. The application of human spinal cord magnetic resonance spectroscopy to clinical studies: a review. *Semin Ultrasound CT MRI.* 38:153–62.

Zidan N, Sims C, Fenn J, Williams K, Griffith E, Early PJ, Mariani C, Munana KR, Guevar J, Olby NJ. 2018. A randomized, blinded, prospective clinical trial of postoperative rehabilitation in dogs after surgical decompression of acute thoracolumbar intervertebral disc herniation. *J Veter Intern Med.* 32: 1133–114.