**Universidade de Lisboa**

**Faculdade de Medicina de Lisboa**

# Effects of Dehydroepiandrosterone on Cognition: an Electrophysiological Approach

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Orientadores: Professor Doutor João Maria Martin Martins

Professor Doutor Carles Enric Escera Micó

Doctoral Program in Metabolic Disorders and Eating Behavior

**Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, Especialidade Endocrinologia**

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- Doutor João Maria Martin Martins, Professor Auxiliar Convidado da Faculdade de Medicina da Universidade de Lisboa; (*Orientador*).

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**As opiniões expressas nesta publicação são da exclusiva responsabilidade do seu autor.**

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*À minha família:*

*aos que sucedo, aos que precedo*

*e em especial ao meu marido.*

*Liberdade*

Aqui nesta praia onde
Não há nenhum vestígio de impureza,
Aqui onde há somente
Ondas tombando ininterruptamente,
Puro espaço e lúcida unidade,
Aqui o tempo apaixonadamente
Encontra a própria liberdade.

*Sophia de Mello Breyner Andresen, in "Mar"*

"We know accurately only when we know little; doubt grows with knowledge."

*Johann Wolfgang von Goethe*

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# Summary

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) physiologic role remain controversial. Central nervous system and behavioral effects have been described. DHEA and DHEAS have been hypothesised to play a role in cortical organization and brain maturation and suggested to have memory, attention and mood enhancement effects in humans. A neuro-stimulatory action and an anti-cortisol mechanism of action may contribute to those effects. Moreover, the balance between DHEA and DHEAS may modulate their effects in the central nervous system. The objective of this thesis was to explore behavioral correlates of DHEA and DHEAS in humans, in particular regarding personality, stress response, working memory and emotion. Relations at the performance and brain processing levels were explored. DHEA response to the performance of cognitive tasks was also analyzed.

In study I we explored the relation between DHEAS and both pituitary-adrenal axis reactivity and personality in human subjects of both genders. This was a retrospective study of 120 patients assisted at the endocrine outpatient department of a public central Portuguese hospital, before medical treatment. Personality was evaluated with the Minnesota Multiphasic Personality Inventory (MMPI) and the pituitary-adrenal axis reactivity was assessed with the corticotrophin releasing hormone (CRH) test. Baseline serum DHEAS was inversely related to peak/baseline cortisol response to CRH infusion. DHEAS reactivity in the CRH test was directly related to the Deviant Behavior triad and type A personality. These results suggest higher DHEAS levels may be a protective factor against an excessive cortisol response under stressful situations and personality may be related to DHEAS reactivity.

In studies II and III DHEA, DHEAS and cortisol relations to working memory and distraction were studied by recording the electroencephalogram of 23 young women performing a discriminatory (no working memory load) or 1-back (working memory load) task in an audio-visual oddball paradigm. We measured salivary DHEA, DHEAS and cortisol both before each task and at 30 and 60 min intervals. In study II, we showed that under

working memory load, a higher baseline cortisol/DHEA ratio was related to higher distraction as indexed by an enhanced novelty-P3. This suggests that cortisol may lead to increased distraction whereas DHEA may hinder distraction by leading to lower processing of the distractor. An increased DHEA production with consecutive cognitive tasks was found, and higher DHEA responses attributed to working memory load were related to enhanced working memory processing as indexed by an enhanced visual P300. Overall, the results suggest that in women DHEA may oppose cortisol effects, reducing distraction and that a higher DHEA response may enhance working memory at the electrophysiological level. In study III we used that same group of subjects and protocol to analyze the DHEAS/DHEA ratio relation to involuntary attention during the performance of a working memory task. Higher DHEAS/DHEA ratio was related to enhanced auditory novelty-P3 amplitudes during the performance of the working memory task, but there was no significant relation to visual P300 amplitudes, novelty-P3 latencies or visual P300 latencies. Therefore, a relation between higher DHEAS/DHEA ratio and enhanced involuntary attention to the surrounding world without a deleterious effect on working memory processing is suggested. These results also suggest that the balance between DHEAS and DHEA may modulate attentional resources and the importance of the sulfotransferase and sulfatase activity in the modulation of DHEA and DHEAS central nervous system effects.

In study IV we explored DHEAS, DHEA and cortisol relations to the processing of negative emotional stimuli at behavioral and brain levels by recording the electroencephalogram of 21 young women while performing a visual task with implicit neutral or negative emotional content in an audio-visual oddball paradigm. For each condition, salivary DHEA, DHEAS and cortisol were measured before performing the task, and at 30 and 60 min intervals. DHEA increased after task performance, independently of the implicit emotional content. With implicit negative emotion, higher DHEAS/DHEA and DHEA/cortisol ratios before task performance were related to shorter visual P300 latencies suggesting faster brain processing under a negative emotional context. In addition, higher DHEAS/DHEA ratios were related to reduced visual P300 amplitudes, indicating less processing of the negative emotional stimuli. With this study, we could show that at the electrophysiological level, higher DHEAS/DHEA and DHEA/cortisol ratios

were related to shorter stimulus evaluation times, suggesting less interference of the implicit negative content of the stimuli with the task. Furthermore, higher DHEAS/DHEA ratios were related to reduced processing of negative emotional stimuli which may eventually constitute a protective mechanism against negative information overload.

In conclusion, these studies showed several behavioral correlates of DHEA and DHEAS. Additionally, the results suggest anti-cortisol effects of DHEA and DHEAS and the importance of the balance between DHEAS and DHEA. DHEA levels increased after the performance of cognitive tasks, more so after a working memory load than a no working memory load task, suggesting that cognitive tasks may modulate DHEA levels. Higher baseline DHEAS levels were related to reduced cortisol response to CRH suggesting a protective effect during the stress response. In addition, significant new findings were described regarding DHEA and DHEAS relations to working memory, emotion and attention at the brain processing level. Those results suggest DHEA may eventually oppose cortisol effects reducing distraction while higher DHEAS/DHEA ratios may enhance involuntary attention to the surrounding world during the performance of working memory tasks with no evident deleterious effects on the working memory load task in course. On the other hand, higher DHEA response may enhance working memory at the electrophysiological level. Regarding emotional processing, higher DHEAS/DHEA and DHEA/cortisol ratios may be related to less interference of the implicit negative content of the stimuli and higher DHEAS/DHEA ratios were related to reduced processing of negative emotional stimuli which may eventually protect against negative information overload. In short, results suggest DHEAS levels may be related to personality and reduced cortisol stress response while studies at brain processing level suggest DHEA and/or DHEAS may enhance working memory / attention and reduce the processing of negative information.

**Key-words:** dehydroepiandrosterone; personality; working memory; attention; emotion.

# Resumo

A desidroepiandrosterona (DHEA) é sintetizada nas suprarrenais, mas também nas gónadas e ao nível do sistema nervoso central. É reversivelmente convertida em desidroepiandrosterona-sulfato (DHEAS) por ação de uma sulfotransferase, quer na periferia, quer no sistema nervoso central. DHEA e DHEAS são hormonas abundantes nos primatas, em especial nos seres humanos e a concentração de ambas é maior no sistema nervoso central que na circulação periférica. A forma como é regulada a sua síntese não está definida, mas as suas concentrações aumentam em situações de stress agudo e reduzem em situações de stress crónico. A partir da quarta década de vida as suas concentrações reduzem-se progressivamente e níveis mais reduzidos relacionam-se com maior morbilidade e mortalidade, independentemente da idade.

Os efeitos fisiológicos e os mecanismos de ação da DHEA e DHEAS são ainda controversos. Contudo, muitos estudos têm sugerido efeitos ao nível do sistema nervoso central. Foi colocada a hipótese de contribuírem para a organização cortical e desenvolvimento cerebral e em particular, têm sido sugeridos efeitos benéficos em relação à memória, atenção e humor. Níveis mais elevados de DHEAS, DHEA ou da razão DHEA/cortisol foram relacionados com melhor desempenho cognitivo, menos sintomas depressivos e níveis mais elevados de bem-estar. Tem sido colocada a hipótese de esses efeitos serem mediados em parte, através de mecanismos de ação anti-cortisol e neuro-estimulantes. De facto, a nível molecular a DHEA e sobretudo a DHEAS são agonistas glutamatérgicos e antagonistas gabaminérgicos. Para além disso, a razão entre níveis de DHEA e DHEAS poderá eventualmente modular os efeitos destas hormonas ao nível do sistema nervoso central. Os efeitos eletrofisiológicos da DHEA e DHEAS em seres humanos são essencialmente desconhecidos e a sua relação com a personalidade ou fenótipo endócrino de resposta ao stress não estão bem definidos.

O objetivo desta tese foi explorar relações da DHEA ou DHEAS com variáveis comportamentais em seres humanos adultos. Em particular foram estudadas relações com a personalidade, resposta ao stress, memória de trabalho e emoções. Foram estudadas relações ao nível do desempenho e do processamento cerebral. Foram

exploradas eventuais relações opostas às do cortisol, bem como os efeitos do balanço entre os níveis de DHEAS e DHEA. Foram ainda analisadas as respostas da DHEA à realização de tarefas cognitivas.

No estudo I explorámos a relação da concentração de DHEAS com a reatividade hipófise-suprarrenal e o perfil de personalidade em adultos de ambos os géneros. Foi um estudo retrospetivo que incluiu 120 doentes assistidos na consulta externa de um hospital público terciário, antes do início de terapêutica médica. Foi avaliada a personalidade utilizando o Inventário de Personalidade Multifásico do Minnesota (MMPI) e a reatividade do eixo hipófise-suprarrenal utilizando a prova da corticoliberina (CRH). A concentração de corticotrofina (ACTH) relacionou-se diretamente com a concentração sérica de DHEAS basal, e em conjunto com a idade e o género explicaram 34% da variabilidade da concentração de DHEAS. A concentração de DHEAS basal relacionou-se inversamente com a resposta pico/basal do cortisol na prova de CRH. A reatividade da DHEAS na prova de CRH relacionou-se diretamente com a pontuação obtida na tríade de Problemas de Comportamento e personalidade tipo A. Estes resultados sugerem que concentrações mais elevadas de DHEAS poderão constituir um fator protetor contra uma resposta excessiva do cortisol em situações de stress. Por outro lado, sugerem a existência de uma relação entre a personalidade e a reatividade da DHEAS.

No estudo II explorámos a relação das concentrações de DHEA, DHEAS e cortisol com a memória de trabalho e distração ao nível do desempenho e do processamento cerebral. Com essa finalidade, registámos o eletroencefalograma de 23 jovens adultas do sexo feminino enquanto realizavam uma tarefa discriminativa (sem utilização da memória de trabalho) e outra com a utilização de memória de trabalho. Essas tarefas eram visuais e antes de cada estímulo visual eram administrados estímulos auditivos, 20% dos quais eram novos e tinham o objetivo de causar distração (paradigma de *"oddball"*). Medimos a DHEA, DHEAS e o cortisol salivares antes de cada tarefa e aos 30 e 60 min. Durante a tarefa com utilização de memória de trabalho, razões cortisol/DHEA mais elevadas relacionaram-se com deflexões *"P3-novidade*" (em inglês, *novelty-P3*) mais amplas, traduzindo maior distração. Este resultado sugere que o cortisol poderá causar maior distração enquanto que a DHEA poderá reduzir essa distração através da redução do

processamento desse estímulo distrativo. Encontrámos uma elevação das concentrações de DHEA com a realização das duas tarefas cognitivas consecutivas, maior com a realização da tarefa com memória de trabalho que com a realização da tarefa discriminativa. Para além disso, uma maior resposta da DHEA devida à realização da tarefa com memória de trabalho, relacionou-se com um maior incremento na amplitude da P300 visual devida à memória de trabalho, traduzindo assim um melhor processamento dessa memória de trabalho. Globalmente, estes resultados sugerem que nas mulheres, a DHEA poderá opor-se aos efeitos do cortisol, reduzindo a distração e que maiores reatividades da DHEA se relacionam com melhor memória de trabalho ao nível eletrofisiológico.

No estudo III utilizámos o protocolo anterior para analisar a relação entre a razão DHEAS/DHEA e a atenção involuntária durante a execução de uma tarefa envolvendo memória de trabalho. Verificámos que razões DHEAS/DHEA mais elevadas se relacionaram com amplitudes maiores da deflexão auditiva *P3-novidade* durante a realização de uma tarefa visual envolvendo memória de trabalho. Contudo, a razão DHEAS/DHEA não se relacionou com a amplitude da P300 visual, a latência da *P3-novidade* ou a latência da P300 visual. Estes resultados sugerem que o balanço entre DHEAS e DHEA pode modular a atenção involuntária. Especificamente, os resultados sugerem que razões DHEAS/DHEA mais elevadas podem aumentar o processamento involuntário de estímulos auditivos sem um efeito deletério no processamento da memória de trabalho visual em curso. A manutenção da atenção involuntária em relação ao mundo envolvente durante a realização de tarefas envolvendo a utilização de memória de trabalho pode ser um mecanismo protetor importante. Os recursos da atenção são limitados e estes resultados sugerem que o balanço entre DHEAS e DHEA pode modular esses recursos bem como sugerem a importância da atividade da sulfotransferase e sulfatase na modulação desses efeitos.

No estudo IV explorámos as relações da DHEAS, DHEA e cortisol com o processamento de estímulos com conteúdo emocional negativo, ao nível do comportamento e do processamento cerebral. Foi registado o eletroencefalograma de 21 jovens adultas enquanto realizavam uma tarefa com conteúdo emocional implícito neutro

ou negativo, utilizando um paradigma de "*oddball*" audiovisual. A tarefa alvo era visual e os estímulos auditivos, dos quais 20% eram sons novos, pretendiam causar distração. Em cada condição, foram doseadas a DHEA, DHEAS e cortisol antes da realização da tarefa e aos 30 e 60 min. A concentração de DHEA aumentou após a realização da tarefa, independentemente do conteúdo emocional. Razões DHEAS/DHEA e DHEA/cortisol mais elevadas antes da execução da tarefa com conteúdo emocional implícito negativo relacionaram-se com latências mais curtas da P300 visual, sugerindo um processamento cerebral mais rápido em contexto emocional negativo. Para além disso, razões DHEAS/DHEA mais elevadas relacionaram-se com amplitudes da P300 visual mais reduzidas, indicando menor processamento do estímulo emocional negativo. Com este estudo mostrámos que ao nível eletrofisiológico, razões DHEAS/DHEA e DHEA/cortisol mais elevadas se relacionaram com tempos de avaliação do estímulo mais curtos, sugerindo menor interferência pelo conteúdo implícito negativo dos estímulos. Também, razões DHEAS/DHEA mais elevadas relacionaram-se com menor processamento dos estímulos negativos, o que pode constituir um mecanismo protetor contra o excesso de informação negativa.

Concluindo, estes estudos revelaram relações da DHEA e DHEAS com variáveis comportamentais. Os resultados sugerem efeitos anti cortisol da DHEA e DHEAS e a importância do balanço entre os níveis de DHEAS e DHEA. As concentrações de DHEA aumentaram com a execução de tarefas cognitivas, sugerindo que essas tarefas podem modular as concentrações de DHEA. Concentrações basais mais elevadas de DHEAS relacionaram-se com menor resposta do cortisol à CRH sugerindo um efeito protetor durante a resposta ao stress. Encontrámos também relações entre DHEAS e o perfil de personalidade. Para além disso, encontrámos resultados novos no que respeita às relações da DHEA e DHEAS com o processamento cerebral. Esses resultados sugerem que ao nível eletrofisiológico, a DHEA poderá opor-se aos efeitos do cortisol, reduzindo a distração; que razões DHEAS/DHEA mais elevadas poderão aumentar a atenção involuntária em relação ao meio envolvente, sem um efeito deletério no processamento da memória de trabalho; e que uma maior resposta da DHEA poderá melhorar o processamento dessa memória de trabalho. No que respeita ao processamento emocional, os resultados sugerem que razões DHEAS/DHEA e DHEA/cortisol mais

elevadas se relacionam com menor interferência do conteúdo implícito negativo dos estímulos e que razões DHEAS/DHEA mais elevadas se relacionam com menor processamento do conteúdo emocional negativo, o que poderá constitui um mecanismo protetor contra os efeitos deletérios do excesso de informação negativa. Portanto, os resultados sugerem que a DHEAS se relaciona com a personalidade e menor resposta do cortisol ao stress, enquanto os estudos ao nível do processamento cerebral sugerem que a DHEA e/ou DHEAS poderão melhorar a memória de trabalho / atenção e reduzir o processamento de informação com conteúdo emocional negativo.

**Palavras-chave:** desidroepiandrosterona (DHEA); personalidade; memória; atenção; processamento emocional.

# List of Original Publications in Relation to the Present Thesis

In agreement with the official edict 388/70, art. 8º, paragraph 2, the results presented in this thesis were published or are currently being prepared for publication.

- **Dehydroepiandrosterone-sulphate (DHEAS) is related to Personality and Stress Response**. Sónia do Vale, João Martin Martins, Maria João Fagundes, Isabel do Carmo. *Neuro Endocrinol Lett.* 2011;32(4):442-8.

- **The Relationship between Dehydroepiandrosterone (DHEA), Working Memory and Distraction - a Behavioral and Electrophysiological Approach.** Sónia do Vale, Lenka Selinger, João Martin Martins, Ana Coelho Gomes, Manuel Bicho, Isabel do Carmo, Carles Escera. *PLoS ONE* 2014; 9(8): e104869.

- **Hormonal Modulation of Novelty Processing in Women: enhanced under Working Memory load with high DHEAS/DHEA ratios.** Sónia do Vale, Lenka Selinger, João Martin Martins, Manuel Bicho, Isabel do Carmo, Carles Escera. Submitted for publication.

- **Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone-sulphate (DHEAS) and Emotional Processing – a Behavioral and Electrophysiological Approach.** Sónia do Vale, Lenka Selinger, João Martin Martins, Manuel Bicho, Isabel do Carmo, Carles Escera. *Hormones and Behavior* 2015; 73: 94-103.

# Abbreviations

| | |
|---|---|
| **ACh** | Acetylcholine |
| **ACC** | Anterior cingulate cortex |
| **AASH** | Adrenal androgen stimulating hormone |
| **ACTH** | Adrenocorticotropic hormone |
| **AMPA** | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| **BDNF** | Brain-derived neurotrophic factor |
| **BMI** | Body mass index |
| **AMPc** | Cyclic Adenosine Monophosphate |
| **CASH** | Cortical Androgen Stimulating Hormone |
| **C/EBP** | CCAAT/enhancer-binding protein |
| **CRH** | Corticotrophin Releasing Hormone |
| **DA** | Dopamine |
| **DHEA** | Dehydroepiandrosterone |
| **DHEAS** | Dehydroepiandrosterone-sulphate |
| **DHEA(S)** | Dehydroepiandrosterone and Dehydroepiandrosterone-sulphate |
| **DMPC** | Dorsomedial prefrontal cortex |
| **DNA** | Deoxyribonucleic acid |
| **EEG** | Electroencephalogram |
| **EGF** | Epidermal growth factor |
| **eNOS** | Endothelial nitric-oxide synthase |
| **ERPs** | Event-Related Potentials |
| **FDG-PET** | Fluorodeoxyglucose - positron emission tomography |
| **fMRI** | Functional Magnetic Resonance Imaging |
| **GABA-A** | γ-aminobutyric acid type A |
| **GSH** | Glutathione |
| **HDLc** | High density lipoprotein cholesterol |
| **HFS** | High-frequency stimulation |
| **HLA** | Human leukocyte antigen |
| **HPA** | Hypothalamic–pituitary–adrenal axis |

| | |
|---|---|
| **HSD** | Hydroxysteroid dehydrogenase enzyme |
| **IGF** | Insulin-like growth factor |
| **IL** | Interleukin |
| **IP3** | Inositol triphosphate |
| **$K_d$** | Binding affinity |
| **$K_{eq}$** | Equilibrium constant |
| **K(m)** | Michaelis constant |
| **LORETA** | Low-resolution brain electromagnetic tomography |
| **LTP** | Long-term potentiation |
| **MAP2C** | Microtubule-associated protein 2C |
| **MAPK** | Mitogen-activated protein kinase |
| **MCR** | Metabolic clearance rate |
| **MMN** | Mismatch negativity |
| **MMPI** | Minnesota Multiphasic Personality Inventory |
| **MRI** | Magnetic resonance imaging |
| **MMSE** | Mini-Mental State Examination |
| **Nov-P3** | Novelty-P3 |
| **NMDA** | *N*-methyl-D-aspartate (NMDA) |
| **OATP** | Organic Anion Transporting Polypeptide |
| **OST** | Organic Solute Transporter |
| **PAP** | 3',5'-diphosphoadenosine |
| **PAPS** | 3'-phosphoadenosine 5'-phosphosulfate |
| **PET** | Positron Emission Tomography |
| **PFC** | Prefrontal cortex |
| **PPARα** | Peroxisome proliferator-activated receptor |
| **PRL** | Prolactin |
| **PTSD** | Post-traumatic stress disorder |
| **r** | Correlation coefficients |
| **$r_s$** | Spearman's Rank Order correlation coefficients |
| **RON** | Reorienting negativity |
| **SAPK3** | Stress-activated protein kinase 3 |

| | |
|---|---|
| **SCC** | Cholesterol side chain cleavage enzyme |
| **SF** | Steroidogenic factor |
| **SOAT** | Sodium-dependent Organic Anion Transporter |
| **StAR** | Steroidogenic acute regulatory protein |
| **STAI** | State-Trait Anxiety Inventory |
| **STS** | Steroid-sulfatase, steroid sulfohydrolase |
| **SULT** | Hydroxysteroid sulfotransferase enzyme |
| **TBPS** | T-butyl-bicyclo-phosphorothionate |
| **TNF** | Tumor necrosis factor |
| **VPP** | Vertex positve potential |
| **WM** | Working memory |
| **$\eta^2$** | Eta squared |

# Introduction/Literature Review: Dehydroepiandrosterone and Dehydroepiandrosterone-sulphate as Neuroactive Steroids

In humans, dehydroepiandrosterone-sulphate (DHEAS) is the most abundant hormone in the peripheral circulation (1; 2). Brain levels of dehydroepiandrosterone (DHEA) and DHEAS are higher than peripheral circulation levels (3; 4) and both forms of the hormone are synthesized in the adrenals, but also in the central nervous system (1). DHEA and DHEAS [DHEA(S)] regulation and its physiological effects are still a matter of debate (5; 6; 7). Nevertheless, stressful stimuli increase DHEA and DHEAS levels in the acute setup (8) whereas chronic stress decreases DHEA and DHEAS levels (9; 10; 11). DHEAS levels are also higher in early adulthood and dramatically decrease with aging (2; 12), reaching a nadir by the time many diseases of aging become more prevalent. Besides, lower DHEAS levels are related to higher morbidity and mortality even after age adjustment (2; 13).

Although DHEA and DHEAS are steroid hormones, no high affinity nuclear receptor has been found for them (5; 6; 7; 14). On the other hand, DHEA and DHEAS effects mediated by binding to cytoplasmic receptors were described. In the brain, these hormones modulate synaptic transmission. They present a general neuro stimulatory effect mainly as glutamatergic agonist and gabaminergic antagonist (1; 3) and general biological actions of DHEA and/or DHEAS involve neuronal survival, neurite growth, neurogenesis, anti-oxidant, anti-inflammatory and anti-glucocorticoid effects (7). It was hypothesised that DHEA and DHEAS may play a role in cortical organization (15) and brain maturation (16). At the behavioral level, higher DHEA, DHEAS or DHEA-to-cortisol ratios were related to improved attention, cognition and mood (17; 18; 19; 20).

The previous findings raised the hypothesis that restoring DHEA to youthful levels might protect the brain from cognitive decline or improve cognition, improve mood and even extend life span. In this regard, DHEA and DHEAS were evaluated in the treatment of

neuropsychiatric disorders, with published reports appearing as early as 1952 (21; 22). Since the late 1980s, a growing amount of studies addressing pre-clinical aspects and some controlled clinical trials with DHEA administration were performed (23). Regarding the effects of DHEA administration, until the present date, the studies failed to show consistent beneficial effects on cognition but suggest beneficial effects of DHEA administration on mood. Although the knowledge of neurobiological actions of DHEA and DHEAS is rapidly growing, the neurofunctional correlates of DHEA and DHEAS are mostly unknown. In fact, only a very small number of studies addressed the effects of DHEA on brain processing, both at the electrophysiological level (mostly since the late 1990s) (24) and functional neuroimaging level (since the early 2010s) (25).

This review will address the following DHEA and DHEAS aspects as neurosteroids: 1) molecular structure, synthesis and distribution; 2) concentrations along the life span; 3) molecular mechanisms of action and cellular effects; 4) cognitive and neuropsychiatric effects; 5) electrophysiological and neuroimaging correlates.

# Synthesis and distribution

Dehydroepiandrosterone (also designated as prasterone) is a steroid hormone. It is derived from the cyclopentanoperhydrophenanthrene structure, which is composed of three cyclohexane rings and a single cyclopentane ring (figure 1.A). Its International Union of Pure and Applied Chemistry (IUPAC) designation is 3β-hydroxyandrost-5-en-17-one or 5-androsten-3β-ol-17-one (26). DHEA possesses a hydroxyl group at position C3 (figure 1.B) while DHEAS has a sulphate group in that position (figure 1.C).

DHEA and DHEAS are synthesized in the adrenals, gonads and central nervous system. The adrenals are responsible for most of its synthesis in both genders, but while in women, the gonads are a minor source, in adult men, the testes secrete approximately 10-25% of DHEA and 5% of DHEAS (27; 28). The brain is believed to produce only small amounts of peripheral DHEA and DHEAS levels (27; 28; 29).

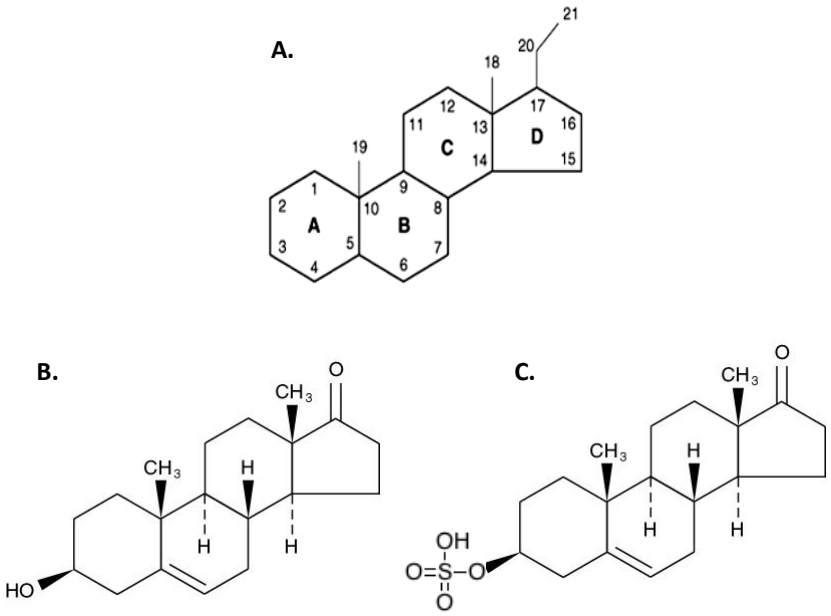


Figure 1: Cyclopentanoperhydrophenanthrene structure (A), dehydroepiandrosterone (B) and dehydroepiandrosterone-sulphate (C) chemical structure.

**DHEA synthesis in the adrenals and gonads**

In the periphery, DHEA is mostly synthesized in the adrenals but also in the gonads. In the adrenals it is synthesized in the adrenal cortex, mostly in the zona reticularis (to a lesser degree it can be synthesized in the zona fasciculata). In the ovary, it is produced in the thecal cells (30) and in the testis it is produced by the Leydig cells.

Cholesterol is the precursor of all adrenal and gonadic steroids, either internalized in steroidogenic cells by receptor mediated endocytosis or synthesized de novo within those cells (31). Within the steroidogenic cells (adrenal cortical cells, theca cells, theca-interstitial cells and Leydig cells), the intracellular cholesterol is transported from the outer to the inner mitochondrial membrane, a process that is mediated by the steroidogenic acute regulatory protein (StAR) (32). This transport of cholesterol across the mitochondrial membrane is the initial rate-limiting step in adrenal steroidogenesis (33; 34). The placental steroidogenic cells are an exception because they do not express StAR and directly utilize the mitochondrial cholesterol side chain cleavage enzyme (a cytochrome P450 enzyme) to initiate steroidogenesis (the protein MLN64 is one candidate for a factor mediating the transport of cholesterol into placental mitochondria) (35). In the mitochondria of adrenal and other steroidogenic cells in general, the cholesterol is converted to pregnenolone by cytochrome P450 side chain cleavage enzyme (P450scc, desmolase or CYP11A1). The steroidogenesis involves the action of other cytochrome P450 enzymes. The cholesterol side-chain cleavage enzyme and the 11β-hydroxylase (CYP11B) enzymes are mitochondrial enzymes and require an electron shuttle system to hydroxylate steroids. The 17α-hydroxylase (CYP17) and 21-hydroxylase (CYP21A2) are microsomal-endoplasmic reticulum enzymes and involve a distinct electron shuttle system (cytochrome $b_5$, a flavoprotein) (34; 35).

The pathway for DHEA synthesis is mostly identical in the adrenals and gonads. After the synthesis of pregnenolone by the action of the P450scc (CYP11A1) enzyme, CYP 17α-hydroxylase hydroxylates pregnenolone to form 17-hydroxypregnenolone. Then, the CYP17 also possesses 17,20-lyase activity and this activity converts 17-hydroxypregnenolone into DHEA, which is a C19 androgen (figure 2) (34). The synthesis of adrenal androgens occurs mostly in the reticularis zone. Additionally, DHEA is sulfated to

DHEAS in the adrenal zona reticularis. DHEA is liposoluble and can easily cross biological membranes, but DHEAS, having a sulfate group, does not (2; 36).

Besides androgens, the adrenals produce two other main types of hormones: glucocorticoids and mineralocorticoids, see figure 3 (34; 35; 37).


Figure 2: Biochemical pathway for DHEA synthesis. SCC - cholesterol side chain cleavage enzyme (CYP11A1); 17α-OH - 17α-hydroxylase enzyme; 17,20-lyase - 17,20-lyase enzyme.

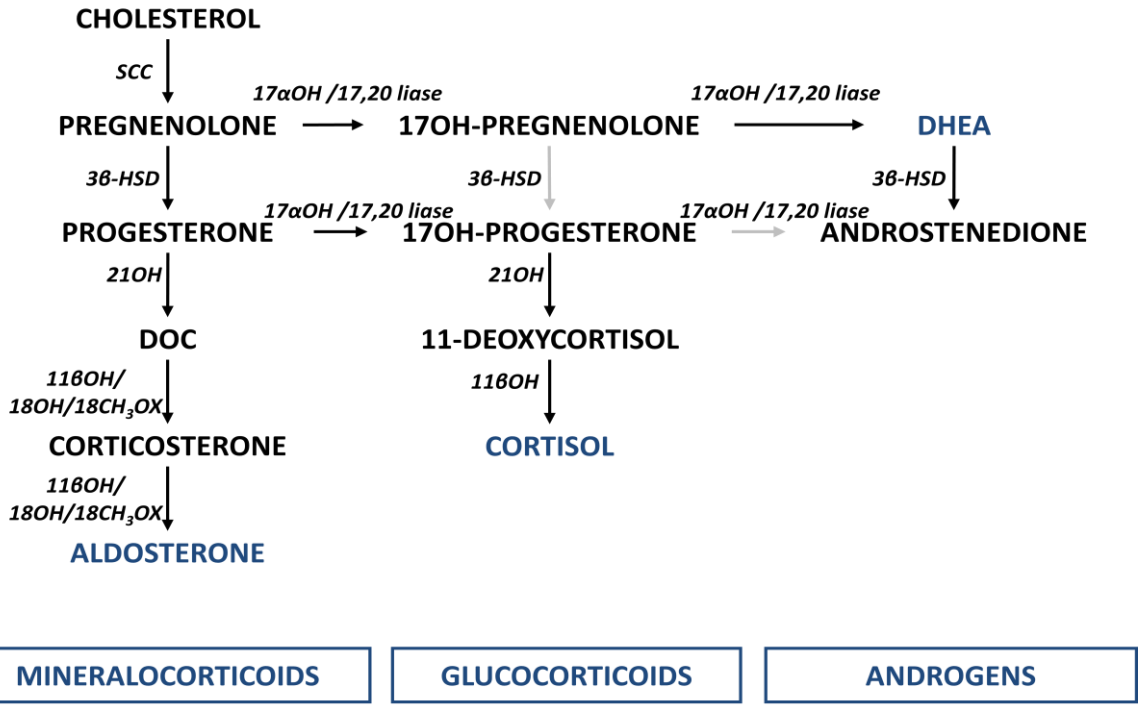


Figure 3: Adrenal steroidogenesis. Three main types of hormones are produced by the adrenals: glucocorticoids, mineralocorticoids and androgens. SCC - cholesterol side chain cleavage enzyme (CYP11A1); 17α-OH/17,20-lyase - 17α-hydroxylase/17,20-lyase enzyme (CYP17); 3β-HSD - 3β-hydroxysteroid dehydrogenase enzyme (HSD3B); 21OH - 21-hydroxylase enzyme (CYP21A2); DOC – deoxycorticosterone; 11βOH - 11-hydroxylase (CYP11B1); 11βOH/18OH/18$CH_3$OX – aldosterone synthase (CYP11B2), which has 11β-hydroxylase (11βOH), 18-hydroxylase (18OH) and 18-methyl oxidase (18$CH_3$OX) activity; black arrows indicate the predominant pathways.

In the **fetal adrenal**, because of relative lack of 3β-HSD and high sulfotransferase activity, DHEA and DHEAS are the main steroids produced by the adrenal. The fetal adrenal has considerable sulfotransferase activity but little steroid sulfatase activity, also favoring conversion of DHEA to DHEAS. The resulting DHEAS is secreted, 16α-hydroxylated in the fetal liver (by the hydroxilase CYP3A7) (38; 39), and then acted on by placental 3βHSD1, 17βHSD1, and P450 aromatase to produce estriol. Small amounts of DHEA and DHEAS bypass the liver and are not 16α-hydroxylated, and hence yield estrone and estradiol. Estrogens in turn, further inhibit adrenal 3βHSD activity, providing a feedback system to promote production of DHEAS (40; 41).

As mentioned, **the gonads** secrete DHEA although in much lower levels then the adrenals. The main hormonal products of the normal ovaries of reproductive age women are progesterone and estradiol. Accordingly, the preferential pathways are to convert pregnenolone into progesterone and estradiol (figure 4) (30). In the testis, testosterone is the main steroid produced (35; 42; 43), see figure 5.

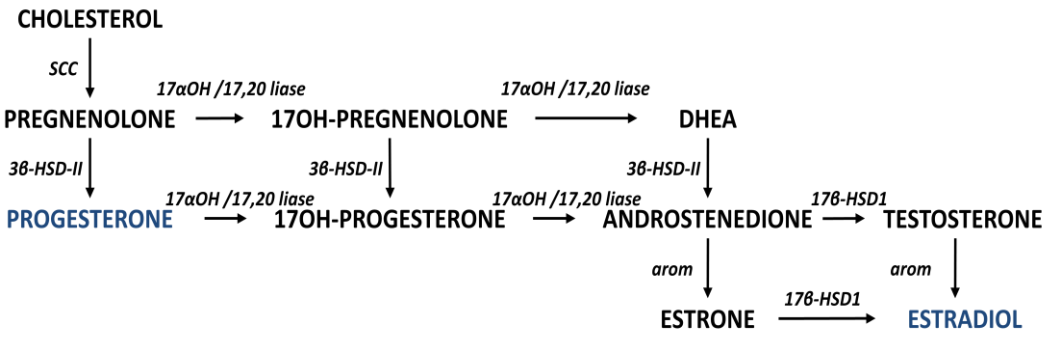


Figure 4: Steroidogenic pathway in the ovary. DHEA is synthesized as an intermediary metabolite. SCC - cholesterol side chain cleavage enzyme; 17α-OH/17,20-lyase - 17α-hydroxylase/17,20-lyase enzyme; 3β-HSD - 3β-hydroxysteroid dehydrogenase enzyme type II; 17βOH – 17β-hydroxysteroid dehydrogenase type 1 enzyme; arom – aromatase.

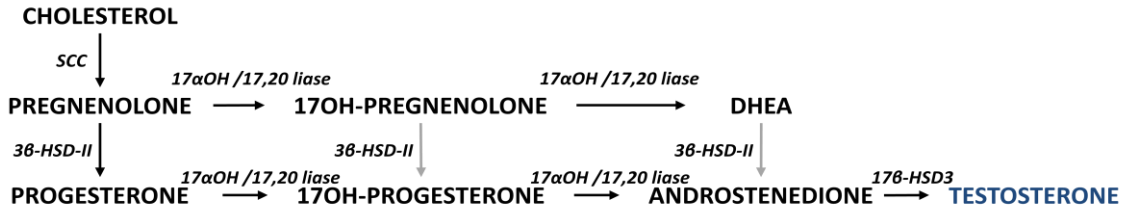


Figure 5: Steroidogenesis in human testis. SCC - cholesterol side chain cleavage enzyme; 17α-OH/17,20-lyase - 17α-hydroxylase/17,20-lyase enzyme; 3β-HSD - 3β-hydroxysteroid dehydrogenase enzyme type II; 17βOH – 17β-hydroxysteroid dehydrogenase type 3 enzyme; black arrows indicate the predominant pathway in humans.

**DHEA and DHEAS interconversion, secretion, peripheral metabolism and excretion**

As mentioned, DHEA is sulfated to DHEAS in the adrenal reticularis zone. This sulfation reaction is reversibly mediated by the hydroxysteroid sulfotransferase enzyme (SULT2A1), which is a cytosolic enzyme (44; 45). Human cytosolic sulfotransferases transfer the sulfuryl group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the hydroxyl group of DHEA (46). The equilibrium constant for this reaction strongly favours DHEAS synthesis ($K_{eq}$ = 1.3 x $10^3$), DHEA is the concentration-limiting substrate and nucleotide (3',5'-diphosphoadenosine, PAP) release is the rate-limiting step (46). In the presence of increasing DHEA concentration, there is partial substrate inhibition by DHEA: in that condition, DHEA excess causes it to add more quickly to the enzyme-PAP complex, trapping a greater fraction of PAP in a dead-end complex (DHEA-enzyme-PAP). Therefore, as the DHEA concentration increases, the rate determining step in this reaction shifts from PAP release from the enzyme-PAP complex ($K_{off}$ = 1.2 $s^{-1}$ ) to PAP release from the dead-end complex (DHEA-enzyme-PAP) ($K_{off}$ = 0.48 $s^{-1}$) (46).

Of note, DHEA-sulfotransferase (SUL2A1) is also expressed in liver (hepatocytes) and a smaller amount is expressed in the gastrointestinal tract (small intestine mucosa and parietal cells of the gastric glands) (47; 48; 49; 50). SUL2A1 expressed in the human liver is responsible for sulfation of bile acids and circulating hydroxysteroids (51; 52; 53; 44), including DHEA. The DHEA-sulfotransferase present in human adrenal and liver show similar physical, biochemical and kinetic properties (47). In humans, the sulfotransferases expression in non-gonadal tissues is identical in both genders (in contrast, rodents show

sex-related differences) (48). Several nuclear receptors might regulate SULT2A1 expression in liver (peroxisome proliferator-activated receptor-α; pregnane X receptor; constitutive androstane receptor, vitamin D receptor, liver X receptors, farnesoid receptor, retinoid-related orphan receptors) while Estrogen-Related Receptor-α is suggested to play a major role in the regulation of SULT2A1 expression in the human adrenal (48).

As mentioned, DHEA can easily cross biological membranes. On the contrary, the transport mechanism for adrenal cell export of DHEAS into the circulation is not well understood. The Organic Solute Transporter α - Organic Solute Transporter β (OSTα-OSTβ) is a heteromeric carrier abundantly expressed in the human adrenal gland which may play a role in the export of DHEAS from the adrenal cell (54). It is also expressed in a variety of other tissues, including the small intestine, colon, liver, biliary tract and kidney (54). In polarized epithelial cells, OSTα-OSTβ protein is localized in the basolateral membrane and functions in the export or uptake of bile acids and steroids (54).

DHEAS may be converted back to DHEA by a steroid-sulfatase (STS) (steroid sulfo-hydrolase) enzyme. The steroid-sulfatase gene is X-linked and escapes X inactivation in humans (55). Its expression was observed in the adrenal, liver, adipose tissue, brain, placenta, gonads, uterus, skin and other peripheral tissues (56; 57; 58; 59; 60). STS is localized in the endoplasmic reticulum, with evidence suggesting that its active site is located within the endoplasmic reticulum membrane and that facilitated transport of DHEAS across the ER membrane may not be required for STS activity (61). Therefore, cycling of DHEA and DHEAS (and other steroids and their sulfates) may occur as a result of steroid sulfotransferases and steroid sulfatases activity. Higher concentration of phosphate reduces the velocity of STS reaction and other sulfated steroids (namely pregnenolone sulfate) functioning as competitive inhibitors of this enzyme (58; 62; 63).

In young adults, the adrenals secrete about 4mg of DHEA and 25mg of DHEAS daily (64). DHEA and DHEAS in the peripheral circulation do not bind significantly to sex hormone binding globulin. They bind to albumin, although DHEAS binding to albumin seems non-specific (64). Overall, in the adrenals and other peripheral tissues, 64-74% of the DHEAS produced each day is converted back to DHEA, while only 6-13% of the DHEA

produced is metabolized to DHEAS (28; 64; 65; 66). DHEA and DHEAS can be converted to other steroids in the adrenals, ovaries and testis and released into the peripheral circulation (64; 67). Secreted DHEA and DHEAS can also be converted to other steroids in several peripheral target tissues (like the skin, adipose tissue, liver, bone), although in many cases, probably with no significant release into the general peripheral circulation (intracrinology) (5; 64). In particular, DHEA and DHEAS can be converted into androgens (Δ4-androstenedione, testosterone, 5α-dihydrotestosterone), estrogens (Δ5-androstenediol and those resulting from the aromatization of testosterone and Δ4-androstenedione - estradiol, estrone) and other DHEA derivatives (16α-, and 7α/β-oxygenated DHEA derivatives) (36; 64).

As a result of the previous findings, circulating DHEAS may be seen as a reservoir for DHEA, with conversion by sulfotransferases occurring in several tissues (5). Besides that, the fact that DHEAS and DHEA may be converted to other active steroids gave rise to the hypothesis that DHEA and DHEAS effects could be mediated by its conversion to androgens, estrogens and other metabolites and not direct effects of DHEA or DHEAS (5; 68). To note, the high DHEA and DHEAS concentrations found in human peripheral circulation are not reproduced in other animals. The peripheral concentration of DHEA and DHEAS in monkeys is much lower than that in humans and in most other laboratory animals, particularly rodents, the peripheral concentration of these hormones is very low (7; 64). This is also suggestive that DHEA and DHEAS might play a partcular role in the human species.

Again, although DHEA is expected to freely cross membranes of target cells, another issue was whether DHEAS, a hydrophilic steroid, can enter the specific target cells. To that concern, transport systems were described. The Organic Anion Transporting Polypeptide (OATP) family has broad and overlapping substrate specificities and in particular, some members of the Organic Anion Transporting Polypeptide (OATP) family were described to occur in the liver, kidney, testis, small intestine, thymus, placenta and other tissues and mediate the trans-epithelial / transmembrane uptake of DHEAS and estrone sulfate (69; 70; 71). Besides, a specific steroid-sulfate transporter, the sodium-dependent Organic Anion Transporter (SOAT), can mediate DHEAS cellular inward in the

testis (69). SOATs are expressed in other tissues namely in vesicular structures in neurons of the central and peripheral nervous system, but a transport function was not yet detected, namely no transport of DHEAS by SOATs was detected in those cells (72). Besides, OATPs were detected in salivary glands, but no transport of DHEAS by OATPs in those cells was described (73). On the other hand, it was described that DHEAS squeezes through the tight junctions between salivary glands and DHEAS concentrations in saliva are therefore dependent on serum concentration and salivary flow rate (74).

Renal and biliary excretion of DHEA and DHEAS do occur. Renal excretion accounts for 51-73% of the elimination of DHEAS and its metabolites (28). Besides the direct excretion of DHEAS and DHEA, renal excretion products include androsterone sulfate, etiocholanolone sulfate, androsterone glucuronoside and etiocholanolone glucuronoside (75; 76). The half-life of DHEA is similar to cortisol and most other steroid hormones. It is estimated to take 15-30 min, for a metabolic clearance rate (MCR) of approximately 2,000 L / day. On the contrary, the half-life of DHEAS is much longer, 7-10 h, and the MCR is low, 5-20 L/day (36; 64). As a consequence, the plasma concentration of DHEA is not much different from that of other adrenal steroids, but it is several times less abundant than DHEAS. DHEA concentration is in several nmol/L (2-9 µg/dL, 7-31 nmol/L), while the concentration of DHEAS is in several µmol/L (50-250 µg/dL, 1.3-6.8 µmol/L) (64; 77). Of note cortisol molar concentration in the peripheral circulation is about 10 times higher than DHEA concentration (morning cortisol: 5-25 µg/dL, 140-690nmol/L) (77).

DHEAS concentration is 20-30% lower in women, therefore the DHEAS/DHEA ratio is also lower in women (64). The ratios for the conversion of DHEAS and androsterone sulfate to DHEA, are both significantly higher for women than men (78). Plasma levels of DHEA are only a little higher in women than in men [in Bird *et al.* study (78), levels were 8.50 ± 0.95 and 8.75 ± 1.01 ng/ml for men and women, respectively]. Concluding from the above stated, the X-linked sulfatase activity, which escapes X-inactivation, may explain or contribute to this difference. There is no sex difference in the binding of DHEA and DHEAS to plasma proteins and this is also reflected in the lack of sex difference in the MCRs (78). Nevertheless, women probably excrete more DHEAS and DHEA through urine when compared to men (73±5.5% in women and 51±3.5% in men), while men excrete more

DHEAS and DHEA though the bile than women (79). Circadian rhythm of DHEA is identical to that of cortisol, eventually related to ACTH stimulation of DHEA synthesis. DHEAS levels on the contrary, due to its long half-life, remain stable 24h a day under normal conditions (64).

**DHEA synthesis in the brain**

Neurosteroids are steroids that can be synthesized *de novo* in the nervous system from sterol precursors (1). This notion was proposed in 1981. Interestingly, this came to light after the observation that DHEA and DHEAS were present in the adult rat brain. At that time, it was an unexpected finding since the rodents steroidogenic glands do not secrete significant amounts of DHEA. As a sequence to that observation, the nervous system steroidogenesis was discovered (80) and DHEA and DHEAS were known to be neurosteroids. However, it is still not clear where and how DHEA and DHEAS are synthesized in the central nervous system.

Corpéchot *et al.* (80) performed a series of experiments showing that DHEAS levels in brain were independent of its peripheral synthesis. DHEAS concentrations in the brain tissue largely exceeded DHEA concentrations in the brain tissue and DHEAS concentrations in plasma. Furthermore, there is an anterior to posterior DHEA and DHEAS brain gradient (3; 80; 81). DHEA concentrations are higher in the anterior brain, while DHEAS concentrations are higher in the posterior brain. In a post-mortem study of human brains, subjects 76 to 93 years old (nine women and one man), DHEA concentrations were found to be higher in the prefrontal lobe (29 nmol/kg) than in other brain regions (16 nmol/kg in the parietal lobe, 13 nmol/kg in the temporal cortex, 17 nmol/kg in the cerebellum and 19 nmol/kg in the corpus callosum) (4). Mice also have higher DHEA concentrations in the anterior brain (0.42±0.10 ng/g in anterior brain and 0.12±0.03 ng/g in posterior brain) (80). On the contrary, DHEAS concentration in rats are higher in the posterior (4.89±1.06 ng/g) than in the anterior brain tissue (1.58±0.14 ng/g) (3; 80) and peripheral concentrations of DHEAS in rats are lower than the central ones (0.26±0.13

ng/mL) (80). In humans, DHEA levels are also about 6.5 time higher in brain than in the plasma of subjects of similar age (4).

Of note, DHEA concentrations in human brain tissue (homogenates) are higher than plasma concentrations, but cerebrospinal fluid concentrations are lower than plasma concentrations, about 5.4% of plasma concentrations (cortisol levels in the cerebrospinal fluid are also about 5.8% of plasma concentrations) (82). Despite the fact that DHEAS is expected to penetrate less into the cerebrospinal fluid than DHEA (0.03% of circulating DHEAS is expected to cross the blood brain barrier) (83), in adults, the levels of DHEAS in the cerebrospinal fluid are still higher than those of DHEA (82). DHEA-to-DHEAS molar ratio was estimated to be about 0.01 in plasma and 0.52 in the cerebrospinal fluid (in the peripheral circulation DHEAS levels are 100 or more times higher than those of DHEA and about 5-10 times those of cortisol; DHEAS levels in the cerebrospinal fluid are about 1/3 of cortisol levels) (82). Gooddyer *et al.* studied the relative levels of DHEA, DHEAS and cortisol in the blood and saliva of adolescents (84), and found a similar relationship to that described by Guazzo *et al.* between blood and cerebrospinal fluid levels (82), hence suggesting that transport into the brain and saliva might be, to this extent, comparable, and that salivary levels may therefore give a reasonable representation to those in the cerebrospinal fluid (82).

Several studies suggest that central nervous system DHEAS levels do not depend on adrenal secretion. Injections for 3 days of long-acting preparations of corticotrophin, to stimulate adrenal steroidogenesis, or of dexamethasone to inhibit endogenous adrenocorticotropic hormone (ACTH) secretion, were not accompanied by clear-cut changes in brain DHEAS (1; 80). Also, brain DHEAS was unchanged one day after castration, whereas testosterone completely disappeared from the brain. Also, no difference was observed in brain DHEAS levels when castrated adrenalectomized male rats were compared 15 days after operation with sham operated controls (1; 80). Taking into account that the rat steroidogenic glands do not secrete significant amounts of DHEA and peripheral concentrations of DHEA and DHEAS are very low (7; 64), these results suggest that in rats, DHEA and DHEAS may be produced mainly in the brain. Moreover, mice have higher DHEA concentrations in brain than in plasma (7).

The same conclusion cannot be directly applied to humans, since DHEA and DHEAS are abundant in brain but also in peripheral circulation. In human beings, brain DHEA might be derived from both local synthesis and peripheral synthesis (7). DHEA may cross the blood-brain barrier and be converted to DHEAS in the brain. On the contrary, DHEAS can enter and leave the brain through specific transport systems but the flux of DHEAS is believed to be mainly from the brain to the peripheral circulation and not the opposite (85). Accordingly, in subjects not taking steroid medication, DHEA and DHEAS levels in blood and cerebrospinal fluid were directly related (82). DHEA levels were shown to decrease in plasma and cerebrospinal fluid in subjects receiving steroid therapy while DHEAS levels decreased in the plasma but not in the cerebrospinal fluid in subjects also receiving steroid therapy (82). Furthermore, in those subjects receiving steroid therapy, cortisol levels in plasma were directly related to cerebrospinal fluid levels, but there was no relation between plasma and cerebrospinal fluid levels of DHEA or DHEAS (82), further suggesting that DHEA and DHEAS may be synthesized in the central nervous system.

The synthesis of DHEA in the central nervous system is believed to follow essentially the same metabolic pathway (3; 86) as the peripheral synthesis. Pregnenolone is derived from cholesterol after side chain cleavage by cytochrome P450scc. Then, cytochrome P450 CYP17, a 17α-hydroxylase with 17,20-lyase activity catalyses the conversion of pregnenolone to 17α-hydroxypregnenolone and DHEA. Finally, hydroxysteroid sulfotransferase enzyme (SULT2A1 and SULT1E1) convert DHEA to DHEAS. This is a reversible reaction, hence, DHEAS can also be converted back to DHEA (by the activity of steroid sulfatase enzyme) in the central nervous system.

Whether or not StAR also participates in brain steroidogenesis, or whether brain steroidogenesis, like that in the placenta, is independent of StAR, remains controversial (35). The mRNAs for StAR and P450scc are co-localized in several regions of the rat brain (87), and StAR mRNA is found in various regions of the human brain (88). In the developing rat, StAR mRNA is found glial and neuronal steroidogenic cells, in the hippocampus, thalamus, cortex, pons and the striatum, with an intracellular pattern, consistent with a mitochondrial localization. In the adult, StAR protein was detected in the same tissues (89). P450scc was also expressed in the same cells (89).

Mutations in StAR resulted in adrenal insufficiency (lipoid congenital adrenal hyperplasia) and is lethal (89), but neither StAR knockout mice nor human patients with lipoid congenital adrenal hyperplasia have a phenotype attributable to altered central nervous system (CNS) function, therefore suggesting the existence of a StAR-independent mechanism for steroidogenesis. As mentioned before, the placental steroidogenic cells do not express StAR and utilize a StAR independent pathway. One proposed hypothesis is that a proportion of steroids may be generated from oxysterols in the brain (89). The brain produces significant levels of oxysterols (like 24-hydroxycholesterol) and these can not only regulate StAR expression but also freely diffuse into the mitochondria and be directly converted to steroids (89; 90; 91). This could represent an alternative pathway for the synthesis and regulation of neurosteroid levels (89; 90; 91).

Pregnenolone and its sulfate ester concentrations in the rat brain are about 10 times higher than DHEA concentrations and about 10 times higher than plasma pregnenolone concentrations (92). The presence of immunoreactivity for P450scc protein (which converts cholesterol to pregnenolone) was initially found in the white matter in rat and human brain, and in glial cell cultures of the newborn rat forebrain (93) and later in several regions of the brain, especially neurons in the hippocampus (86; 87), this was established since 1987. N-methyl-D-aspartate (NMDA) stimulation promoted $Ca^{2+}$ influx and the synthesis of pregnenolone in rat hippocampal neurons, suggesting that pregnenolone synthesis in the hippocampus may be stimulated and regulated by glutamate mediated synaptic communication (86).

P450 CYP17, the key enzyme in the production of DHEA, is found throughout the developing mouse brain (94; 95; 96). Zwain and Yen (97) demonstrated for the first time in 1999 that the neonatal rat brain expressed P450 CYP17 steroidogenic enzyme. More precisely, they demonstrated that hypothalamic and cortical astrocytes *in vitro* expressed the P450 CYP17 steroidogenic enzyme and were able to synthesize and secrete DHEA and metabolize this hormone to testosterone and estradiol in a dose-dependent manner. These cortical neurons *in vitro* expressed a very low level of P450 CYP17 mRNA and produced a small amount of DHEA (97). Hypothalamic astrocytes produce DHEA at a level three times higher than that produced by cortical astrocytes (97). On the contrary,

oligodendrocytes neither express the messenger RNA nor produce DHEA (97). In the adult mouse brain, P450 CYP17 was found in the hippocampus (98) and spinal cord (99). In the hippocampus, it was localized to pyramidal neurons and granule cells of the dentate gyrus (in both cases, localized in pre- and post-synaptic locations and in the endoplasmic reticulum) (98). In the spinal cord, cytochrome P450c17 was found in neurons and glial cells (99). Of note, mRNA transcripts of the several steroidogenic enzymes necessary for DHEA synthesis were found in the hippocampus, although in very low levels (86). P450 CYP17 mRNA transcripts in the embryonic mouse brain (94) and in the hippocampus of adult male rats are low, about 1/200th of the expression found in the testis (7; 86; 98). Similar to the stimulation of pregnenolone synthesis, the activity of P450 CYP17 in the hippocampus was enhanced by exposing neurons to NMDA (98). The synthesis of DHEA from pregnenolone in frog brains was also inhibited by ketoconazole, a known inhibitor of the P450 CYP17 (7).

People with P450 CYP17 gene mutations have sexual infantilism in phenotypic females (due to lack of sex steroid precursors and 46,XY subjects also have female infantile external genitalia), hypertension, and hypokalemia (100; 101). There are no reported neonatal neurological problems in those subjects, eventually because they obtain sufficient quantities of 17-hydroxylated steroids from their mothers during prenatal development. Adults with P450 CYP17 gene mutations are not well studied with regards to neuropsychiatric illness, although this could be complicated with the possible psychological effects of disturbed sexual development. In mouse, studies with knock out of this gene were also uninformative, as the P450c17−/− mice died by embryonic day 7, and the cause of this early lethality was unknown (102).

An **alternative pathway** for DHEA synthesis in the brain might exist. In that respect, Prasad *et al.* (1994) (103) and Cascio *et al.* (1998) (104) suggested an alternative pathway for DHEA synthesis in the brain, which was independent of P450scc activity and involved hydroperoxide intermediates. Prasad *et al.* found evidence of the presence of sterol hydroperoxides or peroxides in brain extracts, that in the presence of $FeSO_4$, increased DHEA concentrations (103). That $Fe^{2+}$ effect is observed even in the absence of CYP17 activity (105). To a much lesser extent, several reagents like triethylamine, HCl,

$FeCl_3$, $Pb(OAc)_4$ also increased DHEA concentrations (103). Prasad *et al.* proposed a *"hydroperoxide pathway",* in which an unknown cholesterol metabolite present in brain would serve as the precursor for pregnenolone and DHEA synthesis (103; 104).

Prasad *et al.* also proposed that this transient complex could serve as a precursor of an organic soluble sterol hydroperoxide, sterol cyclic peroxides, and/or other di-substituted sterol peroxides, steroid-O-O-R (where the radical R is not H) (103). Then, the authors proposed that one possibility was that this complex could be converted into the isolable 20-hydroperoxide, the 20,22-cycloperoxide, or the 17,20-cycloperoxide by enzymes that could be specific for hormone synthesis. Possibly through the action of a different enzyme, the 17-hydroperoxide of cholesterol could be the product of the sterol and 17,20-dioxetane, which eventually could be the source of the 17-ketosteroid dehydroepiandrosterone (103). Therefore, in this hypothesized pathway, the peroxidation of cholesterol could be catalyzed by enzymes, different from the cytochrome P450scc. These authors also propose that if the path cholesterol - cholesterol peroxide - dehydroepiandrosterone does exist in the brain or even in the steroid-producing endocrine glands, then it would undoubtedly be associated with its own regulatory system (trophic factors, etc.) and could therefore represent a new aspect of steroidogenesis (103).

Cascio *et al.* (104) used rat glioma tumor cells and found that $FeSO_4$ induced the synthesis of DHEA (and pregnenolone) in those cells, even in the presence of specific inhibitors of P450scc and/or P450c17. The authors suggested that the synthesis of DHEAS and pregnenolone might result from the fragmentation of in situ-formed tertiary hydroperoxides. When exogenous pregnenolone along with $FeSO_4$ were added to those tumor cell microsomes, the amount of DHEA formed was (5 to 10 times) higher than in controls, indicating that $Fe^{2+}$ facilitated the conversion of pregnenolone to DHEA. Treatment of those cells with KI, $NaBH_4$ or $HIO_4$ also resulted in increased DHEA synthesis, suggesting that the precursor of DHEA in those cells was a steroid in which C17 and C20 were oxygenated (1; 104). In that alternative pathway, for instance, the precursor 17-hydroperoxide of pregnenolone (17-hydroperoxi-pregnenlone), would convert to 17-hydroxy-pregnenolone by the addition of KI; then when treated with NaBH4 would result

in pregn-5-en-3β,17,20-triol; and when treated with $HIO_4$ would result in the formation of DHEA (104). Contrary to the observations made in brain extracts, the treatment of rat adrenal or testis extracts with $Fe^{2+}$ did not increase pregnenolone or dehydroepiandrosterone production (103), suggesting that the effect was tissue specific. Moreover, the enhancement of DHEA formation by $Fe^{2+}$, was somehow specific, as it was not observed for progesterone (104).

In accordance with the previous alternative pathway, oxidative stress is proposed to contribute to DHEA synthesis in Alzheimer´s Disease and other neurodegenerative diseases linked to oxidative stress (106). DHEA levels in Alzheimer Disease central nervous system and cerebrospinal fluid are significantly higher than in age-matched controls, although serum levels are lower than in the cerebrospinal fluid, and not significantly different from age-matched controls (107). $FeSO_4$ increases DHEA synthesis, more in controls than in Alzheimer Disease patients, suggesting the presence of a precursor of DHEA in controls (107). It is proposed that in the brain with Alzheimer Disease, DHEA is formed by oxidative stress metabolism of that precursor (107). A lower DHEA formation in response to $FeSO_4$ would be a marker of Alzheimer Disease (107). Also, the DHEA variation after oxidation was correlated with the patients' cognitive and mental status (106). Besides ferrous sulfate, beta-amyloid peptide (a pro-oxidant) also enhanced the synthesis of DHEA by CYP17 independent pathway (107; 108; 109). This also points to the hypothesis that DHEA synthesis may involve oxygenated hydroxyperoxides, as free radicals and oxidative stress enhance DHEA synthesis while anti-oxidants reduce that synthesis (109; 110).

Conversion of DHEA into DHEAS has been shown to occur in rats, monkeys and human brains. In fact, a low activity of DHEA sulfotransferase (SULT2A1) was detected in several regions of rat brain (pons, hypothalamus, olfactory bulb, cortex, hippocampus, thalamus, basal ganglia and cerebellum), with the highest level found in the hypothalamus and pons (111; 112; 113). The question remains to be answered, whether this low activity of DHEA sulfotransferase is responsible for the formation of the high DHEAS concentrations found elsewhere in the brain. This sulfotransferase (SULT2A1) catalyzes the conversion of DHEA to DHEAS and it is identical to the one expressed in the

liver. Another sulfotransferase, SULT2B1a, is also expressed in rat (114) and human brain (115). SULT2A1 and SULT2B1 are both encoded in chromosome 19 (19q13.3) (116; 45). In contrast to the substrate specificity of SULT2A1, SULT2B1a and SULT2B1b (SULT2B1a is expressed mostly in the brain and spinal cord, while SULT2B1b is expressed in the skin and several other peripheral tissues and weakly expressed in the CNS) catalyze the sulfonation of several 3beta-hydroxysteroids with high catalytic efficiency (cholesterol, pregnenolone, dehydroepiandrosterone, dihydrotestosterone). As mentioned, DHEAS levels in rats did not change after corticotrophin, dexamethasone medication, adrenalectomy or orchiectomy (80). On the contrary, stress conditions increased DHEAS levels in brain, even after adrenalectomy plus orchiectomy, thus suggesting that DHEAS formation in the rat brain may depend on local mechanisms independent of peripheral glands (80).

Due to its hydrophilic nature, DHEAS does not readily cross the blood brain barrier. This transport, in both directions, is mediated by organic anion transporting peptides (OATPs) (85), which, in brain, are expressed in the blood-brain barrier endothelial cells and blood-cerebrospinal fluid barrier epithelial cells (117). DHEAS is predominantly transported outside of the brain. In one study, only 0.03% of peripherally injected DHEAS reached the rat brain (83) while the efflux of DHEAS outside of the brain was tenfold greater than its influx (efflux was a saturable process with a Michaelis constant, K(m) of 32.6 µM, efflux clearance was 118 µl/min.g of brain; influx had a K(m) of 34.4 microM, influx clearance was 11.4 µl/min.g of brain) (85). That DHEAS efflux was inhibited by taurocholate, cholate, sulfobromophthalein, and estrone-3-sulfate and DHEAS influx was inhibited by digoxin (and DHEAS inhibited digoxin influx) (85). The influx of DHEAS (in mouse brain capillary endothelial cells) after a 30 min interval was significantly increased under ATP-depleted conditions, further suggesting that the efflux may be an energy-dependent process (85). The fact that DHEAS is predominantly transported out of the brain, also suggests that DHEAS found in the brain may be most likely due to local synthesis (7).

As stated before, DHEA can be further metabolized to androgens and estrogens in the brain. P450 aromatase activity was found in hypothalamic tissues (118) and

hypothalamic astrocytes were shown to be three times more active than cortical astrocytes in the synthesis and metabolism of DHEA to estradiol (97), thus suggesting that DHEA may be involved in the regulation of hypothalamic neuronal function, particularly gonadotrophin releasing hormone (GnRH) neurons, whether directly or indirectly through its metabolite, estradiol.

Brain tissues are further able to metabolize DHEA to its hydroxylated metabolites, 7α-hydroxy-DHEA and 7β-hydroxy-DHEA, leading to the formation of androstenediol and androstenetriol (81). Astrocytes were reported to be involved in the formation of DHEA metabolites in the brain (81). Besides that, CYP7B1, which converts DHEA into 7-hydroxy-DHEA in brain, was found to be highly expressed in pyramidal neurons in human hippocampal sections (119).

Thus, DHEA is synthesised in the adrenals and gonads, but also in the central nervous system (1; 2; 68; 120; 121; 122). A sulfotransferase reversibly converts DHEA into DHEAS in the adrenals, liver and brain (80; 111; 112; 113; 115). DHEAS is the most abundant hormone in the human peripheral circulation and DHEAS concentrations largely exceed DHEA concentrations both in the peripheral circulation and in brain tissue (80). DHEA and DHEAS concentrations in the central nervous system are higher than their concentration in the peripheral circulation, with higher DHEA concentrations in prefrontal than posterior brain regions, whereas DHEAS concentrations are higher in the posterior than in the anterior brain tissue (3; 4; 80). DHEA is lipid soluble and may easily cross biologic membranes (2; 122) whereas DHEAS does not. DHEA has a short half-life, whereas DHEAS has a long half-life (5; 36; 123).

# Changes along the life span and regulation

## Fetal life

In the fetus, the adrenal steroidogenesis occurs primarily within the inner fetal zone (124; 125). In that period of life, there is a relative lack of 3β-HSD and $\Delta^{4,5}$ isomerase activity and high sulfotransferase activity (126; 127). Accordingly, the fetal adrenals cannot synthesize progesterone and in that period DHEA and DHEAS are the major products of adrenal steroidogenesis. Part of the DHEAS originating from the adrenals, is transported to the fetal liver for 16-hydroxylation. DHEA, DHEAS and 16-hydroxy-DHEAS, are then aromatized to estrogens by placental trophoblast [DHEA is a substract for estrone ($E_1$) synthesis, DHEAS is converted to estradiol ($E_2$), and 16-hydroxy-DHEAS is converted to estriol ($E_3$)].

Proliferation into the fetal and definitive adrenal zones are stimulated by fibroblast growth factor and epidermal growth factor (EGF). The fetal adrenal expresses high levels of insulin-like growth factor-II (IGF-II) mRNA and protein, which respond to ACTH (128; 129). IGF-II augments ACTH-stimulated expression of CYP11A1 (P450scc), CYP17 and 3β-HSD and stimulates cortisol and DHEAS synthesis in the fetal cortex, suggesting a role in adrenal regulation during fetal and postnatal life (128). Nevertheless, according to the pattern of adrenal maturation, it is also suggested that cortisol is synthesized the novo from cholesterol in the adrenal, but only after the $30^{th}$ week of gestation (128).

ACTH concentrations are higher throughout gestation than in postnatal life and its levels fall near the term of gestation. Midgestation fetal ACTH are high (about 250 pg/mL, 55 pmol/L) and stimulate maximum fetal steroidogenesis. Thus, ACTH stimulates the adrenal cortex considerably to produce pregnenolone, DHEA and their sulfate conjugates (130). Prolactin (PRL) and other peptides were also suggested to selectively stimulate adrenal androgen production (131). Low amounts of cortisol are produced by the fetal adrenal. Fetal cortisol is converted to cortisone mostly through 11β-HSD in fetal tissues,

and also levels of cortisone in the fetus at midgestation were found to be fourfold to fivefold higher than cortisol levels (130). Cortisone is a relatively inactive glucocorticoid, and this metabolism protects the anabolic milieu of the fetus because cortisol can retard both placental and fetal growth (132). On the other hand, studies with animal cultures suggest that DHEA and DHEAS might influence neocortical organization (15).

The ACTH feedback control of adrenal steroidogenesis matures during the second half of gestation and the early neonatal period. Dexamethasone can suppress the human fetal pituitary-adrenal axis at term but not at 18-20 weeks of gestation (131). With advancing gestation, increased production of estradiol in the placenta, induces placental 11β-HSD activity resulting in a decrease in cortisol levels of maternal origin reaching the fetus and culminating in the ontogenesis of fetal ACTH production and *de novo* production of cortisol by the fetal adrenal gland (131). Cortisol is necessary to prepare the fetus for extra uterine survival (namely for lung surfactant synthesis, hepatic enzymes maturation, maturation of transport processes of the small intestine, ductus arteriosus closure, increased methylation of norepinephrine to epinephrine, increased conversion of T4 to T3) (130), therefore, during the last 10 weeks of gestation there is an increase in cortisol levels in the fetus as a result of increased secretion and reduced conversion of cortisol to cortisone (130). Then, at birth ACTH, DHEAS, cortisol and cortisone levels sharply decrease (130). The failure of the rising cortisol to suppress plasma ACTH during the last weeks of gestation may be due to altered sensitivity of glucocorticoid negative feedback (133), but presently little is understood about the regulation of hypothalamic-pituitary-adrenal (HPA) function during late gestation and at term (133).

**Childhood and adolescence**

After birth, DHEAS levels decline rapidly during the first year of life and are maintained at a minimum level until around 6-8 years old (134). Concerning DHEA, its levels are relatively high after the first month of life and then decline more slowly, to reach a minimum level in girls between 5 and 7 years and in boys between 5 and 9 years of age (134). DHEA and DHEAS levels remain low until adrenarche starts, around six to

eight years of age (skeletal age 6 to 8 years). In contrast to the zona fasciculata, the expression of 3β-HSD and $\Delta^{4,5}$ isomerase type 2 and their activity in the zona reticularis is very low, whereas the expression and activity of the sulfotransferase is high. Around 6 to 8 years of age, the DHEA and DHEAS are secreted from the zona reticularis of the adrenal cortex and their serum concentrations then begin to rise (135; 136). Those levels continue to rise through early adulthood (137). Around 14-16 years old, boys reach higher DHEAS levels than girls (137), but DHEA levels are not higher in boys than in girls (134). In fact, higher DHEA levels were found in girls 11-15 years old when compared to boys in the same age group (134). In the peripheral circulation, DHEA has a diurnal rhythm similar to that of cortisol while DHEAS shows less variation and is a practical biochemical marker of adrenarche (135; 136).

During that period, there is a parallel increase in DHEA and DHEAS levels in blood and the cerebrospinal fluid (82). Guazzo *et al.* compared DHEAS-to-DHEA ratios in subjects below 18 years old (3-16 years old) and adults and found DHEAS-to-DHEA ratios of 124 and 136 in the peripheral circulation, 1.6 and 2.9 in the cerebrospinal fluid (CSF), in subjects below 18 years old and adults respectively (82). Those authors found a decrease in cortisol-to-DHEA and cortisol-to-DHEAS ratios in the CSF during adolescence and young adulthood, contrary to the increased ratios found in old subjects (82). However, although there are low levels of DHEA and DHEAS during early childhood, they also found lower levels of cortisol in the CSF during early childhood when compared to young adults and higher CSF cortisol levels in the elderly when compared to young adults (82). Therefore, the increased CSF cortisol observed in the elderly were not observed during early childhood (82).

The adrenal androgens contribute to the growth of pubic and axillary hair. Besides that, the elevation of DHEA prior to the first signs of puberty led to the suggestion that DHEA may play a role in the maturation of the hypothalamic-hypophyseal-gonadal axis (138). By extraglandular metabolism, the adrenal androgens contribute to physiologically active testosterone and estradiol. However, the mechanism that triggers the secretion of DHEA is not known and there is no consistent support for a role of adrenal androgens in determining the onset of puberty. In fact, most patients with premature adrenarche do

not have precocious puberty; children with adrenal insufficiency usually have normal onset and progression of puberty when given adequate glucocorticoid and mineralocorticoid replacement therapy (139; 140). Also the transient increase in height velocity that occurs in middle childhood (6 to 7 years) cannot be attributed to adrenarche. This spurt lasts about two years and then terminates, while DHEAS continues to increase. Instead, it is hypothesized that that spurt is probably related to the cyclic pattern of prepubertal growth and/or genetic factors (141).

Although its meaning is an unsolved issue, the changes in DHEA and DHEAS levels through life are unique and do not match other steroid hormones. Focal islands of reticular tissue and then a continuous reticular zone develop during adrenarche (140). DHEAS concentrations are related to the growth of the zona reticularis and the increase in adrenal volume with age (140). This last step in the adrenal zonation is characterized by: 1) low level of expression of 3β-HSD/$\Delta^{4,5}$ isomerase type 2 and cytochrome P450 CYP21 mRNAs and enzyme activities (142; 143); 2) high activity of the DHEA sulfotransferase enzyme (143); 3) increase in 17,20-lyase activity of P450 CYP17, the enzyme that catalyzes both the activity and synthesis of cytochrome b5; and 4) expression of major histocompatibility complex class II (human leukocyte antigen DR, HLA-DR) antigens (144). The fetal adrenal cortex contains the first three characteristics, while the HLA-DR antigens were not expressed.

Several epidemiologic related associations with DHEA levels have been found raising interesting hypothesis about DHEA teleological meaning, but in most cases, causal relationships are not established. DHEA is a neurosteroid, and along with adrenarche, there is a simultaneous parallel increase in cortical maturation from approximately age six to the middle twenties, suggesting that adrenarche may influence brain development. DHEAS may increase the activity of the amygdala and hippocampus and promote synaptogenesis within the cortex. It may influence memory, emotions, anxiety, resilience and increase social interaction with unfamiliar individuals. In summary, it might eventually play a role in human brain maturation (16). Of note, besides humans, adrenarche was shown to occur only in great apes and to some extent, also in Old World

monkeys (145; 146; 147), also suggesting that it evolved to promote brain development (147).

The zona reticularis has an increased ratio of 17,20-lyase to 17α-hydroxylase activity. That ratio increased by increased phosphorylation of serine and threonine residues on the CYP17enzyme (148) and by the abundance of redox partners such as cytochrome P450 oxidoreductase and cytochrome *b*5, which preferentially promotes 17,20-lyase activity by allosterically affecting the interaction between CYP17 and P450 oxidoreductase (149; 150). These studies suggest a mechanism that may be involved in the relative increment of 17,20-lyase activity in the zona reticularis, although not in its regulation.

No definitive major factors regulating DHEAS synthesis have been identified. Nevertheless, ACTH may be a necessary stimulus for DHEA adrenal secretion. This is evidenced by the low levels of DHEA found in cases of ACTH deficiency or resistance (151). In fact, CRH stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for cortisol and DHEA secretion (152). CRH also directly stimulates the expression of CYP17 by fetal adrenal cortical cells (153) as well as fetal, pubertal and adult adrenal androgen secretion. This was evidenced by studies showing that CRH stimulated dexamethasone-suppressed adrenal to secrete DHEA and DHEAS within three hours (154; 155). Nevertheless, there is a 20-fold increase in DHEAS levels between the onset of adrenarche and midpuberty but there is no concomitant increase in cortisol levels.

Evidence of the dissociation between adrenal androgen and glucocorticoid regulation and secretion are the differential suppression by dexamethasone and differential changes in adrenarche (only DHEA increases), aging (only DHEA decreases), starvation, anorexia nervosa and chronic illness (DHEA decreases with no change or increase in cortisol levels) as well as in the age related response to ACTH (only DHEA and DHEAS response decline with age). Accordingly, the action of an unidentified factor is hypothesized, which would be an adrenal androgen stimulating hormone (AASH, also called cortical androgen stimulating hormone, CASH) (140; 156; 157). This factor could originate from the pituitary, the adrenal or an extra-adrenal source and might explain the dissociation of adrenal androgen and glucocorticoid secretion.

Leptin in vitro also stimulates 17,20-lyase activity and 17α-hydroxylase activity, suggesting a role in adrenarche (158), although there is no clinical evidence to suggest a pivotal role of leptin in adrenarche. Nevertheless, an increase in body mass index (BMI) was related to an increase in DHEAS levels during adrenarche, suggesting that the nutritional status may play a role in the regulation of adrenarche (159).

**Adulthood and old age**

In the adult human DHEAS is the most abundant steroid in the peripheral circulation and, as mentioned, brain tissue levels are even higher than in peripheral circulation ones. Its levels in peripheral circulation are about 300 times higher than those of DHEA, in relation to its longer half-life, difficulty to cross plasma membranes and lower clearance rate (36; 160). Curiously, most laboratory animals have low circulating concentrations of DHEA and DHEAS. Concentrations comparable to those in humans are found only in great apes and Old World monkeys (161; 162), and not all primates share this characteristic (1; 2; 163). Nevertheless, the rodent brains still have appreciable concentrations of DHEA, although lower than those found in human brains (1.5 and 0.4 nmol/kg in the anterior and posterior rat brain; 29.4 and 16.9 nmol/kg in anterior and posterior human brain) (4; 80).

During adulthood and old age, DHEAS levels are higher in males than in females. Unlike DHEAS, unconjugated DHEA levels are not higher in men (134). On the contrary, significantly higher levels of DHEA were found in women in the premenopausal period (between 36 and 45 years of age) and in the older group (after 60 years of age) (134). The age- and sex-related dependencies are different between DHEAS and DHEA, suggesting an eventual variable secretion and dynamics of their interconversion (134). In both genders, DHEA and DHEAS levels both in plasma and cerebrospinal fluid peak in early adulthood (in the mid-twenties) and then generally, but progressively decreasing with age (82; 164). Its mean levels decrease to about 1 to 4% per year in men (12; 165) and about 2% per year in women between 40 and 80 years old (12). By 65 to 70 years old, DHEA and DHEAS levels are about 20% of those in early adulthood (12). This progressive decline in

circulating levels of DHEA and DHEAS has been called by several authors as *adrenopause* (166; 167). Of note, one study suggested that 15% of women and 5% of men showed an increase instead of a decrease in DHEAS levels over a 10 to 14 year follow up (12). Besides, a transient increase in DHEAS concentrations was described in women transitioning into menopause along with its associated increase in gonadotrophins (168; 169). And a DHEA increase was also found in women in the premenopausal period (36-45 years of age) (134).

Along with the age-related decline in DHEA and DHEAS levels, there is a parallel decline in CSF levels of DHEA and DHEAS (82). On the contrary, there is an increase in CSF cortisol-to-DHEA molar ratio with aging. Those ratios for 70 years old were four times those for 20 years old (79). Nevertheless, there may be an attenuated decline in CSF levels of DHEA and DHEAS with age when compared to the peripheral circulation decline, and the age decline in CSF levels may be much lower for DHEAS when compared to DHEA (82). Guazzo *et al.* found that the CSF levels of DHEA in the 60 to 85 years old group of subjects was half those in the 19 to 40 years old group, while the blood levels declined to about one third of those in the 19-40 years old group; but concerning DHEAS, in the oldest group of subjects, those levels in the CSF were 80% of those in the younger adults (82). In older individuals with relatively high CSF cortisol and low DHEA(S), the molar ratio was 5-10 times higher than in young adults aged around 20 years old (82).

The CYP3A group of enzymes (which includes CYP3A4) are abundantly expressed in liver, being responsible for the metabolism of several pharmacologic medications. CYP3A7 enzyme is expressed mostly in human fetal liver and is normally silenced after birth. This enzyme carries a similar role in fetus as CYP3A4 in adults. It oxidizes several compounds, including steroids, fatty acids and xenobiotics, and it plays an important role in the 16α-hydroxylation of DHEA and DHEAS. There is a common polymorphism in this gene (CYP3A7*1C) that causes the persistence of its enzymatic activity during adult life. 6 to 8% of the population are heterozygous carriers of this polymorphism resulting in about half the serum DHEAS levels and identical serum DHEA levels compared with homozygous carriers of the reference allele (170). Nevertheless, although those subjects have lower

DHEAS levels than controls, there is no evidence that they have an earlier beginning in diseases related to aging.

DHEA and DHEAS levels reach a nadir by the time many diseases of old age peak its prevalence. Moreover, lower DHEA and DHEAS levels were related with higher morbidity and mortality even after age adjustment (2; 13). In a long time (27 years) period follow-up study, lower baseline DHEAS levels were also predictive of shorter longevity independently of other risk factors such as age, blood pressure or fasting glucose levels (171). Either specific effects or a cortisol antagonism has been evoked to account for those associations (17; 172). Importantly, DHEA and DHEAS levels gradually decrease over time whereas corticotrophin secretion and cortisol levels remain largely unchanged in older subjects. Accordingly, the ratio of DHEA to cortisol and DHEAS to cortisol decline with age. Some authors proposed that this decline in DHEA and DHEAS occur due to a reduction in the number of functional zona reticularis cells in the adrenal cortex rather than due to regulation by some central hypothalamic pacemaker of aging (173).

The sulfotransferase activity is downregulated during infection, inflammation and the euthyroid sick syndrome (174; 175; 176). The acute-phase response and T3 levels have also been shown to modulate the DHEA sulfotransferase activity. TNF and IL-1 mediated inhibitory effects of lipopolysaccharides (LPS) on SULT2A1 at the mRNA level (174) could provide a possible mechanism by which infection and inflammation are associated with reduced serum levels of DHEAS. On the other hand, SULT2A1 enzyme has been reported to be regulated by two transcription factors, the steroidogenic factor 1 (SF1) and GATA in the human adrenal gland. SF1 on the other hand was also found to be indirectly up-regulated by T3 (175). This could eventually contribute to the low DHEAS levels that we usually find in patients with euthyroid sick syndrome. Additionally, cAMP may enhance the synthesis of cortisol, DHEA and the sulfotranstransferase activity (176) while, in patients with chronic liver disease, the dehydroepiandrosterone-sulfotransferase concentrations and its activity in liver are reduced (177).

As mentioned before, DHEA and DHEAS are precursors to peripherally synthesized androgens and estrogens in target tissues such as the brain, bone, skin and adipose

tissue. In this regard, some authors propose that DHEA and DHEAS are inactive precursors and that their actions are mainly exerted after conversion to active androgens and estrogens in peripheral target tissues (intracrinology) (178; 179). In this case, these actions are exerted inside the cells where the active steroids are synthesized and those active steroids are not released to the general circulation.

# Mechanisms of action and cellular effects

## Molecular effects

Steroid hormones in general act by binding to nuclear or cytoplasmic receptors. Cytoplasmic receptors usually translocate into the nucleus and for both cytoplasmic and nuclear receptors, the complex hormone-receptor bind to steroid responding elements in the deoxyribonucleic acid (DNA) (7). Nevertheless, to date, no nuclear steroid receptor with high affinity for either DHEA or DHEAS has been found and the exact molecular mechanisms by which DHEA and DHEAS operate are not fully understood (7). Also, there is no evidence that DHEA or its active metabolites bind to the glucocorticoid receptor or interfere with glucocorticoid hormone binding with its receptor (180; 181). Some of DHEA and DHEAS effects may be mediated after conversion to estrogens and other androgens in target tissues, that later have effects on their respective receptors (7). Besides, although no specific membrane receptor was found for DHEA and DHEAS, these molecules were shown to have affinity for several membrane receptors that could affect their action (3; 7).

In cell membranes in the brain, DHEA and DHEAS modulate ion-gated channel neurotransmitter receptors *N*-methyl-D-aspartate (NMDA) and γ-aminobutyric acid type A (GABA-A) receptors (86; 110). DHEA and DHEAS present in general a neurostimulatory effect: presynaptic actions include glutamate, acetylcholine and norepinephrine release and postsynaptic actions include sigma 1 receptor agonism, *N*-methyl-D-aspartate (NMDA) receptor agonism, γ-aminobutyric acid type A (GABA-A) receptor antagonism and inhibition of voltage-gated calcium currents (1; 3). Although DHEA and DHEAS may influence the release of several neurotransmitters at the synaptic terminals, they act primarily through postsynaptic receptors (1).

Presynaptic actions of DHEAS include spontaneous glutamate release and inhibition of serotonin (5HT)-evoked glutamate release in the prelimbic cortex, as well as spontaneous glutamate, acetylcoline (Ach) and NMDA-evoked norepinephrine (NE)

release in the hippocampus. Post-synaptic actions of DHEAS include GABA-A receptor antagonism with reduction of inhibitory postsynaptic currents and reduction of decay time constants at the ventral midbrain, sigma-1 receptor agonism with inhibition of persistent sodium currents in the prefrontal cortex, pain facilitation at the spinal cord, NMDA-induced neuronal excitability in the hippocampus and inhibition of voltage-gated calcium currents in the hippocampus CA1 region (3).

The GABA-A receptor complex contains a central chloride anion conduction pore. Several substances interact with this receptor complex. It binds GABA and drugs like muscinol, gabaxadol and bicuculline. Also, besides DHEA and DHEAS, other steroids and drugs like picrotoxin, benzodiazepines, barbiturates and ethanol bind at allosteric sites of the GABA-A receptor, modulating its activity. The NMDA receptor is an ionotropic glutamate receptor (ion channel), nonselective to several cations, allowing the voltage-dependent flow of $Na^+$ and small amounts of $Ca^{2+}$ into the cell and $K^+$ out of the cell. Again, numerous substances are known to interact with the NMDA receptor. It has got several regulatory and functional binding sites, including the glutamate (recognition) site, the glycine (co-activator) site, and a site within its ion channel that binds phencyclidine and the noncompetitive antagonist MK-801 (dizocilpine) (182; 183). Agonists include NMDA (which selectively binds to this receptor), aspartic acid, glutamic acid, serine, alanine, nebostinel, DHEA, DHEAS and pregnenolone sulfate (120) amongst others; antagonists include ethanol, nitrous oxide, methadone and tramadol; and modulators include $Na^+$, $K^+$, $Ca^{2+}$ and $H^+$ concentrations. Sigma-1 receptors modulate $Ca^{2+}$ release and voltage gated $K^+$ channels amongst its other actions. Tryptaminergic amines, DHEA, DHEAS, pregnenolone and pregnenolone sulfate may activate this receptor.

By acting as GABA-A receptor antagonists and sigma-1/NMDA receptor agonists in neuronal membranes, several intraneuronal effects of DHEA and DHEAS were described (7). DHEA inhibits $Ca^{2+}$ influx into the mitochondria; DHEA increases kinase activity of Akt and decreases apoptosis, while DHEAS decreases Akt and increases apoptosis; DHEAS increases tyrosine hydroxylase mRNA and protein, leading to increased catecholamine synthesis; DHEA and DHEAS stimulate actin depolymerization and submembrane actin filament disassembly, thereby increasing secretion of catecholamines dopamine and

norepinephrine from secretory vesicles; DHEA and DHEAS inhibit reactive oxygen species (ROS) activation of transcription mediated by NF-κB; DHEA inhibits nuclear translocation of the glucocorticoid receptors; these hormones also inhibit stress-activated protein kinase 3 (SAPK3) translocation and 11β-hydroxysteroid dehydrogenase type 1 activity (7).

DHEA and DHEAS may either directly act at the NMDA receptor level or bind to sigma-1 receptors with posterior NMDA receptor activation (7). Several intracellular actions of sigma-1 receptor activation by DHEAS were suggested. DHEAS activation of sigma-1 receptor was found to inhibit persistent sodium currents, an effect that was mediated through Gi protein and protein kinase C (3; 7). Other pathways might also be involved in sigma-1 signaling, namely inositol triphosphate (IP3) receptors and $Ca^{2+}$ signaling, mitogen-activated protein kinase (MAPK), phospholipase C, c-Jun N-terminal kinase, Ras/Raf/MAPK pathways, and norepinephrine release (3).

DHEA and DHEAS act as noncompetitive antagonists at the GABA-A receptor (184; 185; 186; 187) and as positive allosteric modulators of the NMDA receptor (188; 189). Binding assays indicate that DHEA can bind with high affinity on membranes isolated from rat hippocampal cells (Kd=61.9 nM) (DHEA bind normal human adrenal chromaffin cells with a Kd=0.1 nM) (211). DHEA and DHEAS actions at GABA-A and NMDA receptors were observed at micromolar concentrations (and some at nanomolar concentrations) (29; 190; 191). DHEA and DHEAS may act at distinct sites of the GABA-A receptor (192). DHEAS may bind at the barbiturate site of the GABA-A receptor complex (187). Also, DHEAS but not DHEA, competitively inhibits the binding of TBPS to GABA-A, suggesting that the noncompetitive inhibition of DHEAS on GABA-A responses may be mediated by attaching to the picrotoxin/TBPS (T-butyl-bicyclo-phosphorothionate) binding site of the GABA-A receptor complex (187).

DHEAS produces a concentration-dependent block of GABA-induced currents with IC50 of 13 μM and 10 μM respectively, in cultured neurons from the ventral mesencephalon (192; 193) and cortical neurons (187). DHEA was much less potent in inhibiting GABA-induced currents: $Cl^-$ influx was reduced by 13% at 10 μM and by 33% at 100 μM concentrations (187). The presence of the γ subunit in GABA-A receptor may enhance the efficacy of DHEAS without altering its binding affinity (194). A saturating

concentration of DHEAS blocked approximately 75 % of currents mediated by GABA-A receptor, which is composed of human α1, β1, and γ2S subunits, whereas the inhibition was only 35 % in GABA-A receptor containing only α1 and β1 subunits (194). Long-term exposure of GABA-A receptors to DHEAS did not produce changes in receptor expression and functional coupling, thus suggesting that DHEAS does not induce tolerance and dependence by acting at this receptor (195).

High micromolar concentrations of DHEAS inhibited muscimol and flunitrazepam binding to GABA-A receptors in rat brain membranes, primarily by reducing their binding affinities (192). On the other hand, pregnenolone sulfate, pentobarbital and phenobarbital interacted with the GABA-A receptor, inhibiting the binding of DHEAS (192; 120). Concerning sigma-1 receptors, at nanomolar concentrations, DHEAS enhanced and pegnenolone sulfate reduced the sigma-1 mediated response to NMDA (196). Haloperidol (a sigma antagonist) and progesterone prevented that effect of DHEAS on sigma-1 mediated NMDA-evoked response (norepinephrine release, in that study) (196). Pertussis toxin, which inactivates sigma-1 mediated Gi function, also suppressed the effect of DHEAS (196). Besides, chronic treatment with nandrolone may also change the affinity of DHEAS to the sigma-1 receptor (197).

DHEAS effect on the NMDA receptor may facilitate long-term potentiation induction and increase the number of NMDA binding sites in the hippocampus and cortex of the rat brain (3). Nevertheless, in some non-hippocampal regions, DHEA and DHEAS may inhibit glutamate transmission also through sigma transmission, as sigma transmission was shown to reduce NMDA-induced effects in the striatum (198); and DHEAS may also inhibit serotonin-evoked glutamate release via activation of sigma-1 receptor in the pyramidal cells of the prelimbic cortex (199).

Acute and chronic effects of DHEA and DHEAS in the modulation of the synaptic function may be mediated by different receptors: DHEAS induces short-term potentiation (STP) in rat hippocampus through postsynaptic effects at the metabotrophic glutamate receptor (200), whereas long-term potentiation (LTP) is induced in rat hippocampus and cortex through sigma-1 and NMDA postsynaptic receptors and NMDA-induced $[Ca^{2+}]_i$ increase (201; 202; 203; 204). DHEA and DHEAS effect may also vary according to their

concentration. As an example, low DHEAS concentrations may inhibit presynaptic serotonin-evoked glutamate release in the prelimbic cortex, whereas higher DHEAS concentrations can promote spontaneous glutamate release (205). Therefore, the effects of DHEA and DHEAS on neurotransmitter release are complex, depending on factors such as the brain region involved, presynaptic functional state and hormone concentration (3; 206).

Sulfated steroids in general possibly act as endogenous neuromodulators (207) and the balance between DHEAS and DHEA has been suggested to influence brain functioning. Bearing this in mind, it is important to note that DHEAS has a much more potent excitatory action than DHEA (NMDA agonism and specially gabaminergic antagonism) and that it may account for some differential effects of both forms of the hormone (1; 184; 196). DHEAS, but not DHEA, also enhances cholinergic function. In fact, it increases acetylcholine (ACh) release from rat hippocampal neurons (208) and this effect may also be mediated through sigma 1 receptors, because it is blocked by a sigma 1 antagonist (209). Inhibiting the conversion of DHEAS to DHEA in rats also increased the hippocampal ACh release (210). Notably, several studies suggest that the balance between DHEAS and DHEA may influence brain functioning (7; 207). Hence, the simultaneous evaluation of DHEA and DHEAS may uncover more information than the individual examination of either form of that hormone.

Working memory rely on glutamatergic transmission (211), raising the hypothesis that DHEA and DHEAS might play a role on working memory. On the other hand, DHEA and DHEAS antidepressive effects might be partially mediated via GABAergic receptor antagonism (212) and sigma 1 receptors agonism. In fact, the GABAergic system can mediate depression (213) and other selective sigma-1 receptor agonists are known to cause antidepressant-like effects on rats (214). As mentioned, in this regard, DHEAS has a much more potent NMDA agonism and specially gabaminergic antagonism action than DHEA (1; 184; 196).

The alteration in the mechanisms involving the activity of the calcium-phospholipid-dependent protein kinase C (PKC) is believed to be an important cellular change associated with brain aging (namely regarding the decline in memory and

attention) (215). There is defective activation of PKC-dependent pathways during aging which is due to a defective mechanism of translocation of the kinase due to reduced levels of the major anchoring protein RACK-1 (receptor for activated C kinase) and there is also experimental evidence that DHEA can revert the alteration of RACK-1 anchoring protein expression (215).

Besides the above mentioned receptors, DHEA and DHEAS effects on other receptors were described. There is evidence of DHEA binding with high affinity ($K_d$=49nM) receptors on endothelial cell plasma membranes, where its actions seem to be mediated through Gi protein-coupled receptors, activating the endothelial nitric-oxide synthase, eNOS (191; 216). DHEA may also act on cytoskeleton components. Regarding this, DHEA was found to bind to microtubule-associated protein 2C (MAP2C). This protein is expressed at early development stages and also in the adult retina and the olfactory bulb. Of note, in these two tissues, neurogenesis persists in the adult (217). DHEA and DHEAS may also act at the peroxisome proliferator-activated receptor (PPARα), pregnane receptor, androstanol receptor and estrogen receptor β (6; 218; 219).

Besides the classical slow genomic effects, rapid non-genomic effects of glucocorticoids were observed at the hypothalamus, hippocampus, amygdala and prefrontal cortex (220). These effects involve mineralocorticoid and glucocorticoid receptors and membrane-associated mechanisms, which are not direct but conditional effects, facilitating or inhibiting the signaling of ion channels, receptors and neurotransmitters, thus modulating the threshold of neuronal activation (220).

The mainly neuro-excitatory effects of DHEA and DHEAS on GABA-A and NMDA receptors do not suggest a direct anti-cortisol or anti-stress effect at this level. In that respect, cortisol effects at GABA-A and NMDA receptor binding sites are less evident than those found in DHEA and DHEAS. Most studies failed to find any effect of glucocorticoids on GABA-A receptor mediated responses (120), although a study by Majewska (190) suggested that glucocorticoids may play a role in the modulation of neuronal excitability via interactions with the GABA receptor complex. T-butyl-bicyclo-phosphorothionate (TBPS) blocks the GABA-A receptor mediated $Cl^-$-current response (221) and Majewska also found that at low nanomolar concentrations of glucocorticoids potentiate TBPS

binding (20-50% above control), whereas at high nanomolar and micromolar concentrations glucocorticoids slightly reduced TBPS binding (190). Regarding NMDA receptors, a low dose of dexamethasone (which caused a decrease in serum cortisol levels) reduced by 20% the NMDA affinity for the antagonist MK-801 in the newborn lamb (but not at other developmental stages of the lamb), thus suggesting that exposure to corticosteroids during a critical period could have significant effects on the developing brain (182). Other studies found either an increase in the apparent number of NMDA receptors (with unchanged $K_d$) (222; 223) or no effects of glucocorticoids on NMDA receptor binding (224; 225).

DHEA and DHEAS binding to a NMDA and GABA-A receptor negative neural crest-derived rat sympathoadrenal cells was observed to occur with high affinity, possible at a Gi coupled receptor (IC50 of 1.3nM for DHEAS and 1.5nM for DHEA) (191). Glucocorticoids (cortisol and dexamethasone) and androgens (testosterone and dihydrotestosterone) affinity for that receptor was also tested and found to be 10 to 15-fold lower than that of DHEA, displacing 70% and 60% of DHEA binding at a concentration of 1µM respectively (191). Also, the anti-apoptotic effect that was observed for DHEA and DHEAS in that study, was not observed for those steroids (191). That observation suggest the hypothesis that cortisol and androgens may act as weak endogenous antagonist of DHEA at that non-NMDA and non-GABA-A receptor level (191).

**Neuronal effects**

Biological actions of DHEA and DHEAS involve neuroprotection, neurogenesis, neurite growth and apoptosis. Several studies suggest a neuroprotective effect of DHEA and DHEAS. After spinal cord injury, mice treated with DHEA had better locomotor recovery, more white matter at the site of injury and a reduced area of reactive gliosis surrounding the lesion (226). Rats submitted to forebrain ischemia showed reduced hippocampal CA1 injury if they were treated with DHEA when compared to rats treated with placebo (227). Similarly, rabbits treated with DHEAS also had increased tolerance to ischemia (228). In the like manner, *in vitro* studies found DHEA and DHEAS protective

effects after oxygen-deprivation (229; 230). It was therefore proposed that this effect was mediated though GABA-A receptor antagonism (228) and that the effect of DHEAS is dose-dependent, with higher concentrations providing more neuroprotective effects (230).

Another mechanism for DHEA and DHEAS neuroprotective effect was shown to be the protection from glutamate, NMDA excitotoxicity (230; 231; 232). This last finding was unexpected since DHEA and DHEAS also have NMDA receptor agonist activity. However, it was proposed that these hormones may be neuroprotective against NMDA toxicity through other pathways (233; 234). Although not completely understood in the case of DHEA, studies suggest that the protection might involve the inhibition of nitric oxide production and the reduction of intracellular $Ca^{2+}$ overload (thus preventing $Ca^{2+}$ influx into the mitochondria) whereas DHEAS protective effect could somehow be mediated via the sigma 1 receptor (235).

It is also important to note that while low doses ($10^{-8}$ – $10^{-7}$ M) of DHEA increased neuronal survival, intermediate doses ($10^{-6}$ – $10^{-4}$ M) had less effect, and high DHEA concentrations (500nM and micromolar concentrations) were neurotoxic in rat primary cortex and hippocampus. This deleterious effect may be mediated through DHEA inhibition of the respiratory chain (236; 237). On the contrary, other studies found that DHEAS had no effect on the neuronal cultures viability (238) and when incubated with neuroblastoma cells, DHEAS antagonized the neurotoxic effect of DHEA (238). Given the higher concentrations of DHEAS than DHEA in the central nervous system, the authors assume that the higher concentrations of DHEAS (10-100 nM) *in vivo* may prevent DHEA induced neurotoxicity (238). We don't know how this DHEAS effect was mediated nor do we know if the effect of DHEA and DHEAS was mediated by different receptors. Nevertheless, these data suggest that in some situations DHEA and DHEAS may have distinct and sometimes opposing effects (7).

Both DHEA and DHEAS were also shown to promote neurogenesis, neurite growth and neuronal survival. DHEA promoted neurogenesis in the dentate gyrus (239). It is not known whether this effect is mediated through brain-derived neurotrophic factor (BDNF), but a study did reveal that DHEA and DHEAS administration modulated the levels of

BDNF, which is a nerve growth factor. In this regard, the effects of each hormone were different: after DHEA administration, the BDNF levels decreased in the hippocampus, did not change in the amygdala but increased BDNF in the hypothalamus; after DHEAS administration, the BDNF levels were initially reduced in the hippocampus and then they increased in the hippocampus and amygdala and reduced in the hypothalamus (240). DHEA and DHEAS increased neuronal differentiation and reduced astroglial proliferation rates in cultures of mouse brain cells (241).

In what concerns neurite growth, DHEA and DHEAS effects also differed: using embryonic neocortical neurons, DHEA preferentially increased axonal length whereas DHEAS increased dendritic growth (15). In the case of DHEA, it was suggested that this effect was mediated through NMDA receptors (15). The DHEA and DHEAS effects in neurite growth may lead to the formation of specific neuronal synapses networks in the rat neocortex (242). Noting that P450 CYP17 is expressed in the cortical subplate, a region enrolled in guiding the thalamic fibers to their cortical targets, Compagnone and Mellon purposed that DHEAS might play a role in the brain development of the rat embryo (189).

In adult rats, DHEA increased synapse formation in the hippocampus (243), although this effect was likely mediated through aromatization to estradiol (243). In what concerns neuronal survival, DHEA and DHEAS increased neuronal survival of embryo and adult cultures of rat and human neurons, namely from the human cortex (244; 241; 245). In the case of DHEA, the results suggest that the effect may be mediated through NMDA and sigma 1 receptors (246). DHEA and DHEAS enhancement of glial survival in rat embryo cell cultures was also observed (245).

DHEA and DHEAS may also modulate apoptosis. The activation of the serine-threonine protein kinase B or Akt has been previously shown to inhibit apoptosis, enhancing neuronal survival (247). In this regard, DHEA activated the serine-threonine protein kinase B protecting from apoptosis; on the contrary, DHEAS decreased the serine-threonine protein kinase B increasing apoptosis (248). In the same way, DHEA but not DHEAS, decreased neuronal death due to NMDA neurotoxicity (249). Once again, this suggests that the balance between DHEA and DHEAS is important for the maintenance of the nervous system. On the contrary, both DHEA and DHEAS promoted chromaffin and

pheocromocytoma cells survival (250). Of note, these cells do not have functional GABA-A or NMDA receptors and mechanisms other than NMDA or NOS inhibition may be involved, namely coupled G-protein mechanisms (216).

**Chromaffin cell effects**

DHEA and DHEAS where shown to stimulate the secretion of catecholamines from rat pheocromocitoma cells (251) and DHEAS was shown to stimulate dopamine (DA) release from rat hypothalamic cultures (252). Besides stimulating secretion, DHEAS but not DHEA, stimulated catecholamine production (253) and DHEAS was shown to increase tyrosine hydroxylase protein concentration (this last effect was not studied for DHEA) (253). Accordingly, non-transcriptional effects of DHEA in the chromaffin tissue and non-transcriptional as well as transcriptional effects of DHEAS in that tissue were suggested.

DHEA and DHEAS do not directly stimulate the proliferation of chromaffin cells, but they modulate the proliferation induced by growth factors. They increase the proliferation induced by some growth factors and decrease the proliferation induced by other growth factors (122; 254). Besides, DHEA and DHEAS may also play a role in chromaffin tissue differentiation: using rat pheocromocitoma cells, they inhibited neuronal proliferation and promoted differentiation towards a more neuroendocrine phenotype (251).

Overall, the findings suggest that DHEA and DHEAS production in the adrenal cortex might increase catecholamine production as well as release and modulate the proliferation and differentiation of chromaffin cells in adrenal medulla. Some authors proposed that DHEA and DHEAS might eventually have similar effects in catecholamine producing neurons in the central nervous system (7). These results are unexpected and counter-intuitive if we take into account the anti-cortisol and anti-stress effects of DHEA and DHEAS. On the other hand, most effects of DHEA and DHEAS at neuronal membrane receptors were also neurostimulant and the effects of these hormones were probably more than just anti-cortisol.

**Antioxidant and anti-inflammatory effects**

Antioxidant, anti-inflammatory and immunomodulatory effects of DHEA and DHEAS have also been proposed. In hippocampal tissues (from rat and human species), DHEA protected against toxicity induced by hydrogen peroxide, sodium nitroprusside and ferrous sulfate (255). In Alzheimer's disease, DHEA protected against oxidative stress and amyloid β protein toxicity (231; 255; 256). Studying diabetes models, DHEA decreased oxidative stress radicals, increased the antioxidant glutathione (GSH) and antioxidant enzymes (GSH-peroxidase and catalase) in the rat hippocampus (257). Likewise, DHEA inhibited the transcription factor NF-kB (which is pro-inflammatory) in those diabetic rats (257). DHEA and DHEAS administration to PPARα -/- knockout mice had less antioxidant and anti-inflammatory effects than its administration to wild type PPARα +/+ mice suggesting that DHEA and DHEAS antioxidant and anti-inflammatory effects might be mediated through PPARα (258).

The administration of DHEA to rats decreased the proinflammatory cytokine tumor necrosis factor (TNF) α (259). In rat brain glial cell cultures, DHEA inhibited the production of TNFα and interleukin (IL)-6 (another proinflammatory cytokine) (259). DHEA and DHEAS inhibits NF-kB transcription in hepatocyte cell cultures, both basally and through TNFα stimulation (260). On the other hand, TNFα also induced NF-kB and this pathway inhibits the nuclear receptor steroidogenic factor (SF)-1 activation of P450 CYP17 transcription (261). This is due to competition between NF-kB and SF-1 for binding to the P450 CYP 17 promoter. Thus, the proinflammatory cytokines TNFα and NF-kB probably inhibit DHEA production while DHEA and DHEAS inhibit TNFα and NF-kB transcription.

**Anti-glucocorticoid effects**

Several levels of evidence point towards anti-glucocorticoid effects of DHEAS and particularly DHEA. However, the mechanism of this anti-glucocorticoid effect is not clear. Possible mechanisms accounting for this DHEA effect are the inhibition of 11β-

hydroxysteroid dehydrogenase (11β-HSD) type 1 and up-regulation of 11β-HSD type 2, therefore reducing the conversion of cortisone to cortisol (which is the active glucocorticoid) and enhancing the conversion of cortisol to cortisone, respectively (262; 263). In fact, DHEA inhibited 11β-HSD type 1 mRNA expression and enzyme activity but up-regulated 11β-HSD type 2 mRNA expression and enzyme activity (262; 263).

The mechanisms for this effect may involve the modulation of the phosphatidylinositol-3 kinase/Protein kinase B-dependent pathway with a shift in expression from the transcription factor CCAAT/enhancer-binding protein (C/EBP)-α to C/EBP-β (262; 263). C/EBP-α is a strong activator of *HSD11B1* and a weak activator of *HSD11B2* whereas C/EBP-β acts in an opposite way and preferentially stimulates *HSD11B2* expression (262; 263). Of note, these effects were not in any way mediated by DHEA metabolism to estradiol or to testosterone (262). On the other hand, in the brain, DHEA can be converted into 7α-hydroxy-DHEA or 7β-hydroxy-DHEA. In vitro, these metabolites can act as anti-glucocorticoids through competitive inhibition of 11β-HSD1, thus limiting the conversion of cortisone into cortisol (264). 11β-HSD type 1 is widely expressed (in liver, adipose tissue, muscle, pancreatic islets, adult brain, inflammatory cells, and gonads) (265). Interestingly, its expression is elevated in the ageing brain, increasing active glucocorticoid levels and 11β-HSD type 1 deficiency or selective inhibition of 11β-HSD type 1 improving cognitive function with ageing (265).

Contrary to what might be expected, there is no evidence that DHEA or its active metabolites interfere with glucocorticoid hormone binding with its receptor (180; 181) and there is also conflicting evidence regarding the interference of DHEA on cellular trafficking of the glucocorticoid receptor into the nucleus (181; 231). Nevertheless, prolonged treatment with DHEA may decrease the relative concentration of the glucocorticoid receptor (266) and reduce its baseline glucocorticoid-receptor dependent transcriptional activity (267). In that regard, it was suggested that maybe DHEA could interfere with glucocorticoid receptor binding to glucocorticoid responsive element sequence, counteracting cortisol effects (268).

At the cellular level, several other studies showed that DHEA is protective against the neurotoxic effects of corticosterone (262), both *in vitro* and *in vivo*. Corticosterone

treatment decreased neurogenesis in the rat dentate gyrus whereas DHEA administration antagonized that effect (239). Corticosterone induced hippocampal neurotoxicity in primary rat tissue cultures, whereas DHEA prevented that neurotoxicity (236). In that study, DHEA reduced the corticosterone-induced nuclear translocation of stress-activated protein kinase 3 (SAPK3), which might be important in the sequence of events leading to either neuronal death or its survival (236).

In another *in vitro* study, DHEA protected against glutamate toxicity (231). Neurons treated with glutamate showed increase nuclear localization of glucocorticoid receptors (GR) and DHEA treatment suppressed the nuclear localization of glucocorticoid receptors (GR) in that condition. These results suggest that the inhibition of GR translocation into the nucleus is a possible mechanism of DHEA's anti-glucocorticoid effects (231). Low levels of glucocorticoids are known to facilitate plasticity and hippocampus-dependent memory mostly through mineralocorticoid activation, while the absence or very high levels of glucocorticoids inhibit plasticity, mostly through glucocorticoid receptors effects (220) while DHEAS antagonized the memory deteriorating neurotoxic effects of cortisol in the hippocampus (3; 269). Hence, the simultaneous evaluation of DHEA, DHEAS and cortisol may uncover more information than the individual examination of either steroid.

**Reactivity to psychological stimuli**

As mentioned, DHEA is synthesized in response to ACTH, even if another unknown stimulating factor may exist. Accordingly, several studies have shown that DHEA and DHEAS are synthesized in response to acute psychological stressful stimuli. Nevertheless, acute and chronic stress have different effects on DHEA and DHEAS levels: acute stress is related to an increase in DHEA and DHEAS levels (8; 270; 271) while chronic stress decreases baseline DHEA and DHEAS levels (9; 10; 272; 273; 274). Also, in subjects under chronic stress, the acute DHEAS response to a superimposed psychological stress is reduced (11). Of note, although DHEAS has a long half-life and is present in high

concentrations in humans, short term increases in peripheral levels of DHEAS have been described in response to acute stimuli (8; 11; 271; 275).

DHEA and DHEAS response to emotional stimuli is mostly unexplored, but a study by Hamilton and Meston (275) showed that DHEAS levels rose after the visualization of a movie with positive emotional content. A direct influence of cognitive processing on DHEAS and DHEA levels or the influence of negative emotional stimuli on DHEA or DHEAS levels were mostly unexplored. Interestingly, in a previous study we showed that corticotrophin releasing hormone (CRH) levels increased with the visualization of emotionally significant movies, more so in a movie of negative rather than positive emotional content (276). Of note, although in that study we found no significant relation between baseline CRH and ACTH, cortisol or DHEAS levels (276) [eventually because most CRH in the peripheral circulation may have originated from central extra-hypothalamic origins (277)], hypothalamic CRH is expected to stimulate corticotrophin secretion and in turn corticotrophin is a stimulus for cortisol and DHEA secretion by the adrenal (152).

Although not completely understood, a differential regulation of DHEA and cortisol responses exist, suggesting that DHEA may be an anti-stress hormone. In that concern, Boudarene *et al.* (278) described a direct relation between DHEAS and cortisol responses during the performance of cognitive tasks but nevertheless those differential responses of each hormone depended on the subjects' anxiety level. Most studies addressing pathological conditions, described that higher anxiety levels were related to higher cortisol-to-DHEA or cortisol-to-DHEAS ratios (279; 280; 281). Boudarene *et al.* (278) also studied subjects without mental disorders and varying levels of anxiety, and found that the level of anxiety was related to the following profile of endocrine response after the performance of cognitive tasks: subjects with high anxiety levels in the State-Trait Anxiety Inventory (STAI) (282) had increased cortisol reactivity and subjects with low anxiety levels showed an exclusive increase in DHEAS levels. The authors suggested that the antagonism in DHEAS and cortisol might be related to a competition in their synthesis and release by the adrenal gland. In a different setting, military subjects who reported fewer psychological symptoms of dissociation when exposed to an acute stress, also

showed higher DHEAS-to-cortisol ratios (270) and increased DHEA response during an acute stress (283).

## Cognitive and neuropsychiatric effects

DHEA and DHEAS levels decline with aging, chronic stress, inflammation and illness (82; 284; 285). Although lower DHEA and DHEAS are related to higher morbidity and mortality independent of age (2; 171; 286), it is not known whether DHEA and DHEAS are pathophysiologically directly related to the manifestations of aging and illness. Allostasis refers to the state in which the physiological systems of the body fluctuate to meet demands from external forces (287). It emphasizes the dynamic behavioral and physiological mechanisms that are used to anticipate or cope with environmental change in order to maintain body function (288). Allostasis involves mostly the sympathetic nervous system and HPA axis responses (allostatic responses) (287). These responses are activated whenever the body faces a challenge (such as infection, danger or any other physical or psychological stress) and stop when the danger passes away (287). Nevertheless, body responses may be beneficial, impose significant costs or cause harm (288). In particular, long time duration stressors (chronic stress) change neural and neuroendocrine responses, dysregulating allostatic mechanisms and resulting in deleterious effects on the body (287; 288; 289).

Allostatic load is a measure of the cumulative physiological burden to the body, resulting from that repeated or chronic stress over time (290). McEvan and Stellar proposed that higher allostatic load is responsible for increased "wear and tear" of the body, thus increasing the chances of developing disease (289). The term allostatic overload is also used to refer to the point above which the body starts to suffer negative physiological consequences due to the activation of allostatic mechanisms (288; 291). Ten biological parameters are included to calculate allostatic load scores: DHEAS, cortisol, epinephrine, norepinephrine, high density lipoprotein cholesterol (HDLc), total cholesterol, waist-to-hip ratio, glycated hemoglobin, systolic and diastolic blood pressure (292). Notably, low DHEAS and high cortisol levels contribute to higher allostatic load scores (111; 292) and people with higher allostatic load at baseline have a higher probability of experiencing declines in physical and cognitive functioning and higher incidence of cardiovascular disease (287). Also, higher allostatic load scores, strongly predict cardiovascular disease, in fact more so, than the use of traditional cardiovascular

risk factors alone (111; 292). Besides the general relation of DHEAS to allostatic load, several other interesting relations of DHEA and DHEAS to cognitive and neuropsychiatric parameters were also described. Unfortunately, in most cases those relations are not systematic nor conclusive, hence they do not elucidate the exact physiological effect of DHEA and DHEAS and also do not establish a cause-effect relation.

**Memory, cognition and quality of life**

Some studies suggest that DHEA and particularly DHEAS might be implicated in age-associated memory deficits. One clue came from the fact that DHEA and DHEAS decrease with aging, reaching the lowest levels to the time when memory and other cognitive capacities deteriorate. Of note, cortisol levels do not decrease with age. With advanced age, higher cortisol-to-DHEA and cortisol-to-DHEAS levels are found, raising the hypothesis that the balance between DHEA or DHEAS and cortisol might be relevant to memory and cognition. In fact, several studies found correlations between higher DHEA, DHEAS, DHEA-to-cortisol or DHEAS-to-cortisol ratios and better cognitive functioning in healthy adults and aging individuals (293; 294; 295) and these relations were independent of age.

Higher DHEAS levels were correlated to better executive functioning, working memory and concentration capacity in a group of 295 women aged 21 to 77 years old (293), whereas higher cortisol-to-DHEA ratios were related to more confusion and poorer visuospatial memory performance in older males (295). Also, greater cognitive deterioration was observed in elderly men and women who showed larger decreases in plasma DHEAS-to-cortisol ratios over time, although changes in DHEAS concentrations alone were not significantly correlated with cognitive change (296). A group of frail, institutionalized elderly people did not differ from independent community-dwelling controls in serum concentrations of cortisol or DHEAS, but did have significantly lower DHEAS-to-cortisol ratios (294). Again, this evidence suggests the importance of the balance between DHEA or DHEAS and cortisol, therefore implying anti-cortisol effects of DHEA and DHEAS.

Nevertheless, other studies found no relation between DHEA or DHEAS levels alone or their decline and cognitive function. This occurred in studies for both genders, adults as well as aging subjects (18; 297; 298). Finally, some studies in elderly women also found significant relationship between higher DHEA or DHEA levels and worse cognitive function (299; 300).

Many studies found a direct relation between lower DHEA or DHEAS levels and poor quality of life, namely poor life satisfaction, psychosocial stress and functional limitations (2; 20; 298; 300; 301; 302; 303; 304; 305; 306; 307; 308; 309). Lower DHEAS levels were also related to higher perceived stress scores (274; 306). On the other hand, individuals with higher DHEAS levels had a higher probability of living independently and men with higher DHEAS levels had lower probability of having organic brain syndromes (310). Higher DHEAS levels were also related to higher vitality ratings in pre-menopausal women, but were not related to well-being scores in post-menopausal women (113). In male subjects, higher DHEAS levels were related to indulging in more leisure activities and greater enjoyment in these activities (311) while females with higher DHEAS levels had greater sexual gratification (312; 313).

DHEAS effects on cognition could be mediated through several actions. The administration of a sulfatase inhibitor was shown to increase DHEAS levels (by 88%), with consequent increases in acetylcholine release in the hippocampus and a reduction of scopolamine-induced amnesia (208). This study suggests that DHEAS enhances cholinergic function and that could eventually be a mechanism to enhance memory (208). Although the study did not test it, it simultaneously raises the hypothesis that higher DHEAS-to-DHEA ratios could eventually benefit memory. DHEA and DHEAS could also potentially improve memory through antiglucocorticoid actions (as DHEA and DHEAS antagonize the deleterious effect of cortisol on memory) (314) or sigma-1 receptor agonism (as sigma-1 antagonists can impair memory) (209; 315). Besides, cognitive effects could also be mediated through NMDA agonism or GABA-A antagonism (316).

Higher DHEA, DHEAS or DHEA-to-cortisol levels were related to improved attention (317), lower perceived stress and improved performance under stressful conditions (17; 283; 318), again suggesting the importance of the cortisol/DHEA ratio (7;

269; 319). On the other hand, concerning attention, steroid sulfatase inhibition in mice (reducing the conversion of DHEAS into DHEA), was shown to impair accuracy under attention demanding conditions (320), and subjects with steroid sulfatase deficiency had higher rates of attention deficit hyperactivity disorder, with predominant inattentive symptoms (55; 321; 322). Nevertheless, methylphenidate administered to boys with attention deficit hyperactivity disorder has been shown to increase serum levels of both DHEAS and DHEA and simultaneously produce marked clinical improvement (323). Therefore, a relation between higher DHEAS-to-DHEA ratio or reduced DHEA and reduced attention was suggested, while the previous studies concerning memory suggested a relation between higher DHEAS levels and enhanced memory raising the hypothesis to test an eventual relationship between higher DHEAS-to-DHEA ratio and enhanced memory.

Concerning the potential effects of treatment, DHEAS administration to mice, enhanced memory retention, meliorated the effects of several memory-blocking agents (309) and improved working memory (324). Interestingly, in mice, DHEA and DHEAS administration demonstrated a dose-dependent inverted U-shaped effect on memory, with the lowest doses of DHEA or DHEAS showing no effect on memory, intermediate doses improving memory retention and the highest doses showing no effect at all (325). Nevertheless, in humans there is no evidence of a beneficial effect of DHEA supplementation on cognitive performance in healthy middle-age or elderly subjects (295; 326; 327). In fact, DHEA administration to human subjects showed inconclusive results on memory and attention (328; 329; 319; 330; 331). There is still the hypothesis that DHEA supplementation may eventually protect stress induced impairments in memory. In that concern, in one study of elderly subjects, DHEA supplementation impaired the recall of material learned before the stress but had an enhanced effect on attention after stress (327). These results are however, difficult to interpret and do not support a direct anti-glucocorticoid effect of DHEA on hippocampal mediated memory functions (327). It is also important to note that high quality studies using DHEA treatment for more than one year and involving a large number of participants, that could eventually detect long term cognitive effects of DHEA in humans, are essentially missing (329; 332).

Other authors tested the effects of DHEA administration to patients with Addison's disease. In that condition, results are not consensual. Some studies found positive results with improvements in overall well-being (333), mood, anxiety, depression, obsessive-compulsive traits, hostility and exhaustion (334), or improvements in self-esteem, mood and fatigue, but not in cognitive function (335). Those positive results were found only after three to four months' treatment (334; 335). DHEA treatment for six months to patients with hypopituitarism also resulted in improved psychological well-being compared to placebo (336). Nevertheless, another study was even longer (9 months) but did not find any positive results of DHEA administration to patients with Addison's disease (337). Interestingly, this study however used 25mg DHEA whereas the previously mentioned studies pertaining to Addison's disease and hypopituitarism used 50mg DHEA daily.

**Personality**

Personality trait characteristics of each individual develop in childhood and remain relatively stable over time (338). The same occurs with the HPA axis phenotype, concerning both baseline levels and reactivity patterns (339), and some relations between personality traits and HPA axis phenotype have been described in what concerns the ACTH and cortisol reactivity (339; 340; 341; 342; 343; 344; 345; 346; 347; 348; 349). In general, positive psychological traits were related to reduced HPA axis reactivity to an acute stress (350). Subtlety to profound changes may however occur in personality according to individual experiences (338) and, the HPA axis may show endocrine plasticity according to those experiences. For instance, neonatal stress or repeated stress could change this axis activity in the long term (351; 352). However, the relation between personality trait and DHEA or DHEAS responses to stress are not known.

Over a period of months to a few years, DHEAS levels during adulthood are also relatively stable in the same individual while showing large interindividual differences (353; 354). Also, DHEA and DHEAS are believed to influence cortical organization. Yet, the relationship between DHEA or DHEAS and personality is mostly unknown. Low plasma

DHEA and DHEAS levels have been related to chronic stress states but the relation between DHEA or DHEAS and anxiety trait is not established. Nevertheless, individuals with low anxiety trait scores were shown to have an increase in DHEAS levels after a psychological stress (without a concomitant increase in cortisol levels), whereas high anxiety trait individuals showed mostly an increase in cortisol levels (282).

One study found lower DHEAS levels in individuals with higher Type A behavior scores (355). Of note, Type A personality individuals have hypothalamic-pituitary-adrenocortical axis and sympathetic nervous system hyperactivity, and accordingly, they have higher cortisol and catecholamine levels, which might be the mediators of the higher cardiovascular risk, related to Type A personality (355). Interestingly, while chronic stress reduces DHEAS levels (274), a stress reducing program was also shown to increase DHEAS levels (356).

Another study also suggested that DHEAS might contribute to greater sensation-seeking and monotony-avoidant traits. In that study, individuals with higher DHEAS levels had higher scores in impulsivity and sensation-seeking-related personality scales, suggesting more disinhibited behavior in the social sphere, increased interest in sports and activities involving some danger with an increased need for change (357).

**Depression**

DHEA and DHEAS antidepressive effects may be partially mediated via GABAergic receptor antagonism (212) and sigma 1 receptors agonism. In fact, the GABAergic system can mediate depression (213), and other selective sigma 1 receptor agonists can cause antidepressant-like effects on rats (214). As noted, DHEAS has a much more potent excitatory action than DHEA (NMDA agonism and specially gabaminergic antagonism) and that may account for some differential effects of both forms of the hormone (1; 184; 196). But besides glutamate, sigma-1 and gabaminergic modulatory effects, DHEA and DHEAS effects in neurogenesis and neuroprotection, catecholamine, anti-glucocorticoid, anti-inflammatory and anti-oxidant effects could also contribute to or underlie the

relationship between these hormones and depression (121; 358; 359; 360; 361; 362; 363; 364).

Higher DHEAS concentrations and DHEA-to-cortisol ratios have been related to lower prevalence of depression, lower depression ratings and higher well-being scores (18; 20; 365; 366; 367; 368). Low serum DHEAS levels were also found in dysthymic patients (369). There are also several inconsistent and contradictory results in the literature, showing either reduced, unaltered (370; 371; 372; 373) or increased (374; 375) levels of DHEA or DHEAS in depressed patients. In fact, the evidence for a relation between DHEA levels alone and depression is less consistent than the evidence that suggest a relation between higher DHEAS concentrations or DHEA-to-cortisol ratios and lower depression ratings. Lower concentrations of both DHEAS and DHEA were found in peri-menopausal depressed women (376) and lower DHEA concentrations during pregnancy and postpartum period were found in relation to higher postpartum ratings of depression (377). But also, in a study assessing both DHEA and DHEAS, depressed patients had low DHEAS but normal DHEA concentrations (308). Yet in another study, patients with depression had increased DHEA plasma concentrations, which paralleled elevations in plasma cortisol (374). These results point towards the importance of DHEA-to-cortisol and DHEAS-to-DHEA balance.

In fact, several groups have found that DHEA-to-cortisol ratios, rather than concentrations of either hormone alone, discriminated more accurately depressed from non-depressed individuals, with lower DHEA-to-cortisol ratios seen in depression (84; 304; 365; 366; 378; 379; 380), in untreated depressed patients and in patients who remained depressed after several months (381; 382). It was hence suggested that elevated DHEA and DHEAS relative to cortisol levels, may counteract the negative effects of high cortisol on mood (360; 381). Moreover, controlled trials of DHEA therapy have reported significant and positive effects on mood (362; 383; 384; 385). Those mood improvements were related to increases in the circulating concentrations of DHEA and DHEAS and to increases in their ratios with cortisol, again suggesting anti-glucocorticoid effects of DHEA. Although controlled trials of DHEA administration in depression have consistently reported significant antidepressant effects, large-scale, long-term and

comparative studies (with established antidepressant drugs) are still missing. Such studies will be necessary before an eventual role for DHEA administration in depression management can be established.

**Anxiety**

DHEA and DHEAS gabaminergic antagonist effects could underline the relation between DHEA and DHEAS and anxiety disorders (185; 186; 386). Higher concentrations of DHEA or DHEAS-to-cortisol ratios were reported in patients with panic disorder (387; 388) than in healthy individuals. Nevertheless, no relation was found between social anxiety disorder (social phobia) and DHEA or DHEAS concentrations (389).

Several studies consistently found increased DHEA and DHEAS concentrations (193; 390; 391) as well as higher DHEA-to-cortisol and DHEAS-to-cortisol ratios (193; 390; 392; 393) in patients with post-traumatic stress disorder (PTSD). Nevertheless, these studies suggest that the increase in DHEA and DHEAS in PTSD and other stress conditions may be salutary. In that regard, in men with PTSD, higher DHEA and DHEAS levels were related to symptom improvement and better coping (390). In women with PTSD, increased DHEA response to ACTH was correlated with less PTSD symptoms (393). Also pointing to a beneficial effect of higher DHEA levels, subjects whose DHEA concentrations increased during treatment had decreased symptoms, whereas those whose DHEA levels decreased during treatment, did not improve (394). Apart from PTSD, in other stress conditions, higher DHEA, DHEAS, DHEA-to-cortisol, DHEAS-to-cortisol levels or DHEAS increases, were related to fewer dissociative symptoms and better performance under stress (270; 395; 396). Overall, higher DHEA and DHEAS levels are proposed to be adaptive, contributing to resilience, less deleterious effects of stress and better performance under stress (270; 318; 397; 398; 390).

Five patients with treatment-resistant PTSD and low DHEAS concentrations were treated with 7-keto-DHEA in an open label study (7-keto-DHEA is not converted to testosterone or estrogen and it has higher anti-glucocorticoid activity than DHEA). All five

patients showed improved symptoms within days (399). On the contrary, DHEA supplementation did not have beneficial results during stressful military training (400).

**Schizophrenia**

There are reports of decreased (401; 402; 403) and increased (404; 405; 406) concentrations of DHEA and DHEAS in patients with schizophrenia. Some studies also found increased levels of DHEA and low levels of DHEAS (407) whereas other studies showed opposite results, i.e. decreased DHEA and increased DHEAS levels (408). So, it is difficult to draw conclusions regarding DHEA and DHEA alterations in schizophrenia. One study measured post-mortem tissue DHEA concentrations and found higher DHEA concentrations in the posterior cingulate and parietal cortex of schizophrenics than in control subjects (409).

There are several types of schizophrenia, with different symptoms and this could account for the incongruent results described. In schizophrenic patients, correlations were specifically found between lower DHEA and DHEAS and negative symptoms (410), cognitive impairment (411; 412), depressive and hostility symptoms (280) and anxiety symptoms (406). Two studies suggest that administering DHEA to schizophrenic patients significantly decreased negative symptoms (independently of anxiety or depression) and did not change positive symptoms (413; 414). A few studies also suggest that DHEA treatment may have a beneficial effect in extrapyramidal motor symptoms and parkinsonian symptoms (414; 415) whereas another study did not confirm those beneficial results (416), although in this last study, the patients presented higher baseline DHEA concentrations than in the previous studies.

**Addiction**

Drug addiction is characterized by loss of control over drug consumption. Drug addicts present a chronic stress pattern of the HPA axis function (with increased CRH,

decreased ACTH, increased cortisol and impaired cortisol feedback loop) (417; 418; 419). After cocaine discontinuation, cortisol concentrations decreased while DHEAS levels increased (420) and cocaine abusers who relapse had lower DHEAS levels than the ones who did not relapse (421). Moreover, patients with higher DHEAS levels showed better treatment responses (422). In fact, DHEAS may play a role in morphine tolerance and dependence: DHEA administration to rats prevented the development of tolerance to the antinociceptive effect of morphine and prevented morphine dependence (423; 424).

DHEAS modulates dopamine, glutamate, sigma-1 and GABA-A receptors. Both these receptors may be relevant to drug abuse. Hence, DHEAS effects on addiction could be mediated through modulation of these receptors. Besides, DHEA is also a negative modulator of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, which may attenuate behavioral sensitization, drug self-administration and contribute to glutamate homeostasis (425; 426).

**Dementia**

DHEA and DHEAS effects in dementia could be mediated through their effects on sigma-1 receptors (209), cholinergic transmission (208; 427), and amyloid β protein (256). Again, divergent results exist concerning DHEA and DHEAS levels in dementia. Increased (428; 429; 430; 431; 432; 433; 434), unchanged (435; 436; 437) and decreased (438; 439; 440; 441) levels of DHEA and DHEAS were reported in serum/plasma and cerebrospinal fluid in patients with dementia (mostly patients with Alzheimer's disease). On the other hand, DHEA-to-cortisol and DHEAS-to-cortisol ratios more frequently discriminate demented patients from controls, than the levels of either hormone alone (442; 443; 444; 445; 446) and DHEAS-to-DHEA ratios may also be more relevant than either hormone alone (447). Lower DHEA-to-cortisol ratios were correlated with more cognitive impairment (441; 446; 448) and smaller hippocampal volume, as evaluated by magnetic resonance imaging (MRI) (448). Also, lower DHEAS-to-DHEA ratios were found in the cerebrospinal fluid of demented patients when compared to controls (447).

Hippocampal volume and hippocampal perfusion are correlated to peripheral DHEAS concentrations (449; 450). Hippocampal perfusion was also found to be directly related to DHEAS-to-cortisol ratios but this relation was dependent on DHEAS concentrations and not on cortisol concentrations alone (450). Thus, it is hypothesized that diminished DHEAS concentrations in the central nervous system are involved in the pathophysiology of Alzheimer's disease. DHEAS concentrations in the hypothalamus, striatum and cerebellum of Alzheimer's disease patients were also lower than in controls (451). On the contrary, increased DHEA concentrations (cerebrospinal fluid and tissue concentrations) were found in Alzheimer's patients, in the hippocampus, hypothalamus and frontal cortex (107) and these findings may point towards the importance of DHEAS-to-DHEA ratios. These findings agree with preclinical findings in which DHEAS antagonized the neurotoxic effect of DHEA in neuroblastoma cells (238) and inhibiting the conversion of DHEAS to DHEA resulted in increased acetylcholine concentrations in the hippocampus and improved memory in rats (209; 210; 452). These findings also point towards the importance of the sulfotransferase activity. Reduced activity of the sulfotransferase would lead to a reduced synthesis of DHEAS (453) and this could be involved in the pathophysiology of Alzheimer's disease.

It is also interesting to note that Alzheimer's disease and other neurodegenerative disorders present an increased oxidative stress (106). Pro-oxidant agents, such as amyloid-β peptide may induce an alternative pathway to DHEA synthesis (independent of CYP17 enzyme activity) in these patients (105; 106; 108). This could explain the higher DHEA levels found in Alzheimer's disease patients and the fact that treatment of Alzheimer disease patient's serum with $Fe^{2+}$ induced a much smaller increase in DHEA levels when compared to control patients (105; 106; 108). Also, DHEA variation after oxidation correlated with the patients' cognitive and mental status (108).

Some authors also hypothesize that the conversion of DHEA to 7α-hydroxy-DHEA is relevant in the pathophysiology of Alzheimer's disease. 7α-hydroxy-DHEA has more potent anti-glucocorticoid and neuroprotective effects than DHEA (454) and lower expression of the gene coding for the enzyme that converts DHEA to 7α-hydroxy-DHEA (455), as well as lower plasma 7α-hydroxy-DHEA concentrations were found in patients

with Alzheimer's disease (456). Also interesting, 7α-hydroxy-DHEA does not interfere with glucocorticoid binding to its receptor but was found to be a substrate for 11β-hydroxysteroid dehydrogenase type 1 with kinetic parameters favoring the conversion of 7α-hydroxy-DHEA to 7β-hydroxy-DHEA over the production of active glucocorticoids (457).

To the present date, there is not enough evidence to support the use of DHEA supplementation in dementia. Studies are scarce, with a small number of participants and inconsistent results. In one open-label study with DHEAS administration, three out of seven patients with multi-infarct dementia had an improvement in their daily activities and emotional disturbances, and in two of the four patients the improvement was reflected in the electroencephalogram (EEG) (428). In another randomized study, 58 patients with Alzheimer's disease were treated with DHEA (50 mg twice daily) or placebo. After three to six months' treatment, there was only a trend towards DHEA superiority on cognitive ratings (458). On the other hand, a study of 27 women with mild to moderate cognitive impairment (Mini-Mental State Examination, MMSE) showed positive results of DHEA treatment (25 mg once daily during six months). In this case, DHEA administration increased cognitive scores and maintained basic activities of daily living score, while cognition and basic activities of daily living deteriorated in the control group. More precisely, among the cognitive domains, DHEA treatment improved verbal fluency (459).

# Electrophysiological and neuroimaging correlates

## Auditory distraction and visual event-related potentials (ERPs) correlates of working memory and emotion

Auditory ERPs are characterized by brainstem potentials, followed by middle latency potentials and then cortical potentials. Brainstem potentials occur within a short latency of 1 to 10 ms and consist of several positive waves recorded at the vertex. These potentials are elicited by the presentation of external sensory stimuli, independent of subjects' collaboration and correspond to surface measurements of the sequential activity of the auditory nerve, the cochlear nuclei, the superior olives, the lateral lemniscus, the inferior colliculus and the medial geniculate nucleus (460; 461; 462). Middle latency potentials appear between 10 and 50 ms after the stimulus onset (463) and probably correspond to the neural activity of the thalamic nuclei and initial stages of the temporal region of the auditory cortex activity. Finally, cortical auditory potentials occur from about 60 to 300 ms after the beginning of the stimulus (see figure 6). Typically, these potentials initially include N1 and P2 components, which are sensorial or perceptive (464; 465), corresponding to the activity in the primary and associative areas of the temporal and parietal cortex and reflecting the analysis of the physical characteristics of the stimulus. When individuals are performing cognitive tasks involving the allocation of attention resources, there are negative potentials superimposed to N1 and P2. These negative potentials are designated attention-related negativity or processing negativity (466; 467). The P3 component (P300) is a cognitive, late, positive potential, occurring between 250 and 1000 ms after the stimulus (465; 464).

The P300 family of components reflects the cognitive updating of information, activation of working memory, the process of categorization of comparing with models stored in the memory and the contextual updating of information (468; 469; 470). These cognitive potentials are dependent of the individual experience and may occur even without stimulus (471). The contextual updating or memory recovery can be an automated process or a controlled process (465). Whereas the automated processes occur without the subject's attention, the controlled processes are voluntary and elicit

much more increased P300 amplitudes than automated processes (472). The P300 latencies depend on the process of stimulus classification. It can be correlated to reaction times (473) or not (474), because the reaction times may depend on the selection of the motor response and the time to carry it out (475).

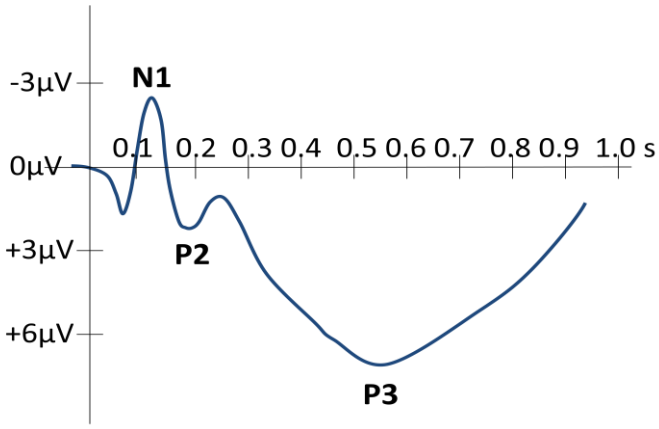


Figure 6: Typical cortical components elicited to an auditory stimulus. s – seconds after stimulus presentation.

Auditory distraction event-related potentials (ERPs) characteristically present an auditory N1/mismatch negativity (N1/MMN) enhancement, reflecting the brain response to a detectable difference in a repetitive acoustic stimulus that leads to attention capture, being elicited even if the auditory stimulus is irrelevant for the task (476; 477; 478). It is followed by a novelty-P3 (nov-P3) reflecting the effective orientation of attention (477; 479; 480). There is an early (P3a) and a late (P3b) phase of this component. The P3a is prominent on the frontal area of the brain in response to unexpected novelty which suggests an orientation reflex and the P3b is prominent in the parietal areas of the brain in response to stimuli that are of unlikely probability of being presented and thus, require reiteration of the categorization process (481). The P3, particularly the P3b, is sensitive to attentional (477; 482) and emotional context manipulations (483). Subsequently, the re-orienting negativity (RON) reflects the re-orientation of attention back to the task (484; 485; 486; 487).

In visual event-related potentials (see figure 7), the potentials derived from subcortical structures (superior colliculus, lateral geniculate nucleus of the thalamus) are observed only to a limited extent. Around 40 ms a small amplitude negative polarity peak can be observed reflecting the stage of subcortical analysis. A second peak around 80 ms (also designated first component, C1 or PN/80), of positive or negative polarity reflects the processing in the primary visual area. It is followed by the P1 sensory component, around 100 to 140 ms after the stimulus, and is believed to reflect the activity of the extrastriate visual cortex (488; 489; 490; 491; 492) and fusiform gyrus (493). C1 and P1 amplitude and latency can be modulated by the stimulus frequency, quadrant of stimulation, luminance and contrast (494; 495; 496). P1 shows hemispheric asymmetry and its amplitude is modulated by attention and expectancy (497; 498).

After P1, a negative sensory N1 and other negative deflections (N2) follow. There are several visual N1 subcomponents. The earliest subcomponent peaks around 100 to 150 ms at anterior electrode sites. There are at least two posterior N1 components that typically peak 150 to 300 ms after stimulus presentation, one originating from parietal cortex and the other from lateral occipital cortex. They are strongly affected by attention (497; 498; 499; 500; 501) and were designated as *selection negativity* or *processing negativity* (467; 502). In particular, faces elicit a particular potential between 150 and 200 ms, the vertex positive potential (VPP) (503) and a more negative potential at lateral occipital electrode sites (the N170, as it peaks around 170 ms), especially over the right hemisphere (504; 505), when compared to non-face stimuli. Although not fully ascertained, it is hypothesized that the VPP and N170 may be the opposite sides of the same dipole (505; 506).

The cognitive components related to visual target processing, include the N2b and P300. The N2b is a negative deflection originated by the task-relevant stimulus. Then, the P300 (visP300), starting around 300 ms after the visual stimulus, reflects the conscious processing of the visual stimulus (472; 507; 508) and is sensitive to attention allocation, cognitive and motivational factors (472; 509). The general characteristics mentioned above regarding the P300 family, apply to visual P300. Of note, increased attention allocation elicits increased P1, N1, N2 and P300 amplitudes, whereas irrelevant visual stimuli elicit deflections of reduced amplitude.

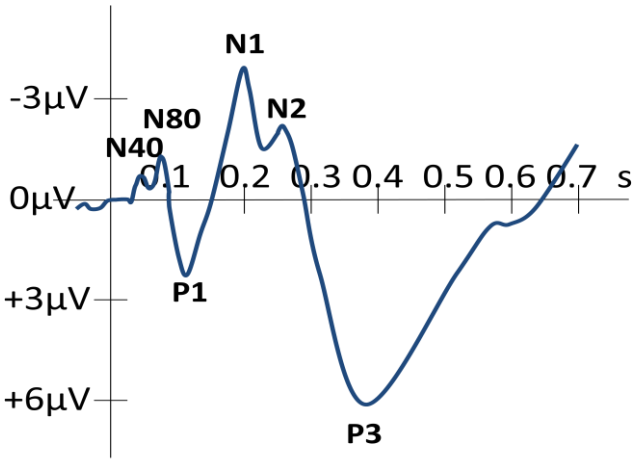


Figure 7: Typical event related potential components elicited to a visual stimulus. s – seconds after stimulus presentation.

Attention is a limited resource (510; 511; 512). There are voluntary or endogenous (top-down) and involuntary or exogenous (bottom-up) mechanisms of control of attention. Exogenous mechanisms are driven by the stimulus. They play a role for instance, when a salient stimuli irrelevant to the task captures attention (513). On the other hand, voluntary mechanisms modulate brain responses related to target stimuli (514; 515). But there are interactions between these endogenous and exogenous mechanisms of attention control. Involuntary attention to exogenous stimuli disrupt responses to target stimuli in a bottom-up way (477; 479; 509; 516) while automatic/exogenous processes can also be modulated in a top-down way (517; 518).

In particular, working memory functions based on prefrontal cortex may provide top-down signals to other brain structures (519; 520; 521) and modulate involuntary attention processing at primary sensory areas of the cortex (for example, reducing early responses to a distractor) (514; 522; 523). Another factor that may modulate involuntary attention in a top-down way is the emotional context. Several studies suggest that ventral prefrontal areas play a role on emotional processing (509; 524; 525) and in the top-down regulation of attention (526; 527).

Working memory load processing is expected to be more difficult than no working memory load processing, leading to worse performance and a reduced P300. This decrease in P300 is thought to reflect less processing of the task-relevant stimuli (472; 507; 508). As noted, working memory load can also modulate involuntary auditory distraction. In several studies, working memory load resulted in either increased (528; 529; 530; 531; 532) or decreased auditory distraction (508; 533). During no working memory load processing, control mechanisms reduce interferences from distractors, whereas higher cognitive load processing, namely working memory load processing, may override that capacity (528).

Emotional stimuli (in particular of threatening, fearful or anger content) elicit stronger and faster attention capture than non-emotional ones (509; 534; 535; 536; 537; 538; 539) and consequently interfere with the processing of concomitant stimuli, either within the same sensory modality (534; 540) or across sensory modalities. Accordingly, task-irrelevant emotional stimuli deteriorate performance (509). Nevertheless, the visual P300 is enhanced by the emotive content of images, as subjects remain engaged in the emotional content processing at the expense of processing the relevant aspects of the task (509). Emotional faces and International Affective Picture System (IAPS) images have been reported to elicit similar hemodynamic responses in emotion-sensitive areas, such as the amygdala, ventromedial prefrontal and visual cortices (541). Nevertheless, in studies using faces, some authors have found visual P300 enhancement by fearful faces (542), while others reported no changes in P300 amplitude (543). The type of emotional content of the stimuli is also relevant. Carretié *et al.* (537) found that the N2 was significantly elicited only by positive or non-emotional stimuli and not by negative stimuli. Nevertheless, contrary to what might be initially expected, a negative emotional context may enhance involuntary auditory distraction (early and late novelty-P3) processing (483; 509; 544). This suggests that acoustic novelty processing is enhanced under a potentially harming environment (544). On the contrary, auditory distraction (involuntary attention to novel auditory stimuli) deteriorates performance under an emotional context (477; 479; 509; 516).

**Neural networks of memory and emotions**

The **memory** processes involve information encoding, storing and retrieval. Encoding includes receiving and processing the information, implying that the information is transformed into physical and chemical stimuli. Then the information is stored into short term (working memory) or long term memory. Retrieval refers to the process by which stored information is recalled, coming back to consciousness in response to some trigger point.

Sensory memory lasts less than ten seconds after the sensory stimulus is delivered. It is an automatic (not cognitive) process (545) and the decay of this memory information can be prevented by rehearsal (545). Short-term memory allows recall for several seconds to about one minute. This type of memory is limited to about 3 to 5 items (546) and may be increased by chunking (grouping) items, a concept that was initially purposed by Miller in 1956 (547). Although this author proposed that people can remember about seven chunks in short term memory, it is now believed that the correct number is four (546).

In 1974, Baddeley and Hitch proposed a three-component model of working memory: the central executive, the phonological loop and the visuo-spatial sketchpad (548). Latter, Baddeley introduced a fourth component into this model: the episodic buffer (548). In this model, the central executive is a supervisory system that channels information to and from the other three components; the phonological loop stores verbal content (by rehearsing sounds or words in a continuous loop) and the visuo-spatial sketchpad stores visuo-spatial data. The episodic buffer is dedicated to linking information across domains (like verbal, visual and spatial) and is attributed to have links to long-term memory. Working memory has limited capacity and provides temporary storage of information held in a multimodal code, which is capable of binding information from the subsidiary systems and from long-term memory, into a unitary episodic representation. Conscious awareness is assumed to be the principal mode of retrieval from the buffer. In this modified model, working memory is used both to process information and to allow the transient storage of information (549).

Contrary to short term memory, long term memory can store large quantities of information for a long period of time (sometimes the whole life). There are different kinds of long-term memory representation (549). Declarative (explicit) memory implies the conscious recall of information and it includes semantic, episodic and visual memory among others (549). Non-declarative memory (procedural, implicit) uses unconscious aspects of previous experiences and is involved in several motor skills. Accordingly, multiple memory systems exist, not just two kinds (550). Non-declarative memory includes skills and habits, simple forms of conditioning, emotional learning, priming and perceptual learning, as well as forms of behavioral plasticity such as habituation and sensitization (550). Non-declarative memories are shaped by past events and influence our behavior conferring each person dispositions, habits and preferences that are inaccessible to their conscious recollection (550).

The internal representation of memory includes the expression of memory at the conscious or behavioral level and the underlying physical and chemical changes at the neuronal level. Short-term memory is supported by transient patterns of neuronal communication whereas long-term memory is maintained by more stable and permanent changes in neural connections widely spread throughout the brain. Encoding of working memory involves spiking of individual neurons induced by sensory input, which persists after the sensory input extinguishes (551; 552). Memory consolidation, referring to the process by which a newly formed and unstable memory (working memory) is transformed into a stable long-term memory, involves underlying physical and chemical changes in the neural tissue, called memory engrams. Some authors propose that a specific pattern of connectivity of engram cells may be crucial for memory consolidation and that strengthened synapses in these cells critically contribute to the memory retrieval process (553). This internal representation of memory is also subject to reactivation and reconstruction. Upon their retrieval, consolidated long-term memories may enter a transient special state, in which they might become prone to change (reconsolidation) (554).

At the cellular level, pyramidal cells and interneurons within the cerebral cortex interact during working memory processes. Most interneurons use the inhibitory

neurotransmitter GABA as their neurotransmitter, whereas pyramidal cells use the excitatory amino acids and pyramidal–nonpyramidal interactions, which may be critical to the formation of memory fields. Dopamine innervation, which is also abundant in the prefrontal cortex, has inhibitory actions on prefrontal cortical neurons by interacting with those other neurotransmitter systems, notably with the excitatory neurotransmitter glutamate, the inhibitory neurotransmitter GABA, and the cholinergic neurotransmitters, thereby regulating working memory (555).

Long-term memory involves long-term potentiation (LTP) or spike-timing-dependent plasticity (STDP) with long term changes in synaptic transmission and neurons (552). Synaptic changes in long-term memory consolidation may involve protein synthesis in the medial temporal lobe (MTL), allowing MTL-independent memory processes to spread through a large portion of the brain that could act over months to years. In this process, consolidation is probably a reiterated process with repeated activation of the memories instead of a one-time process (556). As mentioned, after consolidation, long-term memories become labile and are again updated during retrieval, thus requiring another phase of protein synthesis to be maintained (557). Whenever a memory is reactivated this reconsolidation process is necessary for the memory to persist (557). Evidence comes from post-retrieval studies, in which the treatment with protein synthesis inhibitors during that period, lead to amnestic states (554; 556; 557). Long-term memory formation and maintenance may also involve DNA methylation (558). DNA methylation is associated with transcriptional silencing, it is involved in lifelong molecular information storage during development and it was shown to be dynamically regulated in the adult nervous system (558).

Short-term memory depends on regions of the temporal lobe (in particular the hippocampus), the frontal lobe (especially dorsolateral prefrontal cortex) and the parietal lobe. Consolidation depends on several structures of the medial temporal lobe and diencephalic midline region and lesions within those structures impair memory causing amnesia (550). These medial temporal lobe structures include the hippocampus (dentate gyrus, CA3, CA1 region and subicular complex) and the adjacent entorhinal, perirhinal, and parahippocampal cortices, which make up much of the parahippocampal gyrus; and

midline diencephalic regions include the dorsomedial nucleus of the thalamus and the mammillary bodies of the hypothalamus (550).

The hippocampus is essential for learning new information, meaning the consolidation of information from short-term to long-term memory. It does not seem to store information itself, but without the hippocampus, new memories are unable to be stored into long-term memory (559) and attention would be reduced. The hippocampus may be involved in changing neural connections during the months after the initial learning. It is also involved in memory retrieval processes (560). The hippocampus receives information from different parts of the cortex (secondary and tertiary sensory areas that have already processed the information) and sends information, to different parts of the brain. Hippocampal lesions damage memory storage and cause memory loss, namely loss of memory for the events that happened shortly before the hippocampal lesion.

A volume loss in the hippocampus of ~40% or above (for instance after an anoxic event) impairs memory and this reduction in hippocampal volume (estimated by MRI), may indicate a nearly complete loss of hippocampal neurons (550). Moreover, within the hippocampus, specific CA1 lesions also confer important memory impairment (550). These CA1 field lesions, importantly disrupt hippocampal function, as information processing, that begins at the dentate gyrus and ends in the subiculum and entorhinal cortex must pass through this region of the hippocampus (550). The CA3a,b subregion of the hippocampus plays an important role in the encoding of new spatial information within short-term memory with a duration of seconds to minutes (561).

The PFC and the hippocampus interact during working memory, particularly with increased task demands (562). That hippocampus-PFC pathway involves a monosynaptic projection between these two structures and multisynaptic connections (involving the nucleus reuniens, nucleus accumbens, basolateral amygdala and ventral tegmental area) (562). Pyramidal and local GABAergic neurons in the medial PFC are directly targeted by hippocampal and thalamic glutamatergic afferents (562). Those afferents cause excitatory and/or inhibitory postsynaptic potentials in neocortical pyramidal neurons. The hippocampus-PFC pathway has synaptic plasticity, as repetitive stimulation in the ventral

subiculum or CA1 region of the hippocampus induces LTP or long-term depression in the PFC (562). The hippocampus inputs to prelimbic/infralimbic PFC may be essential for spatial information processing. Of note, the hippocampus and PFC are required for spatial learning, and the PFC is more important in primates than in rodents (562).

Pathologies like posttraumatic stress disorder, depression, autism and Alzheimer's disease are believed to share disruptions in the hippocampus-PFC functional connectivity (562; 563). Glucocorticoid and mineralocorticoid receptors are heavily expressed in the hippocampus, medial PFC and amygdala. Stress also modulates the hippocampus-medial PFC interactions during working memory: increased corticosteroids after stress cause a shift of memory retrieval from the dorsal hippocampus (non stress condition) to the medial PFC (stress condition) (562). Depressive patients show reduced hippocampus-PFC connectivity and reduced plasticity in the hippocampus-PFC pathway (562). In sum, the hippocampus-PFC connectivity is important in cognitive processes like working memory, memory consolidation and emotion regulation and stress may modulate that connectivity (562; 563).

Working memory encompasses both processing and storage functions and there may be multiple working memory domains, each located in a different anatomical subdivision of the prefrontal cortex of both human and nonhuman primates, each having its own specialized processing and content specific storage mechanism. As mentioned, long-term memory records also involve multiple regions and each neocortical region operates within a very specific domain, meaning that each region stores only specific features of an experience (550). For instance, neurons in visual area store the visual memory of a multisensory experience while neurons in auditory areas store the auditory memory of that experience (550). Then, remembering involves the coordinated reactivation of those distributed neocortical regions (550; 564). The same cortical areas are consistently activated in humans during immediate experiences (working memory) or when the person accesses information from long-term storage in the same domain (555). For newly formed memories, the reactivation process depends on the hippocampus and related structures, but for the memories that are fully consolidated, the reactivation process can occur independently in the neocortex (550). Whereas the medial temporal

lobe structures encode and consolidate broad type of memories, each neocortical region stores specific features of an experience. Therefore, a lesion in a specific region of the neocortex simultaneously causes an anterograde and retrograde memory impairment in the respective specific domain (for instance achromatopsia, amusia or prosopagnosia-impaired facial recognition) (550).

Medial temporal lobe structures are thought to be important for declarative memory, specifically for the formation of memory and maintenance of that type of memory for a period of time after learning (550). That consolidation process can take years (550). It is not certain whether these structures are necessary for other domains of memory like working memory or perceptual memory (550). Medial temporal lobe structures are not the ultimate storage sites for acquired memories. Memories that initially require the integrity of medial temporal lobe structures are reorganized as time passes after learning and gradually become independent of these structures (550). This was evidenced by findings in a patient with bilateral medial temporal lobe resection, previously carried out due to epilepsy (550). He retained memory for events that occurred more than three years before the surgery. The period of three years retrograde amnesia provided an indication of how long that process takes (550).

Patients with medial temporal or diencephalic lesions both exhibit memory deficits, and some authors hypothesize it might be because diencephalic nuclei and tracts (the medial dorsal nucleus and the adjacent internal medullary lamina; the mammillothalamic tract and its target, the anterior thalamic nuclei) are anatomically related to the medial temporal lobe (550). The perirhinal cortex originates projections to the medial dorsal nucleus that enter through the internal medullary lamina, while the hippocampal formation projects both to the anterior nuclei and mammillary nuclei (550). One cause of diencephalic amnesia is alcohol related Korsakoff's syndrome (550).

Recognition memory involves recollection and familiarity. Familiarity means that a component of an experience was presented, but the subject may have no other additional information about that component (550; 565). Some authors propose that the hippocampus and the perirhinal cortex contribute to recollection and familiarity (550; 566) whereas others propose that the hippocampus selectively support recollection and

that perirhinal cortex selectively supports familiarity (550; 567). There is an age-related cognitive decline in operations dependent on the hippocampus and prefrontal cortical circuits (568). Nevertheless, that age-related memory decline is different in normal aging and in Alzheimer patients. One of Alzheimer's Disease characteristics is loss of episodic memory and it was suggested that damage of the connections between the entorhinal cortex and hippocampus could play an important role in the memory impairment of Alzheimer's Disease (569; 570). Specifically, the CA1 region of the hippocampus was suggested to be the minimal region that is required for acquisition of episodic memory and some pathways were proposed that may connect the CA1 region of the hippocampus to the entorhinal cortex, namely through the dentate gyrus (571). Notably, neurogenesis in the dentate gyrus was also suggested to be involved in the formation of new memories in adults (570; 571).

Damage to the entorhinal cortex and hippocampal region in Alzheimer Disease is associated with the appearance of senile plaques and neurofibrillary tangles. Nevertheless, the relation between the appearance of those plaques and memory decline, is not clear, as some subjects have plaques but no cognitive impairment and the plaques could eventually reflect a protective, possibly antioxidant response to the primary pathogenesis (570). To explain that discrepancy, some authors proposed that the maintenance of an alternative neural circuitry could eventually prevent cognitive decline in some subjects with plaques and that the proportion of Aβ or tau protein molecules present in different areas of the brain could be important for the maintenance of cognitive functions (570).

Stress involves the activation of the sympathetic nervous system and the HPA axis. The sympathetic nervous system results in rapid release (essentially immediate) of epinephrine and norepinephrine from the adrenal medulla while the HPA axis results in a somehow slower (within minutes) release of corticosteroids from the adrenal cortex (572; 573). Catecholamines and corticosteroids influence the hippocampus, prefrontal cortex (PFC), and amygdala, thereby influencing memory. The hippocampus and the PFC (which is responsible for working memory and higher-order cognitive function), both have high density corticosteroid receptors, making them susceptible to the effects of stress (573).

Glucocorticoids bind to mineralocorticoid and glucocorticoid receptors (574). They bind mineralocorticoid receptors with 6-10 times higher affinity than glucocorticoid receptors (574). The result is that in humans, during the afternoon, glucocorticoids occupy more than 90% of mineralocorticoid receptor, but only 10% of glucocorticoid receptors (574). Yet, during stress or the morning peak of glucocorticoid secretion, mineralocorticoid receptors are saturated and 67-74% of glucocorticoid receptors are also occupied (574). This is also relevant, due to the distribution of these two types of receptors in the brain: mineralocorticoid receptors are present in the limbic system (hippocampus, parahippocampal gyrus, entorhinal and insular cortices) and glucocorticoid receptors are present in subcortical (paraventricular nucleus and other hypothalamic nuclei, the hippocampus and parahippocampal gyrus) and cortical structures, with a preferential distribution in the prefrontal cortex (574).

There is a U-shaped relationship between glucocorticoid levels and hippocampal plasticity and learning. Moderate levels of glucocorticoids are believed to enhance these parameters whereas high glucocorticoid levels, as the ones following stress, may impair hippocampal plasticity and memory storage (575; 576). Excessive stress or long term rises in glucocorticoids and catecholamines were shown to impair encoding processes in the hippocampus and memory recall (577; 578). Glucocorticoid excess result in excessive calcium influx and negative effects on cellular function, which may be both rapid non-genomic effects and slow gene-dependent effects (573).

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic efficacy that results from high-frequency stimulation (HFS) of afferent fibers and is believed to underlie memory formation (573; 579). Memory formation involves the strengthening of neural connections and a lasting pattern of altered synaptic weights (573). LTP-inducing events in close proximity may compete for memory formation but the brain, having limited resources will select the more important information (573). Stress induced LTP, identical to that necessary for memory formation, allows the formation of the memory related to the stress experience (573). In fact, in the hippocampus, stress and LTP increase gene induction, increase NMDA and AMPA receptor activity, increase levels of neurotrophins (BDNF) and increase glutamate and intracellular calcium levels (573).

Stress may then impair subsequent LTP induction and learning, but the memory for the stressful event remains intact (573; 580). As mentioned, NMDA receptor antagonists, impair LTP induction and also prevent stress effects on hippocampus-dependent learning and LTP (581).

Glucocorticoids rapidly raise hippocampal CA1 neurons excitability via nongenomic mineralocorticoid receptors, probably in pre- and postsynaptic membranes. Hours later, gene-mediated effects result in enhanced calcium influx and impaired LTP, which slowly normalize hippocampal activity and preserve stress induced information. Noradrenaline also interact with glucocorticoids in the hippocampus for these effects (582). Therefore, the effects of stress on memory formation are time dependent. Stress facilitates hippocampal memory function during the first minutes after stress onset. In that period there is stress induced glutamate release and glutamatergic receptors are over-stimulated, leading to a posterior refractory period, caused by glutamatergic receptors desensitization and glucocorticoids gene-dependent activity, and during which LTP induction is less probable (573). This may be adaptive, as it allows the brain to form a solid memory of the stress related information without the interference of other cognitive processes (573). Accordingly, stress will enhance memory when a learning event occurs in the same context as the stress event, and this results from shared neural circuits simultaneously forming memories for the learning event and stress event.

On the contrary, if a learning event occurs outside the context of a stress event, or if the events are temporally separated, encoding for this unrelated information would be impaired as the neural circuits necessary for memory formation being previously saturated would compromise the hippocampus into a refractory state (573; 579). This will simultaneously protect the hippocampus from glutamate excess neurotoxicity (573). Stress effect on the retrieval of previously learned information may also result from the interference of the formation of the stress memory with retrieval of another memory (573). In the same way, LTP may produce retrograde amnesia for previously learned information (583).

In 1884, **emotions** were defined by William James as subjective experiences. He proposed that stimuli trigger activity in the autonomic nervous system, and that activity

would produce an emotional experience in the brain. In his theory, the physiological response was the emotion (584). Carl Lange proposed a similar theory and argued that "we feel sad because we cry, angry because we strike, afraid because we tremble, and neither we cry, strike, nor tremble because we are sorry, angry, or fearful, as the case may be" (585; 586). This was the James-Lange theory of emotions. Currently, emotions are believed to involve several components, such as subjective experiences, cognitive processes, expressive behaviors, psychophysiological changes and instrumental behaviors (586). The processing of complex emotional information in one sensory modality can strongly affect emotion processing of another modality during very early stages of neural processing, as well as self-reported emotions (587). The concept of multimodality, apply to emotional stimuli processing, such as audiovisual stimuli (587).

Paul Ekman found that basic emotions could be identified by facial expressions across different cultures. In the 1970s, he proposed a classification that included six groups of basic emotions: anger, disgust, fear, happiness, sadness and surprise (588). Besides facial expressions, emotions may be expressed by gaze and tears and autonomic responses such as pupil-dilation, eye blinks, blushing and sweating that can hardly be controlled or regulated by the sender (589). These autonomic expressions of arousal such as pupil dilation are less prone to cognitive control than facial expressions (589). Thus, facial muscle actions, although unconscious, are possible to control or regulate by 'top–down' cognitive processes (589). Instead, autonomic expressions of arousal are much harder to control and more driven in a bottom-up fashion (589).

Emotional processing is related to the limbic system. This was initially suggested by the work of Broca in 1878 (590), Papez in 1937 (591) and MacLean in 1952 (592). The limbic system includes the hypothalamus, cingulate cortex and hippocampus amongst other structures. A large amount of evidence subsequently confirmed that limbic structures are directly related to emotion, but non-limbic structures have also been found to play an important role in emotional processing. The following limbic structures are currently thought to be involved in emotion (593): amygdalae, thalamus, hypothalamus, hippocampus, fornix, mammillary bodies, olfactory bulbs and cingulate gyrus. Besides, the following non-limbic structures are also believed to be involved in emotion: basal ganglia,

prefrontal cortex, ventral striatum, insular cortex and cerebellum (594; 595; 596; 597; 598).

In particular, the amygdalae are involved in detecting, evaluating and learning emotionally important aspects of our surroundings, particularly those involving fear and other negative emotions (560; 599; 600); the thalamus is involved in relaying sensory and motor signals to the cerebral cortex; the hypothalamus synthesizes and releases neurotransmitters which can affect mood, reward and arousal (601); the hippocampus is mainly involved in memory and is implicated in memory retrieval that is used to evaluate affective stimuli (560); the cingulate gyrus (different parts of this structure have different functions) is involved in affect, visceromotor control, response selection, skeletomotor control, visuospatial processing and memory access. Of note, the anterior cingulate cortex (part of the cingulate gyrus) may be involved in conscious emotional awareness and in the start of motivated behavior (602). Besides, the anterior cingulate cortex is also thought to play a central role in attention (603) and demanding cognitive tasks (602).

Concerning non-limbic structures involved in emotion processing, the basal ganglia are thought to play a role in motivation (594); the orbitofrontal cortex may be involved in decision making taking into account the influence of emotion (595); the prefrontal cortex is believed to play a critical role in the regulation of emotion and behavior by anticipating the consequences of our actions as well as in maintaining emotions over time and organizing behavior towards specific goals (597); the nucleus accumbens is part of the ventral striatum, which is thought to be involved in the experience of goal-directed positive emotion, namely, individuals with addictions, experience increased activity in this area when they encounter the object of their addiction; insular cortex is thought to play a critical role in the bodily experience of emotion, as it is connected to other brain structures that regulate the body's autonomic functions; the cerebellum is involved in emotional regulation (598), it is hypothesized that it may be involved in the activation of redundant alternative limbic structures in response to fearful stimuli or that it is involved in the regulation of the neural response to the rewarding stimuli (604; 605; 606).

The amygdala is involved in emotional memory and emotions also have a memory enhancement effect (emotionally charged events are better remembered than non-

emotionally charged ones) (607; 608). Accordingly, patients with amygdala lesions don't have an emotional enhancement of memory (573). Whereas a stress inherent to the experience enhances memory (609; 610), variable and more complex results were found for stress extrinsic to the learning experience (573; 611). Considering extrinsic stress, it showed variable effects on encoding (612; 613), beneficial effects on consolidation (614) and deleterious effects on long-term memory retrieval (573). The amygdala also facilitates memory operations in other regions, including the hippocampus and prefrontal cortex and emotion–memory interactions occur at various stages of information processing, from the initial encoding and consolidation of memory, to their long-term retrieval (607).

Hormones and neurotransmitters are involved in emotions' modulation, namely cortisol, noradrenaline, dopamine, serotonin, oxytocin and GABA (573; 615). Mild and short lasting increases in glucocorticoids enhance short and long-term memory for both emotionally arousing and emotionally neutral information (220; 574). Stress effect at the amygdala level preferentially facilitates memory for arousing, potentially more important emotional information, at the cost of less memory for neutral, potentially less important information (573; 615). Short-term increases in the stress hormones cortisol and epinephrine affect the amygdala, enhancing the storage of information. Lesions of the amygdala and the stria terminalis block the effects of post-training administration of epinephrine and glucocorticoids on memory (616). This supports the notion that stress hormones affect the amygdala to enhance memory related to emotions (616). In rats, memory is enhanced by post-training intra-amygdala infusions of drugs that activate beta-adrenergic and glucocorticoid receptors as well as injections of GABAergic antagonists and opioid receptor antagonists (616). On the contrary, infusion of beta-adrenergic blockers into the amygdala blocked the memory-modulating effects of epinephrine, glucocorticoids and drugs affecting opiate and GABAergic systems (616). Also interesting is the fact that metyrapone, an 11β-hydroxylase inhibitor (therefore inhibiting cortisol synthesis), prior to training, also block the memory-enhancing effects of epinephrine, thus suggesting some interaction between cortisol and epinephrine (617). In women, differences in sexually dimorphic tasks along the menstrual cycle are small and difficult to replicate, but emotion-related changes are found in relation with

progesterone, with higher levels associated with increased amygdala reactivity and increased emotional memory (618).

The mesocorticolimbic system, consisting, at its core, of the ventral tegmental area, the nucleus accumbens, and medial prefrontal cortex, is involved in positively motivated behaviors and reinforcement learning (reward) (619). Dopamine neurons in the ventral tegmental area and their targets in the nucleus accumbens and the medial prefrontal cortex are believed to play a central role in this reward system (619). More recently, this system is also recognized to be involved in punishment and negative reinforcement and it is assumed that different subtypes of nucleus accumbens neurons (Dopamine D1-receptor vs Dopamine D2-receptor expressing neurons) might exert opposing influences over reward or punishment directed behavior (619). Besides dopamine input, the nucleus accumbens also receives glutamate input from regions including the prefrontal cortex, amygdala, and hippocampus. Also, nucleus accumbens GABAergic neurons (which account for the majority of the neurons in this region) might contribute to the encoding of rewarding and aversive states (620). Oxytocin appears to modulate the dopaminergic activity within the mesocorticolimbic dopamine system, being important not only for reward and motivated behavior but also for the expression of affiliative behaviors (621). The endocannabinoid system modulates neural activity and synaptic functions in brain regions involving rewards (ventral tegmental area, striatum, amygdala, and prefrontal cortex) (622).

Rewarding behaviors such as sexual activity, eating, nursing, parenting, social interactions and play activity are strongly conserved in evolution and they are essential for development and survival (622). Dysfunction of the mesocorticolimbic reward system is involved in addiction, schizophrenia, depression and other mood disorders (619). In particular, the dopaminergic system is involved in neurologic and psychiatric pathologies showing impaired emotional processes like Parkinson disease, schizophrenia, autism, Attention Deficit Hyperactivity Disorder, Huntington disease and frontal lobe lesions (623). Additionally, the administration of dopaminergic agonists/antagonists in those conditions, also modulate those emotional processes (623).

**Electrophysiological correlates of DHEA and DHEAS**

DHEA and DHEAS relations to memory processing at the electrophysiological level are little known as only a few studies in humans, focused on DHEA and DHEAS electrophysiological effects in what concerns memory. Also, DHEA and DHEAS relations to emotional processing at the electrophysiological level are not known. Wolf *et al.* (24) studied the effects of DHEA replacement on short term memory ERPs. The participants were old men and the authors reported an increase in P3 amplitude after DHEA replacement, reflecting an enhancement of information updating. Alhaj *et al.* (624) administered DHEA to young men and used low-resolution brain electromagnetic tomography (LORETA) imaging. In this study, DHEA treatment reduced cortisol levels, improved episodic memory recollection and mood and modified the ERPs associated with retrieval. Another study found that lower DHEA levels were correlated to longer P300 latencies (625) in women aged 40 to 69 years old and men aged 60 to 69 years old. In this study, the authors found no significant relation between DHEA levels and memory or attention scores. Studying sleep, Friess *et al*. (626) found that DHEA administration increased rapid eye movement sleep and electroencephalogram (EEG) power in the sigma frequency range, suggesting a role of DHEA in memory storage.

Studies in rats further suggest an effect of DHEAS on synaptic plasticity. A study with evoked field responses and single-unit activity in anesthetized rats suggested that DHEAS application suppresses hippocampal recurrent inhibition and synchronizes neuronal activity to theta rhythm (627). Of note, rat theta rhythms are likely involved in mechanisms of learning and memory (628; 629). They are very strong in rodent hippocampus and entorhinal cortex during learning and memory retrieval, and are believed to be vital to the induction of long-term potentiation, a potential cellular mechanism of learning and memory (630; 631). The entorhinal cortex is the rat's main interface between hippocampus and neocortex, playing an important role in memory formation and consolidation.

Besides, DHEAS was shown to reduce the threshold pulse number for long-term potentiation (LTP) in hippocampal CA1 pyramidal cells through the amplification of NMDA receptor signaling (201; 202). In contrast, DHEA had no facilitating effect on the induction

of LTP. Thus, it is suggested that chronically administered DHEAS (but not DHEA) plays a priming role in inducing a facilitated synaptic plasticity, probably via chronic activation of sigma1 receptor in rat hippocampal cells (201). Then, the authors evaluated the effects of DHEAS administration in rat ischemic forebrains. In this study, the repetitive administration of DHEAS after reperfusion prevented the impairment of LTP produced by ischemia and this effect was possibly mediated through sigma-1 activation (203). On the contrary, Diamond *et al*. (632) found an enhancement of primed burst but not of LTP after a single dose administration of DHEAS to rats. The enhanced primed burst was achieved after the administration of intermediate doses of DHEAS but not of the lower or highest dose of DHEAS tested. These results also suggest that non-repetitive administration of DHEAS could enhance memory by facilitating the induction of neural plasticity, although through this different mechanism.

Concerning glucocorticoids, whereas mild or short-lasting increases in cortisol due to stress may protect the body, promote adaptation and have beneficial effects on attention and memory, higher cortisol levels or long term increases are related to poorer executive functioning, poorer learning and memory and less cognitive flexibility (220; 633; 634; 635; 636; 637; 638). Working memory (WM) depends on prefrontal cortex activity, and this is modulated by glucocorticoids: prefrontal cortex-dependent working memory is enhanced by acute stress and inhibited by chronic stress (220; 639; 640; 641).

At the electrophysiological level, cortisol administration increased attention in healthy subjects, as indicated by increased amplitudes of the vertex components of the auditory evoked potentials (642). Also, higher cortisol levels during acute stress were related to reduced auditory distraction as indexed by reduced MMN amplitudes (643). Regarding memory, higher cortisol levels were related to impaired working memory (decreased frontal theta activity) (644), modified anterior-posterior distribution of the memory-related electrophysiological responses (increased P600 amplitude in anterior leads and reduced amplitude over posterior leads; P600 is an electrophysiological index of recognition memory and hippocampal activity) (645) and modified laterality of episodic memory retrieval (500-1400 ms) (646). Cortisol also interferes with error detection processes: a single dose of cortisol administration (but not episodic administration)

increases error rates in a choice response task with associated quantitative changes in incorrect-response ERPs (647). Concerning emotional processing, hydrocortisone administration increased the processing of angry faces in highly anxious individuals as indicated by increased amplitudes of early (P150) and late (P3) event-related potentials (648).

**Neuroimaging correlates of DHEA, DHEAS and cortisol**

A vast number of neuroimaging studies were performed in the last decade using mostly functional Magnetic Resonance Imaging (fMRI) and Positron Emission Tomography (PET). These studies provided important information regarding the neurobiology and neurochemistry of memory, emotions and other aspects pertaining to the functioning of the human central nervous system. In contrast, only a few studies addressed the neural network correlates of DHEA and DHEAS.

Magri *et al.* (448) studied human participants and found that higher cortisol-to-DHEAS ratios were correlated with cognitive impairment and smaller hippocampal volume, as measured by magnetic resonance imaging (MRI). In the study by Alhaj *et al.* (624) in which DHEA was administered to young men and resulted in modified ERPs and improved episodic memory recollection, low-resolution brain electromagnetic tomography (LORETA) imaging also showed that DHEA treatment led to a trend towards an early differential activation of the anterior cingulate cortex (ACC). The authors concluded that those effects of DHEA appear to be via neuronal recruitment of the steroid sensitive ACC, which may be involved in pre-hippocampal memory processing.

DHEA and DHEAS effects on cerebral regions specifically involved in emotional processing including the amygdale, hippocampus, insula and anterior cingulated cortex have also been suggested (240; 649; 650). However, little research has explored the neural correlates of DHEA and DHEAS with respect to emotion and mood. Concerning DHEA relations to emotional neuroimaging, Sripada *et al.* (25) performed a functional MRI study to investigate the effect of DHEA administration on emotion regulation neurocircuits. The DHEA administration reduced the activity in the amygdala and

hippocampus, increased the connectivity between these two regions and enhanced the activity in the rostral anterior cingulated cortex during emotion processing. Moreover, the DHEA administration reduced the negative affect and the memory accuracy for emotional stimuli. Hence, those results suggest that DHEA may downregulate the negative emotions induced by aversive stimuli by reducing the activity in regions associated with the generation of negative emotions (amygdala and hippocampus) and enhance the activity in regions linked to regulatory processes. The authors did not exclude whether the effects of DHEA administration were mediated by other DHEA derivatives, namely DHEAS and androsterone. Of note, DHEA administration produced an increase in DHEA and DHEAS in serum and salivary levels, without a simultaneous change in cortisol levels. Sripada *et al.* (651) also studied the effects of DHEA in resting networks and found that DHEA administration to humans reduced the connectivity between amygdala and periamygdala and between amygdala and insula (in fMRI). In this study, reductions of amygdala to precuneus connectivity were associated with less self-reported negative affect. Therefore, DHEA effects on emotion processing neurocircuits differed from its effects on resting networks and so the authors purposed that DHEA may shift the balance between salience network and default network, a finding that could provide insight into the neurocircuitry of anxiety related psychopathology.

Adrenarche coincides with the emergence of the pro-social and neurobehavioral skills of middle childhood and may therefore represent a human-specific stage of development. DHEA appears to modulate the process of cortical maturation during middle childhood: DHEA levels were associated with increases in cortical thickness of the left dorsolateral prefrontal cortex, right temporoparietal junction, right premotor and right entorhinal cortex between the ages of 4-13 years, a period marked by the androgenic changes of adrenarche (652). DHEA and testosterone were also related to cortical thickness of the right cingulate cortex and occipital pole and this was most significant in prepubertal subjects (652).

Important brain remodeling pursued during adolescence, was shown by longitudinal studies using MRI (653; 654). It has been hypothesized that these changes may be related to increases in adrenal and gonadal hormones (655; 656; 657; 658) and

gonadal hormones are known to have organizational and activation effects on limbic structures and association cortex of animals (659). Concomitantly, social behavior, change dramatically during adolescence: there is increased self-consciousness, increase in complex and important relationships, including romantic and sexual relationships, and improvement in relation to understanding others (660; 661). Mentalizing, meaning the ability to recognize and interpret the feelings, intentions, beliefs and desires of others (662) is important for all social behaviors. Social emotions (like guilt or embarrassment) are emotions that require mentalizing about others and their reactions to one's actions. In contrast, basic emotions (like disgust or fear) do not require mentalizing. The network of brain regions recruited during mentalizing tasks comprises the dorsomedial prefrontal cortex (DMPFC), posterior superior temporal sulcus at the temporo-parietal junction and the anterior temporal cortex. The signal in the DMPFC during mentalizing tasks decreases with age across adolescence, while signal in temporal regions increases during the same period (663; 664; 665; 666).

Goddings *et al.* (667) compared brain regions activated during social emotion and a basic emotional condition. The authors used fMRI and measured testosterone, estradiol and DHEA. In this study, the contrast between social versus basic emotion resulted in activity within the social brain network, including dorsomedial prefrontal cortex (DMPFC), the posterior superior temporal sulcus, and the anterior temporal cortex in both hemispheres. Increased hormone levels (independent of age) were associated with higher left anterior temporal cortex activity during social emotion processing. More specifically, this relation was significant for testosterone and there was a trend for DHEA and estradiol. More advanced age (independent of hormone levels) was associated with lower DMPFC activity during social emotion processing (667). Klapwijk *et al*. (668) studied adolescent girls and found enhanced functional connectivity between the DMPFC and the left anterior temporal cortex during social relative to basic emotion processing independent of age. These authors also found that estradiol concentrations were associated with increased functional connectivity between the DMPFC and the right temporo-parietal junction during social related to basic emotion processing, independent of age but they did not find any relation between DHEA levels and the mentalizing network.

Concerning glucocorticoids' effects, as mentioned, mild or short-lasting increases in cortisol due to stress have beneficial effects on attention and memory, whereas higher cortisol levels or long term increases are related to poorer learning and memory and less cognitive flexibility (220; 633; 634; 635; 636; 637; 638). Increased cortisol levels during perception or encoding led to better memory performance and brain regions involved in the modulation of this process were medial temporal lobe regions such as amygdala and hippocampus, the anterior cingulate cortex and other parts of the prefrontal cortex (PFC) (669). Increased cortisol levels during the maintenance of items in working memory also increased the activity in prefrontal cortex and posterior parietal cortex (670).

Corticosteroids' slow effects appear to improve working memory performance and increase neuronal activity during working memory performance in the dorsolateral prefrontal cortex depending on working memory load. Thereby, up to a certain level, the slow actions of corticosteroids seem to facilitate adequate higher-order cognitive functioning, which may support recovery in the aftermath of stress exposure (671). On the contrary, in this study, no effects of corticosteroids' rapid actions were observed during working memory performance in the dorsolateral prefrontal cortex (671). Nevertheless, other studies observed rapid, non-genomic effects of glucocorticoids, namely at the hypothalamus, hippocampus, amygdala and prefrontal cortex (220). These effects may involve mineralocorticoid and glucocorticoid receptors and membrane-associated mechanisms, but are not directly induced by glucocorticoids (220). Instead, glucocorticoids play permissive or conditional effects, in facilitating or inhibiting the signaling of ion channels, receptors and neurotransmitters, thus modulating the threshold of neuronal activation (220).

Contrary to the effects of mild and short lasting increases in cortisol levels, very high or sustained increases in cortisol levels may be deleterious for memory processes. While prefrontal cortex-dependent working memory is enhanced by acute stress, it is inhibited by chronic stress (633; 639; 640; 641). Important stress accompanied by very high cortisol levels during retrieval led to impaired memory retrieval (669) and the administration of cortisol also delayed and impaired implicit learning performance (672). Moreover, higher cortisol levels were correlated to lower hippocampal volume formation

(673). It is hypothesized that glucocorticoids that are released during stress, damage neurons that are located in the hippocampal region of the brain. The CA1 neurons found in the hippocampus are destroyed due to glucocorticoids, decreasing the release of glucose and the reuptake of glutamate. This high level of extracellular glutamate allows calcium to enter NMDA receptors which in return kills neurons.

Finally, glucocorticoids may also modulate the brains emotional network. High cortisol levels during consolidation of emotional material involve increased amygdala and hippocampus activation (669). Nevertheless, acute increases in cortisol levels due to stress seem to reduce hippocampal activation to fearful faces (674), increasing the activity in the dorsal anterior cingulate cortex and Brodmann's area 8 (675) and reducing the amygdala-hippocampal connectivity (676) [on the contrary, as noted before, DHEA administration increased the connectivity between these two regions (651)]. Regarding amygdala responsivity, cortisol can rapidly desensitize amygdala responsivity to negative stimuli and this was related to an altered coupling of the amygdala with the medial prefrontal cortex. There is a posterior slow normalization of amygdala responsivity and amygdala to prefrontal cortex connectivity. These results reveal a temporarily fine-tuned mechanism that is critical for avoiding amygdala overshoot during stress and enabling adequate recovery thereafter (677).

Contrary to short time and small increases in cortisol levels, the effects of chronic increases in cortisol levels, have desensitizing and deleterious effects on brain emotional processes that could be related to depression and anxiety disorders (678). Repetitive glucocorticoid administration increased self-reported negative emotions (679). Depressed subjects showed decreased cortisol sensitivity and simultaneous subgenual hyperactivity while glucocorticoids administration to these subjects decreased subgenual cingulate activity evoked by sadness (680). Cushing's syndrome patients made more errors in categorizing facial expressions and had less activation in left anterior superior temporal gyrus, a region important in emotion processing. Thus they showed altered activation of brain structures relevant to emotion perception, processing and regulation, similar to the performance decrements and brain regions shown to be dysfunctional in major depressive disorder (681). Using Fluorodeoxyglucose (FDG)-positron emission

tomography (FDG-PET) to study anxious temperament monkeys, Shackman *et al*. (682) found that elevated activity in the lateral anterior hippocampus was selective to individuals with high levels of hypothalamic–pituitary–adrenal (HPA) axis activity. Increased distress and anxiety and high cortisol levels were also correlated to increased PFC activation in pregnant women (683). Cortisol effects in the activation of brain memory and emotional processes also showed some lateralization effects and differences according to gender (669; 684; 685).

In sum, higher baseline DHEA levels were related to higher hippocampal volume, increases in cortical thickness of the prefrontal cortex, temporoparietal junction, premotor and entorhinal cortex, cingulate cortex and occipital pole. During episodic memory recollection, DHEA administration was related to an early differential activation of the anterior cingulate cortex. Regarding emotional processing, DHEA administration was related to reduced activity in the amygdala and hippocampus, increased connectivity between these two regions and enhanced activity in the rostral anterior cingulated cortex whereas in the resting state DHEA administration reduced the connectivity between amygdala and periamygdala and between amygdala and insula. There was also a trend towards a relation between higher DHEA levels during adolescence and increased left anterior temporal cortex activity during social emotion processing.

On the other hand, cortisol modulates memory processes enhancing activity in the prefrontal cortex, amygdala, hippocampus, anterior cingulate cortex and posterior parietal cortex. Prefrontal cortex-dependent working memory is enhanced by small acute cortisol increases, but inhibited by chronic cortisol increases and higher cortisol levels are correlated to lower hippocampal volume. Cortisol increases amygdala, hippocampus and anterior cingulate cortex activation during the processing of emotional material but acute physiological increases in cortisol also reduce hippocampal activation to fearful stimuli, reduce the amygdala to hippocampal connectivity and amygdala to prefrontal cortex connectivity. Chronic increased cortisol levels were related to more errors in the categorization of emotional stimuli, less activation in left anterior superior temporal gyrus, increased activity in the lateral anterior hippocampus and altered prefrontal cortical function during processing of fearful stimuli.

**Overall**, research results suggest that DHEA and DHEAS might play a role in cortical organization, enhance memory and attention and protect against depression. Nevertheless, results are not consensual. Moreover, DHEA and DHEAS relations to personality are not established and their electrophysiological effects concerning attention, working memory and emotional processing are mostly unexplored. Neurostimulant and anti-cortisol effects of DHEA and DHEAS have been proposed to contribute to their central nervous system effects. Also, the balance between DHEAS and DHEA may influence those effects, but most studies did not address this parameter. Finally, DHEA and DHEAS increase in response to acute stress but it is not known whether working memory or emotional load influence DHEA and DHEAS levels.

# Aims and Hypothesis

The objective of this doctoral thesis was to explore behavioral and electrophysiological correlates DHEA and DHEAS. In particular, we aimed to explore relations to personality profile and cognitive and emotional processing at the performance and electrophysiological level. Additionally, we wanted to explore DHEA and DHEAS responses to stress and to the performance of cognitive and emotionally loaded tasks. In every case, we wanted to explore evidence of anti-cortisol effects of DHEA and DHEAS and effects of DHEAS-to-DHEA balance.

## Study I

Personality traits are individual behavioral characteristics which are established early in childhood and remain relatively stable over time (338). The same occurs with the HPA axis phenotype, meaning that each person has characteristic and relatively stable baseline levels and reactivity pattern (339). Stressful events experienced in the neonatal period or early years of life may have long-term effects on the HPA axis phenotype, personality, and upon the future development of psychosomatic diseases (351; 352; 574; 686). Also, personality has been related to hypothalamic-pituitary-adrenal axis phenotype (339; 343; 344; 345; 346; 347; 348; 349) in what concerns the ACTH and cortisol reactivity (340; 341; 342; 344) with positive psychological traits generally being related to reduced cortisol reactivity to an acute stress (350).

Previous evidence of DHEA and DHEAS effects on cortical organization (15), cortical maturation (16), synaptic transmission (3; 7; 120) and behavior (18; 20; 317; 365; 366) raise the hypothesis that DHEA and DHEAS may be related to personality profile. There is however, scarce evidence showing a relationship between DHEAS and personality (355; 357). There is also evidence suggesting that DHEA and DHEAS levels may increase in response to an acute psychological stress (8; 270; 271) and evidence suggesting anti-cortisol effects of DHEA and DHEAS (17; 687), but the relation between

pituitary-adrenal axis (HPA) phenotype in what concerns the DHEA and DHEAS response and personality has not been established.

The objective of this study was to explore the relation between DHEAS and both personality traits and pituitary-adrenal axis reactivity in humans. To address this objective, personality was evaluated with the Minnesota Multiphasic Personality Inventory (MMPI) (688) and the pituitary-adrenal axis reactivity was assessed with the CRH test. The main hypotheses were that higher DHEAS levels and/or DHEAS reactivity may be related to lower Type A personality and Neurotic Triad scores and to higher Deviant Behavior scores. DHEAS opposite to cortisol reactivity to CRH infusion is expected.

## Study II

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) have been suggested to have memory enhancement effects in humans (317; 451). A neuro-stimulatory action and an anti-cortisol mechanism of action may contribute to that relation (17; 283; 318). The aim of this study was to test whether DHEA and DHEAS levels are related to distraction and Working Memory at the electrophysiological level, as evidence for their neurophysiologic effects using Event-Related Brain Potentials (ERPs) is scarce (24; 624; 645) and to test whether these endocrine levels are modulated by Working Memory (WM) load. The specific *a priori* hypotheses were: 1) higher endogenous DHEA and DHEAS levels may prevent involuntary distraction and enhance cognitive performance; 2) DHEA and DHEAS putatively beneficial effects may be translated at the neurophysiological level; 3) DHEA and DHEAS effects may be largely antagonistic from those of high baseline cortisol; and 4) cognitive tasks using Working Memory load may be a stimulus for DHEA and DHEAS production.

To test these hypotheses, we measured the relation between DHEA, DHEAS and cortisol on one hand, and the cognitive performance and brain responses, using a well-established auditory-visual distraction paradigm (477; 479; 689) on the other hand. The

protocol included task irrelevant sounds, some of which were aimed to cause distraction and a visual task including working memory manipulation. In this paradigm, unexpected auditory novel sounds caused distraction and were expected to deteriorate performance and elicit a novelty-P3 enhancement (477; 479; 480). Typically, the task with working memory load is more difficult for the subjects, leading to worse performance and a reduced P300, reflecting less processing of the task-relevant visual stimulus (472; 507; 508).

## Study III

Higher DHEA and DHEAS levels have been related to enhanced working memory and attention (317; 451). However, the administration of DHEA returned inconclusive effects on working memory and attention (319; 328; 329; 330; 331) and the relation between both forms of the hormone may modulate their effects in the central nervous system (447). The aim of this study was to test whether DHEAS-to-DHEA ratio modulates working memory and involuntary attention during the performance of a working memory task at the electrophysiological level. Our hypotheses were that higher DHEAS-to-DHEA levels may enhance working memory and/or involuntary attention, and that these effects translate at the electrophysiological level.

To test these hypotheses, we measured baseline DHEAS and DHEA and brain responses, using a well-established auditory-visual distraction paradigm (477; 479; 689). The protocol included task irrelevant sounds, some of which were novel and aimed to cause involuntary attention and a visual task including working memory manipulation. Involuntary distraction by novel sounds elicits a novelty-P3 enhancement and was expected to deteriorate performance (477; 479; 480). The processing of the relevant visual stimulus elicits a P300 component. Working memory load tasks, being more difficult than discriminative tasks, led to worse performance, typically eliciting a reduced P300 and also reduced involuntary attention (472; 507; 508).

## Study IV

DHEA and DHEAS may have mood enhancement effects: higher DHEAS concentrations and DHEA/cortisol ratio have been related to lower depression scores (18; 20; 365; 366) and controlled trials of DHEA administration have reported significant antidepressant effects (364; 383; 384).

The aim of this study was to explore whether DHEA and DHEAS levels were related to involuntary attention and emotional stimuli processing at the performance and brain levels and, on the other hand, if an emotional challenge would alter DHEA and DHEAS levels. Furthermore, we wanted to examine the relation of DHEA and DHEAS with cortisol levels. The *a priori* hypotheses were: 1) higher endogenous DHEAS and DHEA levels as well as higher DHEA-to-cortisol and DHEAS-to-DHEA levels may protect from involuntary distraction and enhance brain processing and performance under a negative emotional context; 2) DHEAS and DHEA effects may be largely antagonistic from those of baseline cortisol; 3) in the short term, a negative emotional context might be a stimulus for DHEA and cortisol secretion.

To test these hypotheses, we used a visual task with a neutral or negative emotional context and unexpected auditory novel sounds aimed to cause distraction. In this paradigm, the negative emotional context was expected to elicit an increased attention capture when compared to non-emotional faces (534) and consequently deteriorate performance (483). Furthermore, auditory distraction by novel sounds was expected to elicit a novelty-P3 component in the electroencephalogram (EEG) and also deteriorate performance (477; 479; 509; 516). We recorded performance parameters, the EEG and took saliva samples in order to determine the hormonal levels before and after the task. We explored DHEA, DHEAS, cortisol, DHEA/cortisol ratio and DHEAS/DHEA ratio relations to distraction and implicit negative emotion at the performance and electrophysiological levels.

# Study I

## Plasma Dehydroepiandrosterone-Sulfate is Related to Personality and Stress Response

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## Abstract

Objectives: Dehydroepiandrosterone-sulphate (DHEAS) physiologic relevance remains controversial. However, several central nervous system and behavioural effects of DHEAS have been described. We explored the relation between DHEAS and both pituitary-adrenal axis reactivity and personality in human subjects. Design: We studied 120 consecutive patients assisted at the out patient endocrine department of a public central hospital before medical treatment. Personality was evaluated with the Minnesota Multiphasic Personality Inventory (MMPI) and the pituitary-adrenal axis reactivity was assessed with the CRH test. Results: Baseline DHEAS was inversely related to peak/basal cortisol (parcial r=–0.454, $p<0.05$) response to CRH infusion. DHEAS reactivity in the CRH test was directly related to the Deviant Behaviour triad (BD) (r=0.257, $p<0.05$) and type A personality (AP) (r=0.295, $p<0.05$). Basal ACTH was directly related to baseline DHEAS (r=0.366, $p<0.001$) and together with age and gender explained 34% of DHEAS variability. Conclusions: DHEAS may be a protective factor against an excessive cortisol response when people are under stress situations. Personality may be related to DHEAS reactivity.

**Key words:** DHEAS; cortisol; personality; stress; DHEAS reactivity; CRH test.

**Resumo**

Objetivos: Os efeitos fisiológicos da desidroepiandrosterona-sulfato (DHEAS) são ainda controversos. Contudo, têm sido descritos efeitos ao nível do sistema nervoso central e efeitos comportamentais. Explorámos a relação entre os níveis de DHEAS e a reatividade do eixo hipófise-suprarrenal e a personalidade. Participantes e Métodos: Estudámos 120 doentes consecutivos, assistidos na consulta externa de um serviço de endocrinologia de um hospital público central, antes de qualquer tratamento médico. A personalidade foi avaliada com o Inventário de Personalidade Multifásico do Minnesota (MMPI). A reatividade hipófise-suprarrenal foi avaliada pela prova da corticoliberina (CRH). Resultados: Os níveis basais de DHEAS relacionaram-se inversamente com a resposta pico/basal do cortisol (r parcial=-0,454; p<0,05) na prova da CRH. A reatividade da DHEAS na prova da CRH relacionou-se diretamente com a tríade de Problemas do Comportamento (r=0,257; p<0,05) e com a personalidade tipo A (r=0,295; p<0,05). Os níveis de ACTH basais relacionaram-se diretamente com os níveis de DHEAS (r=0,366; p<0,001). A idade, género e os níveis de ACTH em conjunto, explicaram 34% da variabilidade nos níveis de DHEAS. Conclusões: A DHEAS poderá ter um papel protetor contra uma resposta excessiva do cortisol em situações de stress. Os resultados sugerem ainda que a personalidade se relaciona com a reatividade da DHEAS.

**Palavras chave:** DHEAS; cortisol; personalidade; stress; reatividade da DHEAS; prova da CRH.

**Abreviations**: AP - Type A Personality; AUC - Area Under the Curve; AUC/h - Area Under the Curve/hour; BD - Behaviour Deviant Triad; GABA-A - Gamma-Aminobutyric Acid Type A; Receptor K-S - Kolmogorov-Smirnov; MMPI - Minnesota Multiphasic Personality Inventory; NMDA - N-methyl-D-aspartate receptor; NT - Neurotic Triad; PD - Psychotic Dyad; sd - standard deviation.

## Introduction

In humans dehydroepiandrosterone-sulphate (DHEAS) is the most abundant hormone in the peripheral circulation. Its normal levels (2–10 μmol/L) are more than 20 times those of cortisol or thyroxine, more than 100 times those of testosterone, growth hormone and prolactin and more than 10,000 times those of aldosterone, estradiol or insulin. On the other hand, most laboratory animals have only negligible amounts of DHEAS. Even primates have much lower levels than humans (Berr *et al.* 1996).

DHEA is mostly synthesised in the adrenals and gonads, either as the final androgen-like compound or as an intermediate in the synthesis of androgens. However, it is also synthesised in the central nervous system (Paul & Purdy 1992; Berr *et al*. 1996; Baulieu & Robel 1998; Reddy & Kulkarni 1998; Labrie *et al.* 2003; Sicard *et al.* 2007). A sulphotransferase reversibly converts DHEA into DHEAS, and the sulphated form is by far the most abundant in the peripheral circulation. Nevertheless, the nonsulphated form is, however, much more lipossoluble and may easily cross biologic membranes presenting a much larger distribution that includes the central nervous system (Berr *et al.* 1996; Sicard et al. 2007). DHEA has a short half-life – 1 to 3 hours – as opposed to DHEAS that has a long half-life – 10 to 20 hours (Legrain *et al.* 2000; Muniyappa *et al.* 2006; Komesaroff 2008).

No definitive factors regulating DHEAS synthesis have been so far identified. DHEAS physiological effects and teleological meaning are unclear and controversial. At the clinical level DHEAS levels are higher in males and dramatically decrease with age – in the seventh decade they are about 20% of those in the third decade (Berr *et al.* 1996; Kimonides *et al.* 1998; Laughlin & Barrett-Connor 2000; Tannenbaum *et al.* 2004; Sicard *et al.* 2007). Furthermore, DHEAS levels relate to morbidity and mortality even after age correction (Berr *et al.* 1996; Gruenewald *et al*. 2006; Sicard *et al.* 2007). Either specific effects or more generally a cortisol antagonism has been invoked to account for those associations (Akinola & Mendes 2008; Wemm *et al*. 2010).

Regarding DHEAS effects, initial evidence resulted mainly from large observational studies on the elderly and post-menopausal women – The Rancho Bernardo Study,

Baltimore Longitudinal Study of Aging and Personnes Agées Quid (PAQUID) – studies with DHEAS replacement in the elderly – DHEAge Study – and smaller clinical studies of patients with primary adrenal failure (Barrett-Connor & Edelstein 1994; Berr *et al.* 1996; Wolf *et al*. 1997; Barrett-Connor *et al.* 1999; Baulieu *et al*. 2000; Schlienger *et al.* 2002; Legrain & Girard 2003; Hougaku *et al*. 2006; O'Donnell *et al.* 2006). Nevertheless, during the last years, more and more evidence regarding DHEAS effects in adolescents and adults has been collected.

Similar to other neurosteroids, specific central nervous system effects have been described for DHEAS. DHEAS seems to modulate cognitive function and higher levels of this hormone are related to better results regarding memory, learning and resilience (Barrett-Connor & Edelstein 1994; Morley *et al.* 1997; Compagnone & Mellon 1998; Reddy & Kulkarni 1998; Morrow 2007; Sicard *et al.* 2007; Wemm *et al.* 2010); to higher well being scores and less depression (Moralès *et al.* 1994; Wolf *et al.* 1997; Barrett-Connor *et al.* 1999; Schlienger *et al.* 2002; Dallman *et al.* 2003; Akinola & Mendes 2008) and to higher resistance to the deleterious effects of a stressful situation (Reddy & Kulkarni 1998; Morrow 2007; Morgan *et al.* 2009; Yoon *et al.* 2009).

At the molecular level DHEAS has a general neurostimulatory effect via gabaminergic antagonism [Gamma-Aminobutyric Acid Type A Receptor (GABAA) antagonism] and glutaminergic agonism [N-methylD-aspartate receptor (NMDA) sigma-1 agonism] (Paul & Purdy 1992; Baulieu & Robel 1998; Reddy & Kulkarni 1998; Morrow 2007). Neurotrophic effects have also been described and DHEA and DHEAS may contribute to neocortical organization (Baulieu & Robel 1998; Compagnone & Mellon 1998; Beck & Handa 2004; Suzuki *et al.* 2004). Moreover, DHEA and DHEAS neuroprotective effects after hypoxia have been documented and lower levels of those hormones in older adults could contribute to the enhanced cerebral vulnerability to vascular lesion or other neural insults (Kimonides *et al.* 1998).

The concept of endocrine phenotypes in endocrine research assumes that with regard to hormone levels, intersubject variability is much larger than intrasubject variability throughout the time (Bertagna *et al.* 1994; Coste *et al.* 1994). It is also postulated that these endocrine phenotypes are established at the beginning of one's life.

A second related concept is that one of endocrine plasticity, meaning that endocrine responses nonrandomly change throughout the time according to previous experiences (Chrousos *et al.* 1988; Chrousos 1992; Levine 1993). In what concerns behavioural endocrine research personality traits are also individual features established quite early in life. A relation between cortisol and the hypothalamic-pituitary-adrenal axis reactivity and personality has been established (Chrousos *et al.* 1988; Chrousos 1992; Kirshbaum *et al.* 1992; Levine 1993; Bertagna *et al.* 1994; Castanon & Morméde 1994; George *et al.* 2010; Wirtz *et al.* 2010); however, this relation was less studied in what concerns DHEAS (Thomas *et al.* 1994).

The Minnesota Multiphasic Personality Inventory (MMPI) is well validated in the general population and, in addition to its importance in the psychiatric setting, it can also be used to interpret classic personality dimensions (Costa *et al.* 1986). In the corticotropin-releasing hormone (CRH) test, pituitary-adrenal response to CRH administration is classically measured with ACTH and cortisol determinations as an index of the stress response. The CRH test has been extensively used in psychoneuroendocrine research of several psychiatric and psychosomatic disorders (Gold *et al*. 1986; Demitrack *et al.* 1991; Chrousos & Gold 1992) and previous studies from our research team found out significant relations between personality and pituitary-adrenal response to CRH administration in common clinical disorders (Martins *et al.* 2001; Martins *et al.* 2002; Martins *et al.* 2004).

The above evidence suggests the existence of a relation between DHEAS and behaviour, namely with personality and stress response. In the current study we explored the relation between DHEAS and both personality traits and pituitary-adrenal axis reactivity in humans.

## Patients *and* Methods

We analysed retrospectively the records of 120 consecutive patients in which CRH test and personality evaluation with the MMPI were included in the clinical workout.

Patients belong to several diagnostic groups. Clinical condition was not further taken into account for this study but diagnostic group was retained as a baseline variable in the statistical analysis, so that only significant results after correction for this potential confounding variable are reported. The study protocol was approved by the Hospital Ethical Committee and informed written consent was obtained for every patient. The data used was obtained before beginning medical treatment.

Age, gender, height and weight without shoes or coats were recorded. The Portuguese translation of the MMPI (Montenegro 1982) was filled by the participants after one of the authors' complete and detailed instructions. The participants remained alone in a quiet room for the entire procedure. Scores were obtained according to MMPI authors' instructions. To avoid the use of multiple comparisons, only conventionally defined type A personality (AP) and superordinate traits were used – neurotic triad (NT): hypochondriasis (Hs) + depression (D) + hysteria (Hy); psychotic dyad (PD): paranoia (Pa) + schizophrenia (Sc) and behaviour-deviant triad (BD): psychopathic deviate (Pd) + masculinity-femininity (Mf) + hypomania (Ma) (Butcher *et al.* 1990; Greene 1991). The original MMPI version was used instead of the revised one (MMPI-2) since the MMPI-2 translation had not been validated yet.

The CRH test was performed the following week, after an overnight fast. In all cases a venous line was obtained in the antecubital region, the participant lying in the supine position; after a 15 to 20 min period of adaptation a venous blood sample was collected – time 0. CRH was then slowly infused in 1 to 2min (human CRH, 100 µg, CRH Ferring GmHb, Kiel, Germany); further blood samples were obtained at 15, 30, 60 and 120 min. All samples were immediately refrigerated at +4 °C and sent to the Endocrine Laboratory after test completion. ACTH and cortisol were measured at 0, 15, 30, 60 and 120 min, DHEAS was measured at 0, 30 and 60 min and prolactin (PRL) was only measured at baseline.

Immunoradiometric assay (IRMA) and enzymelinked immunoassay (ELISA) methods were used for ACTH (IRMA, Nichols Institute, San Juan Capistrano, CA), PRL (IRMA, Diagnostc Products Corporation, Los Angeles, CA), DHEAS and cortisol (ELISA, Diagnostic Products Corporation, Los Angeles, CA) measurements which were performed

in the hospital central laboratory. Intra- and interassay variation coefficients were less than 10% in every case.

Statistical analysis was carried out with the use of the Statistical Package for the Social Sciences Program (SPSS, version 16.0). Results are presented as the mean±standard deviation (sd) or as percentage as appropriate. The area under the curve (AUC) was computed according to the trapezoidal rule (Rowland & Tozer 1995). Mean values per hour were computed. The normal distribution of continuous variables was verified by the Kolmogorov-Smirnov (K-S) goodness of fit test. Non normal distributed variables were log transformed prior to analysis. However, for the sake of simplicity when no differences were found, results regarding the non-transformed variables are presented. The Chi-Square test was used to compare the distribution of non-continuous variables between selected groups, factorial analysis of variance (ANOVA) to compare continuous variables between selected groups and Multiple linear regression analysis to explore the relation between continuous variables. The limit of statistical significance was selected at 0.05.

## Results

All patients' results are summarized in Tables 1–3. Basal DHEAS was different among diagnostic groups – $F_{(4,119)}=3.959$, $p<0.005$ – with hirsute subjects presenting significantly higher basal DHEAS values – 237±113 μg/dL – when compared to all other diagnostic groups. There were no differences in ACTH, cortisol and PRL among diagnostic groups.

Mean baseline DHEAS was 158±99 [20–554] μg/dL and presented a distribution not significantly different from the normal one, K-S test Z=0.862, ns. DHEAS levels were higher in males when compared to females – 207±87 μg/dL vs 151±99 μg/dL, t=2.088, $p<0.05$. DHEAS levels were inversely related to age – r=0.444, $p<0.001$, even after gender correction, the regression equation being DHEAS=272.493–3.736 × age. Together gender and age accounted for 27% of DHEAS variability. DHEAS levels were not significantly related to body mass index (BMI) or body weight.

Table 1. Clinical characteristics and baseline endocrine values.

| | Reference interval | Mean±sd [min-max] or % |
|---|---|---|
| Age (years) | | 33±12 [18-57] |
| Gender F/M (%) | | 87/13 |
| BMI (kg/m$^2$) | | 27.7±9.0 [14.5-60.1] |
| DHEAS (µg/dL) | 35-430 | 158±99 [20-554] |
| ACTH (pg/mL) | 0-46 | 19±14 [1-90] |
| PRL (ng/mL) | 2-29 | 11±7 [2-33] |
| Cortisol (µg/dL) | 4-23 | 18±9 [4-51] |

Table 2. ACTH, cortisol and DHEAS levels in the CRH test.

| | ACTH (pg/mL) | Cortisol (µg/dL) | DHEAS (µg/dL) |
|---|---|---|---|
| 0’ | 21±16 | 19±9 | 177±106 |
| 15’ | 52±74 [b] | 22±8 [c] | - |
| 30’ | 50±58 [c] | 23±8 [c] | 177±114 |
| 60’ | 31±31 [a] | 24±9 [c] | 176±107 |
| 120’ | 16±18 [a] | 18±10 | - |
| AUC | 32±36 [b] | 22±8 [c] | 176±107 |

AUC units are pg/mL.h for ACTH, µg/dL.h for cortisol, and µg/dL.h for DHEAS.

Value different from basal, [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$.

Table 3. Superordinate traits and AP score (MMPI).

| | Mean±sd |
|---|---|
| Neurotic Triad (NT) score | 164±30 |
| Psychotic Dyad (PD) score | 115±22 |
| Behaviour-Deviant triad (BD) score | 158±23 |
| Type A Personality (AP) (%) | 38±16 |

DHEAS levels were significantly related to ACTH – r=+0.366, p<0.001 – and PRL – r=+0.233, p<0.05 – but not to cortisol. However, ACTH and PRL were interrelated and when both variables were included in the analysis only ACTH remained as a significant factor. This relation persists even after age, gender and diagnostic group correction. Age, gender and ACTH account for 34% of DHEAS variability.

Baseline ACTH was significantly related to the peak ACTH (r=+0.490, p<0.001) and peak cortisol (r=+0.246, p<0.05) responses during the CRH test. Baseline cortisol was strongly related to the peak cortisol response (r=+0.782, p<0.001). Baseline DHEAS was not related to the peak ACTH or peak cortisol response in the CRH test but baseline DHEAS was inversely related to the peak/baseline cortisol response (parcial r=–0.454, p<0.05) after age, gender, baseline ACTH and baseline cortisol correction (multiple linear correlation including peak/baseline cortisol, age, gender, baseline ACTH and baseline cortisol, total r=0.631, p<0.005) (Figure 1). DHEAS was not related to peak/baseline ACTH response.

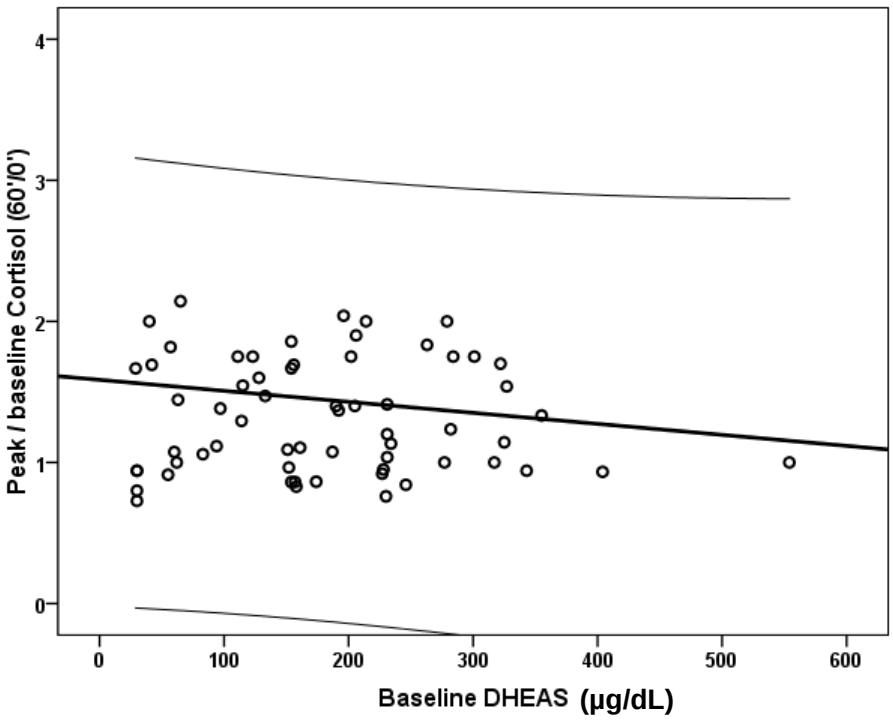


Figure 1: Relation between baseline DHEAS and peak/baseline cortisol response in CRH test.

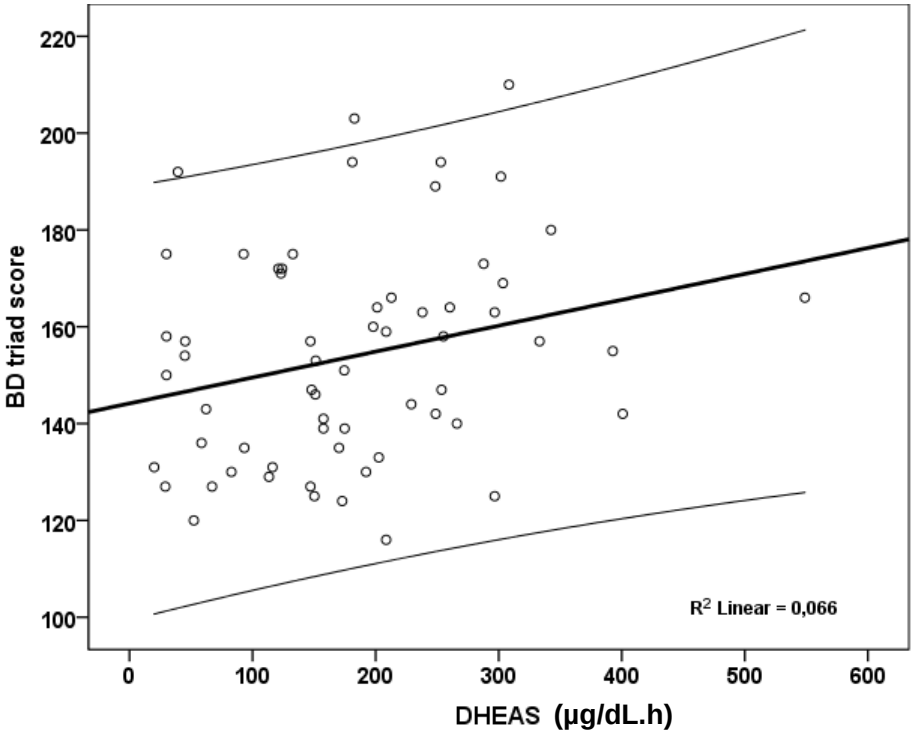


Figure 2: Relation between DHEAS reactivity and BD triad.

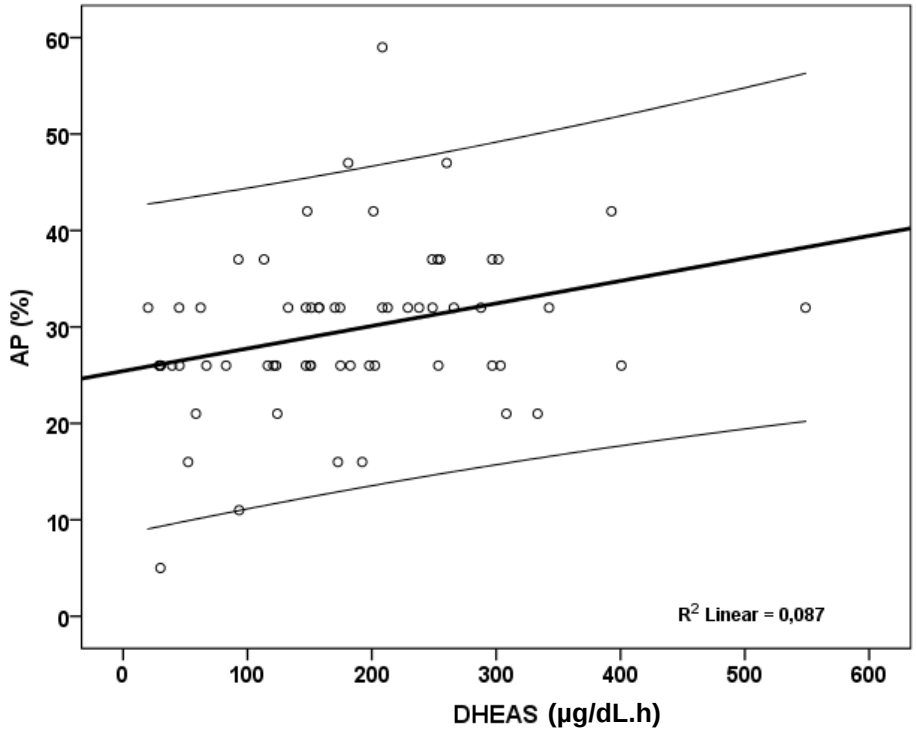


Figure 3: Relation between DHEAS reactivity and type A Personality.

Baseline DHEAS was inversely related to NT score – r=–0.355, p<0.001 – but not to PD or BD triad. However, this relation was no longer significant when age correction was carried out; in fact, age was directly related to NT – r=+0.443, p<0.001. Nevertheless, the DHEAS response in the CRH test (evaluated as the AUC) was significantly and directly related to BD triad and type A personality – simple linear correlation respectively r=+0.257, p<0.05 and r=+0.295, p<0.05 (Figure 2 and 3) – and that relation remains significant even after age and diagnostic group correction. As noted, DHEAS average levels have not significantly changed after the CRH test. Despite this, DHEAS response was highly variable in what concerns the individual level (–73 μg/dL to +317 μg/dL).

## Discussion

This is an observational retrospective study, whose objective is to explore the relation between DHEAS and both personality and pituitary-adrenal axis reactivity in adult humans.

We used several specific diagnostic groups. As noted before only results remaining significant independently of age and diagnostic group are reported. Only DHEAS and not DHEA measurements were used. We expected a stronger relation between DHEAS and more stable parameters as age, gender, basal hypothalamic-pituitary-adrenal (HPA) axis activity and personality as that hormone has a longer half-life. On the contrary, acute changes might be more easily detected with DHEA, namely after CRH administration.

The predominance of female participants (87%) reflects the population assisted in the Endocrine outpatient department. Even so, DHEAS levels were significantly higher (37%) in males, as it was expected (Berr *et al.* 1996; Baulieu *et al.* 2000; Laughlin & Barrett-Conner 2000; Tannenbaum *et al.* 2004; Gruenewald *et al.* 2006).

The mean age was 33 years old (the sample was 18 to 57 years old) and DHEAS levels were inversely related to age as it has extensively been described in the literature (Berr *et al.* 1996; Lane *et al*. 1997; Morley *et al.* 1997; Kimonides *et al.* 1998; Legrain & Girard 2003; Sicard *et al.* 2007). We found a 1.4% mean decline in DHEAS levels per year

while other authors had previously described a decline of about 2% per year during adulthood and higher decline rates in post-menopausal women and older men (Laughlin & Barrett-Connor 2000; Tannenbaum *et al.* 2004; Labrie *et al*. 2005).

Age explained 20% of DHEAS variability, gender explained 4% of DHEAS variability and age and gender together explained 27% of DHEAS variability. Both factors have been extensively identified as relevant factors for DHEAS levels.

DHEAS levels were also significantly and directly related to basal ACTH independently of age, gender and diagnostic group; ACTH explains 14% of DHEAS variability. Age, gender and ACTH accounted for 34% of DHEAS variability. This is a relatively new finding since ACTH had not been generally considered as a relevant factor both for DHEAS and adrenal androgen production. In fact, chronic stress is generally associated with increased cortisol levels and decreased DHEAS levels. Moreover, it should be noted that despite the ACTH response, no DHEAS response was found in the CRH test, even if dexamethasone suppression decreases both cortisol and DHEAS. All this points out the complexity of the relation between ACTH and DHEAS until a putative cortical androgen stimulating hormone (CASH) is still to be identified.

As a group, and despite the ACTH response, mean DHEAS levels did not change in the CRH test suggesting no acute effect of ACTH on DHEAS levels. Taking into account the high DHEAS levels and long DHEAS half-life, it may be necessary a longer ACTH rise to increase DHEAS levels (Berr *et al.* 1996). However, individually DHEAS response was highly variable. Baseline ACTH was strongly and directly related to the peak ACTH response and was also directly related to the peak cortisol response and baseline cortisol was strongly related to the peak cortisol response suggesting that baseline HPA axis activity is a strong determinant of the response to CRH. In more detail, baseline ACTH levels seem to be a major determinant of either ACTH or cortisol response.

An interesting finding is the fact that baseline ACTH is related to both ACTH or cortisol peak levels but not to ACTH or cortisol peak/ baseline ratio suggesting that ACTH does not modulate the intensity of the response, but only that those subjects with higher baseline ACTH and cortisol levels naturally reach higher peak ACTH and cortisol levels.

The influence of DHEAS seems much more subtle. Baseline DHEAS was not related to the peak ACTH or peak cortisol response in the CRH test. Similarly, it was not related to peak/baseline ACTH response in CRH test but it was inversely related to the peak/baseline cortisol response (after age, gender, baseline ACTH and baseline cortisol correction). Those results suggest that DHEAS may reduce the magnitude of the cortisol response independently of baseline ACTH or cortisol levels. There is some previous evidence that DHEAS may indeed down-regulate cortisol levels (Kimonides *et al.* 1998; Gruenewald *et al.* 2006; Morrow 2007; Akinola & Mendes 2008).

Lower DHEAS was related to higher NT scores. The relation between DHEAS and NT score persisted after diagnostic group correction. However, that relation was no longer significant after age correction, and in fact, age was directly related to NT score. This is rather surprising not only because personality is generally considered as a stable personal characteristic established at a very early stage of life but also because this is a rather young sample. On the other hand, it seems plausible that aging, bringing about morbidity and mortality of both the patient and their relationships, should be associated with increased hypochondriac and depressive scores, two of the three components of the neurotic triad. Whatever the reason may be, aging is associated with increased NT scores (Zuckerman 1994) and decreased DHEAS baseline levels and that seems to be the reason for the spurious relation between DHEAS and NT. Disappointingly, baseline DHEAS levels were not significantly related to any of the selected psychometric variables and this points out the insensitivity of baseline endocrine levels.

However, DHEAS reactivity in the CRH test is significantly related to BD triad and Type A personality and both relations persist independently from age, gender and diagnostic group. As noted before neuroendocrine reactivity in the CRH test (regarding the ACTH and cortisol response) has been previously shown to be related to other psychometric variables. The apparent paradox is that although there does not seem to be any DHEAS response in the CRH test (evaluated by the mean), the DHEAS response is significantly related to psychometric variables. In fact, although the mean DHEAS does not change in the CRH test, individually considered peak DHEAS – baseline DHEAS changes deeply from –73 to + 317 μg/mL. In short, higher DHEAS responses are

associated with Type A behaviour and BD triad (psychopathic deviation + hypomania + masculinity-femininity) and more specifically with Pd score (data not shown).

To **conclude**, we found that DHEAS significantly changes according to gender and age. Moreover, we also noticed that: 1) baseline DHEAS is significantly modulated by ACTH; 2) baseline DHEAS significantly modulates the intensity of the cortisol response in the CRH test; 3) DHEAS reactivity is a factor for BD triad and Type A behaviour. In short, baseline DHEAS relates to stress response and DHEAS reactivity relates to personality.

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**Author contributions**

JMM designed and performed the experiments. SV and JMM designed the present study protocol. SV and JMM analyzed the data. SV wrote the first draft of the manuscript. JMM, MJF and IC provided critical revision of the manuscript. JMM – João Martin Martins, SV- Sónia do Vale, MJF – Maria João Fagundes, IC – Isabel do Carmo.

# Study II

## The Relationship between Dehydroepiandrosterone (DHEA), Working Memory and Distraction – A Behavioral and Electrophysiological Approach

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## Abstract

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulphate (DHEAS) have been reported to have memory enhancement effects in humans. A neuro-stimulatory action and an anti-cortisol mechanism of action may contribute to that relation. In order to study DHEA, DHEAS and cortisol relations to working memory and distraction, we recorded the electroencephalogram of 23 young women performing a discrimination (no working memory load) or 1-back (working memory load) task in an audio-visual oddball paradigm. We measured salivary DHEA, DHEAS and cortisol both before each task and at 30 and 60 min. Under working memory load, a higher baseline cortisol/DHEA ratio was related to higher distraction as indexed by an enhanced novelty P3. This suggests that cortisol may lead to increased distraction whereas DHEA may hinder distraction by leading to less processing of the distractor. An increased DHEA production with

consecutive cognitive tasks was found and higher DHEA responses attributed to working memory load were related to enhanced working memory processing as indexed by an enhanced visual P300. Overall, the results suggest that in women DHEA may oppose cortisol effects reducing distraction and that a higher DHEA response may enhance working memory at the electrophysiological level.

**Key-words:** dehydroepiandrosterone; dehydroepiandrosterone-sulphate; cortisol; working memory; auditory distraction; dehydroepiandrosterone response; event-related-potentials.

## Resumo

Têm sido descritos efeitos benéficos da desidroepiandrosterona (DHEA) e desidroepiandrosterona-sulfato (DHEAS) na memória em seres humanos. Uma ação neuroestimulante bem como efeitos anti-cortisol da DHEA e DHEAS, poderão contribuir para essa relação. O objetivo deste estudo foi avaliar as relações da DHEA, DHEAS e cortisol com a memória e a distração. Para isso, gravámos o eletroencefalograma de 23 jovens do género feminino, enquanto realizavam uma tarefa discriminativa (sem memória de trabalho) e outra tarefa com memória de trabalho, usando um paradigma audiovisual (de tipo *"oddball"*), em que a tarefa alvo é visual e a distração é auditiva. Medimos a DHEA, DHEAS e cortisol na saliva, antes de cada tarefa e aos 30 e 60 min. Durante a realização da tarefa com memória de trabalho, uma razão cortisol/DHEA basal mais elevada, relacionou-se com uma deflexão “P3-novidade” mais ampla, indicando maior distração. Esta relação sugere que o cortisol se poderá aumentar a distração enquanto que a DHEA poderá reduzir essa distração, através da redução do processamento do estímulo distrativo. Verificou-se uma maior secreção de DHEA com a realização das duas tarefas cognitivas consecutivas. Uma maior a reatividade da DHEA atribuível à memória de trabalho, relacionou-se com uma maior amplitude da deflexão

P300 visual, sugerindo um maior processamento da memória de trabalho. Globalmente, estes resultados sugerem que nas mulheres, a DHEA poderá opor-se aos efeitos do cortisol, reduzindo a distração e que uma maior resposta da DHEA, poderá melhorar a memória de trabalho, ao nível eletrofisiológico.

**Palavras-chave:** desidroepiandrosterona; desidroepiandrosterona-sulfato; cortisol; memória de trabalho; distração auditiva; reactividade da desidroepiandrosterona; potenciais evocados.

## Introduction

Dehydroepiandrosterone-sulphate (DHEAS) is the most abundant steroid in the peripheral circulation and it is much more abundant in humans than in any other species [1,2,3,4,5,6,7]. Circulating levels dramatically decrease with aging. Moreover, lower levels are related to higher morbidity and mortality ratios even when corrected for age [2,8].

DHEA is mostly synthesized in the adrenals whereas the gonads represent a minor source of this hormone. However, DHEA is also synthesized in the central nervous system [3], where its concentrations are higher than in the peripheral circulation [3,9,10]. In both peripheral and central compartment, a sulpho-transferase reversibly converts DHEA to DHEAS, restricting its distribution and prolonging its half-life [2,3].

Several central effects concerning cognitive performance and stress response have been described for DHEA and DHEAS. Higher levels were related to increased memory and attention scores [9,11] and improved performance in stressful conditions [12,13,14], whereas low levels were found in Alzheimer's disease [15]. However, DHEA administration in older subjects showed inconclusive results [5,16,17,18,19].

On the other hand, stressful stimuli also modulate DHEA and DHEAS levels: acute stress is related to an increase in DHEA and DHEAS levels [20,21,22,23] whereas chronic

stress decreases baseline DHEA and DHEAS levels [24,25,26,27,28], as well as the acute DHEAS response to a superimposed psychological stress [29]. Yet, a direct influence of cognitive processing on DHEA or DHEAS levels has not been studied.

At the molecular level, DHEA and DHEAS have a general neuro-stimulatory effect: presynaptic actions include glutamate, acetylcholine and norepinephrine release and postsynaptic actions include sigma 1 receptor agonism with subsequent N-methyl-D-aspartate (NMDA) receptor activation, gabaminergic antagonism and inhibition of voltage-gated calcium currents [3,9,10]. Nevertheless, DHEA and DHEAS molecular effects are not exactly the same and several studies suggest that the balance between them may influence brain functioning [9,30]. As an example, DHEAS has more potent antagonistic effects at the c-aminobutyric acid type A receptor (GABA-A receptor) than DHEA [31,32]. Hence, the simultaneous evaluation of DHEA and DHEAS may uncover more information than the individual examination of either form of that steroid.

Concerning glucocorticoids, whereas mild or short-lasting increases in cortisol due to stress may protect the body, promote adaptation and have beneficial effects on attention and memory, higher cortisol levels or long term increases are related to poorer executive functioning, poorer learning and memory and less cognitive flexibility [33,34,35,36,37,38,39]. In particular, working memory (WM) depends on prefrontal cortex activity, which is modulated by glucocorticoids: prefrontal cortex-dependent working memory is enhanced by acute stress and inhibited by chronic stress [35,40]. The relation between glucocorticoids and cognitive functioning is bidirectional: glucocorticoids impact cognitive function and cognitive processing has been shown to influence glucocorticoid secretion [41].

Several levels of evidence suggest that DHEA and DHEAS may counterbalance cortisol effects: a higher DHEA/cortisol ratio has been related to better performance under stress [14] and DHEAS antagonized the memory deteriorating neurotoxic effects of cortisol in the hippocampus [10,42]. More generally, DHEA and DHEAS decrease with aging, whereas cortisol does not. Consequently, the cortisol/DHEA ratio increases and may be involved both in the cognitive impairments and in the particular vulnerability to stress damage that seems to characterize the elderly [5,9,42].

The aim of the present study was to test whether DHEA and DHEAS levels are modulated by WM load and whether these endocrine levels are related to distraction and WM at the electrophysiological level, as evidence for their neurophysiologic effects using Event-Related Brain Potentials (ERPs) is scarce [43,44,45]. The specific a priori hypotheses were: 1) higher endogenous DHEA and DHEAS levels may prevent involuntary distraction and enhance cognitive performance; 2) DHEA and DHEAS putatively beneficial effects may be translated at the neurophysiological level; 3) DHEA and DHEAS effects may be largely antagonistic from those of high baseline cortisol; and 4) WM load may be a stimulus for DHEA and DHEAS production.

To test these hypotheses, we measured the relation between DHEA, DHEAS and cortisol on one hand and the cognitive performance and brain responses, using a well-established auditory-visual distraction paradigm [46,47,48] on the other. The protocol includes task irrelevant sounds, some of which are aimed to cause distraction and a visual task including working memory manipulation.

## Subjects and Methods

### Ethics Statement

The experimental protocol was approved by the ethical committees of the University of Barcelona and Lisbon Medical School and it was conducted according to the principles of the Declaration of Helsinki. All the subjects gave their written informed consent before entering the study.

### Subjects

28 healthy female volunteers (undergraduated Psychology students) performed the study protocol. In order to ensure a higher homogeneity in androgen levels, only

women were included. The subjects were young (18 to 25 years old, mean 20±0.5 years old) and presented a normal body mass index (21.8±0.5 kg/m2). All the participants had a normal or corrected to-normal vision and none reported auditory deficits. None of the participants reported a past history of neurologic, psychiatric, endocrine or oral diseases. All the subjects were right-handed. Four subjects were under hormonal contraception. No other medications were allowed. Regular or binge alcohol consumption were exclusion criteria and subjects were asked not to consume alcohol in the twelve hours before the experimental protocol. Regular tobacco consumption as well as illicit drug consumption were further exclusion criteria. Prior to the experimental session, subjects completed the State-Trait Anxiety Inventory [49] and all showed a normal range of state and trait anxiety levels. Five subjects were discarded from the analysis due to technical problems with electroencephalogram (EEG) recordings or endocrine measurements.

**Task and Procedure**

The experimental sessions were held in the afternoon, beginning at 2–3 pm. An adapted version of a well-established auditory-visual distraction task [46,47,50] was presented, based on the protocol used by SanMiguel *et al.* (2008) [51]. In this protocol, two visual tasks were performed: one task without working memory load (WM0) and another one with working memory load (WM1). In the present experiment, the two tasks were performed two hours apart from each other (from onset to onset) and the order of the tasks was counterbalanced across participants. Each task lasted about 15 minutes, and consisted of two blocks of 250 trials each (plus five initial trials that were excluded from the analyses). A short pause was allowed between blocks.

Participants sat in a comfortable chair in a dimly lit and electrically and acoustically shielded room. In the discrimination task (WM0) subjects had to decide whether the two digits appearing on the screen were the same (11 and 22) or different (12 and 21), see figure 1A. In the WM1 task (1-back task) subjects had to decide whether the left or right digit (counterbalanced across subjects) on the screen was the same as the left or right digit of the previous trial (figure 1B). Thus, they had to keep one digit in working memory until the next trial, answering to every trial, except for the first one. Responses were given

through a mouse button (one mouse button for ‘‘same’’ and the other button for ‘‘different’’), also counterbalanced across subjects. The subjects were specifically instructed to respond as quickly and accurately as possible while ignoring the sounds. In order to reduce artifacts originating from eye-blinks and movements during EEG recording, subjects were asked to minimize blinking and to focus on a central fixation cross between the two digits. Before each task, subjects performed practice blocks (composed by 10 trials) without any auditory stimuli until they reached a hit rate of at least 80% in each task.

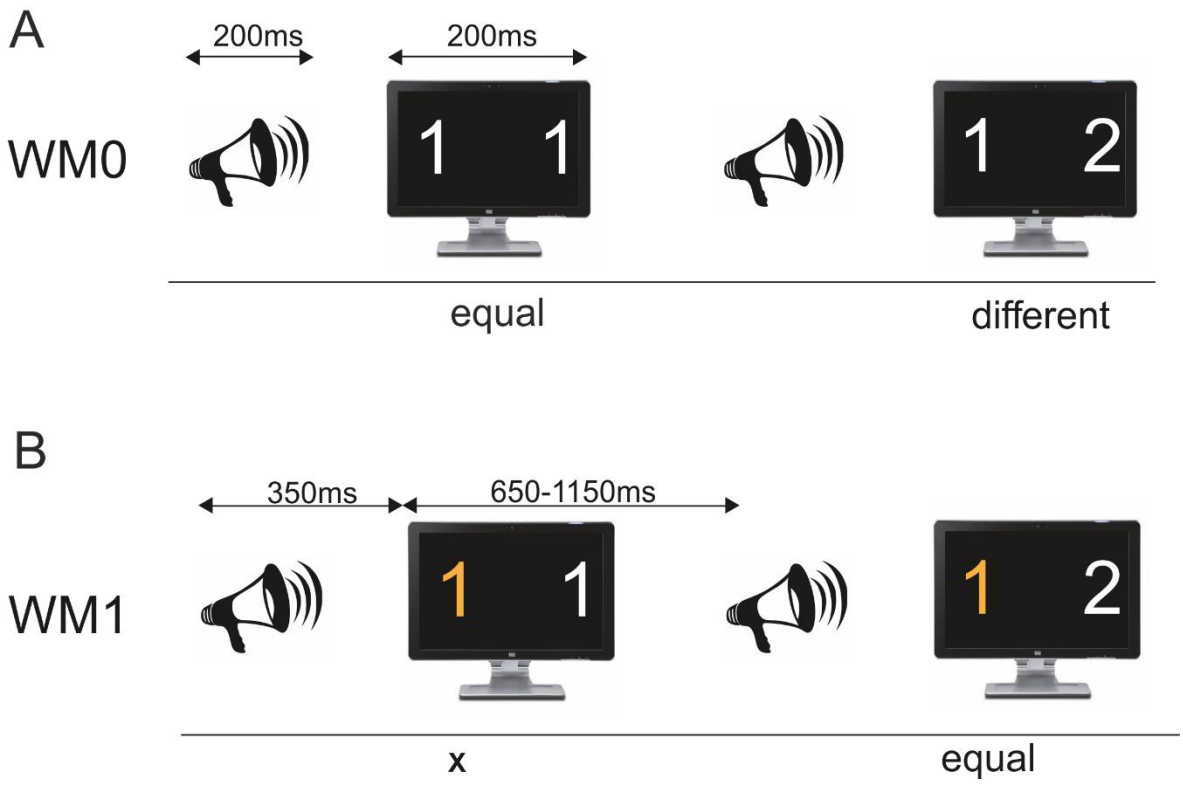


**Figure 1. Example of trials stimulation sequence (above the line) and correct answers to the tasks (below the line) for the two tasks.** A) Discrimination task (WM0), in which subjects had to decide whether the two digits on the screen were equal or different. B) Working memory task (WM1), in which the subjects had to compare the left digit on the screen with the left digit of the previous trial. WM0 – discrimination task; WM1 – working memory task.

Each trial consisted of an auditory stimulus, irrelevant for the task, followed by a visual imperative stimulus after 350 ms (onset to onset), see figure 1. The total trial length varied from 1000 to 1500 ms (1250 ms on average; jitter +/–250 ms). The auditory

sequence consisted of repetitive standard tones (200 ms, including fade-in and fade-out of 10 ms each; 600 Hz; 85 dB; 80% probability), occasionally replaced by environmental novel sounds selected from a sample of 100 different exemplars (edited to have a duration of 200 ms, including fade-in and fade-out of 10 ms each; digitally recorded, low-pass filtered at 10,000 Hz; 85 dB; 20% probability), such as those produced by a drill, hammer, rain, door, telephone ringing, and so forth (50). All sounds were randomly delivered binaurally through headphones (Sennheiser HD 202), and the only restrictions were that the first four stimuli of each block were standard tones, that two novel sounds never appeared consecutively and that each novel sound occurred only once in each task. The visual stimuli consisted of pairs of combinations of the digits 1 and 2 (11, 12, 21 or 22), presented on a computer screen for 200 ms. The appearance probability was the same for every digit combination. The picture size was 357x441 pixels, with a vertical angle of 8º and a horizontal angle of 18º, accounting for two pictures presented simultaneously with the fixation cross in between. The distance from the subjects' eyes to the screen was 100 cm. Overall, there were 400 standard trials and 100 novel trials in each of the two WM conditions.

We recorded response time and hit rates for each trial with Presentation® (Neurobehavioral Systems, Inc). A correct response within the response window (until the sound onset of the subsequent trial) was counted as a hit. We computed distraction as the difference in hit rate or response time between auditory stimulus types (hit rate: WM0standard trials - WM0novel trials and WM1standard trials - WM1novel trials; response time: WM0novel trials - WM0standard trials and WM1novel trials - WM1standard trials) and working memory load costs as the difference in hit rate or response time between the WM load and the discrimination task (hit rate: WM0standard - WM1standard and WM0novel - WM1novel; response time: WM1standard - WM0standard and WM1novel - WM0novel).

**EEG Recording and Analysis**

Electrophysiological activity was continuously recorded during task performance, from 64 scalp Ag/AgCl electrodes following the extended 10/10 convention. Elastic caps

with sintered electrodes and shielded wires were used. The horizontal and vertical electrooculograms (HEOG and VEOG) were recorded with electrodes placed at the outer canthus and below the right eye, respectively. An electrode placed on the tip of the nose was used as the common reference and the ground was located at the AFz position. The EEG and electrooculogram (EOG) were amplified and digitized at a sampling rate of 512 Hz (Eemagine, ANT Software b.v., Enschede, the Netherlands). Impedances were kept at 5 kΩ or below during the whole recording session. Recording was performed with an ANT amplifier of 64 channels (gain 20x; A/D resolution 22 bits, 71.526 nV per bit; filtering 0–138.24 Hz; CMRR>90 dB).

A digital finite impulse response (FIR) bandpass-filter from 0.01 to 30 Hz was applied using a Hamming window. ERPs were averaged offline for each auditory stimulus type and working memory condition, for an epoch of 1000 ms, including a 200 ms pre-auditory-stimulus baseline. The first five epochs of each block and the epochs following a novel trial were excluded from averaging. Only epochs that corresponded to trials with correct responses were averaged.

EOG correction was performed by manually selecting a large number of typical artifacts and accordingly applying a regression algorithm to compute propagation factors (Eeprobe 3.1, ANT Software BV, Enschede, the Netherlands). After EOG correction, epochs that contained EEG activity exceeding ±100 μV peak-to-peak amplitudes were rejected from further analyses. Since we included only trials with correct answers and hit rate was smaller for WM1, the final number of trials was smaller for WM1. The total number of trials included in the averages for each condition and auditory stimulus type was: 246 trials with standard sounds and 81 with novel sounds in WM0 and 213 trials with standard sounds and 64 trials with novel sounds in WM1.

In this paradigm, subjects are specifically instructed to ignore the sounds. Hence, any related effects are necessarily involuntary or lead by exogenous attention. ERPs recorded during this auditory-visual distraction paradigm typically present first an auditory N1/mismatch negativity (N1/MMN) enhancement, reflecting a detection mechanism that leads to attention capture [46], followed by a novelty-P3 (nov-P3) that reflects the effective attention orientation [46,47,52]. Finally, the re-orienting negativity

(RON) reflects the attention re-orientation back to the task [53]. The target is visual and visual ERPs yield sensory (visual P1 and N1) and cognitive components related to target processing (N2b and P300). The N2b is a negative deflection originated by the relevant stimulus and the P300 reflects the processing of the task-relevant visual stimulus [51,54,55]. Typically, the task with working memory load is more difficult for the subjects, leading to a reduced P300 [51,54,55].

To analyze distraction effects at the electrophysiological level, difference waves (dw) were calculated by subtracting the ERPs elicited in standard trials from those elicited in novel trials. These difference waves revealed an early-onset, long-lasting positive deflection that we assimilated to the novelty-P3. Novelty-P3 was measured as the mean amplitude in a time window ranging from 250 to 380 ms at F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrodes.

To analyze WM effects, we compared ERP measures only in standard trials. Specific auditory and visual components were elicited during task-performance: the auditory N1 and P2 and visual P1 and N1. Yet, since cognitive processing was of interest for the present study, we only analyzed N2b (560–645 ms, 210– 295 ms from visual stimulus presentation) and P300 (650–910 ms, 300–560 ms from visual stimulus presentation): F3, Fz, F4, C3, Cz and C4 for N2b and P3, Pz and P4 for P300. Moreover, since the P300 latency was different across WM conditions [$F_{(1,21)}$ =5.683, p=0.027; 753±62 ms for WM1; 787±36 ms for WM0], we analyzed the amplitude of this component at different time windows for each condition (WM1: 650–875 ms; WM0-670– 910 ms).

**Endocrine Measurements**

We collected saliva samples by means of passive drool, using a short straw. Unstimulated whole saliva was used. We collected samples for DHEA, DHEAS and cortisol measurement before each task [before task (BT)], at 30 min [after task (AT)] and 60 min [washout (WO)]. The samples collected before the first task (i.e. before both tasks) were considered as the baseline. We chose the time points to collect the saliva samples in accordance to known cortisol raise and recovery times - raise 10 min after appropriate

stimulus, peak at 20–30 min and recover at 45– 60 min. Furthermore, synchronous 24 h profiles were described for DHEA and cortisol [56].

Unbound DHEA and cortisol in the peripheral circulation penetrate into the saliva via intracellular mechanisms and salivary concentrations reflect serum concentrations [57,58]. DHEAS is not lipid soluble and cannot penetrate into the saliva by passive diffusion through cell membranes. Instead, it squeezes through the tight junctions between salivary glands. DHEAS concentrations in saliva are therefore dependent on serum concentration and salivary flow rate [57].

Samples were refrigerated at 2–8ºC within 30 minutes after collection and they were stored at -20ºC within 4 h and until assayed. Each sample was measured in duplicate by using enzyme linked immunoassays: salivary DHEA and DHEAS enzyme immunoassay kits (Salimetrics Europe®, Ltd, Newmarket Suffolk, UK) and high sensitivity salivary cortisol enzyme immunoassay kits (Salimetrics®, LLC, State College, PA, USA). DHEA was measured in pg/mL and cortisol was measured in µg/dL. Due to the influence of saliva flow rates on DHEAS levels, the concentration of DHEAS (pg/mL) was multiplied by the flow rate (mL/min) and the corrected results were obtained as DHEAS measured per unit of time (pg/min). Intra- and interassay variation coefficients were less than 10% and 15%, in every case, respectively. Cortisol and DHEA were expressed as pmol/L by using the conversion factors 27590 and 3.467, respectively, and DHEAS was expressed as pmol/h by using the conversion factor 0.16284 (system of international units = conventional units x conversion factor).

**Statistical Analysis**

The Statistical Package for the Social Sciences Program (IBM SPSS Statistics, version 21) was used for data analyses. Results are presented as the mean ± standard error of the mean (SEM). The normal distribution of continuous variables was verified by the Kolmogorov-Smirnov Goodness of Fit Test and non-normal distributed variables were log (ln) transformed prior to the analysis. For the sake of simplicity, the results of non-transformed variables are presented whenever we did not find any differences.

To explore the effects of WM load and auditory distraction on performance we performed repeated measures analyses of variance (ANOVAs) on hit rate and response time, including the within-subjects' factors task (WM0 and WM1) and sound (standard and novel). Regarding electrophysiological responses, we examined the auditory ERPs to explore distraction effects and the visual ERPs to explore WM effects. To investigate the effects of auditory distraction on ERPs, we carried out repeated measures ANOVAs on the mean amplitude of the auditory P3 in the time window and electrodes considered above, with the within-subjects' factors task (WM0 and WM1) and sound (standard and novel). To investigate WM effects on distraction ERPs, we carried out an ANOVA on the mean amplitude of the novelty-P3 in the time window and electrodes considered above with task (WM0 and WM1) as within-subjects factor. To investigate WM effects on electrophysiological responses, we included only standard trials and conducted ANOVAs on N2b and visP300 mean amplitude in the time windows and electrodes considered above, with task (WM0 and WM1) as within-subjects factor and task order (simply referred as order in the results) as between-subjects factor (WM0-WM1 or WM1-WM0; this factor is included because we used a blocked protocol, one task consisting exclusively in WM0 trials and the other consisting exclusively in WM1 trials, counterbalanced across subjects).

To investigate the effects of WM manipulation on endocrine levels, we performed repeated measures ANOVAs on DHEA, DHEAS and cortisol levels, including the within-subjects' factors task (WM0 and WM1) and time (before task, after task and washout) and the between-subjects factor task order (WM0-WM1 or WM1-WM0). The endocrine relation to distraction and WM load effects at the behavioral and electrophysiological levels was investigated by repeated measures ANOVAs of behavior and ERP parameters, including baseline DHEA, DHEAS, cortisol or cortisol/DHEA ratio as covariates. Because the DHEA response (determined by the ratio after task/before task) differed between the two WM conditions, DHEA response in WM1 – DHEA response in WM0 (Δ DHEA response) was used as a measure of DHEA response attributed to WM load. This new variable was also tested as a covariate. Whenever the endocrine parameters covariated with the performance or ERP parameters, for each type of auditory stimuli or WM condition, we used Spearman's Rank Order correlation coefficients to select the relevant

endocrine factors and understand the relations direction. DHEAS relations to performance and ERP parameters were not significant and therefore those results are not mentioned in the results section.

ANOVA results were Greenhouse–Geisser corrected whenever the assumption of sphericity was violated. *Post hoc* tests were carried out wherever there were significant interactions between main factors. The Bonferroni correction was used for multiple comparisons. The limit of significance chosen was $\alpha=0.05$.

## Results

### Performance

Behavioral results for each task and auditory stimulus are presented in Figure 2. Overall hit rate was 83±2% in WM0 and 70±2% in WM1 (figure 2A) and this difference in hit rate was significant, as reflected by a main effect of task [$F_{(1,22)}=27.279$, $p<0.001$]. Additionally, there was a main effect of task on response times [$F_{(1,22)}=8.760$, $p=0.007$] with longer response times in WM1 [467±13 ms] than in WM0 [440±9 ms], see figure 2B. Thus, the WM load resulted in lower hit rates and longer response times.

Regarding the effects of auditory distraction on performance, the results showed a main effect of auditory stimulus both on hit rate [$F_{(1,22)}=9.577$, $p=0.005$, with lower hit rates for novel sounds (74±2%) than for standard ones (79±1%), see figure 2A], and response times [$F_{(1,22)}=43.451$, $p<0.001$, with longer response times for novel sounds (476±11 ms) than for standard ones (431±10 ms), see figure 2B]. This means that novel sounds resulted in auditory distraction reflected by lower hit rates and longer response times.

The interaction between task and auditory stimulus also had notable significant effects on hit rates [$F_{(1,22)}=5.557$, $p=0.028$]. *Post hoc* analyses on each WM condition yielded significant effects of auditory distraction only in the working memory load task

[$F_{(1,22)}$=8.697, p=0.007], see figure 2C. In that condition, hit rates were lower for novel sounds (65±4%) than for standards (74±1%).

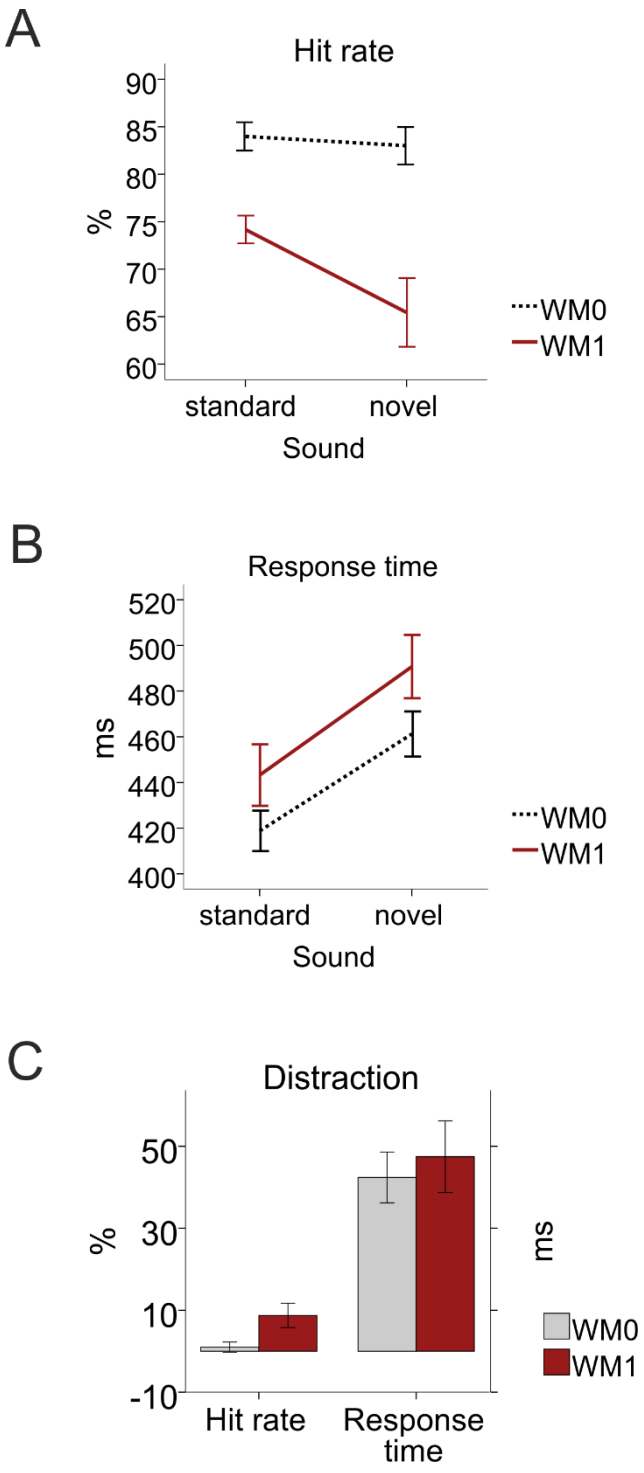


**Figure 2. Performance results.** A) Mean hit rate for each task and auditory stimulus type. B) Mean response time for each task and auditory stimulus type. C) Distraction costs for each task. Distraction = hit rate in standard minus novel trials or response time in novel minus standard trials. WM0 – discrimination task; WM1 – working memory task. Bars represent +/- standard error of the mean (SEM).

**Event-Related Potentials**

**Distraction effects.** As can be seen in figure 3A, novel sounds elicited larger auditory P3 mean amplitudes when compared to standard sounds, both in WM0 [$F_{(1,22)}$=63.696, p<0.001; -3.3±0.5 μV in standard and +1.6±0.7 μV in novel trials] and in WM1 [$F_{(1,22)}$=98.333, p<0.001; -4.1±0.4 μV in standard and +1.9±0.6 μV in novel trials], see figure 3B. This supports that a significant novelty P3 was elicited by novel sounds (figure 3C). However, WM load did not influence the novelty-P3, as its amplitude was similar in both tasks [$F_{(1,22)}$=3.381, p=0.079, 5.0±0.6 μV in WM0, and 5.9±0.6 μV in WM1]. Neither clear N1-enhancement/MMN nor RON was elicited. In sum, significant novelty-P3 responses were elicited by novel sounds in both conditions, but without any significant difference between conditions.

**Working Memory Effects.** The waveforms elicited by standard trials in the two tasks are presented in Figure 4. The N2b significantly increased in WM1 as compared to WM0 [$F_{(1,21)}$=6.738, p=0.017; -1.8±0.9 μV in WM0 and -3.3±0.6 μV in WM1] (see figure 4A and 5B).

The analysis of visP300 revealed a task x order interaction [$F_{(1,21)}$=10.184, p=0.004], see figure 4C. Further analyses revealed a visP300 enhancement in the second task [t(22)=-3.163, p=0.005; 2.6±0.6 μV in the first task, and 4.8±0.8 μV in the second task], independently of the WM load content of that task (figure 4D).

Overall, N2b was enhanced by WM load while the visP300 was enhanced in the second task, independently from working memory load.

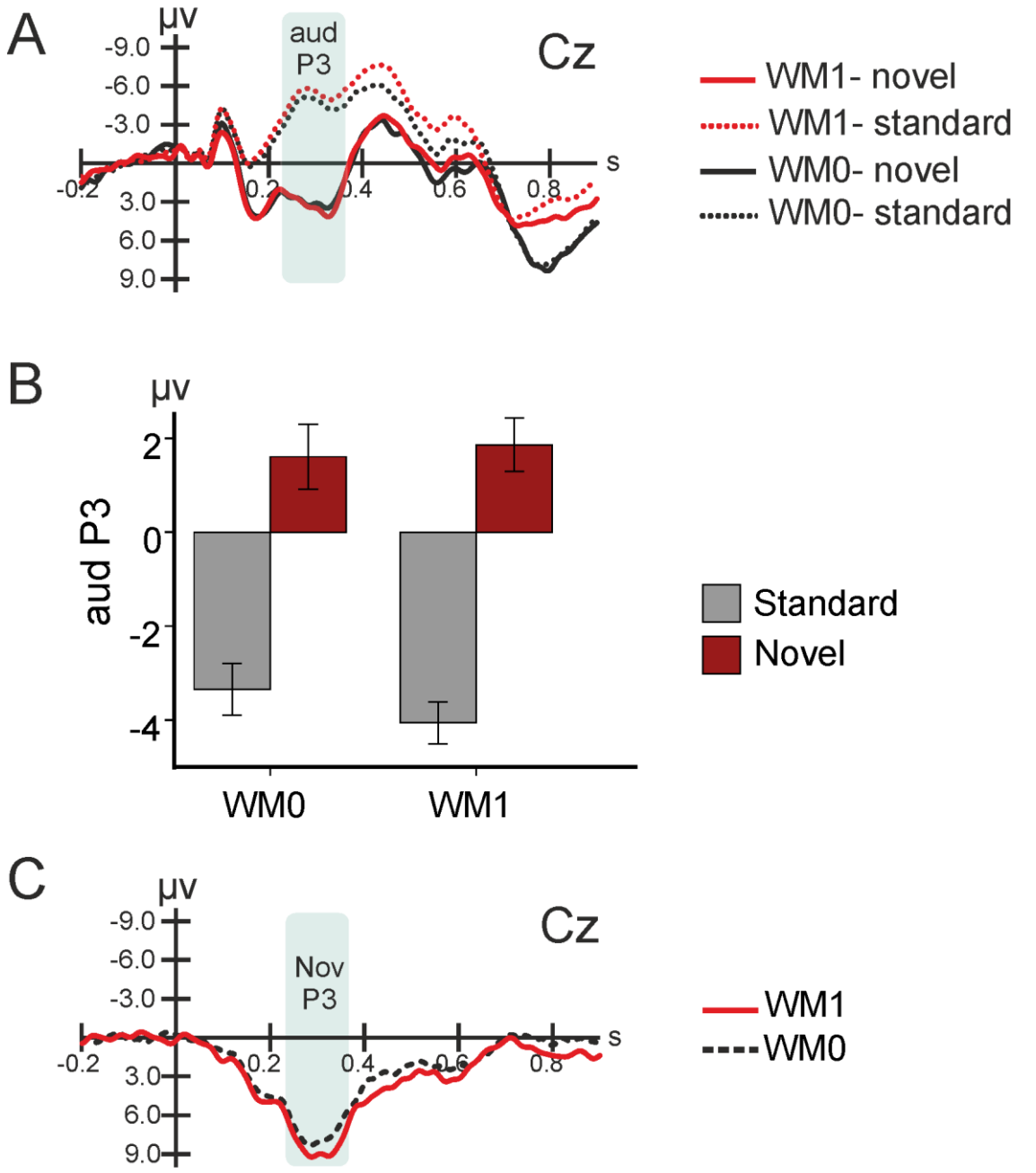


**Figure 3. Event Related Brain Potentials (ERPs).** A) Grand average waveforms at Cz for both tasks (WM0 and WM1) and type of sound (standard and novel). B) Auditory P3 (aud P3) amplitude for each task (WM0 and WM1) and type of sound (standard and novel). C) Grand-average of novel minus standard difference waves at Cz. WM0 – discrimination task; WM1 – working memory task; NovP3 – novelty P3; s - seconds. Bars represent +/- standard error of the mean (SEM).

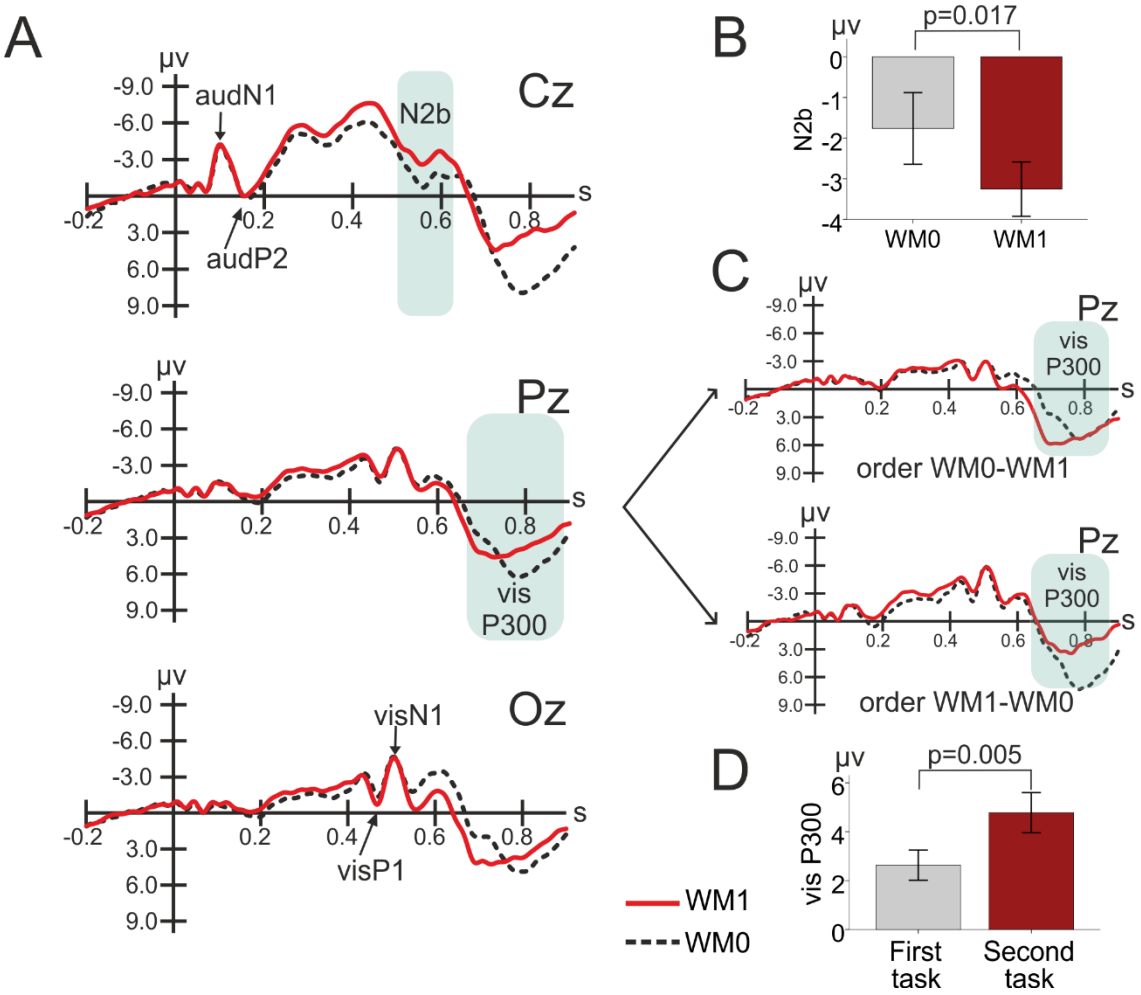


**Figure 4. Standard Event-Related Potentials (ERPs) waveforms in the discrimination and working memory tasks.** A) Standard ERPs in both tasks (both task orders). B) Mean N2b amplitude for each task. C) Standard ERPs in both tasks at Pz, according to the tasks order. D) Mean visual P300 amplitude according to the temporal sequence of tasks (first and second). WM0 – discrimination task; WM1 – working memory task; s – seconds; order WM0-WM1 – the discrimination task performed firstly and the working memory task performed secondly; order WM1-WM0 – the working memory task performed firstly and the discrimination task performed secondly. Bars represent +/- standard error of the mean (SEM).

### Endocrine Baseline Levels and Response

Baseline endocrine levels were: DHEA 790±155 pmol/L, DHEAS 984±120 pmol/h and cortisol 3412±622 pmol/L, with a normal distribution and no significant relation between them. These parameters were not significantly related to age and body mass index (BMI, kg/m$^2$), and did not differ significantly according to the menstrual cycle phase (follicular, peri-ovulatory and luteal, based on self-reported menstrual cycle day) or between subjects taking and not taking hormonal contraception. DHEA and cortisol levels during the experimental procedure are shown in Figure 5.

In contrast to DHEAS levels, which were not affected by any of the factors (task, time or task order), and therefore won't be reported any further, repeated measures ANOVA on DHEA levels revealed a task x order interaction [$F_{(1,20)}$=6.215, p=0.022] and a task x time x order interaction [$F_{(2,40)}$=9.839, p=0.002]. Further analyses revealed that DHEA levels rose after the performance of the second task as indicated by a time effect [$F_{(2,40)}$=8.415, p=0.003; 596±94 pmol/L before task; 839±135 pmol/L after task; 929±156 pmol/L at washout], see figure 5A.

Regarding WM effects, *post hoc* analyses revealed that when the order was WM0-WM1, DHEA levels were higher after WM1 than after WM0 [$F_{(1,10)}$=9.041, p=0.013, 711±139 pmol/L after WM0 and 1064±222 pmol/L after WM1]. The DHEA response [$F_{(1,10)}$=10.676, p=0.008, 0.88 for WM0 and 1.60 for WM1] was also higher in WM1 than in WM0 (figure 5B). Nevertheless, when the order was WM1-WM0, DHEA levels after the second task [$F_{(1,10)}$=6.006, p=0.034] and DHEA response [$F_{(1,10)}$=4.855, p=0.052] were similar in the WM0 (the second task) and in the WM1 task.

An repeated measures ANOVA on cortisol levels revealed a task x time x task order interaction [$F_{(2,40)}$=11.809, p=0.002]. *Post-hoc* analyses for each task order separately showed that cortisol levels decreased after WM0 when the order was WM0-WM1. In fact, for this order, there was a time effect for WM0 [$F_{(2,22)}$=9.544, p=0.007; cortisol levels were 3725±855 pmol/L before task; 2704±579 pmol/L after task; 1766±359 pmol/L at washout], see figure 5C. In spite of this, the cortisol after task/before task ratio in WM0 was not significantly different from the after task/before task ratio in WM1.

DHEA and cortisol changes were directly related for both the WM0 [after task/before task: $r_s$=0.615, n=22, p=0.002, figure 5D; washout/before task: $r_s$=0.852, n=22, p<0.001] and WM1 task [after task/before task: $r_s$=0.654, n=22, p=0.001, figure 5E; washout/before task: $r_s$=0.656, n=22, p=0.001].

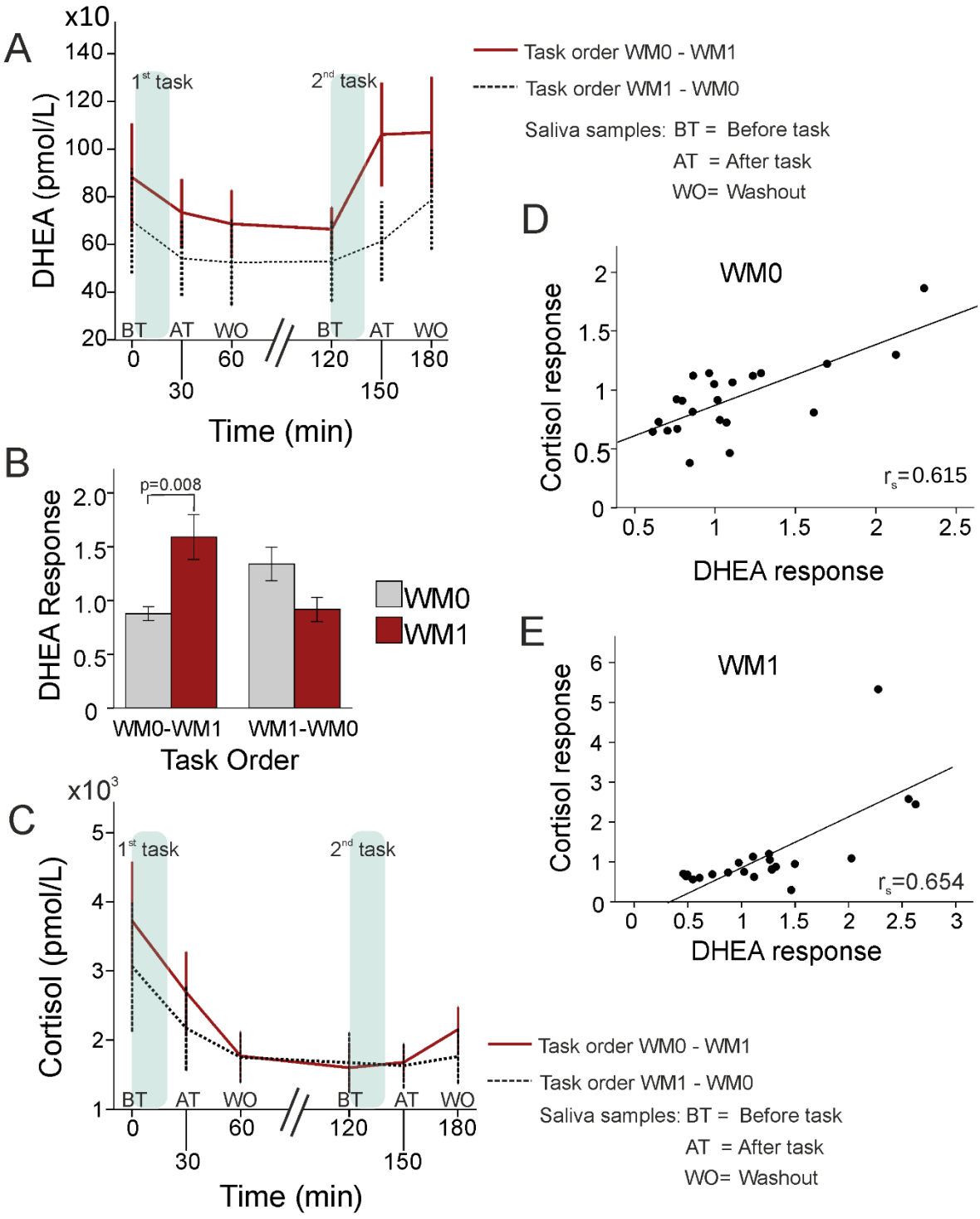


**Figure 5. Endocrine results.** A) Mean DHEA levels for each task and order. B) DHEA response for each task and order. C) Mean cortisol levels for each task and order. D) DHEA and cortisol responses (after task/before task ratios) were directly related in the discrimination task. E) DHEA and cortisol responses were directly related in the working memory task. WM0 – discrimination task; WM1 – working memory task; DHEA response: DHEA after task/before task ratio; Cortisol response: Cortisol after task/before task ratio; order WM0-WM1 – the discrimination task performed firstly and the working memory task performed secondly; order WM1-WM0 – the working memory task performed firstly and the discrimination task performed secondly. Error bars represent +/- standard error of the mean (SEM).

**Endocrine Relations to Performance**

ANOVAs on hit rate and response time were used to select the endocrine parameters that covariated with performance (results are presented as supporting information, Table S1): baseline cortisol, baseline cortisol/DHEA ratio and Δ DHEA response covariated with hit rates and Δ DHEA response covariated with response times. Nevertheless, post hoc Spearman's Rank Order correlations showed no significant relations between those endocrine parameters and performance.

**Endocrine Relations to Event-Related Potentials**

**Distraction Effects.** Endocrine relations to distraction were explored by using the novel minus standard difference waves for WM0 and WM1. There were no endocrine relations to novelty-P3 in WM0. However, in WM1, the novelty-P3 amplitude was enhanced in relation to higher baseline cortisol/DHEA ratios ($r_s$=0.796, n=22, p<0.001; see figure 6).

**Working Memory Effects.** The results showed that visP300 change between tasks was directly related to Δ DHEA response as supported by a significant interaction between visP300 amplitude x Δ DHEA response [$F_{(1,20)}$=9.244, p=0.006] and a direct relation between visP300 enhancement and DHEA response increase attributed to WM load ($r_s$=0.587, n=22, p=0.004; see Figure 7). The difference of visP300 latency between tasks was not related to the endocrine parameters.

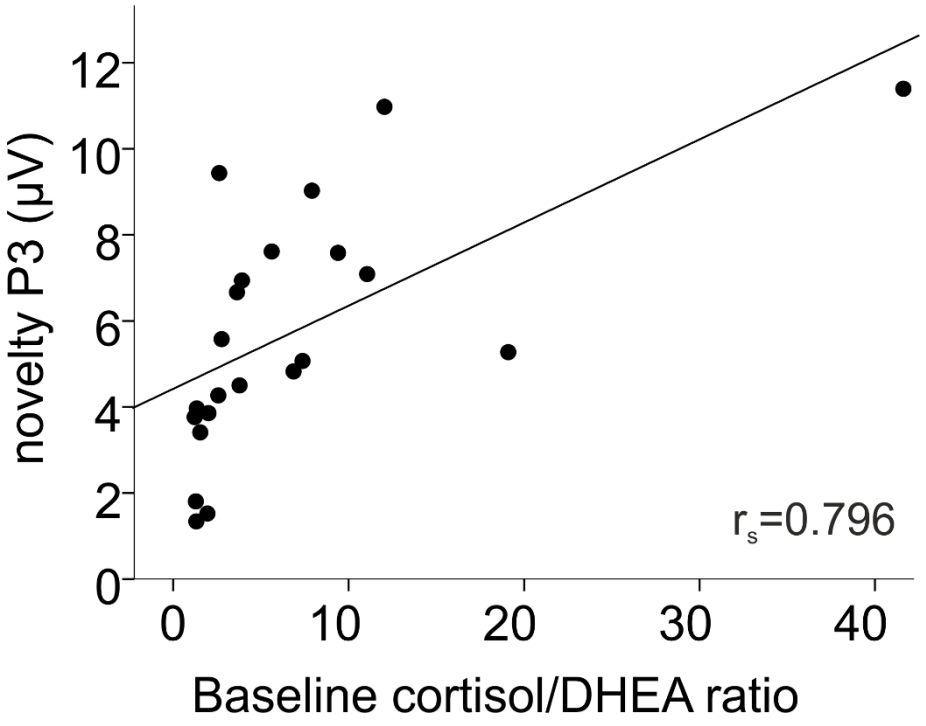


**Figure 6. Baseline cortisol/DHEA ratio was directly related to novelty-P3 under Working Memory load.**

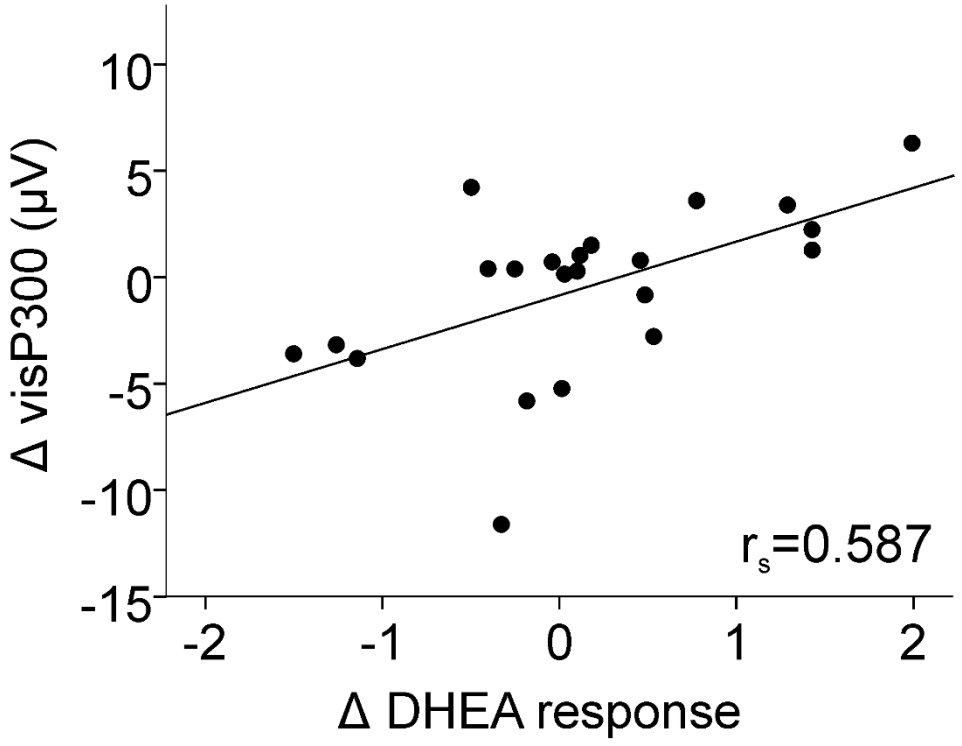


**Figure 7. The visP300 amplitude changed between tasks (WM0, WM1) in direct relation to DHEA response.** WM0 – discrimination task; WM1 – working memory task. Δ DHEA response = DHEA response in WM1 – DHEA response in WM0; Δ visP300 = mean visual P300 in WM1 - mean visual P300 in WM0.

## Discussion

The present study explores the relationships between cognitive performance and endogenous DHEA, DHEAS and cortisol. As expected, the audio-visual distraction paradigm including a manipulation of working memory, yielded typical effects observed in previous studies. Indeed, the WM load task was harder to perform, as revealed by lower hit rates and longer reaction times. Likewise, novel sounds distracted the participants, as reflected by lower hit rates and longer reaction times. Regarding brain responses to novel sounds in both WM0 and WM1 tasks, the results revealed an enhanced P3 deflection, indicating that novel sounds caused distraction when compared to standards. The N2b elicited by the task-relevant visual stimuli was enhanced under WM load as expected [51].

DHEA and cortisol responses were directly related (independently of the WM load content of the task). This is in agreement with the fact that corticotrophin releasing hormone (CRH) stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for DHEA secretion [59,60], even if indirectly through the action of an unidentified DHEA androgen stimulating hormone [61,62,63].

Nevertheless, we found important differences between DHEA and cortisol responses with WM load manipulation. DHEA levels increased with the performance of the second task independently of the task, suggesting either a cumulative effect or a latent interval before the response. Still, the increase in DHEA levels was more pronounced with WM load. Thus, the effect of a greater cognitive effort or specific effects of WM load on DHEA levels are suggested. Interestingly, this response is specific for DHEA and does not occur with cortisol and DHEAS. In fact, regarding cortisol, a decrease was found when the subjects were performing the discrimination task (WM0). Whatever the specific mechanisms may be, there is an interesting point: the distinctive pattern of cortisol and DHEA responses. Thus, cortisol decreases after a simple task if this task comes first and DHEA increases after a second cognitive task when this is a challenging task.

Stangl *et al.* [64] demonstrated that DHEA administration increased DHEA levels and enhanced performance in a visual same-different task (without WM load) while cortisol levels remained constant. Also, those authors described baseline cortisol relations

to performance. However, other studies failed to provide systematic evidence that DHEA and DHEA administration enhanced short-term memory at the performance level [16,19]. In the present study, endogenous levels of DHEA, DHEAS and cortisol were measured and no significant relations were found with the accuracy or latency of the response. Nevertheless, a bigger sample of subjects may be necessary to uncover eventual relations.

On the other hand, the present study demonstrated endocrine relations to the electrophysiological recordings. In the WM task, higher baseline cortisol/DHEA ratio was related to more processing of the distracting stimuli, as indexed by an enhanced novelty-P3. This suggests that baseline cortisol enhances, whereas baseline DHEA prevents auditory distraction, and simultaneously suggests an anti-cortisol effect of DHEA. The fact that this relation became evident in the most stressful situation (WM load) agrees with previous evidence showing that DHEA has anti-cortisol effects under stress [12,14] or that these effects are more evident under stress.

Regarding ERPs to the visual target stimuli, DHEA effects on WM load pointed towards increased visP300 amplitudes. In fact, the DHEA raise due to WM load was related with enhanced P300 amplitudes indicating enhanced memory update and suggesting a rapid DHEA behavioral effect.

Endocrine responses to stimuli are commonly used and they usually provide higher sensitivity than baseline levels to detect pathological conditions or inter-subjects differences [29,65,66]. As an example, glucocorticoids responses to different stimuli can be used to measure the adrenals functional reserve (predicting their response to stress) or to characterize different phenotypes of the stress response (which were related to personality traits and pathological conditions) [67,68,69,70,71]. Accordingly, in the present study, the parameter related to working memory processing was DHEA response and not baseline DHEA. Also, apart from its slow genomic effects, corticosteroids are known to have rapid non-genomic central nervous system effects [35,39,40].

Alternatively, the relation between visual P300 amplitude and DHEA response could suggest that subjects with enhanced working memory update are the ones with higher DHEA responses. In that regard, other authors demonstrated that chronic stress

and higher cortisol levels were related with poorer memory [34,37,38] and reduced DHEAS response to a superimposed psychological stress [29]. Nevertheless, we found no relation between baseline cortisol and visual P300 amplitude, and therefore, an effect of chronic stress is not suggested.

Wolf *et al*. [43] studied the effects of DHEA replacement on short term memory ERPs. They reported an increase in P3 amplitude after DHEA replacement, which reflects an enhancement of information updating. This is in accordance with our results, as we also observed that the physiological DHEA increase was related to an enhanced visP300.

A short term increase in cortisol can damage hippocampal neurons and may impair memory [42]. This may be an oversimplification since specific types of hippocampal mediated memory may be impaired by stress but others may not [72,73]. Yet, and hypothesizing that DHEA might prevent memory impairment under stress, Wolf et al [74] found that DHEA replacement enhanced attention but did not prevent the decline in visual memory under an acute psychosocial stress. This result did not support the idea of a direct anti-glucocorticoid effect of DHEA in hippocampal mediated memory functions. In another study DHEA protected hippocampal neurons against excitatory amino acid-induced neurotoxicity [75]. Our results also support the idea of DHEA anti-cortisol effects in distraction, but regarding working memory, we found relations with DHEA, not cortisol. Moreover, normal ranges of baseline cortisol were observed and cortisol levels did not rise with WM load, they just did not decrease.

Recent results also suggest that repeated stress and consequent activation of the glucocorticoid receptors dampens prefrontal cortex glutamatergic transmission. Actually, it facilitates glutamate receptor turnover, which has a detrimental effect on prefrontal cortex-dependent cognitive processes [76] like WM. The present results agree with the known action of DHEA on glutamatergic receptors as well as with the idea that DHEA opposes cortisol detrimental effects during the performance of working memory tasks under stress. This last relation was evidenced by inverse relations of DHEA and cortisol to distraction.

Nevertheless, working memory effects were related to the DHEA response but not to cortisol. Thus, regarding WM effects, an anti-cortisol effect of DHEA is not so evident

and other specific effects of DHEA may be present. As mentioned, besides their anticortisol effects, DHEA has Gamma Aminobutyric Acid Type A (GABAA) receptor antagonism and sigma 1 agonist effects [3,9,10] which might underlie or contribute to the effects found. In fact, both gabaminergic antagonism and glutamatergic agonism are known to improve cognitive function.

Eventually in relation to DHEAS' long half-life [2,3], its levels did not change with WM load manipulation. Also, we found no relations between baseline DHEAS and performance or ERPs. Nevertheless, we found no relations between baseline DHEA and performance or ERPs either. Instead, baseline cortisol/DHEA ratio and DHEA response relations to ERPs were found.

DHEA metabolites also include other neuroactive steroids such as estradiol, estrone and testosterone [19,77], which may mediate part of the DHEA effects, namely after DHEA administration [78]. Estrogens, in particular estradiol, enhance working memory in women [79,80] and testosterone supplementation may enhance working memory in older men [81,82]. We did not measure DHEA metabolites, which may mediate some of its effects. Subsequently, we cannot exclude that those steroids may contribute or eventually be responsible for the performance and electrophysiological relations we found. Finally, as our study includes only female participants, the outreach of the results is limited only to women. DHEA and DHEAS are androgens and androgen levels, namely testosterone and DHEAS levels, are higher in men than in women [2,7]. Therefore, another group of participants would be necessary to study the electrophysiological correlates of DHEA and DHEAS in men. For further studies it would be relevant to study whether the results are identical or distinct according to gender.

**In summary,** a higher cortisol/DHEA ratio was related to enhanced processing of the auditory distractor during the performance of a visual working memory task. This suggests that in women, DHEA may oppose cortisol effects in involuntary distraction, reducing the processing of the auditory distractor (novelty-P3). Regarding working memory, DHEA increased with the performance of consecutive cognitive tasks, and a higher DHEA response due to WM load was related to an enhancement of the task-relevant information processing (visual P300). Overall, the results suggest that DHEA may

oppose cortisol effects reducing distraction and a higher DHEA response may enhance working memory at the electrophysiological level.

**Supporting Information**

**Table S1. Endocrine relations to performance.** ANOVAs of the performance parameters that covariated with endocrine measurements. Results represent the interaction between endocrine parameters and working memory (WM) condition and auditory stimulus. Baseline endocrine interactions are significant when $p<0.013$. ΔDHEA response=DHEA response in WM1 – DHEA response in WM0. WM0 – discrimination task; WM1 – working memory task. (DOCX)

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**Author Contributions**

Conceived and designed the experiments: SV LS JMM CE. Performed the experiments: SV LS. Analyzed the data: SV. Contributed reagents/materials/analysis tools: SV LS ACG. Wrote the paper: SV. Provided critical revision of the manuscript: LS JMM ACG MB IC CE.

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**Supporting Information**

**Table S1: Endocrine relations to performance.**

| | | ANOVA | |
|---|---|---|---|
| **Hit Rates** | | | |
| Baseline Cortisol x | Task (WM1, WM0) | F (1,21)=5.956 | p=0.024 |
| | Sound (standard, novel) | **F(1,21)=8.222** | **p=0.009** |
| | Task x Sound | **F(1, 21)=11.751** | **p=0.003** |
| Baseline Cortisol/DHEA ratio x | Task | **F(1,20)=8.186** | **p=0.01** |
| | Sound | **F(1,20)=13.178** | **p=0.002** |
| | Task x Sound | **F(1,20)=17.609** | **p<0.001** |
| Δ DHEA response x | Task | **F(1,20)=8.087** | **p=0.01** |
| | Sound | F(1,20)=1.827 | p=0.192 |
| | Task x Sound | F(1,20)=5.671 | p=0.027 |
| **Response times** | | | |
| Baseline Cortisol x | Task | F(1,21)=1.129 | p=0.300 |
| | Sound | F(1,21)=4.441 | p=0.047 |
| | Task x Sound | F(1,21)=3.038 | p=0.096 |
| Baseline Cortisol/DHEA ratio x | Task | F(1,20)=0.467 | P=0.502 |
| | Sound | F(1,20)=6.489 | p=0.019 |
| | Task x Sound | F(1,20)=2.104 | P=0.162 |
| Δ DHEA response x | Task | F(1,20)=3.304 | p=0.084 |
| | Sound | F(1,20)=2.349 | p=0.141 |
| | Task x Sound | **F(1,20)=10.734** | **p=0.004** |

# Study III

## Hormonal Modulation of Novelty Processing in Women: Enhanced under Working Memory Load with High DHEAS/DHEA ratios

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**Submitted for publication**

## Abstract

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) have been suggested to enhance working memory and attention. However, the administration of DHEA alone did not prove to have any beneficial results on memory or attention. The balance between both forms of the hormone might be crucial regarding the effects that DHEA and DHEAS exert on the central nervous system. We studied the DHEAS-to-DHEA ratio in relation to involuntary attention and working memory processing by recording the electroencephalogram of 22 young women while performing a working memory load task and a task without working memory load in an audio-visual oddball paradigm. DHEA and DHEAS were measured in saliva before each task. We found that a higher DHEAS-to-DHEA ratio was related to enhanced auditory novelty-P3 amplitudes during performance of the working memory task. These results suggest that the balance between DHEAS and

DHEA levels modulates involuntary attention. In particular, a higher DHEAS-to-DHEA ratio might be related to an enhanced acoustic novelty processing without any detrimental effect on working memory processing. Moreover, these results suggest that high DHEAS-to-DHEA ratio might enhance involuntary attention to the surrounding world during the performance of cognitive tasks, probably indicating an important protective mechanism.

**Key-words:** dehydroepiandrosterone, dehydroepiandrosterone-sulfate, dehydroepiandrosterone-sulfate to dehydroepiandrosterone ratio, working memory, involuntary attention, distraction, novelty-P3, event-related potentials, sulfotransferase, sulfatase.

## Resumo

Têm sido sugeridos efeitos benéficos da desidroepiandrosterona (DHEA) e da desidroepiandrosterona-sulfato (DHEAS) ao nível da memória e da atenção. Contudo, a administração isolada de DHEA, não mostrou efeitos benéficos na memória nem na atenção. O balanço entre ambas as formas da hormona poderá ser determinante para os efeitos da DHEA e DHEAS ao nível do sistema nervoso central. Neste estudo, explorámos qual é a relação da razão DHEAS/DHEA com a atenção involuntária e a memória de trabalho. Para isso, registámos o eletroencefalograma de 22 mulheres jovens adultas, enquanto realizavam uma tarefa com memória de trabalho e outra tarefa sem memória de trabalho, usando um paradigma de "oddball" audiovisual. Os níveis de DHEA e DHEAS foram medidos na saliva antes de cada tarefa. Durante a realização da tarefa com memória de trabalho, uma razão DHEAS/DHEA mais elevada relacionou-se com maiores amplitudes da deflexão P3-novidade ("novelty-P3"). Este resultado sugere que a atenção involuntária é modulada pelo balanço entre os níveis de DHEAS e DHEA. Em particular, uma razão DHEAS/DHEA mais elevada poderá estar relacionada com maior processamento da novidade acústica, sem um efeito deletério no processamento da

memória de trabalho. Estes resultados sugerem ainda, que uma razão DHEAS/DHEA elevada poderá promover a atenção involuntária em relação ao meio envolvente, durante a realização de tarefas cognitivas, o que poderá constituir um mecanismo protetor importante.

**Palavras chave:** desidroepiandrosterona, desidroepiandrosterona-sulfato, razão DHEAS/DHEA, memória de trabalho, atenção involuntária, distração, P3-novidade, potenciais evocados, sulfotransferase, sulfatase.

## Introduction

### Dehydroepiandrosterone-sulfate-to-dehydroepiandrosterone balance in relation to memory and attention

The physiological effects and mechanism of action of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) is still a matter of debate, yet evidence has been accumulating concerning their relationship to cognition and the stress response. In particular, higher DHEAS concentrations were related to improved memory whereas low levels were found in Alzheimer's disease (Weill-Engerer *et al.,* 2002). Higher DHEA, DHEAS, DHEA-to-cortisol and DHEAS-to-cortisol levels were related to better cognitive function (van Niekerk *et al.,* 2001; Carvalhaes-Neto *et al.,* 2003; Davis *et al.,* 2008), improved attention (Wolf *et al.,* 1998a), lower perceived stress and improved performance under stressful conditions (Morgan CA 3rd *et al.,* 2009; Wemm *et al.,* 2010; Russo *et al.,* 2012) when compared to lower DHEA, DHEAS, DHEA-to-cortisol and DHEAS-to-cortisol levels. Anti-cortisol effects of DHEA and DHEAS have been hypothesized to be contributing factors to these changes.

Most studies have not explored the effects of the differences between DHEAS and DHEA levels, even though sulfated steroids in general could act as endogenous neuromodulators (Gibbs *et al.,* 2006) and the balance between DHEAS and DHEA levels could influence brain functioning and cognition (Maninger *et al.,* 2009; Gibbs *et al.,* 2006). Subjects with Alzheimer's disease were shown to have reduced concentrations of DHEAS (Weill-Engerer *et al.,* 2002) but increased concentrations of DHEA (Brown *et al.,* 2003) in the central nervous system, or reduced conversion of DHEA into DHEAS and consequently, reduced DHEAS-to-DHEA ratios (Kim *et al.,* 2003). On the other hand, steroid sulfatase inhibition in mice, increased DHEAS-to-DHEA ratios and impaired accuracy under attention demanding conditions (Davies *et al*., 2009). In addition, subjects with steroid sulfatase deficiency have higher rates of attention deficit hyperactivity disorder, with predominantly lack of attention symptoms (Kent *et al*., 2008).

DHEA and DHEAS present in general a neurostimulatory effect. Nevertheless, DHEAS has a much more potent excitatory action than DHEA concerning glutamatergic agonism (Urani *et al*., 1998; Zou *et al.,* 2000) and gabaminergic antagonism (Imamura and Prasad, 1998; Park-Chung *et al.,* 1999), which may account for different effects of either form of the hormone (Monnet *et al.,* 1995; Baulieu and Robel, 1998; Imamura and Prasad, 1998). In particular, working memory relies on glutamatergic transmission (Yuen *et al.,* 2011). Therefore, DHEAS might have more potent enhancing effects on memory than DHEA and the evaluation of the DHEAS-to-DHEA ratio may uncover additional information that otherwise would not be revealed by the individual examination of either form of the hormone.

**Involuntary attention under working memory load**

Selective attention to stimuli in the surrounding world is fundamental for adaptive behavior (SanMiguel *et al.,* 2008). Salient exogenous stimuli are known to capture attention involuntarily and endogenous mechanisms do voluntarily modulate brain responses to confront target stimuli (Arnott *et al.,* 2001; Hopfinger and West, 2006). Nevertheless, attentional resources are limited, as evidenced by dual-task and task-

switching experiments (Pashler, 1993; Pashler, 1994; Rogers and Monsell, 1995; Barcelo *et al.,* 2006), and interactions between exogenous and endogenous mechanisms play a role in attention control (Yantis and Jonides, 1990; Pashler *et al.,* 2001; SanMiguel *et al*., 2008). Salient exogenous stimuli are known to capture attention involuntarily, causing reduced voluntary attention to the task relevant stimuli and therefore disrupting responses to target stimuli in a bottom-up way, and causing a deteriorating effect on performance (Yantis and Jonides, 1990, Escera *et al.,* 1998; Escera *et al.* and 2000; Escera and Corral, 2007). On the other hand, involuntary attention to exogenous stimuli can also be modulated by voluntary top-down mechanisms adjusted to behavioral goals and cognitive load (Pashler et al., 2001; Lavie *et al.,* 2004; Vuilleumier, 2005). The novelty-P3 component elicited by salient exogenous stimuli and denoting the effective orientation of attention towards the distractor, is particularly sensitive to top-down mechanisms of attention (Escera *et al.,* 2003; Escera *et al.,* 1998) and to cognitive load (Lavie *et al.,* 2004; SanMiguel *et al*., 2008).

Working memory may play a special role on attention control. In fact, working memory modulates endogenous and exogenous mechanisms of attention (Hester and Garavan, 2005; SanMiguel *et al.,* 2008), as it provides top-down signals to other brain structures (Miller, 2000; Fuster, 2001; Miller and Cohen, 2001) and also modulates involuntary attention processing at primary sensory areas of the cortex, effectively reducing responsiveness to a distractor (Pinsk *et al*., 2004; Spinks *et al.,* 2004; Hopfinger and West, 2006; Postle, 2006; SanMiguel *et al.,* 2008). Nevertheless, contradictory data exists regarding the direction of this influence, some results suggesting increased involuntary attention (distraction) with increased working memory load (DeFockert *et al*., 2001; Lavie *et al.,* 2004; Lavie, 2005; Lavie and DeFockert, 2005) while other results suggesting reduced involuntary attention during working memory load (Berti and Schroger, 2003; Postle, 2006; SanMiguel *et al.,* 2008). Of note, although attentional resources are limited, being alert to relevant information from the surrounding world during the performance of a working memory task is important. For instance, being alert to signals that indicate danger can be critical for physical integrity.

**DHEAS and DHEA relations to memory and attention at the electrophysiological level**

Studies addressing DHEA and DHEAS effects on memory and attention at the electrophysiological level are scarce. Nevertheless, DHEA administration increased DHEAS levels and enhanced P300 amplitudes, reflecting enhanced information regarding to updating of short-term memory (Wolf *et al.,* 1998b); higher DHEA levels were related to shorter P300 latencies (Braverman *et al.,* 2009) when compared to lower DHEA levels; and higher increases in DHEA levels during the performance of a working memory task were related to enhanced P300 amplitudes (do Vale *et al*., 2014). Therefore, in accordance with results at the clinical level, the results at the electrophysiological level suggest a relationship between higher DHEA and DHEAS levels and improved memory. The electrophysiological correlates of the balance between DHEAS and DHEA with regard to working memory processing are unknown. Besides, the effects of DHEAS-to-DHEA balance in the control of involuntary attention during working memory tasks have yet to be explored.

The aim of this study was to test whether the DHEAS-to-DHEA ratio has an influence on working memory and involuntary attention at the electrophysiological level. Our hypothesis was that the DHEAS-to-DHEA relation would modulate working memory processing and/or involuntary attention, and that these effects would be evident at the electrophysiological level. To test this hypothesis, we measured baseline DHEAS and DHEA and analyzed brain responses during the performance of a well-established auditory-visual distraction paradigm (Escera *et al.,* 1998; Escera *et al.,* 2000; Escera and Corral, 2007; SanMiguel *et al.,* 2008; do Vale *et al.,* 2014). The protocol included task irrelevant sounds, some of which were novel and aimed at causing distraction (involuntary attention) and a visual task which included working memory manipulation (SanMiguel *et al*., 2008; do Vale *et al*., 2014). In this paradigm, the unexpected auditory novel sounds were expected to deteriorate performance and elicit a novelty-P3 enhancement (Escera *et al*., 1998; Escera *et al*., 2000; Friedman *et al*., 2001; do Vale *et al*., 2014). The task with working memory load is more difficult to perform and therefore typically leads to a deterioration in performance and a reduction of the P300, reflecting a

lower processing of the task-relevant visual stimulus (Kok, 2001; Polich, 2007; SanMiguel *et al.,* 2008; do Vale *et al.,* 2014).

## Subjects and methods

### Subjects

28 healthy females (18 to 25 years old, mostly undergraduate Psychology students) (do Vale *et al.,* 2014) participated in the experiment. Because DHEAS levels differ according to gender, only women were included in the study. Non-corrected visual deficits, auditory deficits, neurologic, psychiatric, endocrine or oral diseases were exclusion criteria. Besides hormonal contraception, no other medications were allowed. Regular or binge alcohol consumption as well as smoking or illicit drug consumption were further exclusion criteria and subjects were asked not to consume alcohol in the twelve hours before the experimental protocol. Prior to the experimental session, the participants completed the State and Trait Anxiety Inventory (Spielberger *et al*., 1988) and all showed a normal range of state and trait anxiety levels (do Vale *et al.,* 2014). Six subjects were discarded from the analysis due to technical problems with electroencephalogram (EEG) recordings or endocrine measurements. Therefore, 22 subjects with complete data were retained for the analysis. In this group, mean age was 20±0.5 years, all the participants were right-handed and presented a normal body mass index (21.9±0.5 kg/$m^2$) and only three participants were under hormonal contraception.

The experimental protocol was approved by the ethical committees of the University of Barcelona and Lisbon Medical School and it was conducted according to the principles of the Declaration of Helsinki. All subjects gave their written informed consent before entering the study. During the study protocol, DHEA, DHEAS and cortisol were both measured before and after the task. The results concerning the relationship between DHEA, DHEAS, cortisol and cortisol/DHEA ratio and working memory and

distraction at the behavioral and electrophysiological level were the subject of a previous study and were published elsewhere (do Vale *et al.,* 2014).

**Task and procedure**

We used an adapted version of the paradigm used by SanMiguel *et al.* (2008). It consists of two visual tasks: one a discrimination task (no working memory load, WM0) and another one with a working memory load (WM1). The two tasks were performed two hours apart from each other (from onset to onset) and the order of the tasks were counterbalanced across the participants. Each task lasted about 15 minutes, and comprised of two blocks, containing 250 trials each. A short break was allowed between blocks (do Vale *et al.,* 2014).

The experiment was performed in the afternoon (beginning at 2-3 pm), with the participants sitting in a comfortable chair inside an electrically and acoustically shielded room. In the discrimination task (WM0) participants had to decide whether the two digits appearing on the screen were the same (11 and 22) or different (12 and 21); see figure 1A. In the WM1 task (1-back task) participants had to decide whether the left or right digit (counterbalanced across participants) on the screen was the same as the left or right digit of the previous trial (figure 1B) (do Vale *et al.,* 2014), therefore, keeping one digit in working memory until the next trial and answering for every pair of digits appearing on the screen. Responses were given through a mouse button (one mouse button for "same" and the other button for "different"), also counterbalanced across subjects. The participants were instructed to ignore the sounds and respond as quickly and accurately as possible. Furthermore, they were asked to minimize blinking and to focus on a central fixation cross (appearing between each two digits on the screen), in order to reduce eye-blink and movement artifacts during EEG recording. Before each task, subjects performed practice blocks (composed of 10 trials) without any auditory stimuli until they reached a hit rate of at least 80% in each task (do Vale *et al*., 2014).

Each trial consisted of a task-irrelevant auditory stimulus, followed by a visual target stimulus (the interval between each auditory and visual stimulus was 350ms, onset

to onset); see figure 1. Trial length was 1250ms on average (varying from 1000 to 1500ms; jitter +/-250ms). The auditory stimuli consisted of repetitive standard tones (200 ms, including fade-in and fade-out of 10ms each; 600 Hz; 85dB; 80% probability), occasionally replaced by environmental novel sounds selected from a sample of 100 different exemplars (edited to have a duration of 200ms, including fade-in and fade-out of 10ms each; digitally recorded, low-pass filtered sound at 10,000 Hz; 85dB; 20% probability), similar to those produced by a drill, hammer, rain, door, telephone ringing, and so forth (Escera *et al*., 2003; do Vale *et al*., 2014). All sounds were randomly delivered binaurally through headphones (Sennheiser HD 202), and the only restrictions were that the first four stimuli of each block were standard tones, that two novel sounds never appeared consecutively and that each novel sound occurred only once in each task. The visual stimuli consisted of pairs of digit combinations, with the digits 1 and 2 (11, 12, 21 or 22), presented on a computer screen for 200ms. Appearance probability was the same for every digit combination. The picture size was 357x441 pixels, with a vertical angle of 8º and a horizontal angle of 18º, accounting for two pictures presented simultaneously with the fixation cross in between. The distance from the subjects' eyes to the screen was 100cm (do Vale *et al.,* 2014). Each working memory setup (WM0 and WM1) consisted of 500 trials (400 standard trials and 100 novel trials).

Response time and whether the participant gave the correct answer for each trial was registered with Presentation® (Neurobehavioral Systems Inc., Albany, CA, USA). A hit was considered if the participant pressed the correct mouse button during the response window (from image presentation until the onset of the next trial/sound). We computed distraction as the difference in hit rate or response time between auditory stimulus types (hit rate: WM0standard trials - WM0novel trials and WM1standard trials - WM1novel trials; response time: WM0novel trials - WM0standard trials and WM1novel trials - WM1standard trials). Working memory load costs were computed taking into consideration the difference in hit rate or response time between the WM load and the discrimination task (hit rate: WM0standard - WM1standard; response time: WM1standard - WM0standard) (do Vale *et al.,* 2014).

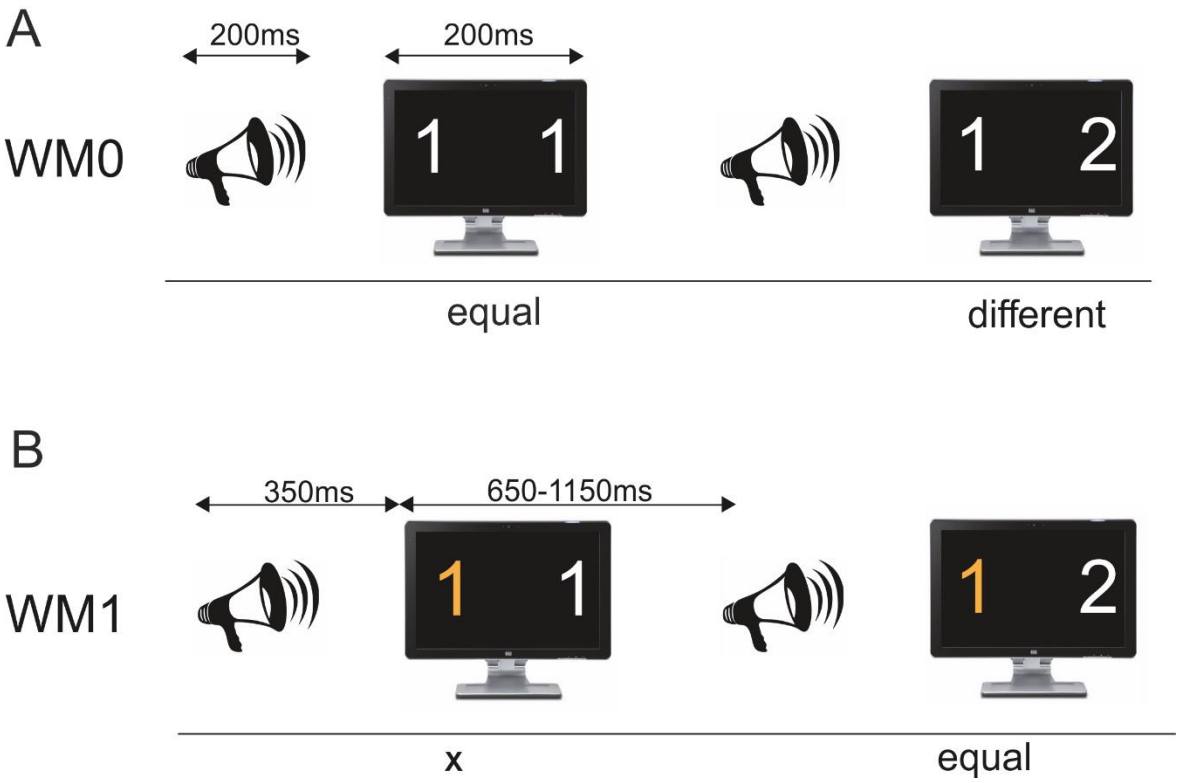


**Figure 1.** Illustration of the stimulation sequence (above the line) and correct responses to the tasks (below the line) for the two conditions. A) Discrimination task (WM0), in which subjects had to decide whether the two digits on the screen were equal or different. B) Working memory task (WM1), in which the subjects had to compare the left digit on the screen with the left digit of the previous trial. WM0 – discrimination task; WM1 – working memory task (do Vale *et al.,* 2014).

**EEG recording and analysis**

The electroencephalogram was continuously recorded during task performance, using 64 scalp Ag/AgCl electrodes contained in elastic caps, following the extended 10/10 convention. The horizontal and vertical electrooculograms were recorded with electrodes placed at the outer canthus and below the right eye, respectively. An electrode placed on the tip of the nose was used as the common reference and the ground was located at the AFz position. The EEG and electrooculogram (EOG) were amplified and digitized at a sampling rate of 512Hz (Eemagine, ANT Software b.v., Enschede, the Netherlands). Impedances were kept at 5kΩ or below during the whole recording session. Recording was performed with an ANT amplifier of 64 channels (gain 20x; A/D resolution 22 bits, 71.526nV per bit; filtering 0-138.24Hz; CMRR >90dB) (do Vale *et al.,* 2014).

Continuous EEG data were bandpass filtered between 0.01 and 30Hz (with a digital finite impulse response filter using a Hamming window). Event-Related Potentials (ERPs) were averaged offline for each auditory stimulus type and working memory condition, for an epoch of 1000ms, including a 200ms pre-auditory-stimulus baseline (do Vale *et al.,* 2014). Only the epochs with correct responses were retained to calculate the average. The first five epochs of each block, the epochs following a novel trial and the epochs with missed or wrong responses were excluded also from the total average.

To perform EOG correction we manually selected a large number of typical artifacts and applied a regression algorithm to compute propagation factors (Eeprobe 3.1, ANT Software BV, Enschede, The Netherlands). After EOG correction, epochs that contained EEG activity exceeding ±100μV peak-to-peak amplitudes were excluded from averaging (do Vale *et al.,* 2014). Because only trials with correct responses were included and the hit rate was smaller for the working memory load task, the final number of trials were smaller for the working memory load when compared with the discrimination task [$t=6.320$, $p<0.001$; 326 trials for the discrimination task and 272 trials for the working memory load task]. The mean number of trials included in the averages for each condition and auditory stimulus type were: 245 trials with standard sounds and 81 with novel sounds in the discrimination task and 210 trials with standard sounds and 62 trials with novel sounds in the working memory load task.

As participants were specifically instructed to ignore the sounds, any related effects were necessarily involuntary or led by exogenous attention. ERPs recorded during this auditory-visual distraction paradigm typically present first an auditory N1-enhancement (mismatch negativity), reflecting a detection mechanism that leads to attention capture (Escera *et al.,* 1998), followed by a novelty-P3 (nov-P3) that reflects the effective attention orientation (Escera *et al.,* 1998; Escera *et al.,* 2000; Friedman *et al.,* 2001). Finally, the re-orienting negativity (RON) reflects the attention re-orientation back to the task (Schröger and Wolff, 1998). The target is visual and visual ERPs yield sensory (visual P1 and N1) and cognitive components related to target processing (N2b and P300). The P300 component reflects the processing of the task-relevant information of the visual stimulus (Kok, 2001; Polich, 2007; SanMiguel *et al.,* 2008). The task with working memory

load is expected to be more difficult to perform, leading to a reduced P300, longer response time and lower hit rates (Kok, 2001; Polich, 2007; SanMiguel *et al*., 2008; do Vale *et al.,* 2014).

To analyze the effects of distraction on ERPs, difference waves were calculated by subtracting the ERPs elicited in standard trials from those elicited in novel trials. These difference waves revealed an early-onset, long-lasting positive deflection that we considered as integrating part of the novelty-P3 (do Vale *et al.,* 2014). The novelty-P3 was measured as the mean amplitude and mean peak latency in a time window ranging from 250 to 380 ms at F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrodes (do Vale *et al.,* 2014). To analyze the effects of working memory we compared ERP measurements in WM0 and WM1, using only standard trials. Specific auditory (N1 and P2) and visual (P1 and N1) components were elicited during task-performance, as expected. Nevertheless, because we aimed to study cognitive processing, we only analyzed the P300. This component was measured as the mean amplitude and mean peak latency in a time window ranging from 650 to 910ms (300–560ms from visual stimulus presentation) at P3, Pz and P4 electrodes. Moreover, since the P300 latency was different for WM0 and WM1 conditions [$F_{(1,21)}$=5.006, $\eta^2$=0.193, p=0.036; 751±13ms for WM1; 783±7ms for WM0], the amplitude of this component was analyzed at different time windows for each condition (WM1: 650-875ms; WM0-670-910ms) (do Vale *et al.,* 2014).

**Endocrine measurements**

Saliva samples were collected by passive drool before each task. Samples collected before the first task (i.e. before both tasks) were considered as baseline (do Vale *et al.,* 2014). Unlike DHEA, which is liposoluble and crosses cell membranes (Vining and McGinley, 1987; Ahn *et al*., 2007), DHEAS is not lipid soluble and penetrates into the saliva through tight junctions. Therefore, DHEAS concentrations in saliva depend on serum concentration and salivary flow rate (Vining and McGinley, 1987).

Samples were refrigerated at 2-8ºC within 30 minutes after collection and stored at -20ºC within 4h until assayed (do Vale *et al.,* 2014). Each sample was measured in

duplicate by using enzyme-linked immunoassays: salivary DHEA and DHEAS enzyme immunoassay kits (Salimetrics Europe®, Ltd, Newmarket Suffolk, UK). DHEA was measured in pg/mL. Due to the influence of saliva flow rates on DHEAS levels, the concentration of DHEAS (pg/mL) was multiplied by the flow rate (mL/min) and the corrected results were obtained as DHEAS measured per unit of time (pg/min). The minimal concentrations that can be distinguished from 0 with the used immunoassays are 5pg/mL for DHEA and 43pg/mL for DHEAS. Intra- and interassay variation coefficients were less than 10% and 15%, in both cases, respectively. DHEA was expressed as pmol/L by using the conversion 3.467, and DHEAS was expressed as pmol/h by using the conversion factor 0.16284 (system of international units = conventional units x conversion factor).

**Statistical analysis**

Statistical analysis was performed with the Statistical Package for Social Sciences Program (IBM SPSS Statistics, version 21). Results are presented as mean ± standard error of the mean (SEM) and the normal distribution of continuous variables was verified by the Kolmogorov-Smirnov Goodness of Fit Test (do Vale *et al.,* 2014). The effects of WM load and auditory distraction on performance were explored by performing repeated measures analyses of variance (ANOVAs) on the hit rate and response time, including the within subjects' factors task (WM0 and WM1) and sound (standard and novel). The effects of WM load and auditory distraction on ERPs were explored by performing ANOVAs on auditory-P3 mean amplitude [including the within subjects' factors task (WM0 and WM1) and sound (standard and novel)], novelty-P3 and visual P300, mean amplitude and peak latency [including the within subjects' factor task (WM0 and WM1)] (do Vale *et al.,* 2014).

Pearson´s correlations were used to explore the relation between DHEAS/DHEA ratios and performance and electrophysiological responses. In both cases involuntary attention and working memory effects were considered. Concerning performance, we explored the relation between DHEAS/DHEA ratio and hit rate as well as response time

distraction (involuntary attention) costs in each condition (WM0 and WM1). We also examined the relation between DHEAS/DHEA ratio before WM1 and hit rate and response time costs of working memory. Regarding electrophysiological responses, in order to explore the relation between DHEAS/DHEA ratio and the effects of involuntary attention to novel sounds, we analyzed the relation between DHEAS/DHEA ratio before the performance of each condition and the respective novelty-P3 amplitudes and latencies. To explore the relation between DHEAS/DHEA ratio and working memory electrophysiological effects, the visual P300 amplitudes and latencies in WM1 minus WM0 were calculated (the resultant variables corresponded to working memory effects on visual P300 amplitudes and latencies) and their relationship to DHEAS/DHEA ratio before WM1 were also analyzed. The limit of significance chosen was $\alpha=0.05$ and the Bonferroni correction was applied for multiple comparisons. In order to explore the relations between endocrine measurements and performance, as well as the relations between endocrine measurements and ERPs, four correlations regarding distraction and two correlations regarding working memory effects were tested. Effect size estimates of the results were expressed as eta squared ($\eta^2$) for ANOVAs and correlation coefficients (r) for regression analyses.

## Results

### Performance and event related potentials

Performance and electrophysiological results were almost identical to those previously published, the main difference being to the fact that the present sample included one subject less (do Vale *et al*., 2014). There were main effects of task and the type of auditory stimulus on performance. Under working memory load, lower hit rates [$F_{(1,21)}=25.899$, $\eta^2=0.552$, $p<0.001$; hit rate was 83±2% in WM0 and 70±2% in WM1] and longer response times [$F_{(1,21)}=8.036$, $\eta^2=0.277$, $p=0.010$, response time was 440±9ms in WM0 and 467±14ms in WM1] were observed when compared to the discrimination task.

Novel sounds when compared to standard sounds, resulted in lower hit rates [$F_{(1,21)}$=8.327, $\eta^2$=0.284, p=0.009; hit rate was 79±1% for standard sounds and 74±2% for novel sounds] and longer response times [$F_{(1,21)}$=39.683, $\eta^2$=0.654, p<0.001; response time was 431±11ms for standard sounds and 476±11ms for novel sounds]. Besides, a significant interaction was observed between task and auditory stimulus type regarding hit rate [$F_{(1,21)}$=5.292, $\eta^2$=0.201, p=0.032], with lower hit rates for novel sounds when compared to standard sounds only in the working memory load task [$F_{(1,21)}$=7.840, $\eta^2$=0.272, corrected p=0.022 (uncorrected p=0.011); hit rate was 74±2% for standard sounds and 65±4% for novel sounds].

At the electrophysiological level, auditory distraction yielded significant novelty-P3 responses, as indexed by a main effect of the type of stimulus on auditory P3 amplitude [$F_{(1,21)}$=96.850, $\eta^2$=0.822, p<0.001; -3.6±0.5µV for standard sounds and +1.6±0.5µV for novel sounds], see figure 2. Working memory manipulation had no significant effect on novelty-P3 amplitudes, novelty-P3 peak latencies or visual P300 amplitudes. As mentioned before, visual P300 peak latencies were shorter in the working memory load task than in the discriminatory task [$F_{(1,21)}$=5.006, $\eta^2$=0.193, p=0.036; 751±13ms in WM1 and 783±7ms in WM0]. In both WM conditions, novelty-P3 amplitudes were not significantly related to visual P300 amplitudes and peak latencies.

**Endocrine levels and DHEAS-to-DHEA ratio relationship to performance and event-related potentials**

Baseline DHEAS, DHEA and DHEAS/DHEA ratio were 984±120 pmol/h, 790±155 pmol/L and 2.1±0.4, respectively. There was no significant difference between DHEAS, DHEA and DHEAS/DHEA ratios before each task. These parameters were not significantly related to age and body mass index (kg/m$^2$), and did not differ significantly to the menstrual cycle phase or between subjects taking or not taking hormonal contraception.

We found no significant relationship between DHEAS/DHEA ratios and performance parameters. Regarding event-related potentials, DHEAS/DHEA ratio before performance on WM1 was directly related to novelty-P3 amplitudes in the WM1 task

[r=+0.574, corrected p=0.010 (uncorrected p=0.005)], see figure 3. On the contrary, DHEAS/DHEA ratio was not related to novelty-P3 amplitudes or latencies in WM0, novelty-P3 latencies in WM1, visual P300 amplitudes or visual P300 latencies in the observed condition. Of note, the DHEAS/DHEA ratio was not related to novelty-P3 amplitudes attributed to working memory load (novelty-P3 in WM1 – novelty-P3 in WM0). Also, novelty-P3 amplitudes in WM1 were significantly related to the DHEAS/DHEA ratio independently of visual P300 amplitudes [novelty-P3 amplitude in WM1 as dependent factor and DHEAS/DHEA ratio before WM1 (partial p=0.008) and visual P300 amplitude in WM1 (partial p=0.487) as independent factors].

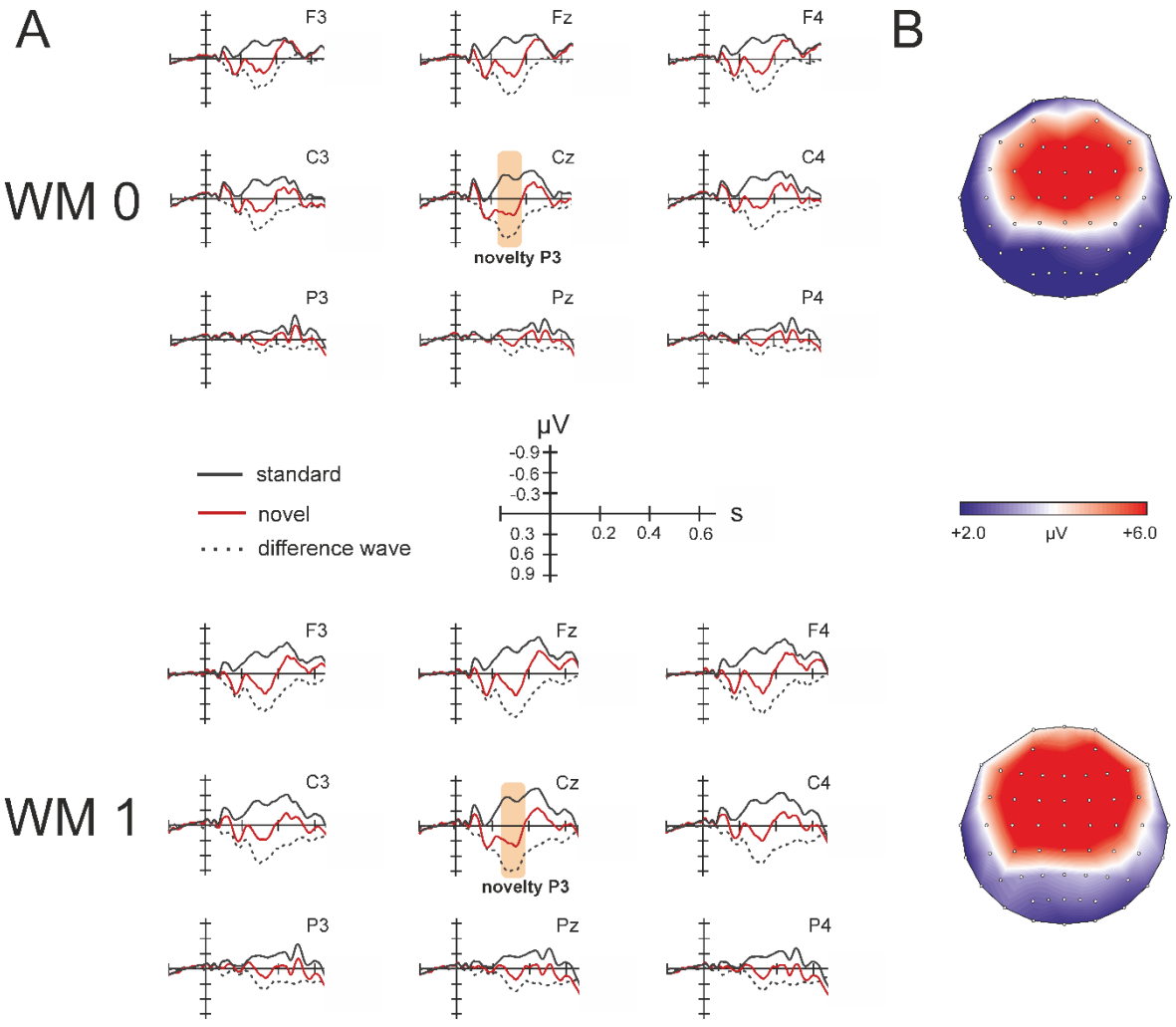


**Figure 2.** Event-Related Potentials. A) Grand-average waveforms for standard sounds, novel sounds and novel minus standard difference waves for both tasks (WM0 and WM1). B) Scalp map distribution of auditory-P3 difference waves (250-380 ms) for both tasks (WM0 and WM1). WM0 - discrimination task; WM1 – working memory task.

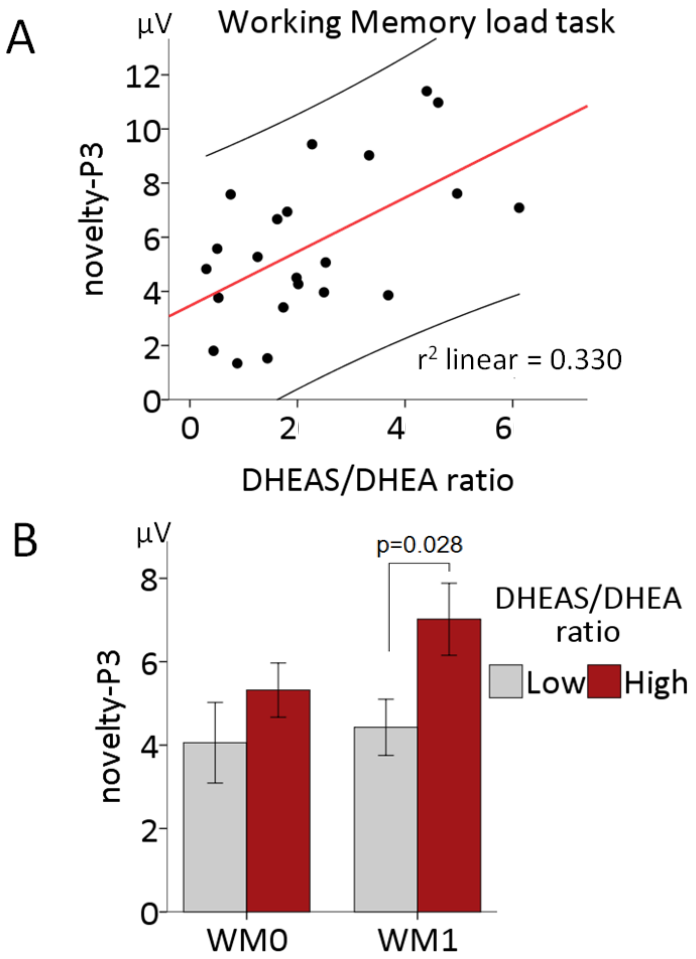


**Figure 3**. DHEAS/DHEA ratio relation to novelty-P3. A) DHEAS/DHEA ratio before the performance of the Working Memory load task was directly related to novelty-P3 amplitude during that task. B) Mean novelty-P3 in subjects with high and low DHEAS/DHEA ratio for each condition. DHEAS/DHEA ratios were dichotomized around the median: values below and above the median were considered as low and high DHEAS/DHEA ratios, respectively. In the working memory task, subjects with high DHEAS/DHEA ratio presented higher novelty-P3 amplitudes than subjects with low DHEAS/DHEA ratio [t=2.366, p=0.028, df=21; novelty-P3 amplitude was +7.0±0.9 µV in subjects with high DHEAS/DHEA ratio and +4.4±0.7 µV in subjects with low DHEAS/DHEA ratio]. In the discrimination task this difference was not significant. WM0 – discrimination task; WM1 – working memory task. Bars represent +/– standard error of the mean.

## Discussion

The present study showed a relationship between DHEAS-to-DHEA balance and brain processing of exogenous stimuli during the performance of a working memory task. We used an auditory-visual distraction paradigm with task-irrelevant sounds and a visual target with working memory manipulation. As published previously (do Vale *et al.,* 2014),

working memory load and auditory distraction deteriorated performance as expected. Hit rate was lower and response times were longer for the working memory task than in the discrimination task and response times were longer when accompanied with auditory distraction (SanMiguel *et al.,* 2008; do Vale *et al.,* 2014). In the working memory task, auditory distraction also caused significantly lower hit rates (do Vale *et al.,* 2014). At the electrophysiological level, significant novelty-P3 deflections were observed in both tasks, inferring enhanced processing of the novel sounds when compared to standard sounds and thus suggesting increased distraction by novel sounds (SanMiguel *et al.,* 2008; do Vale *et al*., 2014). Nevertheless, working memory manipulation had no significant effect on the electrophysiological response to auditory stimuli (do Vale *et al.,* 2014).

**DHEAS-to-DHEA ratio and performance**

Clinical findings in patients with Alzheimer's disease suggested beneficial effects of higher DHEAS-to-DHEA balance concerning memory (Weill-Engerer *et al.,* 2002; Kim *et al*., 2003; Brown *et al.,* 2003; George *et al.,* 2006). Moreover, the inhibition in the conversion of DHEAS to DHEA in rats, enhanced the hippocampal cholinergic function and improved memory (Rhodes *et al.,* 1996; Li *et al*., 1997; Rhodes *et al*., 1997; Urani *et al*., 1998). However, most studies using DHEA administration to humans did not study or control the resultant DHEAS-to-DHEA ratios (Wolf *et al.,* 1998a; Barnhart *et al.,* 1999; van Niekerk *et al*., 2001; Evans *et al.,* 2006; Goel and Cappola, 2011) which could have contributed to the inconclusive results of DHEA administration regarding memory and attention.

On the other hand, reducing the conversion of DHEAS into DHEA in mice impaired accuracy under attention demanding conditions (Davies *et al.,* 2009) and subjects with lower DHEA levels, lower DHEAS levels (Strous *et al.,* 2001) or steroid sulfatase deficiency (and therefore higher DHEAS-to-DHEA ratios) had higher rates of attention deficit hyperactivity disorder (Kent *et al*., 2008). These findings are in agreement with the present results in which a higher DHEAS-to-DHEA ratio was related to an enhanced processing of the auditory distractor. Nevertheless, in the present study the participants had normal DHEAS and DHEA levels and we found no deleterious effects of higher DHEAS-

to-DHEA ratio on working memory processing or performance. Of note, methylphenidate administered to boys with attention deficit hyperactivity disorder showed an increase in serum levels of both DHEAS and DHEA (proportionally more for DHEAS than DHEA) providing for a marked clinical improvement (Maayan *et al*., 2003). Therefore, besides DHEAS-to-DHEA ratio, DHEA and DHEAS levels seem important.

In the present study, no significant direct correlations between DHEAS-to-DHEA ratio and performance were observed. This might be due to the small number of participants. Furthermore, the DHEAS-to-DHEA ratio was related to the processing of the distracting task-irrelevant novel auditory stimuli. On the other hand, the DHEAS-to-DHEA ratio was not related to the task relevant visual stimuli processing, inferring that subjects with a higher DHEAS-to-DHEA ratio show an increased processing of the distracter which does not impact the task relevant processing and therefore does not lead to a deteriorated performance. Different processing strategies leading to a similar outcome might be employed between individuals with a high and low DHEAS-to-DHEA ratio. Individuals with an increased processing of non-task-related novelty might compensate distraction by making a greater effort in concentrating on the task. The increase in novelty-P3 and not the behavioral measures might therefore be a more direct index of DHEAS-to-DHEA balance.

**DHEAS-to-DHEA ratio relations to cerebral processing**

At the electrophysiological level, a significant new finding was described in the present study. Specifically, a higher DHEAS-to-DHEA ratio was related to an enhanced novelty-P3 during the performance of a visual working memory task. This suggests the relation between higher DHEAS-to-DHEA ratio and enhanced acoustic novelty processing during visual working memory tasks and more generally, higher DHEAS-to-DHEA ratios might be related to enhanced involuntary attention to exogenous stimuli during the performance of a cognitively challenging task. In a different context, we had previously found a relation between DHEAS-to-DHEA balance and brain processing under implicit negative emotional content (do Vale *et al.,* 2015a). Of note, DHEAS is the most abundant

hormone in the peripheral circulation and it is even more abundant in the brain. Yet, DHEAS and DHEA physiological effects are not established and their relations to brain processing are largely unknown (Baulieu and Robel, 1998; Komesaroff, 2008; Maninger *et al.,* 2009).

DHEA levels increase in response to stress (Lennartsson *et al.,* 2012) and may have anti-cortisol effects (Browne *et al.,* 1993; Herbert, 1997; Apostolova *et al*., 2005; Hennebert *et al*., 2007; Saponaro *et al.,* 2007; Balazs *et al*., 2008; Sorwell and Urbanski, 2010; Buoso *et al*., 2011; Russo *et al*., 2012). Higher DHEAS levels have been related to lower cortisol levels and reduced peak/baseline cortisol response to stress (do Vale *et al*., 2011; Kimonides *et al.,* 1998; Gruenewald *et al*., 2006). Moreover, an antagonism in DHEAS and cortisol synthesis and release by the adrenal gland in response to stress has also been hypothesized (Boudarene *et al.,* 2002). On the other hand, baseline DHEAS levels were found to be directly related to baseline DHEA/cortisol ratios (do Vale *et al.,* 2015a) and thus, the DHEAS-to-DHEA ratios were inversely related to cortisol levels (do Vale *et al.,* 2015b), suggesting some relation to lower stress levels.

Therefore, higher DHEAS-to-DHEA ratios might be a measure of lower chronic stress and cumulative burden and therefore a lower allostatic load (McEwen, 2004). Hence, the present results suggest that subjects with high DHEAS-to-DHEA ratio might have low chronic stress levels and therefore more attentional resources available during the performance of working memory load tasks. Long term transcriptional effects of high DHEAS-to-DHEA ratio, either directly or related to the inhibition of cortisol transcriptional effects could eventually contribute to a long term increase in attentional resources. Interestingly, we found that cortisol levels alone did not explain these results as they were not inversely related to novelty-P3 processing during the performance of the same working memory load task (do Vale *et al.,* 2014).

The novelty-P3 is an index of the effective orienting of attention towards a distracting event (Escera *et al*., 1998; Escera *et al*., 2000; Friedman *et al.,* 2001; SanMiguel *et al.,* 2008; do Vale *et al.,* 2014). Nevertheless, in the present study, the relation between higher DHEAS-to-DHEA ratio and increased involuntary acoustic novelty processing occurred with no deleterious effect on the visual target stimuli processing. In fact, no

relation between DHEAS-to-DHEA ratio and visual P300 amplitudes was found therefore suggesting that the increased involuntary attention to novel sounds did not distract the subjects from the visual working memory load task. Additionally, there was no relation to increased novelty-P3 or visual P300 latencies suggesting that the increased involuntary attention to novel sounds did not occur at the expense of longer stimulus evaluation times. In this context, the increased attention to unexpected novel sounds may be protective, maintaining an increased alertness to the surrounding world while voluntarily directing attention to the performance of the working memory load task.

Hence, the DHEAS-to-DHEA ratio may be an endogenous factor modulating exogenous attention mechanisms during the performance of cognitive tasks that require an active maintenance of information in working memory. Several studies have shown prefrontal cortex activation in working memory tasks, possibly inhibiting the processing of irrelevant information, leading to a reduction of distraction (Hopfinger *et al.,* 2006; Pinsk *et al.,* 2004; Spinks *et al.,* 2004; Miller, 2000; Jarrold and Towse, 2006). In this regard, it was suggested that distractor stimuli could trigger prefrontal cortex activity and suppress the input of sensory information, thus preserving the contents of working memory from being disrupted by the distractor stimuli (Postle, 2005; SanMiguel *et al*., 2008). Nevertheless, the present results do suggest that high DHEAS-to-DHEA ratio may contribute to enhanced activation caused by irrelevant stimuli while not interfering with the sensory activation related to the task.

The relations of the DHEAS-to-DHEA ratio to brain processes seem to depend on either the type or the difficulty of the cognitive task. The DHEAS-to-DHEA ratio was related to novelty-P3 processing during a visual working memory task, but not during an easier visual discrimination task (no working memory load). In a previous study, using a visual emotionally neutral or negative context we did not find any relation between DHEAS-to-DHEA ratio and involuntary attention to novel sounds or emotionally neutral stimuli processing either, but higher DHEAS-to-DHEA ratios were related to reduced processing of negative emotional stimuli and shorter stimuli evaluation times (do Vale *et al*., 2015a). Taken together, previous findings at the clinical level and our electrophysiological results both point towards an important role of the sulfotransferase

(the enzyme that converts DHEA into DHEAS) and sulfatase (the enzyme that converts DHEAS into DHEA) activity. They also imply that evaluating and manipulating DHEAS-to-DHEA ratios in future studies might be important to understand DHEA and DHEAS physiological effects.

**Limitations of the study**

In the present study, the participants were in different menstrual cycle phases and some were using hormonal contraception. DHEA and DHEAS levels can change along the menstrual cycle and with the use of hormonal contraception (Fern *et al.,* 1978; Wiegratz *et al.,* 2003). Nevertheless, considering the small sample size in the present study, the endocrine levels were not significantly different in relation to the menstrual cycle phase or the use of hormonal contraception. Moreover, given the randomized approach we used, the present results are expected to be independent of the menstrual cycle phase. In any case, even considering that endocrine levels change along the menstrual cycle or with the use of hormonal contraception, the relations we found between endocrine levels and brain processing cannot be invalidated. Finally, only female participants were included, limiting the outreach of the present study only to women. DHEAS levels differ between genders, therefore, another group of participants would be necessary to extend our conclusions also to men. Further studies would be relevant to prove whether the results are also identical in men.

In **summary,** in young women with normal DHEA levels, a high DHEAS-to-DHEA ratio was related to enhanced auditory novelty-P3 amplitudes during the performance of a visual working memory load task but was not related to visual P300 amplitudes, novelty-P3 latencies or visual P300 latencies. These results suggest that higher DHEAS-to-DHEA ratios are related to enhanced acoustic novelty processing with no detrimental effect on working memory processing. Thus, it is proposed that higher DHEAS-to-DHEA ratios might enhance involuntary attention to the surrounding world during the performance of working memory load tasks, which again may be an important protective

mechanism. The present results also suggest the importance of the sulfotransferase/sulfatase activity in the modulation of DHEAS and DHEA effects in brain.

**Abbreviations**

DHEA- dehydroepiandrosterone; DHEAS - dehydroepiandrosterone-sulfate; EEG - electroencephalogram; ERPs - event-related potentials; WM0 - no working memory load (discrimination) condition; WM1 - working memory load condition.

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**Author Contributions**

SV, LS and CE conceived and designed the experiments. SV managed the literature searches. SV and LS managed the subjects' performance of the study protocol, STAI administration, saliva collection and performance and EEG recording. SV performed the endocrine measurements and managed the initial EEG analysis. SV performed the initial statistical analyses and wrote the manuscript's first draft. LS, JMM and CE provided critical revision of the manuscript. SV, LS, JMM, MB, IC and CE contributed to and approved the final manuscript. SV- Sónia do Vale, LS – Lenka Selinger, JMM – João Martin Martins, MB - Manuel Bicho, IC - Isabel do Carmo, CE – Carles Escera.

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**Conflict of Interest Statement**

The authors have the following interests. The material for DHEA measurements was kindly provided by Merck S.A. (www.merck.pt)**.** This funding source had no role in study design; collection, analysis, and interpretation of data; writing of the report; and decision to submit the report for publication.

# Study IV

## Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone-sulfate (DHEAS) and Emotional Processing – a Behavioral and Electrophysiological Approach

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## Abstract

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulphate (DHEAS) may have mood enhancement effects: higher DHEAS concentrations and DHEA/cortisol ratio have been related to lower depression scores and controlled trials of DHEA administration have reported significant antidepressant effects. The balance between DHEAS and DHEA has been suggested to influence brain functioning. We explored DHEAS, DHEA, cortisol, DHEA/cortisol and DHEAS/DHEA ratios relations to the processing of negative emotional stimuli at behavioral and brain levels by recording the electroencephalogram of 21 young women while performing a visual task with implicit neutral or negative emotional content in an audio-visual oddball paradigm. For each condition, salivary DHEA, DHEAS and cortisol were measured before performing the task

and at 30 and 60min. interval. DHEA increased after task performance, independently of the implicit emotional content. With implicit negative emotion, higher DHEAS/DHEA and DHEA/cortisol ratios before task performance were related to shorter visual P300 latencies suggesting faster brain processing under a negative emotional context. In addition, higher DHEAS/DHEA ratios were related to reduced visual P300 amplitudes, indicating less processing of the negative emotional stimuli. With this study, we could show that at the electrophysiological level, higher DHEAS/DHEA and DHEA/cortisol ratios were related to shorter stimulus evaluation times suggesting less interference of the implicit negative content of the stimuli with the task. Furthermore, higher DHEAS/DHEA ratios were related to reduced processing of negative emotional stimuli which may eventually constitute a protective mechanism against negative information overload.

**Key-words:** dehydroepiandrosterone; dehydroepiandrosterone-sulphate; cortisol; emotion processing; performance; dehydroepiandrosterone reactivity; auditory distraction; event-related-potentials.

## Resumo

Alguns estudos sugerem que a desidroepiandrosterona (DHEA) e a desidroepiandrosterona-sulfato (DHEAS) podem ter efeitos benéficos ao nível do humor: concentrações mais elevadas de DHEAS e da razão DHEA/cortisol, têm sido associadas a menos sintomas depressivos e os estudos controlados com administração de DHEA, revelaram efeitos anti-depressivos significativos. Além disso, também tem sido sugerido que o balanço entre os níveis de DHEAS e DHEA pode influenciar o funcionamento cerebral. Neste estudo, explorámos as relações entre os níveis de DHEAS, DHEA, cortisol, DHEA/cortisol e DHEAS/DHEA e o processamento de estímulos emocionais negativos, a nível comportamental e cerebral. Foi gravado o eletroencefalograma de 21 jovens adultas, enquanto realizavam uma tarefa visual com conteúdo emocional implícito neutro ou negativo, usando um paradigma audiovisual (do tipo “oddball”). Para cada condição, foram medidas as concentrações de DHEA, DHEAS e cortisol na saliva, antes da tarefa e

aos 30 e 60 minutos. As concentrações de DHEA aumentaram após a realização das tarefas, independentemente do conteúdo emocional implícito. Razões DHEAS/DHEA e DHEA/cortisol mais elevadas antes da realização da tarefa com conteúdo emocional negativo, correlacionaram-se com latências da deflexão P300 visual mais curtas. Estes resultados sugerem que perante conteúdos emocionais negativos, as razões DHEAS/DHEA e DHEA/cortisol mais elevadas se associam a um processamento cerebral mais rápido. Além disso, razões DHEAS/DHEA mais elevadas, relacionaram-se com amplitudes mais reduzidas da deflexão P300 visual, indiciando um menor processamento dos estímulos emocionais negativos. Neste estudo, mostrámos que ao nível eletrofisiológico, razões DHEAS/DHEA e DHEA/cortisol mais elevadas, se relacionaram com avaliações do estímulo mais rápidas, sugerindo menor interferência do conteúdo emocional negativo, na realização da tarefa. Adicionalmente, razões DHEAS/DHEA mais elevadas relacionaram-se com menor processamento dos estímulos emocionais negativos, o que pode constituir um mecanismo protetor contra o excesso de informação negativa.

**Palavras-chave:** desidroepiandrosterona, desidroepiandrosterona-sulfato, cortisol, processamento emocional, desempenho, reactividade da desidroepiandrosterona, distração auditiva, potenciais evocados.

## Introduction

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulphate (DHEAS) are neuroactive steroids and their concentrations in the central nervous system are higher than in the peripheral circulation (Dong and Zheng, 2011; Lacroix *et al.,* 1987). Although their physiological role and mechanisms of action are still a matter of debate (Komesaroff, 2008), a growing amount of evidence has been accumulating regarding their actions in the central nervous system. Concerning cognitive functions, higher DHEAS levels were related to improved memory (Barrett-Connor and Edelstein, 1994), whereas

low levels were found in Alzheimer's disease (Weill-Engerer et al., 2002). Besides, higher DHEA, DHEAS or DHEA-to-cortisol levels were related to improved attention (Wolf *et al*., 1997), lower perceived stress and also to improved performance under stressful conditions (Morgan *et al*., 2009; Russo *et al*., 2012).

Furthermore, higher DHEAS concentrations and DHEA-to-cortisol ratios have been related to lower prevalence of depression, lower depression ratings and higher well-being scores (Barret-Connor and Edelstein, 1994; Barrett-Connor *et al*., 1999; Michael *et al.,* 2000; Young *et al*., 2002). The relation between DHEA levels alone and depression is less consistent. In fact, several groups have found that DHEA-to-cortisol ratios, rather than concentrations of either hormone alone, discriminated more accurately depressed from non-depressed individuals, with lower DHEA-to-cortisol ratios as seen in depression (Assies *et al*., 2004; Michael *et al.,* 2000), in untreated depressed patients and in patients who remained depressed after several months (Goodyer *et al*., 1998).

It was hence suggested that elevated DHEA and DHEAS relative to cortisol levels, may counteract the negative effects of high cortisol on mood (Goodyer *et al*., 1998; Kaminska *et al.,* 2000). Moreover, controlled trials of DHEA therapy have reported significant positive effects on mood (Bloch *et al.,* 1999; Schmidt *et al*., 2005; Wolkowitz *et al.* 1999). These mood improvements were related to increases in the circulating concentrations of DHEA and DHEAS and to increases in their ratios with cortisol. On the other hand, sulphated steroids in general possibly act as endogenous neuromodulators (Gibbs *et al*., 2006) and the balance between DHEAS and DHEA has also been suggested to influence brain functioning. In a study assessing both DHEA and DHEAS, depressed patients had low DHEAS but normal DHEA concentrations (Scott *et al*., 1999). In a different setting, but also pointing towards the importance of DHEAS to DHEA balance, it has been shown that subjects with Alzheimer's disease have increased levels of DHEA in the central nervous system, but a reduced conversion of DHEA into DHEAS and consequently, reduced DHEAS/DHEA ratios (Kim *et al.,* 2003).

Glucocorticoids' effects are not always deleterious. In fact, glucocorticoids have biphasic effects on fear conditioning: although mild or short lasting increases in glucocorticoids in relation to stress may have beneficial effects on attention and promote

adaptation, higher cortisol levels or long term increases have deleterious effects on executive functioning, attention, learning, memory and cognitive flexibility (Campeau *et al*., 2011; McEwen 2012). Again, anti-glucocorticoid effects of DHEA and DHEAS have been proposed in what concerns cognition and performance.

DHEA and DHEAS present a general neurostimulatory effect, but DHEAS has a much more potent excitatory action than DHEA (Baulieu and Robel, 1998; Imamura and Prasad, 1998; Monnet *et al.,* 1995; Dong and Zheng, 2011). At cellular level DHEAS antagonizes the neurotoxic effect of high doses of DHEA in mouse neuronal cultures (Gil-ad *et al.,* 2001) and DHEA is protective against the neurotoxic effects of corticosterone (Balazs *et al*., 2008). Hence, the simultaneous evaluation of DHEA, DHEAS and cortisol and the ratios of DHEA to cortisol and DHEAS to DHEA may uncover more information than the individual examination of either steroid alone.

DHEA and DHEAS effects on cerebral regions specifically involved in emotional processing including the amygdale, hippocampus, insula and anterior cingulated cortex have been suggested. However, little research has explored the neural correlates of DHEA and DHEAS with respect to emotion and mood. In this regard, a study using LORETA showed that DHEA administration increased the activity in the anterior cingulate cortex (Alhaj *et al*., 2006). Another recent study using functional Magnetic Resonance Imaging (fMRI) found that DHEA reduces the activity in regions associated with the generation of negative emotion and enhances activity in regions linked to regulatory processes (Sripada *et al*., 2013).

DHEA and DHEAS relations to emotional processing at the electrophysiological level are not known. Regarding cortisol, its administration increased the processing of angry faces in highly anxious individuals as indicated by increased amplitudes of early (P150) and late (P3) event-related potentials (van Peer *et al*., 2007). DHEA and DHEAS may modulate attention, cognition and mood while their response to emotional stimuli is mostly unexplored.

The aim of the present study was to explore whether DHEA and DHEAS levels would have an influence on involuntary attention and emotional stimuli processing at the

performance and brain levels and, on the other hand, if an emotional challenge would alter DHEA and DHEAS levels. Furthermore, we wanted to examine the relation of DHEA and DHEAS with cortisol levels. The *a priori* hypotheses were: 1) higher endogenous DHEAS and DHEA levels as well as higher DHEA to cortisol and DHEAS to DHEA levels may protect from involuntary distraction and enhance brain processing and performance under a negative emotional context; 2) DHEAS and DHEA effects may be largely antagonistic from those of baseline cortisol; 3) in the short term, a negative emotional context might be a stimulus for DHEA and cortisol secretion.

To test these hypotheses, we used a visual task with a neutral or negative emotional context and unexpected auditory novel sounds aimed to cause distraction. In this paradigm, the negative emotional context is expected to elicit an increased attention capture when compared to non-emotional faces (Öhman *et al.,* 2001) and consequently deteriorate performance (Domínguez-Borràs *et al*., 2008). Furthermore, auditory distraction by novel sounds is expected to elicit a novelty P3 component in the electroencephalogram (EEG) and also deteriorate performance (Corral and Escera 2008; Domínguez-Borràs et al., 2008; Escera *et al*., 1998, 2000). We recorded performance parameters, the EEG and took saliva samples in order to determine the hormonal levels before and after the task. We explored DHEA, DHEAS, cortisol, DHEA/cortisol ratio and DHEAS/DHEA ratio relations to distraction and implicit negative emotion at the performance and electrophysiological levels.

## Participants and Methods

### Participants

21 healthy female volunteers (university students) from 18 to 26 years (mean 21±1), performed the study protocol. Only women were included as to ensure higher homogeneity in emotional processing (Garcia-Garcia *et al*., 2008) and androgen levels. All had normal or corrected-to-normal vision and none reported auditory deficits. There was

no history of neurologic, psychiatric, endocrine or oral diseases. Subjects were asked to refrain from alcohol intake in the twelve hours before the experimental protocol and tobacco and illicit drug consume were exclusion criteria. All participants gave their written informed consent. The experimental protocol was approved by the ethical committees of the University of Barcelona and Lisbon Medical School and was performed in accordance with the Declaration of Helsinki.

Prior to the experimental session, subjects completed the State-Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1988) and all were within a normal range of state (mean 13±1) and trait (mean 17±1) anxiety levels. Mean body mass index (BMI) was 22.3±0.8 kg/$m^2$ and all participants presented a normal body mass index except one with grade 1 obesity. All except one participant were right-handed. Seven participants were in the follicular phase, three were in the peri-ovulatory phase and six were in the luteal phase, based on self-reported menstrual cycle day. Five subjects were using hormonal contraception, no other medications were allowed.

**Task and Procedure**

The experimental sessions were conducted in the afternoon, beginning at 2-3 pm. An adapted version of a well-established auditory-visual distraction task (Escera *et al.*, 1998, 2000) was presented with two different conditions, one featuring a neutral (NEU) and one featuring a negative emotional content (NEG), as implemented by Domínguez-Borràs *et al.* (2008). Each condition lasted about 15 minutes and conditions were performed two hours apart (beginning to beginning). The order was counterbalanced across subjects. Each condition consisted of two separate blocks of 255 trials with a short interval between them.

Participants sat in a comfortable chair in a dimly lit and electrically and acoustically shielded room. The task consisted of responding as fast and accurately as possible whether the two faces on the screen were equal or different by pressing the correspondent mouse button. The subjects were instructed to ignore the sounds. In order to reduce eye blinks and movements during EEG recording, subjects were asked to blink

as little as possible, and to focus on a central fixation cross between the two pictures. Responses were given through a mouse button (one mouse button for “the same” and the other button for “different”, counterbalanced across subjects) and the probability of both responses was the same. Before each experimental condition, subjects performed practice blocks (composed by 10 trials) using faces with a neutral expression only and without any auditory stimuli, until they reached a hit rate (HR) of at least 80%.

In each trial, a task-irrelevant auditory stimulus was presented, followed after 300 ms (onset-to-onset) by a visual imperative stimulus (figure 1). The total trial length varied from 1200 to 1600 ms (1400 ms on average; jitter +/-200 ms) and the response window until the end of the shortest trial was 1200 ms. The auditory sequence consisted of repetitive standard tones (duration of 200 ms, including fade-in and fade-out of 10ms each; 600 Hz; 85dB; probability of occurrence p = 80%), occasionally replaced by environmental novel sounds (p=20%). These novel sounds were selected from a sample of 100 different specimens (edited to have a duration of 200 ms, including fade-in and fade-out of 10ms each; digitally recorded, low-pass filtered at 10,000 Hz; 85dB), such as those produced by a drill, hammer, rain, door, telephone ringing, and so forth (Escera *et al*., 2003). All sounds were delivered binaurally through headphones (Sennheiser HD 202) in a randomized order with the only restrictions that the first four stimuli of each block had to be standard tones, that two novel sounds never appeared consecutively and that each novel sound occurred only once in each condition. The visual stimuli were pairs of combinations of pictures of faces with either neutral (figure 1.A) or fearful (figure 1.B) expression, presented on a computer screen for 400ms. We used 12 pictures of faces with either neutral (NEU) or fearful (NEG) expression from the Ekman and Friesen (1976) database. All faces had exactly the same probability of occurrence and all had the same valence (NEU or NEG) in each block. The picture size was 356x488 pixels, the vertical angle 9º and the horizontal angle 17º, accounting for two pictures presented simultaneously with a fixation cross in between, and the distance from the subjects’ eyes to the screen was 100cm.


**Figure 1: Trial Structure.** A. Neutral emotional context (NEU). B. Negative emotional context (NEG).

**Performance and EEG Recording and Analysis**

Response time (RT) and whether the button press was correct, wrong or missed were recorded for each trial using Presentation® (Neurobehavioral Systems, Inc). A database was created with the mean response time for correct responses and hit rate (HR), separately for each condition (NEU and NEG) and auditory stimulus type (standard and novel). Distraction by novel sounds was computed as the difference in hit rate and response time between standard and novel auditory stimuli (hit rate: NEUstandard trials – NEUnovel trials and NEGstandard trials – NEGnovel trials; response time: NEUnovel trials – NEUstandard trials and NEGnovel trials – NEGstandard trials). Performance disruption due to the processing of negative emotional stimuli was computed as the difference in hit rate and response time between conditions, as appropriate (hit rate: NEUstandard – NEGstandard and NEUnovel – NEGnovel; response time: NEGstandard – NEUstandard and NEGnovel – NEUnovel).

EEG activity was continuously recorded, from 64 scalp Ag/AgCl electrodes following the extended 10/10 convention. It was amplified and digitalized at a sampling rate of 512 Hz (Eemagine, ANT Software b.v., Enschede, Netherlands). The horizontal and vertical electrooculogram (HEOG and VEOG) were recorded with electrodes placed at the outer canthus and below the right eye, respectively. An electrode placed on the tip of the nose was used as the common reference and the ground was located at the AFz position. Impedances were kept at 5 kΩ or below during the whole recording session. Recording

was performed with an ANT amplifier of 64 channels (gain 20x; A/D resolution 22 bits, 71.526 nV per bit; filtering 0-138.24Hz; CMRR >90dB).

EEG processing was performed off-line by using Eeprobe 3.1 (ANT Software BV, Enschede, Netherlands). A digital finite impulse response (FIR) bandpass-filter from 0.01 to 30 Hz was applied using a Hamming window. ERPs were averaged for each auditory-stimulus trial type and emotional condition, for an epoch of 1400 ms, comprising a pre-auditory-stimulus baseline of 200 ms. The first five epochs of each block and epochs following a novel trial were excluded from averaging. Only epochs corresponding to trials with correct responses were included in further analyses. Electrooculogram (EOG) correction was performed by manually selecting a large number of typical artifacts and accordingly applying a regression algorithm to compute propagation factors (Eeprobe 3.1, ANT Software BV, Enschede, the Netherlands). After EOG correction any epochs containing EEG activity exceeding ±100µV peak-to-peak amplitudes were rejected from further analysis. On average, 84% of epochs (252 epochs) with standard sounds and 87% of epochs (87 epochs) with novel sounds in NEU and 86% of epochs (259 epochs) with standard sounds and 86% of epochs (86 epochs) with novel sounds in NEG were retained for averaging.

In the employed paradigm the participants were specifically instructed to ignore the distraction stimuli (auditory), hence any related effects are necessarily involuntary or led by exogenous attention. ERPs recorded during auditory distraction are typically characterized by an auditory N1/mismatch negativity (N1/MMN) enhancement, reflecting a detection mechanism leading to attention capture, followed by a novelty-P3 (nov-P3) reflecting the effective orientation of attention (Escera *et al*., 1998 and 2000). The nov-P3 component has been shown to be sensitive to the manipulation of the emotional context (Domínguez-Borràs *et al*., 2008) and attention (Escera *et al*., 1998). Subsequently, the re-orienting negativity (RON) reflects the re-orientation of attention back to the task (Schröger and Wolff, 1998). The target stimuli in the present task were visual, and visual ERPs include the P300, which is a cognitive component related to target processing. The P300 (visP300) reflects the conscious processing of the visual stimulus and is sensitive to attention allocation (Domínguez-Borràs *et al*., 2008; Polich, 2007).

To analyze distraction effects, the ERPs elicited to the auditory stimuli were considered and difference waveforms (dw) were calculated by subtracting the ERPs elicited by standards from those elicited by novel sounds. These difference waveforms revealed an early-onset, long-lasting positive deflection that we assimilated to the novelty-P3. We measured the auditory P3 (aud-P3) and novelty-P3 (nov-P3) as the mean amplitude in the 210–470 ms time window at F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrodes.

To analyze emotional effects, ERP measures in standard trials were compared. Specific components were elicited during task-performance, such as the auditory N1 and P2, visual N1 and P2 and the cognitive components N2b and visual P300. Since only cognitive processing was of interest for the present study, the visual P300 (650-1050 ms at F7, F3, FZ, F4, F8, T7, C3, CZ, C4, T8, P7, P3, PZ, P4 and P8, 350-750 ms from stimulus presentation) was analyzed. Mean amplitudes for the considered time windows and electrodes were computed for each deflection.

**Endocrine measurements**

Saliva samples were collected by passive drool, using a short straw. Unstimulated whole saliva was used. For each condition, samples were collected for DHEA, DHEAS and cortisol measurement before task (BF), at 30 min (30 min after the beginning of the task, 10 to 15 min after the end of the task) and 60 min (60 min after the beginning of the task, 40 to 45 min after the end of the task). The samples collected before the first condition (i.e. before both the neutral and the negative condition), were considered as the baseline. Time points to collect the saliva samples were chosen in accordance to known cortisol raise and recovery times (raise 10 min after appropriate stimulus, peak at 20-30 min and recovery at 45-60 min after the end of the stimulus) (Martins *et al*., 2001, 2002 and 2004; do Vale *et al*., 2011). Furthermore, synchronous 24h profiles were described for DHEA and cortisol and DHEA half-life is less than 30min (Rosenfeld et al., 1975). For this reason, the second condition was started two hours after the first one to allow cortisol and DHEA levels to recover from the influence of the first condition (return to a “baseline” level).

Unbound DHEA and cortisol in the peripheral circulation penetrate into the saliva via intracellular mechanisms and salivary concentrations reflect serum concentrations (Ahn *et al*., 2007; Vining and McGinley, 1987). DHEAS is not lipid soluble and cannot penetrate into the saliva by passive diffusion through cell membranes. Instead, it squeezes through the tight junctions between salivary glands. DHEAS concentrations in saliva are therefore dependent on serum concentration and salivary flow rate (Vining and McGinley, 1987).

Samples were refrigerated at 2-8º C within 30 min after collection and were stored at -20ºC within 4h and until assayed. Each sample was measured in duplicate using enzyme-linked immunoassays: salivary DHEA and DHEAS enzyme immunoassay kits (Salimetrics Europe®, Ltd, Newmarket Suffolk, UK) and high sensitivity salivary cortisol enzyme immunoassay kits (Salimetrics®, LLC, State College, PA, USA). DHEA was measured in pg/mL and cortisol was measured in µg/dL. Due to the influence of saliva flow rates on DHEAS levels, the concentration of DHEAS (pg/mL) was multiplied by the flow rate (mL/min) and the corrected results were obtained as DHEAS measured per unit of time (pg/min). The minimal concentrations that can be distinguished from 0 with the used immunoassays are 5pg/mL for DHEA, <0.003µg/dL for cortisol and <43pg/mL for DHEAS. Intra- and interassay coefficients of variation were less than 10% and 15% in every case, respectively.

**Statistical Analysis**

The Statistical Package for the Social Sciences Program (IBM SPSS Statistics, version 21) was used for data analysis. Results are presented as the mean ± standard error of the mean (SEM). The normal distribution of continuous variables was verified by the Kolmogorov-Smirnov goodness of fit test.

To explore the effects of the implicit emotional content and auditory distraction on performance, repeated measures analyses of variance (ANOVA) were performed on hit rate and response time, including the within-subjects' factors emotional condition (NEU and NEG) and type of auditory stimulus (standard and novel). Regarding brain

responses, ERPs time-locked to the auditory stimuli were analyzed to explore distraction effects. ERPs time-locked to the visual stimuli (the faces) were analyzed to explore emotional context effects. To investigate the effects of the emotional context and auditory distraction on brain responses to auditory stimuli, repeated measures ANOVAs were carried out on auditory P3 mean amplitude in the time window and electrodes considered above, including the within-subjects' factors emotional condition (NEU and NEG) and type of auditory stimulus (standard and novel). To investigate the effects of the emotional context on brain responses to visual stimuli, only standards were included and ANOVAs were performed on the visual P300 mean amplitude in the time windows and electrodes considered above, with emotional condition (NEU and NEG) as within-subjects factor. To investigate the effects of emotional context manipulation on endocrine levels, repeated measures ANOVAs were performed on DHEA, DHEAS and cortisol levels, including the within-subjects' factors emotional condition (NEU and NEG) and measurement time (before task, at 30min and 60 min).

To investigate the endocrine relation to distraction and emotional context effects at the behavioral level, the previous repeated measures ANOVAs of behavior parameters were repeated, including baseline DHEA, DHEAS, cortisol, DHEA/cortisol ratio or DHEAS/DHEA ratio as covariates. Whenever the endocrine parameters covariated with the performance or ERP parameters for each type of auditory stimuli or emotional condition, we performed simple or multiple regression analyses to select the relevant independent endocrine factors and/or to understand the direction of the relationship. Lastly, as visual P300 peak latency in both emotional contexts was not directly related, in order to find the relation between visual P300 peak latency and endocrine parameters, linear and multiple regression analyses were used with the endocrine parameters as independent variables.

ANOVA results were Greenhouse–Geisser corrected whenever the assumption of sphericity was violated. The limit of significance chosen was $\alpha$=0.05. *Post hoc* tests were carried out wherever there were significant interactions between main factors and the Bonferroni correction was applied for multiple comparisons. For the endocrine relations to performance or ERPs the alpha therefore was set to 0.01 as the effects were tested for

five variables (cortisol, DHEA, DHEAS, DHEA/cortisol ratio and DHEAS/DHEA ratio). Effect size estimates of the results were expressed as eta squared ($\eta^2$) for ANOVAs and correlation coefficients (r) for regression analyses.

## Results

### Performance

Behavioral results for each condition and trial type are presented in Figure 2. There was a main effect of emotional context on response time [$F_{(1,20)}$=17.51, p<0.001, $\eta^2$=0.47], with longer response times under the emotionally negative context (433±17 ms) than under the neutral one (379±12 ms). Furthermore, there was a main effect of trial type on response time [$F_{(1,20)}$=31.88, p<0.001, $\eta^2$=0.61], with longer response times for novel (423±13 ms) than for standard (389±14ms) trials, indicating that the novel sounds caused distraction of visual task performance (figure 2.A). Overall hit rate was 88±1 % and did not change significantly with the emotional context or trial type (figure 2.B).

### Event-Related Potentials

**Distraction Effects.** No clear N1-enhancement/MMN nor RON was elicited and therefore our analysis focused only on the nov-P3. In fact, the nov-P3 was significantly elicited as supported by the significant differences of the mean amplitudes in the auditory P3 latency window for standard and novel trials [$F_{(1,20}$=169.09, p<0.001, $\eta^2$=0.89; -2.3±0.4 µV in standard and +1.7±0.5 µV in novel trials], see figure 3.A and 3.B. However, there were no effects of emotional context on the auditory P3 (see figure 3.C), as no significant interaction between emotional context and auditory stimulus type was found.

**Emotional Context Effects.** The waveforms elicited by standard trials in the two emotional contexts are presented in figure 3.D. No significant emotional effects were

observed on visual P300 as its amplitude was similar in both conditions. Also, there were no significant latency differences in P300, between emotional conditions.

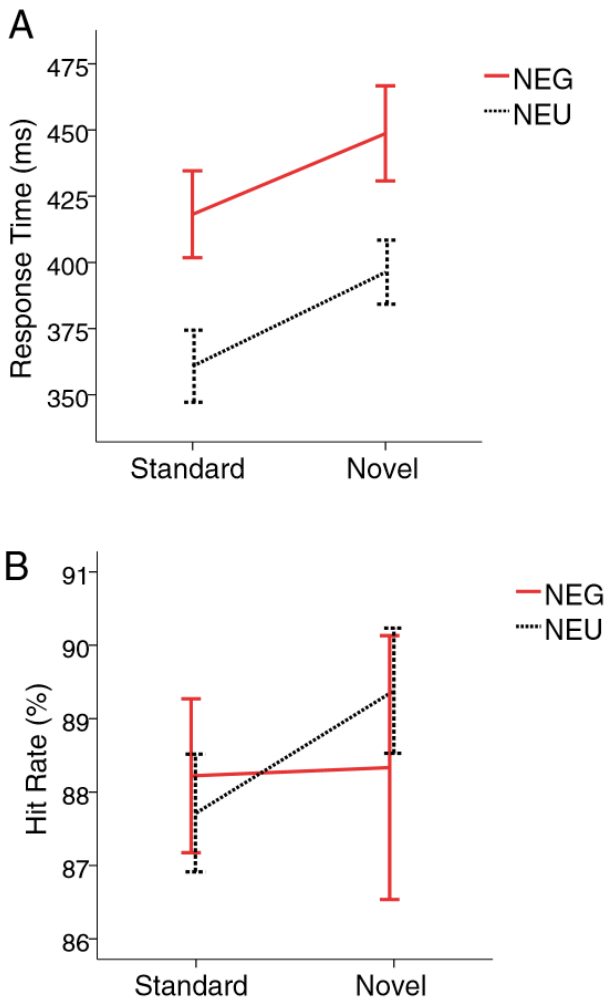


**Figure 2: Performance results.** A. Mean response times for each condition [neutral (NEU) or negative context (NEG)] and auditory stimulus type (standard or novel). B. Mean hit rates for each condition and auditory stimulus type. Error bars represent ±1 standard error of the mean (SEM).

### Endocrine Baseline Levels and Reactivity

Baseline endocrine levels were: DHEA 254±41 pg/mL, DHEAS 5856±690 pg/min and cortisol 764±100 pg/mL, following a normal distribution. Baseline DHEA level was directly related to baseline cortisol level (r=+0.63, p=0.002, n=21). There was no significant relation between baseline DHEAS and DHEA or DHEAS and cortisol level. Nevertheless, baseline DHEAS levels were directly related to baseline DHEA/cortisol ratio (r=+0.56, p=0.008, n=21). Baseline endocrine parameters were not related to age or body

mass index and did not differ according to menstrual cycle phase or between subjects taking and not taking hormonal contraception.

DHEA, DHEAS and cortisol mean levels for each condition and sample time are presented in figure 4. The repeated measures ANOVA on DHEA levels revealed a main effect of measurement time on DHEA levels [$F_{(2,40)}$= 5.94, p=0.007, $\eta^2$=0.24; mean levels were 243±42 pg/mL before task, 258±41 pg/mL at 30 min and 309±45 pg/mL at 60 min], see figure 4.A and 4.B. There was no significant relation between emotional context and DHEA levels. Moreover, there was no interaction between DHEAS (figure 4.C) or cortisol levels (figure 4.D) and the emotional context or measurement time.

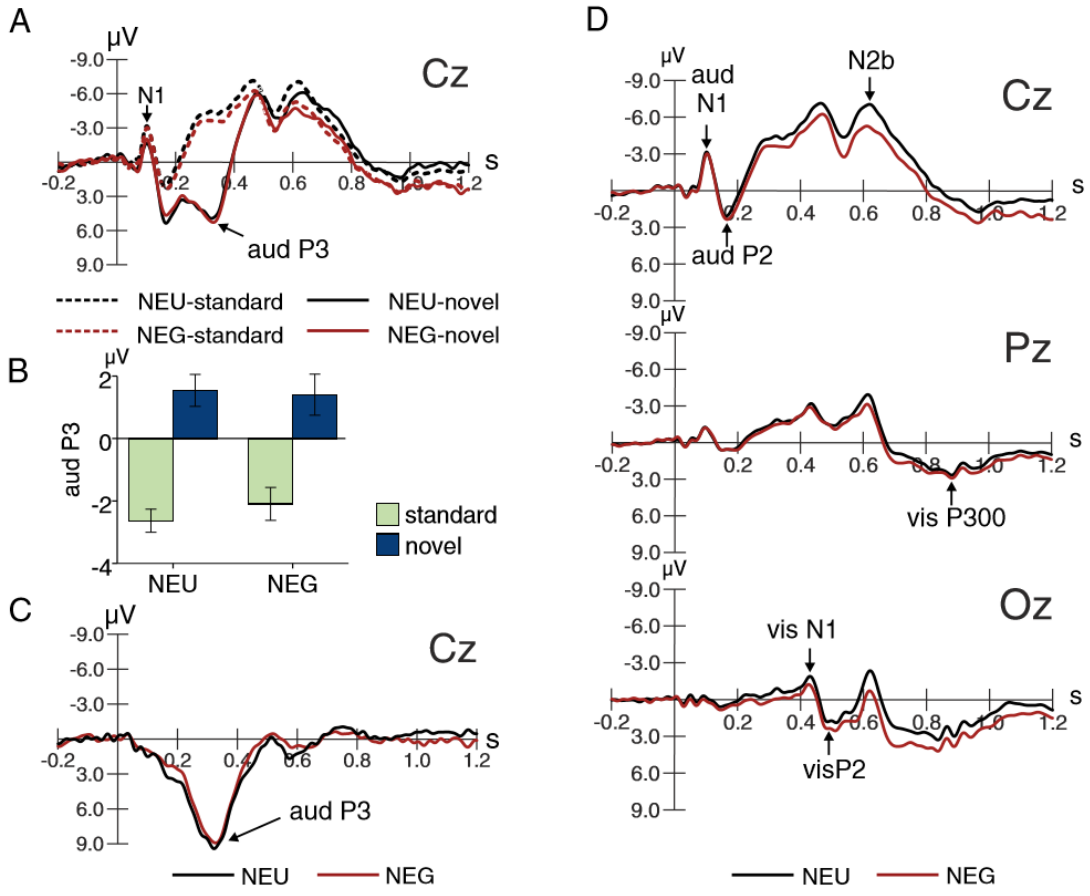


**Figure 3: Event-Related Potentials (ERPs).** A. Grand average waveforms at Cz for each emotional context (neutral and negative) and type of sound (standard and novel). B. Mean auditory P3 (aud P3) amplitude for each emotional context and type of sound. C. Grand average of novel minus standard difference waveforms at Cz for each emotional context. D. Grand average ERPs for neutral and negative context (only standard trials). NEG: negative emotional context; NEU: neutral emotional context; aud: auditory event related potentials; vis: visual event related potentials.

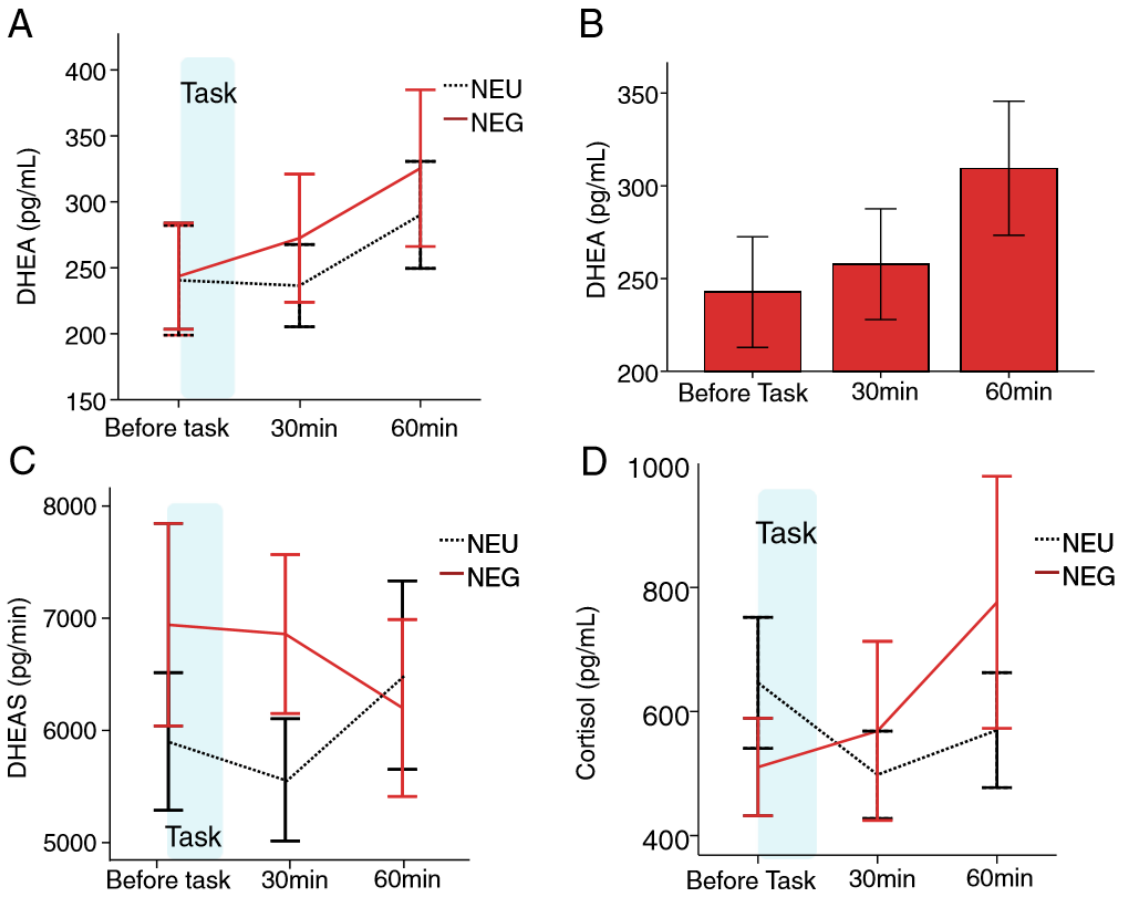


**Figure 4:** Endocrine results. A. DHEA mean levels for each condition and measurement time. B. DHEA levels before task, at 30 min and 60 min. C. DHEAS mean levels for each condition and measurement time. D. Cortisol mean levels for each condition and measurement time. Error bars represent ±1 SEM. The shadow indicates the period of task performance. NEG: negative emotional context; NEU: neutral emotional context.

### Endocrine Relations to Performance and Event-Related Potentials

We found no significant relations between performance (hit rate and response times) and endocrine parameters. Regarding event-related potentials, no significant relations were found between endocrine parameters and distraction effects. Nevertheless, significant relations were found between endocrine parameters and emotional context. Higher DHEAS/DHEA ratios before performing the emotionally negative condition were related to reduced visual P300 amplitudes in this condition. This was revealed by a significant interaction between visual P300 amplitudes and DHEAS/DHEA ratios [$F(1,19)=9.38$, $p=0.006$, $\eta^2=0.33$] with higher DHEAS/DHEA ratios in relation to reduced visual P300 amplitudes attributed to the negative context (r=-0,58,

p=0.006, n=21; see figure 5.A). Concerning visual P300 peak latency, higher DHEA/cortisol (partial r=-0.56, p=0.003, n=21) and DHEAS/DHEA (partial r=-0.60, p=0.004, n=21) ratios before performing the negative emotional context block, were related to shorter visual P300 peak latencies (figure 5.B and 5.C), together explaining 52% of the latency variability. Remarkably, smaller visual P300 amplitudes attributed to the negative context were related to shorter visual P300 peak latencies (r=+0.53, n=21, p=0.015).

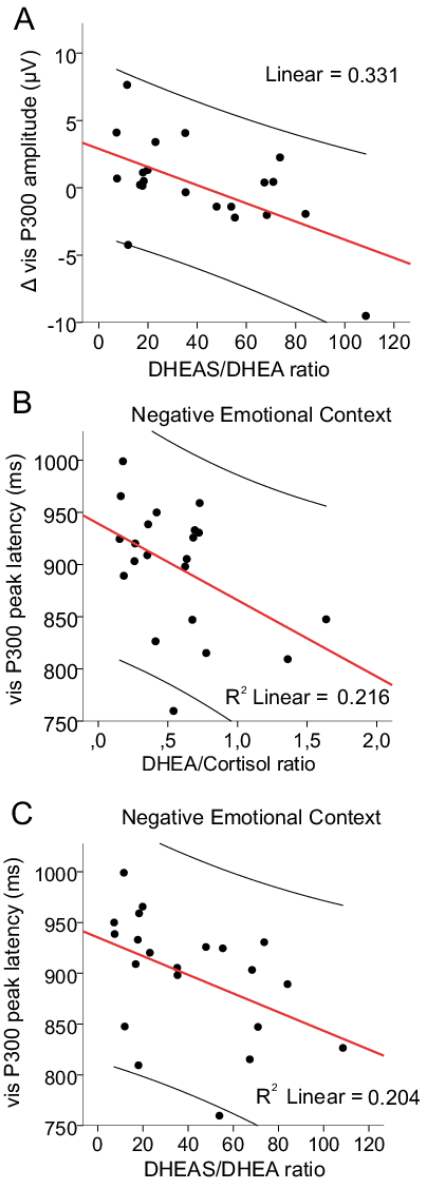


**Figure 5:** Endocrine relations to Event-Related Potentials. A. Higher DHEAS/DHEA ratios before performing the negative emotional context block were related to reduced interference with the task due to the processing of the implicit negative content of the stimuli as shown by reduced visual P300 amplitudes. B and C. Higher DHEA/cortisol and DHEAS/DHEA ratios before performing the negative emotional context block, were related to shorter visual P300 peak latencies. Δ vis P300 amplitude: mean visual P300 amplitude in the negative minus neutral context.

## Discussion

The present study revealed relations between dehydroepiandrosterone (DHEA), its sulphated form (DHEAS) and brain processing under an emotionally negative context induced by images of fearful faces, suggesting that these neurosteroids may modulate the processing of emotionally negative information. Although the sounds as well as the emotional content of the pictures were irrelevant for the task, the results suggest that the subjects were unable to fully ignore them as indicated by significant effects on performance and brain responses. The distraction effect of task irrelevant auditory stimuli as well as the behavioral disruption due to the processing of task irrelevant negative emotional stimuli have been shown before by other authors (Escera *et al*., 1998, 2000; Domínguez-Borràs *et al.,* 2008, 2009; Öhman *et al*., 2001), whereas the relation between the neurosteroids and emotional processing at the brain level is a new finding.

Novel sounds led to distraction as shown by longer response times and the elicitation of a significant novelty P3. Conversely, no clear N1-enhancement/MMN was observed in our results, probably due to a very early P3 onset, causing an overlap between the components. Recent studies have shown that small deviant stimuli, and hence large novel sounds, may activate deviance-detection mechanisms as early as 20 ms from sound onset (see Slabu *et al*., 2010; Escera and Malmierca, 2014) and therefore attention switching may have had occurred before the supratemporal activation giving rise to the typical N1-enhancement/MMN trigger response (Yago et al., 2001). Additionally, in the negative emotional context, response times were longer than in the neutral context which implicates that the novel sounds effectively caused distraction and the emotionally negative context effectively disrupted performance. However, the emotional context did not significantly modulate the electrophysiological response to auditory distraction, contrasting with previous studies (Domínguez-Borràs *et al*., 2008; García-García *et al*., 2008) which found an enhancement of the visual P300 for emotionally negative stimuli (Domínguez-Borràs *et a*l., 2008). Nevertheless, in studies using faces, some authors have found visual P300 enhancement by fearful faces (Luo *et al*., 2010), while others reported no changes in P300 amplitude (Balconi and Lucchiari,

2005) as in the present study. Importantly, in the present study, the emotional content of the images was not relevant for the task.

DHEA levels increased after the performance of both conditions, independent of the emotional context of the task. Interestingly, a previous study showed that corticotrophin releasing hormone (CRH) levels increased with another cognitive task: the visualization of emotionally significant movies (Martins *et al*., 2010). In turn, the present results suggest that cognitive tasks are a stimulus for DHEA secretion and may lead to a higher DHEA/cortisol ratio. Moreover, it suggests that DHEA is not just a stress hormone and that some differential regulation of DHEA and cortisol exists. In fact, CRH stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for cortisol and DHEA secretion (Nieschlag *et al*., 1973). This may explain the direct relation we found between cortisol and DHEA levels.

Boudarene *et al.* (2002) studied subjects without mental disorders and varying levels of anxiety, and found that the level of anxiety was related to the profile of endocrine response after the performance of cognitive tasks: subjects with high anxiety levels in the STAI test had increased cortisol reactivity and subjects with low anxiety levels showed an exclusive increase in DHEAS levels. The authors suggested that the antagonism in DHEAS and cortisol might be related to competition in their synthesis and its release by the adrenal gland. This agrees with the results in the present study, in which all the subjects had low anxiety levels and a DHEA raise but no cortisol response in relation to the cognitive task (in both emotional conditions), was found. For non-pathological conditions and low levels of anxiety, this might eventually represent an adaptive mechanism that includes higher DHEA than cortisol responses and anti-cortisol effects of DHEA. DHEA increase however was identical for both emotional contexts, so that DHEA increase alone is not expected to be related to performance or electrophysiological effects of the emotional context.

No significant relation was found between response times or hit rates and endocrine measurements. A larger sample may be necessary to uncover endocrine relations to performance. Nevertheless, an interesting finding of the present study consists in endocrine relations to brain responses. A higher DHEA/cortisol ratio was

related to lower visual P300 latencies in the negative emotional context, suggesting DHEA to be protective from an interference of the implicit negative content of the stimuli with the task which might represent an anti-glucocorticoid effect of DHEA. In the negative condition, higher DHEAS/DHEA ratios were also related to lower visual P300 latencies, thus, a higher proportion of DHEA respective to cortisol and a higher proportion of sulphated to non-sulphated form of DHEA may protect from any interference with the task caused by the processing of the implicit emotional content of the stimuli.

In agreement, higher DHEA levels have been previously found to be related to shorter P300 latencies (Braverman and Blum, 2003; Braverman *et al.,* 2009). A protective effect of DHEA and DHEAS could eventually be mediated by anti-cortisol effects and/or neuromodulatory effects of these hormones. Wang *et al.* (2013) associating a prolonged P300 latency with an attentional bias to negative stimuli in high trait anxiety subjects, who are expected to have higher cortisol levels. On the contrary, in the present study examining subjects with low trait anxiety, higher DHEA/cortisol ratios were related to shorter P300 latencies in the negative condition. Higher DHEA/cortisol could be related to less interference with the task caused by the implicit stressful content of the stimuli, in agreement with the hypothesis of anti-cortisol effects of DHEA and suggesting a protective mechanism against the deleterious consequences of stress. The present results also agree with studies at the cellular level, in which DHEA was protective against the neurotoxic effects of corticosterone (Balazs *et al*., 2008).

Lastly, the visual P300 amplitude increments in the negative emotional context as compared to the neutral one, were inversely related to DHEAS/DHEA ratios, suggesting that the processing of the task-irrelevant negative content of the stimuli might eventually be reduced by DHEAS. Even though the participants of the study were healthy and depression was not screened by any specific inventory, women in particular have been shown to be specialy sensitive to threatening stimuli and it has been hypothesized that this might be related to the higher prevalence of affective disorders in women (Kemp *et al*., 2004). Enhanced negative affect is an integral characteristic of depressive disorders and negative mood can be experimentally induced using various procedures, among

others by presenting images of faces with emotional expressions (Dyck *et al*., 2011; Schneider *et al*., 1994 and 1997).

Although the mechanisms underlying mood persistence in major depressive disorder remain poorly understood, cognitive theories hypothesize that depressed patients have cognitive biases for emotional information, which help perpetuate depressive symptoms (Beck, 1967; Gotlib and Joormann, 2010). The present results suggest that the processing of negative emotional stimuli may be reduced in relation to higher DHEAS/DHEA levels, which might eventually be involved in protective mechanisms against a negative attention bias and undermine the relation between higher DHEAS levels and lower frequencies of depression, lower depression ratings and better well being scores previously described by other authors (Barret-Connor and Edelstein, 1994; Barrett-Connor et al., 1999). In line with this hypothesis, subjects with higher DHEAS/DHEA ratio would process less and have less memory updating of the implicit negative content of the stimuli and would thus have a reduced attentional bias towards these stimuli, which might promote wellbeing and contribute to protection from depressive states.

Emotional stimuli usually capture attention more effectively than non-emotional ones (Öhman *et al.,* 2001) and have an impact on cognitive functions even when they are task-irrelevant (Domínguez-Borràs *et al*., 2008). On the other hand, it is hypothesized that protective mechanisms filter out irrelevant sensory inputs protecting the higher brain functions from sensory overload (Braff and Geyer, 1990). Taken together, our findings concerning visual P300 latency and visual P300 amplitude under an emotionally negative context showed a relation between higher DHEA/cortisol and DHEAS/DHEA ratios and less processing of the negative emotional stimuli and less disruption by the processing of negative emotional stimuli. These results also suggest that DHEA and DHEAS potentially play a role or contribute to protective mechanisms filtering out negative information overload.

A simultaneous relation to latency reduction and decrease in P300 amplitude may seem contradictory at first sight, nevertheless, as the task consisted in deciding whether two faces with the same emotional expression were equal or different (while the

emotional expression was irrelevant), less processing of the task-irrelevant emotional content – related to higher DHEAS/DHEA ratios - might be reflected in smaller P300 amplitudes and at the same time shorter P300 latencies due to a more efficient processing of the task-relevant visual information and less interference resulting from the task-irrelevant emotion. In other words, the P300 amplitude might be reduced as a reflection of less neuronal recruitment due to less allocation of attentional resources to the task-irrelevant emotion, while P300 latencies might be reduced due to a decreased stimulus evaluation time, likewise due to less processing of the task-irrelevant emotional content of the images. This interpretation is consistent with the direct relation we found between reduced P300 amplitudes and shorter P300 latencies. Furthermore, it is consistent with previous studies showing that emotional stimuli elicit an enhanced P300 as compared to neutral stimuli (Schupp *et al*., 2004) and longer P300 latencies for negative emotional target stimuli (Fichtenholtz *et al.*, 2007) as compared to neutral stimuli. Additionally, emotional stimuli outside the attentional focus have been shown not to elicit an enhanced P300 (MacNamara and Hajcak, 2009).

Endocrine relations to auditory distraction were not found in the present study: DHEA, DHEAS and cortisol were not related to the novelty-P3, suggesting that their levels do not modulate auditory distraction processing under an emotionally negative context. This differs from our previous findings concerning auditory distraction under working memory load, in which we found that the novelty-P3 amplitude was enhanced in relation to higher baseline cortisol/DHEA ratios (do Vale *et al*., 2014). It also suggests that the influence of the endocrine parameters on auditory distraction, may depend on the cognitive task involved.

The present results also highlight the relevance of the balance between DHEA, DHEAS and cortisol concerning the processing of negative emotions. Importantly, these results agree with findings at the clinical level, in which higher DHEAS concentrations and DHEA-to-cortisol ratios but not DHEA levels alone were related to less frequent depression, less depressive mood and higher wellbeing scores (Barrett-Connor *et a*l., 1999; Michael *et al.,* 2000; Young *et al.,* 2002). Although DHEA and DHEAS can be converted into each other and general common effects are expected for the sulphated

and non-sulphated form of the hormone (Baulieu and Robel, 1998; Dong and Zheng, 2011; Maninger *et al*., 2009), several differences exist in their mechanism of action. For instance, DHEAS has a much more potent excitatory action by NMDA agonism and gabaminergic antagonism than DHEA, which may account for some differential effects (Baulieu and Robel, 1998; Imamura and Prasad, 1998; Monnet *et al*., 1995). Moreover, as stated, sulphated steroids in general possibly act as endogenous neuromodulators (Gibbs *et al*., 2006) and the balance between DHEAS and DHEA might influence brain functioning. Previous studies at the cellular and molecular level showed that DHEAS had neuroprotective effects mediated through GABA-A receptor antagonism (Majewska, 1992), DHEAS stimulated dopamine release from rat hypothalamic cells (Murray and Gillies, 1997) and DHEAS antagonized the neurotoxic effect of high doses of DHEA (Gil-ad *et al.,* 2001). These studies suggest potential mechanisms by which DHEAS could have more potent anti-depressant effects than DHEA and agree with the present finding that higher DHEAS/DHEA ratios were related to reduced processing of the negative emotional content.

In the present study, the participants were in different menstrual cycle phases and some were using hormonal contraception. This is a limitation, as DHEA, DHEAS and cortisol levels can change along the menstrual cycle and with the use of hormonal contraception (Fern *et al*., 1978; Wiegratz *et al*., 2003). Nevertheless, eventually in relation to the small sample size, in the present study, the endocrine levels were not significantly different according to the menstrual cycle phase or the use of hormonal contraception. But even considering that DHEA, DHEAS or cortisol levels could change along the menstrual cycle or with the use of hormonal contraception, the relations found between endocrine levels and emotional stimuli processing would not be invalidated. In any case, given the randomized approach we used concerning the menstrual cycle phase, the present results are expected to be independent of the menstrual cycle phase. Finally, the fact that only female participants were included, limit the outreach of the present study only to women. DHEAS levels differ between genders, therefore, another group of participants would be necessary to extend our conclusions also to men. For further studies, it would be relevant to study whether the results are identical in men.

## Conclusions

In a nutshell, in women, during the processing of stimuli with negative emotional content, higher DHEA/cortisol and DHEAS/DHEA ratios were related to shorter visual P300 latencies, suggesting a relation of these endocrine parameters with shorter stimulus evaluation time and less interference with the task at hand due to the processing of the task-irrelevant negative content of the visual stimuli. Additionally, higher DHEAS/DHEA ratios were related to reduced visual P300 amplitudes suggesting less processing of the negative information, which might constitute a protective mechanism against negative information overload.


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The funding sources had no role in the project and study design, in the study execution, in the data analysis and interpretation, in the manuscript writing, or in the decision to submit the paper for publication.


## Conflict of Interest

All authors declare they have no conflicts of interest in what concerns the present work.


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**Author Contributions**

SV, LS, JMM and CE designed the study and wrote the protocol. SV managed the literature searches. SV and LS managed the subjects' performance of the study protocol as well as the personal data collection, STAI administration, saliva collection and performance and EEG recording. SV performed the endocrine measurements and initial EEG analyses. SV performed the initial statistical analyses and wrote the manuscript's first draft. All the authors contributed to and approved the final manuscript. SV – Sónia do Vale, LS – Lenka Selinger, JMM – João Martin Martins, CE – Carles Escera.

# Discussion

In this thesis we studied DHEA and DHEAS behavioral correlates both at the personality, performance and electrophysiological level. Relations to personality, working memory, negative emotional processing and involuntary attention were explored. Furthermore, the relation between baseline DHEAS levels and pituitary-adrenal axis reactivity was tested, as well as the DHEA and DHEAS response to the performance of cognitive tasks.

**DHEA and DHEAS baseline levels and reactivity**

The first study was a retrospective one, in which the relation between DHEAS and pituitary-adrenal axis reactivity in adult humans of both genders was explored. We used several specific diagnostic groups. Nevertheless, only results remaining significant independent of age and diagnostic group were reported. In this study, only DHEAS and not DHEA measurements were used. We expected a stronger relation between DHEAS and more stable parameters like age, gender and basal hypothalamic-pituitary-adrenal (HPA) axis activity as that hormone has a longer half-life. On the contrary, acute changes might have been more easily detected with DHEA, namely after CRH administration.

In this study, DHEAS levels were significantly higher (37%) in males, as expected (2; 12; 13; 690; 691). The mean age was 33 years old (ranging from 18 to 57 years old) and DHEAS levels were inversely related to age as it was also extensively described in the literature (2; 122; 232; 692; 693; 694). We found a 1.4% mean decline in DHEAS levels per year while other authors had previously described a decline of about 2% per year during adulthood and higher decline rates in post-menopausal women and older men (12; 691; 695). Age explained 20% of DHEAS variability, gender explained 4% of DHEAS variability (men were only 13% of the participants in this study) and age and gender together explained 27% of DHEAS variability.

DHEAS levels were also significantly and directly related to baseline ACTH, independent of age, gender and diagnostic group. ACTH explained 14% of DHEAS

variability. Age, gender and ACTH together accounted for 34% of DHEAS variability. ACTH is not a major factor for DHEAS and DHEA production, and is generally not considered as a relevant factor for baseline DHEAS levels. In fact, chronic stress is generally associated with increased cortisol levels and decreased DHEAS levels. Moreover, it should be noted that despite the ACTH response, no DHEAS response was found in the CRH test, even though dexamethasone suppression is known to decrease both cortisol and DHEAS (271). All this point to the complexity of the relation between ACTH and DHEAS, especially taking into consideration that a putative cortical androgen stimulating hormone (CASH) is still to be identified.

Nevertheless, opposite to findings in chronic stress, in the acute set, CRH stimulates corticotrophin and corticotrophin may be a stimulus for both cortisol, DHEA (152; 696) and DHEAS (8; 271) secretion, even if ACTH effects on DHEA and DHEAS are mediated through some unidentified CASH (697; 698; 699). In the present study, despite the ACTH response, mean DHEAS levels did not change in the CRH test, suggesting no acute effect of ACTH on DHEAS levels. Also, ACTH responses were not related to DHEAS responses. Taking into account the high DHEAS levels and long DHEAS half-life, a longer ACTH rise may be necessary to increase DHEAS levels (2), DHEAS may be slower than cortisol response (we only measured DHEAS at 0, 30 and 60min intervals) or the effects of ACTH in DHEAS secretion may be long time effects, as ACTH is also a trophic hormone regarding adrenal androgen secretion (700). However, although DHEAS mean levels did not change in response to CRH, individually DHEAS response was variable (–73 μg/dL to +317 μg/dL, and only two participants showed an increase higher than 37 μg/dL). In most subjects (75%), DHEAS levels changed less than 10%. Only 16% and 5% of the subjects had a variation in DHEAS level that was higher than 15% and 30% of baseline levels respectively.

Due to its long half-life, important short time changes in DHEAS levels were unexpected. Intra and interassay variation coefficients could eventually account for most individual changes in DHEAS concentrations, nevertheless, individual mean values during the CRH test were related to personality traits, which points against that hypothesis. The smaller changes could eventually be justified by adrenal synthesis. Nevertheless, big

changes in DHEAS were not expected to rely only on adrenal synthesis and the two extreme cases could eventually correspond to outliers. On the other hand, besides adrenal synthesis, brain synthesis, transport from brain to periphery and the activity of the sulfotransferase and sulfatase, could eventually contribute to the observed changes in DHEAS levels. In fact, DHEAS concentrations in the brain are several times higher than in the peripheral circulation and the efflux of DHEAS across the blood-brain barrier was determined to be tenfold greater than its influx (701). Hence, the peripheral levels of DHEAS could also be related to its levels in the brain, but only a small portion of central DHEAS is expected to cross the blood brain barrier (0.3%) (83; 85). Regarding the activity of the sulfotransferase/sulfatase, it can rapidly convert DHEA to DHEAS and vice versa (160).

Other authors had previously found that besides individual levels of DHEAS, mean DHEAS levels could also change in response to stressful stimuli (271; 8). Maninger *et al.* (271) studied monkeys and found significant increases in DHEAS mean levels, 30 min after an acute stress (mean levels were around 12 µg/dL at baseline, 15 µg/dL at 30min and more than 20 µg/dL at around 120min, corresponding to mean increases of 25% at 30min and more than 66% at 120min). Lennartsson *et al*. (8) studied men and women and also found significant changes in mean DHEA and DHEAS levels in response to acute stress, with large inter-individual variation in the magnitude of that response. In another study, Lennartson *et al*. (11) also explored DHEA, DHEAS and cortisol responses to stress in human subjects. In the low perceived stress group, they found mean changes in DHEAS levels of about 1 µmol/L (37 µg/dL), with 95% confidence in intervals ranging from 0.60 to 1.8 µmol/L (22 to 66 µg/dL). The relative change (percentage increase) of DHEA was greater than the relative change in DHEAS, but given the fact that DHEAS concentrations were about 300 times higher than DHEA concentrations, the absolute amount of increase in concentration of DHEAS during acute stress was much larger than for DHEA. The delta DHEAS accounted for the majority of the total increase in DHEA and DHEAS put together (by average 98% in that study). Also, in a study of adolescent girls with hyperandrogenism, using CRH infusion over 3h, it was found that DHEA increased 5-fold and DHEAS increased by 46% (on average) during the CRH infusion (154).

The fact that in our study, individual changes in DHEAS responses were related to personality parameters whereas there were no mean changes in DHEAS levels, raises the eventual hypothesis of the existence of different subgroups of DHEAS reactivity. Considering the fact that mean levels did not change, some additional intermediary factor could eventually exist, which we did not measure and which might explain the modulation or even determine the direction of the DHEAS response to CRH. For instance, Boudarene *et al.* (278) found that subjects with low anxiety levels showed an exclusive increase in DHEAS in response to cognitive stress, while subjects with higher anxiety had higher cortisol reactivity. Although we did not evaluate anxiety level in this specific study, cortisol mean levels increased in response to CRH and we found a direct relation between cortisol and DHEAS responses (not considering baseline cortisol and DHEAS levels). In fact, although higher baseline DHEAS levels were related to lower peak/baseline cortisol responses, DHEAS peak/baseline responses to CRH were also directly related to peak/baseline cortisol responses ($r=+0.29$, $p<0.05$) whereas DHEAS peak/baseline responses were not related to peak/baseline ACTH responses. Therefore, to us, a competition in the synthesis and release of DHEAS and cortisol, as Boudarene *et al.* hypothesized (278), does not seem a probable mechanism that could explain the individual differences in DHEAS responses.

In the human fetal and adult pituitary, CRH acts via protein kinase A (PKA). In human fetal adrenal cells, CRH increases messenger ribonucleic acid encoding 17alpha-hydroxylase/17,20 lyase (P450c17), but not 3β-HSD/Δ4-5 isomerase (702; 699). On the other hand, 3β-HSD expression is stimulated by ACTH. PKC, but not PKA inhibitors block CRH-stimulated P450c17 induction, whereas PKA inhibitors block ACTH-stimulated cortisol. Thus, it was suggested that in the human fetal adrenal, CRH is coupled to the phospholipase C-inositol phosphate second messenger system and preferentially induces the expression of P450c17 and DHEAS via PKC. CRH would act through PKA to increase pituitary ACTH response, and then ACTH would stimulate the expression of enzymes involved in cortisol synthesis whereas CRH would more directly induce adrenal enzymes involved in DHEAS synthesis though PKC. This could provide a potential mechanism for CRH stimulation of DHEAS response, independent of ACTH response and therefore distinct of cortisol response (702). Also, there is no significant decline in ACTH or cortisol

responses to CRH with aging whereas there is an ACTH-independent decline in DHEA response to CRH, again suggesting distinct regulatory mechanisms (703). Nevertheless, this is not so simple, because ACTH is also a major tropic hormone for adrenal androgen secretion, and plays a role in increasing cortisol and DHEA responses to CRH (700).

In the CRH test, we found that baseline ACTH was strongly and directly related to the peak ACTH response and was also directly related to the peak cortisol response and in turn baseline cortisol was also strongly related to the peak cortisol response suggesting that baseline HPA axis activity was a strong determinant in the response to CRH. ACTH was not related to ACTH or cortisol peak/baseline ratio suggesting that ACTH does not modulate the intensity of the response, but only that those subjects with higher baseline ACTH and cortisol levels naturally reach higher peak ACTH and cortisol levels. The influence of DHEAS seemed much subtler. Baseline DHEAS was not related to the peak ACTH or peak cortisol response in the CRH test. Similarly, it was not related to peak/baseline ACTH response in CRH test but it was inversely related to the peak/baseline cortisol response (after age, gender, baseline ACTH and baseline cortisol correction). Those results suggest that DHEAS may reduce the magnitude of the cortisol response independently of baseline ACTH or cortisol levels. This finding agrees with previous evidence that DHEAS may down-regulate cortisol levels (13; 232; 687; 704).

The reduction of glucocorticoid receptors, the inhibition of glucocorticoid receptor dependent transcription activity and the inhibition of 11β-HSD type 1 have been hypothesized as possible mechanisms of DHEA anti-cortisol effects. The last one could account for reduced cortisol levels, but would not explain why subjects with higher DHEAS levels would have reduced cortisol responses to CRH. Eventually, one hypothesis could be that long term higher DHEAS levels might modulate the adrenal capacity to respond to an acute stress or that subjects with higher baseline DHEAS levels were the ones that responded to CRH with reduced cortisol response. In fact, other authors found that DHEA acted directly on the rat zona fasciculata-reticularis cells to diminish corticosterone secretion by inhibition of the post cyclic Adenosine Monophosphate (cAMP) pathway, and decreasing the function of steroidogenic enzymes in P450scc as

well as StAR protein expression. More specifically, DHEA attenuated forskolin-stimulated SF-1 phosphorylation to affect StAR protein expression (705).

Then we obtained DHEA, DHEAS and cortisol measurements in two groups of healthy young adult female participants before and after the performance of cognitive tasks, one group with Working Memory load manipulation and another with emotional context manipulation. In these groups, DHEA, DHEAS and cortisol levels were not related to age or body mass index, which was not unexpected given the homogeneity of the sample regarding these two parameters (mean age was 20±0.5 and 21±1 years old and body mass index was 21.8±0.5 kg/$m^2$ and 22.3±0.8 kg/$m^2$).

As expected, the concentrations of DHEA, DHEAS and cortisol were distinct from those found in peripheral blood. We found salivary concentrations of DHEA about 3-11%, concentrations of DHEAS about 0.3-1.7% and concentrations of cortisol about 1.0-4.3% (in molar ratios), when compared to those expected for peripheral blood (using common reference levels). Mean cortisol-to-DHEA molar ratios were about 7 and mean DHEAS-to-DHEA ratios were around 2.1 when using DHEAS in pmol/h and DHEA in pmol/L (molar ratios using pmol/L for both hormones were around 46). Ota and collaborators described mean cortisol-to-DHEA ratios in the saliva between 9 to 21 (706; 707). In the peripheral circulation cortisol-to-DHEA ratios were about 5-10-20 and DHEAS-to-DHEA ratios were 100-300 (82).

DHEA and DHEAS concentrations in human brain tissue (homogenates) were higher than plasma concentrations, but cerebrospinal fluid concentrations were lower than plasma concentrations (82). DHEA was described to be 5% of plasma concentrations and cortisol was described to be 6% of plasma concentrations (82). DHEAS was expected to penetrate less into the cerebrospinal fluid than DHEA (0.03% of circulating DHEAS was expected to cross the blood brain barrier) (83), but the efflux of DHEAS out of the brain was also estimated to be tenfold greater than its influx (85) and in adults, the levels of DHEAS in the cerebrospinal fluid were still higher than those of DHEA (82). DHEAS-to-DHEA molar ratio was estimated to be about 100-300 in plasma and 2 in the cerebrospinal fluid (82). On the contrary, cortisol-to-DHEA ratio was expected to be

similar or a little higher in the cerebrospinal fluid and saliva than in the peripheral circulation (82).

Gooddyer *et al.* studied blood and saliva of adolescents (84) and proposed a similar relationship as that described by Guazzo *et al.* between blood and cerebrospinal fluid levels of DHEA, DHEAS and cortisol (82). It was even suggested that transport into the brain and saliva might be, to some extent, comparable and that salivary levels may thus give a reasonable representation of those found in the cerebrospinal fluid (82). We found a higher DHEAS-to-DHEA molar ratio than those authors. Nevertheless, we used corrected for salivary flow rate levels of DHEAS and in that case a similar DHEAS-to-DHEA ratio was found (12; 82). In fact, it was described that DHEAS may squeeze through the tight junctions between salivary glands (and DHEAS concentrations in saliva are dependent on serum concentration and salivary flow rate) (74). We found saliva-to-blood ratio of DHEA within the expected level when compared to the one described by Guazzo *et al.* (82). Of note, our DHEA levels were within the salivary reference levels but we did not actually measure blood levels in our study, just compared salivary levels with common reference levels.

In the two groups that we used saliva measurements, DHEA and cortisol baseline levels were directly related. On the other hand, baseline DHEAS levels (in saliva) were directly related to baseline DHEA/cortisol ratios and thus, DHEAS/DHEA levels were inversely related to cortisol levels ($r_s$=-0.526, p<0.001, n=43), suggesting a relation between higher DHEAS/DHEA ratios and lower stress levels. It also suggested that higher baseline DHEAS-to-DHEA ratios could eventually be a measure of lower chronic stress or cumulative burden, meaning lower allostatic load (290). It is interesting to note that we found a direct relation between DHEA and cortisol baseline levels, but we did not find a significant relation between DHEAS and cortisol baseline levels, also suggesting a distinct regulation of DHEA and DHEAS levels (considering the correction for the saliva flow rate, DHEAS was not related to DHEA, although if we had considered both measurements in pg/mL, DHEA and DHEAS levels were directly related – r=+0.514, p<0.001, n=44). The sulfotransferase and sulfatase as well as the membrane transport systems may also regulate and modulate DHEAS levels in different compartments contributing to those

differences (48; 54; 58; 62; 63). Besides, we recently found that (in the saliva of these same subjects), baseline DHEAS levels were directly related to baseline CRH (r=+378, p=0.005, n=54) but not to cortisol or DHEA levels, whereas cortisol levels were not significantly related to CRH or DHEAS levels (708).

It was not unexpected that CRH was not a significant factor for cortisol levels, since peripheral CRH is not a measure of hypothalamic levels (277; 709; 710). CRH could eventually stimulate DHEA synthesis through ACTH or another unknown factor contributing to the direct relation between DHEA and cortisol levels. On the other hand, a direct relation between peripheral CRH and DHEAS levels was found, which was independent of cortisol levels (708), therefore suggesting a relation independent of hypothalamic CRH. Chronic stress situations are usually accompanied by increased CRH and reduced DHEAS levels. Nevertheless, CRH and DHEAS have both neurostimulant effects. We studied healthy non-chronically stressed subjects. Besides, as mentioned before, CRH may act through PKA to increase pituitary ACTH response, which stimulates the expression of enzymes involved in cortisol synthesis whereas CRH may more directly induce adrenal enzymes involved in DHEAS synthesis though PKC. This could provide a potential mechanism for CRH stimulation of DHEAS response, independent of ACTH response, and therefore distinct to cortisol stress response (702). Together with our findings, it raises the hypothesis if DHEA might partially respond to ACTH (although the response was not equal to the cortisol response, it was directly related), whereas the conversion of DHEA into DHEAS could have a more distinct regulation. As mentioned, we also found a direct relation between DHEA-to-cortisol ratio and DHEAS levels and an inverse relation between DHEAS-to-DHEA ratio and cortisol. Therefore, higher DHEAS levels could be a measure of the balance between DHEA and cortisol while higher conversion of DHEA into DHEAS would occur in subjects with lower stress/cortisol level.

As mentioned, DHEA and cortisol baseline levels were directly related. Moreover, with the performance of cognitive tasks with Working Memory (WM) manipulation, DHEA and cortisol responses were also directly related, independently of the Working Memory load content of the task. This is in agreement with the fact that CRH stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for DHEA secretion (152;

696), even if indirectly through the action of a hypothesized DHEA androgen stimulating hormone (697; 698; 699). In fact, DHEA levels were shown to increase in response to stress (8) and anti-cortisol effects have been hypothesized to justify it (269; 318; 319). Nevertheless, we found important differences between DHEA and cortisol responses to Working Memory load manipulation. DHEA levels increased with the performance of the second task, independent of the task, suggesting either a cumulative effect or a latent interval before the response. Still, the increase in DHEA levels was more pronounced with WM load. Thus, the effect of a greater cognitive effort or specific effects of WM load on DHEA levels were suggested. Interestingly, this response was specific for DHEA and does not occur with cortisol and DHEAS. In fact, regarding cortisol, a decrease was found when the subjects were performing the discriminatory task (no Working Memory load). Whatever the specific mechanisms involved, there is an interesting point: the distinctive pattern of cortisol and DHEA responses. Thus, cortisol decreases after a simple task if this task comes first and DHEA increases after a second cognitive task when this is a challenging task.

In the group of participants who performed the protocol with emotional context manipulation (one task with neutral and another one with negative emotional context), DHEA levels increased after the performance of both conditions, independent of the emotional context of the task. Interestingly, a previous study showed that CRH levels increased with another cognitive task: the visualization of emotionally significant movies (276). In turn, the present results suggest that cognitive tasks are a stimulus for DHEA secretion and may lead to a higher DHEA/cortisol ratio. Although CRH stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for cortisol and DHEA secretion (152), thus explaining the direct relation we found between cortisol and DHEA levels, these results furthermore suggest that DHEA was not just a stress hormone and that some differential regulation of DHEA and cortisol exists.

Boudarene *et al.* (278) studied subjects without mental disorders and varying levels of anxiety, and found that the level of anxiety was related to the profile of endocrine response after the performance of cognitive tasks: subjects with high anxiety levels in the State-Trait Anxiety Inventory (STAI) test had increased cortisol reactivity and

subjects with low anxiety levels showed an exclusive increase in DHEAS levels. The authors suggested that the antagonism in DHEAS and cortisol might be related to competition in their synthesis and its release by the adrenal gland. Although the underlying mechanism is not clarified, these results agree with the results in the present study, in which all the subjects were found to have low anxiety levels and a DHEA raise but no cortisol response was observed in relation to the cognitive task (in both emotional conditions). For non-pathological conditions and low levels of anxiety, this might eventually represent an adaptive mechanism that includes higher DHEA than cortisol responses and anti-cortisol effects of DHEA. DHEA increase however was identical for both emotional contexts, so that DHEA increase alone was not expected to be related to performance or electrophysiological effects of the emotional context. Opposite to the observed DHEA responses, DHEAS mean levels did not change with working memory load or emotional manipulation.

**DHEAS relation to Personality**

We expected a stronger relation between DHEAS (than DHEA) and more stable parameters as personality as that hormone has a longer half-life. We explored the relations between DHEAS and Type A personality and superordinate trait (Neurotic Triad, Psychotic Dyad and Behavior-Deviant) scores obtained from the MMPI (688). Type A personality was conventionally defined by using the specific group of 11 items selected from the MMPI; Neurotic Triad: hypochondriasis + depression + hysteria; Psychotic Dyad: paranoia + schizophrenia; and Behavior-Deviant triad: psychopathic deviate + masculinity-femininity + hypomania (688). Lower DHEAS levels were related to higher Neurotic Triad scores. However, that relation was no longer significant after age correction, and in fact, age was directly related to Neurotic Triad score. This was surprising, not only because personality is generally considered as a stable personal characteristic established at a very early stage of life but also because our sample was young. Nevertheless, aging was previously described to be related to increased Neurotic Triad scores (711) and decreased DHEAS baseline levels and that seems to be the reason for this spurious relation between DHEAS and Neurotic Triad scores.

Higher DHEAS levels have been previously described in subjects with greater sensation-seeking and monotony-avoidant traits (357) and lower Type A behavior scores (355). In the present study, baseline DHEAS levels were not significantly related to any of the selected psychometric variables, pointing to the lower sensitivity of baseline endocrine levels. However, DHEAS reactivity in the CRH test was significantly related to Behavior Deviant triad and Type A personality and both relations persisted independent of age, gender and diagnostic group. Endocrine reactivity in the CRH test, regarding the ACTH and cortisol response, had been previously shown to be related to other psychometric variables (340; 341; 342; 344; 712; 713). In our study, higher DHEAS responses were associated with higher Type A behavior and Behavior Deviant triad (psychopathic deviation + hypomania + masculinity-femininity) scores and more specifically with psychopathic deviate score. Although the present results point towards a relation between DHEAS and the same group of personality traits that have previously been described, the relations do not follow in the same direction. Nevertheless, the relation between baseline DHEAS levels and DHEAS response is not necessarily direct. On the contrary, subjects with higher cortisol levels in the chronic set may have lower cortisol responses under stress.

**DHEA and DHEAS relations to Working Memory processing and involuntary attention**

We explored the relationships between cognitive performance and endogenous DHEA, DHEAS and cortisol. As expected, the audio-visual distraction paradigm including a manipulation of working memory, yielded typical effects as observed in previous studies. Indeed, the WM load task was harder to perform, as revealed by lower hit rates and longer reaction times. Likewise, novel sounds distracted the participants, as reflected by lower hit rates and longer reaction times. Regarding brain responses to novel sounds in both the discrimination (no WM load) and Working Memory load (WM load) tasks, the results revealed a novelty-P3 deflection, indicating that novel sounds caused distraction when compared to standards. The N2b elicited by the task-relevant visual stimuli was enhanced under WM load as expected (508).

Stangl *et al.* (714) demonstrated that DHEA administration increased DHEA levels and enhanced performance in a visual, same-different task (without WM load) while cortisol levels remained constant. However, other studies failed to provide systematic evidence that DHEA or DHEA administration enhanced short-term memory at the performance level (328; 331). In the present study, endogenous levels of DHEA, DHEAS and cortisol were measured and no significant relations were found to the accuracy or latency of the response. Nevertheless, a bigger sample of subjects may be necessary to uncover eventual relationship at the performance level.

On the other hand, this study demonstrated endocrine relations to the electrophysiological recordings. Rapid effects of DHEA or DHEAS reflected at the electrophysiological level could eventually result from rapid non-genomic effects of these hormones, by acting at ionic channels like NMDA and GABA-A membrane receptors. On the other hand, one could also hypothesize that long term changes in DHEA and DHEAS could eventually promote genomic effects that could then potentially modulate electrophysiological responses. Against these long term effects, no high affinity nuclear receptor of DHEA was identified so far, and direct genomic effects of DHEA and DHEAS are unknown. Nevertheless, DHEA and DHEAS may modulate, for instance glucocorticoids' effects, which are known to have genomic effects. Moreover, DHEA and DHEAS were shown to increase neurite growth and therefore suggested to influence in cortical organization (15).

In the WM task, we found that higher baseline cortisol/DHEA ratio was related to more processing of the distracting stimuli, as indexed by an enhanced novelty-P3. This suggests that baseline cortisol enhances, whereas baseline DHEA prevents auditory distraction (meaning involuntary attention to exogenous novel sounds), and simultaneously suggesting an anti-cortisol effect of DHEA. The fact that this relation became evident in the most stressful situation (WM load) agrees with previous evidence showing that DHEA has anti-cortisol effects under stress (283; 318) or that these effects are more evident under stress.

High cortisol levels or long term increases in cortisol levels are expected to decrease working memory performance whereas mild or short-lasting increases in

cortisol have been described to have beneficial effects on attention and memory (220; 633; 634; 635; 636; 637; 638). We studied healthy subjects with normal anxiety scores (as evaluated by the Stait-Trait Anxiety Inventory) and normal cortisol levels. Nevertheless, higher cortisol-to-DHEA ratios were related to enhanced involuntary attention processing and simultaneously with a trend towards reduced processing of the target stimuli.

We also explored the relationship between DHEAS-to-DHEA balance and brain processing of exogenous stimuli during the performance of a working memory task. Higher DHEAS-to-DHEA ratios were also related to enhanced novelty-P3 during the performance of the visual working memory task. This suggested a relationship between higher DHEAS-to-DHEA ratios and enhanced acoustic novelty processing during visual working memory tasks and more generally, that higher DHEAS-to-DHEA ratios might eventually be related to enhanced involuntary attention to exogenous stimuli (auditory distraction) during the performance of working memory tasks.

Clinical findings in patients with Alzheimer's disease already suggested beneficial effects of higher DHEAS-to-DHEA balance concerning memory (107; 447; 451). In fact, DHEA and DHEAS present a general neurostimulatory effect: presynaptic actions include glutamate, acetylcholine and norepinephrine release and postsynaptic actions include sigma 1 receptor agonism with subsequent *N*-methyl-D-aspartate (NMDA) receptor activation, γ-aminobutyric acid type A (GABA-A) receptor antagonism and inhibition of voltage-gated calcium currents (3; 7; 120). As mentioned, DHEAS has a much more potent excitatory action than DHEA concerning NMDA agonism (209; 315) and gabaminergic antagonism (184; 715) which may account for differential effects of both forms of the hormone (1; 184; 196). Furthermore, inhibiting the conversion of DHEAS to DHEA was shown to enhance cholinergic function in the hippocampus and improve memory in rats (208; 209; 210; 452) and DHEAS antagonized the neurotoxic effect of DHEA in neuroblastoma cells (238). The findings at the clinical level and the ones in our studies both point towards the importance of the sulfotransferase (the enzyme that converts DHEA into DHEAS) activity.

On the other hand, reducing the conversion of DHEAS into DHEA in mice impaired accuracy under attention demanding conditions (320) and subjects with lower DHEA

levels, lower DHEAS levels (716) or steroid sulfatase deficiency have higher rates of attention deficit hyperactivity disorder (322), therefore suggesting a relation between DHEAS deficiency, DHEA deficiency and high DHEAS-to-DHEA ratio and more distraction. These findings agree with the present findings in which a higher DHEAS-to-DHEA ratio was related to an enhanced processing of the auditory distractor. Nevertheless, in the present study the participants had normal DHEAS and DHEA levels and no deleterious effects of higher DHEAS-to-DHEA ratio on working memory processing or performance were found. Besides, methylphenidate [a central stimulant which increases oxidative stress namely lipid peroxidation and brain derived neurotrophic factor amongst others (717; 718)] administered to boys with attention deficit hyperactivity disorder had been shown to increase serum levels of both DHEAS and DHEA and simultaneously causing marked clinical improvement (323).

Novelty-P3 is an index of the effective orienting of attention towards the distracting event (477; 479; 480). We found a trend for a relation between higher cortisol/DHEA ratios and reduced visual P300 amplitudes attributed to working memory load ($r_s$=-0.52, p=0.014, n=22), suggesting that higher cortisol-to-DHEA ratios increased distraction from the visual working memory task. Nevertheless, the relation between higher DHEAS-to-DHEA ratios and increased involuntary auditory novelty processing occurred with no deleterious effect on the visual target stimuli processing. In fact, there was no relation between DHEAS-to-DHEA ratios and visual P300 amplitudes, therefore suggesting that the increased involuntary attention to novel sounds did not distract the subjects from the visual working memory load task. Additionally, there was no increase in novelty-P3 or visual P300 latencies and therefore, the increased involuntary attention to novel sounds did not occur at the expenses of longer stimulus evaluation times. In this context, the increased attention to unexpected novel sounds may be protective, maintaining involuntary attention to the surrounding world while voluntarily directing attention to the performance of the working memory load task.

Hence, DHEAS-to-DHEA balance may constitute an endogenous factor modulating exogenous attention mechanisms during the performance of cognitive tasks that require an active maintenance of information in working memory. The present results suggest

that subjects with higher baseline DHEAS-to-DHEA ratios might eventually have more attention resources available during the performance of working memory load tasks. Long term transcriptional effects of higher DHEAS-to-DHEA ratios, either directly or by inhibiting cortisol transcriptional effects could eventually contribute to long term increase of attentional resources. We also found a significant relationship between higher DHEAS-to-DHEA ratios and lower cortisol levels (719) [therefore suggesting lower chronic stress and lower cumulative burden or lower allostatic load (290) in subjects with higher DHEAS-to-DHEA ratios]. Nevertheless, cortisol levels alone did not have the same meaning as they were not inversely related to novelty-P3 processing during the performance of the same working memory load task (720).

It has been suggested that working memory results in prefrontal cortex activation and, in turn, a reduction of distraction (514; 520; 522; 523; 721). Moreover, it was also suggested that distractor stimuli could trigger prefrontal cortex activity and suppress the input of sensory information, thus preserving the contents of working memory from being disrupted by the distractor stimuli (722). Nevertheless, the present results suggest that higher DHEAS-to-DHEA levels may contribute to enhanced activation caused by irrelevant stimuli while contributing to maintained sensory activation related to the task. Interestingly (although its meaning is not known), working memory, attention and emotion involve prefrontal cortex activity (519; 520; 521; 597), DHEAS concentrations are lower and DHEA concentrations are higher in the prefrontal lobe when compared to the posterior brain areas (4). It has also been suggested relationships between DHEAS and DHEA, working memory, attention and emotional processes (293; 294; 295; 317; 451; 720; 723).

Moreover, the relations of DHEAS-to-DHEA ratio to brain processes seem to depend on the cognitive task involved. DHEAS-to-DHEA ratio was related to novelty-P3 processing during a visual working memory load task, but not during the visual discrimination task (no working memory load task). In the study using a visual emotionally negative context we also found no relation between DHEAS-to-DHEA ratio and involuntary attention to novel sounds, but higher DHEAS-to-DHEA ratios were related to reduced processing of negative emotional stimuli and shorter stimuli evaluation time.

Distinct patterns of receptors and neurotransmitters in different brain structures and circuits as well as circuit interactions may originate different effects of DHEA and DHEAS (233; 234) in different cognitive functions. Also, different DHEAS-to-DHEA gradients may eventually exist in different brain structures (4) involved in different functions, which could also contribute to these differences, but then little is known.

Regarding ERPs to the visual target stimuli, DHEA effects on WM load pointed towards increased visual P300 amplitudes. In fact, the rise in DHEA due to WM load was related to enhanced P300 amplitudes indicating enhanced memory update and suggesting a rapid DHEA behavioral effect. Endocrine responses to stimuli are commonly used and they usually provide higher sensitivity than baseline levels to detect pathological conditions or inter-subject differences (11; 339; 724). As an example, glucocorticoids responses to different stimuli can be used to measure the adrenals functional reserve (predicting their response to stress) or to characterize different phenotypes of the stress response (which were related to personality traits and pathological conditions) (725; 340; 341; 342; 726). Accordingly, in the present study, the parameter related to working memory processing was DHEA response and not baseline DHEA. Also, apart from its slow genomic effects, corticosteroids are known to have rapid non-genomic central nervous system effects (220; 638; 639).

Alternatively, the relation between visual P300 amplitude and DHEA response could suggest that subjects with enhanced working memory update are the ones with higher DHEA responses. In that regard, other authors demonstrated that chronic stress and higher cortisol levels were related to poorer memory (634; 636; 637) and reduced DHEAS responses to a superimposed psychological stress (11). Nevertheless, we found no relation between baseline cortisol and visual P300 amplitude, and therefore, an effect of chronic stress was not suggested. Wolf *et al.* (24) studied the effects of DHEA replacement on short term memory ERPs. They reported an increase in P3 amplitude after DHEA replacement, which reflected an enhancement of information updating. This is in accordance with our results, as we also observed that the physiological DHEA increase was related to an enhanced visual P300.

A short term increase in cortisol can damage hippocampal neurons and may impair memory (574). This may be an oversimplification since specific types of hippocampal mediated memory may be impaired by stress but others may not (727; 728). Yet, and hypothesizing that DHEA might prevent memory impairment under stress, Wolf *et al.* (327) found that DHEA replacement enhanced attention but did not prevent the decline in visual memory under an acute psychosocial stress. This result did not support the idea of a direct anti-glucocorticoid effect of DHEA in hippocampal mediated memory functions. In another study DHEA protected hippocampal neurons against excitatory amino acid-induced neurotoxicity (232). Our results also supported the idea of DHEA anti-cortisol effects in distraction, but regarding working memory, we found a relationship with DHEA, not cortisol. Moreover, normal ranges of baseline cortisol were observed and cortisol levels did not rise with WM load, nor did they decrease.

Recent results also suggest that repeated stress and consequent activation of the glucocorticoid receptors dampens prefrontal cortex glutamatergic transmission. Actually, it facilitates glutamate receptor turnover, which has a detrimental effect on prefrontal cortex-dependent cognitive processes (729) like Working Memory. The present results agree with the known action of DHEA on glutamatergic receptors as well as with the idea that DHEA opposes cortisol detrimental effects during the performance of working memory tasks under stress. This last relationship was evidenced by inverse relation of DHEA and cortisol to distraction.

Nevertheless, working memory effects were related to the DHEA response but not to cortisol. Thus, regarding WM effects, an anti-cortisol effect of DHEA was not so evident but other specific effects of DHEA may be present. As mentioned, besides their anticortisol effects, DHEA has Gamma Aminobutyric Acid Type A (GABA-A) receptor antagonism and sigma 1 agonist effects (1; 3; 7) which might underlie or contribute to the effects found. In fact, both gabaminergic antagonism and glutamatergic agonism are known to improve cognitive function.

Of note, we found no relation between baseline DHEA or DHEAS and performance or ERPs. Instead, baseline cortisol/DHEA ratio, DHEAS/DHEA ratio and DHEA response relationship to ERPs were found. If DHEA and DHEAS have mostly modulatory effects over

ion channels and anti-cortisol mechanisms of action instead of specific receptors and effects, and if DHEAS also function as a pro-hormone regarding these cognitive effects, that by itself might contribute to the relevance of baseline cortisol/DHEA and DHEAS/DHEA ratios instead of baseline DHEA or DHEAS levels alone.

**DHEA and DHEAS relations to emotional processing**

The study with emotional context manipulation revealed relations between DHEA, its sulfated form (DHEAS) and brain processing under an emotionally negative context induced by images of fearful faces, suggesting that these neurosteroids may modulate the processing of emotionally negative information. Although the sounds as well as the emotional content of the pictures were irrelevant for the task, the results suggest that the subjects were unable to fully ignore them as indicated by significant effects on performance and brain responses. The distraction effect of task irrelevant auditory stimuli as well as the behavioral disruption due to the processing of task irrelevant negative emotional stimuli have been shown before by other authors (477; 479; 509; 534; 535), whereas the relation between these neurosteroids and emotional processing at the brain level is a new finding.

Novel sounds led to distraction as shown by longer response times and the elicitation of a significant novelty-P3. Conversely, no clear N1-enhancement/MMN was observed in our results, probably due to a very early P3 onset, causing an overlap between the components. Recent studies have shown that small deviant stimuli, and hence large novel sounds, may activate deviance-detection mechanisms as early as 20 ms from sound onset (730; 731) and therefore attention switching may have had occurred before the supratemporal activation giving rise to the typical N1-enhancement/MMN trigger response (732). Additionally, in the negative emotional context, response times were longer than in the neutral context which implicated that the novel sounds effectively caused distraction and the emotionally negative context effectively disrupted performance. However, the emotional context did not significantly modulate the electrophysiological response to auditory distraction, contrasting with previous studies

(509; 544) which found an enhancement of the novelty-P3 and also found an enhancement of the visual P300 for emotionally negative stimuli (509). Nevertheless, in studies using faces, some authors have found visual P300 enhancement to fearful faces (542), while others reported no changes in P300 amplitude (543) as in the present study. Importantly, in the present study, the emotional content of the images was not relevant for the task.

No significant relation was found between response times or hit rates and endocrine measurements. As mentioned before, a larger sample may be necessary to uncover endocrine relationship to performance. Nevertheless, interesting findings of this study were the endocrine relationships to brain responses. A higher DHEA/cortisol ratio was related to lower visual P300 latencies in the negative emotional context, suggesting DHEA to be protective from an interference of the implicit negative content of the stimuli with the task which might represent an anti-glucocorticoid effect of DHEA. In the negative condition, higher DHEAS/DHEA ratios were also related to lower visual P300 latencies, thus, a higher proportion of DHEA respective to cortisol and a higher proportion of sulfated to non-sulfated form of DHEA may provide protection from any interference with the task caused by the processing of the implicit emotional content of the stimuli.

In agreement, higher DHEA levels have been previously found to be related to shorter P300 latencies (625; 733). A protective effect of DHEA and DHEAS could eventually be mediated by anti-cortisol effects and/or neuromodulatory effects of these hormones. Wang *et al.* (734) associated a prolonged P300 latency with an attentional bias to negative stimuli in high trait anxiety subjects, who were expected to have higher cortisol levels. On the contrary, in the present study, examining subjects with low trait anxiety, higher DHEA/cortisol ratios were related to shorter P300 latencies in the negative condition. Higher DHEA/cortisol could be related to less interference with the task caused by the implicit stressful content of the stimuli, in agreement with the hypothesis of anti-cortisol effects of DHEA and suggesting a protective mechanism against the deleterious consequences of stress. The present results also agree with studies at the cellular level, in which DHEA was protective against the neurotoxic effects of corticosterone (262).

Lastly, the visual P300 amplitude increments in the negative emotional context as compared to the neutral one, were inversely related to DHEAS/DHEA ratios, suggesting that the processing of the task-irrelevant negative content of the stimuli might eventually be reduced by a higher proportion of DHEAS in relation to DHEA. Even though the participants of the study were healthy and depression was not screened by any specific inventory, women in particular have been shown to be especially sensitive to threatening stimuli and it has been hypothesized that this might be related to the higher prevalence of affective disorders in women (735). Enhanced negative affect is an integral characteristic of depressive disorders and negative mood can be experimentally induced using various procedures, among others by presenting images of faces with emotional expressions (736; 737; 738).

Although the mechanisms underlying mood persistence in major depressive disorder remain poorly understood, cognitive theories hypothesize that depressed patients have cognitive biases for emotional information, which help perpetuate depressive symptoms (739; 740). The present results suggest that the processing of negative emotional stimuli may be reduced in relation to higher DHEAS/DHEA levels, which might eventually be involved in protective mechanisms against a negative attention bias and undermine the relation between higher DHEAS levels and lower frequencies of depression, lower depression ratings and better wellbeing scores previously described by other authors (18; 20). In line with this hypothesis, subjects with higher DHEAS/DHEA ratio would process less and have less memory updating of the implicit negative content of the stimuli and would thus have a reduced attentional bias towards these stimuli, which might promote wellbeing and contribute to protection from depressive states.

Emotional stimuli usually capture attention more effectively than non-emotional ones (534) and have an impact on cognitive functions even when they are task-irrelevant (509). On the other hand, it is hypothesized that protective mechanisms filter out irrelevant sensory inputs protecting the higher brain functions from sensory overload (741). Taken together, our findings concerning visual P300 latency and visual P300 amplitude under an emotionally negative context showed a relation between higher DHEA/cortisol and DHEAS/DHEA ratios and lower processing of the negative emotional

stimuli and less disruption by the processing of negative emotional stimuli. These results also suggest that DHEA and DHEAS potentially play a role or contribute to protective mechanisms filtering out negative information overload.

A simultaneous relation to latency reduction and decrease in P300 amplitude may seem contradictory at first sight, nevertheless, as the task consisted in deciding whether two faces with the same emotional expression were equal or different (while the emotional expression was irrelevant), lower processing of the task-irrelevant emotional content – related to higher DHEAS/DHEA ratios - was reflected in smaller P300 amplitudes and at the same time shorter P300 latencies, providing a more efficient processing of the task-relevant visual information, and less interference resulting from the task-irrelevant emotion. In other words, the P300 amplitude might be reduced as a reflection of less neuronal recruitment due to less allocation of attentional resources to the task-irrelevant emotion, while P300 latencies might be reduced due to a decreased stimulus evaluation time, likewise due to less processing of the task-irrelevant emotional content of the images. This interpretation is consistent with the direct relation we found between reduced P300 amplitudes and shorter P300 latencies. Furthermore, it is consistent with previous studies showing that emotional stimuli elicit an enhanced P300 (742) and longer P300 latencies for negative emotional target stimuli (743) when compared to neutral stimuli. Additionally, emotional stimuli outside the attentional focus have been shown not to elicit an enhanced P300 (744).

Endocrine relationship to auditory distraction was not found in the present study: DHEA, DHEAS, cortisol, DHEA-to-cortisol or DHEAS-to-DHEA ratio were not related to the novelty-P3, suggesting that their levels did not modulate auditory distraction processing under an emotionally negative context. This differs from our previous findings concerning auditory distraction under working memory load, in which we found that the novelty-P3 amplitude was enhanced in relation to higher baseline cortisol/DHEA ratios and higher DHEAS/DHEA ratios. As mentioned, it also suggests that the influence of the endocrine parameters on auditory distraction, may depend on the cognitive task involved.

Again, the present results also highlighted the relevance of the balance between DHEA, DHEAS and cortisol, concerning the processing of negative emotions. Importantly,

these results agree with findings at the clinical level, in which higher DHEAS concentrations and DHEA-to-cortisol ratios but not DHEA levels alone were related to less frequent depression, less depressive mood and higher well-being scores (20; 365; 366). As mentioned before, although DHEA and DHEAS can be converted into each other and general common effects can be expected for the sulfated and non-sulfated form of the hormone (1; 3; 7), several differences exist in their mechanism of action. DHEAS has a much more potent excitatory action by NMDA agonism and gabaminergic antagonism than DHEA (1; 184; 196) and sulfated steroids possibly act as endogenous neuromodulators (207). Therefore, the balance between DHEAS and DHEA might influence brain functioning. Previous studies at the cellular and molecular level showed that DHEAS had neuroprotective effects mediated through GABA-A receptor antagonism (185), DHEAS stimulated dopamine release from rat hypothalamic cells (252) and that DHEAS antagonized the neurotoxic effect of high doses of DHEA (238). These studies suggest potential mechanisms by which DHEAS could have more potent anti-depressant effects than DHEA and agree with the present finding that higher DHEAS/DHEA ratios are related to reduced processing of the negative emotional content.

**Integrating the present results with previous knowledge of DHEA and DHEAS regulation and physiological effects**

Some authors defend that there is an unidentified adrenal androgen stimulating hormone (AASH) (697; 698; 699) whereas others defend the notion that adrenal androgen synthesis depends on the relative activities of adrenal enzymes and DHEA sulfotransferase activity and that ACTH alone is involved in this process (152; 696). Concerning the modulation of glucocorticoid and adrenal androgen levels, the literature suggests that in the chronic setting, ACTH may be an adrenal trophic factor, therefore increasing cortisol, DHEA and DHEAS synthesis (700). The present results agree with those results, as baseline ACTH levels explained part of baseline DHEAS levels, even if only a minor part of DHEAS variability was explained by baseline ACTH levels (745). An acute increase in ACTH levels may preferentially stimulate cortisol synthesis and to a lower extend it might also stimulate DHEA synthesis, whereas CRH may stimulate pituitary ACTH

and also directly stimulate adrenal DHEAS synthesis (154; 702). This would also agree with the direct relation we found between cortisol and DHEA, both baseline and reactivity, although the relative responses of cortisol, DHEA and DHEAS were different (719; 720; 723; 745). Because only a minor part of baseline DHEAS variability was explained by baseline ACTH and different responses of DHEA and cortisol were found our results suggest other regulating factor(s) besides ACTH (720; 723; 745).

One hypothesis to integrate the present findings with previous research would be that chronic stress could eventually promote higher chronic CRH levels, with pituitary chronic stimulation of ACTH synthesis, higher adrenal cortisol-to-DHEA production, and therefore lower baseline DHEAS and subjects with higher baseline cortisol would also have lower DHEA to DHEAS conversion. Without chronic stress (low anxiety subjects), low baseline CRH could eventually lead to low baseline ACTH and directly stimulate the adrenal to preferentially produce more DHEA than cortisol and therefore also higher DHEAS levels and higher DHEA to DHEAS conversion. That would also agree with our findings, in which subjects with higher DHEA-to-cortisol ratio were the ones with higher DHEAS levels and subjects with higher cortisol levels had lower DHEAS-to-DHEA ratios (719; 720; 723). As CRH effects may occur at the level of enzymatic synthesis (699; 702), these may be long term effects and it is to be expected that long term stress may affect the acute response of the adrenal to a stressful event (11). Nevertheless, in subjects with low anxiety trait and state, we found that higher baseline CRH was also directly related to higher baseline DHEAS, independently of baseline cortisol or DHEA levels (708). Therefore, it agrees with other studies that suggested that CRH may directly (independently of ACTH) stimulate DHEAS synthesis (702). Nevertheless, we found no rapid changes in CRH (data not shown) or DHEAS levels in response to cognitive tasks when DHEA levels changed, suggesting that CRH may not be the major stimulus for DHEA secretion in these situations.

Subjects with high chronic stress are known to respond with less ACTH surge but increased adrenal cortisol response. Additionally, in the present hypothesis, those subjects could eventually respond with proportionally less DHEA and DHEAS. On the contrary, subjects without chronic stress, would respond with more ACTH but the adrenal

would be prepared to produce proportionally less cortisol and more DHEA in response to an acute ACTH surge as compared to chronic stress subjects, and the CRH surge would also stimulate the adrenal to produce proportionally more DHEAS. Also, higher DHEA and DHEAS would reduce the acute cortisol response to a stressful event (13; 232; 687; 704) by modulating enzyme activity (705), which also agrees with our results (745). The sulfotransferase and sulfatase levels and activity could also contribute to modulating DHEAS-to-DHEA levels. For instance, the sulfotransferase was shown to be down-regulated during infection, inflammation and the euthyroid sick syndrome (174; 175; 176). The liver could also contribute to the balance between DHEAS and DHEA, and the brain, by having higher DHEAS and DHEA levels than the peripheral blood, it could also contribute to peripheral levels of these hormones, in particular concerning peripheral DHEA levels (as this is expected to easily cross the blood brain barrier). In the brain, eventually, higher oxidative stress might induce more DHEA synthesis and decrease the DHEAS-to-DHEA ratio (109; 110).

Stressful stimuli, namely psychological ones, stimulate DHEA and cortisol secretion. Interestingly, we found that DHEA increased in response to cognitive tasks performance (720; 723). This could result from stress inherent to those tasks, but DHEA and not cortisol increase was found, suggesting a preferential DHEA rather than cortisol response in low anxiety subjects (720; 723). Moreover, the increase in DHEA was higher for the working memory load task, also suggesting a possible specific effect of working memory tasks (720). In any case, the present results suggest that cognitive tasks may constitute a stimulus for DHEA secretion and may eventually increase DHEA-to-cortisol ratios. Furthermore, a distinct regulation of DHEA and cortisol secretion also seems apparent.

The rapid effects of DHEA and DHEAS may occur through the modulation of membrane ion channels, receptors and neurotransmitters (3; 7). Cortisol is also known to have rapid effects through such mechanisms (220). DHEA and DHEAS differ in a sulfate group, therefore, different effects on ion channels are expected. On the other hand, baseline levels, may traduce long term effects, even organizational ones (189; 242), either through the modulation of cortisol effects (231; 262; 263) or through the modulation of

enzyme activity or genomic processes (3; 7), even if there is no specific membrane or nuclear receptor (7). For instance, these hormones may modulate cortisol genomic effects (231). Also, the balance between DHEA and cortisol and DHEAS and DHEA may traduce either different or opposite effects of these hormones or eventually that these ratios may involve some unknown regulatory factor. Endocrine phenotypes are established early in life, meaning that each person has a typical baseline and reactivity endocrine pattern, which is relatively stable, while inter-individual differences are higher (339). Personality is also established early in life and relatively stable (338) and we found a relation between DHEAS reactivity and personality profile, in particular related to behavior deviant triad and type A personality.

Concerning central effects, mainly relations to attention, memory and humor have been suggested for DHEA and DHEAS. Although most studies with DHEA reposition failed to find beneficial results in memory, results found in literature suggest that higher endogenous DHEAS, DHEAS-to-DHEA and DHEA-to-cortisol ratios may enhance working memory (7). In particular, lower DHEAS-to-DHEA ratios were found in Alzheimer's disease (107; 447; 451), whereas higher DHEAS-to-DHEA ratios (in sulfatase deficiency) were found in attention deficit disorder (322), thus suggesting that higher DHEAS levels may be important for long term memory but also that high DHEAS or low DHEA may enhance distraction. We found that higher DHEA reactivity was related to enhanced working memory (720). On the contrary, higher cortisol-to-DHEA and DHEAS-to-DHEA were related to enhanced distraction, but (in the present results), whereas higher cortisol-to-DHEA might compromise the processing of a concomitant working memory task, maybe a higher DHEAS-to-DHEA would not. Therefore, anti-cortisol effects of DHEA were suggested regarding distraction whereas specific rapid DHEA effects were suggested regarding working memory processing. We found no relation between DHEAS levels alone and working memory processing.

The present results agree with previous studies which suggest that DHEA, DHEAS and cortisol may modulate memory processes and eventually oppose some deleterious effects of high cortisol levels on memory. Previous studies suggest that mild or short-lasting increases in cortisol due to stress generally have beneficial effects on attention

and memory, whereas high cortisol levels or long term increases are related to poorer prefrontal cortex working memory, poorer executive functioning, poorer learning and less cognitive flexibility (220; 633; 634; 636; 637; 638; 639; 641; 644). Higher DHEA levels have also been related to higher hippocampal volume, increased cortical thickness of the prefrontal cortex and other areas involved in memory processes as well as early activation of the anterior cingulate cortex, whereas higher cortisol levels were correlated to lower hippocampal volume and reduced memory capacities (448). The effects of cortisol on memory also depend on timing, with stress immediately before or after a stimuli enhancing memory, whereas a stress prior to retrieval may impair memory (573). DHEA and DHEAS baseline levels and reactivity might eventually influence different phases of memory processes, but little is known.

Concerning humor, higher DHEAS or DHEA-to-cortisol ratios have been proposed to be related to less depression ratings and small DHEA reposition studies also support that effect (362; 383; 384; 385). Our study suggests that higher DHEA-to-cortisol and higher DHEAS-to-DHEA may protect against interference/disruption by the negative content of the stimuli and higher DHEAS-to-DHEA ratio may be related to less processing of the negative content of the stimuli (723). Less attention to negative stimuli may eventually be protective against depressive states and undermine the relation between higher DHEAS levels and less depression found in clinical studies.

Concerning emotional processing, cortisol increases the processing and memory for emotional stimuli (648). Previous studies associated chronic increases in cortisol levels to more errors in the categorization of emotional stimuli, increased activity in the hippocampus and altered prefrontal cortex function (647). On the other hand, the present study and previous neuroimaging studies, suggest that DHEA and DHEAS may downregulate the negative emotions induced by aversive stimuli (25; 723). During emotional processes, higher DHEA levels were previously related to reduced activity in the amygdala and hippocampus (which were involved in the generation of negative emotions) and an increased connectivity between these two regions (25).

The fact that DHEA and DHEAS have neuro-excitatory and simultaneously anti-stress effects seems contradictory at first sight. Nevertheless, eventually due to its rapid

effects, modulating membrane receptors (norepinephrine release, GABA-A antagonism and glutamatergic agonism for instance) are neuro-excitatory, while simultaneous anti-cortisol effects may be “anti-stress”. It also suggests a different paradigm for these hormones: in part, they would cause the subjects to be “active but without stress” (in what concerns cortisol effects). DHEAS could eventually be more neuro-excitatory (possibly due to its sulfate group; and it is known to have more potent GABA-A antagonism and glutamatergic agonism) whereas DHEA might eventually be more “anti-cortisol” (most opposite to cortisol effects were found for DHEA instead of DHEAS; DHEA and cortisol are lipophilic and can cross cell membranes; molar concentrations of DHEA and cortisol bear similarity much more than DHEAS and cortisol concentrations).

**Limitations of the studies**

In the study exploring DHEAS relations to CRH test and personality the participants belonged to different diagnostic groups. Nevertheless, the diagnostic group was taken into account during the statistical analysis. Moreover, this was a retrospective study and we did not have DHEA measurements, which would be expected to show higher short time changes during the CRH test.

In the studies addressing endocrine relation to working memory, emotional processing and involuntary attention at the performance and electrophysiological level we measured DHEA, cortisol and DHEAS in the saliva and not in the brain. Nevertheless, DHEA and cortisol are lipophilic and saliva levels are expected to be comparable to serum (74; 746), cerebrospinal fluid (747) and brain tissue levels (748). DHEAS is hydrophilic and does not readily cross the blood-brain-barrier or cell membranes. Nevertheless, DHEAS concentrations in the brain are several times higher than in the peripheral circulation and the efflux of DHEAS across the blood-brain barrier was determined to be tenfold greater than its influx (701). On the other hand, only a small proportion of DHEAS is expected to cross the blood brain barrier and most DHEAS in the peripheral circulation has its origin in the adrenals (27; 28). Therefore, we cannot say whether peripheral levels of DHEAS are related to its levels in the brain. Yet, peripheral levels of DHEA and DHEAS levels were

found to be directly related to cerebrospinal fluid levels (82) and as mentioned before, it was suggested by Guazzo *et al.* that salivary levels may give a reasonable representation of DHEA, DHEAS and cortisol levels in the cerebrospinal fluid (82). DHEA metabolites also include other neuroactive steroids such as estradiol, estrone and testosterone (178; 331), which may mediate part of the DHEA effects (749). Estrogens, and in particular estradiol, enhance working memory in women (750; 751). We did not measure DHEA metabolites, which may mediate some of its effects. Subsequently, we cannot exclude that those steroids may contribute or eventually be responsible for the electrophysiological relations we found.

Furthermore, in these studies addressing working memory, emotional processing and attention, the two groups of participants were in different menstrual cycle phases and some were using hormonal contraception. DHEA, DHEAS and cortisol levels can change along the menstrual cycle and with the use of hormonal contraception (752; 753). Nevertheless, eventually in relation to the small sample size, in these studies, the endocrine levels were not significantly different in relation to the menstrual cycle phase or the use of hormonal contraception. But even considering that DHEA, DHEAS or cortisol levels could change along the menstrual cycle or with the use of hormonal contraception, the relations we found between endocrine levels and working memory, emotional stimuli or auditory distraction processing would not be invalidated. In any case, given the randomized approach we used concerning the menstrual cycle phase, the present results are expected to be independent of the menstrual cycle phase. Finally, the fact that only female participants were included in these studies, limit the outreach of the present study only to women. DHEAS levels differ between genders (2; 754), therefore, another group of participants would be necessary to extend our conclusions also to men. For further studies, it would be relevant to study whether the results are identical in men.

# Conclusions

Baseline ACTH levels were directly related to baseline DHEAS levels, suggesting that ACTH modulates DHEAS levels. Baseline DHEAS levels were inversely related to the intensity of the cortisol response in the CRH test (taking into account cortisol baseline levels). Therefore, a relation between higher baseline DHEAS levels and lower magnitude of the cortisol response to stress is suggested. A relation between DHEAS and personality is also suggested as DHEAS reactivity was directly related to Behavior Deviant triad and Type A behavior. On the other hand, in women, DHEA levels increased after the performance of cognitive tasks, independent of the emotional context of the task and to a larger extent after a working memory load rather than on a discriminatory task. These results suggest that cognitive tasks may be a stimulus for DHEA secretion. Moreover, a simple stress response is not suggested as there was no concomitant increase in cortisol levels.

A higher cortisol/DHEA ratio was related to enhanced processing of the auditory distractor during the performance of a visual working memory task. This suggests that in women, DHEA may oppose cortisol effects in involuntary distraction, reducing the processing of the auditory distractor (novelty-P3). Furthermore, higher DHEAS-to-DHEA ratios were related to enhanced auditory novelty-P3 amplitudes during the performance of a visual working memory load task but were not related to visual P300 amplitudes, novelty-P3 latencies or visual P300 latencies. These results suggest that higher DHEAS-to-DHEA ratios may be related to enhanced acoustic novelty processing with no detrimental effect in working memory processing. More generally, it is suggested that higher DHEAS-to-DHEA ratios may enhance involuntary attention to the surrounding world during the performance of working memory load tasks, which may be an important protective mechanism. Regarding working memory, a higher DHEA response due to Working Memory load was related to an enhancement of the task-relevant information processing (visual P300), suggesting an eventual rapid effect of DHEA. Overall, the results suggest that DHEA may oppose cortisol effects reducing distraction, higher DHEAS-to-DHEA balance may increase involuntary attention with no reduction in working memory and a higher DHEA response may enhance working memory at the electrophysiological level.

Also in women, during the processing of stimuli with negative emotional content, higher DHEA/cortisol and DHEAS/DHEA ratios were related to shorter visual P300 latencies, suggesting a relation of these endocrine parameters with shorter stimulus evaluation time and less interference with the task at hand due to the processing of the task-irrelevant negative content of the visual stimuli. Additionally, higher DHEAS/DHEA ratios were related to reduced visual P300 amplitudes suggesting less processing of the negative information, which might constitute a protective mechanism against negative information overload. The present results also suggest the importance of the sulfotransferase and sulfatase activity in the modulation of DHEAS and DHEA brain effects.

# Future research and clinical perspectives

In the future, knowledge of DHEA action and reposition effects may bring additional clinical utility to its measurement and eventually uncover therapeutic usefulness. For future research it would be important to study the relation between DHEA and DHEAS and memory, attention and emotional processing also in men. Men have higher levels of DHEAS in the peripheral circulation while DHEA levels are identical to that in women, so, particular aspects may exist. Furthermore, we found several trends in what concerns the relation between DHEA and performance. Studies with a larger number of participants would be important to confirm the present results and might reveal significant behavioral relations of DHEA and DHEAS with memory, attention and emotions. We did not measure the DHEA response to CRH, which could add some information regarding the relation between these hormones and the ACTH and cortisol responses. We did not measure other hormones that could contribute to the present relations between DHEA or DHEAS and personality or brain processing. Further research could also address DHEA and DHEAS active derivatives and other neurosteroids.

Studies in older people (in whom DHEA and DHEAS levels decrease), in ill and recovering persons (in whom DHEAS levels may change in a short period of time), in patients with adrenal insufficiency (in whom peripheral DHEAS levels are low) and in patients with hepatic failure (in whom sulfotransferase activity may be affected) may provide further evidence of DHEA and DHEAS brain and behavioral effects. Measuring the temporal evolution of DHEA, DHEAS, cortisol and CRH levels during acute medical illness and recovery (during the neuroendocrine adaptation syndrome and recovery) and if possible simultaneously evaluating rapid differences in electrophysiological, neurofunctional or performance parameters may also eventually provide further evidence of short time DHEA and DHEAS changes and their possible effects in illness. It would also be interesting to study short term changes in DHEA-to-cortisol and DHEAS-to-DHEA ratios in those study groups.

Previous reposition studies in humans using oral DHEA were inconclusive or brought insufficient results. In fact, contradictory results exist regarding memory. The

present and previous results point towards an effect of the DHEAS-to-DHEA ratio and that could justify the contradictory results of DHEA reposition studies regarding memory. Therefore, reposition studies measuring not only DHEA but both DHEA, DHEAS and cortisol and their ratios before and after treatment could eventually bring new information regarding the effects of DHEA treatment in memory and particularly in Alzheimer's disease. A different point raised by the present studies is whether the performance of cognitive tasks may raise DHEA levels in the long term and whether this could prevent DHEA decline with age, a point that was also not studied. The present results also suggest that subjects with higher DHEAS levels may have lower cortisol stress responses, therefore, it might be useful to study whether DHEA reposition could protect from deleterious effects of high cortisol levels or cortisol peaks, namely regarding memory, but also concerning other diseases like the cardiovascular ones.

Concerning humor, although coherent and pointing towards positive anti-depressive effects of DHEA reposition, a small number of participants with depression were studied. Therefore, larger reposition studies and also simultaneously measuring DHEA, DHEAS and cortisol levels, might reveal important information. In the future, if the existing preliminary results are confirmed, DHEA treatment could be an anti-depressive drug candidate. If reposition studies point towards an important effect of DHEAS-to-DHEA ratio, then the modulation of the sulfotransferase activity needs to be studied and if possible, addressed.

The present results may help in understanding DHEA and DHEAS physiological effects and future research may establish if there is a place for the use of DHEA and DHEAS levels as clinical indicators for brain processing, personality or the stress response phenotype and if there is a place for the use of DHEA treatment.

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# Annex:

# Published articles included in the Thesis

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ORIGINAL ARTICLE

# Plasma dehydroepiandrosterone-sulphate is related to personality and stress response

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*Key words:* **DHEAS; cortisol; personality; stress; DHEAS reactivity; CRH test**

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## Abstract

**OBJECTIVES:** Dehydroepiandrosterone-sulphate (DHEAS) physiologic relevance remains controversial. However, several central nervous system and behavioural effects of DHEAS have been described. We explored the relation between DHEAS and both pituitary-adrenal axis reactivity and personality in human subjects.
**DESIGN:** We studied 120 consecutive patients assisted at the out patient endocrine department of a public central hospital before medical treatment. Personality was evaluated with the Minnesota Multiphasic Personality Inventory (MMPI) and the pituitary-adrenal axis reactivity was assessed with the CRH test.
**RESULTS:** Baseline DHEAS was inversely related to peak/basal cortisol (parcial r=–0.454, $p<0.05$) response to CRH infusion. DHEAS reactivity in the CRH test was directly related to the Deviant Behaviour triad (BD) (r=0.257, $p<0.05$) and type A personality (AP) (r=0.295, $p<0.05$). Basal ACTH was directly related to baseline DHEAS (r=0.366, $p<0.001$) and together with age and gender explained 34% of DHEAS variability.
**CONCLUSIONS:** DHEAS may be a protective factor against an excessive cortisol response when people are under stress situations. Personality may be related to DHEAS reactivity.

**Abreviations:**

| | |
|---|---|
| AP | - Type A Personality |
| AUC | - Area Under the Curve |
| AUC/h | - Area Under the Curve/hour |
| BD | - Behaviour Deviant Triad |
| GABA-A | - Gamma-Aminobutyric Acid Type A Receptor |
| K-S | - Kolmogorov-Smirnov |
| MMPI | - Minnesota Multiphasic Personality Inventory |
| NMDA | - N-methyl-D-aspartate receptor |
| NT | - Neurotic Triad |
| PD | - Psychotic Dyad |
| sd | - standard deviation |

## INTRODUCTION

In humans dehydroepiandrosterone-sulphate (DHEAS) is the most abundant hormone in the peripheral circulation. Its normal levels (2–10 μmol/L) are more than 20 times those of cortisol or thyroxine, more than 100 times those of testosterone, growth hormone and prolactin and more than 10,000 times those of aldosterone, estradiol or insulin. On the other hand, most laboratory animals have only negligible amounts of DHEAS. Even primates have much lower levels than humans (Berr *et al.* 1996).

DHEA is mostly synthesised in the adrenals and gonads, either as the final androgen-like compound or as an intermediate in the synthesis of androgens. However, it is also synthesised in the central nervous system (Paul & Purdy 1992; Berr *et al.* 1996; Baulieu & Robel 1998; Reddy & Kulkarni 1998; Labrie *et al.* 2003; Sicard *et al.* 2007). A sulphotransferase reversibly converts DHEA into DHEAS, and the sulphated form is by far the most abundant in the peripheral circulation. Nevertheless, the nonsulphated form is, however, much more lipossoluble and may easily cross biologic membranes presenting a much larger distribution that includes the central nervous system (Berr *et al.* 1996; Sicard *et al.* 2007). DHEA has a short half-life – 1 to 3 hours – as opposed to DHEAS that has a long half-life – 10 to 20 hours (Legrain *et al.* 2000; Muniyappa *et al.* 2006; Komesaroff 2008).

No definitive factors regulating DHEAS synthesis have been so far identified.

DHEAS physiological effects and teleological meaning are unclear and controversial. At the clinical level DHEAS levels are higher in males and dramatically decrease with age – in the seventh decade they are about 20% of those in the third decade (Berr *et al.* 1996; Kimonides *et al.* 1998; Laughlin & Barrett-Connor 2000; Tannenbaum *et al.* 2004; Sicard *et al.* 2007). Furthermore, DHEAS levels relate to morbidity and mortality even after age correction (Berr *et al.* 1996; Gruenewald *et al.* 2006; Sicard *et al.* 2007). Either specific effects or more generally a cortisol antagonism has been invoked to account for those associations (Akinola & Mendes 2008; Wemm *et al.* 2010).

Regarding DHEAS effects, initial evidence resulted mainly from large observational studies on the elderly and post menopausal women – The Rancho Bernardo Study, Baltimore Longitudinal Study of Aging and Personnes Agées Quid (PAQUID) – studies with DHEAS replacement in the elderly – DHEAge Study – and smaller clinical studies of patients with primary adrenal failure (Barrett-Connor & Edelstein 1994; Berr *et al.* 1996; Wolf *et al.* 1997; Barrett-Connor *et al.* 1999; Baulieu *et al.* 2000; Schlienger *et al.* 2002; Legrain & Girard 2003; Hougaku *et al.* 2006; O'Donnell *et al.* 2006). Nevertheless, during the last years, more and more evidence regarding DHEAS effects in adolescents and adults has been collected.

Similarly to other neurosteroids specific central nervous system effects have been described for DHEAS. DHEAS seems to modulate cognitive function and higher levels of this hormone are related to better results regarding memory, learning and resilience (Barrett-Connor & Edelstein 1994; Morley *et al.* 1997; Compagnone & Mellon 1998; Reddy & Kulkarni 1998; Morrow 2007; Sicard *et al.* 2007; Wemm *et al.* 2010); to higher well being scores and less depression (Moralès *et al.* 1994; Wolf *et al.* 1997; Barrett-Connor *et al.* 1999; Schlienger *et al.* 2002; Dallman *et al.* 2003; Akinola & Mendes 2008) and to higher resistance to the deleterious effects of a stressful situation (Reddy & Kulkarni 1998; Morrow 2007; Morgan *et al.* 2009; Yoon *et al.* 2009).

At the molecular level DHEAS has a general neurostimulatory effect via gabaminergic antagonism [Gamma-Aminobutyric Acid Type A Receptor (GABA-A) antagonism] and glutaminergic agonism [N-methyl-D-aspartate receptor (NMDA) sigma-1 agonism] (Paul & Purdy 1992; Baulieu & Robel 1998; Reddy & Kulkarni 1998; Morrow 2007). Neurotrophic effects have also been described and DHEA and DHEAS may contribute to neocortical organization (Baulieu & Robel 1998; Compagnone & Mellon 1998; Beck & Handa 2004; Suzuki *et al.* 2004). Moreover, DHEA and DHEAS neuroprotective effects after hypoxia have been documented and lower levels of those hormones in older adults could contribute to the enhanced cerebral vulnerability to vascular lesion or other neural insults (Kimonides *et al.* 1998).

The concept of endocrine phenotypes in endocrine research assumes that with regard to hormone levels, intersubject variability is much larger than intrasubject variability throughout the time (Bertagna *et al.* 1994; Coste *et al.* 1994). It is also postulated that these endocrine phenotypes are established at the beginning of one's life. A second related concept is that one of endocrine plasticity, meaning that endocrine responses nonrandomly change throughout the time according to previous experiences (Chrousos *et al.* 1988; Chrousos 1992; Levine 1993). In what concerns behavioural endocrine research personality traits are also individual features established quite early in life. A relation between cortisol and the hypothalamic-pituitary-adrenal axis reactivity and personality has been established (Chrousos *et al.* 1988; Chrousos 1992; Kirshbaum *et al.* 1992; Levine 1993; Bertagna *et al.* 1994; Castanon & Morméde 1994; George *et al.* 2010; Wirtz *et al.* 2010); however, this relation was less studied in what concerns DHEAS (Thomas *et al.* 1994).

The Minnesota Multiphasic Personality Inventory (MMPI) is well validated in the general population and, in addition to its importance in the psychiatric setting, it can also be used to interpret classic personality dimensions (Costa *et al.* 1986). In the corticotropin-releasing hormone (CRH) test, pituitary-adrenal response to CRH administration is classically measured

with ACTH and cortisol determinations as an index of the stress response. The CRH test has been extensively used in psychoneuroendocrine research of several psychiatric and psychosomatic disorders (Gold *et al.* 1986; Demitrack *et al.* 1991; Chrousos & Gold 1992) and previous studies from our research team found out significant relations between personality and pituitary-adrenal response to CRH administration in common clinical disorders (Martins *et al.* 2001; Martins *et al.* 2002; Martins *et al.* 2004).

The above evidence suggests the existence of a relation between DHEAS and behaviour, namely with personality and stress response. In the current study we explored the relation between DHEAS and both personality traits and pituitary-adrenal axis reactivity in humans.

## PATIENTS AND METHODS

We analysed retrospectively the records of 120 consecutive patients in which CRH test and personality evaluation with the MMPI were included in the clinical workout. Patients belong to several diagnostic groups. Clinical condition was not further taken into account for this study but diagnostic group was retained as a baseline variable in the statistical analysis, so that only significant results after correction for this potential confounding variable are reported. The study protocol was approved by he Hospital Ethical Committee and informed written consent was obtained for every patient. The data used was obtained before beginning medical treatment.

Age, gender, height and weight without shoes or coats were recorded.

The Portuguese translation of the MMPI (Montenegro 1982) was filled by the participants after one of the authors' complete and detailed instructions. The participants remained alone in a quiet room for the entire procedure. Scores were obtained according to MMPI authors' instructions. To avoid the use of multiple comparisons, only conventionally defined type A personality (AP) and superordinate traits were used – neurotic triad (NT): hypochondriasis (Hs) + depression (D) + hysteria (Hy); psychotic dyad (PD): paranoia (Pa) + schizophrenia (Sc) and behaviour-deviant triad (BD): psychopathic deviate (Pd) + masculinity-femininity (Mf) + hypomania (Ma) (Butcher *et al.* 1990; Greene 1991). The original MMPI version was used instead of the revised one (MMPI-2) since the MMPI-2 translation had not been validated yet.

The CRH test was performed the following week, after an overnight fast. In all cases a venous line was obtained in the antecubital region, the participant lying in the supine position; after a 15 to 20 min period of adaptation a venous blood sample was collected – time 0. CRH was then slowly infused in 1 to 2min (human CRH, 100 µg, CRH Ferring GmHb, Kiel, Germany); further blood samples were obtained at 15, 30, 60 and 120 min. All samples were immediately refrigerated at +4 °C and sent to the Endocrine Laboratory after test completion. ACTH and cortisol were measured at 0, 15, 30, 60 and 120 min, DHEAS was measured at 0, 30 and 60 min and prolactin (PRL) was only measured at baseline.

Immunoradiometric assay (IRMA) and enzyme-linked immunoassay (ELISA) methods were used for ACTH (IRMA, Nichols Institute, San Juan Capistrano, CA), PRL (IRMA, Diagnostc Products Corporation, Los Angeles, CA), DHEAS and cortisol (ELISA, Diagnostic Products Corporation, Los Angeles, CA) measurements which were performed in the hospital central laboratory. Intra- and interassay variation coefficients were less than 10% in every case.

Statistical analysis was carried out with the use of the Statistical Package for the Social Sciences Program (SPSS, version 16.0). Results are presented as the mean±standard deviation (sd) or as percentage as appropriate. The area under the curve (AUC) was computed according to the trapezoidal rule (Rowland & Tozer 1995). Mean values per hour were computed. The normal distribution of continuous variables was verified by the Kolmogorov-Smirnov (K-S) goodness of fit test. Non normal distributed variables were log transformed prior to analysis. However, for the sake of simplicity when no differences were found, results regarding the non-transformed variables are presented. The Chi-Square test was used to compare the distribution of non-continuous variables between selected groups, factorial analysis of variance (ANOVA) to compare continuous variables between selected groups and Multiple linear regression analysis to explore the relation between continuous variables. The limit of statistical significance was selected at 0.05.

## RESULTS

All patients results are summarized in Tables 1–3.

Basal DHEAS was different among diagnostic groups – $F(4,119)=3.959$, $p<0.005$ – with hirsute subjects presenting significantly higher basal DHEAS values – 237±113 µg/dL – when compared to all other diagnostic groups. There were no differences in ACTH, cortisol and PRL among diagnostic groups.

Mean baseline DHEAS was 158±99 [20–554] µg/dL and presented a distribution not significantly different from the normal one, K-S test Z=0.862, ns.

DHEAS levels were higher in males when compared to females – 207±87 µg/dL vs 151 ±99 µg/dL, t=2.088, $p<0.05$. DHEAS levels were inversely related to age – r=0.444, $p<0.001$, even after gender correction, the regression equation being DHEAS=272.493–3.736 × age. Together gender and age accounted for 27% of DHEAS variability. DHEAS levels were not significantly related to body mass index (BMI) or body weight.

DHEAS levels were significantly related to ACTH – r=+0.366, $p<0.001$ – and PRL – r=+0.233, $p<0.05$

– but not to cortisol. However, ACTH and PRL were interrelated and when both variables were included in the analysis only ACTH remained as a significant factor. This relation persists even after age, gender and diagnostic group correction. Age, gender and ACTH account for 34% of DHEAS variability.

Baseline ACTH was significantly related to the peak ACTH (r=+0.490, $p<0.001$) and peak cortisol (r=+0.246, $p<0.05$) responses during the CRH test. Baseline cortisol was strongly related to the peak cortisol response (r=+0.782, $p<0.001$). Baseline DHEAS was not related to the peak ACTH or peak cortisol response in the CRH test but baseline DHEAS was inversely related to the peak/baseline cortisol response (parcial r=–0.454, $p<0.05$) after age, gender, baseline ACTH and baseline cortisol correction (multiple linear correlation including peak/baseline cortisol, age, gender, baseline ACTH and baseline cortisol, total r=0.631, $p<0.005$) (Figure 1). DHEAS was not related to peak/baseline ACTH response.

Baseline DHEAS was inversely related to NT score – r=–0.355, $p<0.001$ – but not to PD or BD triad. However, this relation was no longer significant when age correction was carried out; in fact, age was directly related to NT – r=+0.443, $p<0.001$. Nevertheless, the DHEAS response in the CRH test (evaluated as the AUC) was significantly and directly related to BD triad and type A personality – simple linear correlation respectively r=+0.257, $p<0.05$ and r=+0.295, $p<0.05$ (Figure 2 and 3) – and that relation remains significant even after age and diagnostic group correction. As noted, DHEAS average levels have not significantly changed after the CRH test. Despite this, DHEAS response was highly variable in what concerns the individual level (–73 µg/dL to +317 µg/dL).

**Tab. 1.** Clinical characteristics and baseline endocrine values.

| | Reference interval | Mean±sd [min-max] or % |
|---|---|---|
| Age (years) | | 33±12 [18–57] |
| Gender F/M (%) | | 87/13 |
| BMI (kg/m$^2$) | | 27.7±9.0 [14.5–60.1] |
| DHEAS (µg/dL) | 35–430 | 158±99 [20–554] |
| ACTH (pg/mL) | 0–46 | 19±14 [1–90] |
| PRL (ng/mL) | 2–29 | 11±7 [2–33] |
| Cortisol (µg/dL) | 4–23 | 18±9 [4–51] |

**Tab. 2.** ACTH, cortisol and DHEAS levels in the CRH test.

| | ACTH (pg/mL) | Cortisol (µg/dL) | DHEAS (µg/dL) |
|---|---|---|---|
| 0′ | 21±16 | 19±9 | 177±106 |
| 15′ | 52±74 [b] | 22±8 [c] | – |
| 30′ | 50±58 [c] | 23±8 [c] | 177±114 |
| 60′ | 31±31 [a] | 24±9 [c] | 176±107 |
| 120′ | 16±18 [a] | 18±10 | – |
| AUC | 32±36 [b] | 22±8 [c] | 176±107 |

AUC units are pg/mL.h for ACTH, µg/dL.h for cortisol, and µg/dL.h for DHEAS.

Value different from basal, [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

**Tab. 3.** Superordinate traits and AP score (MMPI).

| | Mean±sd |
|---|---|
| Neurotic Triad (NT) score | 164±30 |
| Psychotic Dyad (PD) score | 115±22 |
| Behaviour-Deviant triad (BD) score | 158±23 |
| Type A Personality (AP) (%) | 38±16 |

## DISCUSSION

This is an observational retrospective study, whose objective is to explore the relation between DHEAS and both personality and pituitary-adrenal axis reactivity in adult humans.

We used several specific diagnostic groups. As noted before only results remaining significant independently of age and diagnostic group are reported.

Only DHEAS and not DHEA measurements were used. We expected a stronger relation between DHEAS and more stable parameters as age, gender, basal hypothalamic-pituitary-adrenal (HPA) axis activity and personality as that hormone has a longer half-life. On the contrary, acute changes might be more easily detected with DHEA, namely after CRH administration.

The predominance of female participants (87%) reflects the population assisted in the Endocrine outpatient department. Even so, DHEAS levels were significantly higher (37%) in males, as it was expected (Berr *et al.* 1996; Baulieu *et al.* 2000; Laughlin & Barrett-Conner 2000; Tannenbaum *et al.* 2004; Gruenewald *et al.* 2006).

The mean age was 33 years old (the sample was 18 to 57 years old) and DHEAS levels were inversely related to age as it has extensively been described in the literature (Berr *et al.* 1996; Lane *et al.* 1997; Morley *et al.* 1997; Kimonides *et al.* 1998; Legrain & Girard 2003; Sicard *et al.* 2007). We found a 1.4% mean decline in DHEAS levels per year while other authors had previously described a decline of about 2% per year during adulthood and higher decline rates in post-menopausal women and older men (Laughlin & Barrett-Connor 2000; Tannenbaum *et al.* 2004; Labrie *et al.* 2005).

Age explained 20% of DHEAS variability, gender explained 4% of DHEAS variability and age and gender together explained 27% of DHEAS variability. Both fac-

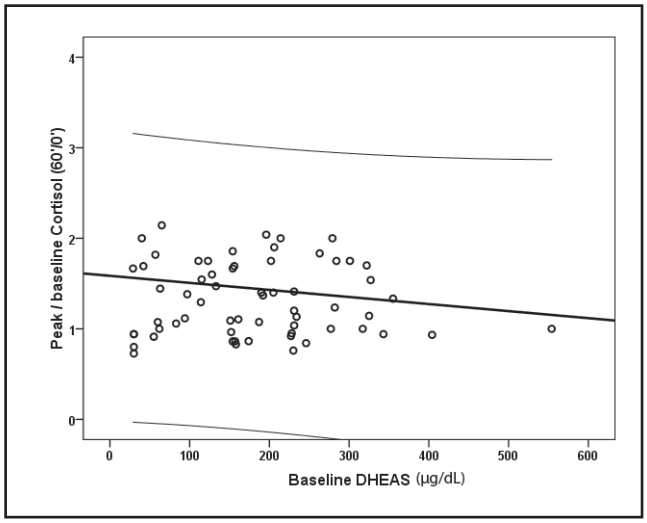


**Fig. 1.** Relation between baseline DHEAS and peak/baseline cortisol response in CRH test.

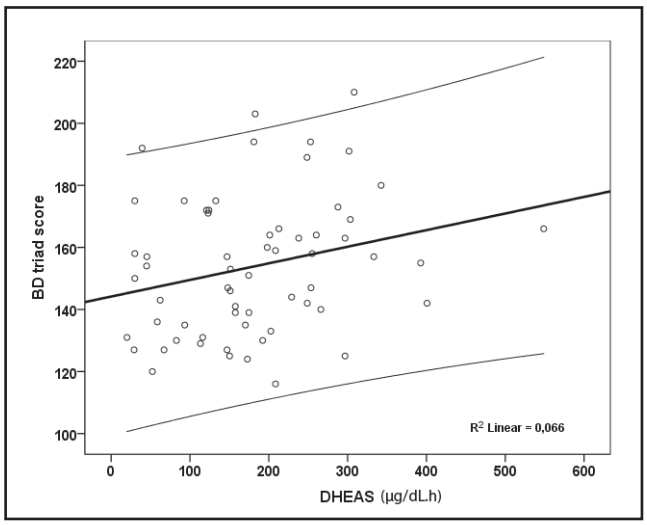


**Fig. 2.** Relation between DHEAS reactivity and BD triad.

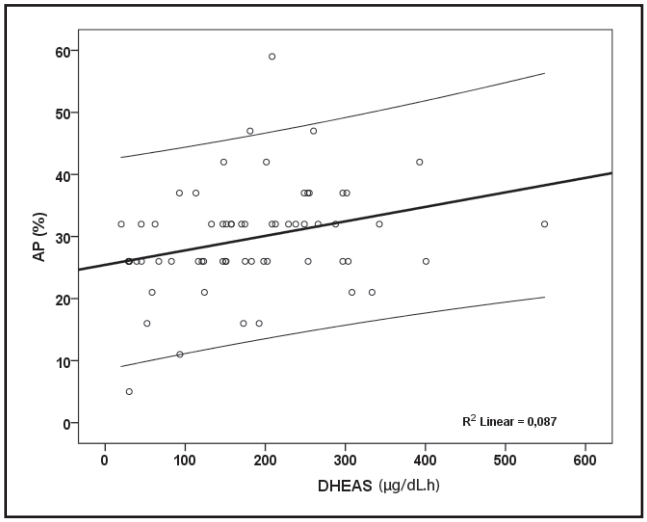


**Fig. 3.** Relation between DHEAS reactivity and type A Personality.

tors have been extensively identified as relevant factors for DHEAS levels.

DHEAS levels were also significantly and directly related to basal ACTH independently of age, gender and diagnostic group; ACTH explains 14% of DHEAS variability. Age, gender and ACTH accounted for 34% of DHEAS variability. This is a relatively new finding since ACTH had not been generally considered as a relevant factor both for DHEAS and adrenal androgen production. In fact, chronic stress is generally associated with increased cortisol levels and decreased DHEAS levels. Moreover, it should be noted that despite the ACTH response, no DHEAS response was found in the CRH test, even if dexamethasone suppression decreases both cortisol and DHEAS. All this points out the complexity of the relation between ACTH and DHEAS until a putative cortical androgen stimulating hormone (CASH) is still to be identified.

As a group, and despite the ACTH response, mean DHEAS levels did not change in the CRH test suggesting no acute effect of ACTH on DHEAS levels. Taking into account the high DHEAS levels and long DHEAS half-life, it may be necessary a longer ACTH rise to increase DHEAS levels (Berr *et al.* 1996). However, individually DHEAS response was highly variable. Baseline ACTH was strongly and directly related to the peak ACTH response and was also directly related to the peak cortisol response and baseline cortisol was strongly related to the peak cortisol response suggesting that baseline HPA axis activity is a strong determinant of the response to CRH. In more detail, baseline ACTH levels seem to be a major determinant of either ACTH or cortisol response. An interesting finding is the fact that baseline ACTH is related to both ACTH or cortisol peak levels but not to ACTH or cortisol peak/baseline ratio suggesting that ACTH does not modulate the intensity of the response, but only that those subjects with higher baseline ACTH and cortisol levels naturally reach higher peak ACTH and cortisol levels. The influence of DHEAS seems much more subtle. Baseline DHEAS was not related to the peak ACTH or peak cortisol response in the CRH test. Similarly, it was not related to peak/baseline ACTH response in CRH test but it was inversely related to the peak/baseline cortisol response (after age, gender, baseline ACTH and baseline cortisol correction). Those results suggest that DHEAS may reduce the magnitude of the cortisol response independently of baseline ACTH or cortisol levels. There is some previous evidence that DHEAS may indeed down-regulate cortisol levels (Kimonides *et al.* 1998; Gruenewald *et al.* 2006; Morrow 2007; Akinola & Mendes 2008).

Lower DHEAS was related to higher NT scores. The relation between DHEAS and NT score persisted after diagnostic group correction. However, that relation was no longer significant after age correction, and in fact, age was directly related to NT score. This is rather surprising not only because personality is generally con-

sidered as a stable personal characteristic established at a very early stage of life but also because this is a rather young sample. On the other hand, it seems plausible that aging, bringing about morbidity and mortality of both the patient and their relationships, should be associated with increased hypochondriac and depressive scores, two of the three components of the neurotic triad. Whatever the reason may be, aging is associated with increased NT scores (Zuckerman 1994) and decreased DHEAS baseline levels and that seems to be the reason for the spurious relation between DHEAS and NT. Disappointingly, baseline DHEAS levels were not significantly related to any of the selected psychometric variables and this points out the insensitivity of baseline endocrine levels.

However, DHEAS reactivity in the CRH test is significantly related to BD triad and Type A personality and both relations persist independently from age, gender and diagnostic group. As noted before neuroendocrine reactivity in the CRH test (regarding the ACTH and cortisol response) has been previously shown to be related to other psychometric variables. The apparent paradox is that although there does not seem to be any DHEAS response in the CRH test (evaluated by the mean), the DHEAS response is significantly related to psychometric variables. In fact, although the mean DHEAS does not change in the CRH test, individually considered peak DHEAS – baseline DHEAS changes deeply from –73 to + 317 μg/mL. In short, higher DHEAS responses are associated with Type A behaviour and BD triad (psychopathic deviation + hypomania + masculinity-femininity) and more specifically with Pd score (data not shown).

To conclude we found that DHEAS significantly changes according to gender and age. Moreover, we also noticed that: 1) baseline DHEAS is significantly modulated by ACTH; 2) baseline DHEAS significantly modulates the intensity of the cortisol response in the CRH test; 3) DHEAS reactivity is a factor for BD triad and Type A behaviour. In short, baseline DHEAS relates to stress response and DHEAS reactivity relates to personality.

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# The Relationship between Dehydroepiandrosterone (DHEA), Working Memory and Distraction – A Behavioral and Electrophysiological Approach

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## Abstract

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulphate (DHEAS) have been reported to have memory enhancement effects in humans. A neuro-stimulatory action and an anti-cortisol mechanism of action may contribute to that relation. In order to study DHEA, DHEAS and cortisol relations to working memory and distraction, we recorded the electroencephalogram of 23 young women performing a discrimination (no working memory load) or 1-back (working memory load) task in an audio-visual oddball paradigm. We measured salivary DHEA, DHEAS and cortisol both before each task and at 30 and 60 min. Under working memory load, a higher baseline cortisol/DHEA ratio was related to higher distraction as indexed by an enhanced novelty P3. This suggests that cortisol may lead to increased distraction whereas DHEA may hinder distraction by leading to less processing of the distractor. An increased DHEA production with consecutive cognitive tasks was found and higher DHEA responses attributed to working memory load were related to enhanced working memory processing as indexed by an enhanced visual P300. Overall, the results suggest that in women DHEA may oppose cortisol effects reducing distraction and that a higher DHEA response may enhance working memory at the electrophysiological level.

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## Introduction

Dehydroepiandrosterone-sulphate (DHEAS) is the most abundant steroid in the peripheral circulation and it is much more abundant in humans than in any other species [1,2,3,4,5,6,7]. Circulating levels dramatically decrease with aging. Moreover, lower levels are related to higher morbidity and mortality ratios even when corrected for age [2,8].

DHEA is mostly synthesized in the adrenals whereas the gonads represent a minor source of this hormone. However, DHEA is also synthesized in the central nervous system [3], where its concentrations are higher than in the peripheral circulation [3,9,10]. In both peripheral and central compartments a sulpho-transferase reversibly converts DHEA to DHEAS, restricting its distribution and prolonging its half-life [2,3].

Several central effects concerning cognitive performance and stress response have been described for DHEA and DHEAS. Higher levels were related to increased memory and attention scores [9,11] and improved performance in stressful conditions [12,13,14], whereas low levels were found in Alzheimer's disease [15]. However, DHEA administration in older subjects showed inconclusive results [5,16,17,18,19].

On the other hand, stressful stimuli also modulate DHEA and DHEAS levels: acute stress is related to an increase in DHEA and DHEAS levels [20,21,22,23] whereas chronic stress decreases baseline DHEA and DHEAS levels [24,25,26,27,28], as well as the acute DHEAS response to a superimposed psychological stress [29]. Yet, a direct influence of cognitive processing on DHEA or DHEAS levels has not been studied.

At the molecular level, DHEA and DHEAS have a general neuro-stimulatory effect: presynaptic actions include glutamate, acetylcholine and norepinephrine release and postsynaptic actions include sigma 1 receptor agonism with subsequent *N*-methyl-D-aspartate (NMDA) receptor activation, gabaminergic antagonism and inhibition of voltage-gated calcium currents [3,9,10]. Nevertheless, DHEA and DHEAS molecular effects are not exactly the same and several studies suggest that the balance between them may influence brain functioning [9,30]. As an example, DHEAS has more potent antagonistic effects at the γ-aminobutyric acid type A receptor ($GABA_A$ receptor) than DHEA [31,32]. Hence,

the simultaneous evaluation of DHEA and DHEAS may uncover more information than the individual examination of either form of that steroid.

Concerning glucocorticoids, whereas mild or short-lasting increases in cortisol due to stress may protect the body, promote adaptation and have beneficial effects on attention and memory, higher cortisol levels or long term increases are related to poorer executive functioning, poorer learning and memory and less cognitive flexibility [33,34,35,36,37,38,39]. In particular, working memory (WM) depends on prefrontal cortex activity, which is modulated by glucocorticoids: prefrontal cortex-dependent working memory is enhanced by acute stress and inhibited by chronic stress [35,40]. The relation between glucocorticoids and cognitive functioning is bidirectional: glucocorticoids impact cognitive function and cognitive processing has been shown to influence glucocorticoid secretion [41].

Several levels of evidence suggest that DHEA and DHEAS may counterbalance cortisol effects: a higher DHEA/cortisol ratio has been related to better performance under stress [14] and DHEAS antagonized the memory deteriorating neurotoxic effects of cortisol in the hippocampus [10,42]. More generally, DHEA and DHEAS decrease with aging, whereas cortisol does not. Consequently, the cortisol/DHEA ratio increases and may be involved both in the cognitive impairments and in the particular vulnerability to stress damage that seems to characterize the elderly [5,9,42].

The aim of the present study was to test whether DHEA and DHEAS levels are modulated by WM load and whether these endocrine levels are related to distraction and WM at the electrophysiological level, as evidence for their neurophysiologic effects using Event-Related Brain Potentials (ERPs) is scarce [43,44,45]. The specific *a priori* hypotheses were: 1) higher endogenous DHEA and DHEAS levels may prevent involuntary distraction and enhance cognitive performance; 2) DHEA and DHEAS putatively beneficial effects may be translated at the neurophysiological level; 3) DHEA and DHEAS effects may be largely antagonistic from those of high baseline cortisol; and 4) WM load may be a stimulus for DHEA and DHEAS production.

To test these hypotheses we measured the relation between DHEA, DHEAS and cortisol on one hand and the cognitive performance and brain responses, using a well-established auditory-visual distraction paradigm [46,47,48] on the other. The protocol includes task irrelevant sounds, some of which are aimed to cause distraction and a visual task including working memory manipulation.

## Subjects and Methods

### Ethics Statement

The experimental protocol was approved by the ethical committees of the University of Barcelona and Lisbon Medical School and it was conducted according to the principles of the Declaration of Helsinki. All the subjects gave their written informed consent before entering the study.

### Subjects

28 healthy female volunteers (undergraduated Psychology students) performed the study protocol. In order to ensure a higher homogeneity in androgen levels, only women were included. The subjects were young (18 to 25 years old, mean $20\pm0.5$ years old) and presented a normal body mass index ($21.8\pm0.5$ kg/m$^2$). All the participants had a normal or corrected-to-normal vision and none reported auditory deficits. None of the participants reported a past history of neurologic, psychiatric, endocrine or oral diseases. All the subjects were right-handed. Four subjects were under hormonal contraception. No other medications were allowed. Regular or binge alcohol consumption were exclusion criteria and subjects were asked not to consume alcohol in the twelve hours before the experimental protocol. Regular tobacco consumption as well as illicit drug consumption were further exclusion criteria. Prior to the experimental session, subjects completed the State-Trait Anxiety Inventory [49] and all showed a normal range of state and trait anxiety levels. Five subjects were discarded from the analysis due to technical problems with electroencephalogram (EEG) recordings or endocrine measurements.

### Task and Procedure

The experimental sessions were held in the afternoon, beginning at 2–3 pm. An adapted version of a well-established auditory-visual distraction task [46,47,50] was presented, based on the protocol used by SanMiguel et al. (2008) [51]. In this protocol, two visual tasks were performed: one task without working memory load (WM0) and another one with working memory load (WM1). In the present experiment, the two tasks were performed two hours apart from each other (from onset to onset) and the order of the tasks was counterbalanced across participants. Each task lasted about 15 minutes, and consisted of two blocks of 250 trials each (plus five initial trials that were excluded from the analyses). A short pause was allowed between blocks.

Participants sat in a comfortable chair in a dimly lit and electrically and acoustically shielded room. In the discrimination task (WM0) subjects had to decide whether the two digits appearing on the screen were the same (11 and 22) or different (12 and 21), see figure 1A. In the WM1 task (1-back task) subjects had to decide whether the left or right digit (counterbalanced across subjects) on the screen was the same as the left or right digit of the previous trial (figure 1B). Thus, they had to keep one digit in working memory until the next trial, answering to every trial, except for the first one. Responses were given through a mouse button (one mouse button for "same" and the other button for "different"), also counterbalanced across subjects. The subjects were specifically instructed to respond as quickly and accurately as possible while ignoring the sounds. In order to reduce artifacts originating from eye-blinks and movements during EEG recording, subjects were asked to minimize blinking and to focus on a central fixation cross between the two digits. Before each task, subjects performed practice blocks (composed by 10 trials) without any auditory stimuli until they reached a hit rate of at least 80% in each task.

Each trial consisted of an auditory stimulus, irrelevant for the task, followed by a visual imperative stimulus after 350 ms (onset to onset), see figure 1. The total trial length varied from 1000 to 1500 ms (1250 ms on average; jitter +/–250 ms). The auditory sequence consisted of repetitive standard tones (200 ms, including fade-in and fade-out of 10 ms each; 600 Hz; 85 dB; 80% probability), occasionally replaced by environmental novel sounds selected from a sample of 100 different exemplars (edited to have a duration of 200 ms, including fade-in and fade-out of 10 ms each; digitally recorded, low-pass filtered at 10,000 Hz; 85 dB; 20% probability), such as those produced by a drill, hammer, rain, door, telephone ringing, and so forth (50). All sounds were randomly delivered binaurally through headphones (Sennheiser HD 202), and the only restrictions were that the first four stimuli of each block were standard tones, that two novel sounds never appeared consecutively and that each novel sound occurred only once in each task. The visual stimuli consisted of pairs of combinations of the digits 1 and 2 (11, 12, 21 or 22), presented on

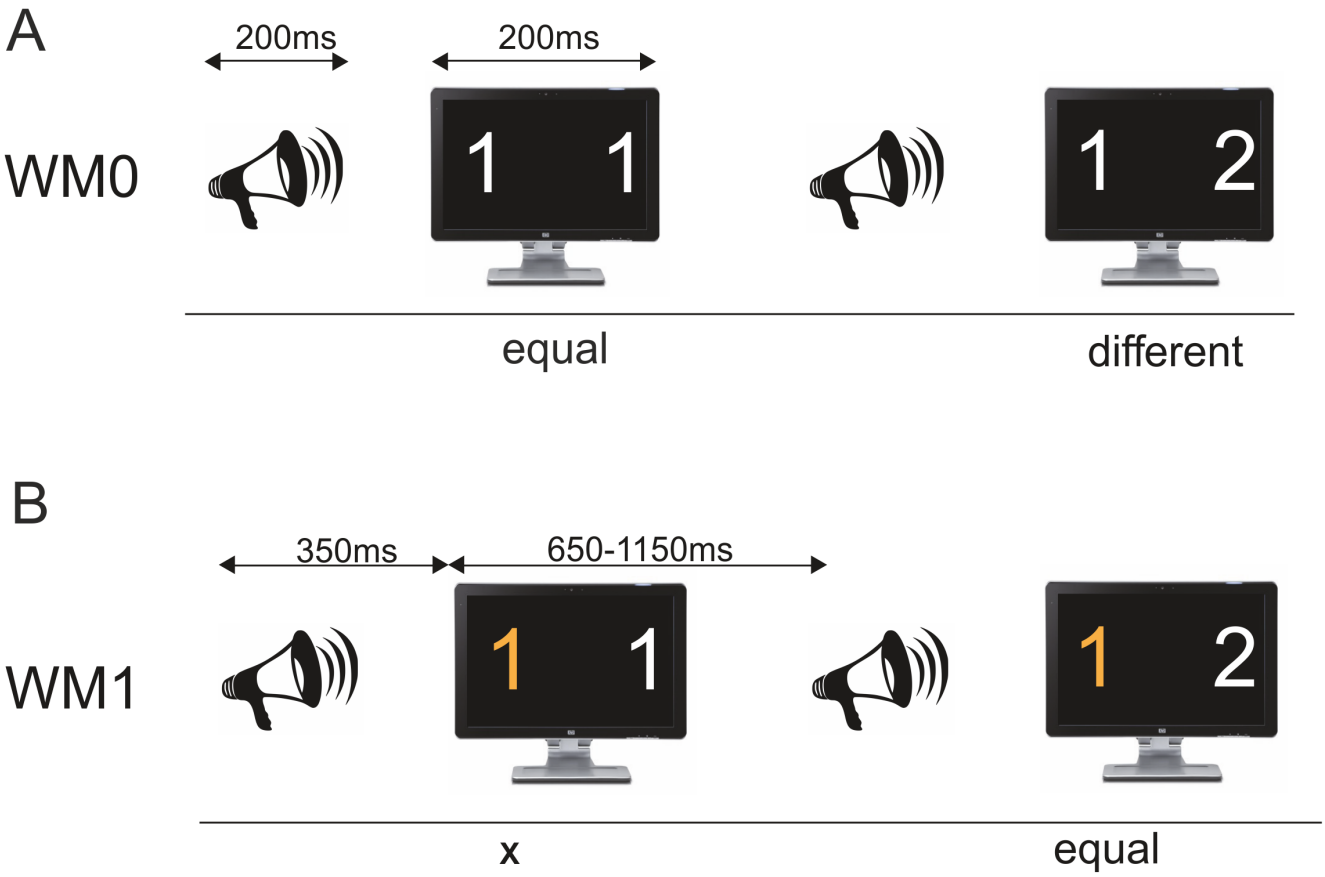


**Figure 1. Example of trials stimulation sequence (above the line) and correct answers to the tasks (below the line) for the two tasks.** A) Discrimination task (WM0), in which subjects had to decide whether the two digits on the screen were equal or different. B) Working memory task (WM1), in which the subjects had to compare the left digit on the screen with the left digit of the previous trial. WM0 – discrimination task; WM1 – working memory task.
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a computer screen for 200 ms. The appearance probability was the same for every digit combination. The picture size was 357×441 pixels, with a vertical angle of 8° and a horizontal angle of 18°, accounting for two pictures presented simultaneously with the fixation cross in between. The distance from the subjects' eyes to the screen was 100 cm. Overall, there were 400 standard trials and 100 novel trials in each of the two WM conditions.

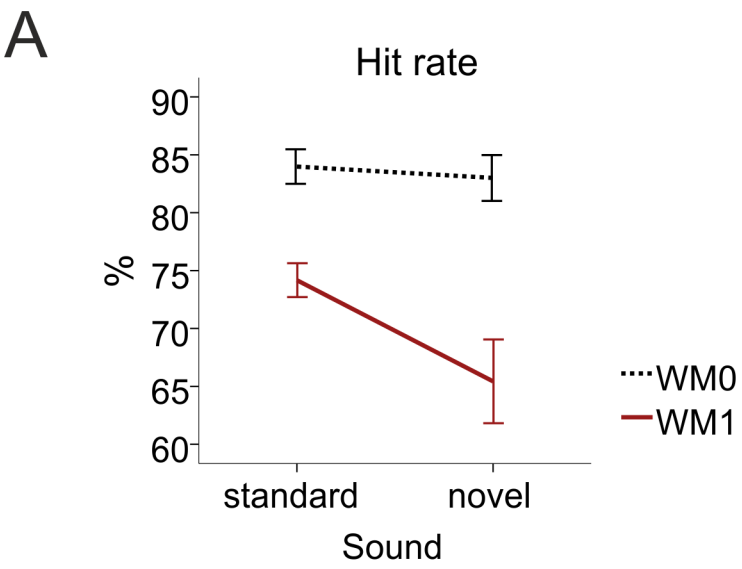
We recorded response time and hit rates for each trial with Presentation® (Neurobehavioral Systems, Inc). A correct response within the response window (until the sound onset of the subsequent trial) was counted as a hit. We computed distraction as the difference in hit rate or response time between auditory stimulus types (hit rate: WM0standard trials − WM0novel trials and WM1standard trials − WM1novel trials; response time: WM0novel trials − WM0standard trials and WM1novel trials − WM1standard trials) and working memory load costs as the difference in hit rate or response time between the WM load and the discrimination task (hit rate: WM0standard − WM1standard and WM0novel − WM1novel; response time: WM1standard − WM0standard and WM1novel − WM0novel).

## EEG Recording and Analysis

Electrophysiological activity was continuously recorded during task performance, from 64 scalp Ag/AgCl electrodes following the extended 10/10 convention. Elastic caps with sintered electrodes and shielded wires were used. The horizontal and vertical electrooculograms (HEOG and VEOG) were recorded with electrodes placed at the outer canthus and below the right eye, respectively. An electrode placed on the tip of the nose was used as the common reference and the ground was located at the AFz position. The EEG and electrooculogram (EOG) were amplified and digitized at a sampling rate of 512 Hz (Eemagine, ANT Software b.v., Enschede, the Netherlands). Impedances were kept at 5 kΩ or below during the whole recording session. Recording was performed with an ANT amplifier of 64 channels (gain 20x; A/D resolution 22 bits, 71.526 nV per bit; filtering 0–138.24 Hz; CMRR>90 dB).

A digital finite impulse response (FIR) bandpass-filter from 0.01 to 30 Hz was applied using a Hamming window. ERPs were averaged offline for each auditory stimulus type and working memory condition, for an epoch of 1000 ms, including a 200 ms pre-auditory-stimulus baseline. The first five epochs of each block and the epochs following a novel trial were excluded from averaging. Only epochs that corresponded to trials with correct responses were averaged.

EOG correction was performed by manually selecting a large number of typical artifacts and accordingly applying a regression algorithm to compute propagation factors (Eeprobe 3.1, ANT Software BV, Enschede, the Netherlands). After EOG correction, epochs that contained EEG activity exceeding ±100 μV peak-to-peak amplitudes were rejected from further analyses. Since we included only trials with correct answers and hit rate was smaller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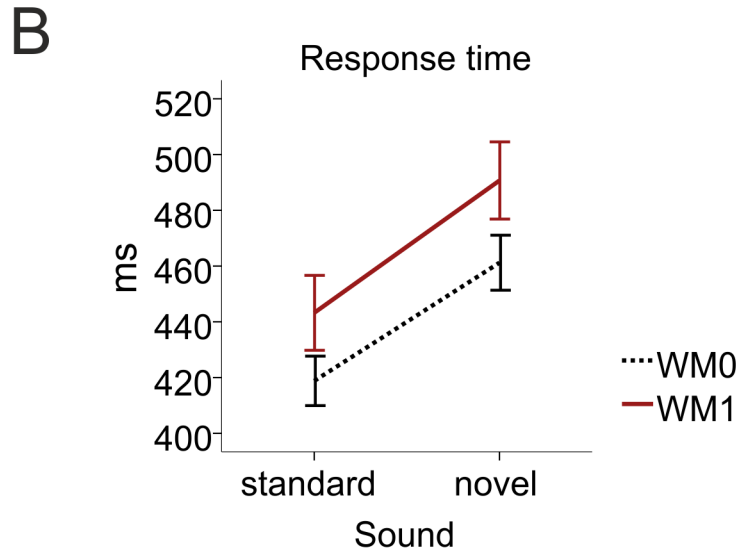
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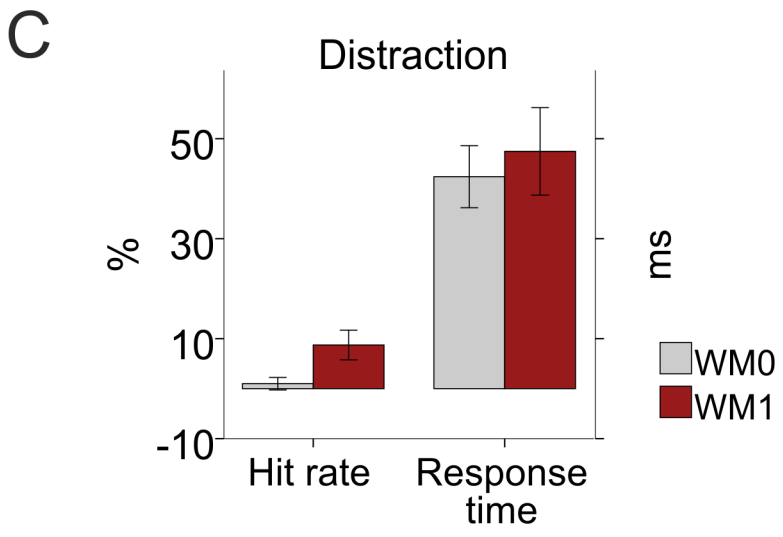
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WM1

**Figure 2. Performance results.** A) Mean hit rate for each task and auditory stimulus type. B) Mean response time for each task and auditory stimulus type. C) Distraction costs for each task. Distraction = hit rate in standard minus novel trials or response time in novel minus standard trials. WM0 – discrimination task; WM1 – working memory task. Bars represent +/– standard error of the mean (SEM).
doi:10.1371/journal.pone.0104869.g002

for WM1, the final number of trials was smaller for WM1. The total number of trials included in the averages for each condition and auditory stimulus type was: 246 trials with standard sounds and 81 with novel sounds in WM0 and 213 trials with standard sounds and 64 trials with novel sounds in WM1.

In this paradigm, subjects are specifically instructed to ignore the sounds. Hence, any related effects are necessarily involuntary or lead by exogenous attention. ERPs recorded during this auditory-visual distraction paradigm typically present first an auditory N1/mismatch negativity (N1/MMN) enhancement, reflecting a detection mechanism that leads to attention capture

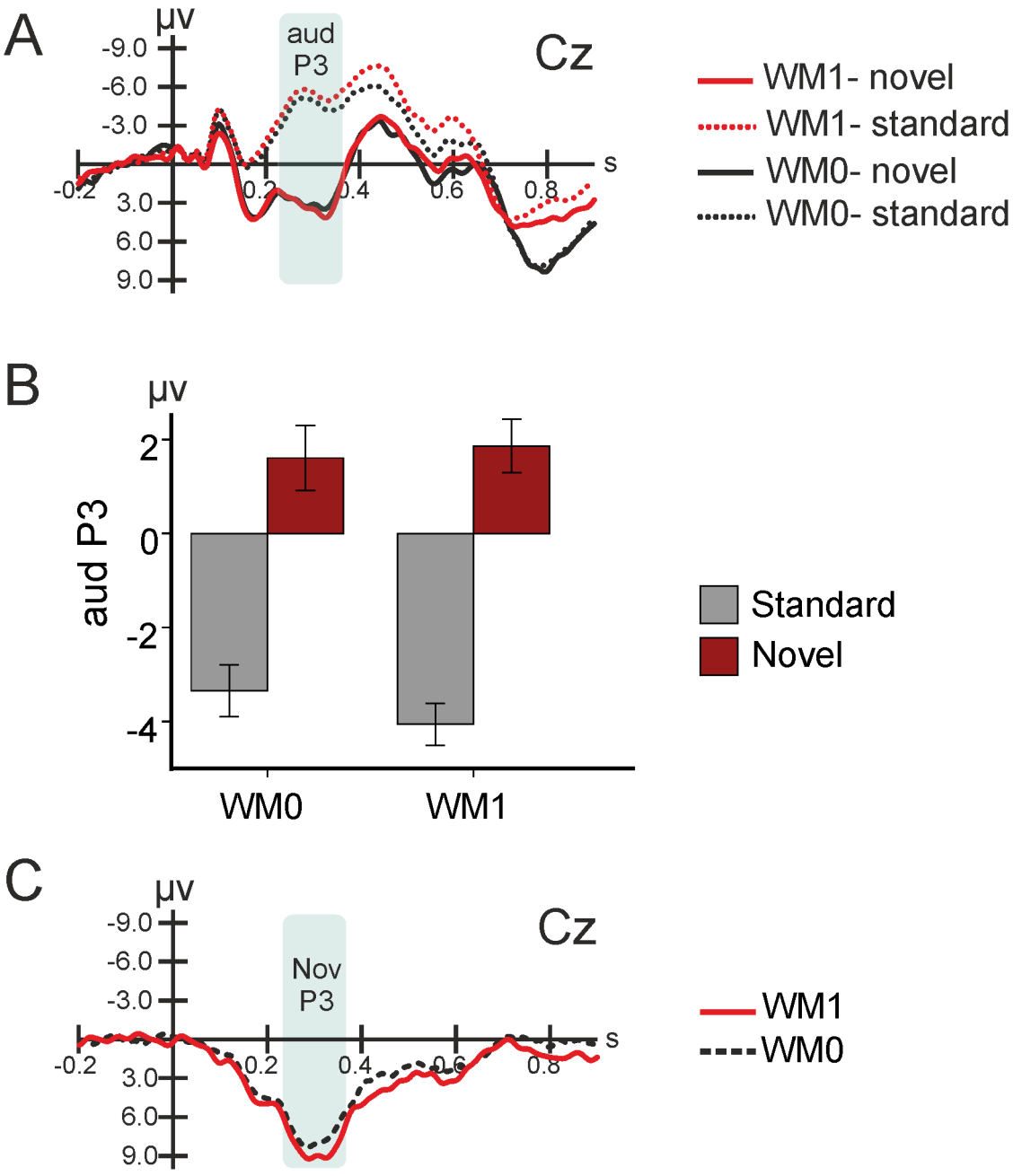


**Figure 3. Event Related Brain Potentials (ERPs).** A) Grand average waveforms at Cz for both tasks (WM0 and WM1) and type of sound (standard and novel). B) Auditory P3 (aud P3) amplitude for each task (WM0 and WM1) and type of sound (standard and novel). C) Grand-average of novel minus standard difference waves at Cz. WM0 – discrimination task; WM1 – working memory task; NovP3 – novelty P3; s - seconds. Bars represent +/– standard error of the mean (SEM).
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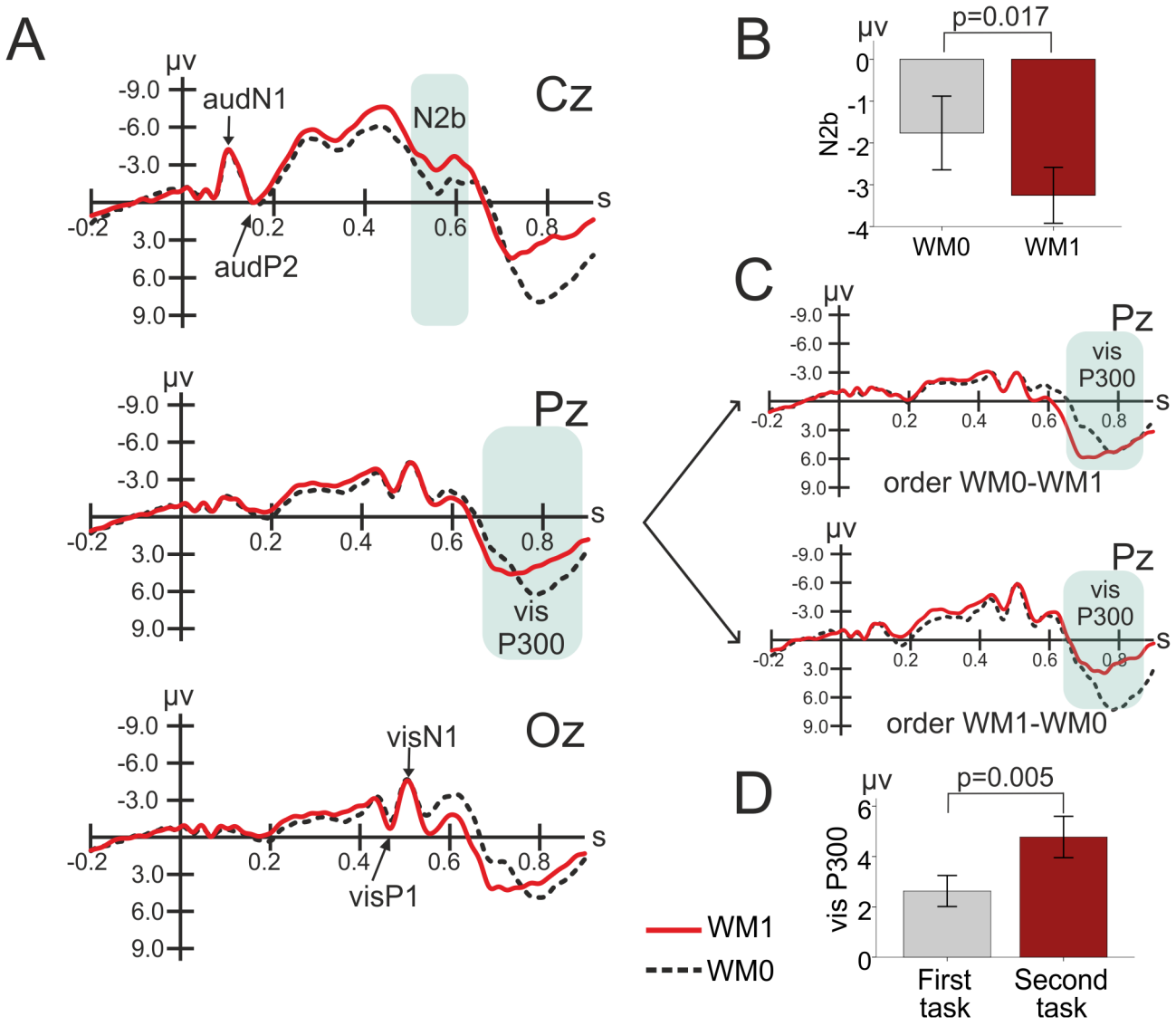


**Figure 4. Standard Event-Related Potentials (ERPs) waveforms in the discrimination and working memory tasks.** A) Standard ERPs in both tasks (both task orders). B) Mean N2b amplitude for each task. C) Standard ERPs in both tasks at Pz, according to the tasks order. D) Mean visual P300 amplitude according to the temporal sequence of tasks (first and second). WM0 – discrimination task; WM1 – working memory task; s – seconds; order WM0-WM1 – the discrimination task performed firstly and the working memory task performed secondly; order WM1-WM0 – the working memory task performed firstly and the discrimination task performed secondly. Bars represent +/− standard error of the mean (SEM).
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[46], followed by a novelty-P3 (nov-P3) that reflects the effective attention orientation [46,47,52]. Finally, the re-orienting negativity (RON) reflects the attention re-orientation back to the task [53]. The target is visual and visual ERPs yield sensory (visual P1 and N1) and cognitive components related to target processing (N2b and P300). The N2b is a negative deflection originated by the relevant stimulus and the P300 reflects the processing of the task-relevant visual stimulus [51,54,55]. Typically the task with working memory load is more difficult for the subjects, leading to a reduced P300 [51,54,55].

To analyze distraction effects at the electrophysiological level, difference waves (dw) were calculated by subtracting the ERPs elicited in standard trials from those elicited in novel trials. These difference waves revealed an early-onset, long-lasting positive deflection that we assimilated to the novelty-P3. Novelty-P3 was measured as the mean amplitude in a time window ranging from 250 to 380 ms at F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrodes.

To analyze WM effects we compared ERP measures only in standard trials. Specific auditory and visual components were elicited during task-performance: the auditory N1 and P2 and visual P1 and N1. Yet, since cognitive processing was of interest for the present study, we only analyzed N2b (560–645 ms, 210–295 ms from visual stimulus presentation) and P300 (650–910 ms, 300–560 ms from visual stimulus presentation): F3, Fz, F4, C3, Cz and C4 for N2b and P3, Pz and P4 for P300. Moreover, since the P300 latency was different across WM conditions [$F_{(1,21)} = 5.683$, $p = 0.027$; 753±62 ms for WM1; 787±36 ms for WM0], we analyzed the amplitude of this component at different time windows for each condition (WM1: 650–875 ms; WM0-670–910 ms).

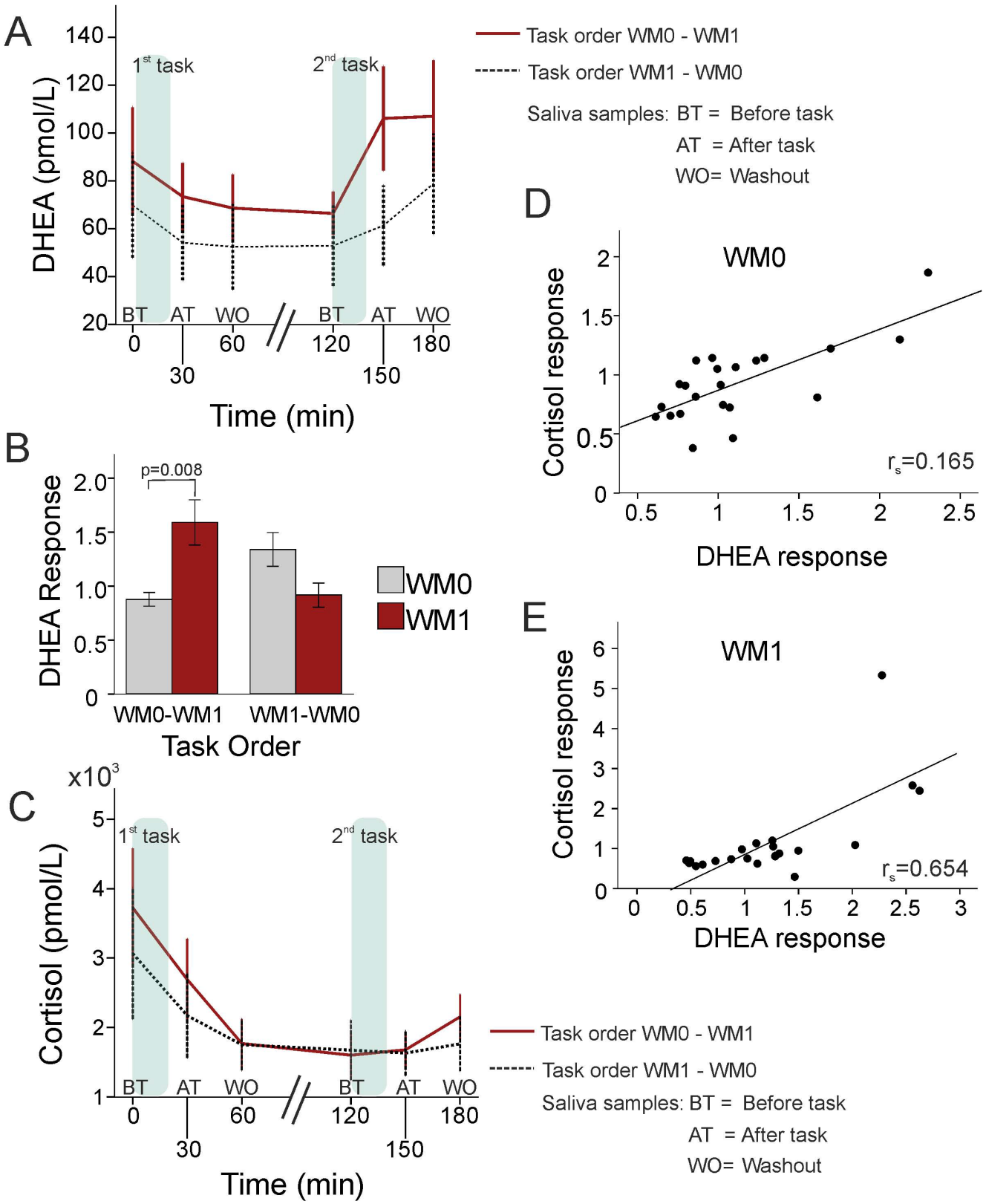


**Figure 5. Endocrine results.** A) Mean DHEA levels for each task and order. B) DHEA response for each task and order. C) Mean cortisol levels for each task and order. D) DHEA and cortisol responses (after task/before task ratios) were directly related in the discrimination task. E) DHEA and cortisol responses were directly related in the working memory task. WM0 – discrimination task; WM1 – working memory task; DHEA response: DHEA after task/before task ratio; Cortisol response: Cortisol after task/before task ratio; order WM0-WM1 – the discrimination task performed firstly and the working memory task performed secondly; order WM1-WM0 – the working memory task performed firstly and the discrimination task performed secondly. Error bars represent +/− standard error of the mean (SEM).
doi:10.1371/journal.pone.0104869.g005

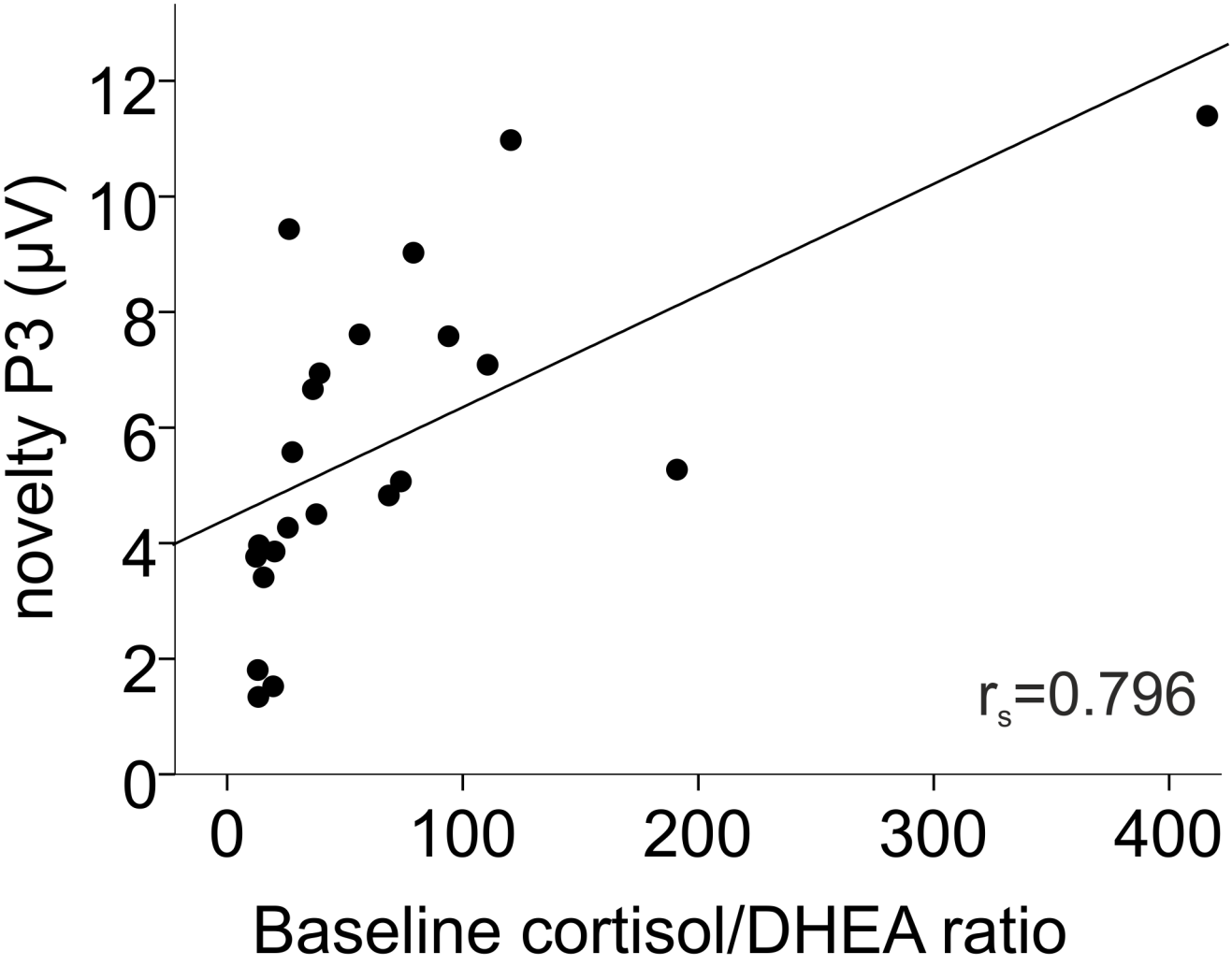


**Figure 6. Baseline cortisol/DHEA ratio was directly related to novelty-P3 under Working Memory load.**
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## Endocrine Measurements

We collected saliva samples by means of passive drool, using a short straw. Unstimulated whole saliva was used. We collected samples for DHEA, DHEAS and cortisol measurement before each task [before task (BT)], at 30 min [after task (AT)] and 60 min [washout (WO)]. The samples collected before the first task (i.e. before both tasks) were considered as the baseline. We chose the time points to collect the saliva samples in accordance to known cortisol raise and recovery times-raise 10 min after appropriate stimulus, peak at 20–30 min and recover at 45–60 min. Furthermore, synchronous 24 h profiles were described for DHEA and cortisol [56].

Unbound DHEA and cortisol in the peripheral circulation penetrate into the saliva via intracellular mechanisms and salivary concentrations reflect serum concentrations [57,58]. DHEAS is not lipid soluble and cannot penetrate into the saliva by passive diffusion through cell membranes. Instead, it squeezes through the tight junctions between salivary glands. DHEAS concentrations in saliva are therefore dependent on serum concentration and salivary flow rate [57].

Samples were refrigerated at 2–8°C within 30 minutes after collection and they were stored at −20°C within 4 h and until assayed. Each sample was measured in duplicate by using enzyme-linked immunoassays: salivary DHEA and DHEAS enzyme immunoassay kits (Salimetrics Europe®, Ltd, Newmarket Suffolk, UK) and high sensitivity salivary cortisol enzyme immunoassay kits (Salimetrics®, LLC, State College, PA, USA). DHEA was measured in pg/mL and cortisol was measured in μg/dL. Due to the influence of saliva flow rates on DHEAS levels, the concentration of DHEAS (pg/mL) was multiplied by the flow rate (mL/min) and the corrected results were obtained as DHEAS measured per unit of time (pg/min). Intra- and interassay variation coefficients were less than 10% and 15%, in every case, respectively. Cortisol and DHEA were expressed as pmol/L by using the conversion factors 27590 and 0.3467, respectively, and DHEAS was expressed as pmol/h by using the conversion factor 0.16284 (system of international units = conventional units × conversion factor).

## Statistical Analysis

The Statistical Package for the Social Sciences Program (IBM SPSS Statistics, version 21) was used for data analyses. Results are presented as the mean ± standard error of the mean (SEM). The normal distribution of continuous variables was verified by the Kolmogorov-Smirnov Goodness of Fit Test and non-normal distributed variables were log (ln) transformed prior to the analysis.

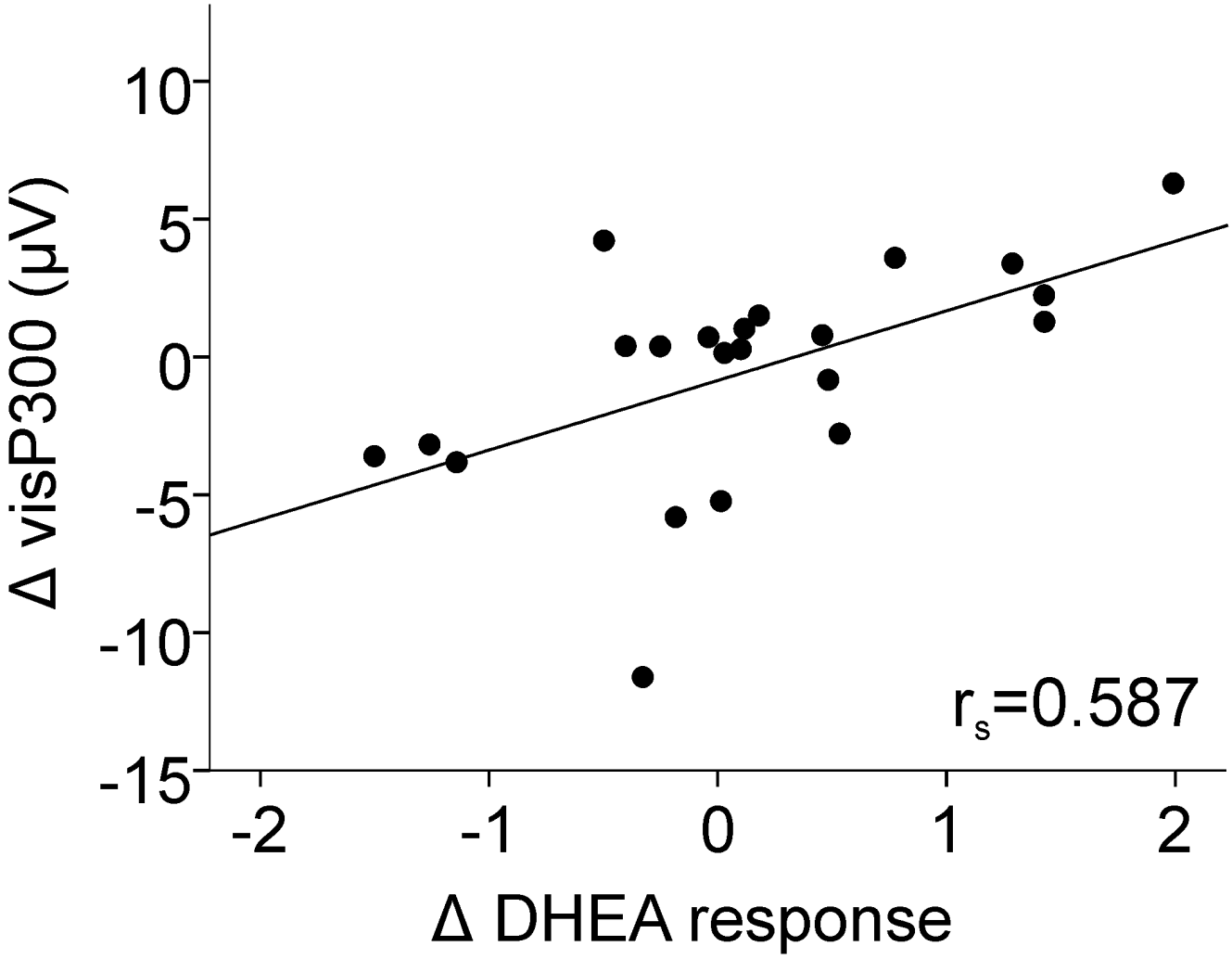


**Figure 7. The visP300 amplitude changed between tasks (WM0, WM1) in direct relation to DHEA response.** WM0 – discrimination task; WM1 – working memory task. Δ DHEA response = DHEA response in WM1 – DHEA response in WM0; Δ visP300 = mean visual P300 in WM1 - mean visual P300 in WM0.
doi:10.1371/journal.pone.0104869.g007

For the sake of simplicity, the results of non-transformed variables are presented whenever we did not find any differences.

To explore the effects of WM load and auditory distraction on performance we performed repeated measures analyses of variance (ANOVAs) on hit rate and response time, including the within-subjects factors task (WM0 and WM1) and sound (standard and novel). Regarding electrophysiological responses, we examined the auditory ERPs to explore distraction effects and the visual ERPs to explore WM effects. To investigate the effects of auditory distraction on ERPs, we carried out repeated measures ANOVAs on the mean amplitude of the auditory P3 in the time window and electrodes considered above, with the within-subjects factors task (WM0 and WM1) and sound (standard and novel). To investigate WM effects on distraction ERPs, we carried out an ANOVA on the mean amplitude of the novelty-P3 in the time window and electrodes considered above with task (WM0 and WM1) as within-subjects factor. To investigate WM effects on electrophysiological responses, we included only standard trials and conducted ANOVAs on N2b and visP300 mean amplitude in the time windows and electrodes considered above, with task (WM0 and WM1) as within-subjects factor and task order (simply referred as order in the results) as between-subjects factor (WM0-WM1 or WM1-WM0; this factor is included because we used a blocked protocol, one task consisting exclusively in WM0 trials and the other consisting exclusively in WM1 trials, counterbalanced across subjects).

To investigate the effects of WM manipulation on endocrine levels, we performed repeated measures ANOVAs on DHEA, DHEAS and cortisol levels, including the within-subjects factors task (WM0 and WM1) and time (before task, after task and washout) and the between-subjects factor task order (WM0−WM1 or WM1−WM0). The endocrine relation to distraction and WM load effects at the behavioral and electrophysiological levels was investigated by repeated measures ANOVAs of behavior and ERP parameters, including baseline DHEA, DHEAS, cortisol or cortisol/DHEA ratio as covariates. Because the DHEA response (determined by the ratio after task/before task) differed between the two WM conditions, DHEA response in WM1 – DHEA response in WM0 (Δ DHEA response) was used as a measure of DHEA response attributed to WM load. This new variable was also tested as a covariate. Whenever the endocrine parameters covariated with the performance or ERP parameters, for each type

of auditory stimuli or WM condition, we used Spearman's Rank Order correlation coefficients to select the relevant endocrine factors and understand the relations direction. DHEAS relations to performance and ERP parameters were not significant and therefore those results are not mentioned in the results section.

ANOVA results were Greenhouse–Geisser corrected whenever the assumption of sphericity was violated. *Post hoc* tests were carried out wherever there were significant interactions between main factors. The Bonferroni correction was used for multiple comparisons. The limit of significance chosen was $\alpha = 0.05$.

## Results

### Performance

Behavioral results for each task and auditory stimulus are presented in Figure 2. Overall hit rate was 83±2% in WM0 and 70±2% in WM1 (figure 2A) and this difference in hit rate was significant, as reflected by a main effect of task [$F_{(1,22)} = 27.279$, $p<0.001$]. Additionally, there was a main effect of task on response times [$F_{(1,22)} = 8.760$, $p = 0.007$] with longer response times in WM1 [467±13 ms] than in WM0 [440±9 ms], see figure 2B. Thus, the WM load resulted in lower hit rates and longer response times.

Regarding the effects of auditory distraction on performance, the results showed a main effect of auditory stimulus both on hit rate [$F_{(1,22)} = 9.577$, $p = 0.005$, with lower hit rates for novel sounds (74±2%) than for standard ones (79±1%), see figure 2A], and response times [$F_{(1,22)} = 43.451$, $p<0.001$, with longer response times for novel sounds (476±11 ms) than for standard ones (431±10 ms), see figure 2B]. This means that novel sounds resulted in auditory distraction reflected by lower hit rates and longer response times.

The interaction between task and auditory stimulus also had notable significant effects on hit rates [$F_{(1,22)} = 5.557$, $p = 0.028$]. *Post hoc* analyses on each WM condition yielded significant effects of auditory distraction only in the working memory load task [$F_{(1,22)} = 8.697$, $p = 0.007$], see figure 2C. In that condition, hit rates were lower for novel sounds (65±4%) than for standards (74±1%).

### Event-Related Potentials

**Distraction effects.** As can be seen in figure 3A, novel sounds elicited larger auditory P3 mean amplitudes when compared to standard sounds, both in WM0 [$F_{(1,22)} = 63.696$, $p<0.001$; −3.3±0.5 μV in standard and +1.6±0.7 μV in novel trials] and in WM1 [$F_{(1,22)} = 98.333$, $p<0.001$; −4.1±0.4 μV in standard and +1.9±0.6 μV in novel trials], see figure 3B. This supports that a significant novelty P3 was elicited by novel sounds (figure 3C). However, WM load did not influence the novelty-P3, as its amplitude was similar in both tasks [$F_{(1,22)} = 3.381$, $p = 0.079$, 5.0±0.6 μV in WM0, and 5.9±0.6 μV in WM1]. Neither clear N1-enhancement/MMN nor RON was elicited. In sum, significant novelty-P3 responses were elicited by novel sounds in both conditions, but without any significant difference between conditions.

**Working Memory Effects.** The waveforms elicited by standard trials in the two tasks are presented in Figure 4. The N2b significantly increased in WM1 as compared to WM0 [$F_{(1,21)} = 6.738$, $p = 0.017$; −1.8±0.9 μV in WM0 and −3.3±0.6 μV in WM1] (see figure 4A and 5B).

The analysis of visP300 revealed a task×order interaction [$F_{(1,21)} = 10.184$, $p = 0.004$], see figure 4C. Further analyses revealed a visP300 enhancement in the second task [$t(22) = -3.163$, $p = 0.005$; 2.6±0.6 μV in the first task, and 4.8±0.8 μV in the second task], independently of the WM load content of that task (figure 4D).

Overall, N2b was enhanced by WM load while the visP300 was enhanced in the second task, independently from working memory load.

### Endocrine Baseline Levels and Response

Baseline endocrine levels were: DHEA 79.0±15.5 pmol/L, DHEAS 984±120 pmol/h and cortisol 3412±622 pmol/L, with a normal distribution and no significant relation between them. These parameters were not significantly related to age and body mass index (BMI, $kg/m^2$), and did not differ significantly according to the menstrual cycle phase (follicular, peri-ovulatory and luteal, based on self reported menstrual cycle day) or between subjects taking and not taking hormonal contraception. DHEA and cortisol levels during the experimental procedure are shown in Figure 5.

In contrast to DHEAS levels, which were not affected by any of the factors (task, time or task order), and therefore won't be reported any further, repeated measures ANOVA on DHEA levels revealed a task×order interaction [$F_{(1,20)} = 6.215$, $p = 0.022$] and a task×time×order interaction [$F_{(2,40)} = 9.839$, $p = 0.002$]. Further analyses revealed that DHEA levels rose after the performance of the second task as indicated by a time effect [$F_{(2,40)} = 8.415$, $p = 0.003$; 59.6±9.4 pmol/L before task; 83.9±13.5 pmol/L after task; 92.9±15.6 pmol/L at washout], see figure 5A.

Regarding WM effects, *post hoc* analyses revealed that when the order was WM0-WM1, DHEA levels were higher after WM1 than after WM0 [$F_{(1,10)} = 9.041$, $p = 0.013$, 71.1±13.9 pmol/L after WM0 and 106.4±22.2 pmol/L after WM1]. The DHEA response [$F_{(1,10)} = 10.676$, $p = 0.008$, 0.88 for WM0 and 1.60 for WM1] was also higher in WM1 than in WM0 (figure 5B). Nevertheless, when the order was WM1−WM0, DHEA levels after the second task [$F_{(1,10)} = 6.006$, $p = 0.034$] and DHEA response [$F_{(1,10)} = 4.855$, $p = 0.052$] were similar in the WM0 (the second task) and in the WM1 task.

An repeated measures ANOVA on cortisol levels revealed a task×time×task order interaction [$F_{(2,40)} = 11.809$, $p = 0.002$]. *Post-hoc* analyses for each task order separately showed that cortisol levels decreased after WM0 when the order was WM0−WM1. In fact, for this order, there was a time effect for WM0 [$F_{(2,22)} = 9.544$, $p = 0.007$; cortisol levels were 3725±855 pmol/L before task; 2704±579 pmol/L after task; 1766±359 pmol/L at washout], see figure 5C. In spite of this, the cortisol after task/before task ratio in WM0 was not significantly different from the after task/before task ratio in WM1.

DHEA and cortisol changes were directly related for both the WM0 [after task/before task: $r_s = 0.615$, $n = 22$, $p = 0.002$, figure 5D; washout/before task: $r_s = 0.852$, $n = 22$, $p<0.001$] and WM1 task [after task/before task: $r_s = 0.654$, $n = 22$, $p = 0.001$, figure 5E; washout/before task: $r_s = 0.656$, $n = 22$, $p = 0.001$].

### Endocrine Relations to Performance

ANOVAs on hit rate and response time were used to select the endocrine parameters that covariated with performance (results are presented as supporting information, Table S1): baseline cortisol, baseline cortisol/DHEA ratio and Δ DHEA response covariated with hit rates and Δ DHEA response covariated with response times. Nevertheless, post hoc Spearman's Rank Order correlations showed no significant relations between those endocrine parameters and performance.

### Endocrine Relations to Event-Related Potentials

**Distraction Effects.** Endocrine relations to distraction were explored by using the novel minus standard difference waves for WM0 and WM1. There were no endocrine relations to novelty-P3 in WM0. However, in WM1, the novelty-P3 amplitude was enhanced in relation to higher baseline cortisol/DHEA ratios ($r_s = 0.796$, $n = 22$, $p < 0.001$; see figure 6).

**Working Memory Effects.** The results showed that visP300 change between tasks was directly related to Δ DHEA response as supported by a significant interaction between visP300 amplitude × Δ DHEA response [$F_{(1,20)} = 9.244$, $p = 0.006$] and a direct relation between visP300 enhancement and DHEA response increase attributed to WM load ($r_s = 0.587$, $n = 22$, $p = 0.004$; see Figure 7). The difference of visP300 latency between tasks was not related to the endocrine parameters.

## Discussion

The present study explores the relationships between cognitive performance and endogenous DHEA, DHEAS and cortisol. As expected, the audio-visual distraction paradigm including a manipulation of working memory, yielded typical effects observed in previous studies. Indeed, the WM load task was harder to perform, as revealed by lower hit rates and longer reaction times. Likewise, novel sounds distracted the participants, as reflected by lower hit rates and longer reaction times. Regarding brain responses to novel sounds in both WM0 and WM1 tasks, the results revealed an enhanced P3 deflection, indicating that novel sounds caused distraction when compared to standards. The N2b elicited by the task-relevant visual stimuli was enhanced under WM load as expected [51].

DHEA and cortisol responses were directly related (independently of the WM load content of the task). This is in agreement with the fact that corticotrophin releasing hormone (CRH) stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for DHEA secretion [59,60], even if indirectly through the action of an unidentified DHEA androgen stimulating hormone [61,62,63].

Nevertheless, we found important differences between DHEA and cortisol responses with WM load manipulation. DHEA levels increased with the performance of the second task independently of the task, suggesting either a cumulative effect or a latent interval before the response. Still, the increase in DHEA levels was more pronounced with WM load. Thus, the effect of a greater cognitive effort or specific effects of WM load on DHEA levels are suggested. Interestingly, this response is specific for DHEA and does not occur with cortisol and DHEAS. In fact, regarding cortisol, a decrease was found when the subjects were performing the discrimination task (WM0). Whatever the specific mechanisms may be, there is an interesting point: the distinctive pattern of cortisol and DHEA responses. Thus, cortisol decreases after a simple task if this task comes first and DHEA increases after a second cognitive task when this is a challenging task.

Stangl et al. [64] demonstrated that DHEA administration increased DHEA levels and enhanced performance in a visual same-different task (without WM load) while cortisol levels remained constant. Also, those authors described baseline cortisol relations to performance. However, other studies failed to provide systematic evidence that DHEA and DHEA administration enhanced short-term memory at the performance level [16,19]. In the present study, endogenous levels of DHEA, DHEAS and cortisol were measured and no significant relations were found with the accuracy or latency of the response. Nevertheless, a bigger sample of subjects may be necessary to uncover eventual relations.

On the other hand, the present study demonstrated endocrine relations to the electrophysiological recordings. In the WM task, higher baseline cortisol/DHEA ratio was related to more processing of the distracting stimuli, as indexed by an enhanced novelty-P3. This suggests that baseline cortisol enhances, whereas baseline DHEA prevents auditory distraction, and simultaneously suggests an anti-cortisol effect of DHEA. The fact that this relation became evident in the most stressful situation (WM load) agrees with previous evidence showing that DHEA has anti-cortisol effects under stress [12,14] or that these effects are more evident under stress.

Regarding ERPs to the visual target stimuli, DHEA effects on WM load pointed towards increased visP300 amplitudes. In fact, the DHEA raise due to WM load was related with enhanced P300 amplitudes indicating enhanced memory update and suggesting a rapid DHEA behavioral effect.

Endocrine responses to stimuli are commonly used and they usually provide higher sensitivity than baseline levels to detect pathological conditions or inter-subjects differences [29,65,66]. As an example, glucocorticoids responses to different stimuli can be used to measure the adrenals functional reserve (predicting their response to stress) or to characterize different phenotypes of the stress response (which were related to personality traits and pathological conditions) [67,68,69,70,71]. Accordingly, in the present study, the parameter related to working memory processing was DHEA response and not baseline DHEA. Also, apart from its slow genomic effects, corticosteroids are known to have rapid non-genomic central nervous system effects [35,39,40].

Alternatively, the relation between visual P300 amplitude and DHEA response could suggest that subjects with enhanced working memory update are the ones with higher DHEA responses. In that regard, other authors demonstrated that chronic stress and higher cortisol levels were related with poorer memory [34,37,38] and reduced DHEAS response to a superimposed psychological stress [29]. Nevertheless, we found no relation between baseline cortisol and visual P300 amplitude, and therefore, an effect of chronic stress is not suggested.

Wolf et al. [43] studied the effects of DHEA replacement on short term memory ERPs. They reported an increase in P3 amplitude after DHEA replacement, which reflects an enhancement of information updating. This is in accordance with our results, as we also observed that the physiological DHEA increase was related to an enhanced visP300.

A short term increase in cortisol can damage hippocampal neurons and may impair memory [42]. This may be an oversimplification since specific types of hippocampal mediated memory may be impaired by stress but others may not [72,73]. Yet, and hypothesizing that DHEA might prevent memory impairment under stress, Wolf et al [74] found that DHEA replacement enhanced attention but did not prevent the decline in visual memory under an acute psychosocial stress. This result did not support the idea of a direct anti-glucocorticoid effect of DHEA in hippocampal mediated memory functions. In another study DHEA protected hippocampal neurons against excitatory amino acid-induced neurotoxicity [75]. Our results also support the idea of DHEA anti-cortisol effects in distraction, but regarding working memory, we found relations with DHEA, not cortisol. Moreover, normal ranges of baseline cortisol were observed and cortisol levels did not rise with WM load, they just did not decrease.

Recent results also suggest that repeated stress and consequent activation of the glucocorticoid receptors dampens prefrontal cortex glutamatergic transmission. Actually, it facilitates glutamate receptor turnover, which has a detrimental effect on prefrontal cortex-dependent cognitive processes [76] like WM. The present

results agree with the known action of DHEA on glutamatergic receptors as well as with the idea that DHEA opposes cortisol detrimental effects during the performance of working memory tasks under stress. This last relation was evidenced by inverse relations of DHEA and cortisol to distraction.

Nevertheless, working memory effects were related to the DHEA response but not to cortisol. Thus, regarding WM effects, an anti-cortisol effect of DHEA is not so evident and other specific effects of DHEA may be present. As mentioned, besides their anti-cortisol effects, DHEA has Gamma Aminobutyric Acid Type A ($GABA_A$) receptor antagonism and sigma 1 agonist effects [3,9,10] which might underlie or contribute to the effects found. In fact, both gabaminergic antagonism and glutamatergic agonism are known to improve cognitive function.

Eventually in relation to DHEAS' long half-life [2,3], its levels did not change with WM load manipulation. Also, we found no relations between baseline DHEAS and performance or ERPs. Nevertheless, we found no relations between baseline DHEA and performance or ERPs either. Instead, baseline cortisol/DHEA ratio and DHEA response relations to ERPs were found.

DHEA metabolites also include other neuroactive steroids such as estradiol, estrone and testosterone [19,77], which may mediate part of the DHEA effects, namely after DHEA administration [78]. Estrogens, in particular estradiol, enhance working memory in women [79,80] and testosterone supplementation may enhance working memory in older men [81,82]. We did not measure DHEA metabolites, which may mediate some of its effects. Subsequently, we cannot exclude that those steroids may contribute or eventually be responsible for the performance and electrophysiological relations we found. Finally, as our study includes only female participants, the outreach of the results is limited only to women. DHEA and DHEAS are androgens and androgen levels, namely testosterone and DHEAS levels, are higher in men than in women [2,7]. Therefore, another group of participants would be necessary to study the electrophysiological correlates of DHEA and DHEAS in men. For further studies it would be relevant to study whether the results are identical or distinct according to gender.

In summary, a higher cortisol/DHEA ratio was related to enhanced processing of the auditory distractor during the performance of a visual working memory task. This suggests that in women, DHEA may oppose cortisol effects in involuntary distraction, reducing the processing of the auditory distractor (novelty-P3). Regarding working memory, DHEA increased with the performance of consecutive cognitive tasks, and a higher DHEA response due to WM load was related to an enhancement of the task-relevant information processing (visual P300). Overall, the results suggest that DHEA may oppose cortisol effects reducing distraction and a higher DHEA response may enhance working memory at the electrophysiological level.

## Supporting Information

**Table S1 Endocrine relations to performance.** ANOVAs of the performance parameters that covariated with endocrine measurements. Results represent the interaction between endocrine parameters and working memory (WM) condition and auditory stimulus. Baseline endocrine interactions are significant when $p<0.013$. Δ DHEA response = DHEA response in WM1 – DHEA response in WM0. WM0 – discrimination task; WM1 – working memory task.
(DOCX)

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## Author Contributions

Conceived and designed the experiments: SV LS JMM CE. Performed the experiments: SV LS. Analyzed the data: SV. Contributed reagents/materials/analysis tools: SV LS ACG. Wrote the paper: SV. Provided critical revision of the manuscript: LS JMM ACG MB IC CE.

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**Supporting Information**

**Table S1: Endocrine relations to performance**

| | | **ANOVA** | |
|---|---|---|---|
| **Hit Rates** | | | |
| Baseline Cortisol x | Task (WM1, WM0) | F (1,21)=5.956 | p=0.024 |
| | Sound (standard, novel) | **F(1,21)=8.222** | **p=0.009** |
| | Task x Sound | **F(1, 21)=11.751** | **p=0.003** |
| Baseline Cortisol/DHEA ratio x | Task | **F(1,20)=8.186** | **p=0.01** |
| | Sound | **F(1,20)=13.178** | **p=0.002** |
| | Task x Sound | **F(1,20)=17.609** | **p<0.001** |
| Δ DHEA response x | Task | **F(1,20)=8.087** | **p=0.01** |
| | Sound | F(1,20)=1.827 | p=0.192 |
| | Task x Sound | F(1,20)=5.671 | p=0.027 |
| **Response times** | | | |
| Baseline Cortisol x | Task | F(1,21)=1.129 | p=0.300 |
| | Sound | F(1,21)=4.441 | p=0.047 |
| | Task x Sound | F(1,21)=3.038 | p=0.096 |
| Baseline Cortisol/DHEA ratio x | Task | F(1,20)=0.467 | P=0.502 |
| | Sound | F(1,20)=6.489 | p=0.019 |
| | Task x Sound | F(1,20)=2.104 | P=0.162 |
| Δ DHEA response x | Task | F(1,20)=3.304 | p=0.084 |
| | Sound | F(1,20)=2.349 | p=0.141 |
| | Task x Sound | **F(1,20)=10.734** | **p=0.004** |

**Correction: The Relationship between Dehydroepiandrosterone (DHEA), Working Memory and Distraction – A Behavioral and Electrophysiological Approach.**

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The factor used to convert DHEA measurements from conventional units to the system of international units should be **3.467** instead of 0.3467 (this error is in the last sentence of the "Subjects and Methods" section, "Endocrine Measurements" subsection). Therefore, DHEA levels in the "Results" section, "Endocrine Baseline Levels and Response" subsection, should read 10 times higher, as follows.

First sentence of the first paragraph: Baseline endocrine levels were: DHEA **790±155** pmol/L, DHEAS 984±120 pmol/h and cortisol 3412±622 pmol/L, with a normal distribution and no significant relation between them.

Last sentence of the second paragraph: Further analyses revealed that DHEA levels rose after the performance of the second task as indicated by a time effect [$F_{(2,40)}$ =8.415, p=0.003; **596±94** pmol/L before task; **839±135** pmol/L after task; **929±156** pmol/L at washout], see figure 5A.

First sentence of the third paragraph: Regarding WM effects, *post hoc* analyses revealed that when the order was WM0-WM1, DHEA levels were higher after WM1 than after WM0 [$F_{(1,10)}$ =9.041, p=0.013, **711±139** pmol/L after WM0 and **1064±222** pmol/L after WM1].

Figure 5.A: DHEA values should read 10 times higher, therefore "**x10**" is missing in the top of DHEA axis.

Figure 6: Baseline cortisol/DHEA ratio axis should be **0, 10, 20, 30 and 40** instead of 0, 100, 200, 300 and 400.

Figure 5.D has got a typographical error: it should be $r_s$=**0.615** (as stated in the text) instead of $r_s$=0.165.

These errors do not significantly affect the results and they do not affect the conclusions of the work. The authors apologize for these errors.

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# Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) and emotional processing — A behavioral and electrophysiological approach

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ABSTRACT

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) may have mood enhancement effects: higher DHEAS concentrations and DHEA/cortisol ratio have been related to lower depression scores and controlled trials of DHEA administration have reported significant antidepressant effects. The balance between DHEAS and DHEA has been suggested to influence brain functioning. We explored DHEAS, DHEA, cortisol, DHEA/cortisol and DHEAS/DHEA ratios relations to the processing of negative emotional stimuli at behavioral and brain levels by recording the electroencephalogram of 21 young women while performing a visual task with implicit neutral or negative emotional content in an audio–visual oddball paradigm. For each condition, salivary DHEA, DHEAS and cortisol were measured before performing the task and at 30 and 60 min intervals. DHEA increased after task performance, independent of the implicit emotional content. With implicit negative emotion, higher DHEAS/DHEA and DHEA/cortisol ratios before task performance were related to shorter visual P300 latencies suggesting faster brain processing under a negative emotional context. In addition, higher DHEAS/DHEA ratios were related to reduced visual P300 amplitudes, indicating less processing of the negative emotional stimuli. With this study, we could show that at the electrophysiological level, higher DHEAS/DHEA and DHEA/cortisol ratios were related to shorter stimulus evaluation times suggesting less interference of the implicit negative content of the stimuli with the task. Furthermore, higher DHEAS/DHEA ratios were related to reduced processing of negative emotional stimuli which may eventually constitute a protective mechanism against negative information overload.



## Introduction

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) are neuroactive steroids and their concentrations in the central nervous system are higher than in the peripheral circulation (Dong and Zheng, 2011; Lacroix et al., 1987). Although their physiological role and mechanisms of action are still a matter of debate (Komesaroff, 2008), a growing amount of evidence has been accumulating regarding their actions in the central nervous system. Concerning cognitive functions, higher DHEAS levels were related to improved memory (Barrett-Connor and Edelstein, 1994), whereas low levels were found in Alzheimer's disease (Weill-Engerer et al., 2002). Besides, higher DHEA, DHEAS or DHEA-to-cortisol levels were related to improved attention (Wolf et al., 1997), lower perceived stress and also to improved performance under stressful conditions (Morgan et al., 2009; Russo et al., 2012).

Furthermore, higher DHEAS concentrations and DHEA-to-cortisol ratios have been related to lower prevalence of depression, lower depression ratings and higher well-being scores (Barrett-Connor and Edelstein, 1994; Barrett-Connor et al., 1999; Michael et al., 2000; Young et al., 2002). The relation between DHEA levels alone and depression is less consistent. In fact, several groups have found that DHEA-to-cortisol ratios, rather than concentrations of either hormone alone, discriminated more accurately depressed from non-depressed individuals, with lower DHEA-to-cortisol ratios as seen in depression (Assies et al.,

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2004; Michael et al., 2000), in untreated depressed patients and in patients who remained depressed after several months (Goodyer et al., 1998).

It was hence suggested that elevated DHEA and DHEAS relative to cortisol levels, may counteract the negative effects of high cortisol on mood (Goodyer et al., 1998; Kaminska et al., 2000). Moreover, controlled trials of DHEA therapy have reported significant positive effects on mood (Bloch et al., 1999; Schmidt et al., 2005; Wolkowitz et al., 1999). These mood improvements were related to increases in the circulating concentrations of DHEA and DHEAS and to increases in their ratios with cortisol. On the other hand, sulfated steroids in general possibly act as endogenous neuromodulators (Gibbs et al., 2006) and the balance between DHEAS and DHEA has also been suggested to influence brain functioning. In a study assessing both DHEA and DHEAS, depressed patients had low DHEAS but normal DHEA concentrations (Scott et al., 1999). In a different setting, but also pointing towards the importance of DHEAS to DHEA balance, it has been shown that subjects with Alzheimer's disease have increased levels of DHEA in the central nervous system, but a reduced conversion of DHEA into DHEAS and consequently, reduced DHEAS/DHEA ratios (Kim et al., 2003).

Glucocorticoids' effects are not always deleterious. In fact, glucocorticoids have biphasic effects on fear conditioning: although mild or short lasting increases in glucocorticoids in relation to stress may have beneficial effects on attention and promote adaptation, higher cortisol levels or long term increases have deleterious effects on executive functioning, attention, learning, memory and cognitive flexibility (Campeau et al., 2011; McEwen, 2012). Again, anti-glucocorticoid effects of DHEA and DHEAS have been proposed in what concerns cognition and performance.

DHEA and DHEAS present a general neurostimulatory effect, but DHEAS has a much more potent excitatory action than DHEA (Baulieu and Robel, 1998; Imamura and Prasad, 1998; Monnet et al., 1995; Dong and Zheng, 2011). At the cellular level DHEAS antagonizes the neurotoxic effect of high doses of DHEA in mouse neuronal cultures (Gil-ad et al., 2001) and DHEA is protective against the neurotoxic effects of corticosterone (Balazs et al., 2008). Hence, the simultaneous evaluation of DHEA, DHEAS and cortisol and the ratios of DHEA-to-cortisol and DHEAS-to-DHEA may uncover more information than the individual examination of either steroid alone.

DHEA and DHEAS effects on cerebral regions specifically involved in emotional processing including the amygdale, hippocampus, insula and anterior cingulated cortex have been suggested. However, little research has explored the neural correlates of DHEA and DHEAS with respect to emotion and mood. In this regard, a study using LORETA showed that DHEA administration increased the activity in the anterior cingulate cortex (Alhaj et al., 2006). Another recent study using functional Magnetic Resonance Imaging (fMRI) found that DHEA reduces the activity in regions associated with the generation of negative emotion and enhances activity in regions linked to regulatory processes (Sripada et al., 2013).

DHEA and DHEAS relations to emotional processing at the electrophysiological level are not known. Regarding cortisol, its administration increased the processing of angry faces in highly anxious individuals as indicated by increased amplitudes of early (P150) and late (P3) event-related potentials (van Peer et al., 2007). DHEA and DHEAS may modulate attention, cognition and mood while their response to emotional stimuli is mostly unexplored.

The aim of the present study was to explore whether DHEA and DHEAS levels would have an influence on involuntary attention and emotional stimuli processing at the performance and brain levels and, on the other hand, if an emotional challenge would alter DHEA and DHEAS levels. Furthermore, we wanted to examine the relation of DHEA and DHEAS with cortisol levels. The a priori hypotheses were: 1) higher endogenous DHEAS and DHEA levels as well as higher DHEA-to-cortisol and DHEAS-to-DHEA levels may protect from involuntary distraction and enhance brain processing and performance under a negative emotional context; 2) DHEAS and DHEA effects may be largely antagonistic from those of baseline cortisol; 3) in the short term, a negative emotional context might be a stimulus for DHEA and cortisol secretion.

To test these hypotheses we used a visual task with a neutral or negative emotional context and unexpected auditory novel sounds aimed to cause distraction. In this paradigm, the negative emotional context is expected to elicit an increased attention capture when compared to non-emotional faces (Öhman et al., 2001) and consequently deteriorate performance (Domínguez-Borràs et al., 2008). Furthermore, auditory distraction by novel sounds is expected to elicit a novelty-P3 component in the electroencephalogram (EEG) and also deteriorate performance (Corral and Escera, 2008; Domínguez-Borràs et al., 2008; Escera et al., 1998, 2000). We recorded performance parameters, the EEG and took saliva samples in order to determine the hormonal levels before and after the task. We explored DHEA, DHEAS, cortisol, DHEA/cortisol ratio and DHEAS/DHEA ratio relations to distraction and implicit negative emotion at the performance and electrophysiological levels.

## Participants and methods

### *Participants*

21 healthy female volunteers (university students) from 18 to 26 years (mean 21 ± 1), performed the study protocol. Only women were included as to ensure higher homogeneity in emotional processing (Garcia-Garcia et al., 2008) and androgen levels. All had normal or corrected-to-normal vision and none reported auditory deficits. There was no history of neurologic, psychiatric, endocrine or oral diseases. Subjects were asked to refrain from alcohol intake in the 12 h before the experimental protocol and tobacco and illicit drug consumption were exclusion criteria. All participants gave their written informed consent. The experimental protocol was approved by the ethical committees of the University of Barcelona and Lisbon Medical School and was performed in accordance with the Declaration of Helsinki.

Prior to the experimental session, subjects completed the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1988) and all were within a normal range of state (mean 13 ± 1) and trait (mean 17 ± 1) anxiety levels. Mean body mass index (BMI) was 22.3 ± 0.8 kg/m$^2$ and all participants presented a normal body mass index except one with grade 1 obesity. All except one participant were right-handed. Seven participants were in the follicular phase, three were in the peri-ovulatory phase and six were in the luteal phase, based on self reported menstrual cycle day. Five subjects were using hormonal contraception, no other medications were allowed.

### *Task and procedure*

The experimental sessions were conducted in the afternoon, beginning at 2–3 pm. An adapted version of a well-established auditory–visual distraction task (Escera et al., 1998, 2000) was presented with two different conditions, one featuring a neutral (NEU) and one featuring a negative emotional content (NEG), as implemented by Domínguez-Borràs et al (2008). Each condition lasted about 15 min and conditions were performed 2 h apart (beginning to beginning). The order was counterbalanced across subjects. Each condition consisted of two separate blocks of 255 trials with a short interval between them.

Participants sat in a comfortable chair in a dimly lit and electrically and acoustically shielded room. The task consisted of responding as fast and accurately as possible whether the two faces on the screen were equal or different by pressing the correspondent mouse button. The subjects were instructed to ignore the sounds. In order to reduce eye blinks and movements during EEG recording, subjects were asked to blink as little as possible, and to focus on a central fixation cross between the two pictures. Responses were given through a mouse button (one mouse button for “the same” and the other button for “different”,

counterbalanced across subjects) and the probability of both responses was the same. Before each experimental condition, subjects performed practice blocks (composed of 10 trials) using faces with a neutral expression only and without any auditory stimuli, until they reached a hit rate (HR) of at least 80%.

In each trial, a task-irrelevant auditory stimulus was presented, followed after 300 ms (onset-to-onset) by a visual imperative stimulus (Fig. 1). The total trial length varied from 1200 to 1600 ms (1400 ms on average; jitter +/− 200 ms) and the response window until the end of the shortest trial was 1200 ms. The auditory sequence consisted of repetitive standard tones (duration of 200 ms, including fade-in and fade-out of 10 ms each; 600 Hz; 85 dB; probability of occurrence p = 80%), occasionally replaced by environmental novel sounds (p = 20%). These novel sounds were selected from a sample of 100 different specimens (edited to have a duration of 200 ms, including fade-in and fade-out of 10 ms each; digitally recorded, low-pass filtered at 10,000 Hz; 85 dB), such as those produced by a drill, hammer, rain, door, telephone ringing, and so forth (Escera et al., 2003). All sounds were delivered binaurally through headphones (Sennheiser HD 202) in a randomized order with the only restrictions that the first four stimuli of each block had to be standard tones, that two novel sounds never appeared consecutively and that each novel sound occurred only once in each condition. The visual stimuli were pairs of combinations of pictures of faces with either neutral (Fig. 1.A) or fearful (Fig. 1.B) expression, presented on a computer screen for 400 ms. We used 12 pictures of faces with either neutral (NEU) or fearful (NEG) expression from the Ekman and Friesen (1976) database. All faces had exactly the same probability of occurrence and all had the same valence (NEU or NEG) in each block. The picture size was 356 × 488 pixels, the vertical angle 9° and the horizontal angle 17°, accounting for two pictures presented simultaneously with a fixation cross in between, and the distance from the subjects' eyes to the screen was 100 cm.

*Performance and EEG recording and analysis*

Response time (RT) and whether the button press was correct, wrong or missed were recorded for each trial using Presentation® (Neurobehavioral Systems, Inc). A database was created with the mean response time for correct responses and hit rate (HR), separately for each condition (NEU and NEG) and auditory stimulus type (standard and novel). Distraction by novel sounds was computed as the difference in hit rate and response time between standard and novel auditory stimuli (hit rate: NEUstandard trials − NEUnovel trials and NEGstandard trials − NEGnovel trials; response time: NEUnovel trials − NEUstandard trials and NEGnovel trials − NEGstandard trials). Performance disruption due to the processing of negative emotional stimuli was computed as the difference in hit rate and response time between conditions, as appropriate (hit rate: NEUstandard − NEGstandard and NEUnovel − NEGnovel; response time: NEGstandard − NEUstandard and NEGnovel − NEUnovel).

EEG activity was continuously recorded, from 64 scalp Ag/AgCl electrodes following the extended 10/10 convention. It was amplified and digitalized at a sampling rate of 512 Hz (Eemagine, ANT Software b.v., Enschede, Netherlands). The horizontal and vertical electrooculogram (HEOG and VEOG) were recorded with electrodes placed at the outer canthus and below the right eye, respectively. An electrode placed on the tip of the nose was used as the common reference and the ground was located at the AFz position. Impedances were kept at 5 kΩ or below during the whole recording session. Recording was performed with an ANT amplifier of 64 channels (gain 20×; A/D resolution 22 bits, 71.526 nV per bit; filtering 0–138.24 Hz; CMRR > 90 dB).

EEG processing was performed off-line by using Eeprobe 3.1 (ANT Software BV, Enschede, Netherlands). A digital finite impulse response (FIR) bandpass-filter from 0.01 to 30 Hz was applied using a Hamming window. ERPs were averaged for each auditory-stimulus trial type and emotional condition, for an epoch of 1400 ms, comprising a pre-auditory-stimulus baseline of 200 ms. The first five epochs of each block and epochs following a novel trial were excluded from averaging. Only epochs corresponding to trials with correct responses were included in further analyses. Electrooculogram (EOG) correction was performed by manually selecting a large number of typical artifacts and accordingly applying a regression algorithm to compute propagation factors (Eeprobe 3.1, ANT Software BV, Enschede, the Netherlands). After EOG correction any epochs containing EEG activity exceeding ± 100 μV peak-to-peak amplitudes were rejected from further analysis. On average, 84% of epochs (252 epochs) with standard sounds and 87% of epochs (87 epochs) with novel sounds in NEU and 86% of epochs (259 epochs) with standard sounds and 86% of epochs (86 epochs) with novel sounds in NEG were retained for averaging.

In the employed paradigm the participants were specifically instructed to ignore the distraction stimuli (auditory), hence any related effects are necessarily involuntary or led by exogenous attention. ERPs recorded during auditory distraction are typically characterized by an auditory N1/mismatch negativity (N1/MMN) enhancement, reflecting a detection mechanism leading to attention capture, followed by a novelty-P3 (nov-P3) reflecting the effective orientation of attention (Escera et al., 1998, 2000). The nov-P3 component has been shown to be sensitive to the manipulation of the emotional context (Domínguez-Borràs et al., 2008) and attention (Escera et al., 1998). Subsequently, the re-orienting negativity (RON) reflects the re-orientation of attention back to the task (Schröger and Wolff, 1998). The target stimuli in the present task were visual, and visual ERPs include the


**Fig. 1.** Trial structure. A. Neutral emotional context (NEU). B. Negative emotional context (NEG).

P300, which is a cognitive component related to target processing. The P300 (visP300) reflects the conscious processing of the visual stimulus and is sensitive to attention allocation (Domínguez-Borràs et al., 2008; Polich, 2007).

To analyze distraction effects, the ERPs elicited to the auditory stimuli were considered and difference waveforms (dw) were calculated by subtracting the ERPs elicited by standards from those elicited by novel sounds. These difference waveforms revealed an early-onset, long-lasting positive deflection that we assimilated to the novelty-P3. We measured the auditory P3 (aud-P3) and novelty-P3 (nov-P3) as the mean amplitude and mean peak latency in the 210–470 ms time window at F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrodes.

To analyze emotional effects, ERP measures in standard trials were compared. Specific components were elicited during task-performance, such as the auditory N1 and P2, visual N1 and P2 and the cognitive components N2b and visual P300. Since only cognitive processing was of interest for the present study, the visual P300 (650–1050 ms at F7, F3, FZ, F4, F8, T7, C3, CZ, C4, T8, P7, P3, PZ, P4 and P8, 350–750 ms from stimulus presentation) was analyzed. Mean amplitudes and mean peak latencies for the considered time window and electrodes were computed for each deflection.

### *Endocrine measurements*

Saliva samples were collected by passive drool, using a short straw. Unstimulated whole saliva was used. For each condition, samples were collected for DHEA, DHEAS and cortisol measurement before task (BF), at 30 min (30 min after the beginning of the task, 10 to 15 min after the end of the task) and 60 min (60 min after the beginning of the task, 40 to 45 min after the end of the task). The samples collected before the first condition (i.e. before both the neutral and the negative condition), were considered as the baseline. Time points to collect the saliva samples were chosen in accordance to known cortisol raise and recovery times (raise 10 min after appropriate stimulus, peak at 20–30 min and recovery at 45–60 min after the end of the stimulus) (Martins et al., 2001, 2002, 2004; do Vale et al., 2011). Furthermore, synchronous 24 h profiles were described for DHEA and cortisol and DHEA half-life is less than 30 min (Rosenfeld et al., 1975). For this reason, the second condition was started 2 h after the first one to allow cortisol and DHEA levels to recover from the influence of the first condition (return to a "baseline" level).

Unbound DHEA and cortisol in the peripheral circulation penetrate into the saliva via intracellular mechanisms and salivary concentrations reflect serum concentrations (Ahn et al., 2007; Vining and McGinley, 1987). DHEAS is not lipid soluble and cannot penetrate into the saliva by passive diffusion through cell membranes. Instead, it squeezes through the tight junctions between salivary glands. DHEAS concentrations in saliva are therefore dependent on serum concentration and salivary flow rate (Vining and McGinley, 1987).

Samples were refrigerated at 2–8 °C within 30 min after collection and were stored at −20 °C within 4 h and until assayed. Each sample was measured in duplicate using enzyme-linked immunoassays: salivary DHEA and DHEAS enzyme immunoassay kits (Salimetrics Europe®, Ltd, Newmarket Suffolk, UK) and high sensitivity salivary cortisol enzyme immunoassay kits (Salimetrics®, LLC, State College, PA, USA). DHEA was measured in pg/mL and cortisol was measured in μg/dL. Due to the influence of saliva flow rates on DHEAS levels, the concentration of DHEAS (pg/mL) was multiplied by the flow rate (mL/min) and the corrected results were obtained as DHEAS measured per unit of time (pg/min). The minimal concentrations that can be distinguished from 0 with the used immunoassays are 5 pg/mL for DHEA, <0.003 μg/dL for cortisol and <43 pg/mL for DHEAS. Intra- and interassay coefficients of variation were less than 10% and 15% in every case, respectively.

### *Statistical analysis*

The Statistical Package for the Social Sciences Program (IBM SPSS Statistics, version 21) was used for data analysis. Results are presented as the mean ± standard error of the mean (SEM). The normal distribution of continuous variables was verified by the Kolmogorov–Smirnov goodness of fit test.

To explore the effects of the implicit emotional content and auditory distraction on performance, repeated measures analyses of variance (ANOVA) were performed on hit rate and response time, including the within-subjects factors emotional condition (NEU and NEG) and type of auditory stimulus (standard and novel). Regarding brain responses, ERPs time-locked to the auditory stimuli were analyzed to explore distraction effects. ERPs time-locked to the visual stimuli (the faces) were analyzed to explore emotional context effects. To investigate the effects of the emotional context and auditory distraction on brain responses to auditory stimuli, repeated measures ANOVAs were carried out on auditory P3 mean amplitude in the time window and electrodes considered above, including the within-subjects factors emotional condition (NEU and NEG) and type of auditory stimulus (standard and novel). To investigate the effects of the emotional context on brain responses to visual stimuli, only standards were included and ANOVAs were performed on the visual P300 mean amplitude in the time windows and electrodes considered above, with emotional condition (NEU and NEG) as within-subjects factor. To investigate the effects of emotional context manipulation on endocrine levels, repeated measures ANOVAs were performed on DHEA, DHEAS and cortisol levels, including the within-subjects factors emotional condition (NEU and NEG) and measurement time (before task, at 30 min and 60 min).

To investigate the endocrine relation to distraction and emotional context effects at the behavioral level, the previous repeated measures ANOVAs of behavior parameters were repeated, including baseline DHEA, DHEAS, cortisol, DHEA/cortisol ratio or DHEAS/DHEA ratio as covariates. Whenever the endocrine parameters covariated with the performance or ERP parameters for each type of auditory stimuli or emotional condition, we performed simple or multiple regression analyses to select the relevant independent endocrine factors and/or to understand the direction of the relationship. Lastly, as visual P300 peak latency in both emotional contexts was not directly related, in order to find the relation between visual P300 peak latency and endocrine parameters, linear and multiple regression analyses were used with the endocrine parameters as independent variables.

ANOVA results were Greenhouse–Geisser corrected whenever the assumption of sphericity was violated. The limit of significance chosen was $\alpha = 0.05$. Post hoc tests were carried out wherever there were significant interactions between main factors and the Bonferroni correction was applied for multiple comparisons. For the endocrine relations to performance or ERPs the alpha therefore was set to 0.01 as the effects were tested for five variables (cortisol, DHEA, DHEAS, DHEA/cortisol ratio and DHEAS/DHEA ratio). Effect size estimates of the results were expressed as eta squared ($\eta^2$) for ANOVAs and correlation coefficients (r) for regression analyses.

## Results

### *Performance*

Behavioral results for each condition and trial type are presented in Fig. 2. There was a main effect of emotional context on response time [$F_{(1,20)} = 17.51$, $p < 0.001$, $\eta^2 = 0.47$], with longer response times under the emotionally negative context (433 ± 17 ms) than under the neutral one (379 ± 12 ms). Furthermore, there was a main effect of trial type on response time [$F_{(1,20)} = 31.88$, $p < 0.001$, $\eta^2 = 0.61$], with longer response times for novel (423 ± 13 ms) than for standard (389 ± 14 ms) trials, indicating that the novel sounds caused distraction of visual task performance (Fig. 2.A). Overall hit rate was 88 ± 1% and

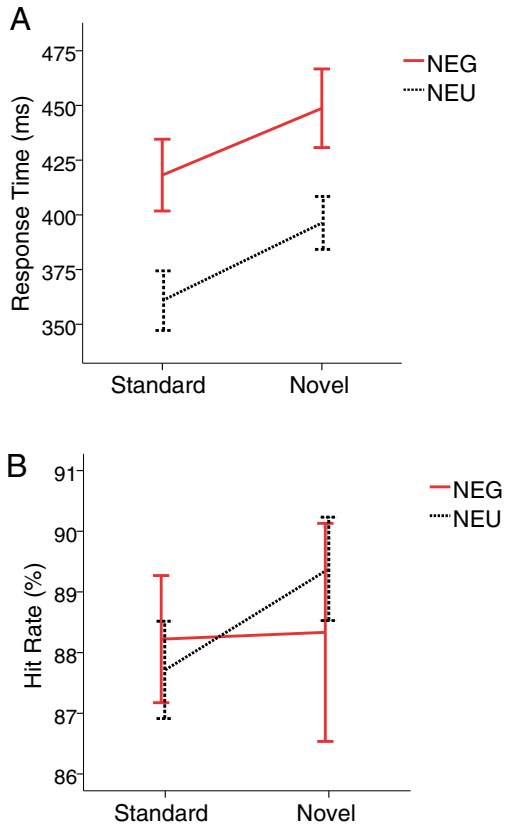


**Fig. 2.** Performance results. A. Mean response times for each condition [neutral (NEU) or negative context (NEG)] and auditory stimulus type (standard or novel). B. Mean hit rates for each condition and auditory stimulus type. Error bars represent ±1 standard error of the mean (SEM).

did not change significantly with the emotional context or trial type (Fig. 2.B).

*Event-related potentials*

*Distraction effects*

No clear N1-enhancement/MMN nor RON was elicited and therefore our analysis focused only on the nov-P3. In fact, the nov-P3 was significantly elicited as supported by the significant differences of the mean amplitudes in the auditory P3 latency window for standard and novel trials [$F_{(1,20)} = 169.09$, $p < 0.001$, $\eta^2 = 0.89$; $-2.3 \pm 0.4$ μV in standard and $+1.7 \pm 0.5$ μV in novel trials], see Fig. 3.A and B. However, there were no effects of emotional context on the auditory P3 (see Fig. 3.C), as no significant interaction between emotional context and auditory stimulus type was found.

*Emotional context effects*

The waveforms elicited by standard trials in the two emotional contexts are presented in Fig. 3.D. No significant emotional effects were observed on visual P300 as its amplitude was similar in both conditions. Also, there were no significant latency differences in P300, between emotional conditions.

*Endocrine baseline levels and reactivity*

Baseline endocrine levels were: DHEA 254 ± 41 pg/mL, DHEAS 5856 ± 690 pg/min and cortisol 764 ± 100 pg/mL, following a normal distribution. Baseline DHEA level was directly related to baseline cortisol level ($r = +0.63$, $p = 0.002$, $n = 21$). There was no significant relation between baseline DHEAS and DHEA or DHEAS and cortisol level. Nevertheless, baseline DHEAS levels were directly related to baseline DHEA/cortisol ratio ($r = +0.56$, $p = 0.008$, $n = 21$). Baseline endocrine parameters were not related to age or body mass index and did not differ according to menstrual cycle phase or between subjects taking and not taking hormonal contraception.

DHEA, DHEAS and cortisol mean levels for each condition and sample time are presented in Fig. 4. The repeated measures ANOVA on DHEA levels revealed a main effect of measurement time on DHEA levels [$F_{(2,40)} = 5.94$, $p = 0.007$, $\eta 2 = 0.24$; mean levels were 243 ± 42 pg/mL before task, 258 ± 41 pg/mL at 30 min and 309 ± 45 pg/mL at 60 min], see Fig. 4.A and B. There was no significant relation between emotional context and DHEA levels. Moreover, there was no interaction between DHEAS (Fig. 4.C) or cortisol levels (Fig. 4.D) and the emotional context or measurement time.

*Endocrine relations to performance and event-related potentials*

We found no significant relations between performance (hit rate and response times) and endocrine parameters. Regarding event-related potentials, no significant relations were found between endocrine parameters and distraction effects. Nevertheless, significant relations were found between endocrine parameters and emotional context. Higher DHEAS/DHEA ratios before performing the emotionally negative condition were related to reduced visual P300 amplitudes in this condition. This was revealed by a significant interaction between visual P300 amplitudes and DHEAS/DHEA ratios [$F(1,19) = 9.38$, $p = 0.006$, $\eta^2 = 0.33$] with higher DHEAS/DHEA ratios in relation to reduced visual P300 amplitudes attributed to the negative context ($r = -0.58$, $p = 0.006$, $n = 21$; see Fig. 5.A). Concerning visual P300 peak latency, higher DHEA/cortisol (partial $r = -0.56$, $p = 0.003$, $n = 21$) and DHEAS/DHEA (partial $r = -0.60$, $p = 0.004$, $n = 21$) ratios before performing the negative emotional context block, were related to shorter visual P300 peak latencies (Fig. 5.B and C), together explaining 52% of the latency variability. Remarkably, smaller visual P300 amplitudes attributed to the negative context were related to shorter visual P300 peak latencies ($r = +0.53$, $n = 21$, $p = 0.015$).

## Discussion

The present study revealed relations between dehydroepiandrosterone (DHEA), its sulfated form (DHEAS) and brain processing under an emotionally negative context induced by images of fearful faces, suggesting that these neurosteroids may modulate the processing of emotionally negative information. Although the sounds as well as the emotional content of the pictures were irrelevant for the task, the results suggest that the subjects were unable to fully ignore them as indicated by significant effects on performance and brain responses. The distraction effect of task irrelevant auditory stimuli as well as the behavioral disruption due to the processing of task irrelevant negative emotional stimuli have been shown before by other authors (Escera et al., 1998, 2000; Domínguez-Borràs et al., 2008, 2009; Öhman et al., 2001), whereas the relation between the neurosteroids and emotional processing at the brain level is a new finding.

Novel sounds led to distraction as shown by longer response times and the elicitation of a significant novelty-P3. Conversely, no clear N1-enhancement/MMN was observed in our results, probably due to a very early P3 onset, causing an overlap between the components. Recent studies have shown that small deviant stimuli, and hence large novel sounds, may activate deviance-detection mechanisms as early as 20 ms from sound onset (see Slabu et al., 2010; Escera and Malmierca, 2014) and therefore attention switching may have had occurred before the supratemporal activation giving rise to the typical N1-enhancement/MMN trigger response (Yago et al., 2001). Additionally, in the negative emotional context, response times were longer than in the neutral context which implicates that the novel sounds effectively caused distraction and the emotionally negative context effectively disrupted performance. However, the emotional context did not

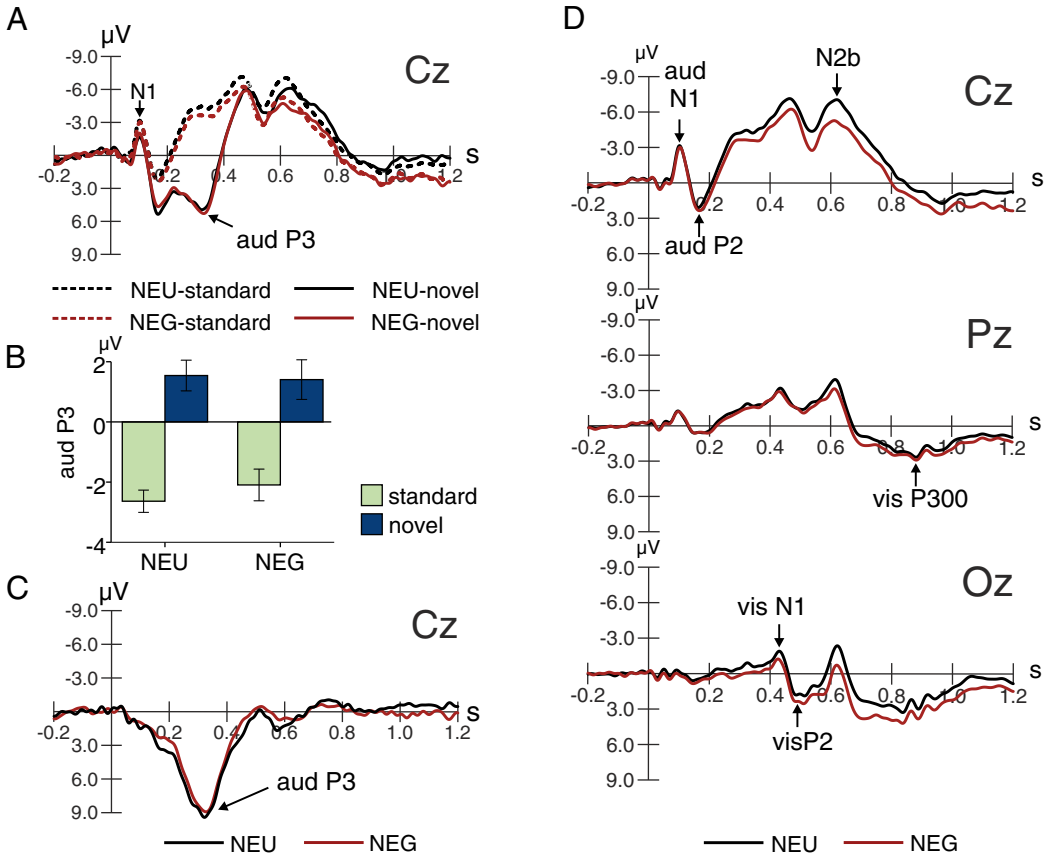


**Fig. 3.** Event-related potentials (ERPs). A. Grand average waveforms at Cz for each emotional context (neutral and negative) and type of sound (standard and novel). B. Mean auditory P3 (aud P3) amplitude for each emotional context and type of sound. C. Grand average of novel minus standard difference waveforms at Cz for each emotional context. D. Grand average ERPs for neutral and negative context (only standard trials). NEG: negative emotional context; NEU: neutral emotional context; aud: auditory event related potentials; vis: visual event related potentials.

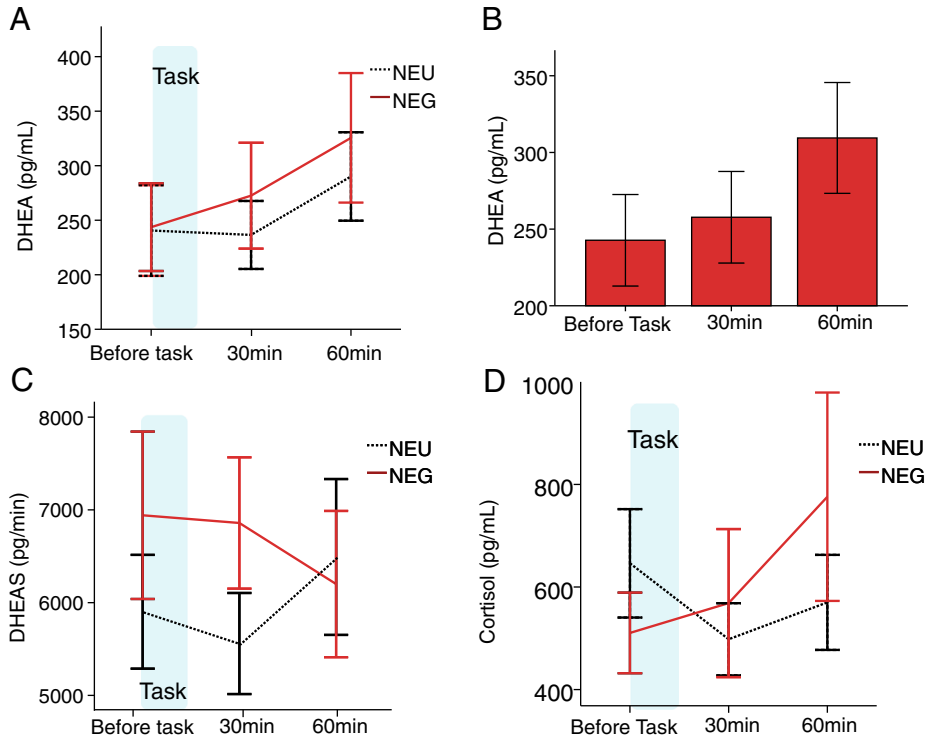


**Fig. 4.** Endocrine results. A. DHEA mean levels for each condition and measurement time. B. DHEA levels before task, at 30 min and 60 min. C. DHEAS mean levels for each condition and measurement time. D. Cortisol mean levels for each condition and measurement time. Error bars represent ±1 SEM. The shadow indicates the period of task performance. NEG: negative emotional context; NEU: neutral emotional context.

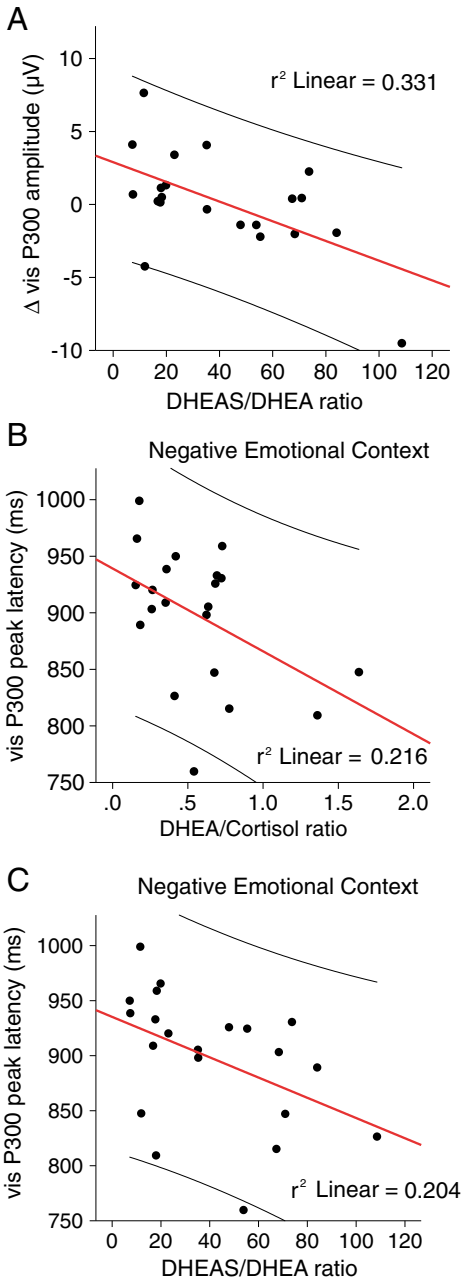


**Fig. 5.** Endocrine relations to event-related potentials. A. Higher DHEAS/DHEA ratios before performing the negative emotional context block were related to reduced interference with the task due to the processing of the implicit negative content of the stimuli as shown by reduced visual P300 amplitudes. B and C. Higher DHEA/cortisol and DHEAS/DHEA ratios before performing the negative emotional context block, were related to shorter visual P300 peak latencies. Δ vis P300 amplitude: mean visual P300 amplitude in the negative minus neutral context.

significantly modulate the electrophysiological response to auditory distraction, contrasting with previous studies (Domínguez-Borràs et al., 2008; Garcia-Garcia et al., 2008) which found an enhancement of the visual P300 for emotionally negative stimuli (Domínguez-Borràs et al., 2008). Nevertheless, in studies using faces, some authors have found visual P300 enhancement by fearful faces (Luo et al., 2010), while others reported no changes in P300 amplitude (Balconi and Lucchiari, 2005) as in the present study. Importantly, in the present study, the emotional content of the images was not relevant for the task.

DHEA levels increased after the performance of both conditions, independent of the emotional context of the task. Interestingly, a previous study showed that corticotrophin releasing hormone (CRH) levels increased with another cognitive task: the visualization of emotionally significant movies (Martins et al., 2010). In turn, the present results suggest that cognitive tasks are a stimulus for DHEA secretion and may lead to a higher DHEA/cortisol ratio. Moreover, it suggests that DHEA is not just a stress hormone and that some differential regulation of DHEA and cortisol exists. In fact CRH stimulates corticotrophin secretion and in turn corticotrophin is a stimulus for cortisol and DHEA secretion (Nieschlag et al., 1973). This may explain the direct relation we found between cortisol and DHEA levels.

Boudarene et al. (2002) studied subjects without mental disorders and varying levels of anxiety, and found that the level of anxiety was related to the profile of endocrine response after the performance of cognitive tasks: subjects with high anxiety levels in the STAI test had increased cortisol reactivity and subjects with low anxiety levels showed an exclusive increase in DHEAS levels. The authors suggested that the antagonism in DHEAS and cortisol might be related to competition in their synthesis and its release by the adrenal gland. This agrees with the results in the present study, in which all the subjects had low anxiety levels and a DHEA raise but no cortisol response in relation to the cognitive task (in both emotional conditions), was found. For non-pathological conditions and low levels of anxiety, this might eventually represent an adaptive mechanism that includes higher DHEA than cortisol responses and anti-cortisol effects of DHEA. DHEA increase however was identical for both emotional contexts, so that DHEA increase alone is not expected to be related to performance or electrophysiological effects of the emotional context.

No significant relation was found between response times or hit rates and endocrine measurements. A larger sample may be necessary to uncover endocrine relations to performance. Nevertheless, an interesting finding of the present study consists in endocrine relations to brain responses. A higher DHEA/cortisol ratio was related to lower visual P300 latencies in the negative emotional context, suggesting DHEA to be protective from an interference of the implicit negative content of the stimuli with the task which might represent an anti-glucocorticoid effect of DHEA. In the negative condition, higher DHEAS/DHEA ratios were also related to lower visual P300 latencies, thus, a higher proportion of DHEA respective to cortisol and a higher proportion of sulfated to non-sulfated form of DHEA may protect from any interference with the task caused by the processing of the implicit emotional content of the stimuli.

In agreement, higher DHEA levels have been previously found to be related to shorter P300 latencies (Braverman and Blum, 2003; Braverman et al., 2009). A protective effect of DHEA and DHEAS could eventually be mediated by anti-cortisol effects and/or neuromodulatory effects of these hormones. Wang et al. (2013) associated a prolonged P300 latency with an attentional bias to negative stimuli in high trait anxiety subjects, who are expected to have higher cortisol levels. On the contrary, in the present study examining subjects with low trait anxiety, higher DHEA/cortisol ratios were related to shorter P300 latencies in the negative condition. Higher DHEA/cortisol could be related to less interference with the task caused by the implicit stressful content of the stimuli, in agreement with the hypothesis of anti-cortisol effects of DHEA and suggesting a protective mechanism against the deleterious consequences of stress. The present results also agree with studies at the cellular level, in which DHEA was protective against the neurotoxic effects of corticosterone (Balazs et al., 2008).

Lastly, the visual P300 amplitude increments in the negative emotional context as compared to the neutral one, were inversely related to DHEAS/DHEA ratios, suggesting that the processing of the task-irrelevant negative content of the stimuli might eventually be reduced by DHEAS. Even though the participants of the study were healthy and depression was not screened by any specific inventory, women in particular have been shown to be specially sensitive to threatening stimuli and it has been hypothesized that this might be related to the higher prevalence of affective disorders in women (Kemp et al., 2004). Enhanced negative affect is an integral characteristic of depressive

disorders and negative mood can be experimentally induced using various procedures, among others by presenting images of faces with emotional expressions (Dyck et al., 2011; Schneider et al., 1994, 1997).

Although the mechanisms underlying mood persistence in major depressive disorder remain poorly understood, cognitive theories hypothesize that depressed patients have cognitive biases for emotional information, which help perpetuate depressive symptoms (Beck, 1967; Gotlib and Joormann, 2010). The present results suggest that the processing of negative emotional stimuli may be reduced in relation to higher DHEAS/DHEA levels, which might eventually be involved in protective mechanisms against a negative attention bias and undermine the relation between higher DHEAS levels and lower frequencies of depression, lower depression ratings and better well-being scores previously described by other authors (Barrett-Connor and Edelstein, 1994; Barrett-Connor et al., 1999). In line with this hypothesis, subjects with higher DHEAS/DHEA ratio would process less and have less memory updating of the implicit negative content of the stimuli and would thus have a reduced attentional bias towards these stimuli, which might promote well-being and contribute to protection from depressive states.

Emotional stimuli usually capture attention more effectively than non-emotional ones (Öhman et al., 2001) and have an impact on cognitive functions even when they are task-irrelevant (Domínguez-Borràs et al., 2008). On the other hand, it is hypothesized that protective mechanisms filter out irrelevant sensory inputs protecting the higher brain functions from sensory overload (Braff and Geyer, 1990). Taken together, our findings concerning visual P300 latency and visual P300 amplitude under an emotionally negative context showed a relation between higher DHEA/cortisol and DHEAS/DHEA ratios and less processing of the negative emotional stimuli and less disruption by the processing of negative emotional stimuli. These results also suggest that DHEA and DHEAS potentially play a role or contribute to protective mechanisms filtering out negative information overload.

A simultaneous relation to latency reduction and decrease in P300 amplitude may seem contradictory at first sight, nevertheless, as the task consisted in deciding whether two faces with the same emotional expression were equal or different (while the emotional expression was irrelevant), less processing of the task-irrelevant emotional content – related to higher DHEAS/DHEA ratios – might be reflected in smaller P300 amplitudes and at the same time shorter P300 latencies due to a more efficient processing of the task-relevant visual information and less interference resulting from the task-irrelevant emotion. In other words, the P300 amplitude might be reduced as a reflection of less neuronal recruitment due to less allocation of attentional resources to the task-irrelevant emotion, while P300 latencies might be reduced due to a decreased stimulus evaluation time, likewise due to less processing of the task-irrelevant emotional content of the images. This interpretation is consistent with the direct relation we found between reduced P300 amplitudes and shorter P300 latencies. Furthermore, it is consistent with previous studies showing that emotional stimuli elicit an enhanced P300 as compared to neutral stimuli (Schupp et al., 2004) and longer P300 latencies for negative emotional target stimuli (Fichtenholtz et al., 2007) as compared to neutral stimuli. Additionally, emotional stimuli outside the attentional focus have been shown not to elicit an enhanced P300 (MacNamara and Hajcak, 2009).

Endocrine relations to auditory distraction were not found in the present study: DHEA, DHEAS and cortisol were not related to the novelty-P3, suggesting that their levels do not modulate auditory distraction processing under an emotionally negative context. This differs from our previous findings concerning auditory distraction under working memory load, in which we found that the novelty-P3 amplitude was enhanced in relation to higher baseline cortisol/DHEA ratios (do Vale et al., 2014). It also suggests that the influence of the endocrine parameters on auditory distraction, may depend on the cognitive task involved.

The present results also highlight the relevance of the balance between DHEA, DHEAS and cortisol concerning the processing of negative emotions. Importantly, these results agree with findings at the clinical level, in which higher DHEAS concentrations and DHEA-to-cortisol ratios but not DHEA levels alone were related to less frequent depression, less depressive mood and higher well-being scores (Barrett-Connor et al., 1999; Michael et al., 2000; Young et al., 2002). Although DHEA and DHEAS can be converted into each other and general common effects are expected for the sulfated and non-sulfated form of the hormone (Baulieu and Robel, 1998; Dong and Zheng, 2011; Maninger et al., 2009), several differences exist in their mechanism of action. For instance, DHEAS has a much more potent excitatory action by NMDA agonism and gabaminergic antagonism than DHEA, which may account for some differential effects (Baulieu and Robel, 1998; Imamura and Prasad, 1998; Monnet et al., 1995). Moreover, as stated, sulfated steroids in general possibly act as endogenous neuromodulators (Gibbs et al., 2006) and the balance between DHEAS and DHEA might influence brain functioning. Previous studies at the cellular and molecular level showed that DHEAS had neuroprotective effects mediated through GABA-A receptor antagonism (Majewska, 1992), DHEAS stimulated dopamine release from rat hypothalamic cells (Murray and Gillies, 1997) and DHEAS antagonized the neurotoxic effect of high doses of DHEA (Gil-ad et al., 2001). These studies suggest potential mechanisms by which DHEAS could have more potent anti-depressant effects than DHEA and agree with the present finding that higher DHEAS/DHEA ratios were related to reduced processing of the negative emotional content.

In the present study, the participants were in different menstrual cycle phases and some were using hormonal contraception. This is a limitation, as DHEA, DHEAS and cortisol levels can change along the menstrual cycle and with the use of hormonal contraception (Fern et al., 1978; Wiegratz et al., 2003). Nevertheless, eventually in relation to the small sample size, in the present study, the endocrine levels were not significantly different according to the menstrual cycle phase or the use of hormonal contraception. But even considering that DHEA, DHEAS or cortisol levels could change along the menstrual cycle or with the use of hormonal contraception, the relations found between endocrine levels and emotional stimuli processing would not be invalidated. In any case, given the randomized approach we used concerning the menstrual cycle phase, the present results are expected to be independent of the menstrual cycle phase. Finally, the fact that only female participants were included, limit the outreach of the present study only to women. DHEAS levels differ between genders, therefore, another group of participants would be necessary to extend our conclusions also to men. For further studies, it would be relevant to study whether the results are identical in men.

## Conclusions

In a nutshell, in women, during the processing of stimuli with negative emotional content, higher DHEA/cortisol and DHEAS/DHEA ratios were related to shorter visual P300 latencies, suggesting a relation of these endocrine parameters with shorter stimulus evaluation time and less interference with the task at hand due to the processing of the task-irrelevant negative content of the visual stimuli. Additionally, higher DHEAS/DHEA ratios were related to reduced visual P300 amplitudes suggesting less processing of the negative information, which might constitute a protective mechanism against negative information overload.

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The funding sources had no role in the project and study design, in the study execution, in the data analysis and interpretation, in the manuscript writing, or in the decision to submit the paper for publication.

## Conflict of interest

All authors declare they have no conflicts of interest in what concerns the present work.

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