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The role of oxytocin in human social behaviour: a multimodal pharmaco-psychophysiological investigation

"Documento Definitivo"

Doutoramento em Engenharia Biomédica e Biofísica

Gonçalo Bairos Cosme

Tese orientada por:
Doutora Diana Prata

Documento especialmente elaborado para a obtenção do grau de doutor

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Abstract

Social interactions are a fundamental aspect of human life, and behaviours like resource sharing, group defence, bonding, and support between peers have been associated with the neuropeptide oxytocin. However, some evidence has failed to support oxytocin's foremost prosocial hypothesis, or that its effects are exclusively social. This stems from a still incomplete knowledge of oxytocin's role on several central and autonomic psychophysiological correlates of social cognition, and from methodological inconsistencies across studies.

This thesis describes four studies aimed to test the effects of intranasal oxytocin (IN-OT) on the central and autonomic psychophysiology. The first study addressed the methodological gap in the literature by describing IN-OT's temporal profile at rest on pupil size and heart rate variability. The second used eye-gaze to test oxytocin's role in visual attention. The third study uses pupillometry, eye-gaze's dwell time and spontaneous eye blink rate during a reinforcement learning task to test two of oxytocin's currently leading hypotheses for its effects on social cognition. Finally, the last study tested whether women's sexualization influenced men's cooperative behaviour and concomitant electroencephalography activity during a social dilemma, and whether IN-OT affected such sexualization bias.

The thesis' results suggest the ideal time-window for future studies aiming to probe the effects of IN-OT on pupillometry and heart rate variability. They also validate the posited effects of oxytocin on salience attribution to social stimuli but only on central psychophysiological correlates, and to rewarding/relevant stimuli mostly on autonomic psychophysiological correlates. Behavior-wise, this thesis' results further corroborate oxytocin's associations with prosocial attitudes. Altogether, the thesis confirms a dopamine by oxytocin interplay in humans, but challenges oxytocin's social specificity. Ultimately, this work may serve as foundation for future research on clinical populations and on the interaction of oxytocin with other neuroendocrinological agents.

Keywords: Socialness, Reward, Eye tracking, Heart rate variability, Electroencephalography

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Resumo

A necessidade de os humanos interagirem socialmente foi, do ponto de vista evolutivo, importante para a sobrevivência da espécie, trazendo inúmeras vantagens como o bem-estar, segurança individual e o aumento do sucesso reprodutivo. Embora muitos dos comportamentos sociais que exibimos não sejam exclusivos aos humanos, a nossa espécie é única na complexidade das sociedades em que vive. Por vivermos constantemente em interação com outros, aprendemos desde cedo a integrar e a processar informação social, como por exemplo, a interpretar as intenções ou emoções dos outros, o que resulta em nós respostas psicofisiológicas centrais e autonómicas. Em alguns distúrbios mentais como é o caso da esquizofrenia, estes processos sociocognitivos estão deficitários, e por ainda não se conhecerem as suas causas mecanísticas, os doentes são muitas vezes diagnosticados e/ou tratados erradamente, ou apresentam grande comorbilidade, acarretando assim um decréscimo acentuado na sua qualidade de vida e também elevados custos para a sociedade em geral. É por isso fundamental estudar de que forma as interações sociais são processadas cognitivamente, quais as respostas psicofisiológicas associadas e quais os agentes neurobiológicos subjacentes.

A oxitocina é um neuropeptido envolvido na cognição social, e o seu sistema no cérebro é suspeito de estar alterado em distúrbios mentais com défices sociocognitivos. A oxitocina modula comportamentos sociais dos mamíferos como a vinculação materno-infantil, reconhecimento social, a aprendizagem, a confiança e os comportamentos cooperativos. É uma proteína composta por nove aminoácidos e sintetizada nos núcleos paraventricular e supraóptico do hipotálamo, que depois de chegar à glândula pituitária (hipófise), pode ser libertada para órgãos periféricos onde tem um papel relevante enquanto hormona em processos relacionados com a homeostase e contrações uterinas durante o parto. No cérebro, a oxitocina comporta-se como um neuromodulador em áreas como o hipocampo, amígdalas, núcleo accumbens, estriado, córtices sensoriais e no tronco cerebral.

Uma das suas funções na cognição social é a de modular o vínculo social entre indivíduos, ao promover especificamente os comportamentos cooperativos, essenciais ao comportamento social humano. Pensa-se que a cooperação surgiu de forma adaptativa para facilitar e aumentar a recompensa de interações repetidas. Contudo, os benefícios das cooperações podem ser ameaçados ao se interagir com indivíduos egoístas, que agem somente para obter vantagens pessoais. Para minimizar esse risco, os humanos ganharam uma tendência natural de favorecer elementos dos seus grupos sociais em oposição a elementos de outros grupos, porque essa

distinção facilita o reconhecimento de indivíduos cuja cooperação é de confiança e recíproca. A oxitocina facilita cooperações com membros do mesmo grupo social, tornando-os motivacionalmente salientes, e criando um viés em favor dos membros do próprio grupo. Esse viés faz com que membros de "fora do grupo" sejam julgados como cooperadores de baixa qualidade, desumanizando-os, que acontece também no fenómeno da objectificação sexual.

Apesar dos efeitos descritos, ainda não se descobriu uma relação causal entre o sistema oxitocinérgico e os sintomas sociocognitivos dos distúrbios mentais. Pensa-se que isso se deve a vários problemas com estudos que recorrem à administração intranasal de oxitocina, como: 1) a variabilidade metodológica entre estudos no que toca à administração, e em particular ao desconhecimento sobre o seu perfil temporal, que dificulta a comparação entre estudos; 2) o fraco conhecimento sobre os efeitos que a administração intranasal da oxitocina tem em certos sinais psicofisiológicos; e 3) não existir consenso relativamente a uma teoria unificadora que explique os efeitos da oxitocina na cognição social. Relativamente ao último ponto, há uma hipótese que sugere que a oxitocina modela a atribuição de saliência motivacional aos estímulos sociais, ao interagir com a dopamina, agindo assim para orientar a resposta atencional até eles. Contudo, ainda não se sabe se essa modulação de saliência acontece apenas para os estímulos sociais por eles serem inerentemente mais relevantes/recompensadores, ou se acontece devido ao seu fator social.

Esta tese apresenta quatro estudos que foram realizados com administração intranasal de oxitocina num ensaio duplamente cego e randomizado com placebo para colmatar as lacunas acima mencionadas, usando os seguintes sinais psicofisiológicos: rastreamento ocular, dilatação pupilar, variabilidade da frequência cardíaca e eletroencefalografia.

No primeiro estudo descreveu-se o perfil temporal da oxitocina intranasal na dilatação pupilar e na variabilidade da frequência cardíaca em repouso, uma vez que, na literatura psicofisiológica autonómica, assume-se 40 minutos como sendo o tempo ideal para a realização de estudos após a administração, com base em medidas periféricas como o sangue ou saliva, ou em estudos efetuados fora de repouso. Vinte participantes masculinos participaram em duas sessões separadas por uma semana, sendo que numa delas foi administrada oxitocina e noutra placebo (ou o reverso), e depois foram registadas as respostas pupilares e de variabilidade da frequência cardíaca em várias janelas temporais distintas. Encontrou-se um efeito significativo da oxitocina sendo que esta diminuiu entre os 65 e 100 minutos o índex de inquietação pupilar, uma medida representativa de sonolência, e aumentou a variabilidade da frequência cardíaca

de alta frequência entre os 80 e 85 minutos, uma medida de ativação do sistema parassimpático. Não houve efeito da oxitocina em medidas representativas da atividade simpática. Este estudo representa uma referência temporal em repouso dos efeitos da oxitocina intranasal para a realização de estudos futuros com sinais psicofisiológicos autonómicos.

Os próximos estudos foram de comparação entre sujeitos. No segundo estudo os participantes visualizaram de forma livre vários vídeos cujo conteúdo continha interações sociais controladas para valência (positivos ou negativos) e para excitabilidade (alta ou baixa). Também foram apresentados vídeos não sociais, que continham paisagens. Usando rastreamento ocular, foi calculado um score de saliência e demonstrou-se que a oxitocina intranasal aumentou significativamente esse score quando comparado com placebo para todos os vídeos exceto um positivo de alta excitabilidade (erótico), onde diminuiu o score, e para outro não-social, onde não houve diferenças significativas.

No terceiro estudo recorreu-se novamente ao rastreamento ocular e a uma tarefa de aprendizagem por reforço para se dissociar o fator relevância/recompensa do estímulos sociais, a fim de se testar se a ação da oxitocina depende do cariz social ou da relevância dos estímulos. Nesta tarefa, os participantes foram instruídos a aprender sobre as contingências de recompensa de estímulos que poderiam ser faces ou frutas, azuis ou vermelhas. As faces e as frutas eram reforçadas em igual probabilidade (50%) enquanto uma das cores era positivamente reforçada (87.5%). Na dilatação pupilar, sinal psicofisiológico autonómico, a oxitocina interagiu com a probabilidade de reforço da figura, sendo que ensaios reforçados (vs. não reforçados) elicitaram maior dilatação pupilar quando comparado com placebo. Na análise do tempo de permanência a olhar para as figuras, sinal psicofisiológico central, utilizou-se ainda o piscar de olho espontâneo como covariável no modelo estatístico por ser uma medida da dopamina tónica. Verificou-se que a oxitocina aumentou os tempos de permanência nas faces (vs. frutas) apenas em participantes com piscar de olho espontâneo elevado. Ambos estes efeitos da oxitocina, tanto na dilatação da pupila como no tempo de permanência ocular, ocorreram somente aquando da expectativa do *feedback* do ensaio, momento em que há a libertação fásica de dopamina. Os resultados deste estudo apoiam a existência de uma interação entre a oxitocina e dopamina no cérebro, e que essa interação modula respostas autonómicas e centrais referentes à saliência motivacional dos estímulos.

Por último, realizou-se um estudo para compreender o efeito da oxitocina no comportamento cooperativo e na resposta eletroencefálica dos participantes no jogo do dilema do prisioneiro,

no contexto da sexualização. Confirmou-se que a oxitocina aumenta a frequência de cooperações no jogo, bem como a probabilidade de cooperar após uma cooperação mútua entre os jogadores. Verificou-se ainda que após uma cooperação não correspondida (traição), os participantes cooperaram mais com as oponentes sexualizadas (vs. não-sexualizadas), contudo, a oxitocina intranasal eliminou esse viés negativo, reforçando o seu papel pró-social. A oxitocina também aumentou a latência do potencial relacionado evocado P3 refletindo tentativamente os resultados comportamentais descritos acima.

No global, este trabalho descreve os efeitos da oxitocina intranasal na cognição social humana, em particular nas respostas psicofisiológicas centrais e autonómicas associadas à saliência motivacional, que tem subjacente uma interação entre a oxitocina e a dopamina.

Palavras-chave: Socialidade, Recompensa, Rastreamento ocular, Variabilidade da frequência cardíaca, Eletroencefalograma

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Acronyms

ANS – Autonomic nervous system
ANOVA - Analysis of variance
AOI – Area of interest

CNS – Central nervous system
CS – Conditioned stimuli

ECG – Electrocardiography
EEG – Electroencephalography
ERP – Event-related potential

FRN – Feedback related negativity

HF-HRV – High-frequency heart rate variability
HRV – Heart rate variability

IN-OT – Intranasal oxytocin
IU – International Units

LF-HRV – Low-frequency heart rate variability

OT – Oxytocin
OTR – Oxytocin receptor

PD – Prisoner's dilemma
PNS – Parasympathetic nervous system
PUI – Pupil unrest index

RP – Reinforcement probability

SampEn – Sample entropy
sSAT – Social salience attribution task
SCZ – Schizophrenia
SD – Standard deviation
sEBR – Spontaneous eye blink rate
SNS – Sympathetic nervous system

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1- Introduction

1.1 Social cognition

Humans are an innately social species whose social capabilities have been shaped throughout evolution by the interplay of nature and nurture. Our cooperative tendencies boost our wellbeing, individual security, reproductive success and prosperity, but prosocial social interactions may also cost fitness due to exposure to conflict or exploitation (De Dreu et al., 2020). Individuals must, therefore, avoid social promiscuity and be selective over their social interactions and partnerships, mechanisms that represent a clear adaptive process in which evolution favoured those that made the best decisions about social interactants.

Many other species show similar capabilities and also live in communities with sophisticated social lives, but humans are unique in their deeply complex cooperative social groups and unusually altruistic and caring behaviours which are essential for our survival. For example, humans' particularly protracted developmental time, where an individual lives in an immature form for a long period of time, demands care, food and protection from an adult which is frequently provisioned in a cooperative-way by a trusted family member (Tomasello, 2020). Since young, human children are also exposed to information and cues about cultural norms that are vital for survival and social development (Tomasello, 2020) often via language which conveys information in a cheap, accurate and flexible fashion (De Dreu et al., 2020; Gelman & Roberts, 2017; House et al., 2013). Interestingly, human children also display a predisposition towards collaboration, sharing intentions, and helping others, a propensity that becomes increasingly sophisticated with age and is driven by the sensitivity to the needs of others. Thus, humans evolved to navigate the complexities of social norms and to cooperate on a large scale, leading to the emergence of diverse cultural practices across societies. And as societies became exposed to different socioecological factors, cultural specificity emerged in social norms and behaviours, whereby, for example, different subsistence styles predict relational mobility, the predisposition of an individual to have more fluid and open interpersonal relationships (Kurzban & Neuberg, 2015; Thomson et al., 2018).

In our daily life we are bombarded with social information. By interacting with others, we rely on social signals like speech, facial expressions and body posture, often automatically, to infer identity, age, future potential actions, social hierarchy, and emotional status of the conspecific. These social signals allow us to experience the world through the others' perspective and

substantially reduce our exposure to dangerous and life-threatening situations. Infants exploit this by observing their mother's emotional expressions to adapt a response when exposed to novel contexts (Frith & Frith, 2007). The perception and processing of these social signals require complex cognitive processes like attention, memory, motivation and emotion, which are influenced by a combination of biological, psychological and environmental processes, and produce neural responses that can be measured via central and/or autonomic psychophysiological correlates of social cognition. Unravelling them and their underpinnings has great implications in a wide range of fields like medicine (Fernández et al., 2018; Goh et al., 2021), sociology (Cerulo et al., 2021), psychology (Salvati & Koc, 2022), economy (de Bruijn & Antonides, 2022; Thompson et al., 2021) and political sciences (Nosek & Riskind, 2012), and may explain personal tendencies and help minimize environmental threats like diseases propagation and warfare. But perhaps the most illustrative example of the importance of researching social cognition comes from the medical field. Schizophrenia (SCZ), affecting approximately 1% of the global population (Kadakia et al., 2022), presents a significant economic burden worldwide (Kadakia et al., 2022) and reduced life expectancy (McCutcheon et al., 2020). Its heterogeneous symptomatology includes, amongst others, obvious social cognition impairments intertwining with psychotic, negative and other cognitive symptoms (Howes & Murray, 2014; McCutcheon et al., 2020; Stilo & Murray, 2019). Understanding the mechanisms underlying SCZ, including the neuroendocrine systems involved and gene-environment interactions, is vital for improving diagnosis accuracy and treatment efficacy, given the disorder's complexity and variability in symptom manifestation.

1.2 The neuropeptide oxytocin

One neuroendocrinological agent known to be involved in social cognition and that is hypothesized to be involved in the mechanisms of SCZ is oxytocin (OT). OT is an evolutionarily conserved neurohypophysial hormone that plays a major role in mammalian behaviour and health (Carter et al., 2020). It has been found to modulate many facets of social cognition like pair and maternal-infant bonding (Carter et al., 2020; Froemke & Young, 2021), maternal nurturing (Carter et al., 2020; Froemke & Young, 2021), social recognition (Skuse et al., 2014), learning (Xu et al., 2019), and cooperative behaviours (X. Chen et al., 2016, 2017; Neto et al., 2020; Rilling et al., 2012), amongst other processes. OT is composed of 9 amino acids and is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus in

magnocellular and parvocellular neurons. Suckling, birth and sex, for example, stimulate magnocellular neurons that transport OT inside vesicles through axonal release to the posterior lobe of the pituitary gland (i.e. hypophysis), which then releases OT to the bloodstream where it peripherally acts as a hormone on uterine constriction, milk let-down and other metabolic, homeostatic and autonomic functions (Quintana & Guastella, 2020). OT can also act centrally as a neuromodulator in the hippocampus, amygdala, nucleus accumbens, striatum, sensory cortices and brain stem (Carter et al., 2020), regions where OT's G protein-coupled OT receptor (OTR) is expressed. OT is released via membrane surface in the dendrites, soma and axon of magnocellular and parvocellular neurons. Crucially, OT's effects are dependent on the ligand availability of the OTR and OTR's expression, which is regulated by epigenetic factors (especially during early life (Kenkel et al., 2019)), transcriptomic factors (Carter et al., 2020; Danoff et al., 2021), and genetic polymorphisms in non-coding regions of the OTR gene (Danoff et al., 2021).

Historically, OT was the first neuropeptide to be sequenced in a laboratory in the 1950s for which Vicent du Vigneaud received a Nobel Prize in Chemistry. Given its known effects on parturition and milk let-down, OT was initially thought to be solely a "female reproductive hormone" but work in the 1970s and 1980s revealed OT also affected learning behaviours. Then, in the 1990s, work done in monogamous praire voles showed that the expression of OTR in rewarding areas of their brains was associated with pair-bonding, whereby more OTRs led to monogamous behaviours. These last highly influential results started a plethora of studies in humans ranging from (epi)genetic to exogenous intranasal OT (IN-OT) administration, to assess the oxytocinergic system's involvement in social behaviour and their psychophysiological correlates, aiming to pinpoint the underpinnings of our innate social capabilities and the pathophysiology of mental disorders like SCZ (Goh et al., 2021; Shilling & Feifel, 2016).

1.3 The role of oxytocin in social bonding and cooperative behaviours

OT aims to promote social bonding, and it achieves this by modulating several processes of human cognition. For example, in order to successfully navigate humans' complex social contexts, several cognitive skills like empathy and theory of mind are important, all of which OT has been implicated in some way (Barchi-Ferreira & Osório, 2021; MacKinnon et al., 2018). OT's tendency to promote prosocial actions may also derive from its modulatory effects

on the hypothalamic-pituitary-adrenal axis, a major endocrine system involved in stress response (Carter et al., 2020) as OT has stimulates social engagement during social stress (Kubzansky et al., 2012), a risk factor for SCZ (Lederbogen et al., 2013).

Another way OT is believed to influence social bonding is via promoting cooperative behaviours. A cooperative act involves an interaction where individuals incur a cost to achieve mutual benefits for themselves and others. Cooperative behaviours are a distinctive feature of human social life and have likely been favoured by natural selection to foster alignment with individuals of the same social group - however not exclusively - to increase survival, reproductive success and wellbeing, to facilitate the transmission of information and enable cultural norm sharing, and to facilitate and increase the gains from repeated interactions. Successful cooperations serve to form and preserve social bonding, and meta-analytic evidence (X. Yang et al., 2021) suggests that IN-OT indeed boosts the frequency of cooperative behaviours (Neto et al., 2020; Rilling et al., 2012).

Yet, the benefits of cooperation are threatened by individuals driven by selfish motives that act to harness the profits of these interactions for personal advantage. Due to these risks, humans gained a natural tendency to favour members of their social group (in-group) in opposition to outsiders (out-group), because it facilitates the identification of trusted individuals with whom cooperation is beneficial and reciprocal. In-group membership affiliations are often set around key salient features like kinship, ethnicity and race, but can also extend to other arbitrary affiliations that are more flexible like profession (e.g. politicians, researchers) and sports clubs. However it is traits like trustworthiness and cooperativeness that are prioritized for group belonging rather than intelligence, extraversion, physical attractiveness and conscientiousness (Kurzban & Neuberg, 2015). It is proposed that OT's effects on cooperative behaviours are contextually dependent on intergroup dynamics whereby it enhances specifically in-group belonging and contributes to the development of in-group biases, preferences and beliefs (De Dreu et al., 2020; De Dreu, 2012a; De Dreu & Kret, 2016). The in-group favouritism phenomenon is prevalent in human societies and is aggravated during competition for resources against an out-group (De Dreu et al., 2020). In-group favouritism may also contribute to outsiders being perceived as low-quality cooperative partners, thus inciting intergroup conflict and prejudice, as individuals from the out-group are often attributed with having less human-like characteristics (Buckels & Trapnell, 2013; Hodson & Costello, 2007; Vaes et al., 2011), compared with in-group ones, a process that is also prevalent during sexual objectification (Bernard et al., 2012, 2018; Cogoni, Carnaghi, Mitrovic, et al., 2018; Vaes et al., 2019),

whereby the objectified target is associated with having less human-like attributes. It is unknown how the sexualization of a partner influences the cooperative tendencies during a socioeconomical game like the prisoner's dilemma (PD) (Axelrod, 1980; Axelrod & Hamilton, 1981), and whether IN-OT has an effect on these cooperative actions during this specific contextual setting. This was tested in the study described in **Chapter 6** of this thesis.

1.4 The interplay of oxytocin and dopamine

The contextually dependent modulatory effects of OT on cooperative behaviours and social bonding strongly suggest a central acting OT-dopamine interaction (J. Kraus et al., 2023), because OT seems to enhance social bonding's motivational relevancy and saliency.

Dopamine, another neurotransmitter, is pivotal in reward-related learning by encoding reward prediction errors — the difference between expected and actual rewards — which affect motivational salience (Berke, 2018). Indeed, phasic dopamine is released shortly after an orienting gaze shift is done towards a stimulus that is anticipated to provide a reward, and if the actual reward differs from expectations, the dopamine system adjusts the prediction to influence future behaviour and motivation (Berke, 2018). If the stimulus is rewarding, dopamine then modulates its motivational salience by increasing its importance and relevance, prioritizing attention and cognitive resources towards it. These processes are central for reinforcement learning, and several brain regions participate in them, namely the ventral tegmental area, amygdala and nucleus accumbens, who belong to the dopamine mesocorticolimbic pathway (Love, 2014) and activate to sustain and maintain these goal-directed behaviours. Importantly, the effects of phasic dopamine release depend on the underlying basal levels of tonic dopamine, highlighting the significant role of individual differences when studying the impact of phasic dopamine on behaviour (Grace, 1991; Shamay-Tsoory & Abu-Akel, 2016).

There is a considerable amount of animal and human research that converges to an apparent modulatory role of OT on these dopaminergic effects. For example, anatomical evidence indicates that dopaminergic pathways are replete with OTRs (Baskerville & Douglas, 2010) and functional neuroimaging evidence also indicates that the striatum activates during reciprocal cooperation (Rilling et al., 2012). OT appears to specifically enhance the motivational salience of social cues, increase attention, cognitive processing, and engagement

with social stimuli (Harari-Dahan & Bernstein, 2014). These ideas help explain the non-prosocial effects that have recently emerged in the literature whereby OT seems to enhance the in-group bias but also the aggression with out-group members (DeWall et al., 2014; Shamay-Tsoory et al., 2009). The effects of IN-OT on visual attention was tested in the study of **Chapter 4** of this thesis.

Ultimately, a unifying and overarching framework that explains OT's vast effects on social cognition is needed. These frameworks could improve our understanding of the oxytocinergic system by directing researchers to test the specific mechanisms by which OT exerts its effects, which can then be tested in more detail in the clinical population to assess the pathophysiology of mental disorders. One such framework for OT's effects on social cognition is the social salience hypothesis (Shamay-Tsoory et al., 2009). It posits that OT modulates the recognition and attention reorienting response towards social stimuli in the environment, regardless of their valence, by interacting with the dopaminergic system. However, it is currently unknown whether OT acts on the salience of social cues because of their socialness, or whether OT increases salience towards social cues because they are inherently more relevant to us. Another proposed framework, the general approach-withdrawal hypothesis, tackles the "social" specificity of OT's role on cognition by proposing that OT increases approach (e.g. emotional engagement towards rewarding stimuli), and reduces withdrawal (e.g. avoidance, anxiety and fear towards aversive stimuli) generally in context, rather than restricting exclusively to social interactions, by acting on the approach/motivation and avoidance/withdrawal mesocorticolimbic dopaminergic circuitry (Harari-Dahan & Bernstein, 2014). Crucially, previous IN-OT studies contrasting its effects on the salience of both social and nonsocial stimuli have not dissociated socialness from their reward/relevancy. This was performed in the study described in this thesis' **Chapter 5**.

1.5 Methodological issues with intranasal oxytocin research

Altogether, the oxytocinergic system is a modulator of social cognition and of complex social phenomena by, apparently, promoting social bonding via in-group biases and affecting the processing of motivationally relevant and rewarding social stimuli. Several frameworks have been proposed to explain OT's multifaceted effects on social cognition, and despite OT being presumably involved in mental disorders like SCZ, the initial hype around its prosocial effects have resulted, however, in a vast literature that has yet to identify SCZ's genetic and neural

biomarkers, and that is notably unsatisfactory regarding the reproducibility of its most influential reports. IN-OT administration in SCZ (Sabe et al., 2021) and other clinical populations (Huang et al., 2021; Neumann & Slattery, 2016) report inconsistent findings and subpar improvements in social cognitive deficits. Consequently, several calls have emerged in the literature regarding the efficacy of the intranasal administration route, whereby some authors question whether IN-OT even crosses the blood-brain-barrier to travel directly to the central nervous system (CNS) and to be central acting (Winterton et al., 2021). Despite these valid concerns, the fact that IN-OT, compared to placebo, has consistently been found to have effects on several social cognitive processes like increasing the frequency of cooperative behaviours (Neto et al., 2020; Rilling et al., 2012), improving facial emotion recognition (Shahrestani et al., 2013) and increasing endogenous levels of OT in cerebrospinal fluid (Winterton et al., 2021) indicates that the delivery route of the drug is adequate.

Findings from animal model studies also rarely translate to humans. Indeed, the collective effort from researching the oxytocinergic system in various animals allows us to expand the repertoire of methods and target manipulations that are otherwise impossible to use in human studies, from genetic manipulations to *in-vivo* single neuron activity recordings, however the 'human-animal model' translation is hindered by the fact that most other species display a range of social behaviours that differ significantly from those exhibited by humans, and by the fact that the heterogeneity of social cognitive deficits in mental disorders are obviously only inferred in animal models and may not correlate with the symptomatology observed in humans.

Another critical and necessary research avenue that may help the translation of IN-OT findings to the clinical setting is to describe and understand the dose response and temporal profile of IN-OT. Currently in human research, the most frequent intranasal administration dose is 24 IU. It is sufficiently large to surpass naturally occurring physiological levels and to increase the baseline concentrations in cerebrospinal fluid, suggesting that this quantity of IN-OT reaches the brain, but it is unclear whether this dose is too low, which might contribute to the many inconsistent findings, or if it is too large such that it also binds to vasopressin receptors, a more ancient but structurally similar neuropeptide, creating ambiguity in the observed effects. Several authors have explored this problem by creating dose-dependent study designs in which several IN-OT doses are administered, ranging from 6 to 48 IU, to assess, usually, the amygdala response to emotional stimuli (Quintana et al., 2021; Winterton et al., 2021). Interestingly, they report a non-linear dose-response, but since they have not assessed these effects during a naturalistic baseline setting like a resting state makes these results difficult to juxtapose to other

research settings. The temporal profile of IN-OT is also a crucial methodological aspect that warrants further exploration in the literature. Most human IN-OT studies assess its effects around 20 to 90 minutes post-administration but are usually conducted in a single time-window unclear when the peak activity occurs, but generally assumed to be around 40 minutes. Those that have tried to describe IN-OT's temporal profile have also done so in non-resting-state settings (Quintana et al., 2021; Winterton et al., 2021). This was addressed in the study of **Chapter 3**.

1.6 Aims and structure of the thesis

The goal of this thesis was to use a pharmacological and multimodal approach to probe the effects of OT on the central and autonomic psychophysiology of human social cognition. Firstly, a methodological gap in the literature was addressed whereby the temporal profile of 24 IU of IN-OT at rest on autonomic psychophysiology was described, to suggest the best timeframe for future IN-OT autonomic psychophysiology studies (**Chapter 3**). Next, the role of IN-OT on visual attention during the free-viewing of social and non-social videoclips was investigated using a salience score computed from eye-gaze of multiple observers (**Chapter 4**). Then, the social salience and the approach-withdrawal hypotheses of OT were contrasted and tested in central and autonomic psychophysiological correlates of salience attribution during a reinforcement learning task (**Chapter 5**). Lastly, the effects of IN-OT and an opponent's sexualization during the PD were explored for their effects on cooperation propensity and on central psychophysiological correlates of social cognition (**Chapter 6**).

For this, overall, I recorded several psychophysiological modalities such as eye-gaze, pupillometry, spontaneous eye blink rate (sEBR), heart rate variability (HRV), and electroencephalography (EEG) , in a range of social cognitive paradigms in between- and within-subjects IN-OT randomized placebo controlled designed studies. Data related to the work reported in **Chapter 3** was collected at the Clinical Research Centre (Centro de Investigação Clínica) of the Lisbon Medical Academic Center (Centro Académico Médico de Lisboa). Data related to the work of **Chapters 4, 5** and **6** were collected at LAPSO lab of ISCTE-IUL (Lisbon, Portugal). Overall, the work was developed primarily at the Institute of Biophyisics and Biomedical Engineering (Instituto de Biofísica e Engenharia Biomédica) of the Faculty of Sciences of the University of Lisbon.

For general context, **Chapter 2** briefly describes relevant literature on three different psychophysiological data modalities: 1) EEG; 2) HRV; and 3) eye tracking, that includes eye-gaze, pupillometry and sEBR; and specifically on the dependent variables associated with social cognition that were used in the four studies reported in this thesis. Previous evidence of IN-OT's effects on them are also highlighted. EEG is the first modality addressed whereby the signal's generation is explained and its strengths and limitations related to social cognitive research are outlined. Then follows a brief summary of oscillation bands and their cognitive associations, but the focal point refers to the event-related potentials (ERPs) and their computation. Heart rate variability (HRV) is the next modality addressed whereby the contributions from the sympathetic (SNS) and parasympathetic nervous system (PNS) are highlighted. A summary of HRV-related metrics extracted with temporal, frequency and non-linear methods is given. Lastly, eye tracking is explored whereby eye-gaze is addressed as an attention orienting response, pupil size as a measure of arousal and cognitive states with contributions from both SNS and PNS, and spontaneous eye blink rate (sEBR) as a proxy of tonic dopamine levels in the CNS.

Chapter 3 includes a study that aims to describe the temporal profile of IN-OT on the autonomic psychophysiology during resting-state. Various efforts have been made to describe such temporal profile on peripheral fluids and on the CNS, but none have focused on the peripheral nervous system, especially the autonomic nervous system (ANS), which is important because the ANS reflects a wide range of social cognitive processes and is more easily accessible than the CNS.

Chapter 4 pertains to a study aiming to investigate the role OT on visual attention by measuring the effects of IN-OT on a salience score calculated from eye-gaze recorded during free-viewing social and non-social videoclips. Crucially, the social stimuli were orthogonalized for their valence (positive, negative) and arousal (high, low) depicting social interactions, whereas the non-social depicted landscapes.

Chapter 5 describes another study aimed to specifically test whether OT's effects on salience attribution are due to fearful faces (social) specifically being, inherently, more rewarding than fruits (non-social). A reinforcement learning task was used which orthogonalized the social and reward features of the social stimuli. Their effects on pupil size and eye-gaze were measured. In eye-gaze in particular, sEBR was used to adjust for tonic dopaminergic levels.

Chapter 6 describes a study aimed at assessing IN-OT's effects on the sexualization bias affecting cooperation in the PD. For this, neurally, the associated event-related potentials (ERP) of EEG were also probed.

Lastly, **Chapter 7** provides overarching concluding remarks to these studies and thesis, and suggestions for potential future work.

1.7 Thesis contributions

The work developed over my PhD embodies biomedical engineering's versatility and multidisciplinary. The studies reported herein address several key gaps in social cognitive neuroscience, while using state-of-the-art methodological techniques and modalities. This thesis contributed with:

- The study of the temporal profile of IN-OT at rest in healthy males on psychophysiological proxies of SNS and PNS activity, extracted from pupil size and HRV **(Chapter 3)**
- Providing the literature with a 'ground truth' on the ideal time-window to conduct IN-OT research of autonomic psychophysiological responses **(Chapter 3).**
- Testing the effects of IN-OT on visual attention during free-viewing of naturalistic and dynamic social and non-social stimuli **(Chapter 4)**
- Investigating whether IN-OT's effects on salience attribution are concomitant with increased (subjective) arousal **(Chapters 4 and 5)**
- Testing OT's social specificity by directly contrasting the social salience and approach-withdrawal hypotheses **(Chapter 5)**
- Investigating whether IN-OT's effects on salience attribution are affected by dopamine (tonic and phasic) **(Chapter 5)**
- Testing if an opponent's sexualization influences cooperative behaviour using the PD, and whether IN-OT influenced this bias **(Chapter 6)**
- Extending the previous research by assessing whether those effects could be detected by EEG's ERPs **(Chapter 6)**

The following list contains the main publications and disseminations of the work related to my PhD which include 1 published journal paper, 3 others in preparation for submission in 2024,

all in international peer-reviewed scientific journals of the first quartile and indexed in PubMed. It also includes 7 posters in international conferences and 2 awards:

Journal publications:

- **Cosme, G.,** Arriaga, P., Rosa, P. J., Mehta, M., Prata, D. (2023) Temporal profile of intranasal oxytocin in the human autonomic nervous system at rest: an electrocardiography and pupillometry study. Journal of Psychopharmacology. https://doi.org/10.1177/02698811231158233
- **Cosme, G.*,** Diogo, V., Prata, D., Oxytocin's role in naturalistic spatio-temporal salience attribution: a pharmaco-eye-gaze study. In preparation.
- **Cosme, G.*,** Esteves, R.*, Diogo, V., Prata, D., Oxytocin's role on central and autonomic psychophysiological correlates of salience attribution: a pupil size and eye-gaze pharmacological study. In preparation.
- **Cosme, G.*,** Cogoni, C.*, Patrocínio, M., Kosilo, M., Prata, D., Intranasal oxytocin reverses negative cooperation bias towards non-sexualized women by men: a pharmaco-electroencephalography study. In preparation.

Poster presentations:

- **Cosme, G.,** Prata, D., (2024) Oxytocin's role on the central and autonomic neurocorrelates of approach and withdrawal behaviours and salience attribution. Organization for Human Brain Mapping (OHBM), Seoul, South Korea
- **Cosme, G.,** Prata, D., (2023) Oxytocin's role on the central and autonomic neurocorrelates of approach and withdrawal behaviours and salience attribution. European College of Neuropsychopharmacology (ECNP), Barcelona, Spain
- **Cosme, G.,** Diogo, V., Prata, D., (2023) Oxytocin increases the spatio-temporal salience of social interactions measured via eye-gaze during free-viewing. International Brain Research Organization (IBRO), Neuroscience Reports 15, S828. https://doi.org/10.1016/j.ibneur.2023.08.1719, Granada, Spain
- **Cosme, G.*,** Esteves, R.*, Diogo, V., Prata, D., (2023) Oxytocin's role on central and autonomic neuro correlates of salience attribution: a pupillometry and eye-gaze study. International Brain Research Organization (IBRO), Neuroscience Reports 15, S828. https://doi.org/10.1016/j.ibneur.2023.08.1718, Granada, Spain

- **Cosme, G.*,** Esteves, R.*, Diogo, V., Prata, D., (2023) Oxytocin's role on central and autonomic neuro correlates of salience attribution: a pupillometry and eye-gaze study. European Brain and Behaviour Society, Amsterdam, Netherlands
- **Cosme, G.,** Esteves, R.*, Diogo, V., Prata, D., Oxytocin's role on central and autonomic neurocorrelates of salience attribution: a pupillometry and eye-gaze study. Federation of European Neuroscience Societies (FENS) Regional Meeting, Albufeira, Portugal
- **Cosme, G.,** Arriaga, P., Rosa, P. J., Mehta, M., Prata, D., (2021) Temporal dynamics of intranasal oxytocin in the sympathetic and parasympathetic nervous systems at rest. European Neuroscience Conference by Doctoral Students (ENCODS). Online

Awards:

- IBRO World Congress Travel Grant 11th meeting, International Brain Research Organization (IBRO), 2023
- Regional FENS meeting Travel Grant, Sociedade Portuguesa de Neurociências (SPN), 2023

* - authors gave equal contribution.

2- Psychophysiology of social cognition

2.1 Electroencephalography

Electroencephalography has been an essential tool in the cognitive neuroscience field ever since it was discovered that the rhythmic oscillations of brain activity, recorded at the scalp, were associated with several mental processes like memory (Jackson et al., 2023) and consciousness (Bai et al., 2021). Nowadays, EEG is used in clinical settings to assess abnormal brain activity associated with head trauma, seizures and strokes, for example. EEG's electrodes measure the voltage potential that is caused by the summation of millions of dipoles originating from cortical pyramidal neurons. These dipoles arise when excitatory or inhibitory neurotransmitters are released from a presynaptic neuron, affecting the apical dendrites of postsynaptic pyramidal neurons. This creates a current that results in a measurable extracellular potential, with a negative charge near the dendrites and a positive charge near the soma. Because pyramidal neurons are aligned perpendicularly to the cortical surface and parallel to one another, their individual dipoles sum to produce a detectable electrical signal (Biasiucci et al., 2019; Müller-Putz, 2020). Due to its millisecond time scale resolution, EEG provides a non-invasive and relatively inexpensive measurement of cognitive functions in real time, although with weak spatial resolution, given that the recorded signal represents the conglomeration of multiple sources of neural activity. Thus, embedded in the raw EEG signal are the neural responses associated with cognitive, sensory and motor activities, whereby a recurrent methodological challenge is to isolate the contributions of each process. However, advancements in experimental design, a deeper understanding of the neurocognitive processes extrapolated from other techniques, and improvements in data science and hardware, have made EEG more reliable and sophisticated than ever before.

Continuous recordings of EEG can be used to detect rhythmic oscillations ranging from below 1Hz to 100Hz, which then are usually divided into segmented bands each with key associations with cognitive and functional processes. For example, the alpha band is a frequency activity within the 8-13Hz interval and with notorious large amplitudes, making it detectable from the raw EEG (i.e. without processing the signal). It is associated with wakefulness in healthy adults and common during resting states (Müller-Putz, 2020). The delta oscillations are another

frequency band in the low-frequency band (1-4Hz interval) often related to deep sleep and pathological neural states like coma or loss of consciousness (Müller-Putz, 2020).

Another form of studying psychophysiological processes using EEG is to average various EEG signals from repeated exposures to the same experimental condition, in order to highlight the peaks of brain activity and average out noise, in a method called ERP (Luck, 2014). A variety of ERP components and their associated underlying mental processes have been described in the literature, most with very precise timing codification. As such, ERPs can be useful in a vast range of experimental manipulations, including drug interventions and stimulus variation (e.g. by comparing faces from in-group vs out-group members). In the classical oddball paradigm, participants are tasked with viewing a frequent stimulus (e.g. the letter 'X'), and infrequently another (e.g. the letter 'O') and press a button after each stimulus presentation. On each trial, the EEG signal is time locked to stimulus onset and then averaged by condition (frequent vs infrequent, **Figure 1**) to create the ERP waveform that is typically a series of positive and negative voltage deflections commonly referred to as components. The naming convention of these components typically join the polarity of the amplitude ('P' for positive or 'N' for negative) with its latency (time interval between stimulus onset and maximal peak), however several other components do not follow these conventions, like the Feedback Related Negativity (FRN). In the oddball paradigm there are several components elicited: the P1, P2, P3, N1 and N2 (**Figure 1**) indicating either a positive or negative deflection at around 100, 200 or 300 ms, and representing the flow of information through the brain (Luck, 2014). The oddball paradigm is a classic example of the ERP technique because it consistently shows larger P3 amplitudes for the infrequent stimuli ('O') compared to the frequent ('X'), with P3 amplitude reflecting the degree of perceived expectation violation (Bell et al., 2015; Hajcak et al., 2005). Interestingly, this finding is irrespective of stimuli type (e.g. letters, sounds or images). In socioeconomical games like the PD, and particularly during its feedback moment, the P3 amplitude has been found to be modulated by the social distance of the opponent, whereby the amplitude was larger when playing (cooperating) against strangers (out-group) compared to friends (in-group) (Y. Chen et al., 2017; Y. Wang et al., 2013). Another ERP, the FRN, is a component that peaks between 250 to 350 ms after stimulus onset on centro-frontal electrodes and is elicited during decision-making and reward processing with contributions from areas like the medial prefrontal cortex (Proudfit, 2014), anterior cingulate cortex and striatum (Hauser et al., 2014). Particularly during performance feedback, the elicited FRN is

related to feedback processing and performance evaluation and is known to show an increased (negative) amplitude to losses vs. gains (San Martín, 2012).

In the IN-OT literature, previous studies have found that relative to placebo, IN-OT increased P3 amplitude in women during an infant facial processing task (Rutherford et al., 2017) and reduced the FRN amplitude difference between positive and negative feedback (with placebo showing larger FRN for negative unfavourable feedback) (Zhuang et al., 2020), which could indicate that OT may enhance learning from positive favourable feedback. The P3 and FRN were assessed in the study reported in **Chapter 6** of this thesis.

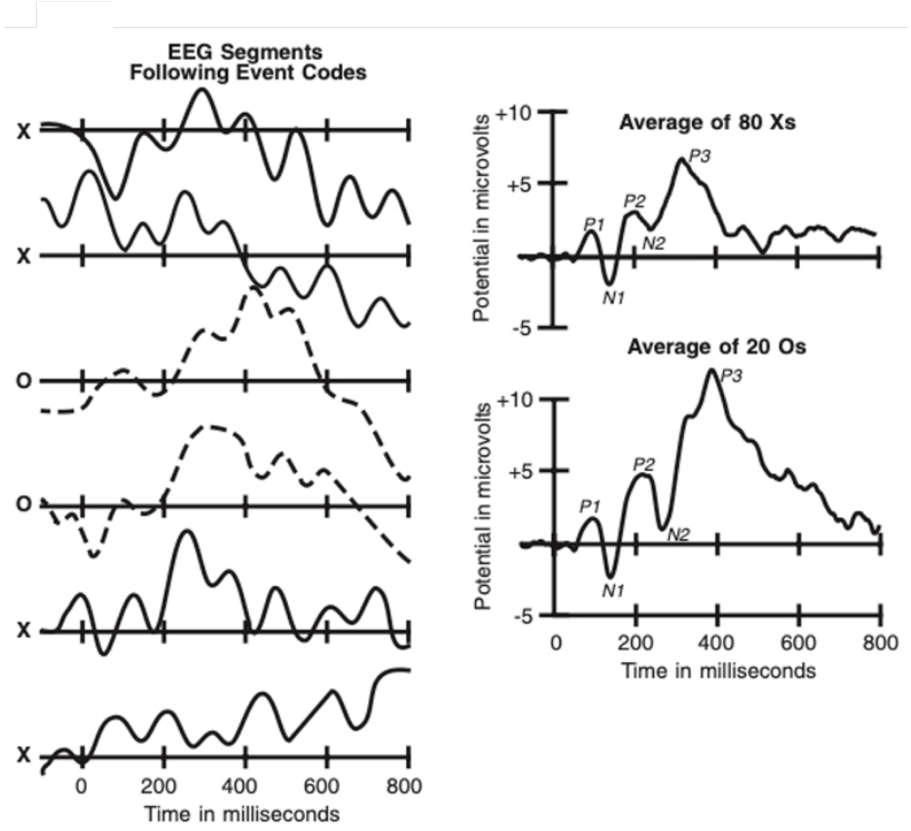

Figure 1 - The ERP method using the oddball paradigm whereby the expected stimulus is the letter 'X' and the unexpected the letter 'O'. Multiple EEG waveforms referenced to the stimulus onset (0ms, left). After averaging multiple repetitions of the same trial type, the ERP components become apparent (right). Adapted from (Luck, 2014).

2.2 Heart rate variability

HRV is the variation of consecutive heartbeats, frequently measured from the peaks of the QRS complex (R-R interval). It has long been used to probe autonomic psychophysiological processes given that activation of the SNS decreases HRV (and increases heart rate) whereas PNS activation increases HRV (and decreases heart rate). There are some clinical applications for HRV in risk stratification, individualized therapies and biofeedback, but its relevance is still questioned (Ernst, 2017). Several physiological factors influence HRV like age, sex, weight and circadian sleep (Shaffer & Ginsberg, 2017). Individuals with anxiety disorders usually exhibit reduced HRV, possibly because of a breakdown in the inhibitory processes of the central autonomic network leading to the continuous state of excessive worry, mirrored by decreased PNS and increased SNS activity (Ernst, 2017). Reduced HRV has also been found in subjects with difficulties in emotion regulation (Mather & Thayer, 2018), in autism spectrum disorders (Lory et al., 2020) and first episode psychosis (Cacciotti-Saija et al., 2018). On the other hand, people with heightened levels of HRV have been found to have increased PNS activity and are linked to better stress responses, adaptability, and motivation towards social engagement (Beffara et al., 2016).

HRV parameters can be extracted from: 1) the time domain; 2) the frequency domain; and 3) non-linear methods. In the time domain, the HRV indices measure the variation in duration between consecutive cardiac cycles, and some of the most common ones are the: 1) SDNN, which is the standard deviation (SD) of N-N peak distances (N-N indicates the use of normal R-R peaks, that is, excluding artifacts due to arrhythmic events) (Ernst, 2017; Shaffer & Ginsberg, 2017); 2) NN50 which represents the number of consecutive N-Ns that differ by more than 50 ms (Ernst, 2017; Shaffer & Ginsberg, 2017); 3) pNN50, considered a proxy of PNS activity, which is the proportion of NN50 divided by the number of N-N peaks over the recording period (Ernst, 2017; Shaffer & Ginsberg, 2017); and 4) RMSSD which is the square root of the mean squared differences of consecutive N-N intervals (Ernst, 2017; Shaffer & Ginsberg, 2017). Temporal indexes have, however, the disadvantage of being heavily influenced by the recording length, with longer periods generating more variability. As a result, comparisons between studies are reliable only when similar recording lengths are used.

Frequency domain parameters describe and quantify the signal energy (i.e. power) of different frequencies bands of the oscillations of the heart rate. Conventionally, there are four bands: 1) ultra-low-frequency ($\leq$ 0.003 Hz), that requires recording periods of over 24 h, and of which

PNS and SNS unique contributions are currently in debate (Ernst, 2017; Shaffer & Ginsberg, 2017); 2) very-low-frequency (0.0033 – 0.04 Hz), which is associated with general health but whose physiological processes underlying its activity are still unknown (Ernst, 2017; Shaffer & Ginsberg, 2017); 3) low-frequency (0.04 – 0.15 Hz) which is modulated by both the SNS and PNS (Ernst, 2017; Shaffer & Ginsberg, 2017); and finally 4) high-frequency (0.15 – 0.40 Hz), which indexes PNS activity associated with the respiratory cycle (Ernst, 2017; Shaffer & Ginsberg, 2017). Frequency methods, however, are sensitive to changes in trends of R-R intervals (Shaffer & Ginsberg, 2017).

Non-linear methods are suitable to measure HRV because they index the signal's unpredictability, and HRV is governed by both stochastic and periodic processes. HRV non-linear indexes can be extracted from Poincaré scatter plots whereby every R-R interval is plotted against its preceding one. Then, an ellipse is fitted to the scatterplot along the y = x axis and the area (S), width (SD1) and length (SD2) of the ellipse are extracted (**Figure 2**). Crucially, SD1 measures short-term HRV and correlates with high-frequency power whereas SD2 measures the opposite, long-term HRV and correlates with low-frequency power (Ernst, 2017; Shaffer & Ginsberg, 2017). Another non-linear method is the detrended fluctuation analysis whereby correlations between consecutive R-R intervals over different time scales are extracted, yielding two scaling exponents, one that describes brief fluctuations and reflects baroreceptor reflex (DFAα1), and another that describes long-term fluctuations (DFAα2) (Ernst, 2017; Shaffer & Ginsberg, 2017).

IN-OT's effects on HRV related metrics are inconsistent as it has been found to both increase, compared to placebo, HF-HRV during a facial emotion recognition task (Gamer & Büchel, 2012) and increase LF-HRV, but decrease HF-HRV in a mental arithmetic task (Tracy et al., 2018). No significant differences have also been reported, but particularly during a social stress task (Kubzansky et al., 2012). At rest, specifically, IN-OT's effects are equally inconclusive as it has decreased DFAα1 (Tulppo et al., 2005) and RMSSD but only in females with positive childhood rearing experiences (Schoormans et al., 2020), increased HF-HRV (Kemp et al., 2012; Kubzansky et al., 2012), and have no influence on HF-HRV, LF-HRV and RMSSD (Tracy et al., 2018). The HF-HRV, DFAα1 and RMSSD are some of the autonomic psychophysiological correlates reported in the study of **Chapter 3** of this thesis.

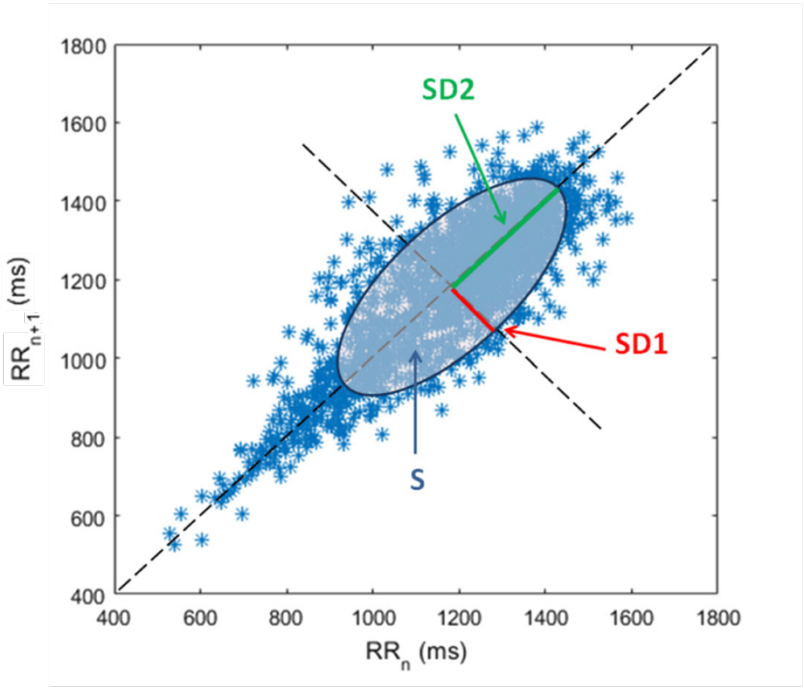

Figure 2 - Poincaré plot of the R-R intervals at index n+1 over the R-R intervals at index n. S represents the area of the fitted ellipse, SD1 represents the ellipse's width and SD2 the ellipse's length. Adapted from (Nardelli et al., 2020).

2.3 Eye tracking

Improvements in eye tracking hardware and analytic approaches have made it emerge as an extremely versatile and easily accessible technique, enticing by its ability to probe central and autonomic psychophysiological correlates of social cognition via measurement of eye-gaze, pupil size and eye blinks. Despite being an indirect measure of brain activity, compared to functional magnetic resonance imaging and EEG, eye tracking is more affordable and easier to use, opening the door for different research questions and more naturalistic study designs.

Our eye-gaze is a fundamental aspect of social cognition, providing numerous clues and signals about the mental and emotional states, and intentions of others. For example, during a conversation with another person, our eye-gaze usually shifts towards the eye region of the interlocutor to pick-up on emotional cues, but can shift instead towards the mouth region to better understand the spoken words (Rogers et al., 2018). When participants are asked to judge the age of several people in a picture, the eye-gaze is significantly more persistent on the peoples' faces, but when asked to judge material wealth, the eye-gaze shifts towards clothing and other clues in the surroundings (M. K. Eckstein et al., 2017). The amount of time spent

looking at different regions, eye scan paths, and other eye-gaze-related behaviours can be quantifiable using an eye tracker, allowing researchers to probe critical information about which social information captures attention and is deemed most salient. As such, eye-gaze behaviour enables the interference of the mental states of participants. For example, IN-OT has been found to increase the fixation count for social and non-social stimuli (vs. placebo) (M. Eckstein et al., 2019), but has also had no effect on overt visual behaviour in an emotional recognition task while, concomitantly, increasing the participants' performance in said task (Lischke et al., 2012).

Importantly, gaze behaviour is altered in mental disorders like autism spectrum disorder who process social cues differently, notoriously exhibiting atypical gaze paths, less frequent eye contacts and different points of focus when looking at faces. IN-OT has been found to promote eye contact in males with autism (Auyeung et al., 2015), to increase the time spent looking at social stimuli (vs. non-social) in people with autistic traits (Le et al., 2020), and in a clinical trial, to increase the fixations on the eye region of a talking face, compared to placebo (Yamasue et al., 2020). Altogether, eye-gaze may provide a deeper understanding of OT's posited underlying mechanisms on salience attribution (Shamay-Tsoory & Abu-Akel, 2016). Eye gaze was used as a central psychophysiological correlate of salience attribution and visual attention in the studies of **Chapters 4** and **5** of this thesis.

Another benefit of the eye tracker is the possibility of measuring pupil size, which is modulated by both light (i.e. pupillary light reflex) and one's cognitive and emotional state. Cognitive modulation of pupil size involves several brain structures, including the pretectal olivary nucleus, superior colliculus, and locus coeruleus. These structures integrate sensory, motor, and cognitive signals to regulate pupil diameter, reflecting cognitive load, shifts in attention, and levels of arousal (Mathôt, 2018). Importantly, the pupil size indexes the ANS because the muscles controlling the pupil, the iris sphincter and dilator muscles, are innervated by the PNS and SNS respectively (M. K. Eckstein et al., 2017; Mathôt, 2018). Generally, activation of the SNS produces an enlargement of the pupil size (i.e. pupil dilation) whereas activation of the PNS produces a reduction of the pupil size (i.e. pupil constriction), however these systems may act in tandem and not just competitively as previously thought. IN-OT (vs. placebo) has enhanced pupil dilation in response to facial emotional stimuli (compared to facial neutral ones) (Leknes et al., 2013; Prehn et al., 2013) and for both social and non-social stimuli, although particularly higher in the former, and for some social stimuli (e.g., sexual) more than others (e.g., parent-child) (M. Eckstein et al., 2019). However, IN-OT has also decreased pupil

dilation for both social (angry, happy and ambiguous facial expressions) and non-social stimuli (geometrical shapes) (Quintana, Westlye, et al., 2019b). At rest, the pupil naturally dilates and constricts in a spasmic and rhythmic fashion (Lüdtke et al., 1998). The pupillary unrest index (PUI) (Lüdtke et al., 1998; Schumann et al., 2020) is a measure of these fluctuations' occurrence, representing the deviation in pupil dilation at low frequencies, and has been positively associated with PNS activity and, in specific, with sleepiness and negatively with alertness (Lüdtke et al., 1998; Schumann et al., 2020). On the other hand, sample entropy (SampEn) is a measure of the pupillary unrest's complexity (Richman & Moorman, 2000) and has been positively associated with SNS activity (Schumann et al., 2020). To date, there have been no reports of the effect of IN-OT on PUI and SampEn, and they were used as psychophysiological correlates of autonomic activity in the study of **Chapter 3** of this thesis.

Finally, sEBR can also be easily measured via an eye tracker and has been found to reflect tonic dopaminergic activity in the central nervous system (Groman et al., 2014; Kaminer et al., 2011; Kotani et al., 2016). The most compelling evidence for this association comes from pharmacological interventions and non-human studies: administration of dopamine receptor agonists increases the sEBR, whereas administration of antagonists decreases sEBR (Groman et al., 2014; Jongkees & Colzato, 2016). It is still unclear which dopamine receptors are linked with sEBR, and there is conflicting evidence regarding the contribution of the two main class of receptors (D1 and D2), but the general consensus is that both can modulate sEBR (M. K. Eckstein et al., 2017; Jongkees & Colzato, 2016). To date, no study has probed the effects of IN-OT on sEBR and their joint association in reinforcement learning and/or other psychophysiological correlates of social cognition. This central psychophysiological correlate was investigated in this thesis' **Chapter 5**.

3- Temporal profile of intranasal oxytocin in the human autonomic nervous system at rest: an electrocardiography and pupillometry study

This work was published in:

Gonçalo Cosme, Patrícia Arriaga, Pedro J. Rosa, Mitul A. Mehta, Diana Prata. Temporal profile of intranasal oxytocin in the human autonomic nervous system at rest: An electrocardiography and pupillometry study. Journal of Psychopharmacology. 2023;37(6):566-576. https://doi.org/10.1177/02698811231158233

My contributions: Performed pupillometry data processing, all statistical analyses and drafted the manuscript.

3.1 Introduction

OT has increasingly gathered the interest of cognitive neuroscientists since it was shown to be implicated in social cognition and behavior in humans (Erdozain & Peñagarikano, 2020) and potentially in the elusive pathophysiology of social symptoms in psychiatric disorders. OT has now been administered to several healthy and clinical populations (Bernaerts et al., 2020; Norman et al., 2011; Shilling & Feifel, 2016) to understand how it modulates neurophysiology and social behavior in a myriad of social cognition tasks. However, there is variability in the methods and inconsistency in the findings (Winterton et al., 2021). IN-OT is, by far, the most frequent route of OT administration in human neuroscience studies, and we have recently summarized these studies (Zelenina et al., 2022). Overall, IN-OT's temporal profile (i.e., across a typical neuroscience experimental session time), at rest, has been characterized by OT measurement in peripheral fluids (i.e. blood plasma, saliva, and urine), and central nervous system OT measurements (i.e. in cerebral spinal fluid) or activity (via blood oxygen level-dependent activation using magnetic resonance imaging, and, by us, microstates using EEG (Zelenina et al., 2022)). However, to the best of our knowledge, in the peripheral nervous system at rest, the temporal profile of IN-OT is still unexamined. Such examination is of crucial importance for neuroscience studies' design and interpretation because the ANS activity is associated with a myriad of social cognitive processes (Jáuregui et al., 2011; Quintana et al., 2012) - as we have recently shown for cognitive empathy (Cosme, Rosa, et al., 2021; Cosme, Tavares, et al., 2021). The ANS is also more easily accessible than the central nervous system in humans. Besides, such knowledge would be useful for the development of biomarkers predictive of IN-OT treatment response (Erdozain & Peñagarikano, 2020; Quintana et al., 2021; Winterton et al., 2021) in preparation for clinical trials.

The effects of OT on social cognition have been linked to both central and peripherally measured nervous system activity, and are integrated into hypotheses such as the social salience (Shamay-Tsoory & Abu-Akel, 2016), the general approach-avoidance (Harari-Dahan & Bernstein, 2014) and the allostatic (Quintana & Guastella, 2020) hypotheses. HRV, for example, has been implicated in social cognition (Park & Thayer, 2014) such that an increase in HRV has been associated with motivation towards social engagement (Beffara et al., 2016), whereas a reduced HRV has been found in subjects with difficulties in emotion regulation (Mather & Thayer, 2018), in autism spectrum disorders (Lory et al., 2020) and first episode psychosis (Cacciotti-Saija et al., 2018) and, at rest, may impair emotion regulation (Park &

Thayer, 2014). What is not known, currently, is how the effects of IN-OT on HRV, and other peripheric measures, at rest, integrate into OT's cognitive hypotheses. As a first step, in this study we aimed to develop understanding of the effects of OT on ANS activity at rest.

OT is produced in the paraventricular, supraoptic and accessory magnocellular nuclei of the hypothalamus (Meyer-Lindenberg et al., 2011) with direct projections to the dorsal brain stem, which regulates cardiovascular activity (Gutkowska et al., 2014), and the amygdala, which regulates ANS response patterns, particularly heart rate (T. Yang et al., 2007), the heart being replete with OTRs (Gutkowska et al., 2014). Altogether, cardiac indices are suitable to assess IN-OT effects on ANS activity, particularly the parasympathetic branch. Research has also shown that increased OT receptor gene methylation (i.e. silencing) is associated with decreased PNS activity at rest, via measurement of this system's well-known positive proxy (Shaffer & Ginsberg, 2017): HF-HRV (Lancaster et al., 2018). High HF-HRV has been associated with increased accuracy in identifying others' positive states, which may encourage longer and more successful social relationships, and approach behaviors (Lischke et al., 2017). At rest, increased HF-HRV has also been found to predict cooperative behavior (Beffara et al., 2016) which, in turn, has been associated with increased OT function (De Dreu, 2012a; Rilling et al., 2012).

Yet, the characterization of IN-OT's impact on the ANS function is still unclear, both during cognitive tasks and at rest. So far, IN-OT's effects on each branch of the ANS have been inconsistent. During tasks, it has been found to: 1) increase PNS activity (specifically, indexed by increased HF-HRV) in a facial emotion recognition task, albeit with no effect on SNS measured by electrodermal activity (Gamer & Büchel, 2012); 2) decrease PNS activity (specifically, indexed by lowered HF-HRV) while also increasing the LF-HRV, whose meaning is still unclear, during a mental arithmetic task (Tracy et al., 2018); and 3) have no effect on PNS (specifically via HF-HRV) but increase SNS activity indexed by decreased pre-ejection period, during a social stress task (Kubzansky et al., 2012). However, the purity of the pre-ejection period as a proxy for the SNS has since been questioned given it has been associated with many other cardiovascular factors (Krohova et al., 2017). During rest, the findings also remain inconsistent by showing that IN-OT: 1) coactivates both PNS and SNS (specifically, via decreasing the nonlinear HRV parameter DFAα1, which has been negatively associated with activation of both branches (Tulppo et al., 2005)) during a 10-min eyes-closed seated rest (Kemp et al., 2012); 2) increases PNS activity (via heightening HF-HRV) (Kemp et al., 2012), and (only) immediately after a social stress task in another study (Kubzansky et al.,

2012); 3) decreases PNS activity (specifically by decreasing the RMSSD) but only in females with positive childhood rearing experiences (Schoormans et al., 2020); and 4) has no influence on resting-state ANS activity (measured by HF-HRV and LF-HRV, R-R interval and RMSSD) (Tracy et al., 2018). All the above-mentioned resting-state studies used the commonly applied 24 IU of IN-OT and a single time-window, with variable lengths, albeit overlapping at 40 to 45 min post-administration. To our knowledge, only one study attempted to characterize the temporal profile, of IN-OT on HRV, but it was task-based, which we discuss later on (Norman et al., 2011).

Pupillary oscillations also reflect ANS activity, however it has not yet been used to help characterize effects of IN-OT at rest. The pupil's constriction is controlled by the sphincter muscle, innervated by the PNS, and its dilation is controlled by the dilator muscle, innervated by the SNS (Mathôt, 2018), thus, the overall pupil size is modulated by the interplay of both branches of the ANS. At rest, the pupil naturally dilates and constricts in a spasmic and rhythmic fashion (Lüdtke et al., 1998). The PUI (Lüdtke et al., 1998; Schumann et al., 2020) is a measure of these fluctuations' occurrence, representing the deviation in pupil dilation at low frequencies, and has been positively associated with PNS activity (using cardiac indices as proxies such as RMSSD) and, in specific, sleepiness but negatively with alertness (Lüdtke et al., 1998; Schumann et al., 2020), and it varies with the time of day (Danker-Hopfe et al., 2001). On the other hand, SampEn is a measure of the pupillary unrest's complexity (Richman & Moorman, 2000) and has been positively associated with SNS activity (as measured by skin conductance indices) (Schumann et al., 2020). In terms of task-based research, two studies have reported increases of emotional faces stimulus-induced mean pupil dilation at 40 min post-administration, one using 24 IU and another 40 IU (Leknes et al., 2013; Prehn et al., 2013). A third study found that 8 IU of IN-OT, when administered via a Breath Powered nasal device, elicits lower facial stimuli-induced pupil dilation compared to 24 IU or placebo (Quintana, Westlye, et al., 2019b) – which may be explained by the dose-effect inverted-U shaped curve previously observed for IN-OT (Borland et al., 2019).

In sum, the temporal profile of IN-OT's effect on the ANS at rest remains to be examined, and its impact at a commonly reported time-window of assessments of around 40 min, is not known. In the present study we aimed to assess the effect of 24 IU IN-OT on ANS activity at rest, across a large neuroscience experimental session duration, in healthy males, with a double-blind randomized placebo-controlled cross-over design, recording their pupillary and cardiac

activity at one baseline time-window pre-administration and at 6 time-windows post-administration (from 15 to 100 min). We report the effect of IN-OT on proxies of PNS and SNS activity, in each time-window, using electrocardiography (ECG) an pupillometry signals: two positive proxies of PNS activity (HF-HRV and PUI) (Schumann et al., 2020; Shaffer & Ginsberg, 2017) and one of SNS activity (SampEn) (Schumann et al., 2020). We specifically chose HF-HRV, a frequency measure of PNS activity, in contrast to time-domain ones (e.g. RMSSD), for comparability with previous IN-OT studies (Kemp et al., 2012; Kubzansky et al., 2012; Norman et al., 2011; Schoormans et al., 2020; Tracy et al., 2018). (However, for completeness, we report the time-domain ones and other indexes in **Annex A**). Our primary hypothesis was that IN-OT would coactivate the PNS and the SNS as reflected in an increased heart rate's HF-HRV (Kemp et al., 2012; Kubzansky et al., 2012) which is considered a robust positive proxy of PNS activity. Aiming at providing converging evidence, we also used as secondary outcomes (more indirect (Schumann et al., 2020) and less studied), PNS and SNS activity which we predicted would increase with IN-OT: pupil size's PUI and SampEn, respectively. These predictions are based on previous (and abovementioned) two studies (one at rest and the other at rest following a social stress task) consistently reporting, with one exception (Tracy et al., 2018), IN-OT to increase PNS and SNS activity measured by nonlinear measures of HRV, via HF-HRV and DFAα1 (Kemp et al., 2012; Kubzansky et al., 2012), whilst no previous pupillometry findings are available. The aim is for our findings to assist in: 1) future study design regarding the selection of the optimal IN-OT neuroscientific experimental sessions length; 2) comparability between previous and future IN-OT findings using different time-windows and data modalities; 3) assessing the potential usefulness of these ANS markers as IN-OT treatment response monitoring tools; and 4) advancing our understanding of the role of OT in human cognition and behavior.

3.2 Materials and methods

Participants

We recruited 20 young (M = 27.4; SD = 3.88, age range = 22 – 34), healthy, male, Portuguese adults, through mailouts and pamphlets in the university community and online social networks. All participants were included in the analysis. We applied standard cognitive neuroscience experiment eligibility criteria, detailed in **Annex A**. All participants gave their written informed consent and received financial compensation for their time. The study was

approved by the Ethics Committee of the Lisbon Medical Academic Center (Centro Académico Médico de Lisboa, CAML) and complies with national and EU legislation for clinical research.

Experimental procedure

The experimental session took place at a quiet room of the CAML's Clinical Research Centre (Centro para Investigação Clínica) in the Hospital de Santa Maria, Lisbon, Portugal. We used a double-blind (throughout data collection up to statistical analysis, inclusive), randomized placebo-controlled, cross-over design, whereby each participant took part in two sessions: one for IN-OT and another for placebo administration, in a counterbalanced order, and at the same time each day (by 2pm). The IN-OT administration of 24 IU was via 3 puffs of 0.1 ml each, in each nostril, from a 40 IU ml^{-1} 5 ml Syntocinon bottle (using the Novartis formula – batch H5148 produced by Huningue Production, France) or an identical placebo bottle (with the same ingredients, except OT – batch 170317.01 produced by VolksApotheke Schffhausen, Switzerland), both supplied by Victoria Apotheke Zürich, Switzerland. 24 IU of IN-OT was used as this dose is sufficient to increase central levels of OT to a functionally relevant degree (Quintana et al., 2021). OT and placebo sessions were approximately seven days apart. Drug storage and administration is further detailed in **Annex A**.

Participants spent 7 time-windows of 5 minutes eyes closed and 5 minutes eyes open (-10 – 0 min before administration and 15 – 27, 30 – 42, 45 – 57 min, 1 hr – 1hr 12 min, 1 hr 15 min – 1 hr 27 min, 1 hr 30 min – 1 hr 42 min after administration) (see **Figure 3**) in which they were asked to stay still, avoid cognitive processes (e.g. mental arithmetic calculations), to relax, and, in the eyes open condition, to fixate their gaze on a fixation cross at the center of a screen. At the end of each time-window the participants filled in three measures with Likert-type scales: alertness (1 – alert, 5 – sleepy), excitement (1 – excited, 5 – calm), and desire to socialize (1 – desire to socialize, 5 – desire to be left alone). EEG data was collected during the same experimental sessions and is reported elsewhere (Zelenina et al., 2022).

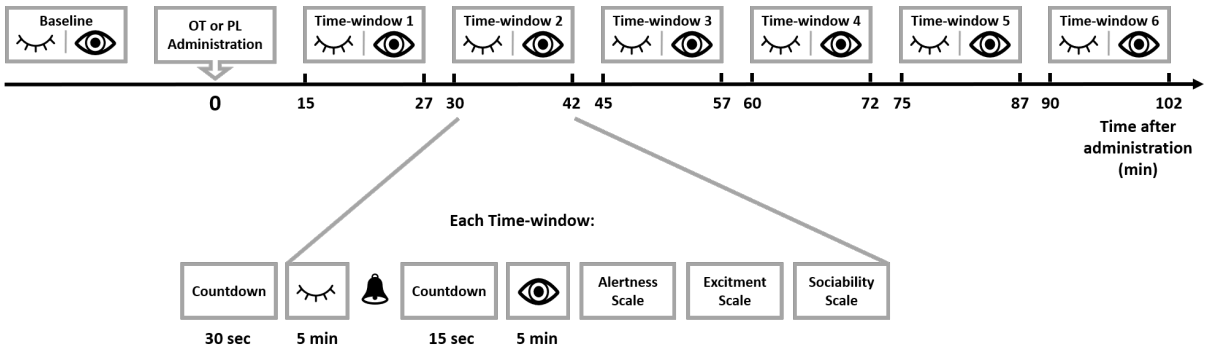

Figure 3 - The resting state task. Psychophysiological data was recorded in eyes closed (HRV) and eyes open (HRV and pupil size) conditions in 7 time-windows, 1 prior to drug administration (baseline) and 6 post-administration. Each time-window was preceded by a 30 sec countdown. Then followed 5 minutes of eyes closed, a beep as an instruction to open eyes, a 15 sec countdown, and finally 5 more minutes of recording. Afterwards, the participants filled in on-screen Likert-type scales for alertness, excitement and sociability. Between time-windows, participants were allowed to rest until the start of the following recording period. OT = oxytocin, PL = placebo, HRV = heart rate variability.

Data acquisition and pre-processing

Pupillary activity. Participants sat comfortably with their chin supported over a chinrest to minimize head movement, at approximately 56 cm away from a Lenovo 23.8-inch screen with 1920x1080 resolution and 60 Hz refresh rate. At 1000 Hz sampling rate, monocular gaze tracking and pupil size of the left eye of every participant was recorded with a SR Research EyeLink 1000 Plus which has an average accuracy of 0.15 visual angle. From the raw pupil size signal, samples 75ms before and after blinks, as identified by the eye tracker, were converted to missing data to remove artifacts caused by partial occlusion of the eye lids (Hershman et al., 2018). Afterwards, using self-written scripts in Python v3.7.4, the signal was filtered using a 3rd order digital filter with 4Hz cut-off frequency. Missing data was linear interpolated if it did not exceed 600ms, as blinks longer than that are considered microsleeps (Caffier et al., 2003; Schleicher et al., 2008; Y. Wang et al., 2011). Finally, if a 5 min time-window had more than 25% of missing data, the time-window was excluded from analysis. From the fully pre-processed pupil size signal two measures were extracted, replicating Schumann and colleagues' work (Schumann et al., 2020): PUI (Lüdtke et al., 1998) and SampEn (Richman & Moorman, 2000) as each are, respectively, positively correlated to indices of PNS and SNS activity (Schumann et al., 2020). To compute the PUI, the absolute

differences of the mean pupil size of consecutive segments lasting 640ms were summed and averaged per minute (Lüdtke et al., 1998; Schumann et al., 2020). The SampEn was computed using the 'pyEntropy' library (Donets et al., 2018) inputting the pupil size signal down sampled to 100 Hz, embedding dimension $m = 5$ and tolerance level, $r = 0.2$ (Schumann et al., 2020).

In order to assess the possible confounding impact of the pupil foreshortening error (Hayes & Petrov, 2016) on pupil size measurements, the Euclidean distance from each sample's location on the screen to the center (i.e. fixation cross) was subjected to the same pre-processing steps as the pupil size (see above). The main effect of drug on the Euclidean distance was not significant, $F(1, 198.72) = 0.18$, $p = .671$, $d = 0.07$], nor was the interaction with time, $F(5, 191.17) = 1.12$, $p = .353$. However, pairwise comparisons, per time-window, indicated a difference in time-window 2 (from 35 to 40 min), $t(192.15) = 2.00$, $p = .047$, $d = 0.66$ (95% CI [0.01, 1.32]) such that the Euclidean distance was increased under IN-OT compared to placebo.

Heart rate variability. The HRV was measured using a BIOPAC MP150 amplifier with the ECG recording module ECG100C-MRI in R wave at 1000 Hz sampling rate, gain as 1000, LP as 35 Hz and HP as 1 Hz (Biopac Systems Inc., Goleta, CA) and AcqKnowledge 4.3 software. Three Ag/AgCl electrodes with 11 mm diameter (EL503 EKG) were placed in a Lead II disposition. The beat-to-beat RR intervals were analyzed using the Kubios Premium software (version 3.2) (Lipponen & Tarvainen, 2019; Tarvainen et al., 2014). A smoothness priors detrending method for trend removal was applied (delta = 500) with interpolation rate of 4 Hz. After visually inspection and correction of missed or misaligned beats, artifact corrections were applied in 8.25% of all data with the very low (0.45 s) or low (0.35 s) thresholds. We used a piecewise cubic spline interpolation method (acceptance threshold 5%) for detecting RR intervals that were considered very different from the average RR interval for each participant (e.g., ectopic beats). To address our main research questions, we analyzed from the frequency domain the HF-HRV (frequency activity in the 0.15 - 0.40 Hz range), calculated by means of nonparametric Fast Fourier transformation absolute power (ms^2), replicating a previous IN-OT administration at rest study (Kemp et al., 2012). As mentioned in our aims' description, we provide in **Annex A** the additional analysis of: 1) RMSSD; 2) Kubios' proprietary PNS and SNS indexes, the first calculated from mean R-R intervals, RMSSD and Poincaré plot index S1 in normalized units, and the later calculated from mean HR, Baevky's stress index and Poincaré plot index S2 in normalized units; and 3) the DFAα1, particularly because it was used in a previous similar study, with statistically significant IN-OT effects (Kemp et al., 2012),

albeit this measure would be more appropriately analyzed in data collected over several hours (Shaffer & Ginsberg, 2017), which we (and the previous study, in fact) have not collected.

Statistical analysis

Statistical analysis was performed in R software 3.6 (R Core Team, 2014). A linear mixed model (LMM) was run for each dependent variable (neurophysiological data: 7 in total, 2 for pupil size related measures and 5 for HRV related measures; behavior data: 3 in total one for each scale) using the package 'lme4' (Bates et al., 2015) with Drug session (IN-OT, placebo), Time (post administration time-window: 1, 2, 3, 4, 5, 6) and their interaction as categorical fixed factors, and participant as random factor. To account for the baseline measurement of each session, the dependent variable measured at the time-window prior to drug administration (time-window 0) was included in the model as a covariate of no interest. For HRV the analysis was performed separately for each condition: eyes open and eyes closed. Naturally, for pupil size, the analysis was only performed for the eyes-open condition. Regarding the mood scales, we have reported on the same analysis earlier (Zelenina et al., 2022) in a sample differing in one subject. LMM are suitable for datasets with missing data and inter-individual random differences (Meteyard & Davies, 2020), and allow for the inclusion of session-varying covariates (such that, as herein, baseline values differed between drug session, per participant). The degrees of freedom and *p*-values were calculated using Type III analysis of variance with the Satterthwaite's method. We report a measure of effect size *d* for LMMs, analogue to Cohen's *d*, for the main effect of drug (Brysbaert & Stevens, 2018), and Cohen's *d* and 95% confidence interval (CI) for statistically significant pairwise comparisons. We considered a main effect of drug statistically significant if its *p*-value was less than 0.017 (after Bonferroni correction, upon diving the standard .05 by 3, three being the number of ANS measures we analyzed). For completeness and to comprehensively assess all time-windows, separately and regardless of the statistical significance of main effects, we ran estimates for each (i.e., pairwise comparisons) on estimated marginal means using EMMEANS package from R (with degrees of freedom estimated using the Kenward-Roger method which is more precise for small samples), besides the main effect of drug estimate. Since the main effect of time is not relevant to our research question, we did not interpret it but report it in **Annex A**, for completeness.

3.3 Results

Heart Rate Variability (HF-HRV)

Eyes closed. The main effect of drug on HF-HRV in eyes-closed, $F(1, 184.30) = 0.18$, $p = .669$, $d < 0.01$, and its interaction with time, $F(5, 171.94) = 0.22$, $p = .955$, were not statistically significant (**Figure 4** and **Table 1**).

Eyes open. The main effect of drug on HF-HRV in eyes-open, $F(1, 175.56) = 2.11$, $p = .148$, $d = 0.30$, and its interaction with time, $F(5, 169.80) = 1.42$, $p = .219$, were not significant. However, exploratory pairwise comparisons in each time-window indicated a significant difference in time-window 5 (from 80 to 85 min), $t(173.08) = 2.28$, $p = .024$, $d = 0.80$, 95% CI [0.10, 1.50], such that HF-HRV increased under IN-OT compared to placebo (**Figure 4** and **Table 1**).

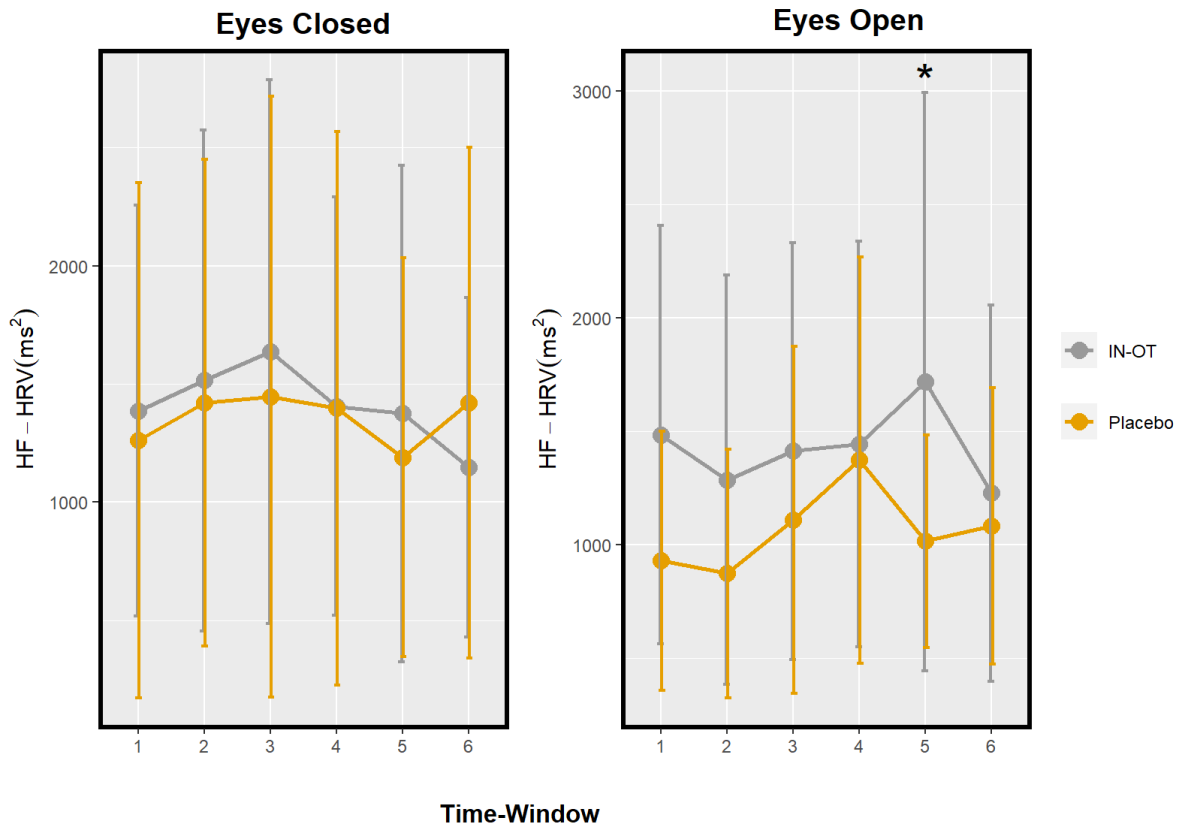

Figure 4 - Profile of HF-HRV after IN-OT in a resting-state paradigm with eyes closed (left) and eyes open (right) conditions. Significant pairwise comparisons (IN-OT vs. placebo) at specific time-windows are marked with an asterisk (*). Eyes closed time-windows: 1 =15 – 20 min; 2 = 30 – 35 min; 3 = 45 – 50 min; 4 = 60 – 65 min; 5 = 75 – 80 min; and 6 = 90 – 95 min. Eyes open time-windows: 1 = 20 – 25 min; 2 = 35 – 40 min; 3 = 50 – 55 min; 4 = 65 – 70 min;

5 = 80 – 85 min; and 6 = 95 – 100 min. Error bars: 95% CI. HF-HRV = high frequency heart rate variability, IN-OT = intranasal oxytocin, HRV = heart rate variability, CI = confidence interval.

Pupillary unrest (PUI and SampEn)

The main effect of drug on PUI was significant, $F(1, 167.92) = 11.42$, $p = .001$, $d = 0.45$, such that PUI was decreased under IN-OT compared to placebo (**Figure 5** and **Table 1**). Pairwise tests show this effect to be significant specifically in the last 3 time-windows (spanning from 65 until 100 min) [respectively, $t(156.61) = 2.69$, $p = .008$, $d = 0.96$, 95% CI [-1.67, -0.25]; $t(158.18) = 2.25$, $p = .026$, $d = 0.85$, 95% CI [-1.61, -0.10]; $t(158.24) = 2.38$, $p = .019$, $d = 0.88$, 95% CI [-1.62, -0.14]]. A drug by time interaction on PUI was not significant, $F(5, 155.84) = 1.60$, $p = .164$. The main effect of drug on SampEn, $F(1, 163.77) = 0.06$, $p = .802$, $d = 0.54$, and its interaction with time were not significant, $F(5, 154.48) = 1.72$, $p = .133$.

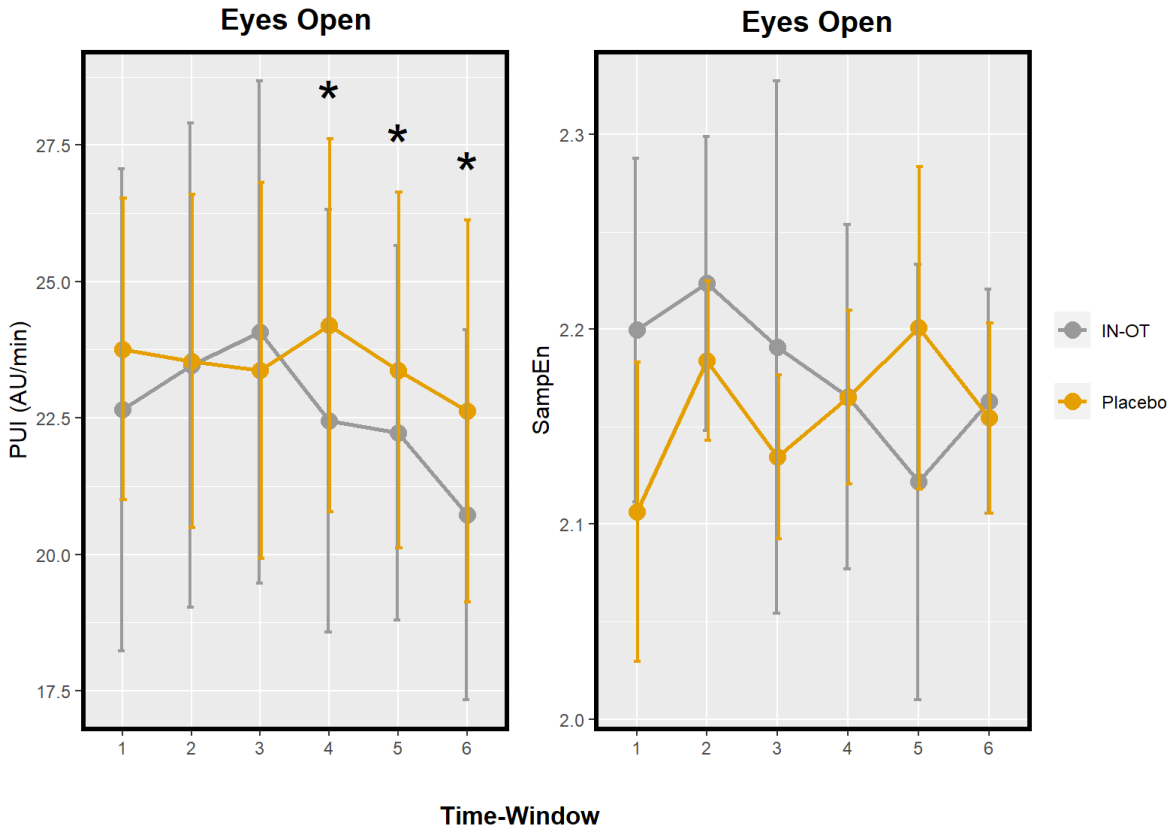

Figure 5 - Profile of two pupil size measures as a function of drug, per time-window: PUI and SampEn; in a resting-state paradigm. Significant pairwise comparisons (IN-OT vs. placebo) at specific time-windows are marked with an asterisk (*). Time-windows: 1 = 20 – 25 min; 2 = 35 – 40 min; 3 = 50 – 55 min; 4 = 65 – 70 min; 5 = 80 – 85 min; and 6 = 95 – 100 min. Error

bars: 95% CI. PUI = pupillary unrest index, SampEn = sample entropy, IN-OT = intranasal oxytocin, CI = confidence interval.

Behavioral – Mood scales

The main effect of drug on excitability, $F(1, 220.37) = 0.76$, $p = .384$, $d = 0.22$, and its interaction with time, $F(5, 202.49) = 1.19$, $p = .313$, were not significant but pairwise comparisons revealed a significant difference between drugs in time-window 5 (from 75 to 87 min), $t(207.44) = 2.35$, $p = .020$, $d = 0.76$, 95% CI [0.12, 1.40], whereby excitability increased under IN-OT compared to placebo. The main effect of drug on sociability, $F(1, 221) = 0.11$, $p = .736$, $d = 0.54$, and its interaction with time, $F(5, 221) = 1.13$, $p = .345$, were not significant; nor for alertness, $F(1, 206.94) < 0.01$, $p = .952$, $d = 0.52$; and $F(5, 202.40) = 1.48$, $p = .199$, respectively.

Table 1 - Summary of the results of the effect of drug on psychophysiological measures. Statistically significant ($p < .05$) main effects are marked with an asterisk (*) and only significant pairwise comparisons are shown.

Neurophysiological measure	Main effects of drug (IN-OT vs placebo)	Pairwise comparisons per TW (if $p < .05$)	Drug effect direction	Tentative ANS response interpretation
Eyes Closed				
HF-HRV	$F(1, 184.30) = 0.18$, $p = .669$, $d < 0.01$	-	-	-
Eyes Open				
HF-HRV	$F(1, 175.56) = 2.11$, $p = .148$, $d = 0.30$	TW 5: $t(173.08) = 2.28$, $p = .024$, $d = 0.80$, 95% CI [0.10, 1.50]	IN-OT ↑	PNS ↑
PUI	$F(1, 167.92) = 11.42$, $p = .001$*, $d = 0.45$	TW 4: $t(156.61) = 2.69$, $p = .008$, $d = 0.96$, 95% CI [-1.67, -0.25]	IN-OT ↓	PNS ↓
		TW 5: $t(158.18) = 2.25$, $p = .026$, $d = 0.85$, 95% CI [-1.61, -0.10]	IN-OT ↓	PNS ↓
		TW 6: $t(158.24) = 2.38$, $p = .019$, $d = 0.88$, 95% CI [-1.62, -0.14]	IN-OT ↓	PNS ↓

SampEn	$F(1, 163.77) = 0.06$, $p = .802$, $d = 0.54$	-	-	-

Footnote: Eyes closed time-windows (TWs): 1 =15 – 20 min; 2 = 30 – 35 min; 3 = 45 – 50 min; 4 = 60 – 65 min; 5 = 75 – 80 min; and 6 = 90 – 95 min. Eyes open TWs: 1 = 20 – 25 min; 2 = 35 – 40 min; 3 = 50 – 55 min; 4 = 65 – 70 min; 5 = 80 – 85 min; and 6 = 95 – 100 min. HF-HRV = high frequency heart rate variability, PUI = pupillary unrest index, SampEn = sample entropy, IN-OT = intranasal oxytocin, CI = confidence interval, ANS = autonomic nervous system.

3.4 Discussion

In this study we aimed, for the first time, to our knowledge, to describe the temporal profile of 24 IU of IN-OT on ANS activity at rest. We included eyes closed and eyes open conditions and multiple time-windows across a typically large neuroscience experiment duration (including a baseline assessment prior to drug administration), where we examined two positive proxies of PNS activity (HF-HRV and PUI) (Schumann et al., 2020; Shaffer & Ginsberg, 2017) and one of SNS activity (SampEn), across two data modalities (Schumann et al., 2020). Contrary to our hypothesis, we found indication that IN-OT may *deactivate* the PNS as reflected by a decrease in PUI - starting from 65 min post-administration until the end of the last window measurement (100 min). However, as predicted, we found an indication that IN-OT may *activate* the PNS, as reflected by an increase in HF-HRV - in the 80 - 85 min post-administration window. Regarding timing, we found our peak effects of IN-OT to be later than the 40 min time-window researched in most studies and lasted longer than previous IN-OT studies' usual session length (up to 90 min) (Norman et al., 2011). Next, we discuss these results and advance that a possible explanation for the seemingly inconsistent PNS findings (between our HRV and pupillary unrest findings) may be the still unclear reliability of the pupillary unrest markers used herein and in other studies. We note that the following interpretations should remain tentative given the early days in IN-OT and ANS association research.

The temporal profile of IN-OT on HRV and pupillary unrest

As abovementioned, the IN-OT effects we found both on HF-HRV (at 80 – 85 min) and on pupillary unrest (at 65-100 min), were detected later than 40 min. Forty minutes is the starting

time point which most previous studies have investigated (Gamer & Büchel, 2012; Kemp et al., 2012; Kubzansky et al., 2012; Leknes et al., 2013; Prehn et al., 2013; Schoormans et al., 2020; Tracy et al., 2018), with variable lengths, except one discussed below which used multiple (Norman et al., 2011), and where they have mostly found significant IN-OT effects with two exceptions (Schoormans et al., 2020; Tracy et al., 2018). Those were specifically on HF-HRV at rest (Kemp et al., 2012; Kubzansky et al., 2012; Tracy et al., 2018), or on HF-HRV (Gamer & Büchel, 2012; Norman et al., 2011) and pupil dilation (Leknes et al., 2013; Prehn et al., 2013) using cognitive tasks. Nevertheless, none has explored IN-OT effects beyond 90 min or with pupillary unrest. Power differences may also explain the variable results since some have different designs (within- vs. between-subject designs) and variable sample sizes (ranging from 21 to 173 subjects) (Gamer & Büchel, 2012; Kemp et al., 2012; Kubzansky et al., 2012; Leknes et al., 2013; Prehn et al., 2013; Schoormans et al., 2020; Tracy et al., 2018). Only one prior study has tested IN-OT effects in multiple time-windows, as we have, albeit with cognitive tasks (Norman et al., 2011). Norman and colleagues (Norman et al., 2011) recorded autonomic cardiac indices during 7 consecutive 15-minute time-windows, from a pre-administration baseline until 90 min post-administration of 20 UI of IN-OT. Direction-wise in line with our results, they found an IN-OT induced increase in HF-HRV as us herein – although theirs was in the 45 to 70 min time-window, whilst ours, in the 80 to 85 min. We thus partially support this finding, and extend it to a resting-state paradigm.

IN-OT decreased PUI at rest: (unexpectedly) suggestive of PNS *deactivation*?

Our finding of IN-OT having an effect on pupil size at rest, i.e. a small ($d = 0.45$) main effect of drug on PUI (but large effects, $d > 0.8$, at specific time-windows), was such that IN-OT, unexpectedly, *decreased* PUI from 65 min until 100 min post-administration. This was our most statistically significant finding, and most novel given the so far only indirect evidence available of PUI's relationship with ANS function (i.e. that PUI had recently been positively associated with RMSSD, a temporal-domain cardiac positive index of PNS activity (Schumann et al., 2020)). Interestingly the same authors also found it to be associated with skin conductance indices, which in turn was found to be a positive proxy of SNS, rather than PNS, activity in healthy controls (Schumann et al., 2017). As such, what PUI is a proxy for, in terms of ANS function, is still unclear.

On the other hand, and more substantially supported by previous evidence, PUI also increases with sleepiness and drowsiness, and decreases with alertness (Lüdtke et al., 1998). This could

be suggested to indicate that IN-OT (by decreasing PUI) increased our study participants' vigilance and attentive state. (This was unaffected by time-of-day variations (Danker-Hopfe et al., 2001), as all recording sessions started at approximately 2.11 pm; as in **Annex A** – Experimental Procedure.) Our behavioral findings did not point to an effect of IN-OT on alertness *per se* but they did on – the somewhat related – excitability, in a positive relationship. More recently, OT has been hypothesized to be associated with attention and orienting responses to external social stimuli in an interplay with the dopaminergic system (Shamay-Tsoory & Abu-Akel, 2016). As such, under the 'salience hypothesis of OT', IN-OT's effects on PUI would not be surprising given the association of PUI with attentive states and alertness (Lüdtke et al., 1998). The sustained increase in attentive state under IN-OT might also be explained by the closely related 'approach-withdrawal hypothesis of OT' which posits it facilitates approach to emotionally relevant stimuli (Harari-Dahan & Bernstein, 2014). OT may serve to maintain alertness in order to promote readiness to eventually engage in approach-mediated social behaviors, or readiness to eventually withdrawal from social stressors (Kubzansky et al., 2012).

IN-OT increased HF-HRV at rest: suggestive of PNS activation?

Our finding of increased HF-HRV under IN-OT at rest, a large effect (d = 0.80) (albeit only in an uncorrected pairwise comparison in the 80 - 85 min time-window), is in the same direction of the two other IN-OT resting-state studies (Kemp et al., 2012; Kubzansky et al., 2012) but at approximately 40 min later. This finding suggests that IN-OT upregulates PNS and may, presumably, be consistent with OT motivating approach behaviors (again in support of the 'approach-withdrawal hypothesis of OT' (Harari-Dahan & Bernstein, 2014)). Alternatively, one previous study found no effect of IN-OT on HF-HRV at rest but found IN-OT to *decrease* HF-HRV during a mental arithmetic task (Tracy et al., 2018), suggesting that, in the presence of a stressor, OT inhibits the PNS rather than triggers it (causing an effect analogous to SNS activation (Chrousos & Gold, 1992)), in order to solve the stressful situation and maintain an optimal internal state (Kemp et al., 2012; Kubzansky et al., 2012; Quintana et al., 2013); while, at rest, OT may induce relaxation and lowered anxiety (Dodhia et al., 2014), such as our results also suggest, in concordance with the 'allostatic hypothesis of OT' (Quintana & Guastella, 2020).

The allodynamic mode of ANS regulation accounts for both its branches to interact in a two-dimensional autonomic surface that allows for coactivation, coinhibition, uncoupled or

reciprocal activation of the branches (Berntson et al., 1991; Berntson & Cacioppo, 2000). Despite them being usually discussed in the same organ systems, there are reports of coactivation on separate ones. For example, fear reactions may lead to both increased heart rate (i.e., SNS activation) and bowel and bladder emptying (i.e., PNS activation) (Berntson et al., 1991). However, we are not aware of literature reporting the same ANS branch's activation in one organ and inhibition in another, as our results seems to suggest (possible PNS activation measured by HF-HRV at the heart, and PNS deactivation measured by PUI at the eyes). We tentatively and speculatively interpreted that although these finding appear contradictory and surprising - both could be consistent with the facilitation of alertness and preparedness for an approach behavior, given previous evidence (Baethge et al., 2019; Beffara et al., 2016; Harari-Dahan & Bernstein, 2017; Kemp et al., 2012). Second, we found no statistically significant effect of IN-OT on SampEn, thus no support for a IN-OT influence on the SNS branch, when measured via pupillometry. We again stress that HF-HRV is one of the most robust proxies of the PNS, backed from practical and theoretical evidence (Acharya et al., 2006; Shaffer & Ginsberg, 2017), and PUI (and SampEn's) association to each branch of the ANS has not been researched as extensively and thus our pupillary unrest findings, although statistically significant, should remain well open to alternative interpretations.

Limitations

Herein we computed the Euclidean distance from each sample's location to the center of the screen (i.e., fixation cross) and subjected this measure to the same statistical analysis of our dependent variables – to assess a, by chance, possible confounding effect of the pupil foreshortening error on our drug effect analyses (Hayes & Petrov, 2016). This was not verified, as this measure was (positively) associated with the drug effect only in the 35 – 40 min time-window (eyes-open time-window 2), where we report no statistically significant effects. Additionally, we recognize variable IN-OT dosages would have allowed us to improve our pharmacokinetic modelling; nevertheless we chose the most commonly administered dosage in the literature for comparability (Zelenina et al., 2022). Although an apparent limitation, we have also not measured OT blood levels as (i) they do not necessarily reflect CNS activity and (ii) they could represent the simulation of endogenous OT release as well as the administered OT (Martins et al., 2020) and (iii) the stress-inducing phlebotomy has noisy effects on ANS activity (Alley et al., 2019; Brown et al., 2016). Finally, it is possible that power limitations may have prevented the detection of significant IN-OT effects on HF-HRV consistently across all time-windows, as others have achieved with a sample size doubling ours (Norman et al.,

2011), however while the latter employed a between-subjects' design, our within-subject design should have been equally powerful with a smaller sample. Indeed, like us, another study reports effects of IN-OT on HF-HRV with approximately 20 subjects in a within-subject design (Kemp et al., 2012), whilst another with an increased sample size (IN-OT N = 87 and placebo N = 86) in a between-subject design, found no such effects (Schoormans et al., 2020). Overall, given the mixed literature, and the early days of ANS and IN-OT research, we cannot so far exclude that the measures we used are not robust markers of IN-OT effects.

3.5 Conclusions

We report herein on the temporal profile of IN-OT in the human ANS at rest using HRV and, for the first time, pupillary unrest measures. Finding evidence of OT increasing HF-HRV (suggesting PNS activation), and decreasing PUI (suggesting PNS deactivation), we speculated that both might be consistent with the facilitation of alertness and preparedness for an approach behaviour. Given the early days in IN-OT and ANS association research, the interpretation of these results remains highly tentative. Nevertheless, we hope our findings assist in future study design, comparability between IN-OT findings across data modalities, and assessing the usefulness of ANS markers for IN-OT response monitoring and human social cognition understanding.

4- Oxytocin's role in naturalistic spatio-temporal salience attribution: a pharmaco-eye-gaze study

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4.1 Introduction

Humans rely on vision to interact with the environment, using eye-gaze to fixate over regions of interest that attract the most attention. Visual attention is mostly divided in two modes: 1) exploration, characterized by an increase in the number of fixations and by their broader spatial distribution; and 2) exploitation, characterized by prolonged fixated eye-gaze, representing scrutinization of a salient cue (Higashi, 2024). Variations in these visual behaviour modes are explained by several bottom-up and top-down processes (Melloni et al., 2012).

Bottom-up mechanisms pertain to the raw sensory signal input of the stimuli which shift (covert) attention automatically and involuntarily towards it (Connor et al., 2004). The low-level physical features of a static picture, for example, like its luminance, colour, saturation and edge information, are sufficient to construct salience maps that predict with high accuracy human eye-gaze behaviour (Itti & Koch, 2001). Dynamic stimuli like videoclips, contain the additional temporal evolution of the scene which provides an expectancy and a narrative that can also modulate attention. Due to these reasons and their more naturalistic features, videoclip-like stimuli are now becoming more recurrent in neuroscience (Sonkusare et al., 2019). Recently, the GLIMPSE algorithm was developed to compute a salience score from eye-gaze data that incorporates the physical spatial salience of *where* attention is allocated, and the physical temporal salience of *when* attention happens, and *how* it evolves over time (Traver et al., 2021). This salience score increases when the gaze-coordinates of multiple observers are consistent over a particular location in a videoclip frame, and over a particular time span.

Top-down mechanisms, on the other hand, suggest longer-term cognitive strategies biasing (overt) attention (Connor et al., 2004), and are modulated by the observer's emotional states, goals, task demands (e.g. free viewing vs. cognitive task) and contextual cues (e.g. stimuli's valence) (Connor et al., 2004; Mohanty & Sussman, 2013). Importantly, rewarding stimuli bias our visual attention via their motivational salience (Love, 2014) which is centrally signalled in the striatum by dopamine (Anderson et al., 2016). In fact, reward-elicited activity in the dopaminergic midbrain causes reward predictive cues to become salient (Hickey & Peelen, 2015) and more arousing (Beeler & Dreyer, 2019; Eban-Rothschild et al., 2016).

Visual attention is predominantly biased to social stimuli across several species (Adachi, 2009; Tanaka, 2003) - including humans (Guillon et al., 2014) - because these stimuli are inherently motivationally salient. They have been found to activate dopaminergic reward circuits despite

their valence (i.e. positive or negative contexts) (Yamaguchi et al., 2017). Importantly, OT, a neuropeptide known to modulate social cognition, has been hypothesized to promote salience attribution to social stimuli, via interacting with the dopaminergic system (Shamay-Tsoory & Abu-Akel, 2016). Several lines of research support this hypothesis, one of which has shown OT to increase activity in the ventral tegmental area, a critical region in central dopaminergic pathways and reward processing, in response to both rewarding friendly faces and punishing angry faces (Groppe et al., 2013). The social specificity of OT's effects, however, has not been established which gave rise to the general approach-withdrawal hypothesis of OT which posits OT to heighten the salience of 'personally relevant or emotionally evocative stimuli', regardless of their socialness, by acting on approach/motivation and avoidance/withdrawal circuitry (Harari-Dahan & Bernstein, 2014).

Most research probing the effects of IN-OT on salience relies on simplified and discretised stimuli in extremely controlled laboratory experiments with debateable ecological validity (Sonkusare et al., 2019). To the best of our knowledge, no study has probed the effects of IN-OT on visual attention, and specifically on the temporal and spatial salience features of social and non-social dynamic stimuli like videoclips. In this study, we aimed to address this gap in the literature by researching IN-OT's effects on the GLIMPSE salience score calculated from the free-viewing eye-gaze of multiple observers (Carvalho et al., 2012; Traver et al., 2021). We hypothesized that IN-OT would increase, relative to placebo, the GLIMPSE salience scores, which would be consistent with the general approach-withdrawal hypothesis of OT (Harari-Dahan & Bernstein, 2014). In order to increase generalizability, we included videoclips that were either social or non-social, and when social, orthogonalized for their valence (positive or negative) and arousal (high or low) and analysed them separately. Furthermore, during a second viewing of the same videoclips, subjects were asked to continuously rate their subjective arousal levels. We hypothesized IN-OT would also increase, compared to placebo, the arousal ratings given that IN-OT has been suggested to attribute salience to motivationally relevant stimuli by interacting with the dopaminergic system (Harari-Dahan & Bernstein, 2014; Shamay-Tsoory & Abu-Akel, 2016), and – as above-mentioned - dopamine renders rewarding stimuli motivationally salient and arousing (Beeler & Dreyer, 2019; Eban-Rothschild et al., 2016; Love, 2014).

4.2 Material and methods

Participants

A total of 62 subjects were recruited through social media and word-of-mouth. Due to unpredictable technical difficulties, the number of participants included in the computation of the GLIMPSE salience score varied for each videoclip (see below). All participants were white Portuguese, healthy males, aged 20 – 35 years old (recruited by design, as OT's effects have shown to be affected by sex and age (Bartz et al., 2011)), right-handed, heterosexual, and had European Portuguese as first language and at least 12 years of education. Exclusion criteria were self-reported premature birth (≤ 36 weeks) with associated health consequences, prior head trauma with loss of consciousness or seizures, prior or current neurological or psychiatric disorders, history of drug or substance abuse, use of psychotropic or hormonal medication in the last 3 months, and colour-blindness. Twenty-four hours before the experiment, participants were asked to abstain from consuming caffeine, alcohol, tobacco or drugs, and intense physical (sports) and sexual activity, as well as to abstain from cannabis consumption for 1 week before the experiment. A drug screening test (for amphetamine, benzodiazepine, cocaine, methamphetamine, morphine/opiates, tetrahydrocannabinol (THC); nal von minden Drug-Screen®) and an interview were conducted on the day of the experiment to confirm pre-requisites were followed.

Experimental procedure

The session took place at the LAPSO lab of ISCTE-IUL (Lisbon, Portugal), was approved by its ethics committee (Ref 19/2019), and was part of an umbrella project involving the collection of other data modalities, not be reported herein, some of which already reported elsewhere (Santiago et al., 2024).

Following the drug test, participants completed some psychological questionnaires and then a between-subjects randomized double-blinded controlled IN-OT/placebo administration was performed. Participants self-administered a nasal spray containing 24 IU of IN-OT (AlfaSigma, Bologna, Italy) or placebo (VolksApotheke Schffhausen, Switzerland), following recommendations described elsewhere (Guastella & MacLeod, 2012). The spray bottles were all identical, blinded in the Santa Maria pharmacy, and were refrigerated until 1 hour maximum before administration. Upon verification of unobstructed participants' breathing and unblocked

nostrils, and 67 minutes before watching the videoclips, participants were instructed to self-administered six puffs (three per nostril, or six in the same nostril if one was partially obstructed) of the nasal spray, resulting in a total of 24 IU of OT or placebo.

Each videoclip lasted 40 seconds and had no sound. Four were non-social, representing landscapes, whilst the other 16 were social and varied in valence (positive or negative) and in arousal (high or low). Thus, the videoclips were categorized in either positive-valence high-arousal (erotic; N = 4), negative-valence high-arousal (gore; N = 4), positive-valence low-arousal (e.g. friends socializing; N = 4) or negative-valence low-arousal (e.g. a couple crying; N = 4). The stimuli were selected based on a previous study that validated the stimuli's arousal and valence levels (Carvalho et al., 2012). The participants were informed that: 1) the videoclips would include scenes from movies represented by actors; 2) there would be gore and erotic scenes; and 3) they could skip the videoclips. Importantly, however, the participants were also instructed to free-view the videos like they were at home or at the cinema, to ensure their observation was naturalistic. At the end of the 20 videos, the participants were in a resting state condition for approximately 6 minutes, after which they moved to another room and observed the same videoclips again. This time, subjects were instructed to quantitatively rate their subjective arousal by pressing the up and down arrows in a keyboard. The arousal ratings were shown in a bar next to the videoclip (see **Figure 6**). Crucially, eye-gaze data was collected only during the first visualization of the videoclips.

Figure 6 - The free-viewing task with the subjective arousal bar during second viewing session. The subjective arousal bar was absent from the screen in the first viewing session.

Eye-gaze data acquisition and pre-processing

The first viewing of the videoclips took place in a quiet and slightly dimmed room, with lighting being constant across participants. Participants were asked to sit in a comfortable position and to stabilize their heads on a chin rest to reduce head movement and to equalize head-to-screen distance at 93 cm and head-to-camera at 45 cm. Eye-gaze data was recorded using an EyeLink Portable Duo (SR Research). Data was collected using corneal reflection and a centroid tracking algorithm binocularly (when not possible, the eye with the best calibration was chosen) at a 2000 Hz rate, with a typical accuracy ranging 0.25 to 0.50 visual angle. The data was pre-processed using SR Research's DataViewer v4.1.63 to exclude bins of 50 ms before and after blinks, thus removing effects of partial occlusion of the eye lid. For each subject and each videoclip, the x- and y-coordinates of their eye-gaze were extracted and normalized, and down sampled to 30 Hz to match the videoclips' framerate.

GLIMPSE

For each drug group we computed the salience score $s(t)$ using the GLIMPSE algorithm (Traver et al., 2021). For a given videoclip, a dataset $\mathcal{P}_t$ was created:

$$\mathcal{P}_t = \{g(o,t) : o \in \{1,\dots,N\}, t \in [t-\theta_t, t+\theta_t]\} \quad (1)$$

where $g(o,t) = (x,y)$ represents the gaze position of observer $o \in \{1,\dots,N\}$ at videoclip frame number $t \in [t-\theta_t, t+\theta_t]$, where θ_t is a hyperparameter temporal threshold. Next, the pairwise Euclidean distance between the ith and jth points in the set $\mathcal{P}_t$ of n gaze points were calculated:

$$s(t) = \frac{2}{n(n-1)} \sum_{\substack{i,j \in \{1,\dots,n\} \\ i \neq j}} \mathbf{1}[d_{i,j} < \theta_s],\ t \in \{1,\dots,T\} \quad (2)$$

where $\mathbf{1}[p]$ is the indicator function that yields, 1 when predicative p is true, and 0 otherwise. The hyperparameter θ_s denotes the spatial scale, a distance threshold, and $2/n(n-1)$ serves as a normalization factor. Equation (2) accounts for the number of paired gaze points that are close enough, in a normalized way, so that $s(t) \in [0,1]$. Larger $s(t)$ values indicate higher spatio-temporal consistency amongst multiple observers, and we empirically set the hyperparameters to θ_t = 5 and θ_s = 0.1 (Traver et al., 2021).

Statistical Analysis

To statistically compare the salience curves between groups, for each videoclip, we: 1) computed the difference between the two salience curves; 2) randomly shuffled the bins of this difference curve, in 250 permutations; 3) computed the empirical *p*-values under the null hypothesis that observed samples come from a given surrogate (Dmochowski et al., 2012); and 4) corrected for multiple comparisons via False Discovery Rate (FDR). This method provides frame intervals that indicate whether the salience score of IN-OT is statistically different than that of the placebo group (**Figure 7**). Lastly, the proportion of frames in which the salience scores were significantly higher in IN-OT (vs. placebo), or higher in placebo (vs. IN-OT), were compared using a proportions z-test (Seabold & Perktold, 2010), and Cohen's h was reported

as a measure of effect size and interpreted as: $h < 0.2$, small effect; $h < 0.5$, moderate effect; and $h \geq 0.8$ large effect (Lee, 2016).

The subjective arousal ratings were averaged across the videoclip and compared between drug groups using t-tests, with Cohen's d as a measure of effect size and interpreted as: $d < 0.2$, small effect; $d < 0.5$ moderate effect; and $d \geq 0.8$ large effect.

4.3 Results

GLIMPSE salience scores

The descriptive statistics and proportion z-tests comparing drug groups on the GLIMPSE salience scores are summarized in **Table 2**. The evolution of the GLIMPSE salience scores across the first videoclip of each category is provided in **Figure 7**. The complete results are shown in **Annex B**, alongside a website link to a repository where the videoclips can be seen alongside the evolution of the GLIMPSE salience scores.

The proportion of videoclip frames where the GLIMPSE salience scores were higher in IN-OT compared to placebo (i.e. 'IN-OT > Placebo'), was significantly larger than the proportion of the videoclip frames where the opposite effect occurred (i.e. 'Placebo > IN-OT'), in most videoclips except two: 1) one belonging to the positive-valence high-arousal category (erotic), where the significant difference was in the opposite direction (i.e. the proportion was significantly larger for 'Placebo > IN-OT'); and 2) for 1 videoclip belonging to the non-social category, where there were no significant differences between the proportions. The largest effect detected was for a negative-valence high-arousal videoclip (Cohen's h = 1.49, Video 18, **Table 2**) and the smallest significant effect detected was for a negative-valence low-arousal videoclip (Cohen's h = 0.09, Video 4, **Table 2** and **Figure 7**).

Table 2 - Mean and standard deviation of the GLIMPSE salience scores for each videoclip and drug group, and percentage of frames where IN-OT salience score is statistically significantly higher than placebo salience scores, and vice-versa, followed by p-values and Cohen's h

obtained from the proportion z-tests comparing such percentages. IN-OT = intranasal oxytocin. SD = standard deviation. * - denotes statistically significance at $p < .05$.

Condition		Salience Scores IN-OT		Salience Scores Placebo		Percentage of Frames IN-OT > Placebo	Percentage of Frames Placebo > IN-OT	Proportion z-test *p*-value	Cohen's h
		Mean	SD	Mean	SD				
Non-social (Landscapes)	Video 1	0.193	0.089	0.170	0.078	42.3	14.5	< .001*	0.64
	Video 2	0.186	0.091	0.179	0.084	36.0	33.1	.133	0.06
	Video 11	0.240	0.086	0.222	0.061	48.7	26.4	< .001*	0.46
	Video 12	0.232	0.069	0.189	0.063	61.3	14.0	< .001*	1.03
High-Arousal Negative-Valence (Gore)	Video 9	0.396	0.129	0.325	0.096	72.2	13.2	< .001*	1.29
	Video 10	0.310	0.100	0.287	0.096	48.3	30.0	< .001*	0.38
	Video 17	0.389	0.115	0.325	0.084	64.8	9.7	< .001*	1.24
	Video 18	0.377	0.147	0.298	0.102	70.4	6.2	< .001*	1.49
High-Arousal Positive-Valence (Erotic)	Video 3	0.282	0.098	0.282	0.085	37.8	27.9	< .001*	0.21
	Video 4	0.249	0.090	0.230	0.084	48.8	25.9	< .001*	0.48
	Video 15	0.275	0.117	0.256	0.087	42.6	23.0	< .001*	0.42
	Video 16	0.269	0.107	0.260	0.100	28.9	33.8 †	.011*	-0.10
Low-Arousal Negative-Valence	Video 5	0.359	0.101	0.351	0.084	33.8	29.4	.022*	0.09
	Video 6	0.312	0.091	0.295	0.100	53.2	26.8	< .001*	0.55
	Video 13	0.356	0.095	0.305	0.087	60.9	13.2	< .001*	1.05
	Video 14	0.382	0.111	0.289	0.086	71.5	6.2	< .001*	1.51
Low-Arousal Positive-Valence	Video 7	0.281	0.073	0.269	0.076	42.2	26.4	< .001*	0.33
	Video 8	0.382	0.135	0.327	0.102	68.8	14.7	< .001*	1.17
	Video 19	0.398	0.138	0.340	0.126	78.1	11.2	< .001*	1.48
	Video 20	0.292	0.086	0.263	0.068	60.2	22.5	< .001*	0.79

Footnote: † - Video 16 is the only one that has a larger proportion of frames for 'Placebo > IN-OT' compared to the proportion of the opposite, of 'IN-OT > Placebo'.

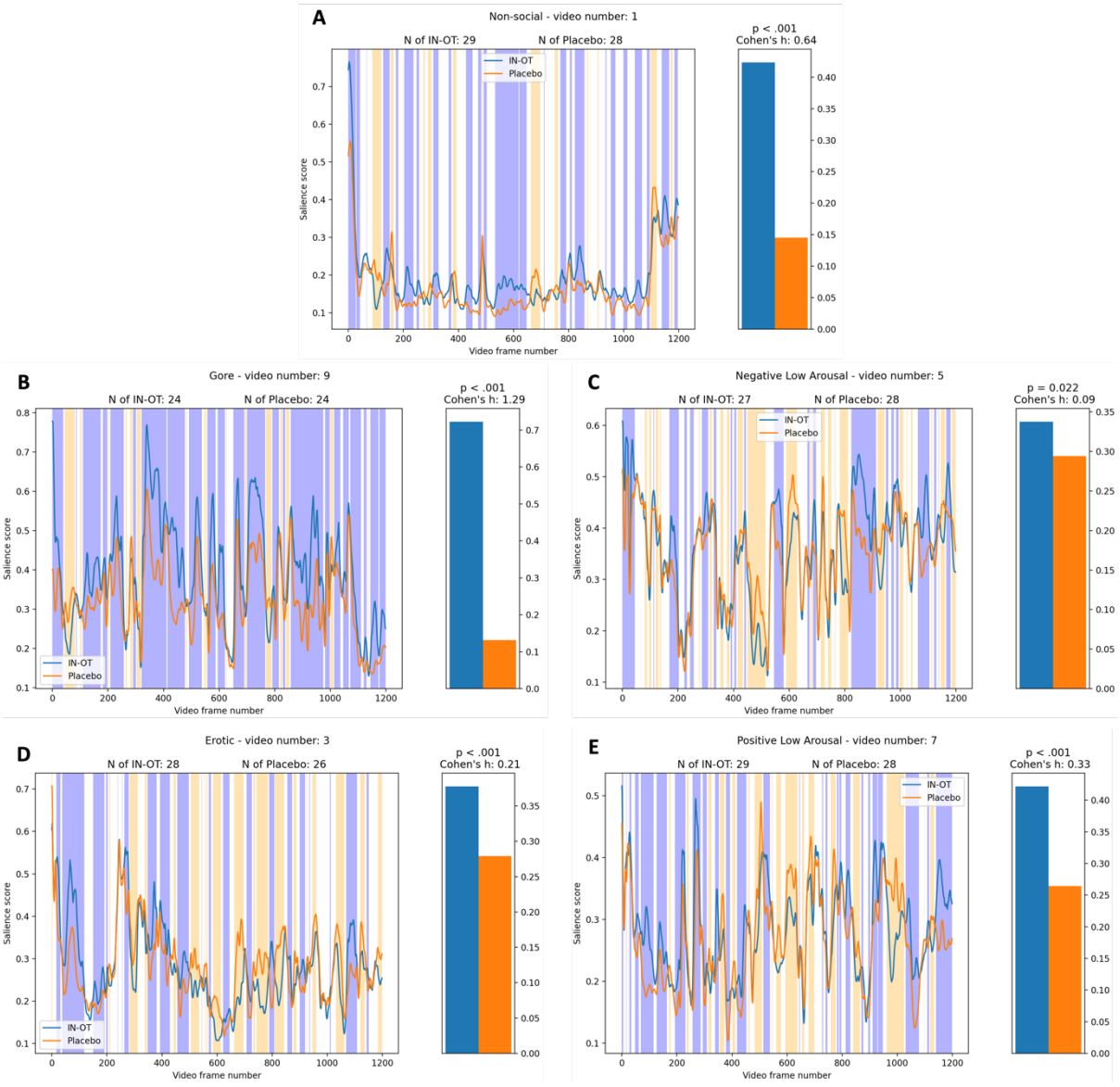

Figure 7 - The GLIMPSE salience scores for each drug group, for the first videoclip shown of each category (as a representative example): **A)** Non-social (landscapes); **B)** negative-valence high-arousal (gore); **C)** negative-valence low-arousal; **D)** positive-valence high arousal (erotic); and **E)** positive-valence low-arousal. The blue shaded areas represent frame intervals in which the salience score of the IN-OT group is significantly higher (FDR-corrected) than that of the placebo group, and orange shaded areas represent the opposite. In the white shaded areas, the difference is not statistically significant. Salience score ranges from 0 to 1. The bar plot indicates the percentage of video frames where the drug difference is statistically significant: blue when 'IN-OT > placebo'; and orange when 'placebo > IN-OT'. The *p*-value and Cohen's h over the bar plot was obtained after conducting proportion a z-test comparing the difference between both drug groups. IN-OT = intranasal oxytocin; FDR = False Discovery Rate.

Subjective arousal ratings

The descriptive statistics and t-tests comparing drug groups on the subjective arousal ratings are summarized in **Table 3**, and the evolution of the subjective arousal ratings across the first videoclip of each category is provided in **Figure 8**. The complete results are shown in **Annex B**.

There was only a moderate effect of IN-OT on subjective arousal ratings which was for a positive-valence high-arousal videoclip (erotic, Cohen's d = 0.73, **Table 3**). The difference between drugs was not significant in any other videoclip (all $p > .05$).

Table 3 - Mean and standard deviation for the subjective arousal of each drug group, per videoclip, following the t-value, degrees of freedom and Cohen's d from the comparison between drug groups. IN-OT = intranasal oxytocin; SD = standard deviation; df = degrees of freedom. * - denotes statistically significance at $p < .05$.

Condition		Subjective arousal IN-OT		Subjective arousal Placebo		t-value (df)	*p*-value	Cohen's d
		Mean	SD	Mean	SD			
Non-social (Landscapes)	Video 1	-0.183	2.213	-0.781	2.796	0.84 (48)	.404	0.24
	Video 2	-0.084	2.384	-0.796	2.730	0.97 (47)	.335	0.28
	Video 11	-0.428	2.639	-1.064	2.344	0.88 (46)	.386	0.26
	Video 12	-0.406	2.407	-0.903	2.090	0.76 (46)	.453	0.22
High-Arousal Negative-Valence (Gore)	Video 9	3.314	1.725	3.055	1.659	0.53 (46)	.600	0.15
	Video 10	3.142	1.749	2.928	1.737	0.42 (46)	.674	0.12
	Video 17	2.897	1.706	2.381	2.000	0.96 (46)	.340	0.28
	Video 18	3.296	1.867	2.516	2.162	1.34 (46)	.186	0.38
High-Arousal Positive-Valence (Erotic)	Video 3	2.222	0.991	1.439	2.135	1.67 (46)	.102	0.46
	Video 4	2.071	1.707	1.398	2.184	1.20 (46)	.237	0.34
	Video 15	2.493	1.304	1.288	1.882	2.61 (46)	.012*	0.73
	Video 16	2.997	1.673	2.521	2.076	0.88 (46)	.384	0.25
Low-Arousal Negative-Valence	Video 5	0.159	1.673	-0.025	2.124	0.34 (46)	.739	0.10
	Video 6	0.783	1.972	-0.023	2.113	1.37 (46)	.179	0.39
	Video 13	0.244	2.166	-0.336	1.956	0.97 (46)	.339	0.28
	Video 14	0.205	1.768	-0.650	2.148	1.51 (46)	.137	0.43
Low-Arousal Positive-Valence	Video 7	1.059	1.634	0.886	1.797	0.35 (46)	.729	0.10
	Video 8	-0.648	2.428	-0.028	1.678	-1.01 (46)	.317	-0.30
	Video 19	-0.790	2.023	-0.161	2.398	-0.99 (46)	.329	-0.28
	Video 20	0.406	2.250	-0.271	2.283	0.87 (32)	.391	0.30

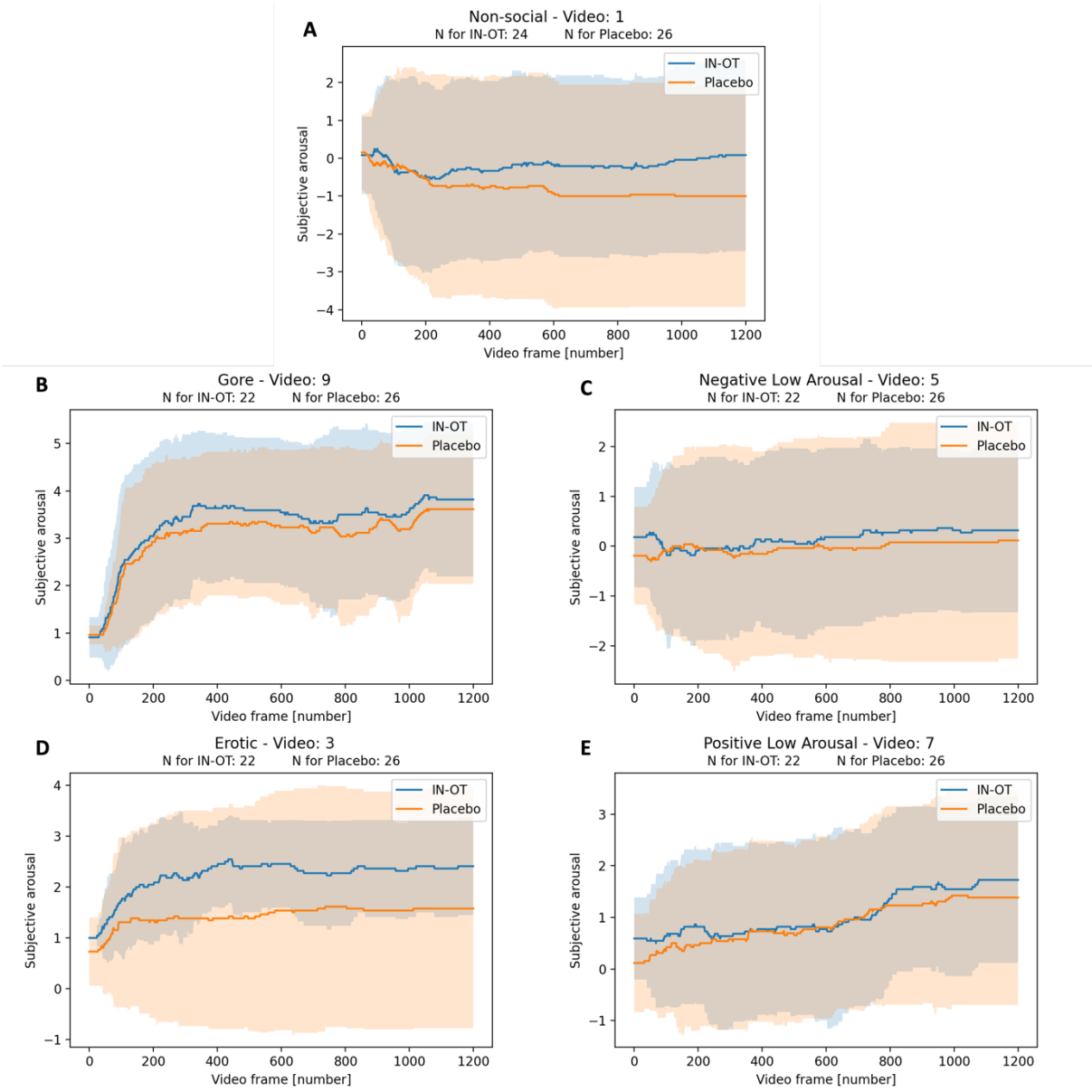

Figure 8 - Mean subjective arousal ratings for each drug group, for the first videoclip shown of each category (as a representative example): **A)** Non-social (landscapes); **B)** negative-valence high-arousal (gore); **C)** negative-valence low-arousal; **D)** positive-valence high arousal (erotic); and **E)** positive-valence low-arousal. Subjective arousal rantings ranged from -5 to 5. Shaded areas represent ± SD. IN-OT = Intranasal oxytocin; SD = Standard Deviation.

4.4 Discussion

In this study we aimed to test the role of IN-OT on the temporal and spatial salience attributed to videoclip scenes, of both social and non-social nature, which, to the best of our knowledge, has never been probed. To this end, we compared the GLIMPSE salience scores between IN-OT and placebo groups, a score that is computed from the free-viewing eye-gaze of multiple observers and that incorporates the spatial and temporal features of a videoclip (Traver et al., 2021). We found, as hypothesized, that IN-OT increased the salience for all 16 social videoclips used, except in one, which was of erotic category (positive-valence high-arousal), where IN-OT decreased the salience score. Additionally, IN-OT increased the GLIMPSE salience scores in all four non-social videoclips, except one where there was no statistical difference between drug groups. On a second watching of the same videoclips, participants continuously rated their subjective arousal levels, and we found significant differences only in one of erotic category (positive-valence high-arousal).

IN-OT increased the GLIMPSE salience scores across social and non-social videoclips

Naturalistic stimuli, such as videoclips, are becoming prevalent in neuroscience because they closely mimic everyday experiences. Visual attention plays a crucial role in processing these stimuli because it monitors the dynamic changes naturalistic stimuli have. They thus provide a more accurate representation of how attention is allocated and maintained in real-world scenarios (Sonkusare et al., 2019). Videoclips of social interactions, in particular, depict the more nuanced features of complex social behaviour that would otherwise be lost in static stimuli, providing a contextual narrative that is now believed to explain some of OT's effects, but that is frequently overlooked in most studies (Bartz et al., 2011; Egito et al., 2020; Marsh et al., 2021). The physical salient properties of these social interactions, along with the unfolding narrative, also demand an interactive processing between bottom-up and top-down mechanisms which together modulate attention (Ludwig et al., 2020; Sonkusare et al., 2019). Using videoclips and IN-OT pharmacological intervention, we confirmed OT's role in attention orienting responses by detecting moderate to large effects (Cohen's h ranging between 0.21 and 1.51, inclusive) that show OT increased the salience for dynamic social as well as non-social scenes - in line with the general approach-withdrawal hypothesis of OT (Harari-Dahan & Bernstein, 2014). Importantly, we found these effects generalize to both positive and negative, and to high and low arousing social scenes. The latter is in line with

recent work, also using eye-tracking, but specifically in binocular visual rivalry, which found that IN-OT increased the salience for human faces with varying emotional expressions (Hovey et al., 2020). Within non-social videoclips, we also found a IN-OT-induced increase in salience scores, except in one videoclip. These stimuli consisted of changing landscapes without any actors, social connotations, or apparent narrative and yet they were made more salient by IN-OT. Indeed, in their validation study, these non-social stimuli were rated more pleasurable than the negative, but less than the positive, irrespective of arousal (Carvalho et al., 2012), potentially revealing that despite being non-social they were still perceived as relevant/rewarding. This is in line with evidence showing that stimuli depicting beautiful natural landscapes are generally perceived as rewarding (Joye et al., 2024), and by studies showing that repeated engagement with natural landscapes are associated with positive feelings of calmness and peacefulness (Richardson & Hallam, 2013), and that being close to nature improves general wellbeing (Ohly et al., 2016).

IN-OT increased subjective arousal ratings for only one erotic videoclip

Regarding subjective arousal ratings, we found IN-OT to increase the arousal perception of only one social interaction of positive-valence high-arousal videoclip category, a moderate effect (Cohen's d = 0.73). This videoclip showed a heterosexual couple engaging in sexual intercourse and was selected, like the other erotic stimuli, for eliciting high arousal as validated in a previous study which also showed that this category elicited the highest skin conductance levels, a robust marker of physiological arousal (Carvalho et al., 2012; Rosebrock et al., 2017; C. Wang et al., 2018), compared to the other categories we report here. Moreover, our result that IN-OT increased subjective arousal perception in response to an erotic dynamic stimuli is consistent with a recent review that highlights OT's role in sexual arousal (Cera et al., 2021), and with other previous evidence supporting OT's involvement in orgasm and sexual behaviour (Alley et al., 2019; Alley & Diamond, 2020).

Limitations

Our study design prioritized social stimuli given OT's well-known role on social cognition (Marsh et al., 2021) which limited the variability in the content of our non-social videoclips portraying landscapes where there was an unbalanced number of social and non-social stimuli

(16 vs. 4). Generalizing the IN-OT effects we found for our non-social stimuli requires caution, as they might not extend to other non-social contexts which warrants future research. Additionally, despite the benefits of our dynamic social stimuli (Sonkusare et al., 2019), we note that they were explicitly fake and not purely naturalistic which might alter their perception and visual inspection. Lastly, OT's effects are also known to be sex-specific and affected by individual characteristics (Bartz et al., 2011), which limits the reach of our results given that our sample only included heterosexual males.

4.5 Conclusions

In this study we aimed to test the effects of IN-OT on the temporal and spatial salience attribution, including both of social and non-social videoclips. From the eye-gaze of multiple observers we calculated GLIMPSE salience scores that incorporate the spatial and temporal physical salience of dynamic videoclips depicting social interactions and landscapes. We found that IN-OT increased the GLIMPSE salience scores during 18 videoclips (out of 20), and decreased them during an erotic stimuli, and showed no differences with placebo during a non-social. Collected during a second viewing of the same stimuli, IN-OT only increased the subjective arousal ratings during an erotic interaction and had no difference in any other videoclip. Altogether, our results validate OT's expected role in heightening the salience for social and non-social stimuli, especially during free-viewing of dynamic stimuli like videoclips.

5- Oxytocin's role on central and autonomic psychophysiological correlates of salience attribution: a pupil size and eye-gaze pharmacological study

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5.1 Introduction

The hypothalamic neuropeptide OT plays a major role in modulating social cognition. Earlier non-human animal research showed that OT facilitates behaviours of approach and socialization by reducing fear and anxiety and heightening stress tolerance. Accordingly, IN-OT in humans has been shown to improve social cognitive processes such as: increased salience attribution to human faces (Prehn et al., 2013); heightened recognition of facial expressions (Rimmele et al., 2009); and prolonged eye-gaze to the eye region of human faces (Guastella et al., 2008). However, IN-OT's effects seem not to be purely prosocial as recently reviewed (Marsh et al., 2021). They depend on contextual setting and individual personal factors given that IN-OT has also been shown to elicit envy, conflict, and prejudice in competitive settings, stemming from individual variations in perception of social threats, attitudes, and response to emotional stimuli. Based on these apparently conflicting findings, the social salience hypothesis of OT emerged which posits OT to facilitate the salience attribution to both positive and negative social stimuli (Shamay-Tsoory & Abu-Akel, 2016). Subsequently, this 'social' specificity of OT's role has also been questioned. Indeed, IN-OT has been shown to: 1) reduce amygdala activation in response to both fear-inducing social and non-social stimuli (even if this effect was stronger for the social stimuli) (Kirsch et al., 2005) and in avoidant behaviours related to emotional and personally relevant social and non-social stimuli (when compared to neutral) (Harari-Dahan & Bernstein, 2017); and 2) to increase the activation of the ventral tegmental area, substantia nigra and the left and right nucleus accumbens (crucial areas in the salience and reward circuity) in non-social rewarding tasks (Love, 2014; Mickey et al., 2016). This, in turn, motivated the general approach-withdrawal hypothesis which states that OT heightens the salience of 'personally relevant or emotionally evocative stimuli', regardless of their socialness, by acting on approach/motivation and avoidance/withdrawal circuitry (Harari-Dahan & Bernstein, 2014). Nevertheless, previous IN-OT studies contrasting its effects on the salience of both social and non-social stimuli (M. Eckstein et al., 2019), have not dissociated socialness from their reward. This is necessary because a stimulus's socialness is commonly confounded by its relevance, especially for faces (Hessels, 2020; Leopold & Rhodes, 2010; Ro et al., 2007; Yarbus, 1967), which are inherently more relevant than non-social stimuli. Accurately testing whether OT exerts its effects on the response to predominantly social or generally motivational/relevant stimuli, by juxtaposing both hypotheses is warranted, and can be achieved by orthogonalizing their social and reward values.

A stimulus' physical salient properties, like colour or shape, may elicit an attention orienting response guided by sensory information in an automatic bottom-up manner (Koenig et al., 2017; Theeuwes & Belopolsky, 2012), but this attentional bias can be further altered by the stimulus' motivational salience, that is, its inherent rewarding or aversive value (Bromberg-Martin et al., 2010; Koenig et al., 2017; Theeuwes & Belopolsky, 2012). OT's role on salience attribution, which is the focus of both above hypotheses, is likely to be explained by its modulation of the dopaminergic system (Bartz et al., 2011; Guastella & MacLeod, 2012; Heinrichs et al., 2009; Landgraf & Neumann, 2004; Meyer-Lindenberg et al., 2011). Indeed, this system is well-known to have a central role in processing aversive, arousing and rewarding stimuli, as well as assigning motivational salience and reorienting attention to stimuli (Berridge, 2007; Love, 2014). These salience attribution processes can be studied with an eye tracker by measuring pupil size, dwell time and spontaneous eye blink rate (sEBR). Pupil size serves as an autonomic nervous system's positive proxy for arousal and cognitive effort (Bradley et al., 2008; C. Wang et al., 2018), dwell time as a central nervous system positive proxy of attention attribution and level of interest in a stimuli (Mahanama et al., 2022; Mele et al., 2014; Nummenmaa & Calder, 2009), and sEBR a central nervous system positive proxy of tonic dopaminergic levels reflecting motivation towards goal and reward oriented behaviours (M. K. Eckstein et al., 2017; Groman et al., 2014; Jongkees & Colzato, 2016).

Pupil size has been shown to increase in the presence of both social (vs. non-social) (Frost-Karlsson et al., 2019; Geangu et al., 2011) and high-reinforcement (vs. low-reinforcement) stimuli (Pietrock et al., 2019). Previous studies have found IN-OT increased pupil size in response to emotional (vs. neutral) facial stimuli (Leknes et al., 2013; Prehn et al., 2013) and for both social and non-social stimuli, although particularly higher in the former, and more for some social stimuli (e.g., sexual) than others (e.g., parent-child) (M. Eckstein et al., 2019). There also exists, however, contradicting evidence where IN-OT decreased pupil size for both social (angry, happy, and ambiguous facial expressions) and non-social stimuli (geometrical shapes, but only for 8 IU and not the commonly used 24 IU) (Quintana, Westlye, et al., 2019b). On the other hand, dwell time has been shown to: 1) increase for both social (vs. non-social) (Chakrabarti et al., 2017; Vernetti et al., 2017); 2) increase for high-reinforcement (vs- low-reinforcement) stimuli, when they are presented as the action cues (before feedback presentation) [34,39]; and, 3) slightly increase for high vs low- reinforcement stimuli when presented as distractors, this difference is not statistically significant (before feedback presentation) (Koenig et al., 2017; Le Pelley et al., 2015; Theeuwes & Belopolsky, 2012;

Watson et al., 2020). IN-OT (vs. placebo) has been shown to increase dwell time for happy faces when shown together with sad, angry and neutral (Boyle et al., 2022). However, as previously mentioned for behavioural findings, these studies with eye tracking psychophysiological correlates also do not ascertain whether it is the social component or the reward component of the stimuli that triggers the response to exogenous OT. To the best of our knowledge, no study has yet probed the effects of IN-OT on sEBR.

In this study, we aimed to test whether OT plays a role in salience attribution to social stimuli (expressing fearfulness) specifically, or to relevant stimuli in general, by orthogonalizing the socialness and reward variables of conditioned stimuli (CS). To achieve this, we applied an attention orienting and reinforcement learning paradigm which is an adapted version of the Salience Attribution Task (SAT) (Roiser et al., 2009) - henceforth referred to as the social SAT (sSAT) (Santiago et al., 2024). This task elicits a focus on a colour-dependent reward (i.e., higher or lower reinforcement probability – RP) conditioned equally to both social and non-social stimuli. We investigated IN-OT's impact on central and autonomic nervous system correlates of motivational salience attribution, as we concomitantly recorded participants' pupil size and dwell time over the CS during the duration of the sSAT (**Figure 9**), and sEBR during a posterior resting state moment. Regarding OT, we predicted an effect on pupil size and dwell time specifically during reward anticipation (i.e. from sSAT's probe offset and until feedback onset) as this was where we presumed a phasic dopaminergic firing response to the CS putatively would elicit motivational salience attribution (Rademacher et al., 2017). Thus, the social salience hypothesis of OT would be supported if IN-OT increased pupil size and dwell time for social more than non-social stimuli (i.e., a 'drug by socialness' interaction) and, on the other hand, the general approach-withdrawal hypothesis would be supported if IN-OT increased pupil size and dwell time for high-RP more than low-RP (i.e., a 'drug by RP' interaction). Lastly, both hypotheses would be supported by a 'drug x socialness x RP' interaction. Specifically for dwell time, we tested these hypotheses while also testing the influence of sEBR, as a proxy of baseline (tonic) dopaminergic levels given that these might influence phasic dopaminergic sensitivity (Grace, 1991). Furthermore, we predicted, as validations of our operationalization, that social (vs. non-social), and high-RP (vs. low-RP) CS presentation would elicit increased pupil size and dwell time, and that gains (upon outcome feedback, i.e. during reward consummation) would elicit increased pupil size and *decreased* dwell time towards the CS.

5.2 Methods

Participants

A total of 62 subjects were recruited through social media and word-of-mouth. All participants were white Portuguese, healthy males, aged 20–35 years old (recruited by design, as OT's effects have shown to be affected by sex and age (Bartz et al., 2011)), right-handed, not-colour blind, had European Portuguese as a first language and at least 12 years of education. Exclusion criteria were self-reported premature birth (≤ 36 weeks) with associated health consequences, prior head trauma with loss of consciousness or seizures, prior or current neurological or psychiatric disorders, history of drug or substance abuse, use of psychotropic or hormonal medication in the last 3 months, and colour-blindness. Twenty-four hours before the experiment, participants were asked to abstain from consuming caffeine, alcohol, tobacco or drugs, and intense physical (sports) and sexual activity; as well as to abstain from cannabis consumption for 1 week before the experiment. A drug screening test (for amphetamine, benzodiazepine, cocaine, methamphetamine, morphine/opiates, tetrahydrocannabinol (THC); nal von minden Drug-Screen®) and an interview were conducted on the day of the experiment to confirm pre-requisites were followed.

Of the 62 participants, 7 participants were excluded due to technical data acquisition problems, plus one at random to exactly match drug groups for the sSAT reward version (i.e., of the red or blue colour being reinforced), totalling a final sample of 54 (IN-OT: N = 28; placebo: N = 26).

Experimental procedure

The session took place at the LAPSO lab of ISCTE-IUL (Lisbon, Portugal), was approved by its ethics committee (Ref 19/2019), and was part of an umbrella project, which involved the collection of EEG and sSAT behavioural data which we have reported elsewhere (Santiago et al., 2024), and two blood collections (spaced +/- 17mn and just before sSAT) not herein analysed. Participants signed a written informed consent and were monetarily compensated for their time, receiving 15-35€ in gift vouchers, depending on their task performance.

Following the drug test, participants completed the Digit Span test from WAIS-III, the anxiety state subscale (Y1) of STAI Y Form and the Empathy Quotient (for approx. 10 min). This was followed by a sSAT tutorial and practice session (see below). A between-subjects randomized

double-blinded controlled IN-OT/placebo administration was performed, 17 min after which, on average, we calibrated the eye tracking device and ran a second sSAT practice session (see below). The main sSAT started, on average, 29 min following drug administration (Cosme et al., 2023; Spengler et al., 2017; Zelenina et al., 2022).

Social Salience Attribution Task

The sSAT, as the non-social Salience Attribution Task (Roiser et al., 2009), is played in two blocks, and required participants to respond as fast as possible (by pressing the space bar on a computer keyboard) following the appearance of a probe (black square) to earn money (**Figure 9**), which was measured as reaction time. For a more detailed description of the tutorial and practice sessions, see **Annex C**. The standard deviation of the fastest half of the trials from the 2nd practice session was used to set the minimum and maximum probe durations during the 1st block of the task (for details, see **Annex C**). Thus, the probe appearance interval is specifically calculated for each participant to adjust task difficulty. On reinforced trials, the reward was dependent on how fast participants responded, with feedback in the centre of the screen indicating how much money they received. (See **Annex C** for a more detailed description of the tutorial, practice sessions, and individual task difficulty and reward calculation). The probability of reinforcement in a given trial was signalled by one of four types of CS, which varied in two orthogonal visual dimensions: colour (blue or red) and socialness (fruit or fearful face) (**Figure 9**, top left). The colour dimension was task-relevant, meaning one colour was reinforced in 35 out of 40 trials (87.5%) and the other colour in 5 out of 40 trials (12.5%), randomised between participants and kept the same across one participant session. The socialness dimension was task-irrelevant, meaning both fruits and faces were reinforced in 20 out of 40 trials (50%). Participants were not informed of these contingencies but were instructed to work out the probability of the reward associated with every stimulus type, and asked to estimate it at the end of each block, as reported in (Santiago et al., 2024).

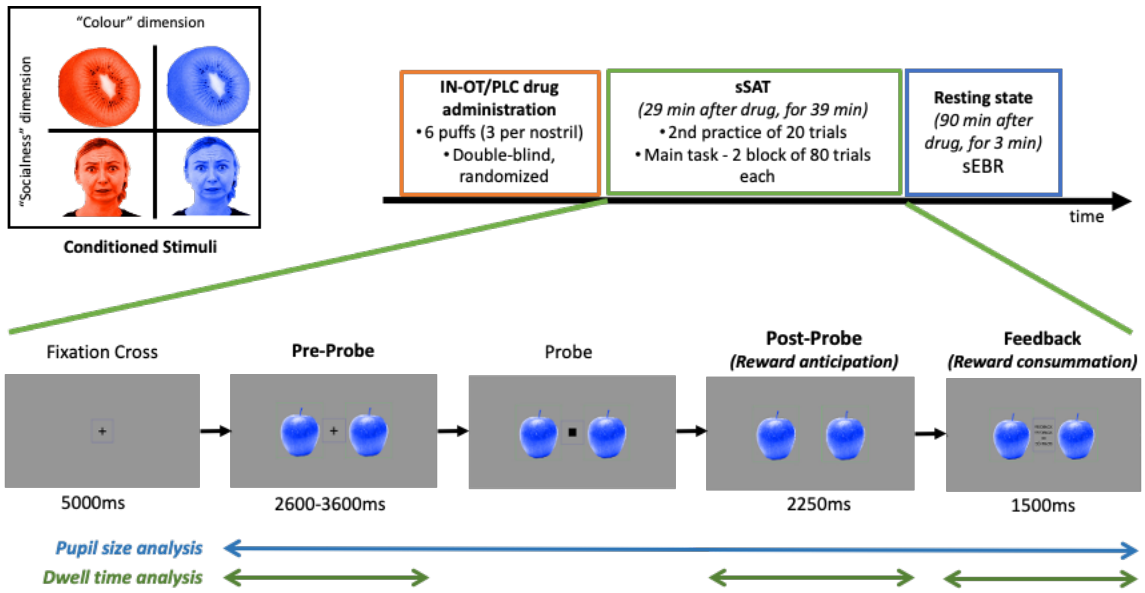

Figure 9 - Outline of an experimental session. Upper-left corner: Example of the four types of CS, divided according to their visual dimensions: colour (blue/red) and socialness (face/fruit), each colour being associated with a high or low (counterbalanced between subjects) reinforcement probability. Rest of figure: Outline of a sSAT trial. Participants were presented with stimuli on either side of the fixation cross. A probe (black square) then replaced the fixation cross, and participants were required to respond to it as quickly as possible via a button press. The participants' sSAT response was followed by a reward anticipation moment (Post-Probe) and then a reward consummation moment (Feedback). Pupil size was analysed from Pre-Probe until Feedback (inclusive); and dwell time was analysed in three separate moments: Pre-Probe, Post-Probe, and Feedback. sEBR was recorded during a posterior resting state window. sSAT – social salience attribution task; IN-OT – intranasal oxytocin; PLC – Placebo; CS – conditioned stimuli; sEBR – spontaneous eye blink rate.

Stimuli

There were two blocks of 80 trials each. Each of the 40 unique stimuli were presented in both blocks, once in each block. The social stimuli consisted of 20 pictures of fearful faces (10 male and 10 female, all white Caucasian), obtained from the "Warsaw Set of Emotional Facial Expression Pictures", selected based on their purity score (i.e., if the emotion being displayed was evident rather than mixed with other emotions) (Olszanowski et al., 2015). We selected the fearful facial expression because – among all emotions - it is the one most repeatedly shown to elicit a response to IN-OT - and has, for this reason, also been commonly the focus in studies

of the neural effects of IN-OT (Domes, Heinrichs, Gläscher, et al., 2007; M. Eckstein et al., 2015; Kanat et al., 2015; Kirsch et al., 2005; Petrovic et al., 2008; Tully et al., 2018, 2023). Adding other emotions would have increased the task length over 40 min which would be too tiring to participants (please see also our "Limitations" section). Non-social stimuli consisted of 20 pictures of different fruits obtained from the Google Images website. We have chosen fruits as non-social stimuli, instead of the commonly used cars and houses, given: 1) their more ubiquitous presence and motivational/survival value across human evolutionary times; 2) their similarity in shape and complexity to faces; and 3) absence of potential anthropomorphic facial characteristics. All pictures were selected and/or equalized (no differences at $p < .05$; female vs. male, faces vs. fruits, and red vs. blue) for: luminance (using the mean value of the image when converted to grayscale with rgb2gray Matlab function, and adjusted in Photopea); complexity (combining several features (Corchs et al., 2016)); and coverage (i.e., percentage of pixels which were not background).

Drug administration

Participants self-administered a nasal spray containing 24 IU of IN-OT (AlfaSigma, Bologna, Italy) or placebo (VolksApotheke Schffhausen, Switzerland), following recommendations described elsewhere (Guastella et al., 2013). The spray bottles were blinded in the Santa Maria pharmacy and were refrigerated until 1 hour maximum before administration. For details of the administration procedure, see **Annex C**. Drug groups differed, by chance, regarding age [t (54) = 0.86, p =.049, d = .237], with the IN-OT group being, on average, 1 year younger than the placebo group (IN-OT: M = 23.64, SD = 3.58; placebo: M = 24.62, SD = 4.61), but did not on the digit span test scores, anxiety state, or empathy scores (total or its domains of cognitive empathy, emotional reactivity, social skills, and empathic difficulties), with results reported elsewhere (Santiago et al., 2024). At the end of the experimental session, participants were unable to guess, above chance-level, whether they received an active agent or placebo (see **Annex C** for further blinding efficacy details).

Pupillometry and eye-gaze data acquisition and pre-processing

The experimental session took place in a quiet and slightly dimmed room, with lighting being constant across participants. Participants were asked to sit in a comfortable position and to

stabilize their heads on a chin rest to reduce head movement and to equalize head-to-screen distance at 93 cm and head-to-camera at 45 cm. Pupil size, and dwell time were recorded using an EyeLink Portable Duo (SR Research). Data was collected using corneal reflection and a centroid tracking algorithm binocularly (when not possible, the eye with the best calibration was chosen) at a 2000 Hz rate, with a typical accuracy ranging 0.25 to 0.50 visual angle. The data was pre-processed using SR Research's DataViewer v4.1.63 to exclude bins of 50 ms before and after blinks, thus removing effects of partial occlusion of the eye lid. Afterwards, pupil area was converted to pupil diameter. In case of binocular acquisition, the average pupil size of both eyes was extracted.

Pupil data was further pre-processed using CHAP toolbox (Hershman et al., 2019) version 1.6 for MATLAB R2022b. Datapoints with Z-scores (measured per trial) larger than 2.5 were considered outliers and removed, so were trials that had more than 20% of missing data. Blinks were detected by CHAP's algorithm (Hershman et al., 2018) and missing data was replaced via linear interpolation. The average pupil size 200ms before the onset of the CS was used for subtractive baseline correction. Pupil temporal analysis ranged from CS onset until the end of the trial (**Figures 9** and **10**).

Dwell time was measured as the total time the eye-gaze was over the two areas of interest which were two rectangles covering the two zones where the visual stimuli appeared. Like pupil size, outlier datapoints larger than 2.5 standard deviations from the mean were excluded. Dwell time was analysed in three separate time windows: 1) pre-probe, from CS onset until probe onset, 2) post-probe, from probe offset until feedback onset, and 3) from feedback onset until feedback offset (**Figure 9**).

Spontaneous eye blink rate

The sEBR was computed from data collected in a resting state period that occurred on average 90 minutes after drug administration in which participants were presented with a fixation cross that lasted 3 min. They were told to look at the fixation cross and just let the mind wander off, and there were no instructions related to blinking behaviour. Blinks were identified by Eyelink's SR Research proprietary blink detection algorithm that uses the abruptness of change in pupil size (SR Research, n.d.) to detect a blink event, thus avoiding classifying random missing data as blinks. The total blink count was divided by 3 to reflect the blink rate per minute (i.e. sEBR), and like for eye-gaze and pupillometry data, outliers exceeding 2.5 standard

deviations from the mean were excluded from analysis. There were no differences in sEBR between drug groups ($t(46) = 1.10$, $p = .277$). Although sEBR was analysed as a continuous covariate, we later categorized sEBR (for the purpose of discussing the results more clearly), into three distinct levels whereby values ranging between: 2.33 and 11.11 blinks/min were considered "low"; 11.11 and 17.22 blinks/min were considered "medium"; and 17.22 and 46.33 blinks/min were categorized "high". These categories were determined using the quartile ranges of the data.

Statistical analysis

All statistical models included Drug (IN-OT, placebo) as between-subject, and Socialness (fearful face, fruit) and RP (high-RP, low-RP) in the pre-probe to post-probe window analyses inclusive, or Outcome (gains, losses) in the feedback window analysis, as within-subjects fixed effects factors. The sEBR was only used as a continuous covariate in the analyses with dwell time. The RP and Outcome variables distinguished high-RP vs. low-RP, or gains vs. losses, respectively, irrespective of the CS colour.

We performed temporal analysis of pupil size by estimating a linear mixed model having participant as random intercept for each time bin, which corresponds to 0.5 ms, in RStudio using the lme4 package (Bates et al., 2015), following pupillometry methodology reported elsewhere (Mathôt et al., 2013, 2017). We considered temporal clusters to have statistically significant main effects or interactions of the above factors when there was a contiguous 200ms period (i.e. 400 time bins) of *p*-values less than .05 (Mathôt et al., 2013, 2017). Degrees of freedom and *p*-values were calculated using type III analysis of variance with the Satterthwaite's method. Within each of these clusters, for the time bin of peak statistical significance (i.e. the highest F-statistic), we performed and report pairwise t-test comparisons to explain significant interaction effects using the RStudio *emmeans* package, but for completeness, we visually represent clusters of pairwise t-test comparisons without reporting their statistic results or discussing them (**Figure 10**).

For dwell time analyses we estimated three linear mixed models - one for each time window: pre-probe, post-probe and feedback (**Figure 9**) - also in RStudio using the lme4 package (Bates et al., 2015). The specific moment of the probe appearance (i.e. from probe onset to probe offset) was excluded from analysis because it was too short as a standalone time window. We then performed pairwise t-test comparisons to explain significant interaction effects using the

RStudio *emmeans* package and calculated degrees of freedom and *p*-values using type III analysis of variance with the Satterthwaite's method.

We report a measure of effect size that is similar to Cohen's *d* but that is only computable for main effects of the linear mixed models (and impossible to calculate for interaction effects with the tools used) (Brysbaert & Stevens, 2018), and Cohen's *d* for all pairwise comparisons. Regarding the behavioural data, such as reaction time (implicit salience) and subjective reward probability (explicit salience), as we have previously reported (Santiago et al., 2024), we found an absence of main or interaction effects of RP, socialness, and drug, except that, as expected by design, participants gave significantly higher subjective reward probability scores to high-RP than low-RP stimuli.

5.3 Results

Pupil size from pre-probe to feedback (inclusive)

We found two temporal clusters where there was a significant main effect of socialness on pupil size. The first and largest cluster started at 314 ms after CS onset and ended at 3940 ms, lasting 3626 ms [peak F-statistic at 663 ms, $F(1, 5925) = 115.46$, $p < .001$, $d = 0.25$]. The second cluster started just before feedback onset, at 4904 ms, and lasted 1446 ms until the end of the trial [peak F-statistic at 5906.5ms, $F(1, 5926) = 30.60$, $p < .001$, $d = 0.13$]. In both these clusters, faces elicited larger pupil dilations than fruits (**Figure 10A** and **Table 4**). Similarly, we found a single cluster with a significant main effect of RP on pupil size that started before probe onset at 1437 ms, and ended 4913 ms after, at the end of the trial [peak F-statistic at 6350ms, $F(1, 5926) = 435.86$, $p < .001$, $d = 0.51$]. High-RP stimuli elicited larger pupil dilations compared to low-RP ones (**Figure 10B** and **Table 4**).

Finally, we found two clusters showing a significant interaction of drug by RP, both occurring after probe offset and before feedback. The first started at 3914 ms, and lasted 417 ms [peak F-statistic at 4029.5 ms, $F(1, 5926) = 7.32$, $p = .007$] and the second started at 4359 ms, lasting 637 ms [peak F-statistic at 4802 ms, $F(1, 5926) = 6.64$, $p = .010$]. Pairwise comparisons revealed that high-RP elicited larger pupil dilations than low-RP in the IN-OT group (1st cluster peak: $t(5925) = 8.23$, $p < .001$, Cohen's $d = 0.30$; at 2nd cluster peak: $t(5925) = 8.07$, $p < .001$, Cohen's $d = 0.30$), more so than in the placebo group (1st cluster peak: $t(5926) = 4.53$, $p < .001$,

Cohen's d = 0.16; at 2nd cluster peak: t(5926) = 4.54, *p* = .002, Cohen's d = 0.17, see **Figure 10C** and **Table 4**).

We did not find any significant clusters for the main effect of drug or for the interactions of socialness by drug, RP by socialness, and drug by RP by socialness across the trial's length.

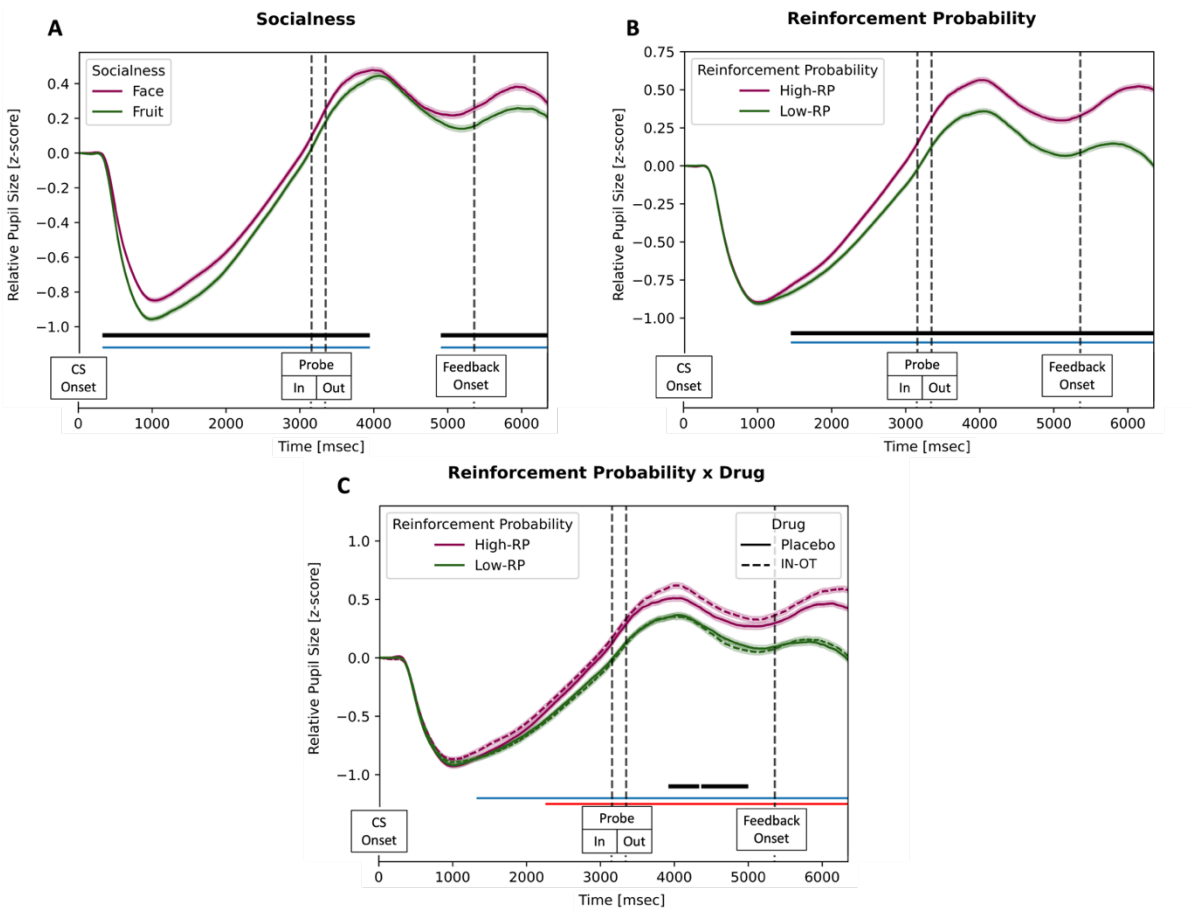

Figure 10 - Temporal profile of the pupil size change from CS onset (0 ms) over the sSAT plotted as a function of the effect of socialness (**A**), RP (**B**) and the RP by drug interaction (**C**). The horizontal black bars represent significant clusters for the overall main and/or interaction effects. For completeness, the blue/red horizontal bars in the bottom plot represent clusters of significant pairwise clusters whereby the blue bar represents the high-RP vs. low-RP contrast within the IN-OT group, and the red bar the same contrast within the placebo group. Descriptively, the pupil size exhibited a steep constriction following CS onset caused by the luminance change in the screen, and thereafter dilated until after probe offset, possibly reflecting cognitive processes related to preparation and execution of fast reaction times and/or reflecting arousal elicited by the CS. After these contributions dissipated, the pupil gradually constricted until the feedback onset where it dilated for a second time. Shaded areas represent

±1 SE. sSAT – social salience attribution task; IN-OT – intranasal oxytocin; CS – conditioned stimuli; RP – reinforcement probability.

Table 4 - Summary of the significant results of the temporal analysis of pupil size. Clusters start, end, duration and peak are in milliseconds. Abbreviations: df – degrees of freedom; den – denominator; num – numerator; RP – reinforcement probability; IN-OT – intranasal oxytocin; SE – standard error.

Temporal analysis of pupil size									
Main effect of socialness									
Cluster #	**Cluster Start**	**Cluster End**	**Cluster Duration**	**Time of Peak F-Statistic**	**F-Statistic**	**df (den, num)**	***p*-value**	**d**	**Direction**
1	314	3940	3626	663	116.46	1, 5925	< .001	0.25	Face > Fruit
2	4904	6350	1446	5906.5	30.60	1, 5926	< .001	0.13	Face > Fruit
Main effect of RP									
Cluster #	**Cluster Start**	**Cluster End**	**Cluster Duration**	**Time of Peak F-Statistic**	**F-Statistic**	**df (den, num)**	***p*-value**	**d**	**Direction**
1	1437	6350	4913	6350	435.86	1, 5926	< .001	0.51	High-RP > Low-RP
Drug by RP interaction									
Cluster #	**Cluster Start**	**Cluster End**	**Cluster Duration**	**Time of Peak F-Statistic**	**F-Statistic**	**df (den, num)**	***p*-value**	**Direction**	
1	3914	4331	417	4029.5	7.32	1, 5926	.007	See below	
2	4359	4996	637	4802	6.64	1, 5926	.010	See below	
Pairwise comparisons for the Drug by RP interaction									
Drug	**Cluster #**	**Mean High-RP**	**SE High-RP**	**Mean Low-RP**	**SE Low-RP**	**t-value**	**p-value**	**Cohen's d**	**Direction**
IN-OT	1	0.62	0.08	0.35	0.08	8.18	< .001	0.30	High-RP > Low-RP
	2	0.37	0.09	0.09	0.09	8.06	< .001	0.30	High-RP > Low-RP
Placebo	1	0.51	0.08	0.36	0.08	4.53	< .001	0.16	High-RP > Low-RP
	2	0.28	0.08	0.12	0.08	4.54	.002	0.17	High-RP > Low-RP

Dwell time during the pre-probe window

We only found a main effect of socialness [$F(1, 2265) = 4.93$, $p = .026$, $d = 0.03$] on dwell time such that participants spent more time observing fearful faces than fruits.

Dwell time during the post-probe window

There was a main effect of socialness [F(1, 5276) = 64.90, $p < .001$, d = 0.23] such that fearful faces elicited longer dwell times than fruits, and a main effect of sEBR [F(1, 41) = 4.34, $p = .043$] such that there was a negative relationship between dwell time and sEBR.

There was also a significant drug by socialness interaction [F(1, 5276) = 9.52, $p = .002$] and socialness by sEBR interaction [F(1, 5275) = 9.53, $p = .002$] on dwell time. Superseding these interactions, there was a three-way interaction between drug, socialness and sEBR [F(1, 5275) = 6.45, $p = .011$] where there was a negative relationship between dwell time and sEBR only in the placebo group and only for faces (β = -13.69, t(44) = 2.84, $p = .007$, Cohen's d = 0.43). Pairwise comparisons also revealed that for sEBR levels under 24.7 blinks/min, participants from both drug groups spent longer times looking at faces (vs. fruits, $p < .05$). However, in sEBR levels above 24.7 blinks/min, the difference only remained significant in the IN-OT group (at sEBR = 24.7, IN-OT: t(5273) = 4.19, $p < .001$, Cohen's d = 0.22; placebo: t(5477) = 1.94, $p = .052$, Cohen's d = 0.14, see **Figure 11** and **Table 5**).

Dwell time during the feedback window

There was a main effect of socialness [F(1, 5275) = 50.54, $p < .001$, d = 0,12] and of Outcome [F(1, 4520) = 56.82, $p < .001$, d = 0.15] on dwell time such that participants spent more time looking at faces compared to fruits, and to the CS during losses than in gains, respectively.

We also found an outcome by sEBR interaction [F(1, 4520) = 4.33, p = .038] in which pairwise comparisons revealed that there was no significant relationship between dwell time and sEBR in gains or losses, but that losses elicited longer dwell times than gains for sEBR levels under 36.6 blinks/min (at sEBR = 36.6, t(4521) = 1.96, $p = .050$, Cohen's d = 0.17; and at average sEBR = 13.91, t(4521) = 10.93, $p < .001$, Cohen's d = 0.33; see **Figure 11** and **Table 5**).

There was also a socialness by sEBR interaction [F(1, 4521) = 5.77, $p = 0.16$] such that there was no significant relationship between dwell time and sEBR in either level of socialness, but pairwise comparisons further revealed that faces elicited longer dwell times compared to fruits only at sEBR levels under 32.2 blinks/min (at sEBR = 32.2, t(4521) = 1.94, $p = .052$, Cohen's d = 0.14; and at average sEBR = 13.91, t(4523) = 9.60, $p < .001$, d = 0.29, see **Figure 11** and **Table 5**).

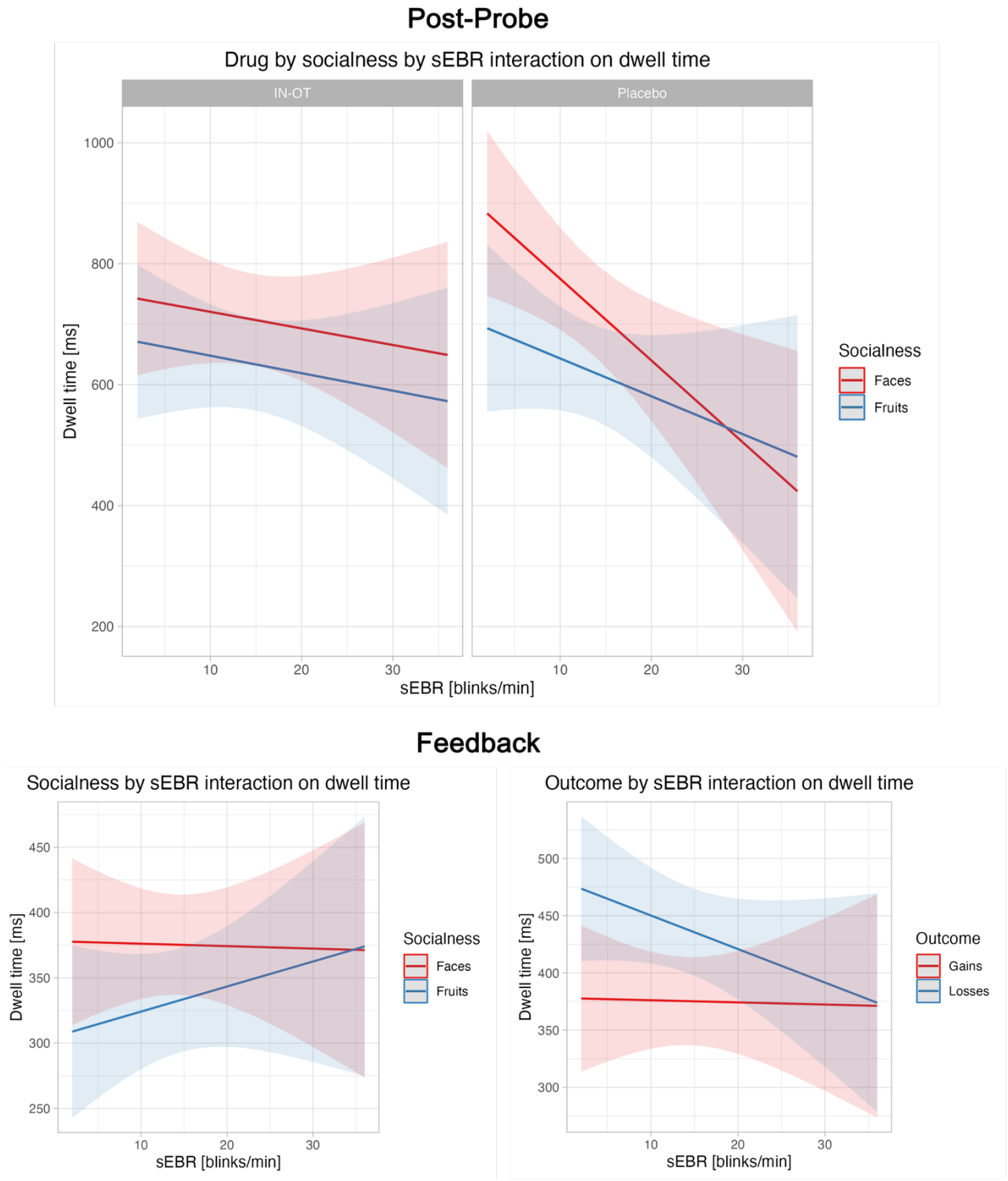

Figure 11 - Dwell time as a function of drug, socialness, RP, Outcome and/or sEBR during specific moments of the sSAT. Top row, during post-probe: The three-way interaction of drug, socialness and sEBR on dwell time. Bottom row, during feedback: the two-way socialness by sEBR (left) and Outcome by sEBR (right) interactions. IN-OT – intranasal oxytocin; sEBR – spontaneous eye blink rate; RP – reinforcement probability; sSAT – social salience attribution test; ms – milliseconds; min – minutes.

Table 5 - Summary of the statistically significant results of dwell time. Abbreviations: df – degree of freedom, num – numerator, den – denominator, RP – reinforcement probability, SE – standard error.

Dwell time during pre-probe screen									
Main effect of Socialness									
F-statistic			**df (num, den)**		***p*-value**	**d**		**Direction**	
4,93			1, 2265		.026	0.03		Faces > Fruits	
Dwell time during post-probe screen									
Main effect of Socialness									
F-statistic			**df (num, den)**		***p*-value**	**d**		**Direction**	
64.90			1, 5276		< .001	0.23		Faces > Fruits	
Main effect of sEBR									
F-Statistic			**df (den, num)**		***p*-value**	**d**		**Direction**	
4.34			1, 41		.043	-		Negative relationship	
Drug by Socialness interaction effect									
F-Statistic			**df (den, num)**		***p*-value**	**d**		**Direction**	
9.52			1, 5276		.002	-		See below	
Pairwise comparisons for the Drug by Socialness interaction									
Drug	**Mean Faces**	**SE Faces**	**Mean Fruits**	**SE Fruits**	**t-value**	**df**	**p-value**	**Cohen's d**	**Direction**
IN-OT	726.3	37.5	644.4	37.6	6.19	5274	< .001	0.24	Faces > Fruits
Placebo	739.1	38.4	625.5	38.7	8.15	5280	< .001	0.33	Faces > Fruits
Socialness by sEBR interaction effect									
F-Statistic			**df (den, num)**		***p*-value**	**d**		**Direction**	
9.52			1, 5275		.002	-		See below	
Pairwise comparisons for the Socialness by sEBR by Socialness interaction									
Socialness	**Regression Coefficient**		**SE**	**t-value**	**df**	***p*-value**	**Cohen's d**	**Direction**	
Faces	-8.15		3.13	2.60	43	.013	0.40	Negative relationship	
Fruits	-4.71		3.14	1.50	44	.141	0.23	-	
sEBR	**Mean Faces**	**SE Faces**	**Mean Fruits**	**SE Fruits**	**t-value**	**df**	**p-value**	**Cohen's d**	**Direction**
At average sEBR = 14.3	732.7	26.8	634.9	27.0	10.17	5278	< .001	0.28	Faces > Fruits
At sEBR = 30.8	598.1	59.1	557.1	59.3	1.94	5274	.052	0.12	-
Drug by sEBR by Socialness interaction effect									
F-Statistic			**df (den, num)**		***p*-value**	**d**		**Direction**	
6.45			1, 5275		.011	-		See below	
Pairwise comparisons for the Drug by sEBR by Socialness interaction									
Socialness	**Drug**	**Regression Coefficient**	**SE**	**t-value**	**df**	***p*-value**	**Cohen's d**	**Direction**	
Faces	IN-OT	-2.61	3.99	0.65	43	.517	0.10	-	
	Placebo	-13.69	4.82	2.84	44	.007	0.43	Negative relationship	
Fruits	IN-OT	-2.00	3.99	0.50	43	.620	0.08	-	
	Placebo	-7.42	4.85	1.53	44	.133	0.23	-	
sEBR	**Drug**	**Mean Faces**	**SE Faces**	**Mean Fruits**	**SE Fruits**	**t-value**	**df**	***p*-value**	**Cohen's d**	**Direction**
At average sEBR = 14.3	IN-OT	726.3	37.5	644.4	37.6	6.19	5274	< .001	0.24	Faces > Fruits
	Placebo	739.1	38.4	625.5	38.7	8.15	5280	< .001	0.33	Faces > Fruits
At sEBR = 24.7	IN-OT	699.2	54.9	623.6	55.1	4.19	5273	< .001	0.22	Faces > Fruits
	Placebo	596.5	66.0	548.1	66.4	1.94	5477	.052	0.14	-
Dwell time during Feedback screen									
Main effect of Socialness									
F-Statistic			**df (den, num)**		***p*-value**	**d**		**Direction**	
50.54			1, 5275		< .001	0.12		Faces > Fruits	

Main effect of Outcome										
F-Statistic				**df (den, num)**		***p*-value**	**d**		**Direction**	
56.82				1, 4520		< .001	0.15		Losses > Gains	
sEBR by Outcome interaction effect										
F-Statistic				**df (den, num)**		***p*-value**	**d**		**Direction**	
4.33				1, 4520		.038	-		See below	
Pairwise comparisons for the sEBR by Outcome interaction										
Outcome	**Regression Coefficient**		**SE**	**t-value**	**df**	***p*-value**	**Cohen's d**		**Direction**	
Gains	1.38		1.55	0.89	47	.379	0.13		-	
Losses	0.22		1.54	0.14	46	.888	0.02		-	
sEBR	**Mean Gains**	**SE Gains**	**Mean Losses**	**SE Losses**	**t-value**	**df**	***p*-value**	**Cohen's d**	**Direction**	
At average sEBR = 13.9	357.9	13.4	430.7	13.2	10.93	4521	< .001	0.33	Losses > Gains	
At sEBR = 36.6	389.2	37.6	425.7	37.3	1.96	4520	.050	0.17	-	
sEBR by Socialness interaction effect										
F-Statistic				**df (den, num)**		***p*-value**	**d**		**Direction**	
5.77				1, 4521		.016	-		See below	
Pairwise comparisons for the sEBR by Socialness interaction										
Socialness	**Regression Coefficient**		**SE**	**t-value**	**df**	***p*-value**	**Cohen's d**		**Direction**	
Faces	-0.34		1.54	0.22	45	.825	0.03		-	
Fruits	1.50		1.56	0.97	48	.339	0.14		-	
sEBR	**Mean Faces**	**SE Faces**	**Mean Fruits**	**SE Fruits**	**t-value**	**df**	***p*-value**	**Cohen's d**	**Direction**	
At average sEBR = 13.9	426.3	13.2	362.3	13.4	9.60	4523	< .001	0.29	Faces > Fruits	
At sEBR = 32.2	420.0	31.0	389.8	31.4	1.94	4521	.052	0.14	-	

5.4 Discussion

In this study we aimed to test the effects of IN-OT on motivational salience attribution, and particularly its social specificity, by using an adapted version of the SAT in which we orthogonalized the social and reward features of stimuli, while concomitantly recording eye tracking autonomic and central nervous system psychophysiological correlates in pupil size and dwell time, respectively. Importantly, in all conducted analyses, we did not find an interaction involving both socialness and RP suggesting that our orthogonalization by design was achieved. As expected, we found social and high-RP stimuli to elicit increased pupil size and longer dwell time than non-social and low-RP, respectively, and that gains (i.e. during reward consummation) would elicit increased pupil dilations and *decrease* dwell time compared to losses (Austin & Duka, 2012; Bourgeois et al., 2018; Chakrabarti et al., 2017; Frost-Karlsson et al., 2019; Geangu et al., 2011; Koenig et al., 2017; Le Pelley et al., 2015; Pietrock et al., 2019, 2019; Theeuwes & Belopolsky, 2012; Vernetti et al., 2017; Watson et al., 2020). Additionally, as expected from the literature (Koenig et al., 2017; Le Pelley et al., 2015; Theeuwes & Belopolsky, 2012; Watson et al., 2020), no RP effect was found for dwell time in the pre- and post-probe windows. Regarding our main aims, we initially hypothesized that we would find support for the social salience hypothesis of OT (Shamay-Tsoory & Abu-Akel,

2016) in the presence of a 'drug by socialness' interaction, or for the approach-withdrawal hypothesis (Harari-Dahan & Bernstein, 2014) in the presence of a 'drug by RP' interaction. We found support for both hypotheses but in different psychophysiological correlates: the social salience hypothesis was supported by dwell time data while concomitantly controlling for individual tonic dopamine levels (using sEBR as a proxy), whereas the approach-withdrawal hypothesis by pupil size data. These effects were also found only during reward anticipation (i.e. post-probe window), as expected, tentatively suggesting IN-OT interacted with phasic dopamine to reinforce reward-motivated saliency which we discuss in more detail next.

Fearful faces (vs. fruits) increased pupil dilation and dwell time

Our prediction that social (vs. non-social) CS presentation would increase pupil dilation was confirmed in two separate clusters, both showing larger dilations for social (fearful faces) than non-social (fruits), as hypothesized, given previous evidence showing the same (Frost-Karlsson et al., 2019; Geangu et al., 2011). The first and largest cluster occurred mostly during the pre-probe window, immediately after CS onset, and the second cluster started just before feedback onset and lasted until the end of the trial. We also found, as initially hypothesized, that participants dwelled longer over fearful faces than over fruits across the sSAT, replicating other studies that found social stimuli to attract more attentional orienting responses than non-social (Chakrabarti et al., 2017; Vernetti et al., 2017). Our successful orthogonalization with RP indicates that it was not their inherent rewarding features that motivated this pupil and eye-gaze behaviour – however we grant some motivational saliency may have persisted for faces over fruits (see Limitations section) – nor their complexity or luminance as these image features were also controlled for, but rather other aspects of fearful faces. Such humans' strong preference for social interaction, stimuli and/or environment is proposed by the social motivation theory (Chevallier et al., 2012) to be a consequence of three distinct factors: 1) social orienting, our inherent and preferable attention orienting response towards anything social; 2) social seeking, our ability to find reward and take pleasure for the social; and 3) social maintaining, our desire to repeatedly engage with others. In line with this theory, we find support in the social orienting factor in which social features of the fearful facial expressions are granted more attentional priority than fruits (Cheng et al., 2021).

It is also noticeable that there was no significant cluster for the effect of socialness on pupil dilation during reward anticipation (i.e. post-probe, **Figure 10**). We presume this occurred because the phasic dopamine released during this anticipatory time window modulated the motivational salience of fruits to match that of faces, as both stimuli were equally reinforced throughout the sSAT. We find, however, an effect of socialness on pupil dilation in the other moments of the task (pre-probe and feedback) because there would putatively be no reward anticipation-dependent phasic dopamine release therein, and thus, presumably, the inherent latent motivational saliency of faces significantly predominated over that of fruits.

Oxytocin increased pupil dilation during reward anticipation in highly reinforced trials

Our hypotheses that high-RP (vs. low-RP trials) during reward anticipation, and gains (vs. losses) during reward consummation, would elicit larger pupil dilations, were confirmed (**Figure 10B**). The effect of RP in particular emerged even before our designated window of reward anticipation, suggesting that this process started earlier than anticipated. Our results suggest: 1) participants learned the contingencies of the task, as the effect of RP emerged even before our designated window of reward anticipation, during CS onset, and confirmed by our behavioural findings reported elsewhere (Santiago et al., 2024); 2) before probe onset (i.e. pre-probe), participants were most likely exerting higher cognitive effort in high-RP (vs. low-RP) trials to minimize their reaction time and maximize their earnings, which is a pupil dilating inducing process (M. K. Eckstein et al., 2017; Skaramagkas et al., 2023); 3) between probe offset and feedback onset, high-RP (vs. low-RP) trials induced stronger reward anticipation, another process known to elicit pupil dilation (Schneider et al., 2018, 2020) and 4) in the feedback window, monetary gains elicited reward consummation which is also known to dilate the pupil (Guath et al., 2023).

Contrasting the temporal profiles of the main effects of socialness and of RP on pupil size in the pre-probe time window highlights an approximate 1000 ms difference between their starting points (**Figure 10A** and **10B**). Importantly, the effect of socialness was relatively early starting around 300 ms after CS onset which suggests the attribution of *perceptual* (i.e. physical) salience to faces (vs. fruits), which is expected given that the salience of faces is more hard-wired than that of fruits (Dekowska et al., 2008). On the other hand, the relatively delayed start of the effect of RP on pupil size suggests the attribution of *motivational* salience to high-RP (vs Low-RP) CS, which is a higher-order cognitive process dependent on contextual

evaluation (Bromberg-Martin et al., 2010). These timing differences corroborate our previous findings of electroencephalography data of the same experimental study whereby socialness modulated earlier event-related potentials, including N170, P2b and P3b, while RP modulated P3b and the late positive potential (Santiago et al., 2024).

Most importantly, we found that the abovementioned effect of RP during reward anticipation (i.e. in the post-probe window) was altered by IN-OT, in support for the approach-withdrawal hypothesis of OT, whereby OT presumably modulated the motivational salience of high- and low-RP stimuli, regardless of their socialness factor. In specific, after IN-OT (1st cluster: Cohen's d = 0.30; 2nd cluster: Cohen's d = 0.30), high-RP trials elicited larger pupil dilations than low-RP, more so than after placebo (1st cluster: Cohen's d = 0.16; 2nd cluster: Cohen's d = 0.17). This finding suggests OT's facilitation of dopamine's mesolimbic action putatively enhanced the subject's motivation to maximize their earnings, and to learn the contingencies of the task, processes that can be indexed by pupil dilation (F. Kraus et al., 2023; Schneider et al., 2018, 2020; Sibley et al., 2011) as an indicator of physiological arousal. Dopamine is a well-known salience attribution mediator, with dopamine striatal phasic bursts during reward, or reward anticipation, rendering CS desirable and arousing (Berke, 2018). Indeed, reward anticipation has been associated with positive arousal related to approach behaviours (Knutson & Greer, 2008) which in turn has been found to increase pupil dilations with concomitant activity in the salience network and enhanced task performance (Schneider et al., 2018). Herein we found support for a modulatory role of dopamine-OT interplay in approach/avoidant motivational processes, as posited by the approach-withdrawal hypothesis of OT (Harari-Dahan & Bernstein, 2017), with OT promoting the dopamine-coded 'wanting' that leads to the approach behaviour.

Oxytocin increased dwell time over social stimuli during reward anticipation, particularly in subjects with high tonic dopamine

We also found support for the social salience of OT hypothesis (Shamay-Tsoory & Abu-Akel, 2016) during reward anticipation but on dwell time and particularly in relation to tonic dopamine as measured with its proxy sEBR **(Figure 11** top row, **Table 5**). Dwell time was negatively associated with sEBR only in placebo and for fearful faces (Cohen's d = 0.43). This finding is consistent with previous evidence proposing that OT reinforces social stimuli, and thus increases their motivational salience, but herein we extend previous studies by showing

that this effect exists even after dissociating the socialness and reinforcement/relevance factors of (fearful) faces. Furthermore, the social salience hypothesis (Shamay-Tsoory & Abu-Akel, 2016) posits that these OT effects are dependent on baseline individual differences. Indeed we demonstrate that OT's effect on social stimuli's motivational salience is reliant on tonic dopaminergic levels, which, assessed via sEBR, has been found to predict positively individual differences in reward-driven behaviours (Jongkees & Colzato, 2016), precisely by promoting and sustaining goal-directed attitudes (Jongkees & Colzato, 2016). Here we found that high-sEBR (but not low- and medium-sEBR) participants, under placebo, were not as attentive to fearful faces (vs. fruits) as were participants under IN-OT, probably because they were more focused on the RP factor of the CS, i.e. on the monetary outcome. Indeed, IN-OT *negated* this tonic dopaminergic effect by rendering fearful faces more salient than fruits, in those putatively highly motivated (i.e. high-sEBR) individuals (Chiew et al., 2016; Jongkees & Colzato, 2016). We suggest that our findings reflect the modulatory effects of OT on the dopamine network towards increasing the social stimulus' reward value, irrespective of the accompanying monetary reinforcement.

Given that we restricted our design to include fearful faces, we cannot ascertain if the above effect is indeed generalizable to all social contexts or emotional expressions, or if fearfulness-specific. One of the most robust findings of IN-OT is its inhibitory effects on amygdala response to fearful faces (Domes, Heinrichs, Gläscher, et al., 2007; Gorka et al., 2015; Kanat et al., 2015; Petrovic et al., 2008; Spengler et al., 2017), and it has also been found that the effects of IN-OT on amygdala's response to social stimuli are modulated by dopamine availability (Sauer et al., 2013). It is thus possible that herein, high-sEBR might have led to an overly stimulated amygdala due to tonic dopamine which could have made them avoid fearful facial expressions – and that this was countered by IN-OT. Nevertheless, this remains speculative and to be ascertained with further research of the sSAT with other emotional expressions and dopaminergic manipulation.

During reward consummation, higher tonic dopamine *negates* the effects of socialness and outcome on dwell time

During the feedback window, as in the previous analysis periods, dwell time was measured as the total time the eye-gaze was over the CS. Importantly, the feedback information was given on the same screen therefore competing for the participant's attention (**Figure 9**). We did not

detect an effect of IN-OT on pupil size or dwell time during this reward consummation moment possibly because our reward was monetary, and a recent review indicates that IN-OT is most effective in increasing the salience of consumed social rewards (J. Kraus et al., 2023).

We did find a negative relationship between sEBR and dwell time during the feedback window (i.e. reward consummation) indicating that higher sEBR levels decreased the attention orienting response to CS (Chiew et al., 2016; Jongkees & Colzato, 2016). We also found a main effect of outcome such that losses elicited longer dwell than gains. This likely occurred because subjects were less engaged in reading the feedback and consuming the reward as it was negative, leading them to prioritize their focus on the CS. Importantly however, these main effects interacted whereby the effect of outcome was mitigated under higher levels of tonic dopamine which might have turned negative and positive outcomes equally motivationally salient. Furthermore, the increased attention (M. K. Eckstein et al., 2017; Wills et al., 2007) to CS during losses in low sEBR is possibly a result of ongoing learning processes (Wills et al., 2007), an idea which is supported by previous evidence that found lower tonic dopamine levels to be associated with a better ability to learn from negative outcomes (Pessiglione et al., 2006; van der Schaaf et al., 2014), a finding also supported by a study using sEBR (Slagter et al., 2015). Similarly, our reported socialness effect on dwell time during reward consummation, whereby fearful faces increased attentional demand, was mitigated by increased tonic dopamine levels. Like the previous finding, these results suggest that high tonic dopaminergic levels modulated attentional responses by making, this time, fruits as equally salient and/or relevant as their social counterparts.

Limitations

We highlight, again, that this work is limited in the generalizability of its findings regarding the socialness of the stimuli. In our operationalization of socialness, we decided for fearful faces only due to robust previous evidence of IN-OT's effects in response to faces expressing that particular emotion, and a single emotion would lead to a more parsimonious statistical model. Furthermore, our consistent main effect of socialness in both dwell time and pupil dilation strongly suggests that despite a successful orthogonalization between socialness and RP, faces are inherently more relevant than fruits and thus more salient and arousing, therefore naturally more evocative of attention reorienting responses. The sEBR covariate was not used in the pupil dilation analysis due to unfortunate methodological setbacks during data collection,

diminishing the scale of this study as it would provide, possibly, a greater understanding of the results reported herein, and to validate previous studies that have probed the effects of sEBR on pupil dilation (Tummeltshammer et al., 2019; Unsworth et al., 2019).

5.5 Conclusions

The social salience hypothesis of OT posits that OT's interplay with dopamine regulates the salience of social cues, depending on baseline individual differences (Shamay-Tsoory & Abu-Akel, 2016). However, the approach-withdrawal hypothesis questions the social specificity of the former by positing the OT-dopamine interaction to modulate the 'wanting' mesocorticolimbic circuitry of approach motivation that is linked to reinforcement learning behaviours in general (Harari-Dahan & Bernstein, 2014). We tested both hypotheses by orthogonalizing the socialness and reward factor of fearful faces using a reinforcement learning paradigm. Our findings support the social salience hypothesis in a psychophysiological correlate of attention orienting responses, eye-gaze's dwell time, while controlling for individual baseline tonic dopaminergic levels via sEBR. Additionally, we also found support for the approach-withdrawal hypothesis but in a correlate of arousal: pupil dilation. Crucially, both of these results only emerged during reward anticipation, which is consistent with it encompassing phasic dopaminergic activity (Lloyd & Nieuwenhuis, 2024). Taken together, our results revealed an expected OT-dopamine interplay which is at the core of both hypotheses.

6- Intranasal oxytocin reverses negative cooperation bias towards non-sexualized women by men: a pharmaco-electroencephalography study

This work is in preparation for submission:

Gonçalo Cosme*, Carlotta Cogoni*, Marta Patrocínio, Maciej Kosilo, Diana Prata. Intranasal oxytocin reverses negative cooperation bias towards non-sexualized women by men: a pharmaco-electroencephalography study. In preparation. * - authors gave equal contribution.

My contributions: Co-performed data collection, ran all statistical analyses and co-drafted the manuscript

6.1 Introduction

Sexualization is commonly defined as the emphasis on the sexual nature of a behaviour or person (Merriam-Webster, n.d.). When it leads to sexual objectification, i.e. the prioritization of seeing and treating others as objects of sexual desire rather than human beings (Beaman & Gurung, 2024), sexualization becomes deleterious (Fasoli et al., 2018; Gurung & Chrouser, 2007; Lamb & Koven, 2019). Sexual objectification is established from physical appearance judgements, and paves the way to sexist, misogynistic and abusive behaviour, women being the most common victims. The World Health Organization reports that, globally, nearly 1 in 3 women aged 15-49 have experienced physical and/or sexual violence by an intimate partner, non-partner or both (World Health Organization, 2021). Numerous visual perception studies have demonstrated that sexualized bodies can be cognitively processed as object-like (Bernard et al., 2012, 2018; Cogoni, Carnaghi, Mitrovic, et al., 2018; Vaes et al., 2019) and we have recently demonstrated that empathy for affective touch (Cogoni et al., 2020) and social pain (Cogoni, Carnaghi, & Silani, 2018) is reduced towards sexually-objectified women. But to our knowledge so far, only one study has tested the effects of sexualization on (ostensible) social interactions (with it having found men to be more aggressive against sexualized vs non-sexualized women after romantic rejection (Blake et al., 2017)). However, sexualization can be enjoyable to the sexualized when appearance-based sexual attention is perceived as positive and rewarding (Liss et al., 2011). Sexualization may also increase attraction and approach (Le Moëne & Ågmo, 2018), which can lead to prosocial behaviours by the opposite heterosexual sex (Iredale et al., 2008; Wilson & Eckel, 2006). As such, the effects of sexualization on social decision-making, i.e. on prosocial or cooperative behaviour (vs. pro-self and competitive) are still unclear. Also, surprisingly little is known about the psychophysiological correlates of sexualization, and its underlying neuroendocrine mechanisms (Cogoni, Monachesi, et al., 2023).

Cooperative/competitive attitude models are usually tested using social dilemma paradigms such as the PD. This game involves two players who choose to either defect or cooperate, and the outcome is dependent upon the combination of the choices made by the two players, where, usually, one opponent's choice is made after having known the choice of the other (sequential PD version). Furthermore, the PD may also be played between the same players in a repeated fashion (iterated PD version) whereby mutual cooperation yields the maximum gain for both players (Axelrod & Hamilton, 1981). Expectedly, social closeness (i.e. familiarity/in-groupness) between the players has been found to increase cooperative behaviour in this

(Majolo et al., 2006) and other social dilemmas (Y. Chen et al., 2017). In addition, objectification may decrease cooperation as has been shown with computers vs. humans (Rilling et al., 2012), as well as with roulettes vs. computers vs. humans (Cogoni, Fiuza, et al., 2023), albeit not consistently as we have shown (Neto et al., 2020). However, whether a sexualized woman is perceived by a heterosexual man as closer (as in more approachable) or as more distant (as in more objectified and thus less-human) – which would hypothetically have opposite consequents in terms of cooperation – is still unclear.

The neuropeptide OT is a known modulator of social bonding whose effects are group-context dependent (i.e. in-group/out-group) (Egito et al., 2020; Kim et al., 2021; Marsh et al., 2021; Triki et al., 2022; Zhang et al., 2019). IN-OT has been shown to increase prosocial abilities like empathy towards ostracized individuals (Riem et al., 2013), cooperation with in-group members (Trumble et al., 2015), and to decrease competition with out-group members especially in individuals with low trait emotional empathy (Schiller et al., 2020). Particularly during PD games, IN-OT has been found to increase the frequency of cooperative behaviours against computers vs. human opponents (treating computers more like humans) but only by female participants (Rilling et al., 2014), whereas in men, OT increased the activity of reward and social bonding brain regions during cooperative interactions (Rilling et al., 2014). More generally, IN-OT has been shown to induce better inference of others' mental states (Domes, Heinrichs, Michel, et al., 2007), better emotion recognition (Lischke et al., 2012), better memory for social stimuli (Rimmele et al., 2009), increased generosity (Zak et al., 2007), and tolerance to betrayal (Baumgartner et al., 2008). Given the above well-supported role of OT in modulating cooperation in social dilemmas like the PD, and furthermore in a group-context dependent manner, it is plausible that OT would modulate the influence of sexualization on cooperation. However, this has never been investigated.

The psychophysiological correlates of cooperation and competition in the iterative and sequential PD are commonly restricted to the moment of outcome evaluation by the players. This moment entails the identification of the salient features useful for learning how to act adaptively in the next interaction, and the player's physiological reactions may be a good indicator of his/her attitude towards the opponent (Y. Wang et al., 2014). Particularly for EEG and during feedback processing, the P3 ERP component is elicited. The P3, a large positive deflection peaking at 300 to 600 ms after stimulus onset in the centro-parietal cortex (Linden, 2005; Verleger, 1988), has been linked to attentional processes, and is considered to reflect the allocation of neural resources and subsequent memory context-updating processes (Hajcak &

Foti, 2020), which are crucial in iterative and sequential social dilemmas such as the PD. Its amplitude increases with the stimuli's degree of arousal, putatively reflecting a higher neural resources allocation (Nieuwenhuis et al., 2005). In accordance, in economic games, P3 amplitude has been shown to increase following unexpected events (Bell et al., 2015; Hajcak et al., 2005), and while gaming against strangers (out-group) vs friends (in-group) (Y. Chen et al., 2017; Y. Wang et al., 2013). Another ERP, the FRN, a negative deflection peaking at 250 to 350 ms (Proudfit, 2014) following stimulus onset and maximal in the fronto-central cortex (Proudfit, 2014), is known to show an increased negative amplitude to unfavourable vs. favourable outcomes (San Martín, 2012). Previous studies revealed that IN-OT, relative to placebo, increased P3 amplitude in women during an infant facial processing task (Rutherford et al., 2017) and reduced the FRN amplitude difference between positive and negative feedback (with placebo showing larger FRN for negative unfavourable feedback) (Zhuang et al., 2020), which could indicate that OT may enhance learning from positive favourable feedback. Given the above, EEG is a promising tool to investigate the neural activity underlying PD's outcome evaluation and behaviour (as previously done (Cervantes Constantino et al., 2021)), and its potential modulation by IN-OT and/or sexualization which, to our knowledge, is still unexplored.

In the present pharmaco-EEG study, we used a 'participant playing first', iterative and sequential PD paradigm (**Figure 12**) and double-blind randomized-controlled IN-OT administration to investigate if the degree of male cooperation with female opponents, and associated outcomes' valence and expectancy ERPs, are influenced by the sexualization of these opponents, and whether these effects are further influenced by IN-OT administration. We hypothesized that we would either find: 1) a bias towards cooperating less with sexualized than with non-sexualized women, as the former may be more regarded as out-group members; worthy of lower empathy (Cogoni, Carnaghi, & Silani, 2018), possessing lower competence and morals (Fasoli et al., 2018; Heflick et al., 2011; Heflick & Goldenberg, 2009; Smith et al., 2018), and consequently dehumanized (Bernard & Wollast, 2019; Vaes et al., 2011) – a behaviour that is often expressed toward to members of out-groups (Bernard et al., 2020; Bernard & Wollast, 2019; Buckels & Trapnell, 2013; Hodson & Costello, 2007); or, by the contrary, 2) a bias towards more cooperation toward sexualized women, as sexual appearance can be perceived as positive and rewarding (Liss et al., 2011), increase attraction and approach , and lead to prosocial behaviours by the opposite heterosexual sex (Iredale et al., 2008; Wilson & Eckel, 2006). Whatever the behavioural finding, we further predicted

that IN-OT would moderate any bias, also in a non-directional hypothesis given previous literature suggesting both that IN-OT increases prosocial behaviour is general but also competitive behaviour with out-group members (Egito et al., 2020; Zhang et al., 2019). Regarding the psychophysiological correlates of the above behaviour, first we predicted a larger P3 amplitude when subjects would play with sexualized women than with non-sexualized women (following evidence of this having been shown against strangers (out-group) vs. friends (in-group) (Y. Chen et al., 2017; Y. Wang et al., 2013)), further influenced by the expectancy of the outcomes (given evidence of P3 increasing for unexpected events (Bell et al., 2015; Hajcak et al., 2005)), and with IN-OT modulating this effect (again, in an unpredicted direction). Second, we also predicted a larger FRN amplitude towards sexualized (vs. non-sexualized), reflecting a negative bias of sexualization, further influenced by outcomes' valence (increased FRN for losses vs. gains), and that these valence effects would be minimized by OT (Zhuang et al., 2020). Thirdly, we also explored (i.e. without a prior evidence-supported hypothesis) if sexualization and OT influenced P3 latency. For all the above effects, behavioural and neural, we have added play-order as a variable of interest, given that we could not assume that playing first with a sexualized vs. non-sexualized opponent in PD, would not influence behaviour with the next opponent. To add interpretability, we also measured the subjects' explicit perceptions of their opponents' characteristics and attitudes, and the subject's emotional intensity upon each outcome of the game.

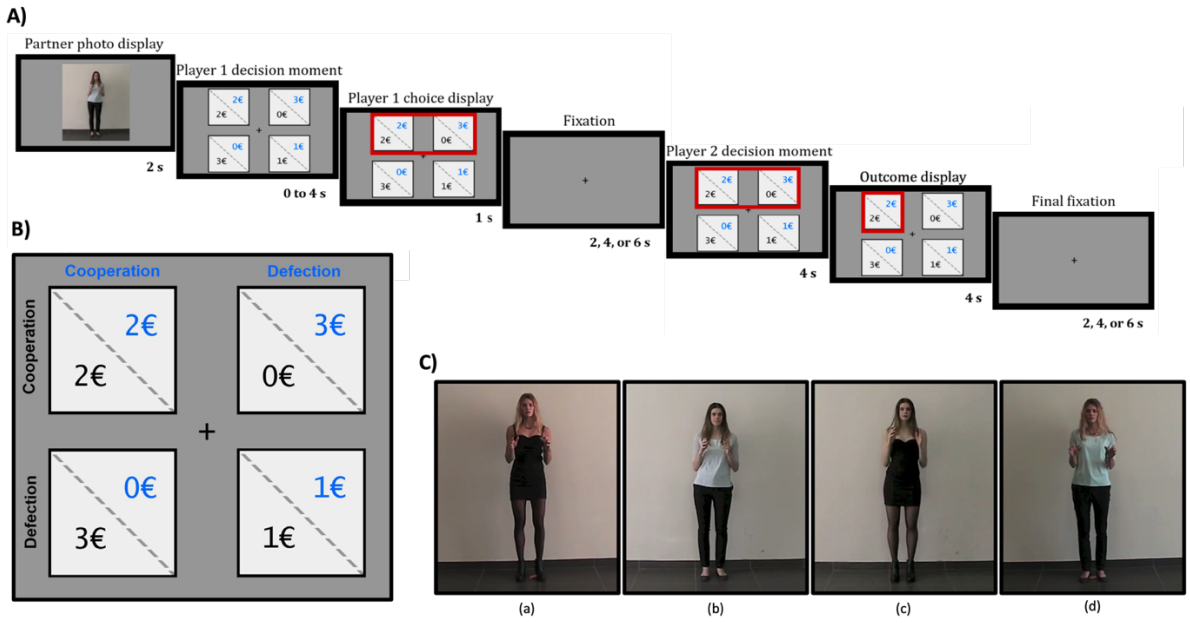

Figure 12 - Prisoner's Dilemma design. **A**) Trial timeline. At the beginning of each trial, the opponent's photo was displayed for 2 s. Player 1 (i.e., the participant) was always the first to

decide in this interaction and had 4 s to decide to cooperate or defect, and his reply was immediately highlighted and displayed for Player 2 for 1 s. A fixation cross with a variable length of 2, 4, or 6 s followed. Then, Player 2 (i.e., the opponent, which had every move established by a preprogrammed algorithm) had 4 s to reciprocate the participant's choice or not. Afterwards, the outcome of the trial was displayed for another 4 s (the possible outcomes being: Cooperation-Cooperation [CC], Cooperation-Defection [CD]; Defection-Cooperation [DC], Defection-Defection [DD]). During the feedback phase, the trial's outcome is highlighted with a red square. In this figure's exemplified trial, the participant selected cooperation, and the opponent reciprocated with another cooperation. Finally, the trial ended with another variable length fixation period of either 2, 4, or 6 s. **B**) Payoff matrix of the PD game. The numbers in black and blue indicate the payoff of the participant and the opponent, respectively. **C**) Opponents. Opponents of the PD game, as used elsewhere (Cogoni, Carnaghi, & Silani, 2018). The participant played the PD game in two blocks, a round against a sexualized opponent and another with a non-sexualized opponent, in a randomly assigned order. The sexualized target had a short dress, heels, and heavy makeup, and could be either blonde (a) or brunette (c); the non-sexualized target was wearing comfortable trousers, a jersey, flat shoes, and light makeup, and could also be either blonde (d) or brunette (b). Each participant saw through the entire game with the same combination of opponents (i.e., if the blonde confederate was wearing a sexually-objectified outfit, the brunette was wearing the non-sexually-objectified ones, and vice-versa).

6.2 Methods

Participants

A total of 55 male participants were recruited for the study. Three participants were excluded from the analysis because they did not meet the study's eligibility requirements on the testing day, another two due to behavioural and one other due to EEG recording errors. Thus, data from 50 male participants aged between 20 and 34 years old (M = 23.5, SD = 3.97) was used for behavioural analysis (N = 24 OT and N = 26 placebo) and 49 for EEG analysis (N = 24 OT and N = 25 placebo). Inclusion criteria were no history of or current psychiatric illness, male gender, heterosexuality, age between 20 and 35 years, native in European Portuguese, and right-handed. All participants gave written informed consent and were compensated for their time with a 15€ to 45€ gift voucher according to their performance during the game. This study

was approved by the ISCTE-University Institute of Lisbon ethics committee (Ref. 94/2019). The sample size was determined based on the effect size $\eta^2 = 0.06$ of a statistically significant ($p < .05$) OT/placebo drug by social group by valence previously reported in the literature (Schiller et al., 2020). Using G-power 3.1 (Faul et al., 2007) (ANOVA: repeated measures, within-between interaction, $\alpha = 0.05, \beta = 0.95$, number of groups = 2, number of measurements = 2, correlation among repeated measures = 0.5, non-sphericity correction = 1) we estimated that around 54 participants would be needed to detect a significant effect.

Experimental procedure

On arrival, participants completed a consent form and a drug screening test to exclude any drug influence (amphetamine, benzodiazepine, cocaine, methamphetamine, morphine/opiates, or tetrahydrocannabinol). They completed the demographics questionnaire, and afterwards, trained for the task and were prepared for the EEG set-up. Upon providing a blood sample (which was relevant for another study), they underwent double-blinded drug administration. Participants randomly received a nasal spray containing OT (synthesized by AlfaSigma, Bologna, Italy), or an equivalent placebo solution lacking the peptide (synthesized by Volksapotheke Schaffhausen, Switzerland). The nasal spray bottles were all identical and blindfolded. Upon verification of unobstructed participants' breathing and unblocked nostrils, and 60 minutes (SD = 10 min) before the PD task, participants were instructed to self-administered six puffs (three per nostril, or six in the same nostril if one was partially obstructed) of the nasal spray, resulting in a total of 24 IU of OT or placebo. A second blood sample was collected (on average, 17 min following drug administration). Participants then completed a battery of tasks (including the PD) and a third blood collection as part of a larger study (not analysed here) and finally, a set of questionnaires (see 'Explicit opponent perceptions ratings' below).

The Prisoner's Dilemma task

The game was a first-player (i.e. the participant plays first in each trial), iterated and sequential-choice PD paradigm, where decisions were made over 45 consecutive trials (about 20 s each) in 2 blocks, one with each opponent, both lasting approximately 15 minutes. The timeline of a single trial is depicted in **Figure 12**. The algorithm controlling the opponent's decisions was designed to mimic a human strategy (Rilling et al., 2012), with a 67% chance of reciprocating cooperation and a 90% chance of reciprocating defection. As an adaptation introduced by us

(Cogoni, Fiuza, et al., 2023) aimed at reducing the heterogeneity of the “first impression" by the participant towards the opponent, opponent decisions followed two constraints: a) cooperation was always the first choice of the opponent, and b) the reciprocation of the participant’s cooperation was ensured for at least the first 4 trials.

Participants were told they would be connected via the internet to two other participants located in another university room. The two opponents were a sexualized woman (either blonde or brunette) and a non-sexualized woman (either blonde or brunette), of similar age, height, and weight (**Figure 12**) already adopted in our previously published research (Cogoni, Carnaghi, & Silani, 2018). Each participant saw through the entire game with the same combination of opponents, which was randomized across participants. In addition, the game had two possible sequences: the participant played the first game with the sexualized target and the second game with the non-sexualized (Play-order S -> NS) or vice versa (Play-order NS -> S).

Explicit perceptions of the opponent

Before the game started, participants were asked to indicate the *Likelihood of cooperation estimation*, i.e. to guess the proportion of rounds in which the opponent would cooperate on a scale from 0 = 0% to 10 = 100%. At the end of the task, participants were also asked to rate the opponents using: 1) the Agency and Experience sub-scales of the Mind Attribution Scale (K. Gray et al., 2011); 2) eight of our study specific questions: how much they consider the opponent to be attractive, sexy, beautiful, moral, intelligent, trustworthy, sexually available in general, and sexually available for the participant; and 3) Anthropomorphism, Likeability, and Perceived Intelligence sub-scales of the GodSpeed Questionnaire (Bartneck et al., 2008). All above questionnaires were answered in a Likert scale from 1= “not at all” to 6= “completely”. Importantly, according to the Mind Attribution Scale (K. Gray et al., 2011), sexual objectification is verified if the perceptions of agency are found to be reduced and those of experience, increased. Lastly, participants were asked to rate the intensity of each emotion (i.e., angry, happy, guilty, and disappointment) after each outcome (i.e., CC, CD, DC, and DD) on a scale from 1= no emotion to 7 = maximum emotion.

EEG acquisition and preprocessing

Electrophysiological data were recorded at a 1000 Hz sampling rate using a 64-electrode actiCHamp system (Brain Products, Munich, Germany). A 10/20 electrode placement system was used, with FCz as the reference and with AFz as the ground electrodes. Electrodes' impedance levels were maintained under 30 kΩ.

ERPs of interest were analysed offline using the EEGLAB toolbox developed for Matlab (Mathworks, Inc., Massachusetts, USA) (Delorme & Makeig, 2004). Continuous data were down-sampled to 250 Hz, filtered with a 0.1 Hz high-pass filter, inspected manually for bad channels and noisy regions removal, and re-referenced offline to the average. An artifact correction was then performed using an independent component analysis (ICA) (*runica* algorithm) for the detection and removal of the non-neural origin signals (e.g., eye-related movement, muscle, and channel noise activity). On average, 3 components were removed per participant (M = 3.2, SD = 1.34) with the help of IClabel (Pion-Tonachini et al., 2019) by visual inspection.

Next, EEG data was divided into epochs time-locked to the outcome presentation moment, from 200 ms pre-stimulus to 800 ms post-stimulus onset. At this stage, data were visually inspected again to remove any artifacts that were not corrected using ICA. The removed electrodes were interpolated, and afterward, a baseline correction was done using the pre-stimulus period (-200 to 0 ms).

The electrodes and time windows for the ERPs of interest were chosen based on visual inspection of grand-averaged data collapsed across experimental conditions - the "collapsed localizers" technique (Luck & Gaspelin, 2017). As such, data from FC1, Cz, FC2, F1, C1, C2, F2, and FCz electrodes were analyzed for the FRN, and data from CP1, Pz, CP2, Cz, C4, FC2, C1, P1, CPz, CPz, CP4, C2, FC4, FCz electrodes were examined for the P3. The FRN amplitude was computed as the mean amplitude between 250-350 ms after the outcome presentation onset and averaged across the chosen electrode sites. The P3 local peaks amplitudes, corresponding to the greatest point in the measurement window surrounded by smaller voltages on both sides, were extracted within the 250–600 ms window after the outcome presentation. Within the interest time window for a specific condition, the peak was found in a trial-averaged waveform for each participant. Then, a mean amplitude was calculated around that peak based on its width, corresponding to a horizontal reference line

positioned at half of the peak's height. The reference line starting point, on the left of the local peak, also served as a measure of the P3 peak's latency.

Statistical analysis

Statistical analyses for the behavioural data were performed in IBM SPSS Statistics version 28.0.0 where the general cooperation frequency (i.e. counts of cooperation choices across a 45-trial round), as well as cooperation after each trial outcome (i.e. counts of cooperation after CC, CD, DC, or DD outcomes) in the PD game were analysed having opponent (sexualized, non-sexualized) as a within-subject factor and drug (OT, placebo) and play-order (Play-order S -> NS, Play-order NS -> S) as between-subject factors. Main effects of opponent, drug and play-order, as well as their interactions with each other were estimated using a generalized estimating equation approach to fit a generalized linear model to each of the behavioural dependent variables. Given the nature of the dependent variables (i.e., counts), a Poisson distribution was considered, with an "exchangeable" correlation structure. In the model for general cooperation, the dependent variable was the number of cooperations across a 45-trial round. In the per outcome models, an offset term corresponding to the number of each outcome type preceding cooperation was included (IBM, 2021; *IBM SPSS Statistics*, 2021). In these models, the cooperation rate corresponded to the expected number of cooperation choices per trial and hence can be interpreted as the transition-to-cooperation probability from a preceding outcome to a cooperation choice.

EEG-wise, the FRN and P3 amplitudes, and the P3 latencies, were tested separately through a mixed repeated-measures analysis of variance (ANOVA), using the *afex* package, version 1.0-1 (Singmann et al., 2021) in R version 4.2.1. (R Core Team, 2021). The neural response during each trial's outcome presentation was analysed, with opponent (sexualized, non-sexualized), and expectancy (expected, unexpected; for P3 amplitude and latency) or valence (gains, losses; for FRN), as within-subject factors; and drug (OT, placebo) and play-order (Play-order S -> NS, Play-order NS -> S) as between-subject factors. Expected outcomes were defined as the reciprocated ones (CC and DD), and unexpected ones as the unreciprocated ones (CD and DC). CC and DC trials were considered gains, which correspond to the participant's second highest and highest payoffs, respectively, and CD and DD were considered losses, which result in a non-reward or low reward to the participant, respectively. Main effects of opponent, drug and play-order, as well as their interactions with each other (and expectancy for P3, or valence for FRN), were estimated.

The subjects' explicit perceptions ratings of their opponent's characteristics, and emotional intensity after each outcome were also analysed with ANOVAs using the *afex* package, version 1.0-1 (Singmann et al., 2021) in R version 4.2.1. (R Core Team, 2021), with opponent (sexualized, non-sexualized) as within-subject factor; and drug (OT, placebo) and play-order (Play-order S -> NS, Play-order NS -> S) as between-subject factors.

Post-hoc analyses were performed in both generalized estimating equation and ANOVA models using the estimated marginal means, only for significant overall (i.e. omnibus) effects. As such, all p-values < .05 were considered statistically significant. For the main effects of the behavioural analyses, the difference between estimated marginal means is reported to represent the magnitude of the cooperation variation (and converted to percentage in the case for the general frequency of cooperation). Cohen's d is reported as a measure of effect size for the pairwise comparisons. Partial eta-squared (η_p^2) and Cohen's d are reported as measures of effect size for the overall effects and pairwise comparisons, respectively, for the EEG and explicit perceptions ratings analyses.

6.3 Results

Behaviour

All overall (omnibus), and post-hoc pairwise tests of significant interactions (with descriptive statistics), on PD behaviour are reported in **Annex D Tables S1** and **S2**, respectively. All statistically significant overall effects, and explanatory pairwise contrasts, are also described below and in **Table 6** with full inferential statistics. There were no statistically significant effects (p < .05) on probability of cooperation after a DC or DD outcome.

General frequency of cooperation. There was a significant main effect of drug on cooperation choice frequency (Wald χ^2 (1, N= 100) = 6.32, p = .012), where the OT group (M = 25.98, SE = 2.33) cooperated 15% more frequently than the placebo group (M = 19.26, SE = 1.51; **Figure 13A**).

Transition-to-cooperation probability after a CC (i.e. mutual cooperation) outcome. We found a main effect of drug on a transition-to-cooperation probability after CC outcome (Wald

χ^2 (1, N= 98) = 3.84, p = .050), where the OT group (M = 0.87, SE = 0.03) was 9% more likely to cooperate than the placebo group (M = 0.78, SE = 0.04; **Figure 13B, Table 6**).

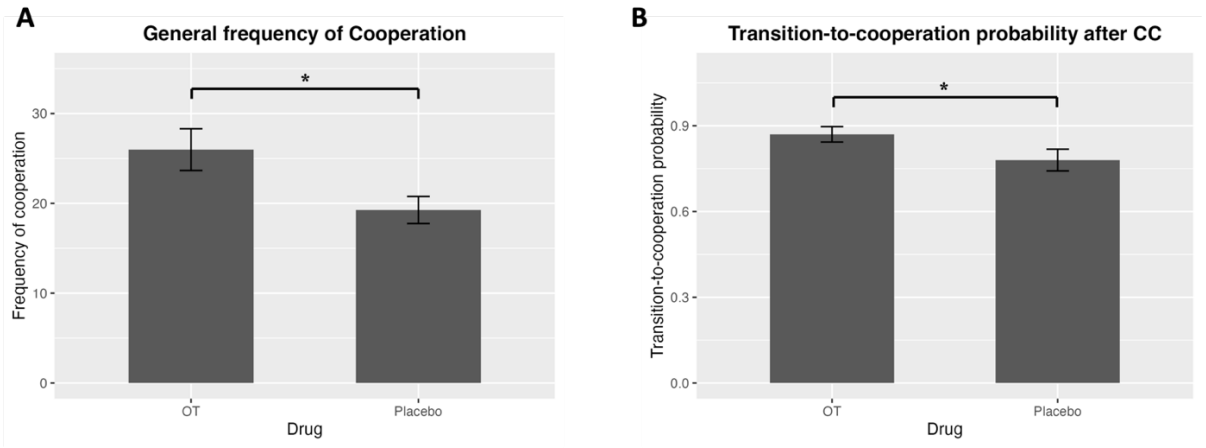

Figure 13 - Main effect of drug on A) the general frequency of cooperation and B) the transition-to-cooperation probability after CC. Error bars indicate the standard error of the mean. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001. OT – Oxytocin; C – Cooperation; CC – Cooperation Cooperation outcome.

Transition-to-cooperation probability after a CD (i.e. unreciprocated cooperation) outcome. The same main effect of drug as above (but with higher statistical significance and magnitude) was also found for cooperation probability after CD outcome (Wald χ^2 (1, N= 98) = 14.15, p < .001), with OT (M = 0.72, SE = 0.07) increasing it by 32% vs. placebo (M = 0.40, SE = 0.05). Additionally, there was a significant opponent by play-order interaction effect (Wald χ^2 (1, N= 98) = 8.80, p = .003), whereby cooperation was 17% higher towards sexualized vs. non-sexualized women (p = .020, d = 0.55) only when participants played first with non-sexualized women. Most importantly, and superseding the above effects, the later interaction moderated the abovementioned effect of drug, since there was a significant three-way interaction (Wald χ^2 (1, N= 98) = 3.85, p = .050). Post-hoc pairwise comparisons revealed that the higher likelihood of OT group (vs. placebo) to cooperate after CD outcomes, was statistically significant for both opponents, but especially higher when playing against non-sexualized (by 49%, p < .001, d = 2.25) rather than sexualized women (by 42%, p = .002, d = 1.31) *and* only when playing with the former in advance of the latter (Play order NS -> S; **Figure 14, Annex D Table S2**).

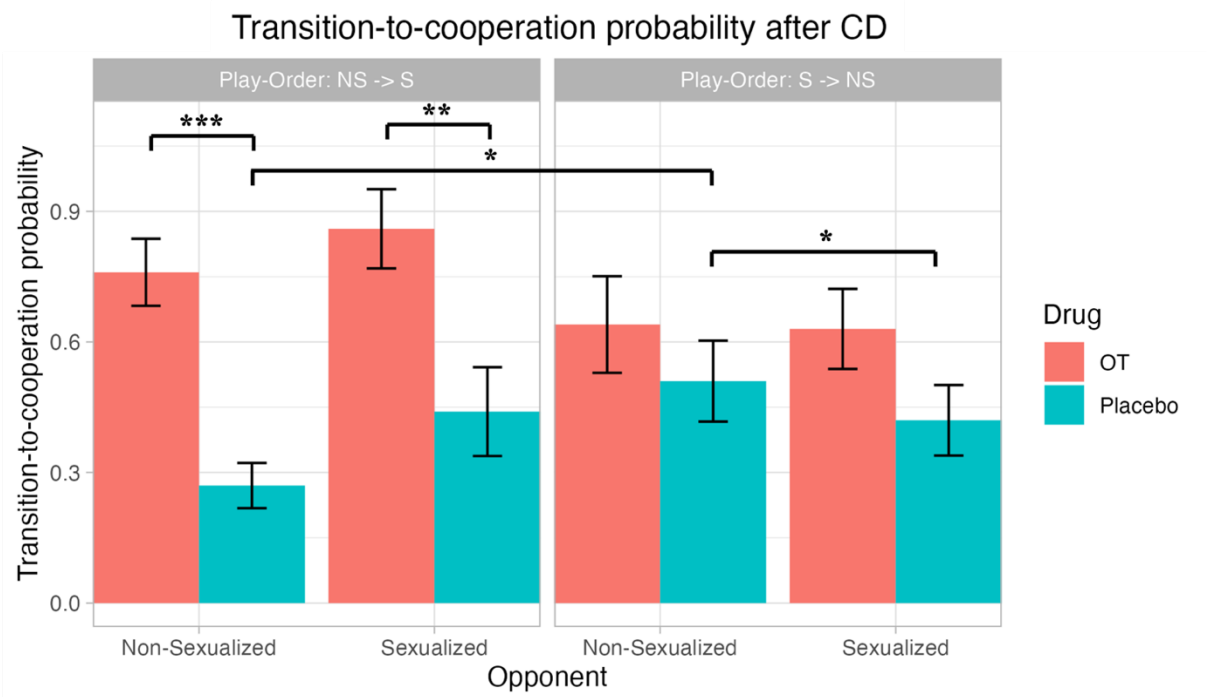

Figure 14 - The transition probability of cooperating following a CD outcome as a function of drug, opponent, and play-order. Error bars indicate the standard error of the mean. Asterisks signal statistically significant effects: *$p < .050$; **$p < .010$; ***$p < .001$. OT – Oxytocin; C – Cooperation; CD – Cooperation Defection outcome. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse.

Table 6 - Overall summary of all (omnibus) behavioural statistically significant results, and respective post-hoc pairwise comparisons. Abbreviations: df – degrees of freedom; F – F-statistic; η_p^2 – partial eta-squared; SE – Standard error; OT – Oxytocin; PL – Placebo; S – Sexualized; NS – Non-sexualized. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001.

BEHAVIOUR							
General Frequency of Cooperation							
Omnibus effects			**Wald Chi-Square**	**df**	**p-value**	**Effect size**	**Direction**
Drug			6.32	1	.012*	15%	OT > PL
Transition-to-cooperation probability after CC outcome							
Omnibus effects			**Wald Chi-Square**	**df**	**p-value**	**Effect size**	**Direction**
Drug			3.84	1	.050*	9%	OT > PL
Transition-to-cooperation probability after CD outcome							
Omnibus effects			**Wald Chi-Square**	**df**	**p-value**	**Effect size**	**Direction**
Drug			14.15	1	< .001***	32%	OT > PL
Play-order x Opponent			8.80	1	.003**	-	See below
Play-order x Opponent x Drug			3.85	1	.050*	-	See below
Opponent x Play-order pairwise comparisons			**Mean difference**	**SE**	**p-value**	**Cohen's d**	**Direction**
Play-order: S -> NS	S – NS		-0.05	0.03	.116	0.14	-
Play-order: NS -> S	S – NS		0.17	0.07	.020	0.55	S > NS
Opponent: S	S -> NS – NS -> S		-0.10	0.10	.300	0.29	-
Opponent: NS	S -> NS – NS -> S		12	0.09	.182	0.38	-
Opponent x Play-order x Drug pairwise comparisons			**Mean difference**	**SE**	**p-value**	**Cohen's d**	**Direction**
Play-order: S -> NS	Opponent: S	OT - PL	0.21	0.12	.089	0.66	-
	Opponent: NS	OT - PL	0.13	0.15	.352	0.35	-
Play-order: NS -> S	Opponent: S	OT - PL	0.42	0.14	.002**	1.31	OT > PL
	Opponent: NS	OT - PL	0.49	0.09	< .001***	2.25	OT > PL
Opponent: S	Drug: OT	S -> NS – NS -> S	-0.23	0.13	.072	0.72	-
	Drug: PL	S -> NS – NS -> S	-0.02	0.13	.864	0.06	-
Opponent: NS	Drug: OT	S -> NS – NS -> S	-0.12	0.14	.366	0.35	-
	Drug: PL	S -> NS – NS -> S	-0.24	0.11	.026*	0.84	S -> NS > NS -> S
Drug: OT	Play-order: S -> NS	S - NS	-0.01	0.06	.889	0.03	-
	Play-order: NS -> S	S - NS	0.10	0.07	.118	0.36	-
Drug: PL	Play-order: S -> NS	S - NS	-0.08	0.03	.014*	0.28	NS > S
	Play-order: NS -> S	S - NS	0.18	0.10	.064	0.63	-

Electroencephalography

All overall (omnibus) effects on EEG are reported in **Annex D Tables S3, S5** and **S7**. Post-doc pairwise tests of statistically significant interactions, including their descriptive statistics, are present in **Annex D Tables S4, S6** and **S8**. All statistically significant overall effects, and explanatory pairwise contrasts, are described below and in **Table 7** with full inferential statistics.

P3 amplitude. There was a main effect of expectancy on P3 amplitude [F(1, 45) = 33.12, p < .001, η_p^2 = .424] such that unexpected outcomes (M = 6.69μV, SE = 0.39) elicited higher amplitudes compared to expected ones (M = 5.47μV, SE = 0.35). This effect was, however, dependent on the opponent, as there was also an opponent by expectancy interaction on P3 amplitude [F(1, 45) = 5.09, p = .029, η_p^2 = .102] whereby pairwise comparisons revealed that only within expected outcomes did sexualized opponents elicit higher P3 amplitudes compared to non-sexualized (p = .004, d = 0.45, **Figure 15**). In other words, expected (vs. unexpected) outcomes generated lower P3 amplitude for sexualized opponents (p < .001, d = 0.56) and non-sexualized (p < .001, d = 0.92). Finally, there was also an opponent by play-order interaction [F(1, 45) = 5.79, p = .020, η_p^2 = .114] where only within Play-order S -> NS did sexualized opponents elicited higher P3 amplitudes than non-sexualized ones (p = .008, d = 0.42).

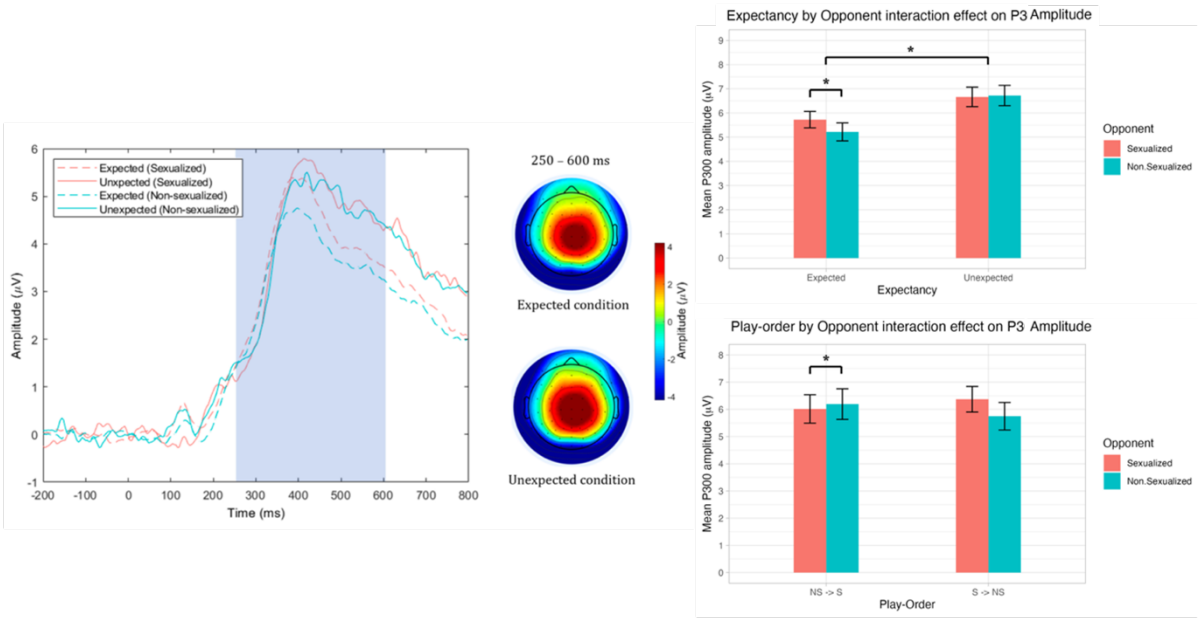

Figure 15 - P3 amplitude. Left: Grand-average ERP waveforms collapsed across electrodes CP1, Pz, CP2, Cz, C4, FC2, C1, P1, CPz, CP4, C2, FC4, FCz shown separately for expected and unexpected outcomes, for each opponent (sexualized vs non-sexualized). The time window used for P3 amplitudes analysis (250-600 ms) is highlighted. The scalp topography presented is of the expected and unexpected conditions within the same time window. Right: Plots of mean P3 amplitude as a function of expectancy, opponent and/or play-order. The error bars indicate the standard error, with confidence intervals of 95%. Asterisks signal statistically significant effects: *$p < .050$; **$p < .010$; ***$p < .001$. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse.

P3 latency. As with amplitude**,** the main effect of expectancy was also found for P3 latencies [$F(1, 45) = 17.23$, $p < .001$, $\eta_p^2 = .277$] such that P3 response during unexpected outcomes (M = 461.10ms, SE = 6.96) was more delayed than during expected ones (M = 419.60ms, SE = 8.10). We found a significant drug by opponent interaction [$F(1, 45) = 4.14$, $p = .048$, $\eta_p^2 = .084$] where the OT group showed longer P3 latencies compared to the placebo only against non-sexualized opponents ($p = 0.12$, d = 0.39). Finally, this interaction was further moderated by drug, as there was a significant drug by opponent by play-order three-way interaction [$F(1, 45) = 4.40$, $p = .042$, $\eta_p^2 = .089$] where pairwise comparisons revealed that only when playing against non-sexualized opponents and when playing with them before the sexualized (i.e. Play-

order NS -> S) did the OT group exhibit longer P3 latencies compared to placebo (p = .003, d = 0.47, **Figure 16**).

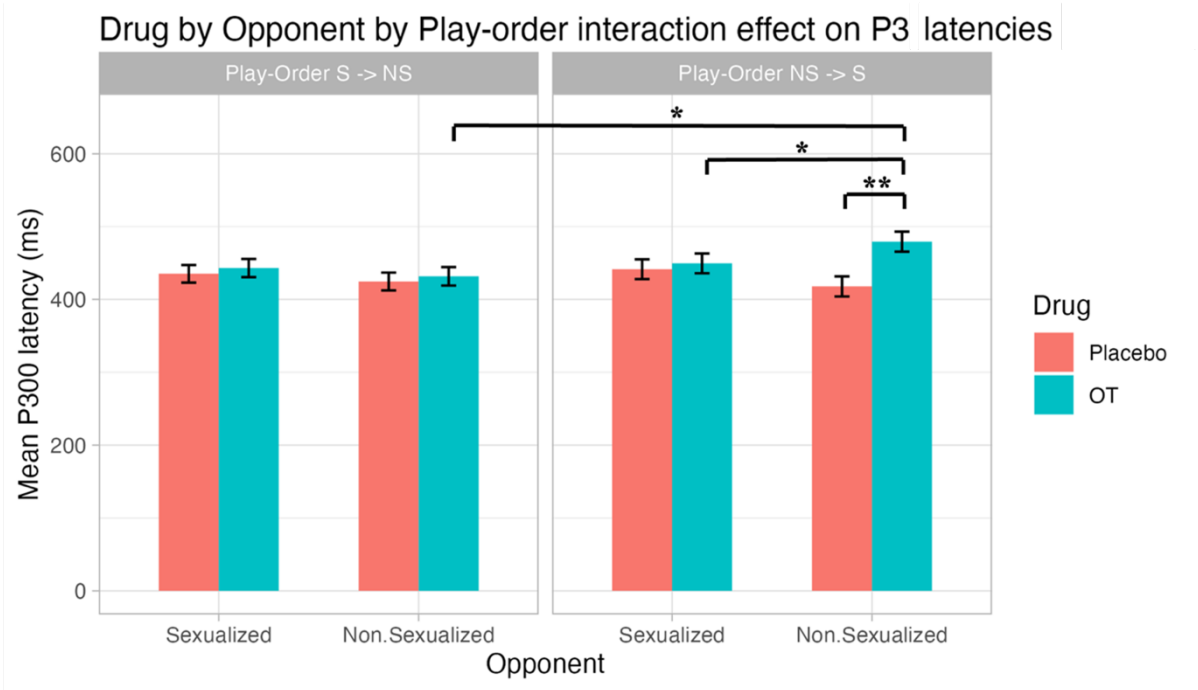

Figure 16 - P3 latency. Mean P3 latency as a function of drug, opponent, and play-order. The error bars indicate the standard error of the mean. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001. OT – Oxytocin. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse.

FRN amplitude. We found a main effect of outcome valence on FRN amplitude [F(1, 45) = 34.78, p < .001, η_p^2 = .436] where gains (M = 5.10μV, SE = 0.36) elicited lower amplitudes than losses (M = 3.89μV, SE = 0.29). There was also an opponent by play-order interaction effect [F(1, 45) = 16.02, p < .001, η_p^2 = .262] whereby only in Play-order S -> NS, the sexualized opponent elicited higher amplitudes than the non-sexualized (p < .001, d = 0.59; **Figure 17**).

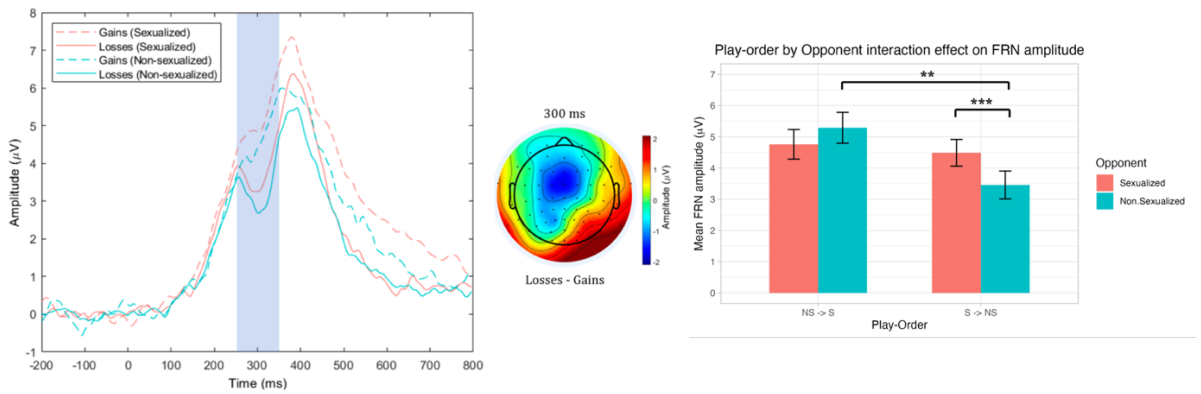

Figure 17 - FRN amplitude. Left: Grand-average ERP waveforms collapsed across FC1, Cz, FC2, F1, C1, C2, F2 and FCz electrodes shown separately according to outcome valence and opponent. The time-windows in which the FRN was identified is highlighted (250-350ms). The scalp topography presented is of the difference between loss and gain trials at 300ms, when it is maximal. Right: Plot of mean FRN amplitude as a function of play-order and opponent. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse. The error bars indicate the standard error, with confidence intervals of 95%. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001.

Table 7 – Overall summary of all (omnibus) EEG statistically significant results, and respective post-hoc pairwise comparisons. Abbreviations: df – degrees of freedom; F – F-statistic; η_p^2 – partial eta-squared; SE – Standard error; OT – Oxytocin; PL – Placebo; S – Sexualized; NS – Non-sexualized. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001.

ELECTROENCEPHALOGRAPHY							
P3 Amplitude							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Expectancy			33.12	1, 45	< .001***	.424	Expected > Unexpected
Play-order x Opponent			5.79	1, 45	.020*	.114	See below
Opponent x Expectancy			5.09	1, 45	.029*	.102	See below
Play-order x Opponent pairwise comparisons			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Play-order: S -> NS	S - NS		2.80	45	.008**	0.42	S > NS
Play-order: NS -> S	S - NS		-0.72	45	.477	0.11	-
Opponent: S	S -> NS – NS -> S		0.51	45	.614	0.08	-
Opponent: NS	S -> NS – NS -> S		-0.59	45	.558	0.09	-
Opponent x Expectancy pairwise comparisons			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Expectancy: Expected	S - NS		3.04	45	.004**	0.45	S > NS
Expectancy: Unexpected	S - NS		-0.24	45	.810	0.04	-
Opponent: S	Expected – Unexpected		-3.77	45	< .001***	0.56	Unexpected > Expected
Opponent: NS	Expected - Unexpected		-6.15	45	<.001***	0.92	Unexpected > Expected
P3 Latency							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Expectancy			17.23	1, 45	< .001***	.277	Unexpected > Expected
Opponent x Drug			4.14	1, 45	.048*	.084	See below
Drug x Play-order x Opponent			4.40	1, 45	.042*	.089	See below
Opponent x Drug pairwise comparisons			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Opponent: NS	OT – PL		2.62	45	.012*	0.39	OT > PL
Opponent: S	OT - PL		0.62	45	.541	0.09	-
Drug: OT	S – NS		1.00	45	.323	0.15	-
Drug: PL	S – NS		1.89	45	.066	0.28	-
Opponent x Play-order x Drug pairwise comparisons			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Opponent: NS	Play-order: S -> NS	OT – PL	0.41	45	.687	0.06	-
	Play-order: NS -> S	OT - PL	3.16	45	.003**	0.47	OT > PL
Opponent: S	Play-order: S -> NS	OT - PL	0.46	45	.650	0.07	-
	Play-order: NS -> S	OT - PL	0.42	45	.679	0.06	-
Play-order: S -> NS	Drug: PLC	S - NS	0.88	45	.385	0.13	-
	Drug: OT	S - NS	0.91	45	.367	0.14	-
Play-order: NS -> S	Drug: PLC	S - NS	1.74	45	.089	0.26	-
	Drug: OT	S - NS	-2.20	45	.033*	0.33	NS > S
Drug: OT	Opponent: S	S -> NS – NS -> S	-0.35	45	.731	0.05	-
	Opponent: NS	S -> NS – NS -> S	-2.55	45	.014*	0.38	NS -> S > S -> NS
Drug: PL	Opponent: S	S -> NS – NS -> S	-0.35	45	.730	0.05	-
	Opponent: NS	S -> NS – NS -> S	0.37	45	.715	0.05	-

FRN Amplitude						
Omnibus effects		**F**	**df**	**p-value**	**η_p^2**	**Direction**
Valence		34.78	1, 45	< .001***	.436	Losses > Gains
Play-order x Opponent		16.02	1, 45	< .001***	.262	See below
Opponent x Play-order pairwise comparisons		**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Play-order: S -> NS	S – NS	3.94	45	< .001***	0.59	S > NS
Play-order: NS -> S	S – NS	-1.83	45	.074	0.27	-
Opponent: S	S -> NS – NS -> S	-0.42	45	.674	0.06	-
Opponent: NS	S -> NS – NS ->S	-2.75	45	.009*	0.41	S -> NS > NS -> S

Explicit perception

All overall (omnibus) effects on explicit perceptions are reported in **Annex D Tables S9, S11** and **S13**. Post-doc pairwise tests of statistically significant interactions, including their descriptive statistics, are present in **Annex D Tables S10, S12** and **S14**. All statistically significant overall effects, and explanatory pairwise contrasts, are described below and in **Table 8** with full inferential statistics.

Likelihood of cooperation expectation. We found no effect of opponent, drug, play-order or their interactions on the participant's prediction of opponent's likelihood of cooperation (all $p > .050$).

Opponent Ratings. We found several main effects of opponent such that the sexualized opponent was rated as being more attractive [$F(1, 45) = 9.33$, $p = .004$, $\eta_p^2 = .172$]; sexier [$F(1, 45) = 41.64$, $p < .001$, $\eta_p^2 = .481$]; less moral [$F(1, 44) = 6.58$, $p = .014$, $\eta_p^2 = .130$]; and having higher general sexual availability [$F(1, 45) = 20.28$, $p < .001$, $\eta_p^2 = .311$]. Sexual availability to the participant was moderated by play-order [$F(1, 45) = 4.10$, $p = .049$, $\eta_p^2 = .084$] where sexualized opponents were only rated more sexually available compared to the non-sexualized for participants that played first with the sexualized (Play-order S -> NS; $p = .002$, d = 0.50) and not with non-sexualized first (Play-order NS -> S; $p = .838$, d = 0.03) – superseding a main effect of opponent [$F(1, 45) = 5.45$, $p = .024$, $\eta_p^2 = .108$].

Mind attribution sub-scales. There was a significant drug by opponent by play-order three-way interaction on the agency score [$F(1, 46) = 5.21$, $p = .027$, $\eta_p^2 = .102$] where only participants that took OT and played with non-sexualized opponents first (in Play-order NS -> S) rated these with having more agency than the sexualized counterparts ($p = .004$, d = 0.45, **Figure 18**).

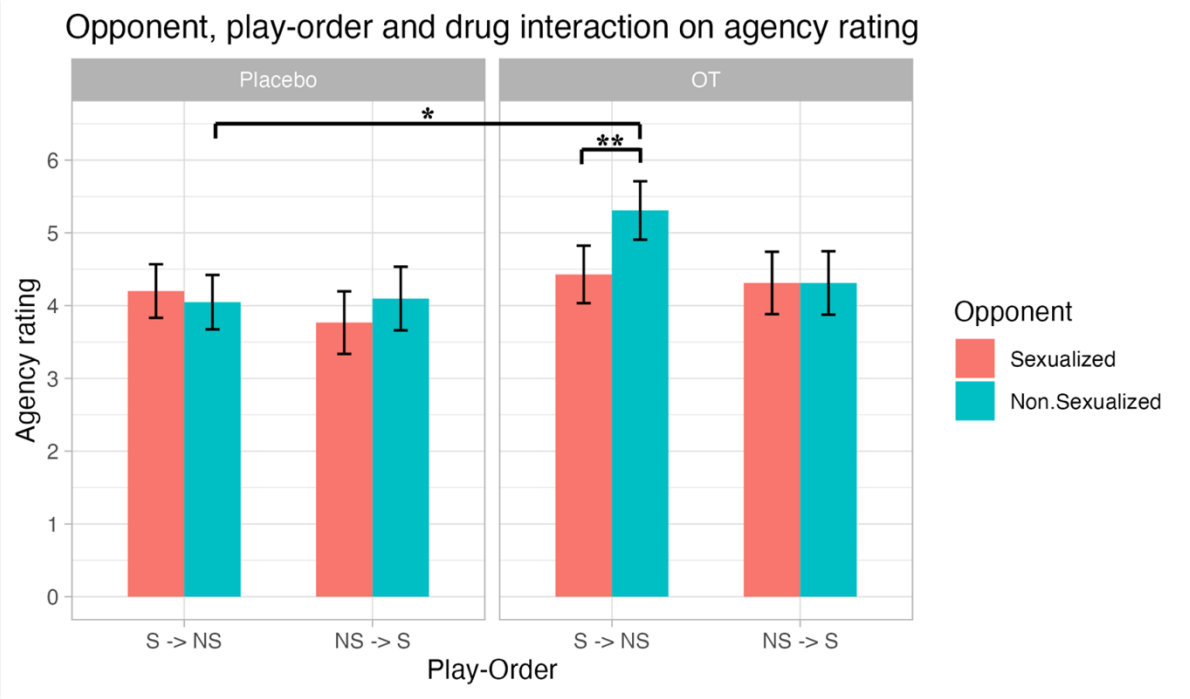

Figure 18 - The opponent by play-order by drug interaction on the explicit perception of opponents' agency from the mind attribution scale. In Play-order S -> NS, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order NS -> S, the non-sexualized woman is the first opponent and sexualized woman the second. OT – Oxytocin; S - Sexualized; NS - Non-sexualized. Asterisks signal statistically significant effects: *$p < .050$; **$p < .010$; ***$p < .001$.

Godspeed Questionnaire. We found a significant opponent by play-order interaction on the likeability score [$F(1, 46) = 4.18$, $p = .047$, $\eta_p^2 = .083$], with pairwise comparisons revealing that sexualized (vs. non-sexualized) opponents were less liked when played with last, and more liked when played with first, albeit none of these pairwise contrasts were significant (all $p > .050$; more detail in **Annex D Table S12**).

Emotion intensity rating of each outcome. On the intensity of angry after a CD outcome, we found a three-way interaction of drug by opponent by play-order [$F(1, 46) = 5.21$, $p = .027$, $\eta_p^2 = .102$], superseding an opponent main effect and an opponent by play order interaction. Pairwise comparisons revealed that only the participants that took OT and played with sexualized opponents first (Play-order S -> NS) reported experiencing anger more intensely against non-sexualized opponents vs. sexualized ($p = .001$, d = 0.50). On happiness intensity

after a DC outcome, we found a main effect of drug such that OT induced less intense happiness than placebo [$F(1, 45) = 6.10$, $p = .017$, $\eta_p^2 = .119$]. On disappointment intensity after the same outcome type, we found a main effect of opponent such that sexualized opponents' responses induced less intense disappointment than non-sexualized [$F(1, 44) = 6.17$, $p = .017$, $\eta_p^2 = .123$].

Table 8 – Overall summary of all (omnibus) explicit perceptions statistically significant results, and respective post-hoc pairwise comparisons. Abbreviations: df – degrees of freedom; F – F-statistic; η_p^2 – partial eta-squared; SE – Standard error; OT – Oxytocin; PL – Placebo; S – Sexualized; NS – Non-sexualized. In Play-order S -> NS, a sexualized woman plays with the participant before a non-sexualized woman; in Play-order NS -> S, the reverse. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001.

EXPLICIT PERCEPTIONS							
Attractiveness							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Opponent			9.33	1, 45	.004**	.172	S > NS
Sexiness							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Opponent			41.64	1, 45	< .001***	.481	S > NS
Morality							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Opponent			6.58	1, 44	.014*	.130	S < NS
General sexual availability							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Opponent			20.28	1, 45	< .001***	.311	S > NS
Sexual availability to the participant							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Opponent			5.45	1, 45	.024*	.108	S > NS
Opponent x Play-order			4.10	1, 45	.049*	.084	See below
Opponent x Play-order Pairwise comparison			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Play-order: S -> NS	S – NS		3.33	45	.002**	0.50	S > NS
Play-order: NS -> S	S - NS		0.21	45	.838	0.03	-
Opponent: S	S -> NS – NS -> S		-0.02	45	.988	< 0.01	-
Opponent: NS	S -> NS – NS -> S		1.62	45	.113	0.24	-
Mind Attribution's Agency							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Drug x Play-order x Opponent			5.21	1, 46	.027*	.102	See below
Opponent x Play-order x Drug pairwise comparison			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Drug: OT	Play-order: S -> NS	S – NS	-3.03	46	.004**	0.45	NS > S
	Play-order: NS -> S	S – NS	0.00	46	> .999	< 0.01	-
Drug: PL	Play-order: S -> NS	S – NS	0.56	46	.576	0.08	-
	Play-order: NS -> S	S – NS	-1.05	46	.299	0.15	-

Opponent: S	Drug: OT	S -> NS – NS -> S	1.68	46	.100	0.25	-
	Drug: PL	S -> NS – NS -> S	0.77	46	.447	0.11	-
Opponent: NS	Drug: OT	S -> NS – NS -> S	0.20	46	.842	0.03	-
	Drug: PL	S -> NS – NS -> S	-0.09	46	.931	0.01	-
Play-order: S -> NS	Opponent: S	OT – PL	0.42	46	.674	0.06	-
	Opponent: NS	OT – PL	2.30	46	.026*	0.34	OT > PL
Play-order: NS -> S	Opponent: S	OT – PL	0.90	46	.374	0.13	-
	Opponent: NS	OT – PL	0.36	46	.730	0.05	-
Godspeed's Likeability							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Play-order x Opponent			4.18	1, 46	.047	.083	See below
Opponent x Play-order pairwise comparison			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Opponent: S	S -> NS – NS -> S		0.24	46	.815	0.03	-
Opponent: NS	S -> NS – NS -> S		-2.26	46	.029	0.33	NS -> S > S -> NS
Play-order: S -> NS	S - NS		1.69	46	.098	0.25	S > NS (non-sign)
Play-order: NS -> S	S - NS		-1.24	46	.223	0.18	NS > S (non-sign)
Anger intensity rating after CD outcome							
Omnibus effects			**F**	**df**	**p-value**	**η_p^2**	**Direction**
Opponent			4.08	1, 46	.049*	.081	S < NS
Opponent x Play-order			4.08	1, 46	.049*	.081	See below
Opponent x Play-order x Drug			5.21	1, 46	.027*	.102	See below
Opponent x Play-order pairwise comparison			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Play-order: S -> NS	S – NS		-3.04	46	.004**	0.45	S < NS
Play-order: NS -> S	S – NS		0.00	46	> .999	< 0.01	-
Opponent: S	S -> NS – NS -> S		0.33	46	.744	0.05	-
Opponent: NS	S -> NS – NS -> S		1.35	46	.185	0.20	-
Opponent x Play-order x Drug pairwise comparison			**t**	**df**	**p-value**	**Cohen's d**	**Direction**
Drug: OT	Play-order: S -> NS	S – NS	-3.42	46	.001**	0.50	S < NS
	Play-order: NS -> S	S – NS	0.93	46	.358	0.14	-
Drug: PL	Play-order: S -> NS	S – NS	-0.79	46	.431	0.12	-
	Play-order: NS -> S	S – NS	-0.93	46	.358	0.14	-
Opponent: S	Drug: OT	S -> NS – NS -> S	-0.47	46	.642	0.07	-
	Drug: PL	S -> NS – NS -> S	0.95	46	.345	0.14	-
Opponent: NS	Drug: OT	S -> NS – NS -> S	1.14	46	.261	0.17	-
	Drug: PL	S -> NS – NS -> S	0.76	46	.452	0.11	-
Play-order: S -> NS	Opponent: S	OT - PL	-1.93	46	.059	0.29	-
	Opponent: NS	OT – PL	-0.72	46	.478	0.11	-
Play-order: NS -> S	Opponent: S	OT - PL	-0.38	46	.705	0.06	-
	Opponent: NS	OT - PL	-1.03	46	.311	0.15	-
Happiness intensity rating after DC							

Omnibus effects	F	df	p-value	η_p^2	Direction
Drug	6.10	1, 45	.017*	.119	OT < PL
Disappointment intensity rating after DC					
Omnibus effects	**F**	**df**	**p-value**	η_p^2	**Direction**
Opponent	6.17	1, 44	.017*	.123	S < NS

6.4 Discussion

In the present study, we aimed to ascertain if the degree of heterosexual male cooperation with female opponents during a socioeconomic game such as the iterative and sequential PD game, and associated ERPs reflecting outcomes' valence or expectancy, is influenced by the manipulated sexualization of these opponents. We also tested whether these biases might be further changed by IN-OT administration with the goal of understanding OT's role in cooperation in the specific context of sexualization. In sum, we found a sexualization bias, which was *negative* towards non-sexualized women, when subjects played with them first and only when having faced the worst possible PD outcome (i.e. a betrayal). IN-OT significantly reverted this bias, and also increased 'cooperation after betrayal' for both sexualized and non-sexualized women, particularly in the same play order (i.e. non-sexualized as first opponent). Moreover, IN-OT significantly increased general cooperation frequency and the transition-to-cooperation probability after a mutual cooperation. These findings support the previously reported prosocial role of OT (Marsh et al., 2021; Martins, Lockwood, et al., 2022; Rilling et al., 2012; Striepens et al., 2011; Tarsha & Narvaez, 2023), even if also contextually dependent (Bartz et al., 2011). At the level of neural activity, we found that sexualization modulated P3 and FRN responses during the game's outcomes, and in particular, P3 latency was increased by OT for plays with non-sexualized women, when they preceded those with sexualized women – tentatively reflecting the above behavioural results. **Tables 1, 2 and 3** provide an overview of all statistically significant results, which are also discussed next in detail.

Opponent sexualization increases 'forgiving' cooperation

Our medium effect (p = .020, Cohen's d = 0.55) finding of a negative male bias towards non-sexualized (vs. sexualized) women when they played first, was seen only after participants has just received an unreciprocated cooperation. This is the most unpleasant and trust-threatening PD outcome, as it leads to the lowest gains for the participant and to feelings of betrayal. Thus, cooperating after a previous unreciprocated cooperation trial

can be interpreted as a signal of forgiveness and tolerance, i.e. a reiteration of a willingness to continue cooperating despite the disappointment, and that trust has not been affected. One of the possible study outcomes was that non-sexualized women would be considered more familiar and of in-group membership, and thus more worthy of cooperation (which was derived from previous evidence of them being cognitively perceived as of higher morals (Heflick et al., 2011; Heflick & Goldenberg, 2009), receiving more empathy (Cogoni, Carnaghi, & Silani, 2018; Cogoni et al., 2020), and less dehumanized (Bernard et al., 2012, 2018; Cogoni, Carnaghi, Mitrovic, et al., 2018; Vaes et al., 2011, 2019)). Indeed, participants rated feeling more disappointment after successfully betraying a non-sexualized opponent (vs. sexualized; medium effect; p = .017, η_p^2 = .123), suggesting some level of regret after exploiting an in-group and/or familiar opponent. However, even though non-sexualized women were considered of higher morals (vs. the sexualized; and independently of play-order; medium effect p = .014, η_p^2 = .130), with no difference in anthropomorphization/dehumanization, trustworthiness or expectations of cooperation, our finding may have been due to their perceived qualities of lower attractiveness, lower sexiness and lower general sexual availability, independently of play-order (all large effects *ps* < .005, η_p^2 > .171). The observation that sexualized women were significantly better rated for these qualities, may have been sufficient to induce a positive approach behaviour towards them, such as 'forgiving' cooperation after betrayal. Interestingly, non-sexualized women were found even less sexually available to participants *specifically*, when participants had just been exposed to playing with sexualized women, a medium sized effect (p = .002, Cohen's d = 0.50) – putatively because this order allowed non-sexualized to be directly contrasted with the latter, who they had already rated as high in attractiveness. This also converges with our finding that non-sexualized (vs. sexualized) women were generally less liked, when played with last (vs. first). Moreover, it converges with our finding that participants became angrier (medium sized effect; p =.049, η_p^2 = .081) against non-sexualized (vs. sexualized) women after betrayal (i.e. unreciprocated cooperation), also when playing last (vs. first) with them (small effect; p = .004, Cohen's d = 0.45), probably because they were judged as more moral.

As suggested above, participants had a less forgiving attitude towards non-sexualized (vs. sexualized) women most likely because they perceived them as less sexually available, less sexy, less attractive, and thus less worthy of approach. Under the idea that males are providers and resource gatherers, some authors have proposed that cooperation (and altruism) serves as a way to attract partners (Bhogal et al., 2017; Roberts, 1998; Zahavi, 1995). Females should

then seek males that have increased altruistic tendencies in order to share their resources with themselves and their offsprings. Indeed, previous studies have shown that males in particular tend to use altruism as courtship displays of their qualities as partners, which then makes them being viewed as more desirable romantic partners (Bhogal et al., 2019; Ehlebracht et al., 2018; Farrelly & King, 2019; Iredale et al., 2008; Phillips et al., 2008), and tend to be more generous and trusting towards attractive females (Eagly & Crowley, 1986; Goldberg, 1995; Iredale et al., 2008; Wilson & Eckel, 2006). These hypotheses are in line with our present results whereby our male participants signalled more cooperative tendencies towards sexualized (vs. non-sexualised) women, and particularly after a betrayal, putatively in order to be sought by the more sexually available and attractive partner. This may in fact be a root cause for the phenomena of women's self-sexualization in several cultures (Gattino et al., 2023). As to why this negative bias was *not* observed when they played after sexualized women did, we speculate that it was due to a positive priming by the latter's presence. This is supported by close examination of pairwise contrasts and descriptive data (**Figure 14**): in the placebo group, forgiving cooperation was identical for all sexualized women, whether they played first or last, but it was much lower towards non-sexualized women when they were not preceded by a sexualized player. Perhaps playing with a (more attractive and available) sexualized women primed participants to be more enthusiastic about a positive social interaction with women in general during the game, and thus more prone towards a forgiving cooperation towards women in general. This converges with the play-order dependence of our finding of participants reporting to be angrier with non-sexualized (vs. sexualized) women after betrayal, but *not* when having played prior with a sexualized women (i.e. after being positively primed by them).

Oxytocin increases cooperation, and eliminates bias against non-sexualised women

As hypothesized, we found IN-OT to have a prosocial effect. It significantly increased both general cooperation across the whole PD game by 15% (p = .012), and specifically the transition-to-cooperation probability after mutual cooperation by 9% (i.e. CC outcomes; p = .050) or unreciprocated cooperation by 32% (i.e. betrayal; CD; p < .001). These results are consistent with a large body of evidence supporting a prosocial effect of the neuromodulator (Marsh et al., 2021; Martins, Lockwood, et al., 2022; Rilling et al., 2012; Striepens et al., 2011; Tarsha & Narvaez, 2023), and particularly during the PD whereby OT also increased the transition-to-cooperation probability after CD (but only when compared with vasopressin administration; (Rilling et al., 2012)), attenuated the BOLD response after

a CD outcome in the amygdala and anterior insula, key brain regions for emotional processing (X. Chen et al., 2016), and reduced betrayal aversion (De Dreu, 2012b). The link between OT and betrayal reaction has also been explored using other economical games whereby OT (Klackl et al., 2013) and variations of the OT receptor gene (Tabak et al., 2014) have been found to modulate behavioural and emotional responses, however there are also reports of no effect of OT on restoring trust in males after betrayal from unknown trustees (Yao et al., 2014).

The specific finding of OT increasing cooperation after mutual cooperation is consistent with OT being known to be highly produced during mutually positive interactions such as social support and bonding (De Dreu, 2012b; Striepens et al., 2011), and to mediate the inherent reward value of these experiences (Clark-Elford et al., 2014; Dölen et al., 2013; Mouchlianitis et al., 2022). Indeed, in PD specifically, OT has been found to increase the activity of brain regions associated with and social bonding during mutual cooperation (Rilling et al., 2012, 2014). This collection of evidence supports the idea that OT acts to maximize social reward and mutual cooperation, thus promoting prosocial behaviour. In addition, our finding that OT reduced the degree of happiness after a 'benevolent cooperation' by the opponent (that which takes place after a defection by the participant, i.e. DC outcome, whereby he gains the maximum reward at the expense of the opponent) may suggest, speculatively, that OT also minimizes the reward value of exploiting others (p = .017, η_p^2 = .123).

Our finding of OT also increasing 'forgiving' cooperation (i.e. after betrayal; CD; very large effects $p < .003$, Cohen's d > 1.31), is particularly in line with evidence of it increasing trust (Klackl et al., 2013; Tabak et al., 2014) after previous betrayal in other social-economic games. This means OT has herein increased the will to forgive and tolerate, to continue cooperating even after disappointment, and to signal trust has not been affected by a previous betrayal. Furthermore, and in line with a commonly believed context-dependent role of OT (Bartz et al., 2011) we found that the positive effect of OT on 'forgiving' cooperation, increasing it by 32%, is in fact also significantly dependent on opponent type and play order - as previously mentioned. Although a positive effect of OT was evident in both play orders and opponent types, OT roughly doubled and tripled 'forgiving' cooperation in sexualized and non-sexualized women, respectively, when non-sexualized women played first. Interestingly, this happened in parallel of OT (vs. placebo) also enhancing, in the same play-order, the agency perception of non-sexualized (small effect; p =

.004, d = 0.45, **Figure 18**). As such, the negative bias towards non-sexualized women observed in this play-order (discussed in the previous section) was completely annulled by OT, even increasing cooperation above that towards sexualized in the placebo context. Contributing to this effect may be that OT increases the perceived attractiveness of faces, as previously reported (Striepens et al., 2014; Theodoridou et al., 2009), and that sexual attraction elicits approach behaviours (Le Moëne & Ågmo, 2018), which, in turn, OT has also specifically been hypothesized to promote by the approach-withdrawal hypothesis of OT (Harari-Dahan & Bernstein, 2014). We note that although OT enhanced the perception of agency for non-sexualized opponents, it did not affect the experience perception ($p > .05$), we have no grounds to believe that the sexualization manipulation in this study lead to objectification of the sexualized opponent (K. Gray et al., 2011). Regarding our previous finding of higher angry ratings after CD outcomes for non-sexualized opponents, but only when playing with them first, we also find it is restricted to the OT group ($p = .001$, Cohen's d = 0.50), consistent with others that found IN-OT to increase the self-reported fear and anger to CD outcomes against human opponents, but only in female participants (X. Chen et al., 2016).

Prosocial effects of OT in PD may take place via numerous intermediate processes. OT may be increasing the participant's capacity to recognize the opponent's *general* intention to cooperate (which was algorithmically set to reciprocate cooperation at 67% chance and reciprocate defection at 90% chance). Such rationale would be in line with OT facilitating cognitive empathy, i.e. interpretation of others' emotions, intentions, and mental states, as has been shown (Abu-Akel et al., 2015; Barchi-Ferreira & Osório, 2021). Indeed, herein OT modulated the perception of agency of opponents by putatively highlighting the non-sexualized opponents' 'human uniqueness' features (H. M. Gray et al., 2007; Haslam & Loughnan, 2014). Alternatively, or additionally, OT's facilitation of a forgiving attitude, which allows the basis for trust and future cooperation in human intercation (Rilling et al., 2012), may be taking place due to its boosting of the reward value of mutual cooperation (which, as mentioned, was also increased by OT). This interaction between the OT and the dopamine reward systems is suggested by a range of evidence: OT receptors are expressed across the dopaminergic mesocorticolimbic system (Peris et al., 2017; Quintana, Rokicki, et al., 2019); OT typically affects dopamine-dependent cognitive processes in learning, salience and cognition (Shamay-Tsoory & Abu-Akel, 2016), and OT rendering caregiving and pare-bonding rewarding (Baskerville & Douglas, 2010; Skuse & Gallagher, 2009).

Opponent sexualization increases P3 and FRN amplitudes

We found that only when playing against sexualized women first, was a significant increase in P3 (p = .008, Cohen's d = 0.42) and a decrease in FRN (p < .001, Cohen's d = 0.59) amplitudes elicited by sexualized (vs. non-sexualized) women. As initially hypothesized, non-sexualized women would be usually perceived as more friendly and similar to the self, while the sexualized ones would be perceived as more distant to the self. This aligns with what we found, given previous evidence of a higher P3 amplitude being associated with playing with strangers vs. friends (Y. Chen et al., 2017; Y. Wang et al., 2014), which probably steams from it also being associated with higher arousing and unexpected stimuli, which need higher attention allocation and cognitive processing (Polich, 2007; Wada et al., 2019), such as stranger (vs. familiar) stimuli. The fact that this effect did not take place when sexualized played last, may – speculatively, as the P3 has been found to decrease with habituation (Friedman et al., 2001) - indicate some habituation/desensitisation, i.e. sexualized women might have been more arousing and unexpected when appearing in a scientific experimental session context when no other types of women had been yet presented. We also found P3 amplitudes were higher for sexualized (vs. non-sexualized) women only when considering expected outcomes (i.e. reciprocated, CC and DD, p = .004, Cohen's d = 0.45). As P3 amplitude is thought to positively reflect the allocation of cognitive resources (Polich, 2007) and subsequent memory context-updating processes (Hajcak & Foti, 2020), our finding corroborates our proposal that participants were attributing more motivational salience to sexualized women's behaviour, in a context of a higher approach attitude (vs. non-sexualized), we have proposed in the first section of this Discussion. The fact that this P3 difference was only detected in expected outcomes, may derive from the fact that these include a majority of CC, and mutual cooperation was the most motivationally relevant (and aimed for) outcome in an approach attitude.

Regarding FRN, which is thought to reflect the interpretation of an outcome as good or bad for the self, depending on personal self-interest (Fredrickson & Roberts, 1997), our finding that FRN was higher for non-sexualized (vs. sexualized; as it was for losses vs. gains) may reflect a negative perception of non-sexualized vs. sexualized women as a cooperating partner in the PD, and the decreased willingness to cooperate with them, which was observed in our behavioural findings. But unlike the behavioural findings, this neural sexualization bias was not reverted with OT administration. The fact that this effect only took place when sexualized women played first (just as for the P3 amplitude, abovementioned) might – speculatively – be

due to participants being therein more disappointed with the 'disappearance' of a women type they preferred to engage with.

Lastly, we note that, as expected and serving as quality-control, both P3 and FRN amplitudes were significantly dependent (as main effects) on expectancy and valence of the PD outcomes, respectively, in the directions predicted by previous evidence.

Oxytocin's increase of P3 latency may reflect OT's elimination of bias against non-sexualised women

Our finding that OT increased P3 latency when participants played against non-sexualized women (small effect; $p = .012$, Cohen's d = 0.39), and when playing with them first (small effect; $p = .003$, Cohen's d = 0.47), may be a reflection of our behavioural finding of OT increasing 'forgiving' cooperation – also more so with non-sexualized – and also in the same play-order. Since longer P3 latencies are indicative of a longer feedback evaluation time (Dickson & Wicha, 2019), they may reflect a higher cognitive load necessary to process outcomes, especially unexpected. As we proposed in the first section of this discussion pertaining to our behavioural results, non-sexualized women, when playing before sexualized women might have 'lost the benefit' of a positive priming by sexualized women which we think motivated a higher approach and cooperation with women from participants. We propose that it is in this situation that IN-OT has the most relevant role (in increasing forgiving cooperation, as observed) and that it may achieve this – at the neural level - by increasing the time (i.e. tagged by P3 latency) participants spent evaluating the previous PD outcome, and thus compensating for the observed negative bias towards non-sexualized women seen in placebo.

Limitations

Limitations of this study must be highlighted. First, others including us have reported sex-differences in the PD behaviour and its modulation by OT (X. Chen et al., 2016; Neto et al., 2020; Rilling et al., 2014), leaving the open question of whether some of the present results are replicable in female (or non-heterosexual) male participants. Second, only the most reported dosage (24 IU) was used in this study, which allows comparability with most of the literature; however to more comprehensively assess OT's influence on neural activity and behaviour, a variation of OT dosages is warranted (Martins, Brodmann, et al., 2022),

which we recommend in future studies. Third, an EEG analysis during decision-making toward sexualized and non-sexualized targets was not possible in our study setup. Fourth, and lastly, we have not analysed our EEG data per PD outcome as we did for the behavioural findings since we were unable to control for the different expositions each participant had to each PD outcome, although some authors have tried (Cervantes Constantino et al., 2021).

6.5 Conclusion

Sexualization biases are pervasive across societies and potentiates sexist, misogynistic and even violent attitudes, particularly – albeit not exclusively - by men. However, the present study challenges the general premise that sexualization bias entails exclusively anti-social or pro-self behaviour, as it reports a positive bias towards higher 'forgiving' cooperation with sexualized women by heterosexual males. Speculatively, this bias seems to supersede any potential out-group equivalence to sexualized women which was suggested by the literature, and might, at least partially, explain the also widespread phenomena, in women, of self-sexualisation. This study also shows a robust prosocial role for OT, supporting frequent previous evidence and existing theories of a role of OT being prosocial or pro-approach behaviour (Marsh et al., 2021; Martins, Lockwood, et al., 2022; Rilling et al., 2012; Striepens et al., 2011; Tarsha & Narvaez, 2023). Given the novel research questions being tested, the present findings, particularly the neural, should be considered preliminary and their interpretation tentative, and generating of finer hypotheses.

7- Conclusions

Social interactions are a crucial element of our daily lives. Since young we have learned to recognize, process and express social signals like speech or facial expressions, which elicit central and/or autonomic psychophysiological responses. OT is a neurobiological agent known to be involved in many of these social processes, mainly by promoting prosocial cooperative and altruistic behaviours. However, accumulating evidence also suggest that OT's prosocial effects depend on contextual and individual characteristics which may lead to antisocial behaviours. Taken together, these findings motivated the formulation of two hypotheses that aim to explain OT's overarching role on social cognition, both suggesting OT interacts with mesocorticolimbic dopamine: 1) the social salience hypothesis, which posits OT to modulate the salience attribution to both positive and negative social stimuli (Shamay-Tsoory & Abu-Akel, 2016); and 2) the approach-withdrawal hypothesis, which questions OT's social specificity and suggests it modulates the approach motivation linked to reward, and the withdrawal motivation linked to fear (Harari-Dahan & Bernstein, 2014).

The main objectives of this thesis were to use a pharmaco-multimodal approach to research the effects of OT on the central and autonomic psychophysiology of human social cognition. These objectives were achieved, and most importantly, this thesis reports on the first evidence of an OT-dopamine interaction during salience attribution on eye-gaze, the leading psychophysiological correlate of attention orienting responses (McKay et al., 2021; Posner, 2016; Sheliga et al., 1994), and on pupillometry, a robust psychophysiological correlate of arousal. This thesis also reports significant IN-OT effects on all tested psychophysiological modalities, except sEBR, in pupillometry, eye-gaze, HRV and EEG ERPs. These multimodal findings were collected at rest, during free-viewing videoclips, during a reinforcement learning paradigm, and while playing a PD game. More concretely, IN-OT had an effect on the following psychophysiological correlates of social cognition: 1) pupil size during the sSAT; 2) PUI at rest; 3) eye-gaze during free-viewing social and non-social videoclips; 4) dwell time during the sSAT; 5) HF-HRV during rest; and 6) P3's latency during the PD. It had no effect on SampEn of pupil size and on sEBR, both measured at rest, and on EEG's P3 amplitude and FRN, during the PD.

Altogether, this work, which is summarized next, provides an encompassing multimodal investigation of IN-OT's effects on the central and autonomic psychophysiology of social cognition, while incisively demonstrating that OT plays a crucial role in motivational salience.

Chapter 3 presented a study of IN-OT's temporal profile at rest on pupillometry and HRV, proxies of ANS activity. In the literature, IN-OT's most frequently used administration dosage of 24 IU was often considered to have its peak activity at around 40 min after administration, which was identified from indirect evidence of measuring OT concentrations in peripheral fluids, or from ANS activity during tasks that possibly confounded IN-OT's temporal profile assessment. In this study, a long experimental session was used where participants' pupillary and cardiac activity at one baseline time-window pre-administration and at six time-windows post-administration (from 15 to 100 min) were recorded. Two positive proxies of PNS activity (HF-HRV and PUI) and one of SNS activity (SampEn) were extracted. The results showed IN-OT *deactivated* the PNS as reflected by a decrease in PUI, starting from 65 min post-administration until the end of the last window measurement (100 min). They also showed IN-OT *activated* the PNS, as reflected by an increase in HF-HRV, in the 80 - 85 min post-administration window. The findings in this chapter provide a valuable reference for other researchers by showing that the peak effects of IN-OT are later than the 40 min time-window researched in most studies, and that they last longer than previous IN-OT studies' usual session length (up to 90 min). This work was published in a first quartile and indexed in PubMed journal and presented as a poster in a conference.

Chapter 4 reported an investigation of IN-OT's effects on GLIMPSE salience scores calculated from the eye-gaze of multiple observers free-viewing dynamic social and non-social interactions. Previous studies investigating the effects of IN-OT on the psychophysiology of attention orienting responses have mostly used unnatural simplified stimuli hindering their generalizability to daily life contexts. This work addressed these setbacks by showing that IN-OT increased the GLIMPSE salience scores for 18 (out of 20) videoclips except for one social positive-valence high-arousal (erotic), where it decreased the salience, and for one non-social (landscapes), where it had no significant differences with placebo. It also reported that IN-OT only significantly increased the subjective arousal ratings on another positive-valence high-arousal video, measured during a second viewing of the same videoclips. Overall, this work provides important contributions to the scientific community by showing that IN-OT's effects on enhancing salience generalize for dynamic social interactions with variable levels of valence (positive and negative) and arousal (high

and low). This work was presented as a poster at three conferences and is in preparation for submission in a first quartile and indexed in PubMed journal.

Several authors had posited that OT's social specificity originated from the fact that social stimuli were inherently more relevant/rewarding than non-social. However, none had directly orthogonalized social stimuli's socialness and reward features. **Chapter 5** presented a study that used a reinforcement learning paradigm to achieve this orthogonalization, and to test IN-OT's effects on pupil size and eye-gaze's dwell time. IN-OT interacted with the socialness of stimuli on dwell time, while being adjusted for individual tonic dopamine levels using sEBR, supporting the social salience hypothesis of OT. IN-OT also interacted with the stimuli's rewarding features on pupil size, supporting the approach-withdrawal hypothesis of OT. These effects were only found during reward anticipation, that is, during phasic dopaminergic release. This work importantly shows that IN-OT interacted with dopamine, which is at the core of both hypotheses of OT, to reinforce motivational saliency. This study was presented as a poster in five conferences and is in preparation for submission in a first quartile and indexed in PubMed journal.

Lastly, sexualization is often associated with nefarious, misogynistic, and abusive behaviours, as the sexualized person may be perceived as belonging to an out-group and as having less human-like attributes. However, it can also lead to courtship behaviours, as interacting with a sexualized person may be seen as positive and rewarding. In this context, the report presented in **Chapter 6** probed whether IN-OT and the sexualization of a PD opponent influenced cooperative behaviours and EEG ERP responses. After a betrayal, participants were found to cooperate less with the non-sexualized opponent. But validating its prosocial effects, the results showed IN-OT increased the overall frequency of cooperations, the probability of cooperating after mutual cooperation, and the probability of cooperating after being betrayed by both sexualized and non-sexualized opponents, negating the negative bias found for the non-sexualized, an effect that was tagged by P3's latency. Taken together, these findings crucially provide the first evidence for an interaction between the sexualization of a PD opponent and OT on cooperative behaviours. This work is in preparation for submission in a first quartile and indexed in PubMed journal.

Future work

The investigation elaborated for this thesis showed that the effects of IN-OT on social cognition are complex and diverse, opening the door for complementary future work. An important aspect to consider when researching the neurobiological substrate of social cognition is that neuropeptides do not act in isolation, as they centrally interact with others. The OT-dopamine interaction is an example, which was confirmed in this thesis. Much work is currently being done on investigating other OT interactions, like for example with testosterone. It has been proposed that these two agents modulate different aspects of intergroup dynamics, with OT being a promotor of social bonding, while testosterone being a promotor of asocial and self-centric behaviours (Cherki et al., 2024; Crespi, 2016; X. Yang et al., 2021). A recent meta-analysis on the effects of exogenous administration of these neuropeptides on cooperative behaviours confirmed a positive effect for OT, as also validated by this thesis, but no effect for testosterone (X. Yang et al., 2021). While this meta-analysis and other work properly factor the opponents' in-group/out-group membership, they overlook the opponents' hierarchical-status. This is warranted because testosterone promotes social dominance and status-seeking behaviours (Casto et al., 2019; Inoue et al., 2017; Nave et al., 2018; van der Meij et al., 2016; Zilioli & Watson, 2014), and because OT's effects in relation to hierarchical status are currently unexplored. Future work should aim to investigate OT-testosterone interaction in the context of hierarchical interpersonal dynamics, possibly using the PD or other socioeconomic games while controlling for the opponent's status.

Reward processing of social interactions is sexual-dependent, with females finding same-sex interactions more rewarding than males (Borland et al., 2019). This is worrying because most OT research, which is conducted in males, may not generalize to females. Indeed, IN-OT's prosocial effects are more evident in men than in females (Procyshyn et al., 2024). This sexual specificity is likely a consequence of differences in: 1) endogenous OT levels, as females have higher baseline OT concentrations in the cerebrospinal fluid (Altemus et al., 1999); 2) the interaction between OT and other hormones, which also have variable sex-specific concentrations; 3) sociocultural gender roles (Procyshyn et al., 2024); and/or 4) neural responses to OT (Borland et al., 2019). Regarding neural responses, there are reports of sex differences on the number of OTRs in rodents, and on the OTR binding in non-human primates (Procyshyn et al., 2024). In humans, methodological limitations hamper the estimation of the density of OTRs in the living brain, and particularly in the subregions associated with reward, but post-mortem assessments have provided minimal

support for a difference (Procyshyn et al., 2024). Importantly, the studies reported in this thesis were conducted with males because logistically, recruiting female participants presents additional challenges as it requires controlling for variables such as the phase of the menstrual cycle and the use of hormonal contraceptives, both of which can influence the outcomes of the studies. Despite these obstacles, including female participants alongside males would also reduce the parsimony of the statistical models, and demand an increase in sample size. As such, in conclusion, a natural recommendation for future work is to replicate the studies reported in this thesis but with a sample that includes female participants to increase the generalizability of the findings.

A multimodal approach was used in this thesis, aiming to best describe the effects of IN-OT on the psychophysiology of social cognition. This proved beneficial given that all studies reported herein demonstrated significant drug effects on all data modalities used, in eye-tracking, ECG and EEG. Despite this, other psychophysiological correlates could have been investigated. Skin conductance response, for example, measures eccrine sweat gland activity which is triggered by acetylcholine release in the SNS, such that its increase is a positive proxy of autonomic sympathetic arousal (Nikula, 1991; C. Wang et al., 2018). In relation to this psychophysiological correlate, IN-OT has been found to: 1) have no effect on its overall amplitude (Daniels et al., 2020; Gamer & Büchel, 2012); 2) enhance its amplitude in response to fearful stimuli (M. Eckstein et al., 2016); 3) decrease its amplitude in response to empathic- and self-embarrassment (Geng et al., 2018); and 4) reduce its recovery time to baseline during eye contact with another individual (Daniels et al., 2020). Given these conflicting and inconsistent findings, the use of skin conductance response in the study reported in **Chapter 3** would have benefited the literature by providing an assessment of the effects of IN-OT during a rest condition, unconfounded by other processes. Moreover, in the work of **Chapter 4**, the use of skin conductance response would have proven valuable to corroborate the findings related to the subjective arousal ratings. Additionally, this thesis showcases that there are clear advantages of using a multimodal approach while investigating the effects of IN-OT, but the segregated analysis of each data modality limited some of the potential benefits. Future work could also use multivariate approaches in order to better describe IN-OT's effects on the psychophysiology of social cognition.

Lastly, and as previously alluded to, OT signalling in the brain is dependent on OTR expression which is regulated by the OTR gene. Epigenetic mechanisms, such as DNA methylation, are known to influence gene expression through environmental factors like

early life adversity which is known to specifically influence the methylation of OTR gene (Danoff et al., 2021; Grimm et al., 2014), and early life adversity has been found to modulate the effects of IN-OT (Londono Tobon et al., 2018). Thus, future work aiming to replicate the studies of this thesis should consider controlling for the methylation of the OTR gene.

Concluding, the results of this thesis provide a pharmaco-multimodal investigation of OT's effects on the central and autonomic psychophysiology of social cognition in eye-gaze, pupillometry, HRV and EEG ERP. Taken together, these findings elucidate on how humans process and integrate social cues, particularly those related to motivational salience and social reward.

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Annex A – Supplemental material for Chapter 3

Temporal profile of intranasal oxytocin in the human autonomic nervous system at rest: an electrocardiography and pupillometry study

Supplementary details for Materials and Methods

Participants' exclusion criteria. history of endocrinological, cardiovascular, or neurological disorders, substance abuse, blocked nose; consumption of cannabis 2 weeks prior to data collection, and alcohol consumption, drugs or medication 24 hours prior, and smoking 2 hours prior to the experimental session; and caffeine consumption or heavy physical exercise or sexual activity on the experiment day.

Experimental procedure. Drug acquisition, storage and randomization of drug administration was performed and controlled by the hospital's pharmacy. IN-OT/placebo administration was at 2:24 pm (SD = 29 min) for all participants, and HRV and pupil recording at 2:11 pm, to restrict the impact of the circadian rhythm on baseline endogenous OT levels . Screening for eligibility was performed via self-report during an initial phone interview and in person via questionnaire, upon arrival to the first session, and their health state was assessed via medical examination, which included heart rate, blood pressure and electrocardiogram measurements. In the second session, only the eligibility questionnaire was administered.

Supplementary Results

Results – Kubio's proprietary PNS and SNS indexes, DFAα1 and RMSSD

Eyes closed. The exploratory analysis of the main effect of drug on Kubios' PNS index, $F(1, 176.68) = 3.22$, $p = .075$, $d = 0.21$, and its interaction with time, $F(5, 169.11) = 0.31$, $p = .909$ were not significant.

The exploratory analysis of the main effect of drug on Kubios' SNS index was statistically significant, $F(1, 175.47) = 4.94$, $p = .028$, $d = 0.32$, indicating that the SNS index increased

under IN-OT compared to placebo. The interaction with time was not significant, $F(5, 169.32) = 0.64$, $p = .668$.

The main effect of drug on DFAα1 $F(1, 182.77) = 0.73$, $p = .396$, $d = 0.08$ and its interaction with time, $F(5, 165.95) = 1.18$, $p = .321$, were not significant. However, pairwise comparisons indicated a significant difference between conditions in the last time-window (from 90 to 95 min), $t(175) = 2.33$, $p = .021$, $d = 0.81$, 95% CI [0.12, 1.51], such that DFAα1 increased under IN-OT compared to placebo.

Lastly, the main effect of drug on RMSSD, $F(1, 177.67) = 2.92$, $p = .089$, $d = 0.19$ and its interaction with time, $F(5, 169.39) = 0.29$, $p = .920$, were not significant.

Eyes open. The exploratory analysis of the main effect of drug on Kubios' PNS index $F(1, 172.63) = 1.13$, $p = .288$, $d = 0.39$, and its interaction with time $F(5, 162.83) = 0.68$, $p = .641$, were not significant.

The exploratory analysis of the main effect of drug on Kubios' SNS index $F(1, 176.04) = 0.04$, $p = .845$, $d = 0.43$, and its interaction with time $F(5, 166.78) = 1.17$, $p = .327$ revealed it was not significant, but exploratory pairwise comparisons indicated a difference in the first time-window (from 20 to 25 minutes) $t(173.61) = 1.98$, $p = .049$, $d = 0.69$, 95% CI [-1.39, 0.00], such that Kubios' SNS index decreased under IN-OT compared to placebo.

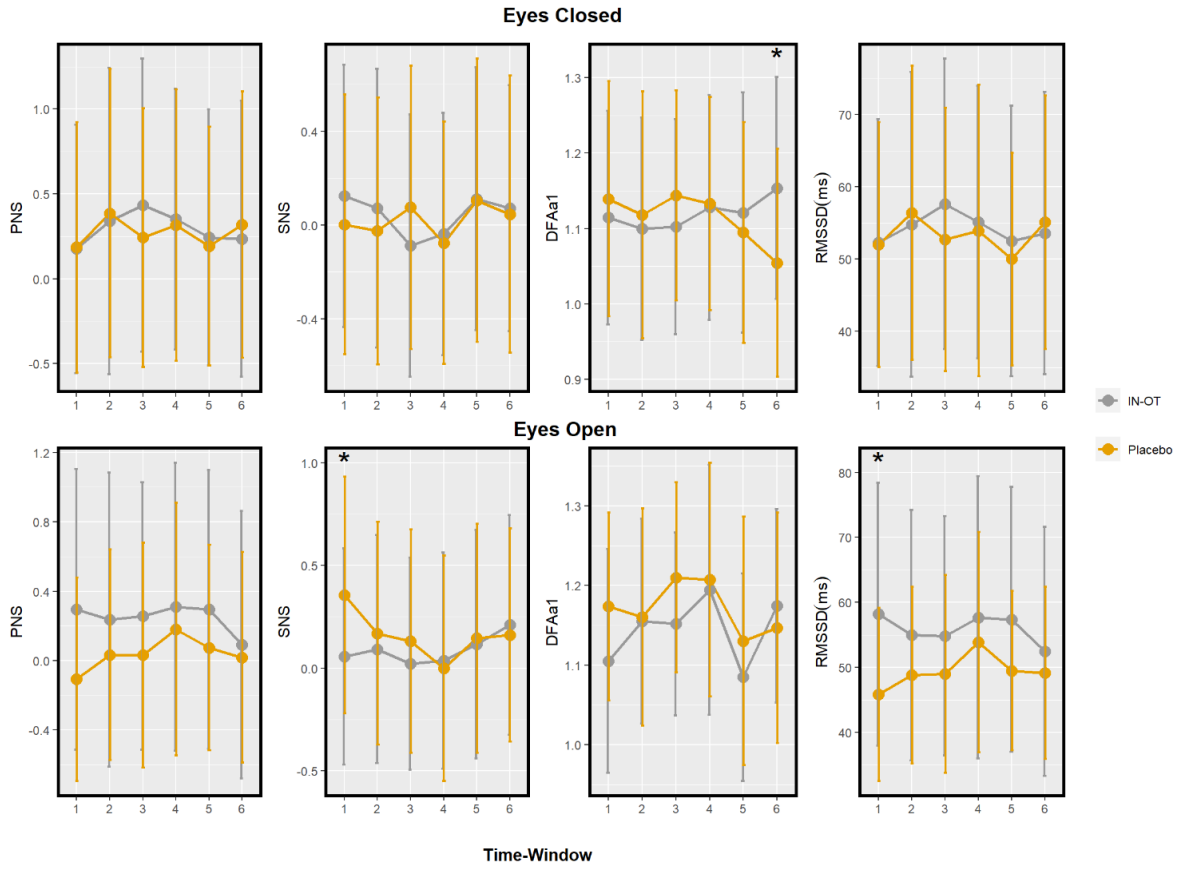
The main effect of drug on DFAα1 $F(1, 183.25) = 3.07$, $p = .081$, $d = 0.43$, and its interaction with time $F(5, 171.04) = 0.54$, $p = .749$, was not significant.

Lastly, the main effect of drug on RMSSD $F(1, 173.66) = 0.76$, $p = .385$, $d = 0.38$, and its interaction with time $F(5, 164,57) = 0.862$, $p = .508$ were not significant. However, exploratory pairwise comparisons indicated a difference in the first time-window (from 20 to 25 minutes) $t(173.49) = 1.99$, $p = .048$, such that RMSSD was increased under IN-OT compared to placebo.

Annex A Table 1 – Summary of the results of the effect of drug on Kubio's proprietary PNS and SNS indexes, DFAα1 and RMSSD. Statistically significant ($p < 0.05$) main effects are marked with and asterisk (*) and only significant pairwise comparisons are shown.

Neurophysiological Measure	All main effects of drug (IN-OT vs placebo)	Pairwise comparisons per TW (if p< .05)	Drug effect direction	Tentative ANS response interpretation
Eyes Closed				
Kubio's PNS index	N.S. - $F(1, 176.68) = 3.22$, $p = .075$, $d = 0.21$	-	-	-
Kubio's SNS index	$F(1, 175.47) = 4.94$, $p = .028$*, $d = 0.32$	-	IN-OT ↑	SNS ↑
DFAα1	N.S. - $F(1, 182.77) = 0.73$, $p = .396$, $d = 0.08$	TW 6 - $t(175) = 2.33$, $p = .021$, $d = 0.81$, 95% CI [0.12, 1.51]	IN-OT ↑	PNS ↓ SNS ↓
RMSSD	N.S. – $F(1, 177.67) = 2.92$, $p = .089$, $d = 0.19$	-	-	-
Eyes Open				
Kubio's PNS index	N.S. - $F(1\ 172.63) = 1.13$, $p = .288$, $d = 0.39$	-	-	-
Kubio's SNS index	N.S. - $F(1\ 176.04) = 0.04$, $p = .845$, $d = 0.43$	TW 1 - $t(173.61) = 1.98$, $p = .049$, $d = 0.69$, 95% CI [-1.39, 0.00]	IN-OT ↓	SNS ↓
DFAα1	N.S. - $F(1, 183.25) = 3.07$, $p = .081$, $d = 0.43$	-	-	-
RMSSD	N.S. – $F(1, 173.66) = 0.76$, $p = .385$, $d = 0.38$	TW 1 – $t(173.49) = 1.99$, $p = .048$, $d = 0.70$, 95% CI [0.00, 1.40]	IN-OT ↑	PNS ↑

Footnote: Eyes closed time-windows (TWs): 1 =15 – 20 min; 2 = 30 – 35 min; 3 = 45 – 50 min; 4 = 60 – 65 min; 5 = 75 – 80 min; and 6 = 90 – 95 min. Eyes open TWs: 1 = 20 – 25 min; 2 = 35 – 40 min; 3 = 50 – 55 min; 4 = 65 – 70 min; 5 = 80 – 85 min; and 6 = 95 – 100 min., TW = time-window, PNS = parasympathetic nervous system, SNS = sympathetic nervous system, DFAα1 = detrended fluctuation analysis scaling exponent, RMSSD = root mean square of successive differences, IN-OT = intranasal oxytocin, CI = confidence interval.



Annex A Figure 1 – Dynamics of 4 HRV measures after IN-OT: Kubio's proprietary PNS index (1st column) and SNS index (2nd column), DFAα1 (3rd column) and RMSSD (4th column); in a resting-state paradigm with eyes closed (top row) and eyes open (bottom row) conditions. A significant pairwise comparison (IN-OT vs placebo) at specific time-windows are marked with an *. Eyes closed condition: time-window 1 =15 – 20 min; time-window 2 = 30 – 35 min; time-window 3 = 45 – 50 min; time-window 4 = 60 – 65 min; time-window 5 = 75 – 80 min; and time-window 6 = 90 – 95 min. Eyes open condition: time-window 1 = 20 – 25 min; time-window 2 = 35 – 40 min; time-window 3 = 50 – 55 min; time-window 4 = 65 – 70 min; time-window 5 = 80 – 85 min; and time-window 6 = 95 – 100 min. Error bars: 95% CI. HF-HRV = high frequency heart rate variability, IN-OT = intranasal oxytocin, HRV = heart rate variability, PNS = parasympathetic nervous system, SNS = sympathetic nervous system, DFAα1 = detrended fluctuation analysis scaling exponent, RMSSD = root mean square of successive differences, CI = confidence interval.

Results – Main effect of time

Eyes closed. The main effect of time on all measure of HRV was not statistically significant: HF-HRV $F(5, 171.94) = 0.32$, $p = .901$; Kubio's proprietary PNS index $F(5, 169.11) = 0.71$, p

= .620; Kubio's proprietary SNS index $F(5, 169{,}32) = 0.87$, $p = .506$; DFAα1 $F(5, 165{,}95) = 0.23$, $p = .948$; and RMSSD $F(5, 169{,}39) = 0.56$, $p = .729$.

Eyes open. The main effect of time on PUI was statistically significant $F(5, 156.00) = 2.33$, $p = .045$ but not on SampEn $F(5, 154.59) = 0.77$, $p = .571$. The main effect of time on all measures of HRV was not statistically significant: HF-HRV $F(5, 169.80) = 1.52$, $p = .185$; Kubio's proprietary PNS index $F(5, 162.83) = 0.98$, $p = .434$; Kubio's proprietary SNS index $F(5, 166{,}78) = 1.39$, $p = .231$; DFAα1 $F(5, 171{,}04) = 1.69$, $p = .139$; and RMSSD $F(5, 164{,}57) = 0.98$, $p = .433$.

Behavioural - Mood Scales. The main effect of time was not statistically significant in any of the mood scales: alertness $F(5, 202.40) = 0.70$, $p = .622$; arousal $F(5, 202.49) = 0.14$, $p = .984$; and sociability $F(5, 221) = 0.48$, $p = .791$.

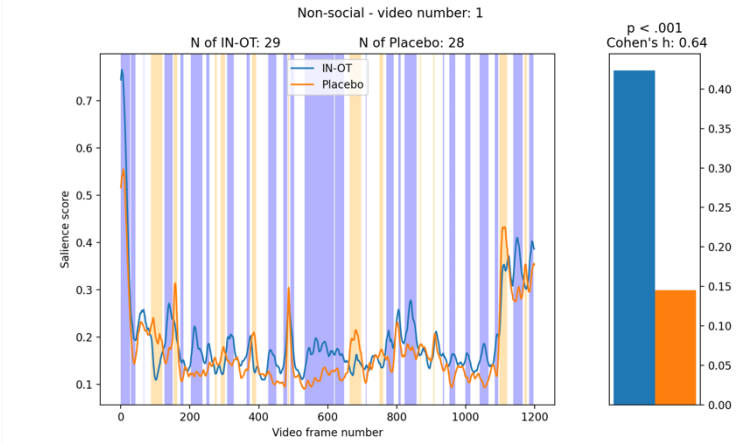
Annex B – Supplemental material for Chapter 4

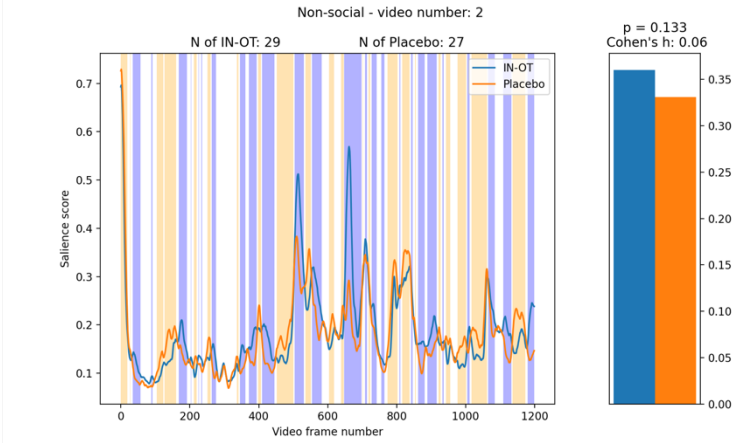
Oxytocin's role in naturalistic spatio-temporal salience attribution: a pharmaco-eye-gaze study

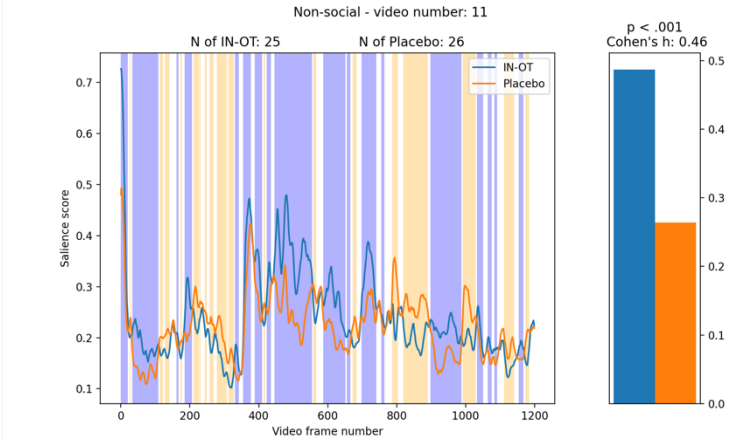
GLIMPSE salience scores

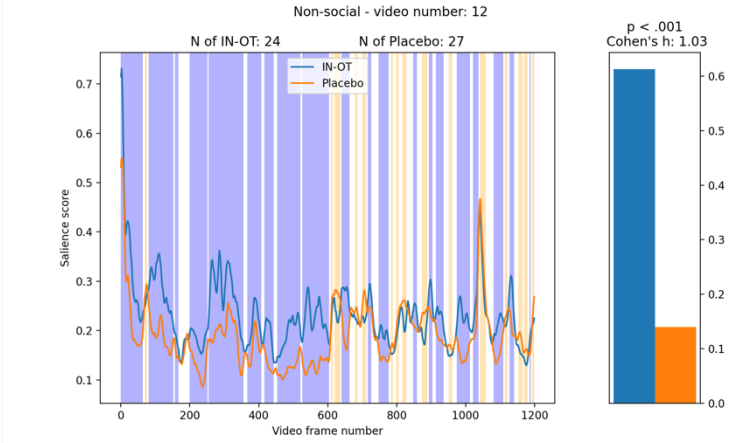
The videoclips and GLIMPSE salience scores are dynamically shown together in https://osf.io/smvfh/?view_only=28f507211a014e0f87abef6ec1cd777a

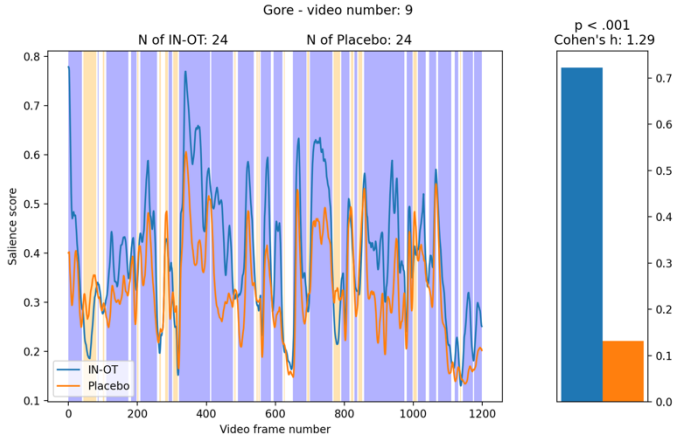
Next follows the plots for the GLIMPSE salience scores for each drug group, for all videoclips shown of each category, in the following order: Non-social (landscapes); negative-valence high-arousal (gore); negative-valence low-arousal; positive-valence high arousal (erotic); and positive-valence low-arousal. The blue shaded areas represent frame intervals in which the salience score of the IN-OT group is significantly higher (FDR-corrected) than that of the placebo group, and orange shaded areas represent the opposite. In the white shaded areas, the difference is not statistically significant. Salience score ranges from 0 to 1. The bar plot indicates the percentage of video frames where the drug difference is statistically significant: blue when 'IN-OT > placebo'; and orange when 'placebo > IN-OT'. The *p*-value and Cohen's h over the bar plot was obtained after conducting proportion a z-test comparing the difference between both drug groups. IN-OT = intranasal oxytocin; FDR = False Discovery Rate. Next follows the average arousal ratings for each video, for each drug group.

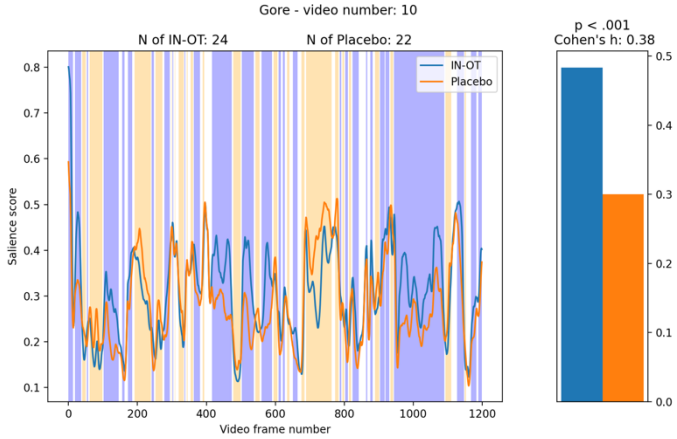

Non-social - video number: 1
N of IN-OT: 29
N of Placebo: 28
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 0.64

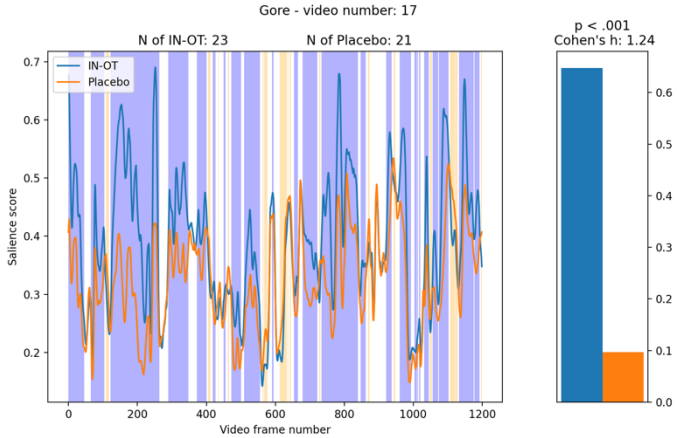

Non-social - video number: 2
N of IN-OT: 29
N of Placebo: 27
IN-OT
Placebo
Salience score
Video frame number
p = 0.133
Cohen's h: 0.06

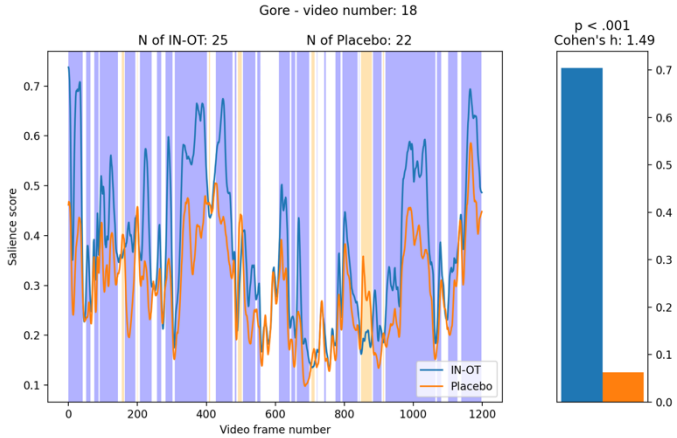

Non-social - video number: 11
N of IN-OT: 25
N of Placebo: 26
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 0.46

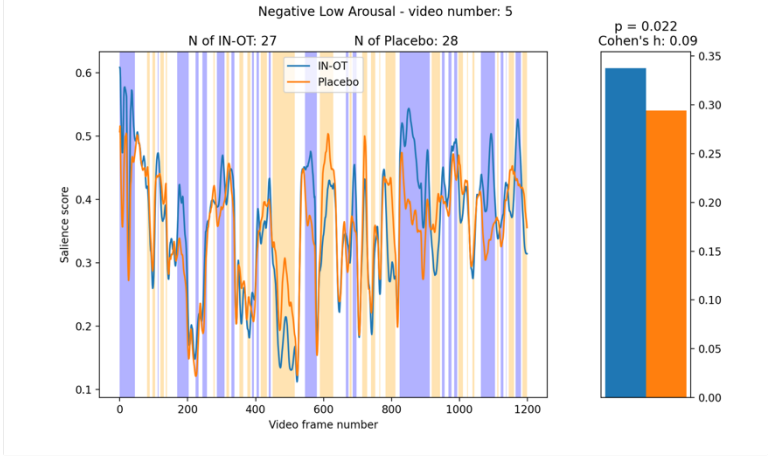

Non-social - video number: 12
N of IN-OT: 24
N of Placebo: 27
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 1.03

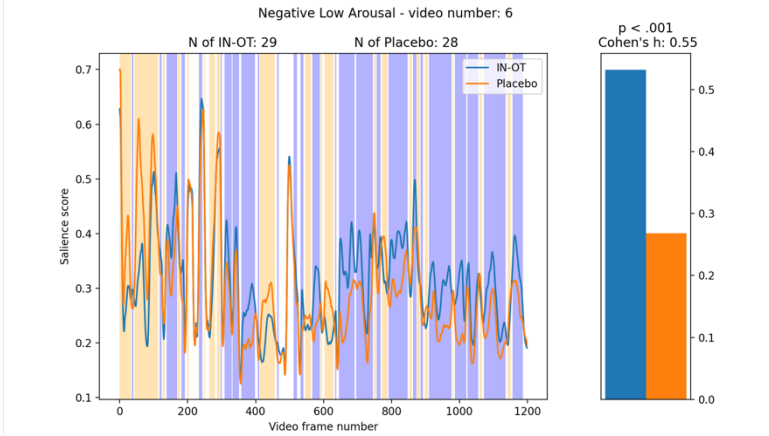

Gore - video number: 9
N of IN-OT: 24
N of Placebo: 24
p < .001
Cohen's h: 1.29
Salience score
Video frame number
IN-OT
Placebo

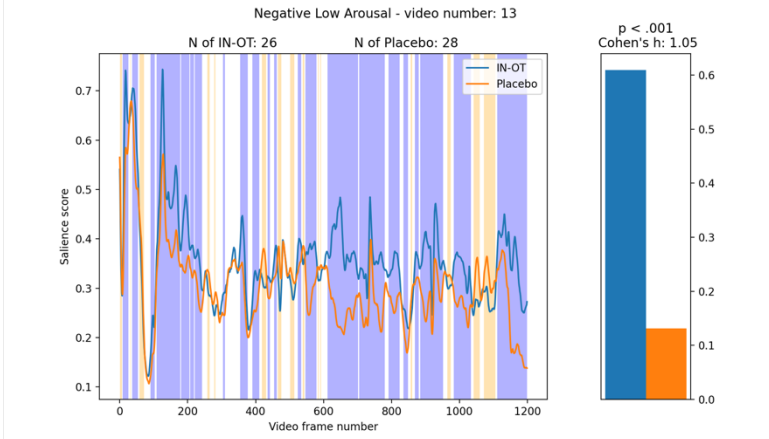

Gore - video number: 10
N of IN-OT: 24
N of Placebo: 22
p < .001
Cohen's h: 0.38
Salience score
Video frame number
IN-OT
Placebo

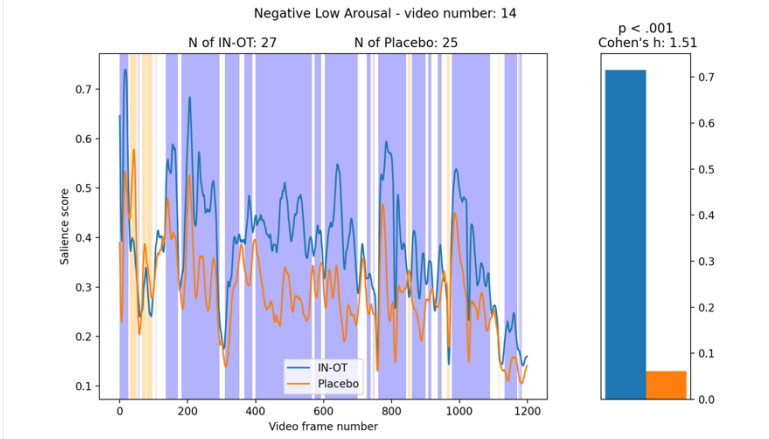

Gore - video number: 17
N of IN-OT: 23
N of Placebo: 21
p < .001
Cohen's h: 1.24
Salience score
Video frame number
IN-OT
Placebo

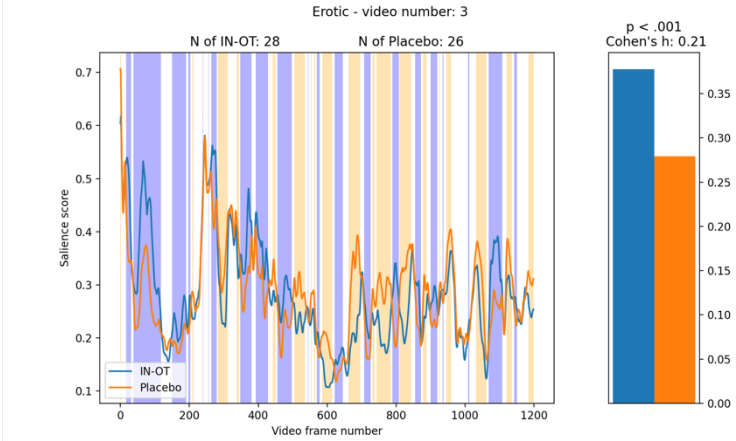

Gore - video number: 18
N of IN-OT: 25
N of Placebo: 22
p < .001
Cohen's h: 1.49
Salience score
Video frame number
IN-OT
Placebo

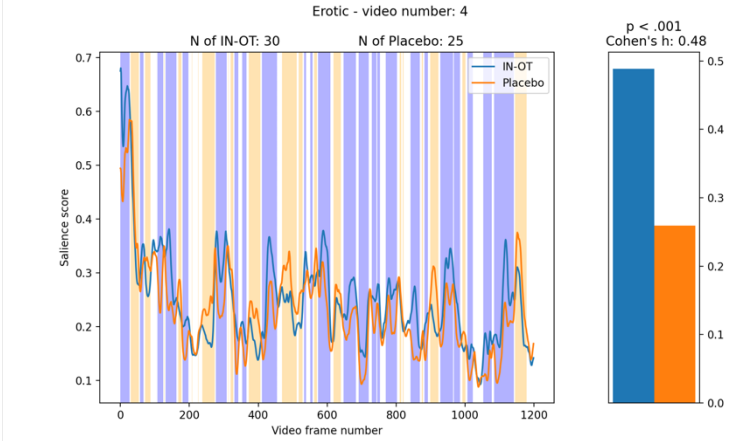

Negative Low Arousal - video number: 5
N of IN-OT: 27
N of Placebo: 28
p = 0.022
Cohen's h: 0.09
IN-OT
Placebo
Salience score
Video frame number

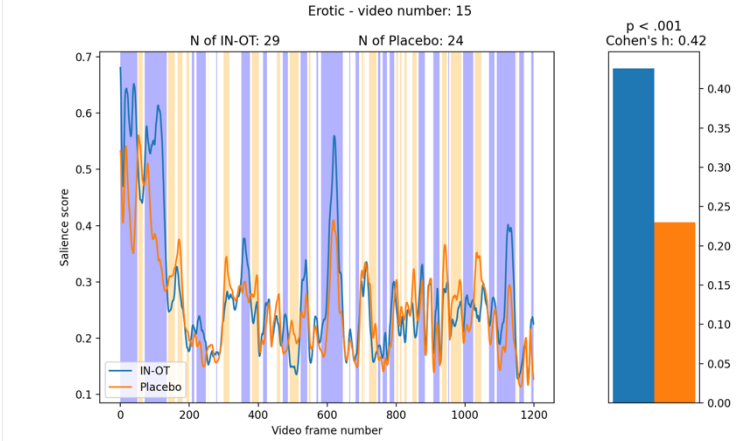

Negative Low Arousal - video number: 6
N of IN-OT: 29
N of Placebo: 28
p < .001
Cohen's h: 0.55
IN-OT
Placebo
Salience score
Video frame number

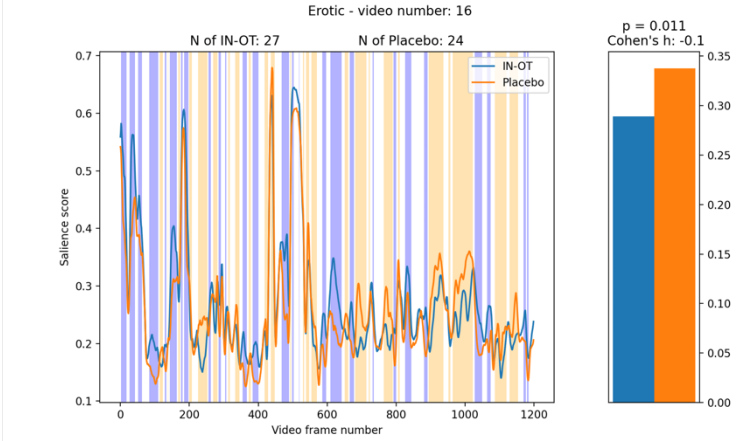

Negative Low Arousal - video number: 13
N of IN-OT: 26
N of Placebo: 28
p < .001
Cohen's h: 1.05
IN-OT
Placebo
Salience score
Video frame number

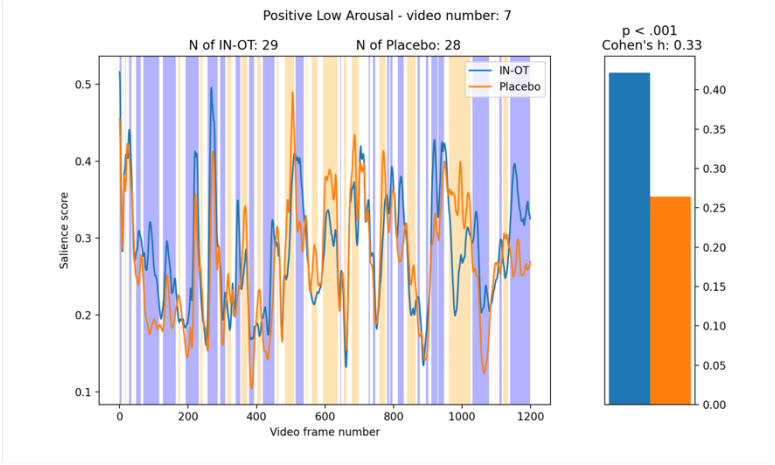

Negative Low Arousal - video number: 14
N of IN-OT: 27
N of Placebo: 25
p < .001
Cohen's h: 1.51
IN-OT
Placebo
Salience score
Video frame number

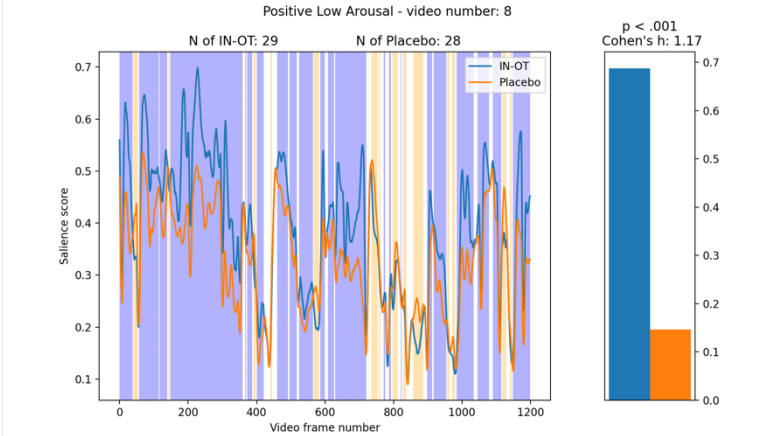

Erotic - video number: 3
N of IN-OT: 28
N of Placebo: 26
p < .001
Cohen's h: 0.21
Salience score
Video frame number
IN-OT
Placebo

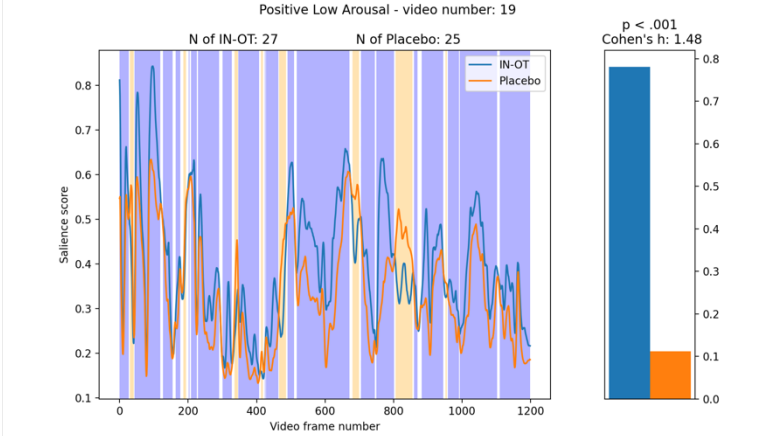

Erotic - video number: 4
N of IN-OT: 30
N of Placebo: 25
p < .001
Cohen's h: 0.48
Salience score
Video frame number
IN-OT
Placebo

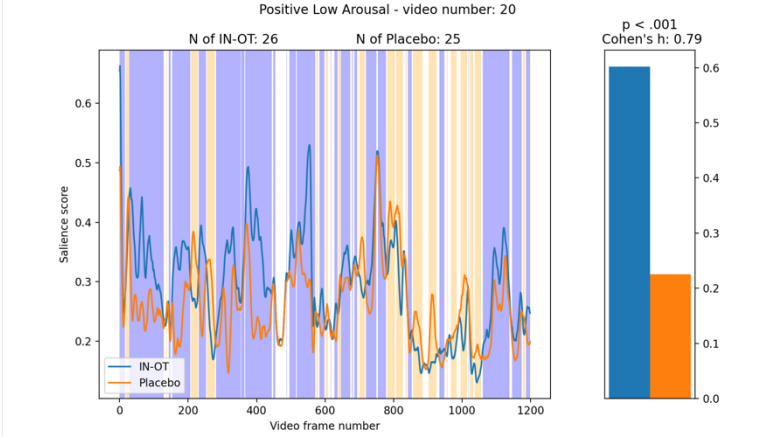

Erotic - video number: 15
N of IN-OT: 29
N of Placebo: 24
p < .001
Cohen's h: 0.42
Salience score
Video frame number
IN-OT
Placebo


Erotic - video number: 16
N of IN-OT: 27
N of Placebo: 24
p = 0.011
Cohen's h: -0.1
Salience score
Video frame number
IN-OT
Placebo


Positive Low Arousal - video number: 7
N of IN-OT: 29
N of Placebo: 28
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 0.33

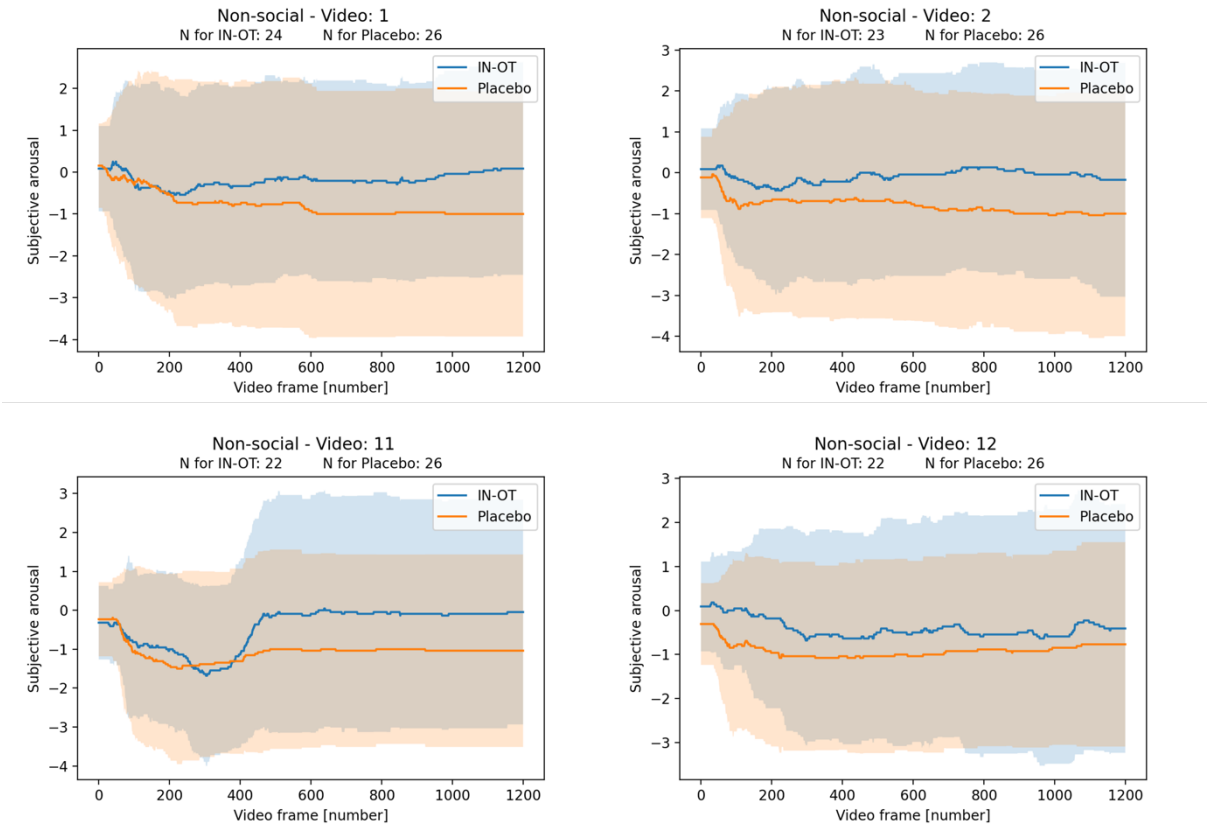

Positive Low Arousal - video number: 8
N of IN-OT: 29
N of Placebo: 28
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 1.17

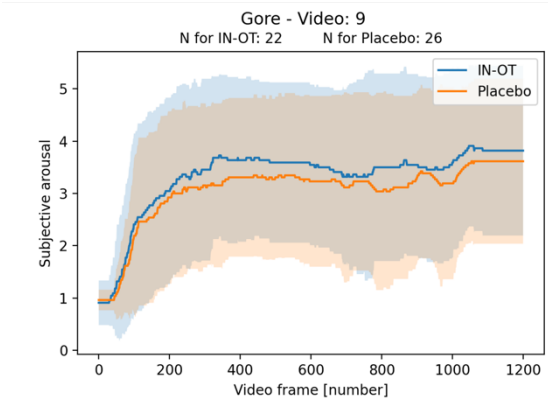

Positive Low Arousal - video number: 19
N of IN-OT: 27
N of Placebo: 25
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 1.48

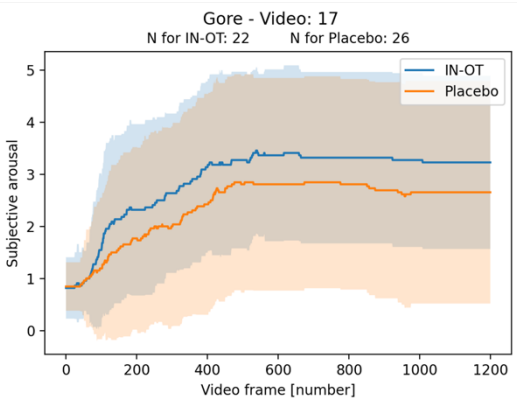

Positive Low Arousal - video number: 20
N of IN-OT: 26
N of Placebo: 25
IN-OT
Placebo
Salience score
Video frame number
p < .001
Cohen's h: 0.79

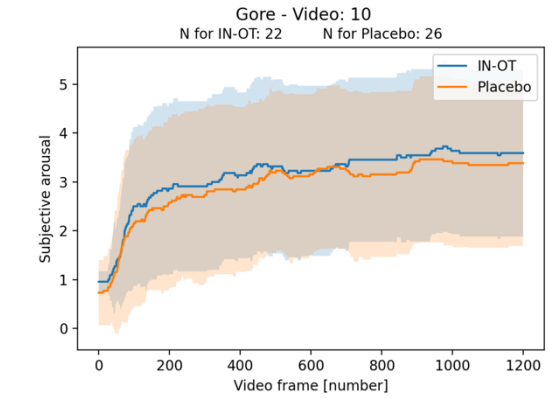
Subjective arousal ratings

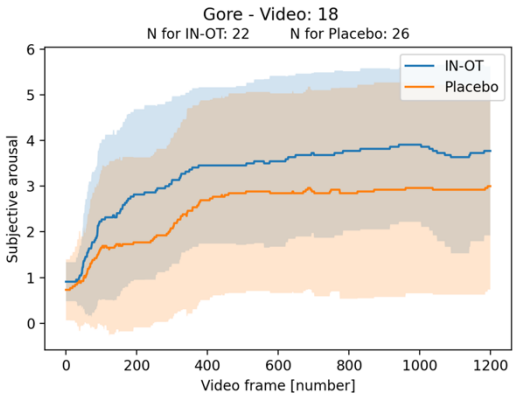
Next follows the mean subjective arousal ratings for each drug group, for all videoclip shown of each category, in the following order: Non-social (landscapes); negative-valence high-arousal (gore); negative-valence low-arousal; positive-valence high arousal (erotic); and positive-valence low-arousal. Subjective arousal rantings ranged from -5 to 5. Shaded areas represent ± SD. IN-OT = Intranasal oxytocin; SD = Standard Deviation.

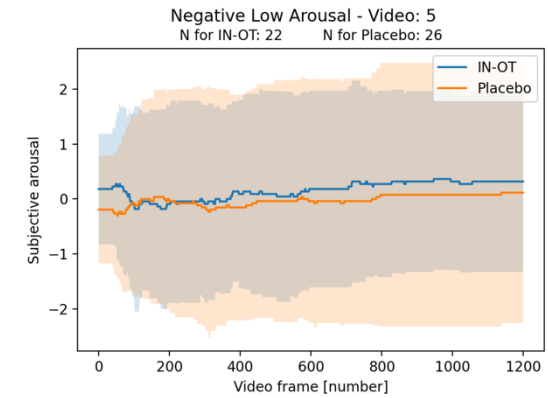


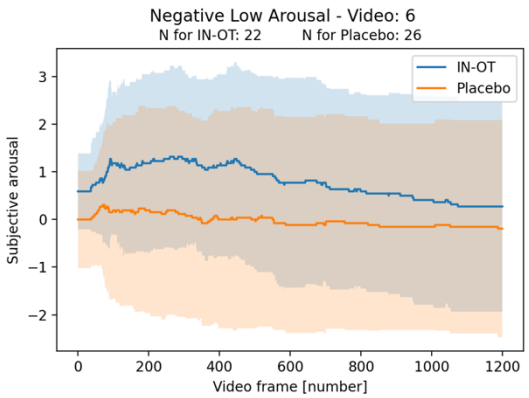

Gore - Video: 9
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

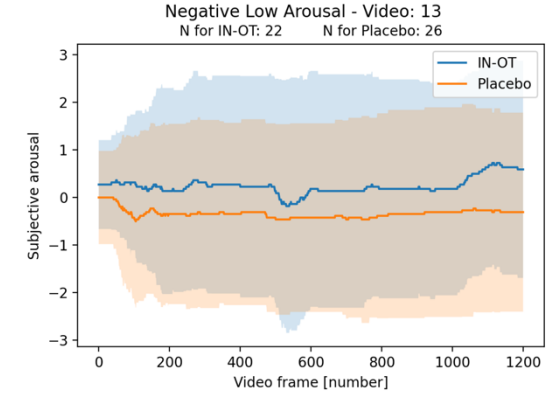

Gore - Video: 17
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

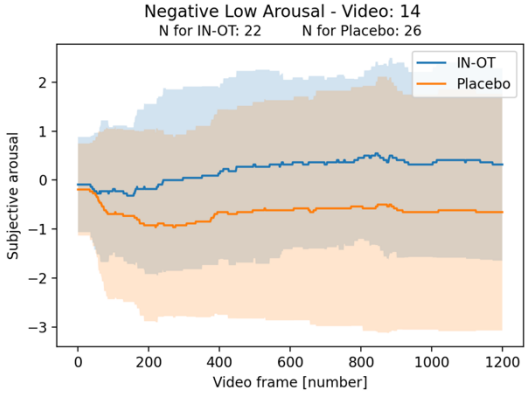

Gore - Video: 10
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

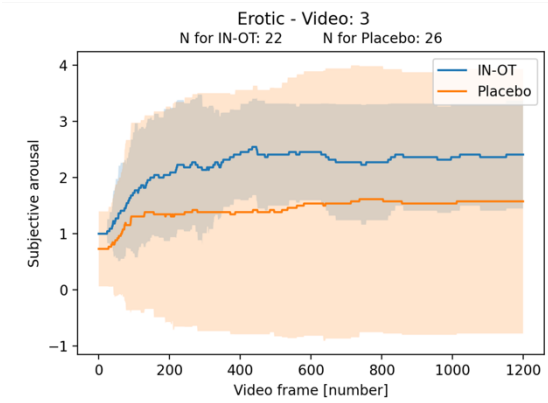

Gore - Video: 18
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

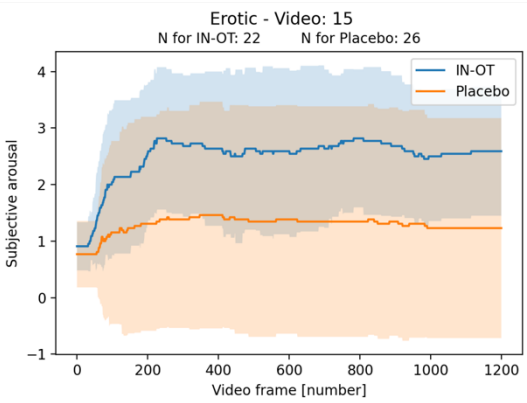

Negative Low Arousal - Video: 5
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

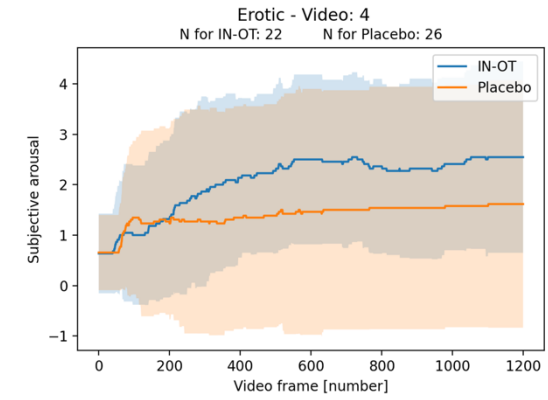

Negative Low Arousal - Video: 6
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

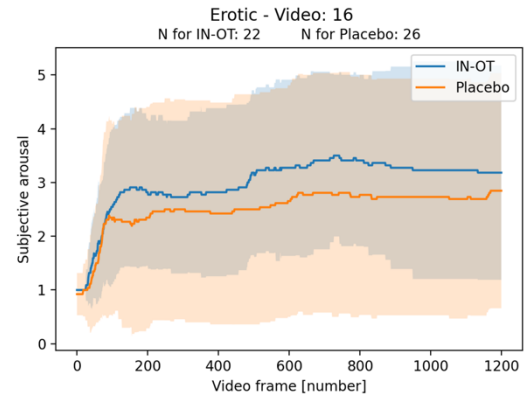

Negative Low Arousal - Video: 13
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

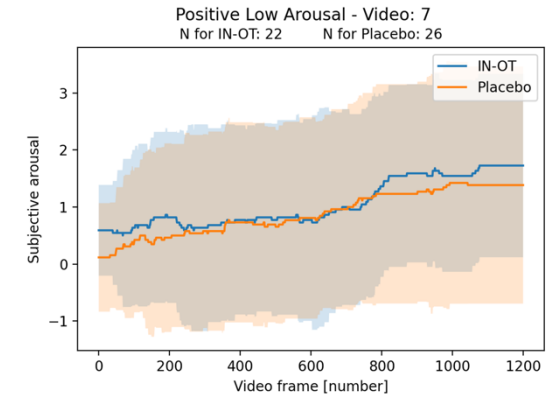

Negative Low Arousal - Video: 14
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

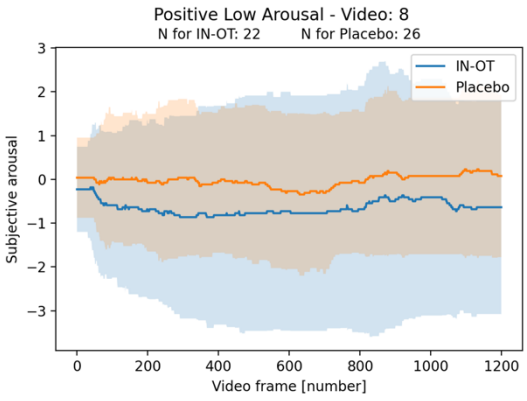

Erotic - Video: 3
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

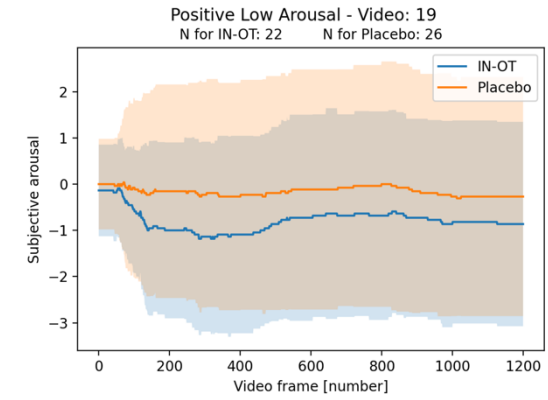

Erotic - Video: 15
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]

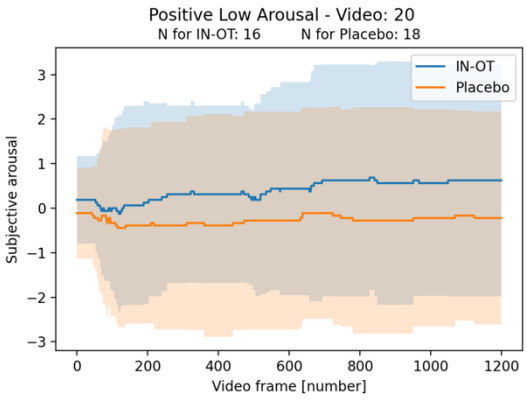

Erotic - Video: 4
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]


Erotic - Video: 16
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]


Positive Low Arousal - Video: 7
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]


Positive Low Arousal - Video: 8
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]


Positive Low Arousal - Video: 19
N for IN-OT: 22 N for Placebo: 26
IN-OT
Placebo
Subjective arousal
Video frame [number]


Positive Low Arousal - Video: 20
N for IN-OT: 16 N for Placebo: 18
IN-OT
Placebo
Subjective arousal
Video frame [number]

Annex C – Supplemental material for Chapter 6

Oxytocin's role on central and autonomic psychophysiological correlates of salience attribution: a pupil size and eye-gaze pharmacological study

sSAT tutorial, practice sessions, and individual task difficulty and reward calculations

Participants first underwent a tutorial that provided a step-by-step breakdown of the task, using example displays, with pink squares instead of the stimuli used in the main experiment. Afterwards, they went through two practice sessions for reaction time (RT) calibration, that consisted in responding to a probe appearing at the centre of the screen (no conditioned stimuli were displayed, contrary to the main experiment).

In the first practice session, which consisted of 20 trials, the probe duration on the screen varied randomly between 300-700 ms. For the second practice session, also consisting of 20 trials, the standard deviation (SD) of the fastest fraction of trials (SDF) from the first session was calculated, with this value then being used to set the minimum and maximum probe durations for the second session (mean RT from first session $\pm$ 2 $\times$ SDF), to ensure participants responded as quickly as possible and to adjust task difficulty to individual performance. Feedback was given at the end of each trial and practice session were not monetarily reinforced. If participants: responded before the probe disappeared, the message "Boa" (transl. "Nice") appeared; answered after the probe disappeared, the message "Tente responder mais rápido" (transl. "Try to respond faster") appeared; responded before the probe appeared, the message "Cedo demais" (transl. "Too early") appeared; did not respond, the message "Nenhuma tecla pressionada" (Transl. "No key pressed") appeared. After the second practice session, the SDF calculations were performed again to obtain the mean, minimum and maximum probe durations for the first block of the task.

In the main task, on reinforced trials, the reward was dependent on how quickly participants responded, calculated using the formula - X = 10 + 90 x (mean RT – trial RT / 3 x SDF) - with a maximum reward of 100 cents. The money earned from each trial added up to the participant's total during the task, with only a percentage of the money being given to the

participants at the end of the study. The feedback was the same as in the practice sessions described above.

Intranasal oxytocin/placebo administration, group baseline statistics and blinding efficacy

The procedure was as follows: participants first blew their noses and rated how clear each nostril was. The experimenter proceeded with both nostrils if both were clear or with only one if the other was blocked (4 out of 54 participants used only one nostril). The experimenter verbally explained the self-application procedure while physically demonstrating the procedure. The spray was then self-administered while participants kept their heads straight (without tilting backwards), once in each nostril, followed by normal breathing and a 30-sec pause. This procedure was repeated three times, for a total of 6 puffs. The spray bottle weight was measured before and after administration to verify if all participants used a similar about of spray.

Drug groups differed, by chance, regarding age [$t(52) = 8.80$, $p = .005$, $d = .393$], with the IN-OT: M group being, on average, 1 year younger than the placebo: M group (IN-OT: M = 23.11, SD = 2.87; placebo: M = 24.62, SD = 4.61). They, however, did not differ significantly in regards to: the digit span test [$t(51) = 0.01$, $p = .929$, $d = .270$] (IN-OT: M = 17.70, SD = 3.90; placebo: M = 16.69, SD = 3.59); anxiety state [$t(52) = 1.52$, $p = .223$, $d = .282$] (IN-OT: M = 67.89, SD = 5.22; placebo: M = 66.15, SD = 6.99); nor in terms of empathy scores: cognitive empathy [$t(52) = 0.43$, $p = .515$, $d = .142$] (IN-OT: M = 11.18, SD = 2.09; placebo: M = 10.85, SD = 2.57), emotional reactivity [$t(52) = 1.78$, $p = .188$, $d = .118$] (IN-OT: M = 10.14, SD = 3.14; placebo: M = 9.81, SD = 2.51), social skills [$t(52) = 0.09$, $p = .763$, $d = .098$] (IN-OT: M = 9.36, SD = 2.53; placebo: M = 9.12, SD = 2.42), empathic difficulties [$t(52) = 0.24$, $p = .624$, $d = .137$] (IN-OT: M = 15.43, SD = 2.89; placebo: M = 15.85, SD = 3.22) or total scores [$t(52) = 1.85$, $p = .180$, $d = .137$] (IN-OT: M = 48.79, SD = 5.04; placebo: M = 47.96, SD = 6.86).

At the end of the experimental session, participants were asked what the chance was they received the active agent vs. placebo: M (scale: 0-10; “0” corresponds to “0% active agent”; “10” corresponds to “100% active agent”), to confirm that they were unable to differentiate between the two. Indeed, their responses were not influenced on the actual random drug group allocation [$\chi^2(1) = .02, p = .879$]. For “believe they received placebo: M”, we counted

participants that answered from 0-4; for “believe they received active agent”, we counted participants that answered from 5-10, with a total of 49 out of the 54 participants analysed having responded to this question.

Annex D – Supplemental material for Chapter 6

Intranasal oxytocin reverses negative cooperation bias towards non-sexualized women by men: a pharmaco-electroencephalography study

Behavioral measurements

Behavioral measurements

Table S1. Overall effects of drug, opponent and play-order on the frequency of cooperation in general (across all trials) and on the rate of cooperation after each outcome. OT – Oxytocin; PL – Placebo. Asterisks signal statistically significant effects: *$p < .050$; **$p < .010$; ***$p < .001$.

General frequency of cooperation across all outcomes			
Effects	**Wald Chi-Square**	**df**	***p*-value**
Drug	6.32	1	.012* (OT > PL)
Opponent	1.57	1	.210
Play-Order	0.58	1	.446
Play-Order x Drug	0.72	1	.396
Play-Order x Opponent	2.89	1	.089
Drug x Opponent	0.15	1	.697
Play-Order x Opponent x Drug	0.05	1	.818
Transition-to-cooperation probability after Cooperation - Cooperation outcome			
Effects	**Wald Chi-Square**	**df**	***p*-value**
Drug	3.84	1	.050* (OT > PL)
Opponent	1.35	1	.246
Play-Order	0.08	1	.782
Play-Order x Drug	0.20	1	.656
Play-Order x Opponent	0.18	1	.669
Drug x Opponent	1.95	1	.162
Play-Order x Opponent x Drug	1.94	1	.164
Transition-to-cooperation probability after Cooperation - Defection outcome			
Effects	**Wald Chi-Square**	**df**	***p*-value**
Drug	14.15	1	< .001*** (OT > PL)
Opponent	2.48	1	.115
Play-Order	0.02	1	.881
Play-Order x Drug	2.94	1	.087
Play-Order x Opponent	8.80	1	.003**
Drug x Opponent	0.59	1	.444
Play-Order x Opponent x Drug	3.85	1	.050*
Transition-to-cooperation probability after Defection - Cooperation outcome			
Effects	**Wald Chi-Square**	**df**	***p*-value**
Drug	1.07	1	.300
Opponent	< 0.01	1	.971
Play-Order	0.18	1	.669
Play-Order x Drug	0.17	1	.685
Play-Order x Opponent	2.65	1	.104
Drug x Opponent	1.95	1	.162
Play-Order x Opponent x Drug	0.12	1	.744
Transition-to-cooperation probability after Defection - Defection outcome			
Effects	**Wald Chi-Square**	**df**	***p*-value**

Drug	1.06	1	.304
Opponent	2.05	1	.152
Play-Order	0.68	1	.410
Play-Order x Drug	0.15	1	.698
Play-Order x Opponent	0.05	1	.825
Drug x Opponent	0.05	1	.823
Play-Order x Opponent x Drug	0.45	1	.503

Table S2. Post-hoc pairwise comparisons of statistically significant interactions on the transition probability of cooperation after a cooperation-defection (CD) outcome (as per **Table S1**). Asterisks signal statistically significant effects accompanied by their direction. OT – Oxytocin; PL – Placebo; SE – Standard Error; emmean – Estimated Marginal Means; S – Sexualized; NS – Non-sexualized; Play-order S -> NS – a sexualized woman is the first opponent and a non-sexualized woman the second; Play-order NS -> S – a non-sexualized woman is the first opponent and a sexualized woman the second.

Transition-to-cooperation probability after CD outcome								
Post-hoc comparisons for interaction of opponent and play-order on the probability of cooperation after CD outcome								
Play-order	**Opponent contrast**	**emmean S**	**emmean NS**	**Mean difference**	**SE**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S - NS	0.52	0.57	-0.05	0.03	.116	0.14	-
NS -> S	S – NS	0.62	0.45	0.17	0.07	.020	0.55	S > NS
Opponent	**Play-order contrast**	**emmean play-order 1**	**emmean play-order 2**	**Mean difference**	**SE**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS – NS -> S	0.52	0.62	-0.10	0.10	.300	0.29	-
NS	S -> NS – NS -> S	0.57	0.45	0.12	0.09	.182	0.38	-
Post-hoc comparisons for interaction of opponent, play-order and drug on the transition-to-cooperation probability after CD outcome								
Play-order	**Opponent**	**Drug contrast**	**emmean OT**	**emmean PL**	**Mean difference**	**SE**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S	OT - PL	0.63	0.42	0.21	0.12	.089	0.66	-
	NS	OT - PL	0.64	0.51	0.13	0.15	.352	0.35	-
NS -> S	S	OT - PL	0.86	0.44	0.42	0.14	.002*	1.31	OT > PL
	NS	OT - PL	0.76	0.27	0.49	0.09	< .001*	2.25	OT > PL
Opponent	**Drug**	**Play-order contrast**	**emmean Play-order 1**	**emmean Play-order 2**	**Mean difference**	**SE**	***p*-value**	**Cohen's d**	**Direction**
S	OT	S -> NS – NS -> S	0.63	0.86	-0.23	0.13	.072	0.72	-
	PL	S -> NS – NS -> S	0.42	0.44	-0.02	0.13	.864	0.06	-
NS	OT	S -> NS – NS -> S	0.64	0.76	-0.12	0.14	.366	0.35	-
	PL	S -> NS – NS -> S	0.51	0.27	-0.24	0.11	.026*	0.84	S -> NS > NS -> S
Drug	**Play-order**	**Opponent contrast**	**emmean S**	**emmean NS**	**Mean difference**	**SE**	***p*-value**	**Cohen's d**	**Direction**
OT	S -> NS	S - NS	0.63	0.64	-0.01	0.06	.889	0.03	-
	NS -> S	S - NS	0.86	0.76	0.10	0.07	.118	0.36	-
PL	S -> NS	S - NS	0.42	0.51	-0.08	0.03	.014*	0.28	S < NS
	NS -> S	S - NS	0.44	0.27	0.18	0.10	.064	0.63	-

Electroencephalography

Table S3. Overall effects of drug, opponent, expectancy and play-order on P3 amplitude. Abbreviations: df, degrees of freedom; F, F-statistic; η_p^2, partial eta-squared. Asterisks signal statistically significant effects: *p < *.050;* **p < *.010;* ***p < *.001.*

P3 amplitude				
Effects	**df**	**F**	**η_p^2**	***p*-value**
Drug	1, 45	0.02	< .001	.882
PlayOrder	1, 45	< 0.01	< .001	.951
Drug x PlayOrder	1, 45	0.05	.001	.819
Opponent	1, 45	1.80	.038	.186
Drug x Opponent	1, 45	0.91	.020	.345
PlayOrder x Opponent	1, 45	5.79	.114	.020*
Drug x PlayOrder x Opponent	1, 45	0.19	.004	.663
Expectancy	1, 45	33.12	.424	< .001*** (unexpected > expected)
Drug x Expectancy	1, 45	1.44	.031	.236
PlayOrder x Expectancy	1, 45	< 0.01	< .001	.967
Drug x PlayOrder x Expectancy	1, 45	0.02	< .001	.881
Opponent x Expectancy	1, 45	5.09	.102	.029* (see **Table S4**)
Drug x Opponent x Expectancy	1, 45	0.01	< .001	.925
PlayOrder x Opponent x Expectancy	1, 45	0.05	.001	.820
Drug x PlayOrder x Opponent x Expectancy	1, 45	0.10	.002	.757

Table S4. Post-hoc pairwise comparisons of statistically significant interactions on P3 amplitude (as per **Table S3**). Asterisks signal statistically significant effects, accompanied by their direction. In Play-order S -> NS, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order NS -> S, the non-sexualized woman is the first opponent and sexualized woman the second. S – Sexualized; NS – Non-sexualized; SE – Standard error, df – degrees of freedom.

P3 amplitude							
Post-hoc comparisons for interaction of outcome expectancy and opponent on P3 amplitude							
Expectancy	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
Expected	S - NS	0.51	0.17	3.04 (45)	.004*	0.45	S > NS
Unexpected	S - NS	-0.06	0.24	-0.24 (45)	.810	0.04	-
Opponent	**Expectancy contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	Expected - Unexpected	-0.94	0.25	-3.77 (45)	< .001*	0.56	Unexpected > Expected
NS	Expected - Unexpected	-1.50	0.24	-6.15 (45)	< .001*	0.92	Unexpected > Expected
Post-hoc comparisons of interaction of opponent and play-order on P3 amplitude							
Play-Order	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S - NS	0.63	0.22	2.80 (45)	.008*	0.42	S > NS
NS -> S	S - NS	-0.18	0.25	-0.72 (45)	.477	0.11	-
Opponent	**Play-Order contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS – NS -> S	0.36	0.70	0.51 (45)	.614	0.08	-
NS	S -> NS – NS -> S	-0.45	0.76	-0.59 (45)	.558	0.09	-

Table S5. Overall effects of drug, opponent, expectancy and play-order on P3 latency. Abbreviations: df, degrees of freedom; F, F-statistic; η_p^2, partial eta-squared. Asterisks signal statistically significant effects: *p < *.050;* **p < *.010;* ***p < *.001.*

P3 latency				
Effect	**df**	**F**	η_p^2	***p*-value**
Drug	1, 45	3.49	.072	.068
Playorder	1, 45	1.40	.030	.243
Drug x playorder	1, 45	1.45	.031	.235
Opponent	1, 45	0.37	.008	.545
Drug x Opponent	1, 45	4.14	.084	.048* (see **Table S6**)
Playorder x Opponent	1, 45	1.18	.026	.283
Drug x playorder x Opponent	1, 45	4.40	.089	.042 (see **Table S6**)
Expectancy	1, 45	17.23	.277	< .001*** (Unexpected > Expected)
Drug x Expectancy	1, 45	2.51	.053	.120
Playorder x Expectancy	1, 45	0.17	.004	.682
Drug x playorder x Expectancy	1, 45	0.38	.008	.541
Opponent x Expectancy	1, 45	0.29	.006	.592
Drug x Opponent x Expectancy	1, 45	0.13	.003	.717
Playorder x Opponent x Expectancy	1, 45	0.31	.007	.578
Drug x playorder x Opponent x Expectancy	1, 45	0.07	.002	.787

Table S6. Post-hoc pairwise comparisons of statistically significant interactions on P3 latency (as per **Table S5**). Asterisks signal statistically significant effects, accompanied by their direction. In Play-order S -> NS, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order NS -> S, the non-sexualized woman is the first opponent and sexualized woman the second. OT – Oxytocin; PL – Placebo; S – Sexualized; NS – Non-sexualized; SE – Standard error, df – degrees of freedom.

P3 latency							
Post-hoc comparisons for interaction of opponent and drug on P3 latency							
Opponent	**Drug contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	OT – PL	7.97	12.94	0.62 (45)	.541	0.09	-
NS	OT - PL	34.29	13.11	2.62 (45)	.012*	0.39	OT > PL
Drug	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
OT	S - NS	9.22	9.23	1.00 (45)	.323	0.15	-
PL	S - NS	17.10	9.07	1.89 (45)	.066	0.28	-
Post-hoc comparisons for interaction of drug, opponent and play-order on P3 latency							
Opponent	**Play-order**	**Drug Contrasts**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS	OT – PL	7.93	17.35	0.46 (45)	.650	0.07	-
	NS -> S	OT - PL	8.00	19.21	0.42 (45)	.679	0.06	-
NS	S -> NS	OT - PL	7.12	17.58	0.41 (45)	.687	0.06	-
	NS -> S	OT - PL	61.45	19.46	3.16 (45)	.003*	0.47	OT > PL
Play-Order	**Drug**	**Opponent Contrasts**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	PLC	S - NS	10.57	12.04	0.88 (45)	.385	0.13	-
	OT	S - NS	11.38	12.49	0.91 (45)	.367	0.14	-
NS -> S	PLC	S - NS	23.64	13.58	1.74 (45)	.089	0.26	-
	OT	S - NS	-29.82	13.58	-2.20 (45)	.033*	0.33	S < NS

Drug	Opponent	Play-Order contrasts	Mean difference	SE	t (df)	*p*-value	Cohen's d	Direction
OT	S	S -> NS – NS -> S	-6.38	18.46	-0.35 (45)	.731	0.05	-
	NS	S -> NS – NS -> S	-47.58	18.70	-2.55 (45)	.014*	0.38	NS -> S > S -> NS
PLC	S	S -> NS – NS -> S	-6.31	18.15	-0.35 (45)	.730	0.05	-
	NS	S -> NS – NS -> S	6.75	18.49	0.37 (45)	.715	0.05	-

Table S7. Overall effects of drug, opponent, play-order, and valence on FRN amplitude. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001. df - degrees of freedom; F - F-statistic; η_p^2 - partial eta-squared.

FRN amplitude				
Effect	**df**	**F**	**η_p^2**	***p*-value**
Drug	1, 45	0.13	.003	.723
PlayOrder	1, 45	2.84	.059	.099
Drug x PlayOrder	1, 45	3.55	.073	.066
Opponent	1, 45	1.65	.035	.206
Drug x Opponent	1, 45	1.36	.029	.250
PlayOrder x Opponent	1, 45	16.02	.262	< .001*** (see **Table S8**)
Drug x PlayOrder x Opponent	1, 45	2.11	.045	.154
Valence	1, 45	34.78	.436	< .001*** (Losses > Gains)
Drug x Valence	1, 45	0.13	.003	.719
PlayOrder x Valence	1, 45	3.46	.071	.069
Drug x PlayOrder x Valence	1, 45	0.25	.005	.623
Opponent x Valence	1, 45	1.63	.035	.209
Drug x Opponent x Valence	1, 45	0.53	.012	.472
PlayOrder x Opponent x Valence	1, 45	0.04	< .001	.847
Drug x PlayOrder x Opponent x Valence	1, 45	2.24	.047	.142

Table S8. Post-hoc pairwise comparisons of statistically significant interactions on FRN amplitude (as per **Table S7**). Asterisks signal statistically significant effects, accompanied by their direction. In Play-order S -> NS, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order NS -> S, the non-sexualized woman is the first opponent and sexualized woman the second. S – Sexualized; NS – Non-sexualized; SE – Standard error, df – degrees of freedom.

FRN amplitude							
Post-hoc comparisons for interaction of play-order and opponent on FRN amplitude							
Play-Order	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S - NS	1.03	0.26	3.94 (45)	< .001*	0.59	S < NS
NS -> S	S - NS	-0.53	0.29	-1.83 (45)	.074	0.27	-
Opponent	**Play-Order contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS - NS -> S	-0.27	0.64	-0.42 (45)	.674	0.06	-
NS	S -> NS - NS -> S	-1.83	0.67	-2.75 (45)	.009*	0.41	S -> NS > NS -> S

Explicit perception

Table S9. Overall effects of drug, opponent and play-order on the ratings of explicit perceptions of the opponent, separately for attractiveness, sexiness, beauty, morality, intelligence, trustworthiness, sexual availability in general, and sexual availability to the participant, total rating of the Agency and Experience sub-scales of the Mind Attribution Scale [62], and the likelihood of cooperation expectation. Asterisks signal statistically significant effects: *$p < .050$; **$p < .010$; ***$p < .001$. Abbreviations: df - degrees of freedom; F - F-statistic; η_p^2 - partial eta-squared.

Statistical results of opponent questionnaire rating					
Analysis	**Effect**	**df**	**F**	η_p^2	***p*-value**
Attractiveness	Drug	1, 45	0.37	.008	.546
	PlayOrder	1, 45	2.82	.059	.100
	Drug x PlayOrder	1, 45	0.28	.006	.596
	Opponent	1, 45	9.33	.172	.004** (S > NS)
	Drug x Opponent	1, 45	0.19	.004	.668
	PlayOrder x Opponent	1, 45	0.19	.004	.668
	Drug x PlayOrder x Opponent	1, 45	0.19	.002	.760
Sexiness	Drug	1, 45	0.11	.002	.740
	PlayOrder	1, 45	0.53	.012	.472
	Drug x PlayOrder	1, 45	0.27	.006	.604
	Opponent	1, 45	41.64	.481	< .001*** (S > NS)
	Drug x Opponent	1, 45	0.07	.002	.795
	PlayOrder x Opponent	1, 45	0.36	.008	.551
	Drug x PlayOrder x Opponent	1, 45	0.72	.016	.400
Beauty	Drug	1, 45	1.23	.027	.273
	PlayOrder	1, 45	1.47	.032	.231
	Drug x PlayOrder	1, 45	2.47	.052	.123
	Opponent	1, 45	0.59	.013	.445
	Drug x Opponent	1, 45	0.59	.013	.445
	PlayOrder x Opponent	1, 45	0.03	< .001	.871
	Drug x PlayOrder x Opponent	1, 45	0.03	< .001	.871
Morality	Drug	1, 44	0.45	.010	.508
	PlayOrder	1, 44	0.02	< .001	.895
	Drug x PlayOrder	1, 44	2.07	.045	.157
	Opponent	1, 44	6.58	.130	.014* (S < NS)
	Drug x Opponent	1, 44	3.05	.065	.088
	PlayOrder x Opponent	1, 44	0.07	.001	.800
	Drug x PlayOrder x Opponent	1, 44	0.32	.007	.576
Intelligence	Drug	1, 45	0.48	.011	.490
	PlayOrder	1, 45	0.22	.005	.638
	Drug x PlayOrder	1, 45	1.35	.029	.252
	Opponent	1, 45	0.37	.008	.544
	Drug x Opponent	1, 45	0.07	.002	.792
	PlayOrder x Opponent	1, 45	0.07	.002	.792
	Drug x PlayOrder x Opponent	1, 45	0.37	.008	.544
Trustworthiness	Drug	1, 45	0.01	< .001	.930
	PlayOrder	1, 45	0.69	.015	.412
	Drug x PlayOrder	1, 45	0.83	.018	.366
	Opponent	1, 45	1.41	.030	.242
	Drug x Opponent	1, 45	0.48	.011	.490
	PlayOrder x Opponent	1, 45	1.90	.040	.175
	Drug x PlayOrder x Opponent	1, 45	0.59	.013	.445
Sexual availability in general	Drug	1, 45	0.94	.020	.338
	PlayOrder	1, 45	0.07	.002	.796
	Drug x PlayOrder	1, 45	0.94	.020	.338
	Opponent	1, 45	20.28	.311	< .001*** (S > NS)
	Drug x Opponent	1, 45	0.39	.009	.538
	PlayOrder x Opponent	1, 45	0.70	.015	.408
	Drug x PlayOrder x Opponent	1, 45	0.20	.004	.657
	Drug	1, 45	3.30	.068	.076
	PlayOrder	1, 45	0.59	.013	.445

Sexual availability to the participant	Drug x PlayOrder	1, 45	0.11	.002	.746
	Opponent	1, 45	5.45	.108	.024 * (S > NS)
	Drug x Opponent	1, 45	0.14	.003	.706
	PlayOrder x Opponent	1, 45	4.10	.084	.049 * (see **Table S10**)
	Drug x PlayOrder x Opponent	1, 45	0.48	.010	.493
Experiece (sub-scale)	Drug	1, 46	2.49	.051	.121
	PlayOrder	1, 46	< 0.01	<.001	.964
	Drug x PlayOrder	1, 46	1.10	.023	.300
	Opponent	1, 46	0.07	.001	.795
	Drug x Opponent	1, 46	0.65	.014	.423
	PlayOrder x Opponent	1, 46	0.64	.014	.436
	Drug x PlayOrder x Opponent	1, 46	2.56	.053	.117
Agency (sub-scale)	Drug	1, 46	2.18	.045	.147
	PlayOrder	1, 46	0.96	.021	.331
	Drug x PlayOrder	1, 46	0.23	.005	.635
	Opponent	1, 46	3.14	.064	.083
	Drug x Opponent	1, 46	1.38	.029	.247
	PlayOrder x Opponent	1, 46	0.44	.009	.511
	Drug x PlayOrder x Opponent	1, 46	5.21	.102	.027 * (see **Table S10**)
Likelihood of cooperation expectation	Drug	1, 46	0.05	.001	.830
	PlayOrder	1, 46	0.88	.019	.354
	Drug x PlayOrder	1, 46	0.38	.008	.541
	Opponent	1, 46	0.09	.002	.768
	Drug x Opponent	1, 46	0.04	< .001	.836
	PlayOrder x Opponent	1, 46	0.07	.001	.797
	Drug x PlayOrder x Opponent	1, 46	0.10	.002	.751

Table S10. Post-hoc pairwise comparisons of statistically significant interactions on ratings, per question, of the Mind Attribution Scale (as per **Table S9**). Asterisks signal statistically significant effects), accompanied by their direction. In Play-order S -> NS, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order NS -> S, the non-sexualized woman is the first opponent and sexualized woman the second. S – Sexualized; NS – Non-sexualized; OT - Oxytocin, PL - Placebo. SE - standard error; SE - standard error, df – degrees of freedom.

Sexual availability to the participant							
Interaction of opponent and play-order on opponent's sexual availability to the participant							
Play-Order	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S - NS	0.57	0.17	3.33 (45)	.002*	0.50	S > NS
NS -> S	S - NS	0.04	0.20	0.21 (45)	.838	0.03	-
Opponent	**Play-Order contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS - NS -> S	-0.01	0.42	-0.02 (45)	.988	< 0.01	-
NS	S -> NS - NS -> S	-5.40	0.33	1.62 (45)	.113	0.24	-
Agency							
Interaction of opponent, play-order and drug on opponent's agency rating							
Drug	**Play-order**	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
PL	S -> NS	S - NS	0.15	0.27	0.56 (46)	.576	0.08	-
	NS -> S	S - NS	-0.33	0.32	-1.05 (46)	.299	0.15	-
OT	S -> NS	S - NS	-0.88	0.29	-3.03 (46)	.004*	0.45	S < NS
	NS -> S	S - NS	0.00	0.32	0.00 (46)	> .999	< 0.01	-
Drug	**Opponent**	**Play-Order contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
PL	S	S -> NS - NS -> S	0.43	0.57	0.77 (46)	.447	0.11	-
	NS	S -> NS - NS -> S	-0.05	0.57	-0.09 (46)	.931	0.01	-
OT	S	S -> NS - NS -> S	0.12	0.58	0.20 (46)	.842	0.03	-
	NS	S -> NS - NS -> S	1.00	0.59	1.68 (46)	.100	0.25	-
Play-Order	**Opponent**	**Drug contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S	OT - PL	0.23	0.54	0.42 (46)	.674	0.06	-
	NS	OT - PL	1.26	0.55	2.30 (46)	.026*	0.34	OT > PL
NS -> S	S	OT - PL	0.55	0.61	0.90 (46)	.374	0.13	-
	NS	OT - PL	0.21	0.62	0.39 (46)	.730	0.05	-

Table S11. Overall effects of drug, opponent and play-order on the ratings of explicit perceptions of the opponent, as measured by the Anthropomorphism, Likeability, and Perceived Intelligence sub-scales of the Godspeed Questionnaire (Bartneck et al., 2008). Asterisks signal statistically significant effects: *$p < .050$; **$p < .010$; ***$p < .001$. Abbreviations: df - degrees of freedom; F - F-statistic; η_p^2 - partial eta-squared.

Godspeed Questionnaire					
Analysis	**Effect**	**df**	**F**	η_p^2	***p*-value**
Antropomorphism	Drug	1, 46	0.08	.002	.781
	PlayOrder	1, 46	1.89	.039	.176
	Drug x PlayOrder	1, 46	0.03	< .001	.857
	Opponent	1, 46	1.80	.038	.186
	Drug x Opponent	1, 46	0.02	< .001	.880
	PlayOrder x Opponent	1, 46	0.02	< .001	.903
	Drug x PlayOrder x Opponent	1, 46	1.14	.024	.292
Likeability	Drug	1, 46	0.48	.010	.491
	PlayOrder	1, 46	1.44	.030	.236
	Drug x PlayOrder	1, 46	0.18	.004	.674
	Opponent	1, 46	0.04	< .001	.843
	Drug x Opponent	1, 46	0.56	.012	.457
	PlayOrder x Opponent	1, 46	4.18	.083	.047* (see **Table S12**)
	Drug x PlayOrder x Opponent	1, 46	1.25	.027	.269
Perceived Intelligence	Drug	1, 46	0.02	< .001	.886
	PlayOrder	1, 46	0.19	.004	.668
	Drug x PlayOrder	1, 46	0.44	.009	.513
	Opponent	1, 46	1.93	.040	.171
	Drug x Opponent	1, 46	0.02	< .001	.882
	PlayOrder x Opponent	1, 46	0.38	.008	.540
	Drug x PlayOrder x Opponent	1, 46	0.85	.018	.361

Table S12. Post-hoc pairwise comparisons of statistically significant interactions on ratings of the GodSpeed Questionnaire (as per **Table S11**). In Play-order 1, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order 2, the non-sexualized woman is the first opponent and sexualized woman the second. S = Sexualized; NS = Non-sexualized; SE - standard error, df – degrees of freedom.

Likeability							
Interaction of play-order and opponent on opponent's likeability rating							
Opponent	**Play-order contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS – NS -> S	0.05	0.22	0.24 (46)	.815	0.03	-
NS	S -> NS – NS -> S	-0.45	0.20	-2.26 (46)	.029*	0.33	NS -> S – S -> NS
Play-order	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S - NS	0.28	0.16	1.69 (46)	.098	0.25	S > NS (non-sign)
NS -> S	S - NS	-0.23	0.18	-1.24 (46)	.223	0.18	NS > S (non-sign)

Table S13. Overall effects of drug, opponent and play-order on the ratings emotion intensity after each Prisoner's Dilemma outcome type. Asterisks signal statistically significant effects: *p < .050; **p < .010; ***p < .001. Abbreviations: df - degrees of freedom; F - F-statistic; η_p^2 - partial eta-squared.

Opponent Questionnaire Rating					
After Cooperation-Cooperation					
Emotion	**Effect**	**df**	**F**	**η_p^2**	***p*-value**
Angry	Drug	1, 46	0.26	.006	.612
	PlayOrder	1, 46	1.02	.022	.318
	Drug x PlayOrder	1, 46	0.01	< .001	.930
	Opponent	1, 46	0.05	.001	.828
	Drug x Opponent	1, 46	1.84	.038	.182
	PlayOrder x Opponent	1, 46	0.05	.001	.828
	Drug x PlayOrder x Opponent	1, 46	1.84	.038	.182
Happy	Drug	1, 46	< 0.01	< .001	.986
	PlayOrder	1, 46	1.36	.029	.249
	Drug x PlayOrder	1, 46	0.02	< .001	.893
	Opponent	1, 46	2.03	.042	.161
	Drug x Opponent	1, 46	0.03	< .001	.875
	PlayOrder x Opponent	1, 46	0.31	.007	.582
	Drug x PlayOrder x Opponent	1, 46	1.06	.022	.309
Guilty	Drug	1, 46	0.03	< .001	.863
	PlayOrder	1, 46	0.36	.008	.550
	Drug x PlayOrder	1, 46	0.55	.012	.462
	Opponent	1, 46	2.30	.048	.136
	Drug x Opponent	1, 46	0.03	< .001	.863
	PlayOrder x Opponent	1, 46	0.36	.008	.550
	Drug x PlayOrder x Opponent	1, 46	0.55	.012	.462
Disappointed	Drug	1, 46	0.56	.012	.457
	PlayOrder	1, 46	0.08	.002	.773
	Drug x PlayOrder	1, 46	0.94	.020	.337
	Opponent	1, 46	3.57	.072	.065
	Drug x Opponent	1, 46	0.29	.006	.593
	PlayOrder x Opponent	1, 46	0.33	.007	.569
	Drug x PlayOrder x Opponent	1, 46	0.60	.013	.442
After Cooperation - Defection					
Emotion	**Effect**	**df**	**F**	**η_p^2**	***p*-value**
Angry	Drug	1, 46	2.10	.044	.154
	PlayOrder	1, 46	0.81	.017	.374
	Drug x PlayOrder	1, 46	0.11	.002	.742
	Opponent	1, 46	4.08	.081	.049*
	Drug x Opponent	1, 46	0.10	.002	.751
	PlayOrder x Opponent	1, 46	4.08	.081	.049* (see **Table S14**)
	Drug x PlayOrder x Opponent	1, 46	5.21	.102	.027* (see **Table S14**)
Happy	Drug	1, 46	< 0.01	< .001	.994
	PlayOrder	1, 46	1.10	.023	.299
	Drug x PlayOrder	1, 46	< 0.01	< .001	.994
	Opponent	1, 46	0.09	.002	.762
	Drug x Opponent	1, 46	0.63	.014	.430
	PlayOrder x Opponent	1, 46	0.26	.006	.610
	Drug x PlayOrder x Opponent	1, 46	0.63	.014	.430
Guilty	Drug	1, 46	0.48	.010	.493
	PlayOrder	1, 46	< 0.01	< .001	.996
	Drug x PlayOrder	1, 46	0.16	.003	.694
	Opponent	1, 46	0.09	.002	.764
	Drug x Opponent	1, 46	1.19	.025	.281
	PlayOrder x Opponent	1, 46	0.77	.017	.383
	Drug x PlayOrder x Opponent	1, 46	2.78	.057	.102
Disappointed	Drug	1, 46	< 0.01	< .001	.969
	PlayOrder	1, 46	0.62	.013	.436
	Drug x PlayOrder	1, 46	0.10	.002	.749

	Opponent	1, 46	1.04	.022	.314
	Drug x Opponent	1, 46	0.49	.011	.486
	PlayOrder x Opponent	1, 46	0.31	.007	.581
	Drug x PlayOrder x Opponent	1, 46	0.06	.001	.810
After Defection - Cooperation					
Emotion	**Effect**	**df**	**F**	**η_p^2**	***p*-value**
Angry	Drug	1, 45	0.05	.001	.824
	PlayOrder	1, 45	0.37	.008	.547
	Drug x PlayOrder	1, 45	< 0.01	< .001	.954
	Opponent	1, 45	0.04	< .001	.834
	Drug x Opponent	1, 45	0.37	.008	.546
	PlayOrder x Opponent	1, 45	0.23	.005	.636
	Drug x PlayOrder x Opponent	1, 45	0.76	.017	.387
Happy	Drug	1, 45	6.10	.119	.017* (OT < PL)
	PlayOrder	1, 45	< 0.01	< .001	.995
	Drug x PlayOrder	1, 45	0.94	.020	.339
	Opponent	1, 45	0.36	.008	.552
	Drug x Opponent	1, 45	0.21	.005	.647
	PlayOrder x Opponent	1, 45	0.17	.004	.680
	Drug x PlayOrder x Opponent	1, 45	1.32	.029	.256
Guilty	Drug	1, 45	0.19	.004	.666
	PlayOrder	1, 45	0.19	.004	.666
	Drug x PlayOrder	1, 45	< 0.01	< .001	.982
	Opponent	1, 45	2.20	.047	.145
	Drug x Opponent	1, 45	0.73	.016	.398
	PlayOrder x Opponent	1, 45	3.32	.069	.075
	Drug x PlayOrder x Opponent	1, 45	0.10	.002	.751
Disappointed	Drug	1, 44	< 0.01	< .001	.960
	PlayOrder	1, 44	0.04	< .001	.848
	Drug x PlayOrder	1, 44	0.02	< .001	.878
	Opponent	1, 44	6.17	.123	.017* (S < NS)
	Drug x Opponent	1, 44	0.69	.015	.410
	PlayOrder x Opponent	1, 44	1.91	.042	.174
	Drug x PlayOrder x Opponent	1, 44	1.27	.028	.265
After Defection - Defection					
Emotion	**Effect**	**df**	**F**	**η_p^2**	***p*-value**
Angry	Drug	1, 46	1.65	.035	.206
	PlayOrder	1, 46	0.27	.006	.605
	Drug x PlayOrder	1, 46	0.81	.017	.374
	Opponent	1, 46	0.82	.018	.369
	Drug x Opponent	1, 46	0.12	.003	.729
	PlayOrder x Opponent	1, 46	1.16	.025	.288
	Drug x PlayOrder x Opponent	1, 46	2.67	.055	.109
Happy	Drug	1, 46	0.03	< .001	.862
	PlayOrder	1, 46	1.34	.028	.253
	Drug x PlayOrder	1, 46	1.85	.039	.180
	Opponent	1, 46	0.30	.006	.588
	Drug x Opponent	1, 46	0.11	.002	.737
	PlayOrder x Opponent	1, 46	0.72	.015	.401
	Drug x PlayOrder x Opponent	1, 46	< 0.01	< .001	.971
Guilty	Drug	1, 46	0.46	.010	.501
	PlayOrder	1, 46	0.19	.004	.667
	Drug x PlayOrder	1, 46	0.20	.004	.658
	Opponent	1, 46	0.06	.001	.802
	Drug x Opponent	1, 46	< 0.01	< .001	.986
	PlayOrder x Opponent	1, 46	0.06	.001	.802
	Drug x PlayOrder x Opponent	1, 46	< 0.01	< .001	.986
Disappointed	Drug	1, 46	1.09	.023	.302
	PlayOrder	1, 46	0.04	< .001	.836
	Drug x PlayOrder	1, 46	0.21	.004	.653
	Opponent	1, 46	0.65	.014	.424
	Drug x Opponent	1, 46	0.76	.016	.388
	PlayOrder x Opponent	1, 46	0.65	.014	.424
	Drug x PlayOrder x Opponent	1, 46	< 0.01	< .001	.985

Table S14. Post-hoc pairwise comparisons of statistically significant interactions on emotion intensity ratings (as per **Table S13**). Asterisks signal statistically significant effects, accompanied by their direction. In Play-order S -> NS, the sexualized woman is the first opponent and non-sexualized woman the second; in Play-order NS -> S, the non-sexualized woman is the first opponent and sexualized woman the second. S – Sexualized; NS – Non-sexualized; OT - Oxytocin, PL - Placebo. SE - standard error, df – degrees of freedom.

Emotion intensity ratings after Cooperation - Defection outcomes								
Interaction of opponent and play-order on angry ratings after Cooperation - Defection								
Play-order	**Opponent contrast**		**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S - NS		-0.56	0.19	-3.04 (46)	.004*	0.45	S < NS
NS -> S	S - NS		0.00	0.21	0.00 (46)	> .999	< 0.01	-
Opponent	**Play-Order contrast**		**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S	S -> NS – NS -> S		0.16	0.48	0.33 (46)	.744	0.05	-
NS	S -> NS – NS -> S		0.72	0.54	1.35 (46)	.185	0.20	-
Interaction of drug, opponent and play-order on angry ratings after Cooperation - Defection								
Drug	**Play-order**	**Opponent contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
PL	S -> NS	S - NS	-0.20	0.25	-0.79 (46)	.431	0.12	-
	NS -> S	S - NS	-0.27	0.29	-0.93 (46)	.358	0.14	-
OT	S -> NS	S - NS	-0.92	0.27	-3.42 (46)	.001*	0.50	S < NS
	NS -> S	S - NS	0.27	0.29	0.93 (46)	.358	0.14	-
Drug	**Opponent**	**Play-Order contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
PL	S	S -> NS – NS -> S	0.64	0.67	0.95 (46)	.345	0.14	-
	NS	S -> NS – NS -> S	0.54	0.74	0.76 (46)	.452	0.11	-
OT	S	S -> NS – NS -> S	-0.32	0.69	-0.47 (46)	.642	0.07	-
	NS	S -> NS – NS -> S	0.84	0.77	1.14 (46)	.261	0.17	-
Play-order	**Opponent**	**Drug contrast**	**Mean difference**	**SE**	**t (df)**	***p*-value**	**Cohen's d**	**Direction**
S -> NS	S	OT - PL	-1.23	0.64	-1.93 (46)	.060	0.29	-
	NS	OT - PL	-0.51	0.71	-0.72 (46)	.478	0.11	-
NS -> S	S	OT - PL	-0.27	0.72	-0.38 (46)	.705	0.06	-
	NS	OT - PL	-0.82	0.80	-1.03 (46)	.311	0.15	-