# Monitoring the effect of oxytocin on the neural sensitivity to emotional faces via frequency-tagging EEG: a double-blind, cross-over study

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**Short title**: Oxytocin effect on the neural sensitivity to emotional faces

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# Abstract

The neuropeptide oxytocin (OXT) is suggested to exert an important role in human social behaviors by modulating the salience of social cues. To date, however, there is mixed evidence whether a single dose of OXT can improve the behavioral and neural sensitivity for emotional face processing. To overcome difficulties encountered with classic event-related potential studies assessing stimulus-saliency, we applied frequency-tagging EEG to implicitly assess the effect of a single dose of OXT (24 IU) on the neural sensitivity for positive and negative facial emotions. Neutral faces with different identities were presented at 6 Hz, periodically interleaved with an expressive face (angry, fearful, and happy, in separate sequences) every fifth image (i.e. 1.2 Hz oddball frequency). These distinctive frequency tags for neutral and expressive stimuli allowed direct and objective quantification of the neural expression-categorization responses. The study involved a double-blind, placebo-controlled, cross-over trial with 31 healthy adult men. Contrary to our expectations, we did not find an effect of OXT on facial emotion processing, neither at the neural, nor at the behavioral level. A single dose of OXT did not evoke social enhancement in general, nor did it affect social approach-avoidance tendencies. Possibly ceiling performances in facial emotion processing might have hampered further improvement.

# 1. Introduction

Being able to quickly and adequately read faces and facial expressions is a key component for successful everyday social interactions, as it allows for understanding one's feelings, reactions and intentions (Elfenbein & Ambady, 2002). Moreover, proficient recognition of emotional faces has been found to be related to more prosocial behavior (Kaltwasser et al., 2017).

An important biological modulator of social behavior and socio-cognitive processes is endogenous oxytocin (OXT; MacDonald & MacDonald, 2010). OXT is a neuropeptide that is produced in the hypothalamus and functions as a hormone and neuromodulator (Wigton et al., 2015). Central OXT levels can, however, be manipulated by administering exogenous OXT (Martins et al., 2020). Extensive animal research has demonstrated the potential of exogenous OXT in modulating social behavior when delivered directly to the brain (for a review, see Quintana et al., 2018), which encouraged numerous researchers over the past decades to study its effect on human sociality. The effects of intranasally administered OXT on social functioning have increasingly been investigated in neurotypical populations, as well as in psychiatric conditions that are characterized by social difficulties (Keech et al., 2018; Kendrick et al., 2018; Peled-Avron et al., 2020), such as anxiety disorders (Naja & Aoun, 2017), schizophrenia (Shilling & Feifel, 2016) and autism spectrum disorder (Guastella & Hickie, 2016). Despite mixed results, generally, the findings summarized in these reviews suggest the effectiveness of exogenous OXT to ameliorate social symptoms (Guastella & Hickie, 2016; Naja & Aoun, 2017; Shilling & Feifel, 2016).

Mechanistic models suggest that OXT may exert its complex social effects by regulating the saliency of social cues and/or by modulating (social) stress and anxiety (Churchland & Winkielman, 2012; Pehlivanoglu et al., 2020; Shamay-Tsoory & Abu-Akel, 2016). As these effects can be of particular interest for facial emotion processing, many behavioral studies have sought to elucidate how OXT affects this ability. Despite the overall notion of OXT enhancing emotion recognition, closer inspection of the findings reveals rather weak and inconsistent results, demonstrating OXT's variable nature and

the modulating impact of contextual and person-specific characteristics (for reviews and a meta-analysis, see Bartz et al., 2011; Evans et al., 2014; Leppanen et al., 2017; Shahrestani et al., 2013; Van IJzendoorn & Bakermans-Kranenburg, 2012). For example, whereas some studies found an overall improvement of facial emotion recognition, irrespective of the valence of the expressions (Guastella et al., 2010; Lischke et al., 2012), others have reported an OXT effect for positive (Di Simplicio et al., 2009; Schulze et al., 2011) or negative (Fischer-Shofty et al., 2010) emotions only. In a similar vein, OXT has been found to modulate approach-avoidance motivational tendencies, by enhancing sensitivity for emotional stimuli that elicit approach-related behavior (Kemp & Guastella, 2011) and attenuating sensitivity for negative emotional stimuli (Ellenbogen, 2018) that may elicit social withdrawal (Kemp & Guastella, 2011). Likewise, exogenous OXT administration has been found to increase the perceived salience of social cues in a context-specific manner (Shamay-Tsoory & Abu-Akel, 2016). For instance, in addition to the enhanced prosocial behavior often encountered in positive and cooperative contexts, increased negative emotions and behavior have also been reported after OXT administration in competitive and aggressive contexts (Shamay-Tsoory et al., 2009). Furthermore, some studies showed that the OXT induced improvement depends on task difficulty (Guastella et al., 2010) and on individual characteristics, with more robust effects being observed in those individuals who initially (i.e. before OXT treatment) scored poorer in terms of social-cognitive competence (Bartz et al., 2010) or displayed lower levels of happiness when watching images of neutral faces (Pavarini et al., 2019). Indeed, in addition to regulating the saliency of social cues or modulating the motivational tendencies, OXT has also been found to enhance spontaneous facial mimicry in neurotypical adults (Korb et al., 2016; Pavarini et al., 2019). This enhanced facial mimicry might in turn improve emotion recognition based on sensorimotor simulation of the perceived expression in the brain (Wood et al., 2016), as a neural feedback process elicits the corresponding emotion in the observer, supporting the perceptual recognition of this perceived expression (Hess & Fischer, 2014; McIntosh, 1996).

Effects of a single dose of OXT on emotion recognition have also been investigated at the neural level, mostly using functional magnetic resonance imaging (fMRI). In general, these studies showed

attenuated amygdala activity, mostly in response to negative social stimuli (for meta-analyses, see Grace et al., 2018; Wang et al., 2017), which is considered to reflect a reduction in social anxiety. Yet, similar to the behavioral data, results vary. For example, while attenuated amygdala activity has been reported during implicit and explicit processing of both positive (Domes et al., 2007) and negative (Domes et al., 2007; Gamer et al., 2010) emotions, enhanced amygdala activity for happy faces has also been reported (Gamer et al., 2010). Furthermore, in women, a single dose of OXT enhanced activity in different brain regions in relation to specific facial emotions (Domes et al., 2010): increased activation for angry faces was reported in the inferior frontal gyrus and ventro-lateral prefrontal regions, for happy faces in the inferior frontal gyrus and the fusiform gyrus, and for fearful faces in the medial and superior temporal cortex and the bilateral fusiform gyrus. In addition, OXT also augmented functional connectivity between the amygdala and the reward system (Wang et al., 2017) or the salience network (Grace et al., 2018; Wang et al., 2017). Moreover, a recent meta-analysis proposed increased activity in the superior temporal gyrus resulting from OXT administration as the main factor for improved emotion recognition (Grace et al., 2018).

To date, few studies have investigated the effects of a single dose of OXT on facial emotion processing using electroencephalography (EEG) (for a review, see Pehlivanoglu et al., 2020). Event-related potential (ERP) studies investigating the modulatory effect of OXT reported shorter latencies of the N170 component (Tillman et al., 2019), as well as increased amplitudes of the N170 (Peltola et al., 2018) and the vertex positive potential (VPP; Huffmeijer et al., 2013), indicating enhanced sensitivity and improved neural efficiency to process emotional faces. Yet, results were inconsistent for the late positive potential (LPP): some studies reported OXT-induced increases in LPP amplitudes (Huffmeijer et al., 2013), whereas others reported no effects of OXT on this component (Peltola et al., 2018). Possibly, differences in task demands, or differently chosen time windows to capture the ERP components might account for the contrasting findings across studies, as selecting specific time windows to accurately measure the ERP component(s) of interest is one of the most challenging aspects of classic ERP studies (Kappenman

& Luck, 2016). In addition, the low signal-to-noise ratio (SNR) of ERP measurements requires many trials, resulting in long EEG recordings.

Given the challenges and limitations of classic ERP research, here, we applied a relatively novel but highly sensitive EEG approach to investigate the modulatory effect of a single dose of OXT on facial emotion processing: frequency-tagging EEG in combination with fast periodic visual stimulation. Frequency-tagging EEG relies on the principle that brain activity synchronizes to a periodically flickering stimulus (Adrian & Matthews, 1934) and elicits a brain response at exactly the same frequency of stimulation (Norcia et al., 2015). This technique allows to reliably pinpoint individual differences in sensitivity for various socio-communicative cues, such as faces versus non-social cues or facial expressions and identities in infants (de Heering & Rossion, 2015), children (Van der Donck et al., 2019; Vettori, Dzhelyova, et al., 2020; Vettori, Van der Donck, et al., 2020) and adults (Leleu et al., 2019; Poncet et al., 2019). Accordingly, frequency-tagging EEG has demonstrated to differentiate robustly between control populations and clinical populations that are characterized by social impairments, such as children with autism spectrum disorder (Van der Donck et al., 2019; Vettori, Dzhelyova, et al., 2020; Vettori, Van der Donck, et al., 2020) and adults with 22q11 deletion syndrome (Leleu et al., 2019).

Similar to previous studies (Dzhelyova et al., 2017; Van der Donck et al., 2020), we applied frequency-tagging EEG with a facial expression oddball paradigm. In particular, neutral faces were periodically presented in a rapid stream at 6 Hz (i.e. base rate) and were periodically interleaved with emotional faces every fifth stimulus (i.e. at 1.2 Hz oddball rate). The neutral faces act as forward and backward masks for the emotional faces, allowing us to selectively isolate the sensitivity to the expressions by putting the emotional face processing system under tight temporal constraints (i.e. discarding influences of mechanisms other than fast and automatic emotion extraction). The periodic presentation at predefined, yet, different, frequency rates generates distinguishable frequency tags for the base and oddball stimuli, allowing direct quantification of the neural responses, indicating the discrimination of expressive faces amongst neutral faces. This makes frequency-tagging EEG a highly objective measure.

In addition, the rapid presentation enables a fast acquisition of many neural responses indexing expression discrimination in only a few minutes of recording, with a high SNR. Previous research showed that frequency-tagging oddball paradigms have a high test-retest reliability (Dzhelyova et al., 2019) and are able to sensitively pinpoint differences in facial expression processing (Van der Donck et al., 2019, 2020), making them highly suited to monitor subtle changes in facial expression sensitivity, as for example induced by intranasal OXT administration.

Accordingly, in the present study, we investigated the effect of a single dose of OXT on the neural sensitivity to brief changes in facial expression. In separate trials, we included angry, fearful, and happy faces as oddball stimuli in rapidly presented streams of neutral faces, in order to monitor possible modulatory effects on both positive and negative facial expressions. To prevent expression discrimination based on low-level visual features, we continuously changed the identity of the faces (i.e. every image). Thirty-one healthy adult men participated in a double-blind, placebo-controlled, cross-over trial, where they randomly received either a single dose of OXT or placebo during test sessions with a two-week interval. In addition to the EEG measures, we administered a behavioral facial expression matching task.

Following the social salience hypothesis (Shamay-Tsoory & Abu-Akel, 2016), we might expect to find an overall salience effect of OXT, reflected in enhanced neural responses to facial expressions in general, irrespective of emotion. However, in line with the inconsistencies in the OXT literature (Bartz et al., 2011; Evans et al., 2014; Shahrestani et al., 2013), we anticipate a potential modulation of the OXT treatment response depending on the expression valence. Specifically, and in line with evidence (Domes et al., 2013; Ellenbogen, 2018; Xu et al., 2015) supporting the social approach/withdrawal hypothesis (Kemp & Guastella, 2011), we expect OXT to selectively attenuate the neural response for negative facial stimuli and enhance the neural sensitivity for positive facial stimuli.

# 2. Method

## 2.1. Participants

Thirty-one healthy right-handed male participants, aged between 18 and 32 years (mean age = 22.81 years, SD = 2.38 years), were included in this study. Only male participants were recruited in order to avoid possible gender-dependent differences in response to OXT administration (Domes et al., 2010; MacDonald, 2012) and in facial emotion processing (Kret & De Gelder, 2012). In addition to gender, age (18-35 years old) and right-handedness, inclusion criteria further comprised the absence of any diagnosed psychiatric, neurological or genetic disorders in the participant or a first-degree relative. All participants had normal or corrected to normal vision. One participant reported color blindness, but as he had no difficulties detecting the color changes of the fixation cross, he was not excluded.

The Medical Ethical Committee of the university hospital approved this study. Written informed consent according to the Declaration of Helsinki was obtained from the participants. Participants received a monetary compensation for their participation. The trial was registered with the ClinicalTrials.gov database of the U.S. National Institutes of Health (NCT03096249).

## 2.2. Study design

We performed a randomized, double-blind, within-subjects, cross-over, placebo (PL)-controlled study, with the sessions two weeks apart. More specifically, the experiment consisted of two identical test sessions – except for the nasal spray the participants received – that took place at exactly the same time of the day, 14 days apart. Based on random assignment, half of the participants received the OXT spray (Syntocinon®, Sigma Tau) in the first session and the PL spray (saline solution of sodium chloride in water) in the second session. For the other half of the participants, the order was reversed.

## 2.3. OXT administration

At the start of each session, participants received clear instructions on how to administer the nasal spray themselves (Guastella et al., 2013), applying the widely used single dose of 24 international units (IU; Guastella & MacLeod, 2012; Quintana et al., 2021) of OXT via three puffs of 4 IU per nostril. Based on previous studies investigating the time interval between the intranasal administration of a single dose of OXT and increased peripheral OXT levels (Daughters et al., 2015; Striepens et al., 2013), generally, a 30-45 minute wait-time is implemented post-administration (Guastella & MacLeod, 2012). Consequently, in order to test during peak OXT concentrations, we incorporated an interval of 30 minutes between nasal spray administration and the start of the EEG paradigm. Potential side effects or adverse events due to the OXT administration were monitored throughout the entire session (see Supplementary Table 1).

## 2.4. Procedure

Participants were seated in a dimly lit room at 80 cm viewing distance of an LCD 24-in. computer screen, placed at eye level, on which pictures of facial expressions were presented while recording EEG. During the stimulus presentation, an orthogonal task was implemented to guarantee attentiveness of the participants. A fixation cross, presented on the nasion of the presented faces, briefly (300 ms) changed color from black to red 10 times within every sequence. The participants had to respond as soon and accurately as possible when noticing the color changes. Note that the current study was part of a larger project examining the effect of OXT on neural sensitivity for different subtle socio-emotional cues.

### 2.4.1 FPVS-EEG paradigm

The design was similar to previous studies (Dzhelyova et al., 2017; Van der Donck et al., 2020). Neutral faces from continuously changing identities (i.e., every image) were displayed through sinusoidal contrast modulation (0%–100%) at a 6 Hz base rate, periodically interleaved with an oddball stimulus displaying an expression every fifth image (6 Hz/5 = 1.2 Hz oddball rate). Each sequence started with a

blank screen for a variable duration of 2–5 s. After two seconds of gradually fading in (0%–100%), the images were presented for 60 s, followed by two seconds of gradually fading out (100%–0%). Three conditions were included (i.e., the emotional expressions happiness, anger and fear), and each was presented in a separate sequence and repeated four times, resulting in 12 sequences that were all presented in a randomized order (Fig 1). The facial stimuli varied randomly in size between 80% and 120% of the original size.

[Insert Fig.1 about here]

### 2.4.2 Stimuli

The stimuli comprised full-front, full-color images of 14 individuals (seven females, seven males) from the Karolinska Directed Emotional Faces database (Lundqvist et al., 1998), displaying either a neutral, happy, angry or fearful expression. Mean pixel contrast and luminance of the pictures were equalized. The images were set to a size of 300 x 450 pixels, ~~equalizing~~ corresponding to 5.08° x 6.58° of visual angle, and were placed against a grey background.

### 2.4.3 EEG acquisition

We recorded EEG activity using a BIOSEMI Active-Two amplifier system with 64 Ag/AgCl electrodes and two additional electrodes as reference and ground electrodes (Common Mode Sense active electrode and Driven Right Leg passive electrode). Vertical eye movements were recorded via one electrode above and one below the right eye. One electrode was placed at the corner of both eyes to record horizontal eye movements. We recorded EEG and electrooculogram at 512 Hz.

### 2.4.4 EEG analysis

**Pre-processing.** Pre-processing was performed using 'Letswave6' (http://www.nocions.org/letswave/), a toolbox running in Matlab 2017b (Mathworks). The continuous EEG data were cropped into segments of 70 seconds (4 s before and 6 s after stimulus presentation). We applied a bandpass Butterworth filter (fourth order; 0.1-100 Hz) and resampled the data to 256 Hz. We applied independent component

analysis via the runica algorithm (Makeig et al., 1995) for two participants who blinked on average more than 2SD above the mean (average number of blinks per second across participants = 0.10, SD = 0.09) and we removed the component that accounted for most of the variance. Noisy or artefact-ridden channels were re-estimated via linear interpolation using the three spatially nearest, neighboring electrodes (not more than 5% of the electrodes (i.e. three electrodes) were interpolated). All data segments were re-referenced to a common average reference.

Frequency domain analysis. The pre-processed data segments were then cropped to contain an integer number of 1.2 Hz cycles beginning immediately after the fade-in until approximately 59.22 seconds (72 cycles). After averaging the data in the time domain per condition and for each participant separately, we applied a fast Fourier transformation (FFT), yielding amplitude spectra with a spectral resolution of 0.017 Hz. To obtain a measure of neural sensitivity for facial expressions (Dzhelyova et al., 2017), we calculated baseline-subtracted amplitudes at the oddball frequency and its harmonics (i.e., n*F/5 = 2.4 Hz, 3.6 Hz, etc.), by subtracting the average amplitude level of the 20 surrounding bins from the amplitude of the frequency bin of interest (Retter & Rossion, 2016). These 20 surrounding bins are the 10 bins on each side of the target frequency bin, excluding the immediately neighboring bins and the two bins with the most extreme values. Baseline-subtracted amplitudes express responses in amplitudes (μV) that can be summed across significant harmonics to quantify the overall base and oddball response (Retter & Rossion, 2016). In addition, we calculated signal-to-noise ratio (SNR; by dividing the amplitude value of the target frequency bin by the average amplitude of the 20 surrounding frequency bins) for visualization, as this allows to visualize even small response amplitudes with high SNR (Rossion et al., 2012).

In order to assess the significance of the responses to define the number of base and oddball harmonics to include in the analyses, Z-scores were calculated using the mean and standard deviation of the 20 frequency bins surrounding the bin of interest (Liu-Shuang et al., 2014). Harmonics were considered significant until the Z-score no longer exceeded 1.64 ($p$ < .05), for two consecutive harmonics.

Consequently, we quantified the oddball response as the sum of the responses of the first seven harmonics (i.e. until 7F/5 = 8.4 Hz), excluding the 6 Hz general response. The base rate response (i.e. 6 Hz) was quantified as the summed responses of the base rate and its following two harmonics (2F and 3F = 12 Hz and 18 Hz, respectively).

Additional analyses were performed at the individual subject level by averaging the raw FFT spectrum per ROI and cropping it into segments centered at the oddball frequency and its harmonics, surrounded by 20 neighboring bins on each side that represent the noise level. Similar to previous studies (Van der Donck et al., 2019, 2020), these spectra were summed across the significant harmonics and transformed into an individual Z-score for each condition and for all ROIs.

**Defining regions of interest (ROIs).** Previous research using similar frequency-tagging facial expression processing EEG paradigms (Dzhelyova et al., 2017; Van der Donck et al., 2019, 2020) has consistently identified three regions to be the most responsive for emotion discrimination as assessed by oddball stimulation. Likewise, also in the present study, the highest oddball responses – capturing all relevant oddball activity – were measured over these left and right occipito-temporal (LOT and ROT region) and medial-occipital (MO region) sites. Accordingly, region-of-interest (ROI) analyses were performed to examine OXT-treatment effects within these regions by averaging the summed baseline-subtracted oddball responses over channels P7, P9, and PO7 for the LOT region, over channels P8, P10, and PO8 for the ROT region and over channels Iz and Oz for the MO region (see Fig 2).

While the 1.2 Hz oddball response reflects the strength of neural expression discrimination, the 6 Hz base rate response reflects the contrast between the facial stimuli and the background (Dzhelyova et al., 2017). As this general stimulation response is mainly driven by low-level visual features, it is typically characterized by a medial-occipital topography (Dzhelyova & Rossion, 2014a). ~~As this general visual stimulation response is not the focus of this intervention study, we report the results on this base rate response in Supplementary Fig 1.~~

## 2.5. Behavioral facial expression processing

In order to investigate whether potential prosocial OXT effects at the neural level would also be reflected at the behavioral level, we additionally administered the Emotion-matching task (Palermo et al., 2013) subsequently to the EEG paradigm. This is a computerized facial expression processing task where three faces are shown simultaneously on the screen, and participants have to detect a target face showing a different facial emotion compared to two distractor faces both showing the same expression. All faces display one of the six basic emotions. Participants were instructed to select the target face by pressing the corresponding number (i.e. 1, 2 or 3) on the keyboard. To ensure that all data was gathered within the assumed 75-minutes window of boosted levels of peripheral OXT (Daughters et al., 2015; Striepens et al., 2013), we used the shorter 65-item version of the task, preceded by four practice trials (for specifics, see Palermo et al., 2013).

## 2.6. Statistical analyses

We performed linear mixed models (LMM; 'Afex' package version 0.28-1 in R version 4.0.5 (Singmann et al., 2020)) to assess the modulatory effects of facial expressions and treatment on the participants' **EEG baseline-subtracted amplitudes** recorded over the LOT, ROT and MO regions, with *ROI* (LOT, ROT, MO), *emotion* (anger, fear, happiness), and *treatment condition* (OXT, PL) as fixed effects. Since half of the participants received OXT during their first session, while the other half started with the PL condition, *session order* was added as nuisance covariate to adjust for its effect on the neural responses. We included a random intercept and random slope for each of the fixed factors per participant. Nine outlying data points were detected using the median absolute deviation and removed. Considering the previously reported modulatory effect of expression valence on the OXT treatment response (Evans et al., 2014), additional LMMs were performed for each of the three facial expressions (anger, fear and happiness) separately. Tukey-corrected post-hoc T-tests were performed on the fitted models for all significant effects ('emmeans' package version 1.6.0 (Lenth et al., 2019)). Cohen's d effect sizes were calculated by dividing the least square means difference by the pooled standard deviation.

In keeping with the previous analyses, we also performed LMMs on the **behavioral measures** of the orthogonal fixation cross color chance detection task and the Emotion-matching task, which had no outlying data points. For the orthogonal task, fixed factors were *emotion* (anger, fear, happiness) and *treatment condition* (OXT, PL). For the Emotion-matching task, the LMM only included *treatment condition* as fixed factor. For all behavioral measures, *session order* was added as a nuisance covariate as well. Tukey-corrected post-hoc T-tests were performed for all significant effects.

When the LMMs did not display any treatment effect, Bayes Factors (BFs) were computed to estimate the statistical evidence in favor of the null hypothesis ('BayesFactor' package version 0.9.12-4.2 in R; Morey et al., 2018). By computing the BFs, we compared the fitting of two competing models: the model as described in the LMMs versus the same model without the factor "treatment condition". In line with the heuristic classification scheme of Lee and Wagenmakers (2013), BF-values lower than 1 indicate support for the null hypothesis, whereas values higher than 1 indicate support for the alternative hypothesis. Yet, in general, BF-values in between 3 and 1/3 indicate that the data are ambiguous, making the outcome indecisive. Additionally, we calculated the mean posterior difference between the two treatment conditions for all emotions, as well as their 95% highest density interval (HDI).

~~Since half of the participants received OXT during their first session, while the other half started with the PL condition, we performed secondary analyses accounting for potential effects of 'session order', by adding this factor as a nuisance covariate in all the performed analyses. Overall, the pattern of results was qualitatively similar to the main analyses, confirming no modulatory effect of the factor 'session order' on the reported effects.~~

# ~~4.~~3. Results

## ~~4.1.~~3.1. Reported side effects

Participants were monitored for potential side effects until three hours after the single-dose administration. As can be observed in Supplementary Table 1, side effects were only minimal, non-

treatment specific (cf. insignificant Pearson Chi-square tests), and were possibly due to EEG administration with a tight head cap and fixating on a screen for a prolonged period (e.g. headache reported in 7 OXT and 8 PL participants).

## ~~4.2.~~3.2. Neural responses

All three expressions elicited clear expression-discrimination EEG responses at the oddball frequency and its harmonics (Fig 2), mostly centered over lateral occipito-temporal sites (Fig 3).

[Insert Fig.2 about here]

### ~~4.2.1~~3.2.1 Estimated reliability and power analysis

A power analysis was performed to calculate the power to detect true differences. The highly reliable measurements (with between-session correlations of $r$ = .57, $r$ = .70 and $r$ = .74 for anger, fear and happiness, respectively; all $p < 0.001$) included in this repeated-measures design substantially enhanced the power of this study. Based on the average correlation ($r$ = .67), a power analysis with G*Power 3 (Faul et al., 2007) revealed a power of .91 to detect group differences, even for a small effect size (0.25), indicating that our study design yielded adequate power.

### ~~4.2.2~~3.2.2 Expression discrimination responses

The main analysis across all three facial expressions revealed no main effect of treatment ($F$(1,30.15) = 0.72, $p$ = 0.40), nor emotion ($F$(2,29~~28.99~~) = 2.11~~0~~, $p$ = 0.14~~4~~) or ROI ($F$(2,28.75~~8.99~~) = 1.78~~77~~, $p$ = 0.19~~9~~). Nor did we find a significant effect of sessions order ($F$(1,28.94) = 0.05, $p$ = 0.82). The marginally significant interaction effect between treatment condition and emotion ($F$(2,349.94~~52.5~~) = 2.63~~2~~, $p$ = 0.07), suggested an opposite effect of treatment for angry versus fearful and happy faces. However, subsequent Tukey-corrected post-hoc t-tests failed to reveal any treatment effects for any of the emotions ($M_{anger\ OXT}$ = 0.47 µV, $M_{anger\ PL}$ = 0.50 µV; $M_{fear\ OXT}$ = 0.54 µV, $M_{fear\ PL}$ = 0.50 µV,;$M_{happiness\ OXT}$ = 0.61 µV, $M_{happiness\ PL}$ = 0.54 µV; $t(74.7)_{anger\ OXT\text{-}PL}$ = -0.73, $d$ = 0.09, 95% CI [-0.38 – 0.20]; $t(74.1)_{fear\ OXT\text{-}PL}$ = 1.28, $d$ = 0.13, 95% CI [-0.16 – 0.42]; $t(74.8)_{happiness\ OXT\text{-}PL}$ = 1.46, $d$ = 0.15, 95% CI [-0.14 – 0.45]; all $p > 0.15$).

The significant emotion by ROI interaction ($F(4,3513.4187) = 11.867$, $p < 0.001$) revealed higher neural responses in the MO region for happy (M = 0.65 µV) versus angry (M = 0.33 µV) and fearful (M = 0.46 µV) faces ($t(65.9)_{anger\text{-}happiness} = -5.65$, $p < 0.001$, $d = -0.86$, 95% CI [-1.23 – -0.49]; $t(70.5)_{fear\text{-}happiness} = -3.44$, $p = 0.003$, $d = -0.49$, 95% CI [-0.85 – -0.13]), and for fearful versus angry faces ($t(138.12) = -3.01$, $p = 0.009$, $d = -0.47$, 95% CI [-0.83 – -0.11]). In addition, for angry faces, the neural responses recorded over the MO region were significantly lower than in the LOT (M = 0.55 µV; $t(57.2) = 3.45$, $p = 0.003$, $d = 0.68$, 95% CI [0.32 -1.04]) and ROT (M = 0.56 µV; $t(55.85) = -4.01$, $p < 0.001$, $d = -0.70$, 95% CI [-1.06 – -0.33]) regions. There were no other significant two- or three-way interaction effects (all $p > 0.32$).

The BF analysis provided decisive evidence for a lack of treatment effect: BF = 0.000052. When comparing the neural responses in the OXT versus PL condition, we found a mean posterior decrease for angry faces of 0.057 µV (95% HDI = [-0.13 0.01]), and a mean posterior increase for fearful (0.02 µV; 95% HDI = [-0.05 0.09]) and happy (0.04 µV; 95% HDI = [-0.03 0.11]) faces, all indicating that the treatment effect is negligible.

[Insert Fig.3 about here]

In line with the approach-avoidance hypothesis and the marginally significant emotion by treatment effect, we also calculated LMMs for each facial expression separately. Yet, also these additional LMMs did not reveal any main effect of treatment ($F(1,878.5232)_{Anger} = 0.875$, $F(1,88.778.84)_{Fear} = 1.7782$, $F(1,88)_{Happiness} = 2.59$; all $p > 0.11$), nor any treatment by ROI interaction ($F(2,878.5019)_{Anger} = 1.145$, $F(2,88.88.7784)_{Fear} = 0.854$, $F(2,88)_{Happiness} = 0.09$; all $p > 0.32$). No significant effects of session order were detected (all $p > 0.32$).

Statistical analysis of the individual subject data revealed that the majority of participants showed robust individual expression discrimination responses (i.e. z-scores > 1.64, $p < 0.05$) in at least one of the three ROIs, irrespective of treatment condition (see Supplementary Table 2).

~~[Insert Fig.3 about here]~~

### ~~4.2.3~~3.2.3 General visual base rate responses

In line with previous reports, the LMM of the ~~the~~ general visual base rate response (i.e. 6 Hz) revealed a significant ~~yielded the highest responses in the MO region (~~main effect of ROI (*F*(2,29.94) = 26.11~~21.61~~, *p* < 0.001) and a significant two-way interaction between emotion and ROI (*F*(4,395.57) = 2.96, *p* = 0.02). As expected, we found the highest neural responses over the medial-occipital (MO) region for all three emotions ($M_{anger\ MO}$ = 3.22 µV; $M_{fear\ MO}$ = 3.32 µV; $M_{happiness\ MO}$ = 3.28 µV; $t(30.1)_{anger\ LOT\text{-}MO}$ = -6.85, *d* = -1.59, 95% CI [-1.99 – -1.18]; $t(30.1)_{anger\ MO\text{-}ROT}$ = 4.80, *d* = 1.24, 95% CI [0.85 – 1.62]; $t(30.1)_{fear\ LOT\text{-}MO}$ = -7.26, *d* = -1.67, 95% CI [-2.07 – -1.25]; $t(30.1)_{fear\ MO\text{-}ROT}$ = 5.18, *d* = 1.35, 95% CI [0.96 – 1.74]; $t(30.1)_{happiness\ LOT\text{-}MO}$ = -7.34, *d* = -1.69, 95% CI [-2.10 – -1.28]; $t(30.1)_{happiness\ MO\text{-}ROT}$ = 5.38, *d* = 1.39, 95% CI [1.00 – 1.79]; all *p* < 0.001).~~)~~. Responses in the right occipito-temporal (ROT) region ($M_{anger}$ = 1.97 µV; $M_{fear}$ = 1.97 µV; $M_{happiness}$ = 1.90 µV) were also significantly larger than in the left occipito-temporal (LOT) region ($M_{anger}$ = 1.37 µV; $M_{fear}$ = 1.35 µV; $M_{happiness}$ = 1.25 µV; $t(31.3)_{anger}$ = 2.64, *p* = 0.04, *d* = 0.53, 95% CI [0.17 – 0.89]; $t(31.3)_{fear}$ = 2.67, *p* = 0.03, *d* = 0.56, 95% CI [0.20 – 0.92]; $t(31.4)_{happiness}$ = 2.48, *p* = 0.04, *d* = 0.53, 95% CI [0.17 – 0.89]). N~~As expected, n~~o treatment effects nor any interactions with treatment were observed for the general visual base rate response (for any of the three facial expressions (all *p* > 0.15~~3~~, see ~~Supplementary~~ Fig 4~~1~~)).

As for the oddball analysis, also the BF analysis of the general visual response to faces revealed conclusive evidence in favor of the null hypothesis: BF = 0.000028. For angry faces, across the three ROIs, we found a negligible mean posterior decrease in neural responses in the OXT condition compared to the PL condition (0.12 µV; 95% HDI = [-0.29 0.05]), and a negligible mean posterior increase for fearful (0.06 µV; 95% HDI = [-0.11 0.24]) and happy (0.06 µV; 95% HDI = [-0.11 0.24]) faces.

[Insert Fig.4 about here]

## ~~4.3.~~3.3. Behavioral measures: Orthogonal task and explicit facial emotion processing

Results of the LMM revealed equal performances during the PL and OXT sessions on the fixation cross color change detection task, both in terms of accuracy ($M_{PL}$ = 95%, $SD_{PL}$ = 0.05; $M_{OXT}$ = 95%, $SD_{OXT}$ = 0.06; $F(1,150) = 0.01$, $p = 0.94$, $d = 0.009$, 95% CI [-0.28 – 0.30]) and in terms of reaction times ($M_{PL}$ = 0.43 s, $SD_{PL}$ = 0.05; $M_{OXT}$ = 0.43 s, $SD_{OXT}$ = 0.04; $F(1,150) = 0.26$, $p = 0.77$, $d = -0.13$, 95% CI [-0.42 – 0.16]). These results indicate that the participants were equally attentive to the screen within each treatment session. No other main or interaction effects were found (all $p > 0.27$).

BF analyses of the accuracies and reaction times showed moderate evidence for an absent treatment effect: $BF_{ACC}$ = 0.042 and $BF_{RT}$ = 0.080. On average, participants were slightly better (mean posterior difference = 0.00049; 95% HDI = [-0.011 0.012] and slightly faster (mean posterior difference = -0.0051; 95% HDI = [-0.011 0.0009] in detecting the color change of the fixation cross during the OXT condition versus the PL condition.

In addition, the LMM investigating the participants' performances on the Emotion-matching task also revealed equal accuracy ($M_{PL}$ = 75%, $SD_{PL}$ = 0.07; $M_{OXT}$ = 74%, $SD_{OXT}$ = 0.10; $F(1,30) = 0.12$, $p = 0.73$, $d$ = 0.06, 95% CI [-0.44 – 0.55]) and equal reaction times ($M_{PL}$ = 4.11 s, $SD_{PL}$ = 1.31; $M_{OXT}$ = 4.03 s, $SD_{OXT}$ = 1.16; $F(1,30) = 0.72$, $p = 0.40$, $d = 0.07$, 95% CI [-0.42 – 0.57]). Hence, these results suggest that OXT treatment does not have a modulatory effect on explicit facial expression processing, nor did the order of the treatment conditions ($p_{acc}$ = 0.60; $p_{rt}$ = 0.34).

For the accuracies and reaction times on the Emotion-matching task, the BF analyses only provide anecdotal evidence for the null hypothesis: $BF_{ACC}$ = 0.2739 and $BF_{RT}$ = 0.3712. We found a slightly reduced emotion matching performance in the OXT condition (mean posterior difference = -0.0043; 95% HDI = [-0.030 0.022] compared to the PL condition, as well as a reduction in reaction times (mean posterior difference = -75.59; 95% HDI = [-286.633 128.427].

## ~~5.~~4. Discussion

In the current randomized, double-blind, cross-over, placebo-controlled study, we applied frequency-tagging EEG to investigate the modulating effects of a single dose of OXT on the automatic and implicit neural sensitivity of 31 healthy adult men to brief changes in facial expression.

We did not find an OXT treatment effect, neither at the neural level, nor on the behavioral task assessing the accuracy and speed of matching emotional expressions.

Similar to previous research (Van der Donck et al., 2019, 2020), we showed that neural expression discrimination is mainly driven by EEG responses in higher-level occipito-temporal regions, and that expressions crucially involving the mouth area (i.e. happiness and fear) additionally recruit medial-occipital perceptual regions. Similar to what has been demonstrated before with comparable frequency-tagging EEG paradigms (Dzhelyova et al., 2019), the neural expression-discrimination responses obtained in our study showed a high test-retest reliability. Together with the ability to sensitively identify individuals characterized by socio-communicative impairments such as autism spectrum disorder (Van der Donck et al., 2019, 2020) and 22q11.2 deletion syndrome (Leleu et al., 2019), in principle, these combined characteristics of reliability and sensitivity would have made the frequency-tagging EEG approach perfectly suited to monitor the impact of oxytocin treatment.

### ~~5.1.~~4.1. No general enhancement of emotional salience, nor any modulation of social approach or withdrawal tendencies

In general, our findings argue against a strong account of the social salience hypothesis of OXT (Shamay-Tsoory & Abu-Akel, 2016). This framework posits that OXT uniformly increases the salience of social stimuli, irrespective of their valence. This was not the case in our study, as there was no generally enhanced neural sensitivity to the three displayed emotions.

Neither do our findings align with the social approach/withdrawal hypothesis (Kemp & Guastella, 2011), stating that OXT enhances social approach-related behavior and diminishes social withdrawal. At a neural level, this would have been reflected in increased activation in response to positive facial expressions and reduced responses when processing negative facial expressions. However, as the oxytocinergic effects in response to positive and negative facial expressions are thought to be largely independent of each other (Ellenbogen, 2018), we could have observed either one of these effects.

Although previous studies have reported modulatory OXT effects on the implicit processing of emotional faces (e.g. presented for < 300 ms; Shahrestani et al., 2013), our EEG findings did not reveal such an impact. Possibly, this absence of OXT-induced modulation of neural responses could be explained by the fact that OXT might exert little effect when the emotional sensitivity is already high, as suggested by ~~(~~Leknes and colleagues~~et al.,~~ (2013). Indeed, as OXT has been found to improve facial expression processing in particular in individuals for whom the task is more demanding (Bartz et al., 2011; Mierop et al., 2020), our implicit emotion processing paradigm might not have been fully suited to detect enhanced neural sensitivity in individuals who may already perform well in face processing at baseline. Moreover, while fMRI studies have demonstrated oxytocinergic effects in several (subcortical) brain areas, such as the amygdala (Grace et al., 2018; Wang et al., 2017), scalp EEG might not be optimal to detect these effects. Yet, if underlying brain areas might have been influenced by OXT, this might also have been reflected in improved behavioral emotion matching, which was neither the case in our study.

If OXT would only affect the processing of facial expressions, this would have been reflected in selectively enhanced neural responses to the emotional stimuli, either for specific emotions or for all emotions. However, if OXT would enhance the saliency of social stimuli in general, the effect may not have been limited to emotional faces but may encompass any type of faces, thus also the neutral ones. This would imply also observing higher base rate responses in the OXT versus PL condition, as these reflect the processing of the facial stimuli, both neutral and expressive. Previous studies have indeed provided evidence for a modulatory OXT effect on face processing in general (Andari et al., 2016; Hovey

et al., 2020). In adults with autism, for example, OXT has been shown to enhance neural activity in bilateral occipito-temporal regions during the processing of neutral facial stimuli (Andari et al., 2016). Yet, for the participants included in our sample, this was not the case as the general neural response to faces was not modulated by OXT, not in the occipito-temporal regions, nor in the MO region. Some studies suggest that the modulatory effect of OXT on face processing may rather be attributed to enhanced face memory -as OXT increases the familiarity of previously encountered faces- instead of enhanced facial perception (for a review, see Lopatina et al., 2018; Rimmele et al., 2009). In our study, the rapid presentation rate only allows a single glance at the images, which are all preceded and followed by forward and backward masks, thereby interfering with conscious processing. However, even if OXT would have enhanced the recognition of the facial stimulus as a 'known/familiar' stimulus -even at this presentation rate-, we would rather expect reduced neural responses in the OXT condition due to the larger adaptation effects for familiar stimuli.

To ensure that we did not overlook a possible OXT treatment effect in the latency of the responses -which we would not detect within the frequency-domain- we also performed a time-domain analysis of the waveforms time-locked to the oddball stimuli (i.e. expressive faces; see Supplementary Materials for method and results). Similar to previous studies investigating facial expression processing at the neural level (DaSilva et al., 2016; Dzhelyova et al., 2017), our data yielded a clear tri-phasic response to brief changes in facial expressions. This tri-phasic response consisted of three differential components (P1, N1 and P2), rather that ERP components, as these components already reflecting the contrast between the neutral faces and the expressive faces (Dzhelyova & Rossion, 2014b). One might argue that the time-locked waveforms elicited by these facial expressions may overlap with the tri-phasic OXT-related ERP modulation, as proposed by Pehlivanoglu and colleagues (2020). According to these authors, the ERP modulation effect of OXT starts in the perception stage (100-200 ms), where it enhances the perceived salience of stimuli. Second, during the selection stage (200-300 ms), OXT is thought to facilitate attention towards social stimuli, and, third, during the evaluation stage (>300 ms), the OXT-induced increased sustained attention to motivationally salient information converts to higher-

order cognitive processing (Pehlivanoglu et al., 2020). While the time-windows defined in our study may map perfectly on these postulated latency-dependent OXT modulatory stages, the selection of our time-windows was based on the average waveform of OXT and PL combined. Importantly, in terms of latencies and amplitudes, our results clearly show an identical tri-phasic response in the PL condition as in the OXT condition, thereby refuting the idea of an OXT-specific modulation of the signal. If OXT would indeed have reduced the time required for facial (emotion) processing, we could have expected to find a shorter latency for the expression discrimination responses in the OXT versus the PL condition. However, no significant OXT effects were discovered in the time-domain, neither in the latency of the components, nor in their amplitudes.

## ~~5.3.~~4.2. No modulation of behavioral emotion matching performance in healthy individuals

In addition, we found no improvement of behavioral facial emotion processing after OXT administration. Although OXT has mostly been reported to improve the accuracy of emotion recognition, it might also reduce the time that is required to process the faces in order to recognize emotions, without compromising the accuracy (Hubble et al., 2017). Yet, in our study, both the accuracy and reaction times of the behavioral emotion processing performances seem to be unaffected by a single dose of OXT. However, it should be noted that the participants included in our study were healthy adults with adequate baseline emotion processing abilities (Palermo et al., 2013; behavioral results). Accordingly, the possibility cannot be ruled out that the lack of OXT treatment effects on the behavioral emotion processing task might reflect ceiling performance, allowing no further behavioral improvement (Bartz et al., 2011; Guastella et al., 2010).

In addition, the absence of behavioral OXT effects could possibly be attributed to the task we used in this study. Possibly, OXT-induced enhanced emotion processing depends more on higher-level

processes associated with labeling and recognizing facial expressions, rather than comparing (matching) emotional faces, given that ~~(~~Horta de Macedo and colleagues~~et al.,~~ (2014) ~~neither~~ also did not f~~iou~~nd improved emotion matching performance in healthy adult participants after intranasal OXT administration.

One might argue that the relatively small sample size (N = 31) yields relatively low statistical power to detect main effects of intranasally administered OXT (Mierop et al., 2020). It is to say, the non-significant p-values may either indicate data insensitivity due to a small sample size or they may reflect actual null results (Winterton et al., 2021). Yet, investigating the evidence in favor of the null hypothesis via Bayes Factors, convincingly confirms the absence of an OXT effect on neural and behavioral facial expression processing.

To conclude, we applied frequency-tagging EEG to investigate the effects of a single dose of OXT on the automatic and implicit neural sensitivity for positive and negative facial expressions. Yet, our findings from the frequency- and time-domain showed that OXT did not influence the sensitivity to (specific) facial expressions, nor did it affect the timing of these neural processes. More specifically, our results did not demonstrate enhanced emotional salience, nor did the results reflect a modulatory effect of OXT on social approach-avoidance tendencies. ~~-~~These OXT-induced effects were absent both on the neural and the behavioral level, possibly due to ceiling emotional face processing performances.

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## Funding sources

This work was supported by the Research Foundation Flanders [grant G0C7816N, and the Excellence of Science EOS grant G0E8718N (HUMVISCAT)]; and KU Leuven [grant C14/17/102].

## Acknowledgements

The authors would like to thank all the participants who contributed to this study. The authors would also like to thank Robine Hellemans for her help in conducting the study.

## Conflict of interest

The authors declare no conflict of interest.

## Reprints

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# Figure captions

**Fig 1. Fast periodic visual stimulation oddball paradigm.** Neutral faces are presented sequentially at a fast 6 Hz base rate, periodically interleaved with an expressive face – anger, fear, happiness – every fifth image (1.2 Hz oddball rate). The identity of the faces changes every image. Stimuli shown here: AF02, AF07, AF13, AF15, AF22, AF27, AF29.

**Fig 2. SNR spectra visualizing the expression-discrimination responses.** (Left) Visualization of the ROIs. The three most leftward and three most rightward open circles constitute left and right occipito-temporal (LOT and ROT) regions, respectively. The two central open circles constitute the medial-occipital (MO) region. (Right) SNR spectra visualizing the expression-discrimination responses, recorded over the LOT (upper row), MO (middle row) and ROT (lower row) regions, for each of the expressions and both treatment conditions. The significant first seven harmonics (until 8.4 Hz) are displayed; the dashed line indicates the 6 Hz base rate response.

**Fig 3. Expression-discrimination responses.** Scalp topographies and bar graphs of the summed baseline-subtracted amplitudes evoked during both treatment sessions, displaying the mean expression-discrimination responses for each of the three ROIs and for each facial expression. Error bars reflect standard errors of the mean. No main nor interaction effect of treatment was evidenced. The emotion by ROI interaction entails that medial-occipital (MO) responses for fearful and happy faces are significantly larger than for angry faces, and that for angry faces, the MO responses are significantly lower than the responses in the left and right occipito-temporal (LOT and ROT, respectively) regions. ** < 0.01, *** < 0.001

**Fig 4. General visual base rate responses.** Scalp topographies and bar graphs of the summed baseline-subtracted amplitudes evoked during both treatment sessions, displaying the general visual responses to faces, for each of the three ROIs and for each facial expression. Error bars reflect standard errors of the mean. The main effect of ROI is clearly visualized, with highest responses recorded over medial-occipital (MO) sites, and higher responses in the right occipito-temporal (ROT) region versus the left

occipito-temporal (LOT) region, irrespective of the treatment condition or facial expression. * < 0.05, *** < 0.001