# Title page

**Title**: To mirror or not to mirror upon mutual gaze, oxytocin can pave the way: a cross-over randomized placebo-controlled trial

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# Abstract

The eyes constitute a highly salient cue to communicate social intent. Previous research showed that direct eye contact between two individuals can readily evoke an increased propensity to 'mirror' other peoples' actions. Considering the implicated role of the 'prosocial' neuropeptide oxytocin (OXT) in enhancing the saliency of social cues and modulating approach/avoidance motivational tendencies, the current study adopted the non-invasive brain stimulation technique transcranial magnetic stimulation (TMS) to explore whether a single dose of intranasal OXT (24 IU) modulated (enhanced) a person's propensity to show heightened 'mirroring' or 'motor resonance' upon salient social cues, such as eye contact. The study involved a double-blind, placebo-controlled, cross-over trial with twenty-seven healthy adult men (18-31y). By applying single-pulse TMS over the primary motor cortex during movement observation, it was shown that motor resonance was significantly higher when movement observation was accompanied by direct, compared to averted gaze, but that a single dose of OXT did not uniformly enhance this effect. Significant moderations of the treatment effect were noted however, indicating that participants with high self-reports of attachment avoidance displayed a stronger OXT-treatment effect (enhancement of motor resonance upon direct eye contact), compared to participants with low attachment avoidance. Particularly, while participants with high attachment avoidance initially displayed a reduced propensity to increase their motor resonance upon direct eye contact, a single dose of OXT was able to promote an otherwise avoidant individual's propensity to engage in motor resonance upon a salient social cue such as eye contact.

**Keywords**: Oxytocin; mirror system; eye contact; transcranial magnetic stimulation; biological motion perception.

# 1. Introduction

Interpersonal interactions are extremely complex, involving both approach and avoidance behaviors toward other conspecifics. An important feature of successful social interaction and indicator of social approach is biobehavioral synchrony, or the coordination of biological and behavioral processes between interaction partners (Feldman, 2017). At the neural level, the brain's action observation system or mirror system is anticipated to play a key role in establishing interpersonal synchrony or 'resonance'. Several neuroimaging and neurophysiological studies show that distinct motor regions in fronto- and parietal cortices are increasingly activated not only when performing a particular action, but also when merely observing the same action performed by others, thereby providing a direct 'mirror-motor matching' or 'motor resonance' mechanism (Rizzolatti and Craighero, 2004). Overall, this 'mapping' of observed actions onto the observer's own motor system is suggested to form the basic mechanism by which others' actions, facial expressions or emotional states can be recognized, understood and acted upon (Cattaneo and Rizzolatti, 2009; Rizzolatti and Fabbri-Destro, 2008).

Albeit automatic, the propensity to 'synchronize' with conspecifics is anticipated to depend heavily upon the presented social context and prior social experiences of the individual (Wang and Hamilton, 2012). Among different social cues from the environment, mutual gaze forms a very powerful signal to express communicative intent and attention, and may therefore constitute a salient cue to evoke interpersonal synchrony or approach-related behavior (Grossman, 2017; Senju and Johnson, 2009). In line with this notion, studies from our and other labs showed that eye contact can rapidly and specifically facilitate automatic mirroring of others' actions, indicative of social approach (Prinsen et al., 2017; Wang et al., 2011a, 2011b). Here, we aim to explore the effect of social context (i.e. eye gaze) on motor resonance further and, in particular, whether administration of the 'prosocial' neuropeptide oxytocin (OXT) can modulate this effect.

Endogenous OXT is synthesized in the hypothalamus where neurons of the paraventricular nuclei project to various cortical and subcortical brain areas involved in social behavior and socio-cognitive processes. Since the discovery that central OXT levels can be pharmacologically manipulated by means of intranasal administration of exogenous OXT (Born et al., 2002; Churchland and Winkielman, 2012), an ever-growing body of research has tested the implication of OXT on human sociality. Based on early findings reporting beneficial effects of OXT on social behavior, OXT has gained its 'prosocial' reputation. However, this exclusively prosocial view of OXT has been nuanced by findings showing that the effects of OXT are strongly dependent upon the context in which the social interaction happens (Bos et al., 2012), as it can for example lead to a *decrease* in social cooperation towards members of an 'out-group' (De Dreu et al., 2010).

Although not mutually exclusive, several mechanisms have been proposed by which OXT affects social behavior, namely (i) by enhancing the saliency of social cues; (ii) by modulating reward sensitivity and approach/avoidance motivational tendencies; and (iii) by reducing (social) anxiety (Bartz, 2016; Neumann and Slattery, 2015; Shamay-Tsoory and Abu-Akel, 2016). In particular interest for this study, eye-tracking studies showed that exogenously administered OXT promotes gaze towards the eye region of the communicator (Guastella et al., 2008) and increases eye contact during naturalistic social interactions (Auyeung et al., 2015). Increasing evidence also suggests that OXT can mediate the processing of the communicator's body language (Bernaerts et al., 2016; De Coster et al., 2014; Kéri and Benedek, 2009; Perry et al., 2010). For example, in terms of mapping of bodily cues, a handful of behavioral studies showed that a single dose of OXT reduced reaction times in an imitation task (De Coster et al., 2014) and enhanced biological motion perception or emotion recognition from so-called point-light display's (Bernaerts et al., 2016; Kéri and Benedek, 2009). An initial EEG study showed that OXT induced an increase in *mu*-rhythm suppression during biological motion perception, which is indicative of mirror-neuron activation (Perry et al., 2010).

With the present study, we adopted a novel paradigm to explore the prosocial effects of OXT-treatment on mirror-motor mapping or interpersonal motor resonance from a neurophysiological perspective. Particularly, by using the non-invasive and widely-used brain transcranial magnetic stimulation (TMS) technique, motor resonance upon movement observation was measured in order to obtain an unbiased neurophysiological measure of an individual's propensity to 'synchronize with' an observed model. In the past decade, single-pulse TMS has been used extensively as an assessment tool to measure resonant mirror activity in the observer's motor system during the observation of others' actions (see Fadiga et al., 2005 for a review). In particular, by applying a single magnetic pulse over the primary motor cortex, the underlying cortical neurons are activated, which elicits a motor evoked potential (MEP) from the corresponding contralateral muscles. Fadiga et al. (1995) showed that during the mere observation of others' actions, activity within the primary motor cortex becomes increasingly facilitated, as indicated by significant enhancements in MEP amplitudes elicited by TMS. By measuring the amplitude of the motor evoked potentials (MEPs) elicited by TMS under various experimental conditions, TMS can be used to monitor changes in putative mirror system activity in a relatively high temporal resolution.

As previous research showed that eye gaze provides a salient modulator of motor resonance (Prinsen et al., 2017; Wang et al., 2011a, 2011b), we expected to observe an enhancement of 'synchronization' during movement observation accompanied with direct gaze from the model (indicative of communicative intent), compared to averted gaze (indicative of no or even averted communicative intent). A key objective was to examine whether an individual's propensity to show motor resonance upon direct gaze is modulated from the administration of a single dose of OXT. In line with the implicated role of OXT in enhancing the saliency of social cues and modulating approach/avoidance motivational tendencies, we expected OXT to induce an augmentation of motor resonance or 'approach behavior' upon a salient communicative cue such as direct eye contact (i.e., socially adaptive mirroring). Furthermore, since OXT has been shown to impact viewing behavior towards the eye region, we also

explored whether changes in viewing behavior were related to changes in interpersonal motor resonance. Finally, considering the emerging relevance of person-dependent factors in modulating the prosocial effects of OXT (Bakermans-Kranenburg and van IJzendoorn, 2013; Bartz et al., 2011), we additionally explored whether the observed treatment effects of OXT on motor resonance were moderated by inter-individual differences in social responsiveness or attachment style.

# 2. Materials and methods

## 2.1. General study design

This randomized, double-blind, placebo (PL)-controlled, cross-over trial with a wash-out period of one week was conducted at the Department of Rehabilitation Sciences at the University of Leuven (Belgium) to test single-dose effects of intranasal oxytocin (OXT) administration on interpersonal motor resonance assessed using transcranial magnetic stimulation (TMS) (**Fig 1.A**). Written informed consent was obtained from all participants. Consent forms and study design were approved by the Ethics Committee for Biomedical Research at the University of Leuven (S56327) in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964). The trial was registered with the ClinicalTrials.gov database of the U.S. National Institutes of Health (NCT03010670).

## 2.2. Sample size and participants

A total of 26 participants (age-range: 19-32 years; participants' characteristics see **Table 1**) completed the two sessions of the cross-over trial and were included in the final analyses (see CONSORT flowchart in **appendix A**). Inclusion criteria comprised gender (male); age (18-35 years old); and handedness (right). Only male participants were recruited to avoid potential sex differences in OXT response as well as the potential interaction with the female hormonal cycle. Other exclusion criteria comprised medication use; any diagnosed psychiatric or neuropsychological disorder (e.g., stroke, epilepsy, concussion) or any contraindication for TMS (Rossi et al., 2012).

In one prior clinical trial, a cross-over design was used to assess the effects of single-dose OXT-treatment on a neurophysiological measure of mirror activity (*mu*-rhythm) using EEG. Significant effects (large-size) were reported for a total of 24 participants who completed the OXT/PL cross-over treatment (Perry et al., 2010). Considering this prior cross-over study, the current sample size was set at a comparable sample of 26 participants.

### 2.3. Drug protocol

Participants were randomly assigned to receive the OXT (Syntocinon®, Sigma Tau) or PL (saline solution of sodium chloride in water) nasal spray on the first/second testing session. Both sprays were prepared by the KU Leuven University Hospital pharmacist and were administered in identical amber 15 ml glass bottles with metered pump, such that all research staff conducting the trial and participants were blind to treatment allocation. According to the golden standard in human OXT research (Graustella and MacLeod, 2012), a single dose of 24 international units (IU), delivered as 3 puffs of 4 IU per nostril, was adopted. Participants received clear instructions about the use of the nasal spray prior to self-administration (Guastella et al., 2013).

Studies investigating OXT concentrations in saliva (Daughters et al., 2015) and plasma (Gossen et al., 2012; Striepens et al., 2013) after intranasal administration of a single dose of OXT have indicated that peripheral OXT levels significantly increase approximately half an hour after intranasal administration. The efficacy of this time interval has also been confirmed by animal research (Chang et al., 2012; Neumann et al., 2013). Consequently, in healthy humans, the impact of a single dose of intranasal OXT on social cognition is commonly evaluated using a 30–45 min wait-time before the experimental task (see Graustella and MacLeod, 2012 for a review). Here, a thirty-minute wait-time was incorporated prior to any experimental task in order to test during peak OXT concentrations. All experimental measures were conducted within the assumed 75 minutes time window in which heightened levels of peripheral OXT can be observed (Daughters et al., 2015; Gossen et al., 2012; Striepens et al., 2013) (see **Fig. 1.A**). Participants were monitored onsite for the full experimental procedure (until approximately 1.5 hours after nasal spray administration) and were screened for potential adverse events or side effects. Additionally, the Profile Of Mood States questionnaire (POMS) (Wald and Mellenbergh, 1990) was used at the beginning and end of each session to monitor transient mood levels of participants within and across sessions.

### 2.4. Neurophysiological outcome measure: motor resonance

The primary outcome measure was assessed 30 minutes after nasal spray administration (Daughters et al., 2015), using the non-invasive brain stimulation TMS technique. During the assessment of motor resonance by TMS, participants were seated in a comfortable chair approximately 80 cm in front of a widescreen monitor (resolution: 1920 × 1080 pixels, refresh frequency: 60 Hz) with their hands placed palm-down on a soft cushion on their lap and another cushion placed on top to obstruct vision of the own hands during the experiment. Participants were asked to relax their hand muscles while they spontaneously viewed a random sequence of four different video clips showing a model performing a simple hand movement (hand opening) or no movement (static hand), accompanied with either direct or averted gaze (**Fig. 1.B**). Video clips were identical to those previously adopted in Prinsen et al. (2017) and Wang, Newport, et al. (2011). Each condition was presented five times in blocks of four five-second video clips (total of 20 clips per condition). Video presentation timing was controlled by LabVIEW software (version 14.0, National Instruments, UK).

During observation of the video clips, single-pulse TMS (Magstim-200 stimulator, Magstim Company Ltd., UK) was applied over the left primary motor cortex using a hand-held 70mm figure-of-eight coil and electromyography (EMG) recordings were performed to measure motor-evoked potentials (MEPs) from the contralateral abductor pollicis brevis (APB) muscle, a muscle implicated in the observed hand opening movement. TMS pulses were delivered to coincide with the hand opening phase, i.e., 4.6 seconds after the start of the video clip (see **Fig. 1.C** for an example). Coil placement, optimal location for TMS-stimulation and resting motor threshold were defined for each participant as described in Prinsen et al. (2017). Experimental stimulation intensity was set supra-thresholded at 130%. Signal Software (version 2.02, Cambridge Electronic Design, UK) was used for EMG-recordings and triggering of the TMS-stimulator. All EMG-recordings were sampled (2000 Hz), amplified and band-pass filtered (5-1000 Hz) via a CED Power 1401 analog-to-digital

converting unit (Cambridge Electronic Design, UK). The neurophysiological assessment with TMS lasted approximately 40 minutes.

### 2.5. Secondary outcome measure: eye tracking

After the neurophysiological assessment, a short eye tracking session was conducted to evaluate potential changes in spontaneous viewing behavior of the participants. During this session (duration approximately 5 min, see **Fig. 1.A**), participants sat in front of a Tobii T120 binocular eye tracking device (resolution: 1280 × 1024 pixels, sampling rate: 120 Hz, average precision .5° of visual angle) (Tobii AB, Sweden) and were presented with the same experimental video clips as described above. During eye tracking, the total fixation duration (TFD) or the sum of the durations of all fixations towards a predefined area of interest (AOI) centered over the eye region of the model's face was assessed. Please not that data of two participants was excluded from the final analysis due to technical errors during gaze behavior acquisition.

### 2.6. Assessment of person-dependent factors

To assess inter-individual differences in treatment-effects related to person-dependent factors, participants completed self-report questionnaires assessing social responsiveness (Social Responsiveness Scale for adults, SRS-A) (Constantino and Todd, 2005) and state attachment (State Adult Attachment Measure, SAAM) (Gillath et al., 2009). The SRS-A is a 64-item questionnaire to assess variations in social responsiveness in the typical population and autism spectrum disorders using a four-point Likert-scale. It encompasses four subscales: social communication (22 items), social awareness (19 items), social motivation (11 items) and rigidity/repetitiveness (12 items). Higher scores indicate less social responsiveness. The SAAM is a 21-item questionnaire to assess inter-individual differences in state attachment using a seven-point Likert-scale. The questionnaire comprises three subscales of 7 items assessing attachment security (e.g. "I feel like I have someone to rely on"); attachment anxiety (e.g. "I feel a strong need to be unconditionally loved right now");

and attachment avoidance (e.g. “If someone tried to get close to me, I would try to keep my distance”).

### 2.7. Data analysis and statistics

Based on the recorded EMG data, peak-to-peak amplitudes of the TMS-evoked MEPs were determined to assess condition-induced changes in cortico-motor excitability at the level of M1. Additionally, background EMG was quantified by calculating the root mean square error (RMSE) across the 110 to 10 millisecond interval prior to TMS-stimulation to ensure that subjects were completely relaxed during stimulation. Trials with excessive tonic muscle activity (background EMG exceeding 2.5 standard deviations from the mean) were not included in the final analyses (2.41% of all trials). Further, extreme MEP-amplitudes (exceeding 1.5 interquartile distances from the mean) were removed from the analysis (8.77% of all trials). Note that the number of discarded trials was similar across sessions and observation conditions (all $p > .68$).

As raw MEP amplitude values were not normally distributed, mean MEP amplitudes were natural log-transformed. To explore whether ln-transformed MEPs recorded upon movement observation were modulated by ‘gaze condition’ or ‘treatment’, a two-way repeated-measures ANOVA with the within-subject factors ‘observed eye gaze’ (direct gaze, averted gaze) and ‘treatment session’ (PL, OXT) was conducted.

In subsequent ANCOVA analyses, we explored whether the baseline ‘gaze’ effect at the placebo session was potentially modulated by variations in person-dependent factors. Two separate ANCOVA models were performed, one model in which the subscales of the SRS (n = 4) and one model in which the subscales of the SAAM (n = 3) were inserted as continuous regressors. Similarly, the influence of person-dependent factors on the OXT treatment effect was investigated in a similar way, i.e. by repeating the aforementioned 2-way ANOVA analysis with the additional inclusion of the person-dependent variations in SAAM or SRS questionnaire scores as continuous regressors.

To visualize significant relationships, Pearson correlation coefficients were calculated when a modulatory effect was detected. In order to quantify the baseline 'gaze' effect, the difference in MEP amplitude between direct and averted gaze was calculated ($MEP_{direct}$ – $MEP_{averted}$ difference score) for each subject. The OXT treatment effect was calculated separately for each subject by subtracting the difference score of the PL session from the difference score of the OXT session, divided by the pooled standard deviation ($\Delta Gaze_{OXT}$ – $\Delta Gaze_{PL}$) / sqrt(($SD^2_{OXT}$ + $SD^2_{PL}$)/2) (Cohen's *d* treatment effect; Cohen, 1992).

All statistics were calculated with Statistica 10 (StatSoft, USA) and results were considered significant with a *p*-value lower than .05. The partial Eta square ($\eta^2$) value was calculated as an estimate of effect size.

# 3. Results

## 3.1. Side effect screening

All participants were screened for potential side effects or changes in mood states related to the OXT treatment. As described in detail in the appendices, only minimal, non-treatment specific side effects (see **Table B.1** in **appendix B**) or changes in mood states (**Fig. C.1** in **appendix C**) were reported.

## 3.2. The effect of eye contact on motor resonance and its modulation by oxytocin

In **Fig. 2**, the effect of observed eye gaze on MEP amplitudes (i.e. interpersonal motor resonance) is visualized for the experimental opening hand condition, separately for each session (PL, OXT). The repeated-measures ANOVA analyses on the naturally log-transformed MEP amplitudes with the within-subject factors 'observed gaze' (direct, averted) and 'treatment session' (PL, OXT) revealed a significant effect of 'gaze' ($F(1,25) = 5.79$, $p = .02$, $\eta^2 = .19$), indicating that across treatment sessions, MEP responses were significantly larger for the direct, compared to the averted eye gaze condition. These results are in line with previous reports of an enhancing effect of direct gaze on interpersonal motor resonance during movement observation (Prinsen et al., 2017).

Although the difference between direct and averted gaze was slightly larger in the OXT session (difference: 0.08 mV, Fisher LSD: $p = .04$), compared to the PL session (difference: 0.05 mV, Fisher LSD: $p = .16$), the interaction between 'observed gaze' and 'treatment' was not significant ($F(1,25) = 0.01$, $p = .92$, $\eta^2 < .001$), indicating that across all participants, the facilitating effect of direct eye contact on interpersonal motor resonance was not significantly augmented by the OXT treatment (**Fig. 2**). Note that while the mean MEP amplitudes of the direct eye gaze and averted eye gaze conditions were not significantly different between the PL and OXT session, it appeared that the overall dispersion of the data points around the sample mean (standard deviation) was larger in the placebo ($SD_{direct} = 0.90$, $SD_{averted} = 0.92$), compared to the OXT treatment session ($SD_{direct} = 0.68$, $SD_{averted} = 0.67$).

In a subsequent analysis, we explored whether the high variance in interpersonal motor resonance at the PL treatment session was potentially related to inter-individual variance in person-dependent factors (self-reported social responsiveness (SRS) or attachment style (SAAM)). To do so, repeated-measures ANCOVA analyses with the within-subject factor 'eye gaze' (direct, averted) were conducted with the person-dependent factors included as continuous regressors (separate models for the SAAM and SRS subscales).

For the MEP data recorded at the PL session, a significant interaction was revealed between observed gaze and the subscale attachment avoidance ($F(1,22) = 6.32$, $p = .02$, $\eta^2 = .22$), indicating that the extent of the eye gaze effect on interpersonal motor resonance was significantly modulated by attachment avoidance. In particular, the modulatory interaction indicated that the facilitating effect of direct gaze on interpersonal motor resonance (higher $MEP_{direct} - MEP_{averted}$ difference scores) was *more* pronounced for participants with *low* attachment avoidance scores, compared to participants with *high* avoidance scores ($r = -.50$, $p = .009$; **Fig 3.A**).

No significant modulatory interactions were revealed for the other subscales of the SAAM ('gaze x attachment security' interaction: $F(1,22) = 0.18$, $p = .67$, $\eta^2 = .008$) ('gaze x attachment anxiety' interaction: $F(1,24) = 0.58$, $p = .45$, $\eta^2 = .03$) or for the model assessing modulatory effects by the subscales of the SRS (all $p > .14$), indicating that the modulation of the eye gaze effect at the placebo session was specific for attachment avoidance.

### 3.3. Modulation of the oxytocin treatment effect by person-dependent factors

Considering the modulatory effect of attachment avoidance in the baseline PL session, we further explored the possibility of a modulatory impact of this person-dependent factor on the OXT-treatment response. To do so, the ANCOVA analysis with the within-subject factors 'observed gaze' (direct, averted) and 'treatment session' (PL, OXT) was repeated with the person-dependent factor 'attachment avoidance' inserted as a continuous regressor. Interestingly, a significant tree-way interaction between the factors 'observed gaze',

'treatment session' and 'avoidance' was revealed ($F(1,24) = 8.24$, $p = .008$, $\eta^2 = .26$), indicating that the effect of OXT on the eye gaze effect was significantly modulated by attachment avoidance. In particular, the modulatory interaction with attachment avoidance indicated that while the facilitating effect of direct eye gaze on interpersonal motor resonance was not further augmented by OXT in participants with *low* attachment avoidance, a single dose of OXT was able to induce a significant augmentation of this eye gaze effect in participants with *high* attachment avoidance. **Fig. 3.B** visualizes the significant relationship ($r = .51$, $p = .008$) between attachment avoidance and the individual OXT treatment effect scores (individual Cohen's *d*, higher *d* scores indicate a stronger facilitation of the eye gaze effect by OXT).

Of note, the aforementioned ANCOVA analysis (with the inclusion of the 'attachment avoidance' regressor) also revealed a significant two-way interaction between 'eye gaze' and 'treatment session' ($F(1,24) = 7.20$, $p = .01$, $\eta^2 = .23$, medium effect). This indicates that – across all individuals – a significant OXT-induced augmentation of the eye gaze effect on interpersonal motor resonance was evident when variations related to inter-individual differences in attachment avoidance are regressed out (**Fig. 2**).

Note that no significant modulations of the 'eye gaze x treatment' interaction were revealed when any of the other SAAM or SRS subscales were inserted as continuous regressors. Accordingly, also no significant correlations were revealed between these person-dependent-factors and the individual OXT treatment effect scores (all $p > .07$), indicating that the modulatory effect was specific for attachment avoidance (see **Table D.1** in **appendix D**).

Together, these observations indicate that while participants with high attachment avoidance initially showed a reduced tendency to show enhanced interpersonal motor resonance upon direct eye contact at the PL session, a single dose of OXT was able to induce an augmentation of this effect, particularly for the participants high on attachment avoidance.

### 3.4. The effect of eye contact on gaze behavior and its modulation by oxytocin

Similarly to the analysis on the MEP data, a two-way repeated-measures ANOVA with the within-subject factors 'observed gaze' (direct gaze, averted gaze) and 'treatment session' (PL, OXT) was conducted on the total fixation time (in sec) towards the eye region of the model's face. A significant main effect of observed gaze was revealed (**Fig. 4**), indicating that across treatment sessions (PL or OXT) participants fixated significantly longer at the eye region of the face when the presented model displayed direct compared to averted eye gaze ($F(1,23) = 10.45$, $p = .004$, $\eta^2 = .31$).

Although the difference in gaze time between direct and averted gaze was larger in the OXT session (difference: 2.44 sec, Fisher LSD: $p = .002$), compared to the PL session (difference: 1.61 sec, Fisher LSD: $p = .03$), the interaction between 'observed gaze' and 'treatment session' was not significant ($F(1,23) = 0.67$, $p = .42$, $\eta^2 = .03$), indicating that across participants, the difference in gaze time between the direct and averted eye gaze condition was not significantly enlarged by OXT. Note that, when performing a similar ANCOVA as described for the MEP responses, the 'gaze x treatment' interaction effect on gaze behavior towards the eye region, albeit still not reaching statistical significance, became more pronounced by including 'attachment avoidance' as an additional regressor into the model ($F(1,22) = 2.28$, $p = .14$; $\eta^2 = .09$).

Further, we explored whether the effect of OXT on augmenting interpersonal motor resonance upon direct eye gaze (compared to averted gaze) was paralleled by an increase in gaze time towards the eye region during the direct eye gaze condition (compared to the averted gaze condition). Pearson correlation analyses between the individual OXT treatment effect on the MEP data (Cohen's $d_{\text{MEP-scores}}$) and the OXT treatment effect on the gaze time data (Cohen's $d_{\text{TFD-scores}}$) did not reveal a relationship between these measures (raw correlation: $r = .04$, $p = .84$). The relationship remained insignificant when variance related to attachment avoidance was regressed out (partial correlation: $r = .21$, $p = .34$).

### 3.5. Control static hand condition and EMG background

MEP-amplitudes recorded during the observation of the 'control' static hand condition (i.e., no movement observation) were not significantly modulated by eye gaze ($F(1,25) = .73$, $p = .40$, $\eta^2 = .03$). The effect of eye gaze during the control condition was also not significantly modulated by the administration of a single dose of OXT ('eye gaze' by 'treatment' interaction effect: $F(1,25) = 0.01$, $p = .91$, $\eta^2 < .01$) (see **Fig. E.1** in **appendix E**). No modulations by person-dependent factors (SAAM, SRS) were observed for the eye gaze effect or OXT treatment effect of the MEPs obtained for the control condition.

Furthermore, none of the reported effects on MEP responses were modulated by condition- or session-related differences in background EMG scores (all $p > .61$, see **Fig. E.2** in **appendix E**).

# 4. Discussion

The current study presents results of a double-blind, cross-over, randomized placebo-controlled trial assessing the immediate effects of OXT – a neuropeptide implicated in prosocial behavior – on an individual's tendency to 'synchronize with' or 'approach' an observed model displaying communicative intent (i.e. engaging in direct eye contact) or not (i.e. displaying averted gaze). Particularly, by using the non-invasive brain stimulation tool TMS, an objective neurophysiological index of a person's propensity to show interpersonal motor resonance in different situational contexts was assessed.

### 4.1. The effect of eye contact on motor resonance and its modulation by oxytocin

Similar to previous research (Prinsen et al., 2017; Wang et al., 2011a, 2011b), this study underlines the notion that the observed model's communicative intent provides a salient modulator of mirror-motor mapping, such that 'synchronization' during movement observation is higher when accompanied with mutual gaze between the observer and observed model. These observations support the notion that interpersonal motor resonance is not an isolated automatic process, but can be controlled by a hierarchical 'social top-down response modulation' mechanism (STORM) that is dependent on the social context in which others' actions are observed (Wang and Hamilton, 2012). In this view, instead of automatically simulating all possible movement-related information perceived in a visual scene, salient social cues (such as direct eye contact) may 'direct' the observer's motor system to preferentially process visuo-motor input originating from the most socially salient communicator.

With the current study, we also provide first neurophysiological evidence that a single dose of OXT was able to induce an augmentation of eye contact induced interpersonal motor resonance, specifically for participants high on attachment avoidance (as measured by the SAAM). Particularly, our data demonstrated that while participants with high attachment avoidance initially showed a reduced tendency to increase their interpersonal motor

resonance upon a salient social cue such as direct eye contact, a single dose of OXT was able to induce an augmentation of this effect.

### 4.2. Modulation of the treatment effect by person-dependent factors

While in more 'avoidant' individuals the presence of a social cue such as direct gaze did not unanimously result in enhanced interpersonal resonance (indicative of 'approach'), a single dose of OXT was able to promote the propensity of this otherwise 'avoidant' individual to engage in 'approach behavior' upon a communicative cue such as eye contact.

All in all, our data are in line with prior reports that the induction of prosocial effects by OXT may be more pronounced for individuals with low baseline levels of social proficiency or approach motivation (e.g. avoidantly attached individuals), whereas for individuals with already high baseline levels of approach motivational tendencies (e.g. securely attached individuals), the additional administration of exogenous OXT may not stimulate prosocial behavior further (Bartz, 2016). In a previous study by our lab, young adult men were administered with a daily dose of OXT for a period of two weeks, and significant improvements in self-reports of attachment avoidance (SAAM) and attachment toward peers (measured by the Inventory of Parent and Peer Attachment (IPPA; Armsden and Greenberg, 1987) were revealed (Bernaerts et al., 2017). Interestingly, and similar to the present study, the treatment-induced changes in the latter study were also found to be most pronounced for participants with less secure attachments. Likewise, Buchheim et al. (2009) found that, in insecurely attached adults, a single dose of intranasal OXT is sufficient to induce a significant increase in the experience of attachment security, as measured by the Adult Attachment Projective Picture System (AAP; George and West, 2001).

Aside the observation that treatment effects may be more pronounced in participants with low social proficiency or high attachment avoidance, recent accounts also highlight the possibility of reversed or anti-social effects of OXT for individuals with a high sensitivity towards rejection (e.g. anxiously attached individuals) (Bartz et al., 2015; Bartz et al., 2011).

For example, in individuals with borderline personality disorder OXT was shown to induce a *reduction* in the perception of trust or the likelihood to cooperate (Bartz et al., 2011). In the present study, a measure of inter-individual variation in attachment anxiety was obtained from the SAAM questionnaire, but based on the current sample no moderating effects were revealed  Future studies will however be necessary to address this issue further (e.g., by exploring moderating effects in a priori selected sample of participants with high attachment anxiety).

### 4.3. The effect of oxytocin on spontaneous gaze behavior

Although an increase in mutual gaze after administration of a single dose of OXT has been observed before (Auyeung et al., 2015; Guastella et al., 2008), we only observed a non-significant trend that OXT enhanced spontaneous gaze behavior towards the eye region of the observed model's face. Even though we observed that the effect of the OXT treatment on spontaneous gaze behavior was to some extent more pronounced when variance related to inter-individual differences in attachment avoidance was regressed out, we cannot draw any firm conclusions, since none of the effects of OXT on gaze behavior reached significance.

We would like to note however that, since the experimental design was prioritized for assessing the effects of OXT on interpersonal motor resonance as assessed with TMS, the eye tracking assessments of changes in spontaneous gaze behavior were only performed at the end of the experimental session, i.e. around 70 min post-administration. Although uncertainty exists with respect to the pharmacokinetics of OXT, heightened levels of peripheral OXT have repeatedly been observed until 75-90 min post-administration (Daughters et al., 2015; Gossen et al., 2012; Striepens et al., 2013). However, a more recent study suggested the most optimal time window to lie between 45 and 70 min (Spengler et al., 2017). While the timing of the TMS assessment largely overlapped with this time window, the possibility cannot be ruled out that – perhaps within a subset of individuals – the timing of the eye tracking session might have extended beyond the most optimal pharmacokinetic

time window to assess the single-dose effect of OXT, hence the observation of only tentative effects. To rule out this possibility of a timing effect on the assessed outcome measures, future studies might envisage adopting a randomized order (instead of a fixed order) for the included experimental assessments.

Despite this methodological consideration in terms of the adopted timing, there have been previous studies that were also not able to show a significant modulation of mutual gaze by OXT (Domes et al., 2010; Hubble et al., 2017; Lischke et al., 2012). Further research may therefore be necessary to establish the robustness of the effect of OXT on increasing spontaneous gaze behavior towards the eye region and the establishment of mutual gaze. Furthermore, considering the current observation of a tentative modulation by attachment avoidance, we recommend these future explorations to continue to take variations in person-dependent factors into account.

### 4.4. Relationship between motor resonance and gaze behavior

The encountered inter-individual variability in effects raises questions about the mechanism(s) by which OXT modulates approach behavior in general, and interpersonal motor resonance in particular. On the one hand, it can be suggested that OXT exerted these effects by increasing the 'saliency' of the presented social cue (eye gaze), which is in line with the social saliency hypothesis of OXT (Shamay-Tsoory and Abu-Akel, 2016). In this view, the demonstrated effect of OXT on enhancing socially adaptive motor resonance in avoidant individuals may have been related to OXT-related enhancements of overt viewing behavior towards the eye region of the model. However, although our study was not specifically designed to test this hypothesis, the obtained pattern of results suggests that the relationship between overt viewing behavior and interpersonal motor resonance may be more complex.

First, since the perception of cues in a presented scene may not be limited to the fixated area, the possibility cannot be ruled out that the modulation of the 'saliency' of the presented

social cues by OXT may extend beyond the overt fixated area i.e., involving peripheral vision. Furthermore, Myllyneva and Hietanen (2015) have shown that not continued mutual gaze per se, but rather the knowledge of being looked at by another person may be the pivotal factor in modulating responses to social stimuli. In their study, they manipulated participant's beliefs of whether or not they could be seen by a live person performing direct gaze sitting behind a liquid crystal shutter screen. Notably, only when participants merely believed that the person was able to see him or her through the shutter, enhanced autonomic arousal responses were observed. These results suggest that mental attributions, rather than overt visual attention, are important in modulating the processing of socially relevant information.

Of note, other studies that have not showed a significant modulation of mutual gaze by OXT did encounter OXT-induced improvements in a different measured variable of sociality; i.e. facial emotion recognition (Domes et al., 2010; Hubble et al., 2017; Lischke et al., 2012), suggesting that OXT-induced changes in social cognition can occur independently of modulations in overt visual attention.

Thus, while overt fixations towards the eye region may be equally high in different participants, it appears that the mental evaluation of the perceived eye contact may be considerably different. For some, direct eye contact may readily trigger an increased tendency to 'mirror' the other person, whereas for others, the perceived eye contact may be evaluated as being more unpleasant or intrusive and therefore elicit avoidant related responses (i.e., no increased tendency to mirror). In other words, we speculate that perhaps not the 'saliency' of the eye contact per se, but rather the perceived or evaluated 'approachability' of the presented social cue may have been modulated by OXT (although note the difficulty in strictly delineating these two constructs on a conceptual level).

# 5. Conclusion

To conclude, a single dose of intranasally administered OXT was shown to induce an augmentation of a person's propensity to engage in interpersonal motor resonance or 'approach behavior' upon a salient communicative cue such as direct eye contact, but only in individuals with high reports of attachment avoidance. These results provide neurophysiological support to the implicated role of OXT in modulating approach/avoidance motivational tendencies, and importantly, underscore that inter-individual differences in 'baseline' approach/avoidance tendencies can constitute an important moderating factor.

# 6. Role of the funding sources

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# 7. Conflicts of interest

The authors declare no conflict of interest.

# 8. Acknowledgments

The authors would like to thank all participating subjects and Inne Verwaest and Hanne Vanhaverbeke for their help in conducting the experiment.

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# 10. Tables

**Table 1**. Participants' characteristics.

| Measure | Mean ± SD |
|---|---|
| **Age (years; months)** | 24;4 ± 3;6 |
| **Social Responsiveness – SRS-A** | |
| Social Awareness | 7.50 ± 4.40 |
| Social Communication | 9.96 ± 6.04 |
| Social Motivation | 7.27 ± 4.06 |
| Rigidity and Repetitive Behavior | 7.81 ± 4.04 |
| **State Attachment – SAAM** | |
| Attachment Security | 6.05 ± 0.61 |
| Attachment Anxiety | 3.43 ± 1.42 |
| Attachment Avoidance | 1.96 ± 0.68 |

*Note.* SAAM = State Adult Attachment Scale; SRS-A = Social Responsiveness Scale, adult version. *N* = 26.

# 11. Figure legends

**Figure 1. A.** Overview of the clinical trail procedure and timing schedule. **B.** Factorial design: video stimuli showing a model performing a simple intransitive hand movement (hand opening) or no movement (static hand), accompanied with either direct or averted gaze. The last still of each video clip is depicted. **C.** Example of the timing of the TMS pulse during the direct-open condition. Single-pulse TMS was delivered approximately 4.6 seconds after the start of each video clip, which corresponded to the execution phase of the observed hand opening movement.

**Figure 2.** The effect of eye gaze on naturally log-normalized MEP amplitudes recorded during movement observation. Mean MEP's are displayed separately for each eye gaze condition (averted, direct) and treatment session (PL, OXT). Across treatment sessions, MEP amplitudes where higher when movement observation was accompanied with direct, compared to averted gaze. Although the difference between direct and averted gaze was more pronounced in the OXT session compared to the PL session, primary analysis revealed no significant interaction between 'eye gaze' and 'treatment'. However, when secondary analyses were performed – regressing out variability in treatment responses related to inter-individual differences in reports of attachment avoidance (SAAM) – a significant 'eye gaze' by 'treatment' effect was revealed, indicating an augmentation of eye contact induced interpersonal motor resonance after administration of OXT ($^{\dagger}p < .05$). Horizontal lines show median, boxes denote 25% – 75% of data and vertical lines denote non-outlier range.

**Figure 3.** Modulation by person-dependent factors. **A.** Relationship between inter-individual variations in self-reported SAAM attachment avoidance and the effect of eye gaze on interpersonal motor resonance at the baseline (PL) session. The facilitating effect of direct gaze on motor resonance (higher $MEP_{direct}$ – $MEP_{averted}$ difference scores) was more pronounced for participants with low attachment avoidance scores, compared to participants

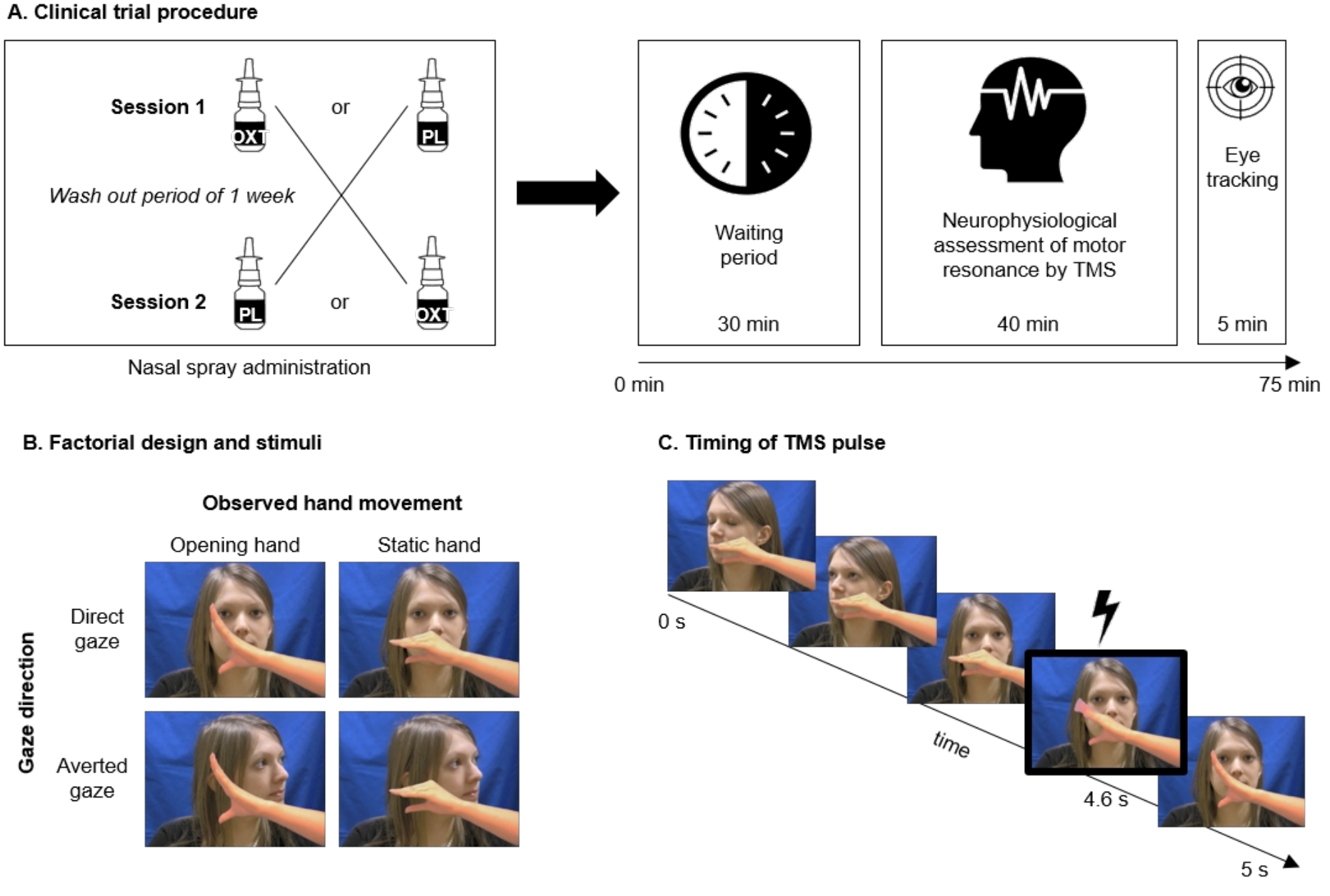
with high avoidance scores. **B.** Relationship between inter-individual variations in self-reported SAAM attachment avoidance and the treatment effect of OXT on interpersonal motor resonance (individual Cohen's *d* scores). The effect of direct eye gaze on motor resonance was further augmented by OXT for participants with high attachment avoidance, not for participants with low attachment avoidance (higher *d* scores indicate a stronger augmentation of the eye gaze effect by OXT).

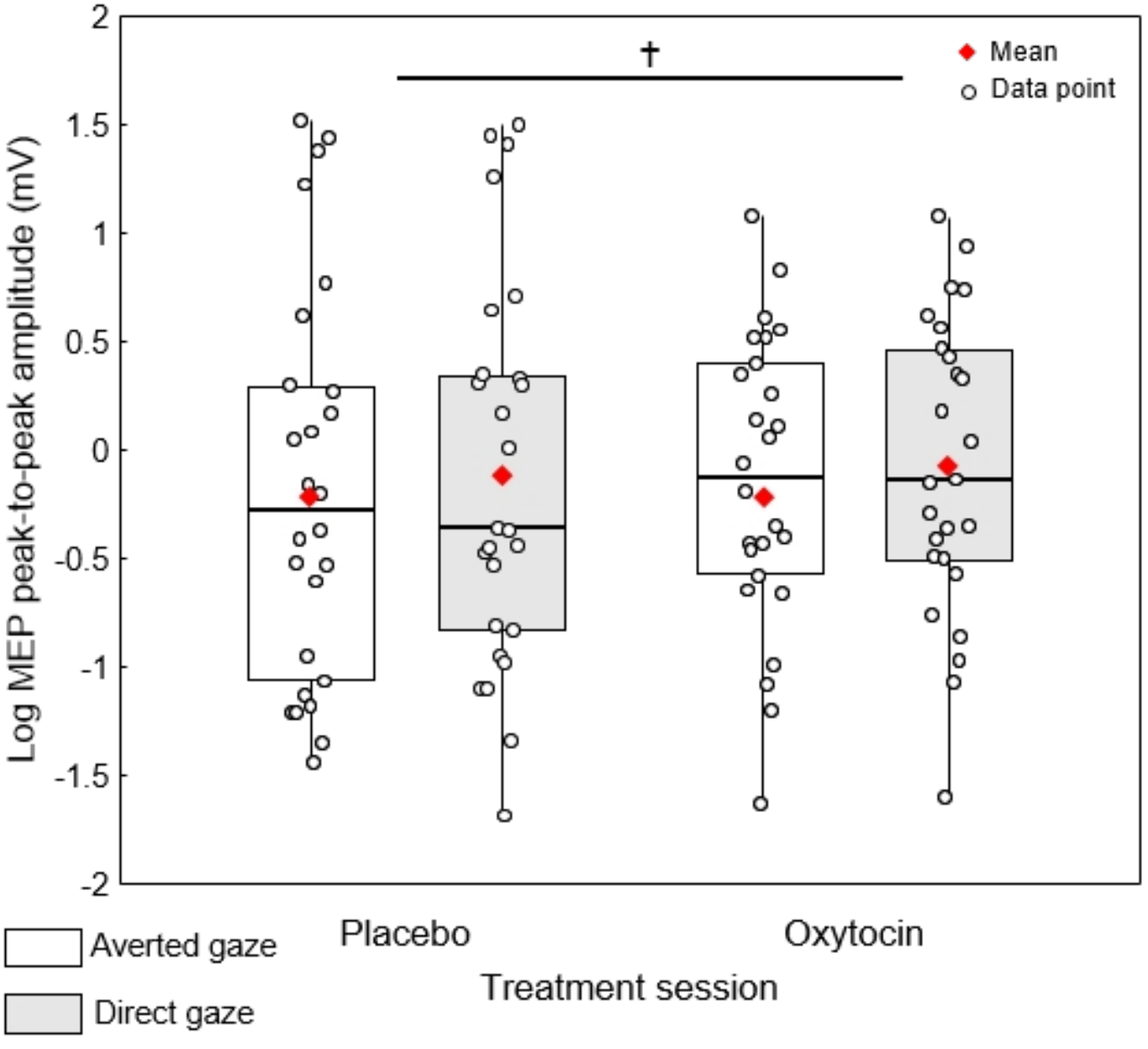
**Figure 4.** The effect of observed gaze direction on the total fixation duration towards the eye region of the model, separately for each treatment session. Participants fixated significantly longer at the eye region of the face when the presented model displayed direct compared to averted eye gaze. Although OXT enhanced spontaneous gaze behavior towards the eye region of the observed model's face (in the direct versus the averted gaze condition), the 'eye gaze' by 'treatment' interaction effect failed to reach statistical significance. Horizontal lines show median, boxes denote 25% – 75% of data and vertical lines denote non-outlier range.

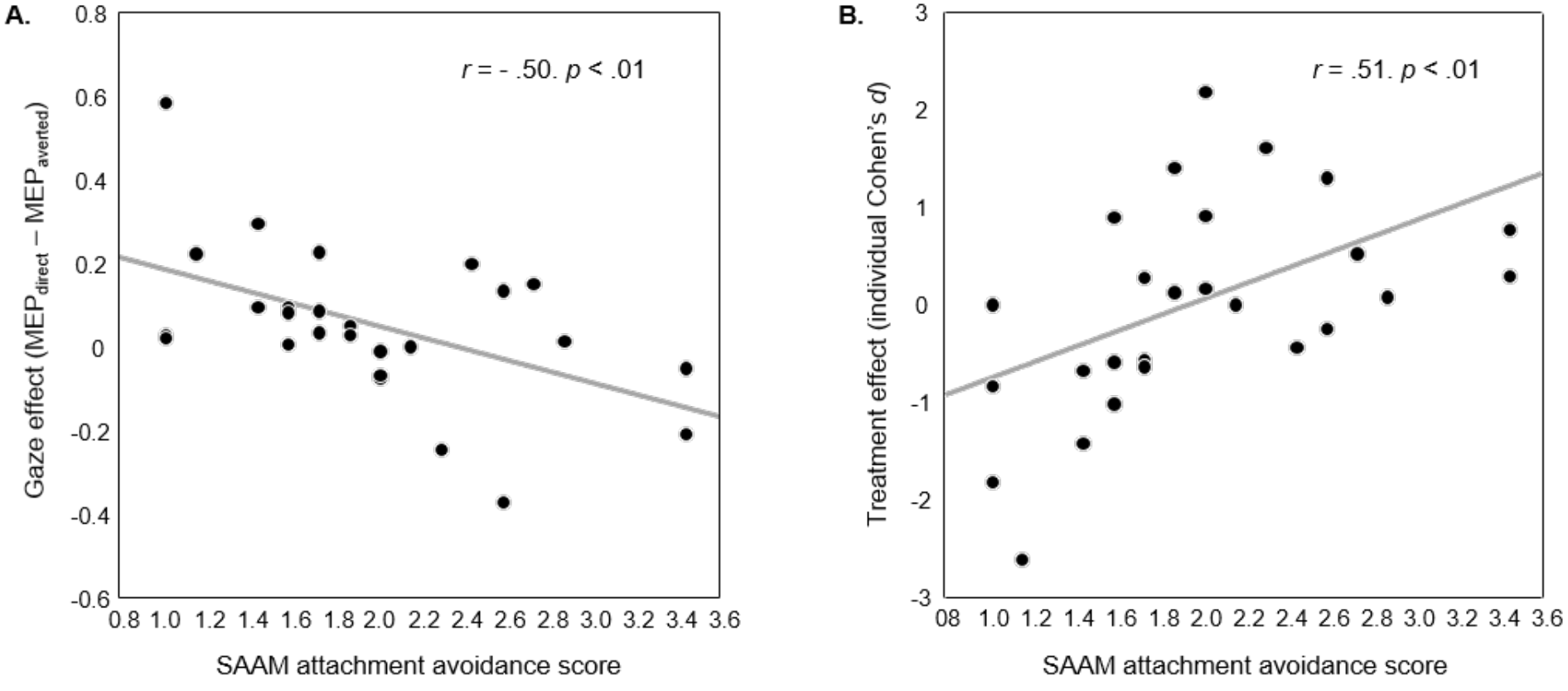
# Appendices

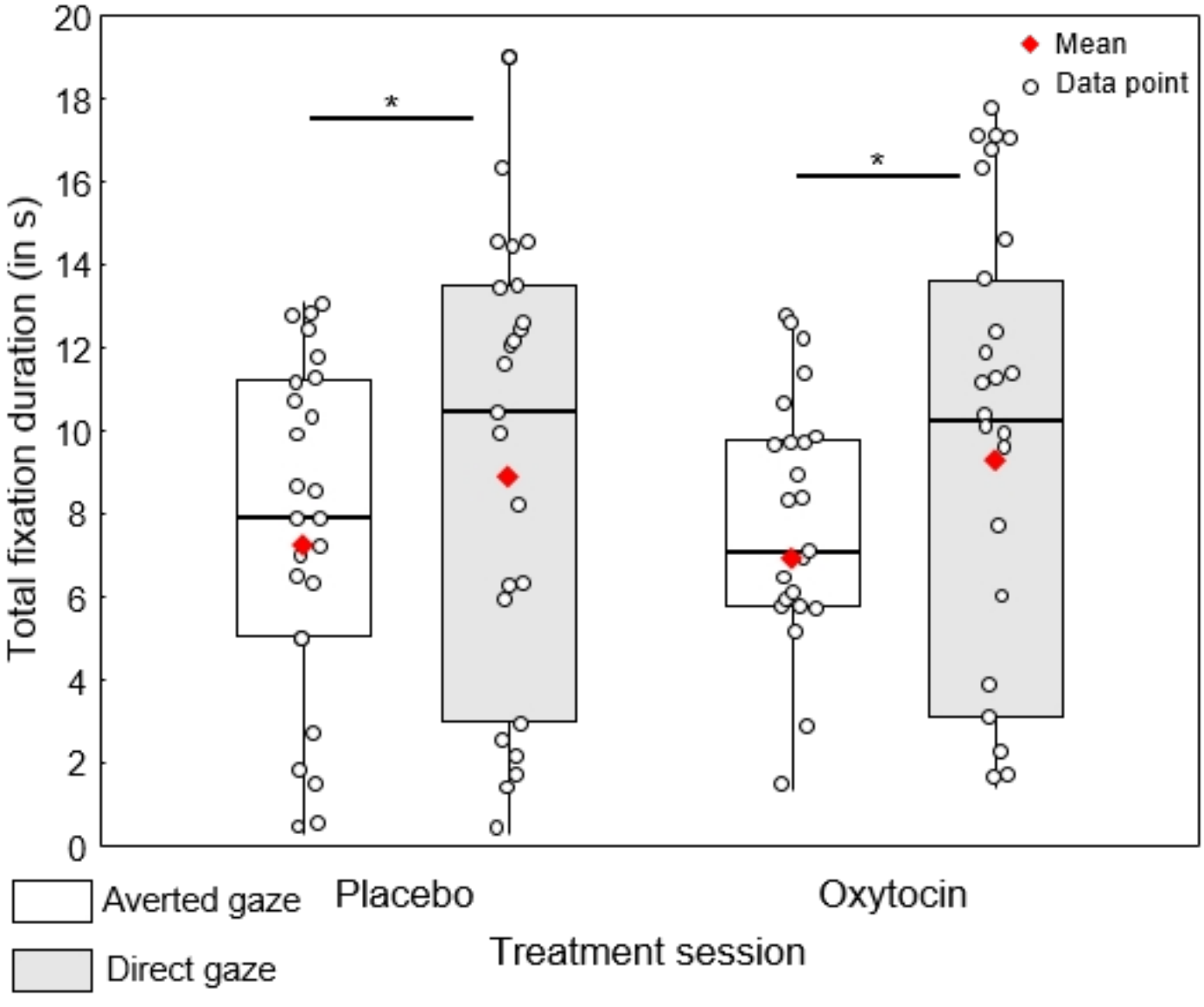
**Title: To mirror or not to mirror upon mutual gaze, oxytocin can pave the way: a cross-over randomized placebo-controlled trial.**

Authors: Jellina Prinsen, Stephanie Brams, Kaat Alaerts

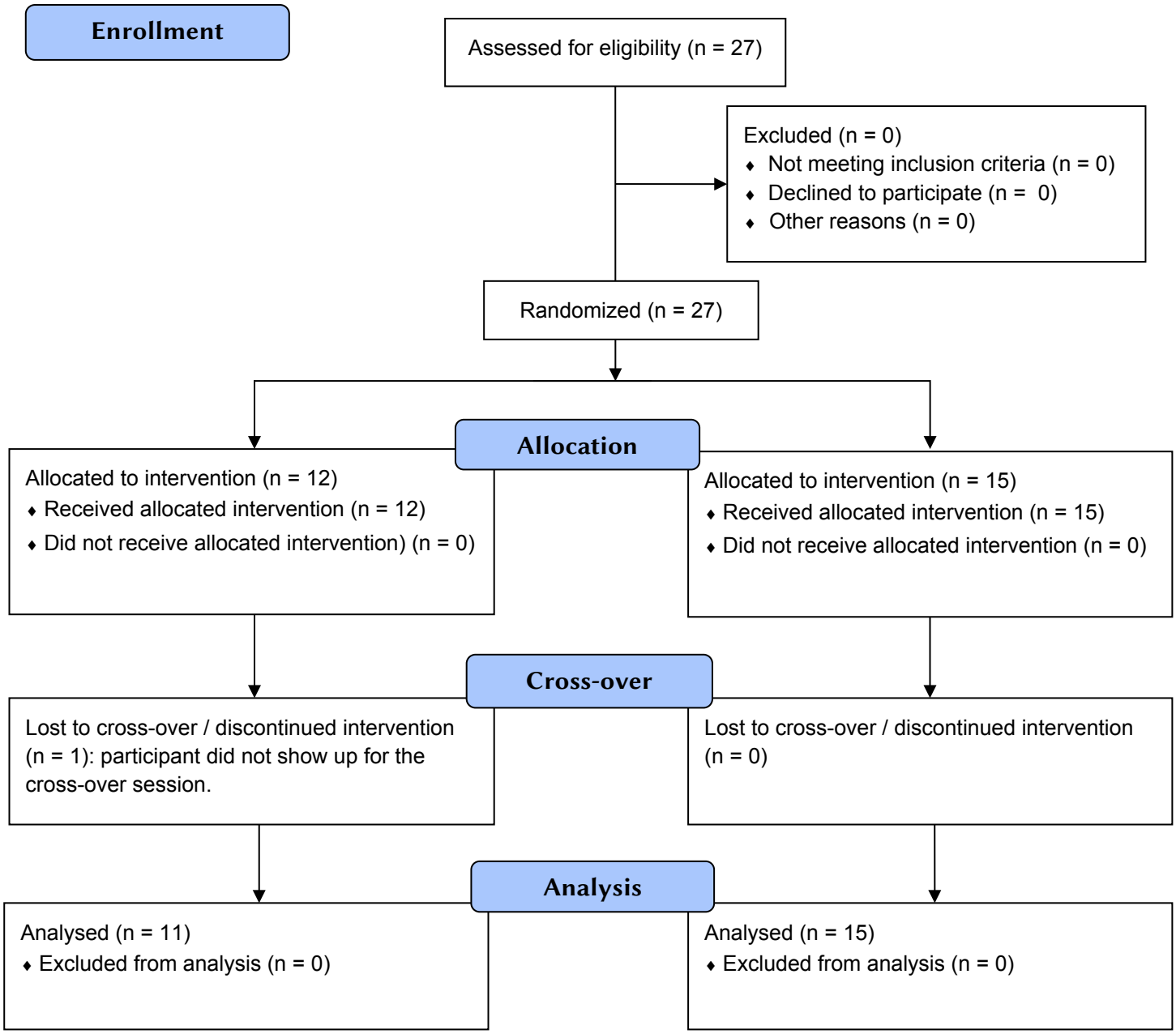

A. Clinical trial procedure
Session 1
OXT
or
PL
Wash out period of 1 week
Session 2
PL
or
OXT
Nasal spray administration
Waiting period
30 min
Neurophysiological assessment of motor resonance by TMS
40 min
Eye tracking
5 min
0 min
75 min
B. Factorial design and stimuli
Observed hand movement
Opening hand
Static hand
Gaze direction
Direct gaze
Averted gaze
C. Timing of TMS pulse
0 s
time
4.6 s
5 s


2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Log MEP peak-to-peak amplitude (mV)
†
Mean
Data point
Placebo
Oxytocin
Treatment session
Averted gaze
Direct gaze


A.
r = - .50. p < .01
Gaze effect (MEP direct – MEP averted)
SAAM attachment avoidance score
B.
r = .51. p < .01
Treatment effect (individual Cohen's d)
SAAM attachment avoidance score


Mean
Data point
Total fixation duration (in s)
20
18
16
14
12
10
8
6
4
2
0
*
*
Averted gaze
Direct gaze
Placebo
Oxytocin
Treatment session

**Appendix A.** CONSORT flowchart.



## **Appendix B.** Reported side effects

At the end of each experimental session (oxytocin and placebo), participants were asked to report whether they experienced any of the listed (or other) side effects and to indicate the severity of the side effect (mild, moderate, or severe). For each session, the number of participants that reported any mild, moderate or severe side effects are listed.

**Table B.1.** Frequency of reported side effects and severity.

| Side effect | Mild | | Moderate | | Severe | |
|---|---|---|---|---|---|---|
| | OXT | PL | OXT | PL | OXT | PL |
| Head ache | 2 | 2 | | | | |
| Drowsiness | 11 | 9 | 2 | 6 | | 1 |
| Dizziness | 1 | 1 | | | 1 | |
| Dry throat/dry mouth | 2 | 1 | | 1 | | |
| Congested nose | 1 | 1 | | | | |
| Sneezing | 1 | | | | | |
| Runny nose | 2 | 3 | 1 | | | |
| Muscle pain/cramps | | 1 | | | | |
| Sweating | 1 | 2 | | | | |
| Blurred vision | 1 | 1 | | | | |

*Note*. OXT = oxytocin, PL = placebo.

## **Appendix C.** Results POMS questionnaire.

A 32-item short version of the Profile of Mood States (POMS) questionnaire (Wald and Mellenbergh, 1990) was used as a measure of transient affective states in order to assess whether mood levels of participants changed over the course of the trial. This instrument comprises 32 emotional adjectives subdivided in five domains: anxiety (6 items), depression (8 items), vigor (5 items), fatigue (6 items) and anger (7 items) rated on a five-point Likert scale. For all participants, the POMS questionnaire was assessed at the start and end of each experimental session (i.e., pre- and post- administration of a single dose of nasal spray). No significant differences in mood states were revealed between the oxytocin and placebo treatment session (Wilcoxon matched pairs test, all $p > .18$).

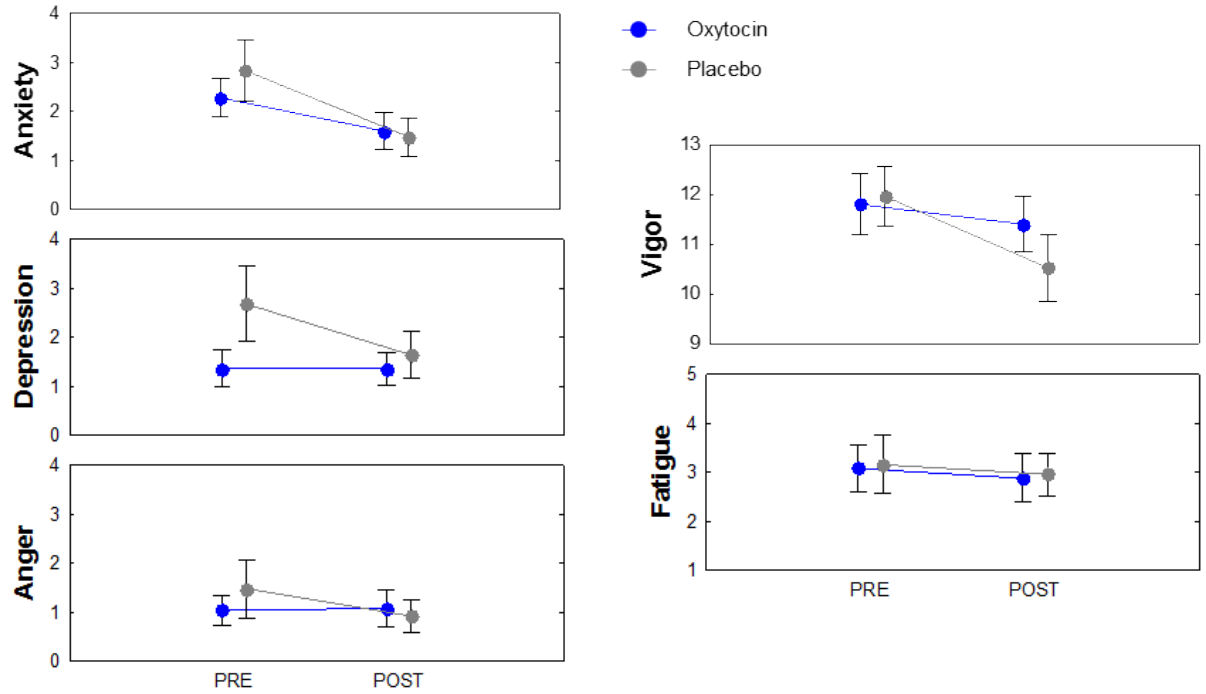



**Figure C.1.** Mean scores at the start and end of each experimental session (i.e., pre- and post-administration of a single dose of nasal spray) for each subdomain of the POMS questionnaire. Vertical bars denote mean ± SE.

**Appendix D.** Relationship between measures of sociality and OXT treatment effect.

**Table D.1.** Pearson correlation coefficients examining the relationship between self-reported social responsiveness (SRS-A) and attachment style (SAAM) on the extent by which treatment with a single dose of OXT modulated gaze-dependent interpersonal motor resonance (individual treatment effect scores; Cohen's *d*) (N = 26).

| | **Individual Cohen's *d*** | |
|---|---|---|
| **Measure** | ***r*** | ***p*** |
| **Social Responsiveness – SRS-A** | | |
| Social Awareness | .07 | .72 |
| Social Communication | .31 | .13 |
| Social Motivation | .36 | .07 |
| Rigidity and Repetitive Behavior | -.14 | .49 |
| **Attachment Style – SAAM** | | |
| Attachment Security | -.27 | .19 |
| Attachment Anxiety | -.06 | .77 |
| Attachment Avoidance | **.51** | **.008** |

*Note.* SRS-A = Social Responsiveness Scale, adult version; SAAM = State Adult Attachment Scale.

**Appendix E.** Control analyses on static hand condition and background EMG scores.

MEP-amplitudes recorded during the observation of the 'control' static hand condition (i.e., no movement observation) were not significantly modulated by eye gaze ($F(1,25) = .73$, $p = .40$, $\eta^2 = .03$). The effect of eye gaze during the control condition was also not significantly modulated by the administration of a single dose of OXT ('eye gaze' by 'treatment' interaction effect: $F(1,25) = 0.01$, $p = .91$, $\eta^2 < .01$) (**Fig. E.1**).

Background EMG was quantified by calculating the root mean square error (RMSE) across the 110 to 10 millisecond interval prior to TMS-stimulation. Since background EMG is known to modulate the size of MEP amplitudes, similar analysis were performed on the background EMG data. Across treatment sessions, the main effect of gaze was not significant, neither for the opening ($F(1,25) = 0.004$, $p = .95$, $\eta^2 < .001$) nor for the static hand ($F(1,25) = 0.16$, $p = .69$, $\eta^2 = .007$). The 'eye gaze' by 'treatment' interaction effects were also not significant, not for the experimental opening hand ($F(1,25) = 0.26$, $p = .61$, $\eta^2 = .01$), nor for the control static hand ($F(1,25) = 0.01$, $p = .91$, $\eta^2 < .001$) condition (**Fig. E.2**).

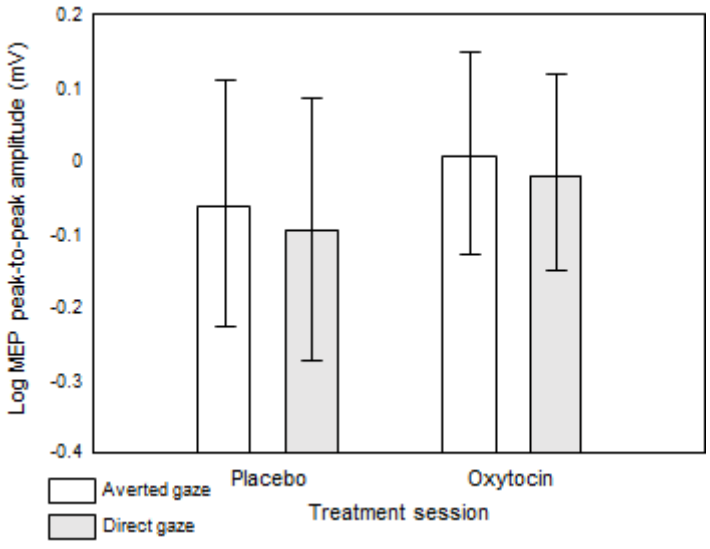



**Figure E.1.** The effect of eye gaze on naturally log-normalized MEP amplitudes recorded during the control static hand condition. Mean MEP's are displayed separately for each eye gaze condition (averted, direct) and treatment session (PL, OXT). No significant effects were encountered. Vertical bars denote SE ± mean.

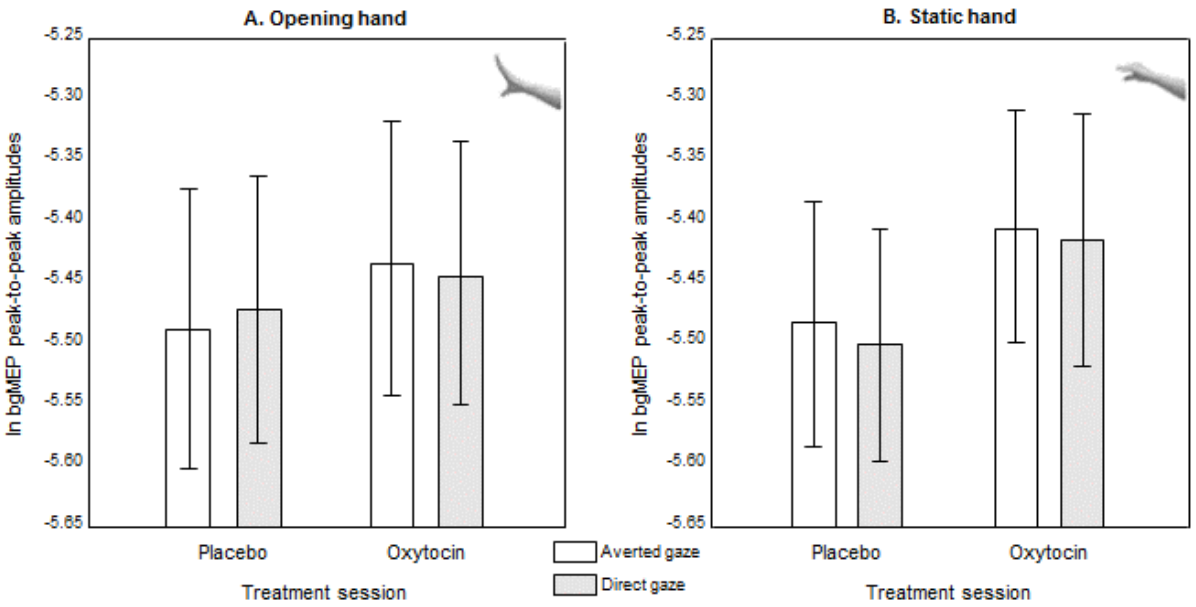



**Figure E.2.** The effect of observed eye gaze (averted gaze, direct gaze) and received treatment (placebo, oxytocin) on the naturally log-transformed background EMG scores, separately for each observed hand (opening hand, static hand). No significant effects were encountered. Vertical bars denote SE ± mean.

# CONSORT 2010 checklist of information to include when reporting a randomised trial *

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| **Title and abstract** | | | |
| | 1a | Identification as a randomised trial in the title | 1 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 2 |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 3-6 |
| | 2b | Specific objectives or hypotheses | 4-5 |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 6 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | / |
| Participants | 4a | Eligibility criteria for participants | 6 |
| | 4b | Settings and locations where the data were collected | 6 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 6-7 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 7-10 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | / |
| Sample size | 7a | How sample size was determined | 6 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | / |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | / |
| | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | / |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | / |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | / |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those | 7-8 |

| | | | |
|---|---|---|---|
| | | assessing outcomes) and how | |
| | 11b | If relevant, description of the similarity of interventions | 7 |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 11-12 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | 11-12 |
| **Results** | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome | Appendix A |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | Appendix A |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | / |
| | 14b | Why the trial ended or was stopped | / |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | 31 |
| Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | Appendix A |
| Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 13-17 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | / |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | 13-17 Appendix C - E |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | Appendix B |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 18-22 |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | 18-22 |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 18-22 |
| **Other information** | | | |
| Registration | 23 | Registration number and name of trial registry | 6 |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | 6 |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 23 |

* We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.

Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.