Title

Oxytocin enhances the recovery of eye-contact induced autonomic arousal: A treatment mechanism study with placebo-controlled design

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Abstract

The neuropeptide oxytocin (OT) is suggested to exert a pivotal role in a variety of complex human behaviors, including trust, attachment, social perception and fear-regulation. Previous studies have demonstrated that intranasal administration of OT reduces subjective and neuroendocrine stress responses and dampens amygdala reactivity. OT has also been proposed to modulate activity of the autonomic nervous system.

Here, a randomized double-blind, placebo-controlled study (with parallel design) was conducted with 56 healthy adult men to investigate whether a single-dose of OT (24 IU) modulates sympathetic autonomic arousal upon live dyadic gaze interactions. To do so, electrodermal recordings of skin conductance were performed during the engagement of eye contact with a live model in a two-person social context.

In accordance to prior research, direct eye gaze elicited a significant enhancement in skin conductance responses, but OT did not specifically enhance or dampen the overall magnitude (amplitude) of the skin conductance response. Administration of OT did facilitate the recovery of skin conductance responses back to baseline (reduced recovery time), indicating a role of OT in restoring homeostatic balance. Notably, the treatment-effect on autonomic recovery was most prominent in participants with low self-reported social responsiveness, indicating that person-dependent factors play an important role in determining OT treatment-responses. Exploratory, it was shown that OT also significantly reduced self-reported feelings of tension and (at trend-level) worrying about how one presents oneself.

Together, these observations add further evidence to a role of OT in modulating activity of the autonomic nervous system, primarily by facilitating a restoration of homeostatic balance after stimulus-induced increases in sympathetically-driven autonomic arousal.

Key words: Oxytocin, Autonomic nervous system, Skin conductance, Arousal, eye contact,

Introduction

The neuropeptide oxytocin (OT) has been implicated to exert a pivotal role in a variety of complex human behaviors, including trust, attachment, social perception and fear regulation. The neuropeptide is synthesized in the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus and through projections to the posterior pituitary gland, it is released to the bloodstream to act as a hormone and impact bodily functions. At the level of the brain, OT acts as an important neurotransmitter/ neuromodulator of a wide-range of complex social behaviors by impacting distinct subcortical and cortical brain structures through direct axonal projections (fast, focal) or continuous diffusion (slow, global) (Stoop, 2012).

Mechanistically, OT's impact on human brain function has been proposed to involve a bottom-up anxiolytic effect to facilitate social approach behavior; and a top-down social salience effect to facilitate attention to, and perception of social signals (Ma et al., 2016; Shamay-Tsoory & Abu-Akel, 2016). In terms of the social salience effect, several intranasal OT (IN-OT) administration studies have consistently shown that OT facilitates the recognition of emotional expressions from faces (Shahrestani et al., 2013; van IJzendoorn & Bakermans-Kranenburg, 2012). Several studies also showed that OT increases the gaze time towards the eye region of faces both from static pictures (Guastella et al., 2008; Eckstein et al., 2019) and during real-time interactions (Auyeung et al., 2015; Hall et al., 2012) (although see Hubble et al., 2017; Lischke et al., 2012; Prinsen et al., 2018 for contradictory results).

With respect to OT's anxiolytic role, several studies have demonstrated decreases in subjective reports of anxiety and reductions in neuroendocrine stress responses (cortisol) after IN-OT (Heinrichs et al., 2003; Meinlschmidt & Heim, 2007; Riem et al., 2019). The diverse neuromodulatory effects of OT have been attributed to be mediated at least partly by OT's influence on amygdala activation, a key neural structure of both fear-processing and (social) salience networks. For example, the seminal study by Kirsch et al. (2005) demonstrated that IN-OT induced an attenuation in amygdala activity during the processing of fearful or threatening visual images and a reduction in the coupling between amygdala and brainstem regions involved in autonomic functions. Initial studies examining the autonomic effects of OT demonstrated an attenuating effect of OT on electrodermal activity or skin conductance (SC), a widely used measure of physiological arousal driven solely by the sympathetic branch of the autonomic nervous system. For example, de Oliveira et al. (2012) demonstrated that IN-OT decreased subjective anticipatory anxiety as well as tonic skin conductance levels before and during a simulated Public Speaking Test (de Oliveira et al., 2012). Likewise, Vietnam veterans with post-traumatic stress disorder showed lowest mean skin conductance levels during personal combat imagery after receiving IN-OT (Pitman et al., 1993). Also associations between plasma OT levels and habituation in skin conductance responses (SCRs) during a trust game have been demonstrated, indicating faster habituation in individuals with higher OT levels (Keri & Kiss, 2011).

In contrast to these studies, Gamer et al. (2012) investigated the effect of OT on sympathetic (SCRs) and parasympathetic (heart rate changes) function during a facial emotion classification task, but only identified an effect of IN-OT on heart rate changes, not on sympathetically driven SCRs (Gamer & Buchel, 2012). Further, in a series of studies, Eckstein et al. (2015, 2016) demonstrated that while IN-OT enhanced the decline in SCRs during late phases of fear extinction (Eckstein et al., 2015), IN-OT

also strengthened Pavlovian fear conditioning during the acquisition phase by enhancing SCRs to fear-associated stimuli (Eckstein et al., 2016). As noted by the authors, these findings speak against a strong account of OT having purely 'arousal reducing' effects, but instead suggest that OT enables a rapid and flexible adaptation for the detection and processing of potential fear signals (Eckstein et al., 2016). Indeed, increases in skin conductance have also been linked to increased attention allocation and heightened perceptual processing (Frith & Allen, 1983). In particular, within the framework of the Orienting Response theory (OR theory), it has been proposed that stimuli with a high significance or importance to a person elicit higher orienting responses, as indexed using SCRs (Boucsein et al., 2012). Accordingly, within this framework, the IN-OT-induced enhancement of SCRs to fear-associated stimuli can be interpreted to reflect an effect of IN-OT on enhancing orienting responses or attention allocation to relevant fear-associated stimuli.

Recent meta-analytic reports of pharmaco-imaging studies have also highlighted a dual mode of action of OT at the level of the amygdala, with some studies showing increased activity after IN-OT, while others show decreased amygdala activity (Grace et al., 2018; Wang et al., 2017). As elegantly proposed by MacDonald et al. (2014), the seemingly dual action of OT on central and autonomic function during processing of social signals may stem from the ambiguous nature of social signals themselves, which in particular contexts, may signal reward, safety and pleasure, but in others, may signal competition, threat and stress (MacDonald & Feifel, 2014). Also evolutionary adaptations in perceiving and processing social cues, particularly eye-region related signals need to be considered to understand their ambiguous nature. For many animal species, a pair of watching eyes forms a highly salient 'threat' cue that reliably activates fear processing circuits and the sympathetic branch of the autonomic system, triggering defensive behavior or fighting responses (i.e. 'flight or fight'), as they most likely signal the presence of a dominant conspecific or predator (Emery, 2000; Skuse et al., 2003). For humans on the other hand, the engagement of direct gaze primarily constitutes an important cue for facilitating cooperative interaction and the formation of bonds as well as signaling one's own and inferring others' emotional states and social intentions (Grossmann, 2017). Still, also in humans, direct eye contact has been consistently shown to form a highly salient cue for eliciting increased sympathetic arousal, i.e., indicating higher SCRs upon direct, reciprocated gaze, compared to unreciprocated gaze (averted gaze or closed eyes) (Helminen et al., 2011; Hietanen et al., 2008; Prinsen & Alaerts, 2019; Prinsen et al., 2019). As outlined by Skuse et al. (2003), increased SCRs upon direct eye gaze have been shown to be predominantly driven by amygdala-centered subcortical circuits for facilitating attention allocation to and processing of the direct gaze cues. However, excessive sympathetic arousal responses (SCRs) upon direct eye contact have been identified in individuals with social anxiety disorders and children with autism spectrum disorder (ASD), indicating that ineffective regulation of eye-contact induced autonomic responses may form an important hallmark of neuropsychiatric conditions in which social function is compromised (Joseph et al., 2008; Kaartinen et al., 2012; Kylliainen et al., 2015).

To date however, it remains unexplored whether and how IN-OT modulates skin conductance responses during the processing of direct eye gaze cues. In the present study, we adopted a double-blind, randomized, placebo-controlled, between-subject design to examine potential modulations in skin conductance responses (both in terms of magnitude (amplitude) and recovery) induced by IN-OT

administration. In line with prior research, the eye contact stimuli were conveyed by a live model in a two-person social context, which has been shown to elicit stronger SCRs as compared to video recordings or pictures of faces (Hietanen et al., 2008; Ponkanen, Peltola, & Hietanen, 2011; Prinsen & Alaerts, 2019).

Considering prior reports of attenuated sympathetic arousal after IN-OT (e.g. reduced skin conductance levels during a public speaking task; de Oliveira et al., 2012) and in support of the anxiolytic, stress-reducing role attributed to OT, IN-OT can be hypothesized to *reduce* the SCR magnitude (amplitude) upon direct eye contact. However, considering that increased sympathetic drive is linked to attention allocation and heightened perceptual processing (in the framework of the OR theory), the possibility cannot be ruled out that IN-OT would induce an *increase* in SCRs upon direct eye contact (similar to Eckstein et al., 2016). This increase in SCRs would be indicative of a predominant effect of IN-OT on enhancing the salience and processing of the (socially relevant) eye contact stimulus.

In addition to characterizing SCR amplitudes, we also included a measure of SCR recovery, characterizing how fast the induced skin conductance response after stimulus presentation declines back to 50% of its initial level (50% recovery time). In line with the hypothesized role of OT in facilitating restoration of homeostatic balance after stress-induced perturbation (Stoop, 2012) and previous reports of enhanced decline in SCRs after IN-OT during late phases of fear extinction (Eckstein et al., 2015), we particularly aimed to investigate whether IN-OT potentially facilitates the recovery of the induced SCRs back to baseline. However, since the effect of IN-OT on SCR recovery has not been directly explored before, its investigation in the present study is considered exploratory.

In addition to the characterization of SCR amplitudes and recovery, also subjective reports of experienced arousal, valence and self-consciousness (during the presentation of direct eye contact) were obtained to explore whether OT-induced changes in SCRs are paralleled by changes in subjective experiences. Furthermore, considering recent notions that OT's treatment responses are susceptibly modulated by person-dependent factors (Bartz et al., 2015; Bartz et al., 2011), we explored whether inter-individual differences in self-reported social functioning (Social Responsiveness Scale) (Constantino et al., 2003) and attachment style (State Adult Attachment Measure, assessing dimensions of attachment security, avoidance and anxiety) (Gillath et al., 2009) potentially modulated identified treatment-responses.

Experimental procedures

Participants

A total of 56 healthy adults (all right-handed, Dutch speaking men) were recruited to participate in this double-blind, randomized, placebo-controlled study with parallel design assessing the effect of IN-OT on electrodermal recordings during the presentation of eye gaze stimuli. Due to technical difficulties, electrodermal (skin conductance) recordings were not available for six participants (3 oxytocin; 3 placebo), such that analyses were performed on a total of 50 participants (25 oxytocin, mean age 22.0 ± 3.20 years; 25 placebo, 22.4 ± 3.42 years). Only male participants were included to avoid the potential confounding gender-dependent effects of OT (Zink & Meyer-Lindenberg, 2012). Participants abstained from alcohol and caffeine 24-hours before testing. Written informed consent was obtained from all participants prior to the study. Consent forms and study design were approved by the UZ / KU Leuven Ethics Committee for Biomedical Research in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki, 2013). The study procedure was pre-registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT03272321). Note that participants were recruited to participate in a larger study, including additional neurophysiological recordings. Overall, the procedures outlined below for nasal spray administration and eye gaze stimuli are similar to those described in (Soriano et al., 2020).

Experimental procedure and stimuli

Participants were seated in front of a 20 × 30 cm voltage-sensitive liquid crystal (LC) shutter screen (DreamGlass Group, Spain) attached to a black frame separating the participant from the live model (similar setup as used in Prinsen et al., 2019). Participants were instructed to observe and pay close attention to the stimuli presented through the screen. The presented stimuli comprised the face of a live female model sitting with eyes open and gazing towards the participant (i.e. showing direct gaze and engaging in mutual eye contact) or sitting with eyes closed. During stimulus presentation, the model bore a neutral expression and kept her face as motionless as possible and tried to avoid eye blinks. Two female experimenters, trained to act similarly towards the participants, served as models. The models were unknown to the participants and only had a brief standardized interaction with the participant before stimuli presentation. For each participant, the same model was presented pre- and post-nasal spray administration. The participant was seated 60 cm in front of the shutter at the same height as the model's face with an overall distance between the participant and the model of 110 cm. Nexus Trigger Interface in combination with E-Prime software was used to trigger the liquid crystal window to shift from an opaque to a transparent state. Each stimulus (eyes open, eyes closed) was presented 10 times with duration of 5 seconds in a semi-random sequence (no more than three consecutive trials of the same type). During all trials, electrodermal recordings (skin conductance) were acquired. The inter-stimulus interval (ISI) varied with a minimum of 20 seconds to allow skin conductance responses to return to baseline. Each recording had a total duration of approximately 10 minutes, performed before (pre) and after (post) nasal spray administration.

Nasal spray administration

Participants were randomly assigned to receive a single dose of OT or placebo (PL) based on a computer-generated randomized order. Except for the manager of randomization and masking of drug administration, all participants and research staff conducting the study were blind to treatment allocation. In correspondence with previous studies (see Guastella & MacLeod, 2012 for a review), participants received 24 IU of OT (Syntocinon®, Sigma-tau) or placebo containing a saline natriumchloride solution. Participants received three puffs per nostril in an alternating fashion with each puff containing 4 IU. Participants were asked to first remove air present in the nasal spray by pumping the spray until a fine mist was observed. For inhalation of the spray, they were instructed to take a deep breath through the nose and to tilt their head slightly backwards during nasal administration in order to minimize gravitational loss of the spray.

Studies investigating OT concentrations in saliva (Daughters et al., 2015) and blood (Striepens et al., 2013) after a single dose of IN-OT have demonstrated reliable increases in peripheral OT levels approximately half an hour after intranasal administration. Consequently, in healthy humans, the impact of IN-OT on social cognition is commonly evaluated using a 30–45 min wait-time before initiation of the experimental task (see Guastella & MacLeod, 2012 for a review). Accordingly, in the current study, a 40-minute wait-time was incorporated prior to initiating the post-session recording, allowing to assess the effect of IN-OT on skin conductance during peak OT concentrations. All participants were monitored onsite until approximately one hour after nasal spray administration and were then screened for potential side effects. Overall, only minimal, non-treatment specific side effects were reported, although note that a larger proportion of participants receiving the OT spray reported a (mild) headache (OT group: 6 out of 28; PL group: 1 out of 27) (see **Supplementary Table 1**). Finally, at the end of the experimental session, participants were asked if they thought they had received OT or PL or whether they were uncertain about the received spray. The majority of participants reported to be uncertain about the received spray (OT: 48%; PL: 48%) or thought they had received the PL nasal spray (OT: 32%; PL: 40%). The proportion of participants that believed they had received the OT treatment was not significantly larger in the actual OT group (20%), compared to the PL group (12%) (Pearson Chi-square 0.60; p = .44).

Electrodermal recordings and data handling

The Nexus-32 multimodal acquisition system and BioTrace+ software (version 2015a, Mind Media, The Netherlands) were used to collect electrodermal recordings (skin conductance). Other neurophysiological recordings (e.g. electroencephalography) were performed simultaneously, but these measures are not part of the current report. Two Ag/AgCl Velcro snap-on electrodes were attached to the palmar surface of the distal phalanxes of the index and middle fingers on the participant's non-dominant hand to record stimuli-specific skin conductance responses (SCR) with a sampling rate of 128 Hz. No filtering or smoothing of the SC signal was applied. Before electrode attachment, the skin was prepared with electrode paste (0.5% saline in a neutral base) (MedCat, The Netherlands).

Stimuli-specific SCRs are typically characterized by a rise from initial level to a peak within 1-4 seconds after stimulus onset, followed by a recovery period of approximately 20–30 seconds in which the electrodermal signal declines back to baseline. For each trial, the baseline was determined as the

average of the two seconds right before stimulus onset. The **SCR amplitude** (measured in µS) was defined as the maximum change in skin conductance relative to baseline during the 5 seconds after stimulus onset. SCR amplitudes of trials with no response (change in skin conductance relative to baseline smaller than 0 µS) were set to zero. To assess **SCR recovery**, the 50% SCR recovery time was determined (SCR rec.t/2, in seconds), defined as the time it takes for the SCR amplitude to decline by 50% (i.e., determined only for trials with SCR > 0 µS) (Boucsein et al., 2012). Recovery times were recorded within the ISI of 20 seconds. Trials for which the 50% recovery was not reached by the end of the ISI, the SCR rec.t/2 was set at 20 seconds (6.5% of the trials).

Self-rated public self-awareness, arousal, valence and mood states

After the presentation of the live gaze stimuli, participants completed three items of the Situational Self-Awareness Scale (SSAS) (Govern & Marsch, 2001) (Dutch translation) to assess public self-awareness in response to seeing a face with direct gaze *("I was concerned about the way I present myself"*, *"I was self-conscious about the way I look"*, and *"I was concerned about what other people think of me"*). Responses were obtained on a visual analog scale from 1 = "completely agree" to 7 = "completely disagree". The answers were reverse scored such that higher scores correspond with more self-awareness. Further, two items of the Self-Assessment Manikin (SAM) (Bradley & Lang, 1994) were adopted to assess the participants' arousal and affective valence to seeing a face with direct gaze (9-point scale; 1 = calm/pleasant, 9 = arousing/unpleasant). Note that subjective ratings were not recorded for two participants (1 OT; 1 PL), such that analyses were performed on a total of 27 OT and 27 PL participants.

Participants also completed the 32-item short version of the Profile of Mood States (POMS) questionnaire (McNair & Lorr, 1964) at the start (before nasal spray administration) and end of the experimental session (post nasal spray administration) as a measure of transient mood levels in five domains: tension (6 items), depression (8 items), anger (7 items), fatigue (6 items) and vigor (5 items). The POMS comprises emotional adjectives for which subjects need to indicate to what extent the word fits their current mood using a five-point Likert scale from "not at all" to "very well". Note that reports of mood states were not recorded for three participants (all placebo), such that analyses were performed on a total of 28 OT and 25 PL participants.

Assessment of person-dependent factors

Prior to the experimental recordings, participants completed the Social Responsiveness Scale (SRS) (Constantino et al., 2003) and the State Adult Attachment Measure (SAAM) (Gillath et al., 2009). The 64-item Dutch adult self-report version of the SRS comprises four subscales examining social communication, social awareness, social motivation and rigidity/repetitiveness, using a four-point Likert-scale. The SAAM comprises three subscales examining attachment security (e.g., *"I feel like I have someone to rely on"*) (7 items); attachment anxiety (e.g., *"I feel a strong need to be unconditionally loved right now"*) (7 items); and attachment avoidance (e.g., "*If someone tried to get close to me, I would try to keep my distance"*) (7 items) using a seven-point Likert-scale.

Statistical Analysis

First, treatment-specific changes in skin conductance responses (SCR amplitude and SCR recovery) were explored. To do so, SCR amplitude and rec.t/2 data were analyzed trial-by-trial, by subjecting the trial-specific scores (10 trials per condition, at each session) to a linear mixed-effect model analysis with the random factor 'subject' and the fixed factors 'treatment' (OT, PL); 'time' (pre, post); 'condition' (eyes open, eyes closed) and trial (trial 1 to trial 10).

Next, self-reported scores of mood states (five states) and subjective ratings of eye contact (self-awareness, pleasantness, arousal) were also subjected to mixed-model analyses with the random factor 'subject' and the fixed factors 'treatment' (OT, PL) and 'time' (pre, post). Given the exploratory nature of these behavioral measures, the significance level for reporting treatment-specific effects was set at $p < .05$, without correction for multiple comparisons.

Finally, Spearman's r correlation analyses were performed to assess whether identified IN-OT treatment effects on SCR were potentially modulated by inter-individual differences in self-reported social functioning (SRS) or attachment style (SAAM).

All statistics were executed with Statistica 13 (StatSoft. Inc. Tulsa, USA).

Results

Effect of IN-OT on skin conductance

Skin conductance response - amplitude. In accordance to prior literature (Helminen et al., 2011; Hietanen et al., 2008; Prinsen & Alaerts, 2019; Prinsen et al., 2019), mixed-model analysis of the SCR amplitude data revealed a main effect of 'condition' ($F(1,1872)= 16.88$; $p< .0001$), indicating that across trials, sessions and treatment groups, SCR amplitudes were higher during the direct eye gaze condition, compared to the eyes closed condition (**Figure 1A**). Also a 'time x condition' interaction effect was revealed ($F(1,1872) = 3.97$; $p = .046$), indicating that the condition effect (eyes open > eyes closed) was most pronounced at the pre-session ($F(1,1872) = 18.61$; $p < .0001$), compared to the post-session ($F(1,1872) = 2.24$; $p = .13$).

Significant main effects were also revealed for the factors 'time' ($F(1,1872) = 55.04$; $p < .0001$) and 'trial' ($F(9,1872) = 12.60$; $p < .0001$), indicating that in both treatment groups, SCR amplitudes significantly declined from the pre- to the post session; and from the first to the last trial. A significant 'time x trial' interaction ($F(9,1872) = 7.44$; $p < .0001$) indicated that the pre-to-post reduction in SCR amplitudes was more pronounced for the first trials, compared to the last trials (see **Figure 1B**, visualizing pre-to-post changes in SCR amplitudes trial-by-trial).

With respect to the effect of IN-OT, no significant 'treatment x time' ($F(1, 1872) = .036$; $p = .85$) or 'treatment x time x condition' interaction were revealed ($F(1,1872) = .04$; $p = .84$), indicating that pre-to-post changes in SCR amplitudes were not significantly different between the OT and PL group (**Figure 1A and B**). Also no main effect of treatment ($F(1,48) = .15$; $p = .70$) or other interactions with the factor 'trial' were revealed (i.e., 'treatment x time x trial' ($F(9,1872) = .75$; $p = .66$); 'treatment x time x condition x trial' ($F(9,1872) = 0.65$; $p = .76$)).

Skin conductance response - recovery time (SCR rec.t/2). Similar to the SCR amplitude data, mixed-model analysis of the SCR rec.t/2 data revealed a main effect of 'condition' ($F(1, 1026) = 23.90$;

$p < .0001$), indicating that across trials, sessions and treatment groups, SCR recovery times were longer for the direct eye gaze condition, compared to the eyes closed condition (**Figure 2A**). The main effects of 'time' ($F(1, 1026) = .73$; $p = .39$) or 'trial' ($F(1, 1026) = .55$; $p = .84$) were not significant.

Notably however, a significant 'treatment x time x condition' interaction was revealed ($F(1, 1026) = 5.44$; $p = .019$) (**Figure 2A**), indicating a significant 'treatment x time' interaction for the direct eye gaze condition ($F(1, 1026) = 3.93$; $p = .047$), but not for the eyes closed condition ($F(1, 1026) = 0.27$; $p = .60$). Compared to PL, OT administration induced a faster recovery (faster decline) of the eye-contact induced SCR back to baseline (**Figure 2A**).

No main effect of treatment ($F(1,48) = 2.66$; $p = .11$) or other interactions with this factor were revealed (i.e., 'treatment x time' ($F(1,1026) = .51$; $p = .47$); 'treatment x time x trial' ($F(9,1026) = 1.09$; $p = .36$); 'treatment x time x condition x trial' ($F(9,1026) = 1.43$; $p = .17$). In **Figure 2B**, pre-to-post changes in SCR recovery time are displayed trial-by-trial. As visualized, the IN-OT treatment effect in the eyes open condition (stronger pre-to-post reduction in recovery time) was evident in most trials, albeit most pronounced at the first trial.

Exploratory assessments of the effect of IN-OT on self-rated mood, public self-awareness, arousal and valence

A significant effect of 'treatment x time' interaction was identified for the mood state 'tension' (assessed with the Profile of Mood States: POMS) ($F(1, 51) = 4.51$; $p = .038$), indicating a pre-to-post reduction in reported feelings of tension in the OT group, compared to the PL group (**Figure 3A**). No significant 'treatment x time' interaction effects were identified for the other POMS mood states (depression, anger, fatigue, vigor) (all, $p > .30$) (see **Table 1**).

A 'treatment x time' interaction effect was evident at trend-level in terms of public self-awareness (assessed with the Situational Self-Awareness Scale; SSAS) ($F(1, 52) = 3.54$; $p = .065$), indicating a relative pre-to-post reduction in self-awareness (less concerned about themselves) after OT administration, compared to PL administration (**Figure 3B**). No treatment-specific pre-to-post changes were evident in terms of self-rated arousal and ratings of valence (pleasantness) (all, $p > .24$) (see **Table 1**).

Modulation of IN-OT effect by variations in person-dependent factors

The effect of IN-OT treatment on SCR recovery (SCR rec.t/2) was modulated by person-dependent factors, indicating that participants of the IN-OT group with higher self-reported scores on the Social Responsiveness Scale (SRS) (more impairment) showed a faster recovery (shorter recovery time) after IN-OT treatment (Spearman $R_{(25)} = -.48$; $p = .015$) (**Figure 4**). Split-group analyses confirmed that pre-to-post reductions in SCR recovery after IN-OT were more pronounced in subgroups with high, compared to low SRS scores based on median-split ($t(23) = 2.01$; $p = .056$) or upper/lower quartiles ($t(11) = 2.86$; $p = .016$) (see **Supplementary Figure 1A-B**). Note that at the pre-session assessment, no significant association was evident between SRS scores and SCR rec.t/2 ($R_{(25)} = -.14$; $p = .48$), indicating that participants with low SRS scores did not already display shorter recovery times at the pre-session.

No significant associations were revealed between the IN-OT treatment-effect on SCR recovery and inter-individual variance in SAAM attachment (security, avoidance or anxiety) (all, $p > .15$). Also no significant associations were revealed between pre-to-post changes in SCR recovery and person-dependent factors in the PL group (all $p > .20$).

Discussion

Sympathetically-driven autonomic arousal upon direct eye contact was shown to be modulated by IN-OT, indicating a faster recovery of skin conductance responses (SCR) back to baseline (reduced recovery time) after IN-OT, but not a treatment-specific effect on the evoked SCR amplitude per se. Notably, the treatment effect on SCR recovery was most prominently observed in participants with low self-reported social responsiveness (higher total scores on the Social Responsiveness Scale; SRS). IN-OT was also shown to induce a general reduction in self-rated 'public self-awareness' and feelings of 'tension' (Profile of Mood States; POMS).

Prior IN-OT studies consistently demonstrated reductions in tonic skin conductance levels during a public speaking test (de Oliveira et al., 2012) and during personal combat imagery in Vietnam veterans (Pitman et al., 1993). In terms of IN-OT effects on phasic SCRs, a more mixed pattern of results was revealed; with one study reporting decreases in SCRs (during late phases of fear extinction (Eckstein et al., 2015)), while other studies reported increases in SCRs (during the acquisition phase of fear conditioning; Eckstein et al., 2016), or no significant changes in SCRs (during a facial emotion classification task) (Gamer & Buchel, 2012). While direct comparisons between studies are difficult, the present identification of no significant IN-OT effects on SCR amplitudes upon presentation of direct eye contact stimuli (presented using a live model), seems to be largely in line with the prior study by Gamer et al. (2012), demonstrating no effect of IN-OT on SCR amplitudes during a facial emotion task (presented with static pictures).

The present study included an additional assessment of the effect of IN-OT on the recovery of induced SCRs, and identified that the SCR 50% recovery time was reduced after IN-OT, indicating a faster decline of the skin conductance response back to baseline levels. The obtained pattern of results can be interpreted within the proposed dual action account of OT, implying both a top-down social salience effect (facilitating attention to, and perception of social signals), combined with a bottom-up anxiolytic effect. Firstly, the lack of a treatment-specific effect on SCR amplitudes may imply that the overall attention allocation to and perception of socially-salient signals such as the eye contact cue was not specifically diminished (or augmented) after IN-OT. However, the identification of a significant IN-OT on SCR recovery provides initial indications that IN-OT may primarily promote the restoration of 'stressor-induced' arousal responses, rather than impacting on the overall magnitude of evoked arousal responses per se.

Rodent research showed that both OT and its closely related neuropeptide vasopressin (VP) play an important antagonistic role in alertness and homeostasis, indicating that while VP primarily increases alertness and sympathetic drive, OT may primarily enhance parasympathetic outflow (Stoop, 2012). In line with this notion, the present pattern of results provides indications that OT may not directly impact sympathetic arousal responses per se, but instead facilitates recovery to initial baseline arousal states, presumably by enhancing autonomic homeostatic output after (stress-induced) perturbation. While speculative, it can be anticipated that by reducing the recovery time of the SCRs, OT may also indirectly contribute to enhancing the signal strength of the stimulus (albeit without impacting on the SCR amplitude per se). As outlined in the Orienting Response theory, stimuli with a high significance or importance are anticipated to elicit higher SCRs for facilitating attention allocation and processing

(Boucsein et al., 2012). It is however well-known that fast presentations of successive stimuli (with short inter-stimulus-intervals) considerably attenuate subsequent SCR amplitudes due to an incomplete recovery of the previous SCRs. A rapid restoration of the signal back to baseline may therefore form an efficient strategy for maintaining signal value, especially when a series of stimuli are to be processed successively. In this view, albeit speculative, by enhancing the recovery of the SCR signal back to baseline, IN-OT may reduce the level of residual noise after induction of the SCR peak, thereby augmenting the overall signal strength/ salience of to-be-processed stimuli. In the current study, relatively long inter-stimulus-intervals (ISI of 20 seconds) were adopted, allowing the SCR to sufficiently decline back to baseline in-between successive stimuli. However, it can be hypothesized that in designs with shorter ISI's, a facilitating effect of OT on speeding recovery time may be instrumental for maintaining larger orienting responses (SCR amplitudes) to the (socially relevant) eye contact stimuli.

In addition to the effect on SCR recovery, exploratory analyses showed that IN-OT additionally induced a relative pre-to-post reduction in self-rated feelings of public awareness and feelings of tension. A previous study from our lab (Bernaerts et al., 2017) also adopted the POMS to evaluate the effects of a two-week multiple-dose IN-OT treatment in neurotypical men, and similarly showed OT-specific reductions in feelings of tension (and also anger). The POMS was also adopted in a recent study evaluating the effects of a four-week IN-OT treatment in adult men with autism spectrum disorder (Bernaerts et al., 2020), and while here, no treatment-specific changes in tension were detected (both groups reported improvements), the POMS revealed OT-specific enhancements in feelings of vigor (feeling more "active", "energetic", "lively").

Notably, the treatment effect on SCR recovery was most prominently observed in participants with low self-reported social responsiveness (higher total scores on the SRS). These observations are generally in line with the notion that IN-OT treatment responses 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/ more socially proficient individuals), the additional administration of IN-OT may not stimulate prosocial behavior further (Bartz et al., 2015). In line with the current observation of a modulatory impact of social responsiveness, Bartz et al. (2010, 2019) showed that OT was able to improve empathic accuracy on an emotion recognition task, but only for less-socially proficient individuals (Bartz et al., 2019; Bartz et al., 2010). Together, these previous studies and our study add to the growing body of evidence that inter-individual differences in (baseline) person-dependent factors may play a pivotal role in determining IN-OT treatment responses. In the current study, however, attachment style as assessed with the SAAM (State Adult Attachment Measure) was not shown to significantly modulate treatment responses, which is at odds with some prior studies identifying SAAM attachment to be a sensitive modulator of IN-OT treatment responses (Bernaerts et al., 2017; Prinsen et al., 2018). For example, significant improvements in self-reports of attachment avoidance (SAAM) and attachment toward peers were shown after a two-week treatment with IN-OT, and these treatment-induced changes were found to be most pronounced for participants with less secure attachments (Bernaerts et al., 2017). Also in an earlier study by De Dreu et al (2012), IN-OT

administration was shown to significantly improve cooperation behavior, but particularly in individuals scoring high on attachment avoidance.

While the current study provides important new insights into the effect of IN-OT on eye-contact induced autonomic arousal, several limitations need to be considered. Since only neurotypical men were included, the current observations of beneficial effects on SCR recovery, self-awareness and feelings of tension cannot be generalized to women. Especially with regard to the adopted live eye gaze stimuli, this is an important issue, considering that prior studies showed that both the participant's and the model's gender may influence gaze processing (Ponkanen et al., 2011; Slepian et al., 2011). For example, Pönkänen et al. (2011) showed that for female participants, a significant effect of eye contact on autonomic arousal was only evident for viewing female, but not male faces. Since the investigation of gender differences was beyond the scope of the present study, future research is warranted to systematically assess whether gender (of the participant and model) impacts the effect of IN-OT on eye-contact induced arousal. Prior IN-OT administration studies have demonstrated increases in ratings of trustworthiness and attractiveness in judgments of both men and women (by either men or women) (Theodoridou et al., 2009; but see Lambert et al., 2014), as well as ratings of attractiveness of romantic partners (Scheele et al., 2016; Scheele et al., 2013; Hurlemann et al., 2017). In light of these observations, it would also have been interesting to explore whether the observed changes in autonomic arousal responses after IN-OT were possibly related to changes in perceived trustworthiness or attractiveness. In this respect, the observed reductions in feelings of public self-awareness and tension in the IN-OT group may indeed provide indications that participants felt more comfortable in the presence of the model, possibly due to an increase in perceived trustworthiness.

Further, in the current study, a fixed dose of 24 IU was adopted, at a dose-response latency of 40 min, which is in accordance to prior studies investigating the effects of IN-OT on social cognition (Guastella & MacLeod, 2012). It cannot be ascertained however that a similar pattern of results would have emerged at a different dose or dose-response latency. In particular, in a prior study investigating a range of doses, reductions in amygdala responses to fear were shown to be most pronounced 45 minutes after a 24-IU dose of IN-OT, but administrations of higher doses (48 IU) were shown to induce opposite effects (i.e., indicating an increase, rather than a decrease in amygdala response) (Spengler et al., 2017). Indeed, it has been suggested that at higher (supraphysiological) doses, OT may occupy vasopressin receptors and therefore produce more vasopressin-like effects (e.g. increasing amygdala reactivity and/or alertness) (Spengler et al., 2017). In this view, the possibility cannot be ruled out that the observed IN-OT induced modulations of skin conductance responses (recovery) will be inversely affected when more high-range doses (> 24 IU) are adopted.

Finally, SCR data were analyzed trial-by-trial, showing a general decrease in SCR amplitudes, irrespective of treatment group, i.e., indicating no treatment-specific modulation of SCR amplitude decreases over time. It should be noted however, that the design of the study was not prioritized to explicitly investigate the effect of IN-OT on habituation over trials. Previously, Keri and Kiss (2011) showed that increases in endogenous OT release during a trust-related social situation (measured in blood plasma) enhanced the habituation of SCRs to a series of auditory tones (Keri & Kiss, 2011). In this view, it would be interesting for future studies to address the effect of IN-OT on habituation to eye

contact cues more explicitly, e.g., by presenting a series of ‘direct eye gaze’ trials without interleaving of ‘eyes closed’ trials.

To conclude, while IN-OT did not specifically enhance or dampen the overall magnitude (amplitude) of sympathetic arousal responses, administration of OT was shown to facilitate the recovery of skin conductance responses back to baseline (reduced SCR recovery time). Together, these observations provide important evidence that OT plays a pivotal role in modulating autonomic arousal responses, i.e., by facilitating restoration of homeostatic balance after (social) stress-induced perturbation.

Figure Legends

Figure 1

Effect of oxytocin on skin conductance amplitude.

In **panel A**, skin conductance response (SCR) amplitude scores are visualized before and after nasal spray administration (pre, post), separately for each group (oxytocin, placebo) and condition (eyes open, eyes closed).

In **panel B**, pre-to-post changes in SCR amplitudes are visualized trial-by-trial. Negative scores indicate a pre-to-post reduction in SCR amplitude.

Vertical bars denote +/- standard errors.

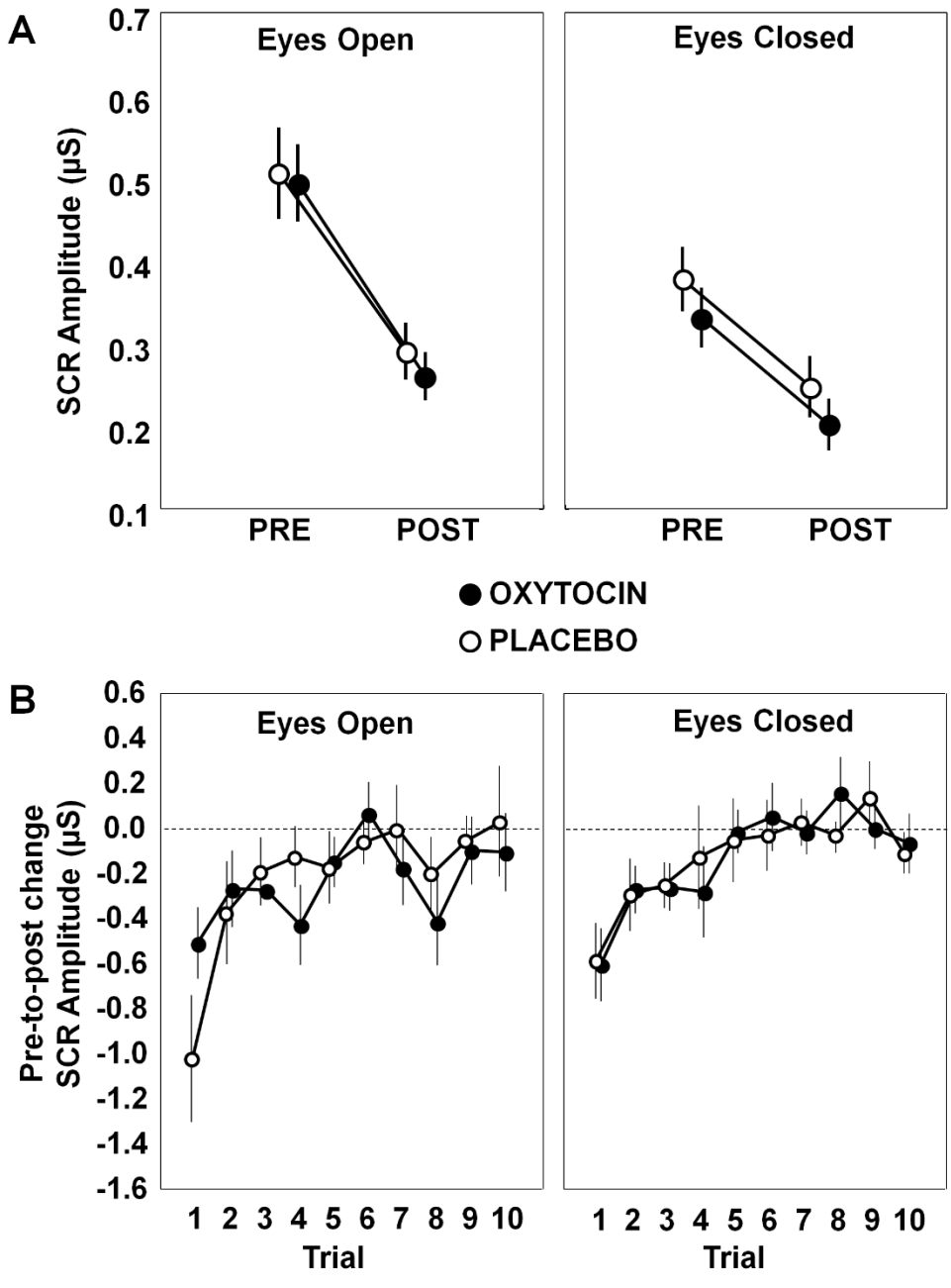


Figure 2

Effect of oxytocin on skin conductance recovery.

In **panel A**, skin conductance response recovery time scores (SCR rec. t/2) are visualized before and after nasal spray administration (pre, post), separately for each group (oxytocin, placebo) and condition (eyes open, eyes closed).

In **panel B**, pre-to-post changes in SCR recovery time are visualized trial-by-trial. Negative scores indicate a pre-to-post reduction in SCR recovery time (faster recovery).

Vertical bars denote +/- standard errors.

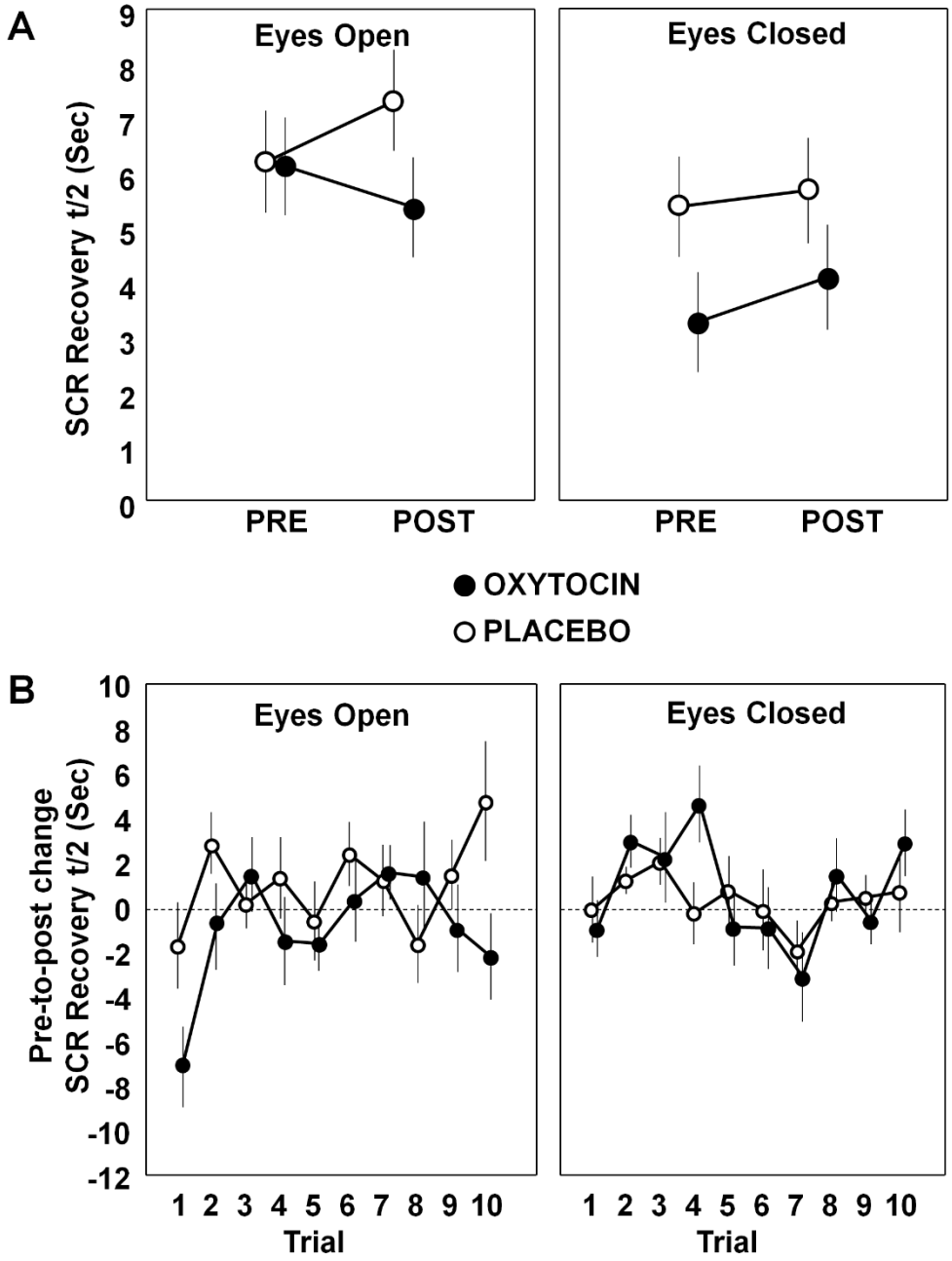


Figure 3

Effect of oxytocin on self-rated mood and public self-awareness. For each treatment group (oxytocin, placebo), self-rated 'tension' (assessed with the Profile of Mood States: POMS) (**A**) and public self-awareness (assessed with the Situational Self-Awareness Scale: SSAS) (**B**) are visualized separately for each assessment session (pre, post). Vertical bars denote +/- standard errors.

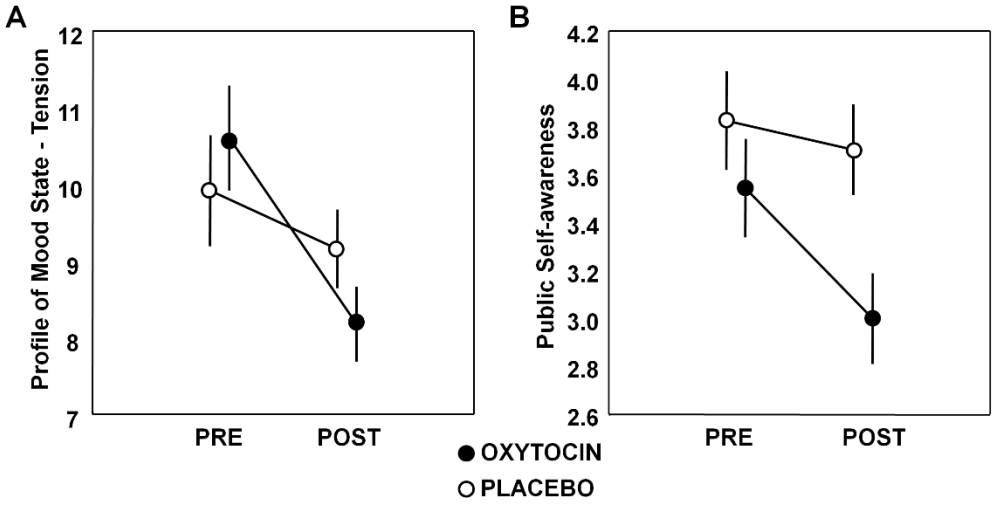
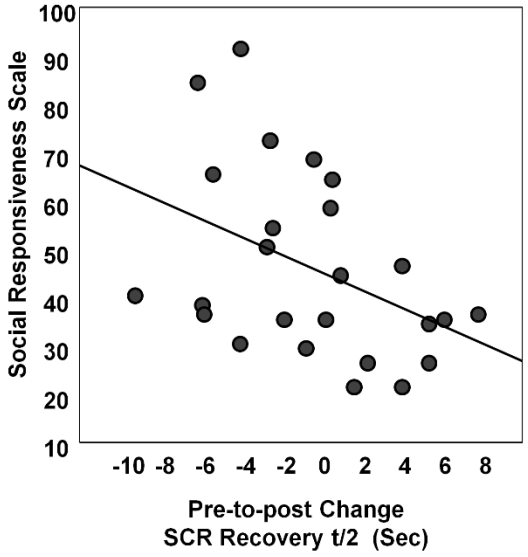


Figure 4

Modulation by person-dependent factors. Visualization of the relationship between scores on the Social Responsiveness Scale (SRS, self-report) and oxytocin-induced pre-to-post changes in skin conductance recovery time (rec. t/2). Participants with higher self-reported SRS-scores (more impairment) showed a stronger treatment-induced reduction in skin conductance recovery time (faster recovery).



Author Disclosures

Role of the Funding Source. Funding for this study was provided by grants from the Flanders Fund for Scientific Research (FWO [KAN 1506716N, KAN 1521313N and G.0401.12] and the Branco Weiss fellowship of the Society in Science - ETH Zurich granted to KA. ND is supported by an internal C1 fund of the KU Leuven [ELG-D2857-C14/17/102]. JP is supported by the Marguerite-Marie Delacroix foundation. The funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributions. Author KA designed the study together with author JP. Author KA and ND managed the literature searches and data collection/ processing. Authors KA, ND and JRS undertook the statistical analysis, and author KA wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest. The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Elisa Maes for her contribution in conducting the experiment. We would also like to thank all participating subjects and students for their help in data collection.

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Table 1

Effect of oxytocin administration on behavioral measures. Mean pre-to-post change scores (± standard error) are listed separately for each treatment group (oxytocin, placebo). T and p values correspond to single-sample t tests assessing within-group pre-to-post changes separately within the oxytocin and placebo group. F and p values correspond to between-group differences in pre-to-post change scores ('time x treatment' interaction).

	Oxytocin				Placebo				Between-group difference	
	N	***Mean ± SE***	***t value***	***p***	***N***	***Mean ± SE***	***t value***	***p***	***F value***	***p***
Profile of Mood State (POMS)										
Tension	28	-2.43 ± 0.54	-4.32	*.0002*	25	-0.76 ± 0.54	-1.40	.17	***4.51***	***.038****
Anger	28	-1.43 ± 0.36	-3.43	*.002*	25	-0.88 ± 0.31	-2.86	*.01*	1.08	.30
Depression	28	-1.68 ± 0.50	-3.84	*.0007*	25	-1.52 ± 0.61	-2.48	*.02*	0.05	.83
Fatigue	28	0.54 ± 0.52	1.13	.27	25	0.76 ± 0.61	1.25	.22	0.09	.77
Vigor	28	-1.43 ± 0.60	-2.28	.03	25	-2.32 ± 0.61	-3.78	*.0009*	1.02	.32
Self-Assessment Manikin (SAM)										
Affective valence	27	-0.52 ± 0.25	-2.05	*.05*	27	-0.67 ± 0.24	-2.73	*.01*	0.18	.68
Arousal	27	-0.30 ± 0.31	-0.95	.35	27	-0.85 ± 0.34	-2.47	*.02*	1.43	.24
Situational Self-Awareness Scale (SSAS)	27	-0.54 ± 0.16	-3.23	*.003*	27	-0.12 ± 0.15	-0.80	.43	3.54	.065

For POMS tension, anger, depression and fatigue; negative scores indicate pre-to-post improvement. For SAM affective valence and arousal, negative scores indicate pre-to-post change to more pleasant/ less arousing ratings. For SSAS, negative scores indicate pre-to-post reduction in self-awareness.

Supplementary Information

Oxytocin enhances the recovery of eye-contact induced autonomic arousal: A treatment mechanism study with placebo-controlled design

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Supplementary Table 1

At the end of the experimental session, participants were asked to report whether they presented any of the listed (or other) side effects and to indicate the severity of the side effect (mild, moderate, or severe). The number of oxytocin participants (out of n=28) or placebo participants (out of n=27) that reported any mild, moderate or severe side effects are listed separately for each side effect. Note that one participant in the placebo group did not fill the side-effect form. A significant group difference (Pearson Chi-square test) was noted for the side effect 'headache', indicating that a larger number of oxytocin participants reported a (mild) headache.

	Mild		**Moderate**		**Severe**		**Total**			
	Oxytocin	**Placebo**	**Oxytocin**	**Placebo**	**Oxytocin**	**Placebo**	**Oxytocin**	**Placebo**	Chi-square	p-value
Headache	6	1	0	0	0	0	6	1	**3.89**	**0.05***
Drowsiness	6	6	6	7	1	0	13	13	0.16	0.90
Dizziness	0	0	1	0	0	0	1	0	0.98	0.32
Fainting	0	0	0	0	0	0	0	0	0.00	1.00
Changes in heart rate or palpitations	1	2	0	0	0	0	1	2	0.39	0.53
Shortness of breath	2	0	0	0	0	0	2	0	2.00	0.16
Fever	0	0	0	0	0	0	0	0	0.00	1.00
Sore throat	0	0	0	0	0	0	0	0	0.00	1.00
Dry throat/dry mouth	3	1	0	1	0	0	3	2	0.18	0.67
Hoarseness	0	0	0	0	0	0	0	0	0.00	1.00
Coughing	1	0	0	0	0	0	1	0	0.98	0.32
Coughing up mucus	1	0	0	1	0	0	1	1	0.00	0.74
Congested nose	1	1	0	0	0	0	1	1	0.00	0.74
Sneezing	2	0	1	0	0	0	3	0	3.06	0.12
Nasal irritation	1	2	0	0	0	0	1	2	0.39	0.49
Runny nose	8	2	0	1	0	0	8	3	2.62	0.01
Watery eyes	0	2	0	1	0	0	0	3	3.29	0.11
Nausea and/or vomiting	0	1	0	0	0	0	0	1	1.06	0.49
Abdominal or stomach pain	0	0	0	0	0	0	0	0	0.00	1.00
Changes in perception of the tongue	0	0	0	0	0	0	0	0	0.00	1.00
Burning sensation in nose and/or ears	0	0	0	0	0	0	0	0	0.00	1.00
Muscle pain/cramps	0	1	0	0	0	0	0	1	1.06	0.49
Skin rash	0	2	0	0	0	0	0	2	2.15	0.24
Sweating	0	2	1	0	0	0	1	2	0.39	0.49
Sensitive to fragrances	1	0	0	0	0	0	1	0	0.98	0.51
Blurred vision	1	1	1	0	0	0	2	1	0.31	0.51

Supplementary Figure 1

Modulation by person-dependent factors. Visualization of pre-to-post changes in skin conductance recovery time (rec. t/2) for participants of the oxytocin group with high versus low scores on the Social Responsiveness Scale (SRS, self-report), with subgroups based on median-split (**panel A**) or upper/lower quartiles (**panel B**).

Participants with higher self-reported SRS-scores (more impairment) display overall stronger oxytocin-induced reductions in skin conductance recovery time (faster recovery), compared to participants with low SRS-scores (median-split: $t(23) = 2.01$; $p = .056$) (upper/lower quartiles: $t(11) = 2.86$; $p = .016$).