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Oxytocin effects on emotion recognition under conditions of unlimited and limited awareness

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Erklärung

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List of Publications

This thesis is based on the following publications which are referred to in the text by their Roman Numerals (Study I-III).

Study I: Lischke, A., Berger, C., Prehn, K., Heinrichs, M., Herpertz, S. C., & Domes, G. (2012). Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected. *Psychoneuroendocrinology*, *37(4)*, 475-481.

Study II: Prehn, K., Kazzer, P., Lischke, A., Heinrichs, M., Herpertz, S. C., & Domes, G. (under review). Effects of intranasal oxytocin on pupil dilation indicate increased salience of socio-affective stimuli. *Psychophysiology*.

Study III: Schulze, L., Lischke, A., Greif, J., Herpertz, S. C., Heinrichs, M., & Domes, G. (2011). Oxytocin increases recognition of masked emotional faces. *Psychoneuroendocrinology*, *36(9)*, 1378-1382.

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List of Abbreviations

AMG	amygdala
ANOVA	analysis of variance
ASD	autism spectrum disorders
BPD	borderline personality disorder
BS	brain stem
CSF	cerebrospinal fluid
IRI	Interpersonal Reactivity Index
IU	international unit
MDBF	Multidimensionaler Befindlichkeitsbogen
OXT	oxytocin
OXT-R	oxytocin receptor
PANAS	Positive and Negative Affect Scale
PLC	placebo
SAD	social anxiety disorder
SC	superior colliculi
SCL-90-R	Symptom Checklist-90-Revised
STAI	State Trait Anxiety Inventory
STAXI	State Trait Anger Expression Inventory
TAS-20	20-item Toronto Alexithymia Scale
TJ	temporoparietal junction
TP	temporal pole
WST	Wortschatztest

1. Introduction

Over the last decade, the neuropeptide oxytocin (OXT) has received considerable attention for its crucial role in social behavior and social cognition (Adolphs, 2010; Ebstein, Israel, Chew, Zhong, & Knafo, 2010; Insel, 2010). Moreover, OXT has been regarded as a promising target for the treatment of mental disorders that are characterized by marked deficits in social behavior and social cognition (Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011), such as autism spectrum disorders (ASD), borderline personality disorder (BPD) or social anxiety disorder (SAD). Motivated by animal studies revealing dramatic effects of OXT on social anxiety, social stress and social recognition (Neumann, 2008), numerous studies have been conducted to investigate whether OXT has similar effects on social cognition and social behavior in humans (Meyer-Lindenberg, et al., 2011).

Most of these studies were limited by methodical constraints inherent in human research. Whereas animal researchers were able to use non-invasive as well as invasive methods in their studies (e.g., central administration of OXT agonists and antagonists, manipulation of OXT and OXT receptor (OXT-R) expression by gene inactivation, assessment of OXT messenger RNA), researchers investigating OXT effects in humans had mainly to rely on non-invasive methods. One prominent method was to correlate markers of OXT activity in urine (e.g., Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005), saliva (e.g., Feldman, Gordon, & Zagoory-Sharon, 2010) or blood (e.g., Zak, Kurzban, & Matzner, 2005) with changes in social behavior and social cognition. However, the relationship between peripheral and central OXT is not well understood (Landgraf & Neumann, 2004), leaving open whether changes in peripheral OXT levels indeed reflect changes in central OXT levels. In consideration of this, researchers used markers of OXT activity in cerebrospinal fluid (CSF) rather than in urine, saliva or plasma in their studies (e.g., Heim et al., 2009). However, assessing central OXT levels is much more invasive than assessing peripheral OXT levels,

rendering this method impracticable in many circumstances. In addition, this method also involves the use of correlation analysis, which precludes any inferences about the causality of the respective findings. To overcome these limitations, researchers used intravenous infusion (e.g., Hollander et al., 2003) or intranasal application (e.g., Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005) of OXT to gain more insights into OXT effects on social cognition and social behavior. Since only a small fraction of OXT passes the blood-brain barrier after intravenous infusion (Kang & Park, 2000) as compared to intranasal application (Born et al., 2002; Chang, Barter, Ebitz, Watson, & Platt, 2012), intranasal application has become the method of choice for researchers investigating the social effects of OXT (Bartz, Zaki, Bolger, & Ochsner, 2011; Guastella & MacLeod, 2012). In addition, researchers genotyped polymorphisms of the OXT-R to elucidate the genetic mechanisms underlying OXT effects on social cognition and social behavior (Ebstein, Knafo, Mankuta, Chew, & Lai, 2012), either alone (e.g., Chen et al., 2011) or in combination with intranasal application of OXT (e.g., Sauer, Montag, Worner, Kirsch, & Reuter, 2012).

Up to now, studies employing the aforementioned methods have revealed striking similarities between OXT effects on social behavior and social cognition in animals and humans (Meyer-Lindenberg, et al., 2011). Most relevant here, OXT also facilitates social recognition (e.g., Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Fischer-Shofty, Shamay-Tsoory, Harari, & Levkovitz, 2010; Lischke, Berger, et al., 2012; Marsh, Yu, Pine, & Blair, 2010; Prehn et al., under review; Schulze et al., 2011) and social memory (e.g., Guastella, Mitchell, & Mathews, 2008; Rimmele, Hediger, Heinrichs, & Klaver, 2009; Savaskan, Ehrhardt, Schulz, Walter, & Schachinger, 2008) in humans, stressing the therapeutic potential of OXT for the treatment of individuals with impairments in social cognition. Notwithstanding these promising findings, it has to be acknowledged that the mechanisms underlying the social effects of OXT are poorly understood (Bartz, et al., 2011).

The present thesis provides a review of previous studies examining the effects of OXT on social cognition and describes a series of recent studies that were explicitly designed to test possible mechanisms accounting for OXT effects on social memory and social recognition[1]. The findings of these studies are discussed in the context of previous studies[2], while considering implications for future studies investigating the therapeutic potential of OXT.

1.1. Effects of oxytocin on social cognition

1.1.1. Social recognition

Domes and colleagues (Domes, Heinrichs, Michel, et al., 2007) performed the first study dealing with OXT effects on social recognition (see Table 1 for an overview of similar studies). Using a double-blind, placebo-controlled within-subjects design, they administered 24 international units (IU) of OXT or placebo (PLC) to 30 males who participated in a well established social cognition task, the Reading the Mind in the Eyes Test (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). During task performance, participants had to infer complex emotional expressions from the eye region of 36 (18 male, 18 female) faces (self-paced). OXT improved participants' recognition accuracy, in particular for those expressions that were most difficult to recognize[3]. Since Domes and colleagues (Domes, Heinrichs,

[1] The focus of this thesis is on studies examining OXT effects on social recognition and social memory in male individuals. Only studies employing face processing tasks and intranasal OXT challenge are considered. Although this thesis primarily deals with OXT effects on social cognition in healthy individuals, studies examining these effects in mentally disordered individuals are also discussed if necessary. Of note, only studies that have been published prior to the 1st of May 2012 are included in the review.

[2] Since this thesis is based on the findings of three studies, a summary of the studies' most important findings rather than a detailed account of each single finding is provided. For a detailed description of the studies' findings, the reader is referred to the respective passages of the corresponding publications (see Appendix).

[3] Similar effects were reported in studies investigating OXT effects on social recognition in individuals exhibiting traits associated with mental disorders (Luminet, Grynberg, Ruzette, & Mikolajczak, 2011) as well as in individuals with mental disorders (Guastella et al., 2010). These findings indicate that OXT may, in fact, be useful for the treatment of individuals with deficits in social cognition and social behavior (Meyer-Lindenberg, et al., 2011).

Table 1. Behavioral studies investigating oxytocin effects on social recognition

Study	Subjects	Design	Application	Task	Stimuli	Results
Domes et al. (2007)	30 males	Double-blind, placebo-controlled, within-subjects design	24 IU of OXT or PLC	Emotion recognition test (complex expression rating)	36 male and females faces (complex expressions) conveying only the eye region, static presentation (self-paced)	OXT enhanced recognition accuracy for complex expressions, in particular difficult ones
Fischer-Shofty et al. (2010)	27 males	Double-blind, placebo-controlled, within-subjects design	24 IU of OXT or NaCl	Emotion recognition test (basal expression rating)	36 male and females faces (6 happy, 6 angry, 6 fearful, 6 disgusted, 6 sad, 6 surprised) conveying only the eye region, dynamic presentation in 1% steps of intensity (100ms)	OXT enhanced recognition accuracy for fearful expressions but had no effect on recognition sensitivity
Di Simplicio et al. (2009)	29 males	Double-blind, placebo-controlled, between-subjects design	24 IU of OXT or PLC	Emotion recognition test (basal expression rating)	250 male and female faces (10 neutral, 40 happy, 40 angry, 40 fearful, 40 disgusted, 40 sad, 40 surprised), static presentation in 10% steps of intensity (500 ms)	OXT had no effect on recognition accuracy but attenuated the misclassification of positive or ambiguous expressions as negative ones
Marsh et al. (2010)	29 males, 21 females	Double-blind, placebo-controlled, between-subjects design	24 IU of OXT or PLC	Emotion recognition test (basal expression rating)	360 male and female faces (60 happy, 60 angry, 60 fearful, 60 disgusted, 60 sad, 60 surprised), static presentation in 10% steps of intensity (500 ms)	OXT enhanced recognition accuracy for happy expressions without exerting any sex-specific effects

Note. OXT = oxytocin; PLC = placebo.

Michel, et al., 2007) only examined whether OXT enhances the recognition of complex emotional expressions, such as desire or hate, Fisher-Shofty and colleagues (Fischer-Shofty, et al., 2010) investigated how OXT affects the recognition of basal emotional expressions, such as fear or anger. In a double-blind, placebo-controlled within-subjects design, 27 males received 24 IU of OXT or PLC before they viewed 36 (18 male, 18 female) eye regions of faces with dynamically changing expressions. Each face was initially presented with a neutral expression that subsequently turned into an emotional one (6 happy, 6 angry, 6 fearful, 6 disgusted, 6 sad, 6 surprise; 100 ms per 1% increase in emotional intensity). Participants had to detect the onset of the developing expression and to identify the respective emotion. OXT specifically enhanced participants' recognition accuracy for fearful expressions, while having no effect on their recognition sensitivity. That is, OXT improved participants' ability to recognize fearful expressions, but did not change the threshold at which these expressions were recognized.

The aforementioned studies revealed that OXT improves emotion recognition from the eye region of faces. However, beneficial effects of OXT on emotion recognition have also been reported during the processing of all facial regions as suggested by Di Simplicio and colleagues (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009) and Marsh and colleagues (Marsh, et al., 2010). In a double-blind, placebo-controlled between-subjects design, Di Simplicio and colleagues (Di Simplicio, et al., 2009) administered 24 IU of OXT or PLC to 29 males who had to identify the expressions of briefly presented faces (500 ms). In total, 250 (125 male, 125 female) faces showing various expressions (10 neutral, 40 happy, 40 angry, 40 fearful, 40 disgusted, 40 sad, 40 surprised) at different intensity levels (ranging in emotional intensity from 0% to 100%) had to be analyzed by the participants. Although OXT had no effect on participants' recognition accuracy, it attenuated the misclassification of positive (happy) or ambiguous (neutral, surprised) expressions as negative (angry, fearful, disgusted,

sad) ones. Since the study suffered from various methodical limitations (Guastella & MacLeod, 2012; Marsh, et al., 2010), Marsh and colleagues (Marsh, et al., 2010) performed a similar study to replicate and extend these findings. In a double-blind, placebo-controlled between-subjects design, 29 males and 21 females received 24 IU of OXT or PLC before they viewed 360 (180 male, 180 female) faces expressing various emotions (60 happy, 60 angry, 60 fearful, 60 disgusted, 60 sad, 60 surprised) at different intensity levels (ranging in emotional intensity from 0% to 100%). Following each face presentation (500 ms), participants had to identify the emotional expression of the respective face. In contrast to Di Simplicio and colleagues (Di Simplicio, et al., 2009), Marsh and colleagues (Marsh, et al., 2010) revealed that OXT specifically enhanced participants' recognition accuracy for happy expressions. Of note, there was no evidence indicating that OXT differentially affected recognition accuracy in males and females, ruling out that OXT exerted sex-specific effects on social cognition as recently suggested (Domes et al., 2010; Lischke, Gamer, et al., 2012a, 2012b).

1.1.2. Social memory

The first study investigating OXT effects on social memory was performed by Guastella and colleagues (Guastella, Mitchell, & Mathews, 2008; see Table 2 for an overview of similar studies). Using a double-blind, placebo-controlled between-subjects design, they administered 24 IU of OXT or PLC to 69 males who had to provide trustworthiness and acceptance ratings for 36 faces (2 s) depicting neutral (6 male, 6 female), happy (6 male, 6 female) or angry (6 male, 6 female) expressions. Twenty-four hr later, participants completed a surprise memory test, which required them to distinguish the 36 faces (self-paced) they had seen previously from 36 faces (12 neutral, 12 happy, 12 angry; self-paced) they had not seen previously on basis of "remember", "know" and "new" judgments. OXT specifically enhanced participants' memory for happy but not neutral or angry expressions in terms of

Table 2. Behavioral studies investigating oxytocin effects on social memory

Study	Subjects	Design	Application	Task	Stimuli	Results
Guastella et al. (2008)	69 males	Double-blind, placebo-controlled, between-subjects design	24 IU of OXT or PLC before encoding	Surprise memory test (remember-know-new rating) after 24 hr	72 male and female faces (24 neutral, 24 happy, 24 angry), static presentation (2 s during encoding, self-paced during recall)	OXT enhanced recognition of happy expressions in terms of familiarity ratings
Rimmele et al. (2009)	44 males	Double-blind, placebo-controlled, between-subjects design	24 IU of OXT or PLC before encoding	Surprise memory test (remember-know-new rating) after 24 hr	120 male and female faces (40 neutral, 40 positive, 40 negative), 120 non-faces (30 houses, 30 objects, 60 landscapes), static presentation (3.5 s during encoding, 5 s during recall)	OXT enhanced recognition of all expressions in terms of familiarity ratings
Savaskan et al. (2008)	18 males, 18 female	Single-blind, placebo-controlled, between-subjects design	20 IU of OXT or NaCl after encoding	Memory test (old-new rating, expression rating) after 30 min and 24 hr	80 male faces (40 neutral, 20 happy, 20 angry), static presentation (10 s during encoding, self-paced during recall)	OXT enhanced recognition of all expressions in terms of recognition ratings

Note. OXT = oxytocin; PLC = placebo.

familiarity ratings. Although the findings by Guastella and colleagues indicated that OXT improves social memory (Guastella, Mitchell, & Mathews, 2008), it remained open whether OXT in fact facilitated social as compared to non-social memory. In consideration of this, Rimmele and colleagues (Rimmele, et al., 2009) examined how OXT affects memory for social and non-social stimuli. In a double-blind, placebo-controlled between-subjects design, 44 males received 24 IU of OXT or PLC before they provided approach ratings for 84 faces (3.5 s) showing neutral (14 male, 14 female), positive (14 male, 14 female) or negative (14 male, 14 female) expressions as well as for 84 images (3.5 s) depicting houses (21 images), objects (21 images) or landscapes (42 images). After 24 hr, participants had to complete a similar surprise memory test as the one administered by Guastella and colleagues (Guastella, Mitchell, & Mathews, 2008). By providing "remember", "know" and "new" judgments, they had to distinguish the 84 faces (5 s) and 84 images (5 s) they had seen previously from 36 faces (12 neutral, 12 positive, 12 negative; 5 s) and 36 images (9 houses, 9 objects, 18 landscapes; 5 s) they had not seen previously. OXT specifically improved participants' memory for faces in terms of familiarity ratings, while leaving these ratings for non-faces unaffected. Moreover, this face-specific memory effect was not restricted to a particular type of expression as reported by Guastella and colleagues (Guastella, Mitchell, & Mathews, 2008) but was present during the processing of all expressions, irrespective of the expressions' emotional valence (neutral, positive, negative).

Although the aforementioned studies revealed beneficial effects of OXT on social memory, these studies only investigated how OXT affects the encoding (Guastella, Mitchell, & Mathews, 2008; Rimmele, et al., 2009)[4] but not the consolidation of facial expressions. To

[4] Although most studies showed that OXT improves the encoding of facial expressions (Guastella, Mitchell, & Mathews, 2008; Rimmele, et al., 2009), one study failed to do so (Di Simplicio, et al., 2009). However, participants of this study completed several social cognition tasks before performing the social memory task (Di Simplicio, et al., 2009), which may have affected their memory performance. For this reason, the findings

address this issue, Savaskan and colleagues (Savaskan, et al., 2008) investigated whether OXT also improves social memory when given during the consolidation rather than encoding of facial expressions. In a single-blind, placebo-controlled between-subjects design, 18 males and 18 females received 20 IU of OXT or PLC after viewing 60 faces (60 males; 10 s) with various emotional expressions (20 neutral, 20 happy, 20 angry). Thirty min and 24 hr later, participants completed a memory test, which required them to distinguish between 30 faces (self-paced) they had seen previously (10 neutral, 10 happy, 10 angry) and 20 faces (self-paced) they had not seen previously. Notably, all faces were shown with neutral expressions during the memory test. Participants were asked to provide "old" and "new" ratings for each face and to indicate whether the face had formerly been presented with a happy or angry expression. OXT improved participants' memory for all emotional expressions, after 30 min as well as after 24 hr. There was some, but not convincing, evidence that this effect may have been more pronounced for angry than happy expressions (Guastella & MacLeod, 2012), leaving open whether OXT affects face memory in an expression-specific way as suggested by Guastella and colleagues (Guastella, Mitchell, & Mathews, 2008).

1.1.3. Summary

Studies investigating OXT effects on social cognition via face processing tasks revealed impressive effects of OXT on social recognition and social memory (Bartz, et al., 2011; Guastella & MacLeod, 2012). According to these studies, OXT improves the recognition (Di Simplicio, et al., 2009; Fischer-Shofty, et al., 2010; Marsh, et al., 2010) as well as the encoding (Guastella, Mitchell, & Mathews, 2008; Rimmele, et al., 2009) and consolidation (Savaskan, et al., 2008) of emotional expressions under various conditions. Although not entirely conclusive (Guastella & MacLeod, 2012), these effects appear to be

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regarding participants' memory performance will not be considered in this review, following the suggestions of previous reviews dealing with OXT effects on social cognition (Guastella & MacLeod, 2012).

more pronounced during the processing of positive (Di Simplicio, et al., 2009; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010) than negative (Fischer-Shofty, et al., 2010) expressions. Notwithstanding these inconsistencies, the aforementioned studies consistently demonstrated beneficial effects of OXT on social cognition (see Table 1 and Table 2 for an overview of the respective studies).

1.2. Mechanisms underlying the effects of oxytocin on social cognition

Animal studies revealed that OXT effects on social cognition crucially depend on the amygdala (Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009; Ferguson, Young, & Insel, 2002). For instance, administration of OXT to the amygdala restores social memory in OXT-R knockout mice (Ferguson et al., 2000) and administration of OXT antagonists or OXT-R antisense DNA to the amygdala impairs social recognition in wild type mice (Choleris et al., 2007; Ferguson, Aldag, Insel, & Young, 2001). Motivated by these findings, Kirsch and colleagues (Kirsch et al., 2005) investigated for the first time whether the amygdala similarly mediates OXT effects on social cognition in humans (see Table 3 for an overview of similar studies). Using a double-blind, placebo-controlled within-subjects, they administered 27 IU of OXT or PLC to 15 males who had to perform a face matching task on 12 faces (5 s) showing angry (3 male, 3 female) or fearful (3 male, 3 female) expressions. OXT attenuated left amygdala activity as well as coupling of the amygdala with the brainstem, a connection essential for physiological reactions to emotional expressions (Adolphs, 2002). Since Kirsch and colleagues (Kirsch, et al., 2005) only showed that OXT attenuated amygdala reactivity to angry and fearful expressions, Domes and colleagues (Domes et al., 2007) investigated whether amygdala reactivity to happy expressions would be similarly affected by OXT. In a double-blind, placebo-controlled within-subjects design, 13 males received 24 IU of OXT or PL before they had to perform a gender discrimination task on 130 (65 male, 65 female) faces (2 s) depicting various expressions (10 neutral, 40 happy, 40 angry, 40 fearful) at different

Table 3. Imaging studies investigating oxytocin effects on social recognition

Study	Subjects	Design	Application	Task	Stimuli	Results
Kirsch et al. (2005)	15 males	Double-blind, placebo-controlled, within-subjects design	27 IU of OXT or PLC	Implicit emotion recognition test (face matching)	12 male and females faces (6 angry, 6 fearful), static presentation (5 s)	OXT attenuated left AMG reactivity to angry and fearful expressions and attenuated AMG-BS connectivity
Domes et al. (2007)	13 males	Double-blind, placebo-controlled, within-subjects design	24 IU of OXT or PLC	Implicit emotion recognition test (gender rating)	130 male and females faces (10 neutral, 40 happy, 40 angry, 40 fearful), static presentation in 25 % steps of intensity (2 s)	OXT attenuated right AMG reactivity to all expressions, attenuated bilateral TP and left TPJ reactivity to fearful and happy expressions
Gamer et al. (2010)	46 males	Double-blind, placebo-controlled, between-subjects design	24 IU of OXT or PLC	Explicit emotion recognition test (basal expression rating), eye-tracking (gaze behavior)	72 male and females faces (24 neutral, 24 happy, 24 fearful), static presentation with focus on the mouth or eye region (150 ms)	OXT attenuated left anterior AMG reactivity to fearful expressions and augmented left anterior AMG reactivity to happy expressions during expression rating; OXT augmented right posterior AMG-SC connectivity during gaze shifts to the eye region; OXT enhanced gaze shifts to the eye region; OXT had no effect on recognition accuracy

Note. AMG = amygdala; BS = brainstem; OXT = oxytocin; PLC = placebo; SC = superior colliculi; TP = temporal pole; TJ = temporoparietal junction.

intensity levels (0%, 25%, 75% and 100% of emotional intensity). OXT attenuated right amygdala reactivity to all emotional expressions, irrespective of the expressions' emotional valence (positive, negative). Moreover, OXT also attenuated activity in the left and right temporal pole and left temporoparietal junction in response to happy and fearful expressions, suggesting that OXT modulates neural activity in various brain regions associated with face processing (Adolphs, 2002). Although these studies revealed that the amygdala also mediates OXT effects on social cognition in humans (Domes, Heinrichs, Glascher, et al., 2007; Kirsch, et al., 2005), they only provided limited insights into the underlying mechanisms of these effects. In consideration of this, Gamer and colleagues (Gamer, Zurowski, & Buchel, 2010) investigated how different subregions of the amygdala contribute to OXT effects on social cognition. In a double-blind, placebo-controlled between-subjects design, 46 males received 24 IU of OXT or PLC before they had to perform an emotion classification task on 72 faces (150 ms) displaying neutral (12 male, 12 female), happy (12 male, 12 female) or fearful (12 male, 12 female) expressions. Importantly, the faces were presented in a way that participants' attention was either directed to the mouth or to the eye region of the expressions. OXT directed participants' attention to the eye region of all expressions as indicated by corresponding changes in gaze behavior. Interestingly, these gaze changes were accompanied by an augmented activation of a right posterior amygdala subregion as well as by an augmented coupling of this amygdala subregion with the superior colliculi, a crucial connection for gaze changes and attention shifts (e.g., Akiyama et al., 2007; Sereno, Briand, Amador, & Szapiel, 2006). In addition, OXT attenuated activity for fearful expressions and augmented activity for happy expressions in a left anterior amygdala subregion. These findings clearly indicate the amygdala as the major site for OXT effects on social cognition (Domes, Heinrichs, Glascher, et al., 2007; Gamer, et al., 2010; Kirsch, et al., 2005)[5], but also

[5] Studies investigating OXT effects on social cognition in other contexts than social recognition or social

reveal that these effects are mediated by local rather than global activity changes within the amygdala (Gamer, et al., 2010): OXT modulated activity in subregions of the amygdala that were differentially engaged during attentional orienting (right posterior amygdala subregion in conjunction with the superior colliculi) and emotion classification (left anterior amygdala subregion).

Taken together, these studies suggest that OXT effects on social cognition involve various mechanisms, including mechanisms related to attention allocation and mechanisms related to emotion recognition (Guastella & MacLeod, 2012). First of all, OXT may generally improve the recognition of and memory for facial expressions by enhancing attention for distinct facial features. Accordingly, OXT directs gaze to the eye region of a face (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008), which conveys the most informative features of an expression (Schyns, Bonnar, & Gosselin, 2002; Schyns, Petro, & Smith, 2007, 2009; Smith, Cottrell, Gosselin, & Schyns, 2005). In this regard, it is not surprising that OXT improves social cognition on tasks that rely on a thorough exploration of the eye region (Domes, Heinrichs, Michel, et al., 2007; Fischer-Shofty, et al., 2010). These gaze-related effects are most likely mediated by activity changes in the amygdala and superior colliculi (Gamer, et al., 2010). However, activity changes in the amygdala and superior colliculi may also account for other attention-related processes than those involving gaze changes. Changes in pupil size, for instance, are also mediated by activity changes in the amygdala and superior colliculi, presumably via projections to the locus coeruleus (e.g., Bouret, Duvel, Onat, & Sara, 2003; Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010; Wang, Boehnke, White, & Munoz, 2012)[6]. OXT, indeed, does not only change gaze behavior (Gamer, et al., 2010; Guastella,

memory have also shown that these effects are amygdala dependent (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Petrovic, Kalisch, Singer, & Dolan, 2008; Rilling et al., 2012).

[6] It should be noted that the exact mechanisms underlying changes in pupil size are not completely understood yet (Laeng, Sirois, & Gredeback, 2012). Although it has been shown that activity changes in the locus coeruleus,

Mitchell, & Dadds, 2008) but also pupil size (Leknes et al., 2012) during face processing, indicating that OXT effects on social cognition involve shifts in overt (changes in gaze behavior) as well as covert (changes in pupil size) attention. Second, OXT may specifically improve the recognition of and memory for positive as compared to negative expressions by decreasing the aversive value of negative expressions and increasing the appetitive value of positive expressions. Accordingly, OXT enhances the processing of positive rather than negative expressions on various social cognition tasks (Guastella & MacLeod, 2012). For instance, OXT attenuates the misclassification of positive expressions as negative ones (Di Simplicio, et al., 2009) and facilitates the recognition and encoding of positive relative to negative expressions (Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010). These emotion-related effects are most likely mediated by differential activity changes in the amygdala (Gamer, et al., 2010), presumably by increased amygdala reactivity to positive expressions and decreased amygdala reactivity to negative expressions. However, whether OXT in fact improves social recognition and social memory by enhancing attention for distinct facial features and by changing the affective value of positive and negative expressions has not been studied yet.

amygdala and superior colliculi are related to changes in pupil size (e.g., Bouret, et al., 2003; Gilzenrat, et al., 2010; Wang, et al., 2012), it remains to be determined how these activity changes lead to pupil constriction, via the sphincter muscle, or pupil dilation, via the dilator muscle.

2. Aim

Studies investigating the effects of OXT on social cognition revealed that OXT improves the recognition of and memory for facial expressions under various conditions (Bartz, et al., 2011; Guastella & MacLeod, 2012). On basis of these studies, it may be assumed that these effects are mediated by attention- and emotion-related activity changes in the amygdala and superior colliculi (Zink & Meyer-Lindenberg, 2012). OXT may generally facilitate face processing by enhancing attention for distinct facial features, probably by initiating shifts in overt (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008) and/or covert (Leknes, et al., 2012) attention. Additionally, OXT may specifically improve the recognition of and memory for positive expressions by enhancing the saliency of positive rather than negative expressions (Di Simplicio, et al., 2009; Gamer, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010). Although previous findings are broadly consistent with these predictions, they have not explicitly been tested yet. In consideration of this, the present thesis investigated, for the first time, whether OXT indeed improves social cognition via the proposed mechanisms.

Three studies were performed to investigate the effects of OXT on facial emotion recognition under conditions of unlimited (Study I, Study II) and limited awareness (Study III). In Study I and Study II, participants' gaze behavior was recorded while they performed an emotion recognition task that involved the presentation of gradually changing expressions. These studies tested whether OXT differentially affected the recognition of positive and negative expressions and whether these effects were related to shifts in overt and/or covert attention. More precisely, Study I tested whether changes in gaze behavior and Study II tested whether changes in pupil size accounted for OXT effects on emotion recognition. In Study III, participants performed an emotion recognition task that involved the masked presentation of static expressions. The expressions were presented very briefly, ruling out that gaze changes

may have occurred during face processing. This study tested whether OXT differentially affected the recognition of positive and negative expressions under conditions that precluded shifts in overt attention. Across all three studies, it was expected that OXT would specifically improve the recognition of positive expressions by initiating shifts in overt and/or covert attention during face processing.

3. Materials and Methods

3.1. Participants

All studies were performed at the Department for Psychiatry and Psychotherapy of the University of Rostock. Each study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethics committee. Forty-seven healthy males participated in the first two studies (Study I, Study II) and 56 healthy males participated in the last study (Study III)[7]. It should be noted, however, that the same participants were included in Study I and Study II, whereas different participants were included in Study III. Exclusion criteria for participation were medical or mental illness, use of medication, substance abuse and smoking. A brief interview and several self-report questionnaires were administered to determine exclusion of participants[8]. The brief interview was used to screen for medical and mental illness, whereas the self-report questionnaires assessed general intelligence (Wortschatztest, WST; Schmidt & Metzler, 1992), general psychopathology (Symptom Checklist-90-Revised, SCL-90-R; Franke, 1995), anxiety (State Trait Anxiety Inventory, STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981), anger (State Trait Anger Expression Inventory, STAXI; Spielberger, 1991), positive and negative affect (Positive and Negative Affect Scales, PANAS; Watson, Clark, & Tellegen, 1988), alexithymia (Twenty-item Toronto Alexithymia Scale, TAS-20; Bagby, Parker, & Taylor, 1994; Bagby, Taylor, & Parker, 1994) and empathy (Interpersonal Reactivity Index, IRI; Davis, 1983). As none of the

[7] It is important to note that only males were included in the studies because it has recently been suggested that OXT effects on social cognition are sexually dimorphic (Domes, et al., 2010; Lischke, Gamer, et al., 2012a, 2012b). However, for the ease of readability, this is not explicitly stated throughout this work when referring to the studies' participants. Consequently, the term "participant" rather than "male participant" is used. Nonetheless, caution is warranted when generalizing the studies' findings to female as compared to male populations.

[8] The interview was administered in all studies, whereas the studies differed with regard to the administered self-report questionnaires (see Appendix).

participants reported a significant illness in the interview or scored in the clinical range of the self-report questionnaires, they were all included in the respective studies. All participants provided written performed consent prior to participation and were reimbursed for their time.

3.2. Experimental procedure

3.2.1. Substance application

Participants were instructed to abstain from alcohol, drugs, nicotine and caffeine on the testing day. After arrival at the laboratory, they were randomly assigned to the OXT or PLC group using a double-blind, placebo-controlled between-subjects design. Following a standardized protocol (Domes, et al., 2010; Lischke, Gamer, et al., 2012a, 2012b), they self-administered 24 IU of OXT (Syntocinon, Novartis, Basel, Switzerland) or PLC (containing all ingredients except for the neuropeptide) 45 min before the start of the experimental session. Substance induced changes in participants' mental state, in terms of wakefulness, calmness and mood, were tracked by repeatedly administering a multidimensional mood questionnaire (Multidimensionaler Befindlichkeitsbogen, MDBF; Steyer, Schwenkmezger, Notz, & Eid, 1997) throughout substance application. In addition, participants were told to report any side-effects they may have experienced following substance application.

3.2.2. Hormone assessment

Blood samples were collected to control for differences in participants' peripheral OXT levels before and after substance application[9]. The samples were drawn into 5 ml EDTA vacutainer tubes, immediately cooled in ice-chilled water at 4° C and later centrifuged at 4000 rpm (for 5 min at 4° C) before they were stored in a freezer at -20° C. After completion of the studies, the samples were shipped on dry ice at -20° C to an external laboratory (Department of Behavioral and Molecular Neuroendocrinology, University of Regensburg, Germany),

[9] Although blood samples were assessed in all studies, they were not analyzed in Study III because of equipment malfunction.

where they were extracted and analyzed using a radioimmunoassay (Landgraf, 1981). The assay detection limit was 0.1 pg/sample, cross-reactivity with other related neuropeptides was < 0.7%, intra-assay precision was 7-10% and inter-assay precision was eliminated by measuring all samples within the same assay.

3.3. Experimental tasks

3.3.1. Dynamic emotion recognition task

The emotion recognition task used in Study I and Study II involved the presentation of computer-manipulated faces, whose expression was gradually changing from a neutral to an emotional one. The faces were selected on basis of a validation study from the FACES database (Ebner, Riediger, & Lindenberger, 2010) and consisted of six male and six female faces showing a neutral, happy, angry, fearful and sad expression. Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA, USA) and Matlab 7.7 (MathWorks Inc., Natick, MA, USA) were used to prepare the faces for the emotion recognition task. At first, the faces were converted into grayscales and equalized in size and cumulative brightness. Thereafter, each face was enclosed within an elliptic mask (411 x 570 pixels) that only revealed the face itself while hiding hair and ears. Finally, Winmorph 3.01 (http://www.debugmode.com/winmorph/) was used to gradually transform each neutral expression into an emotional expression of the same individual face, resulting in expressions whose emotional intensity ranged from 0% (100% neutral) to 100% (0% neutral). During the emotion recognition task, the faces were presented on a 20” computer screen (resolution: 1024 x 768 pixels) against a gray background. The viewing distance was held constant at 55 cm by fixating participants’ head on a chin rest. Each trial started with a fixation cross (1 s), followed by a face with a gradually changing expression (800 ms per 5% increase in emotional intensity). Participants were instructed to watch the face and to press a stop button as soon as they detected the onset of an emotional expression. After stopping the presentation, they had to identify the particular

expression (self-paced) by making a forced-choice between four possible emotion labels (happy, angry, fearful, sad). For statistical purposes, the proportion of correctly recognized expressions (recognition accuracy) and the corresponding intensity levels of the expressions (recognition sensitivity or recognition threshold) were determined. The experiment was run on a Microsoft Windows XP operating system using Presentation 12.1 (Neurobiobehavioral Systems Inc., Albany, CA, USA).

3.3.2. Static emotion recognition task

The emotion recognition task used in Study III involved the masked presentation of faces with static expressions. The faces were selected on basis of a validation study from the KDEF database (Goeleven, De Raedt, Leyman, & Verschuere, 2008) and consisted of eight male and eight female faces showing a neutral, happy or angry expression. One male and one female face with a neutral expression were also added to the faces set. The faces were prepared for the emotion recognition task using Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA, USA) and Matlab 7.7 (MathWorks Inc., Natick, MA, USA). After converting the faces into grayscales, they were equalized in size and cumulative brightness. Finally, they were enclosed in a rectangular mask (286 x 269 pixels) that only revealed the face itself while hiding the hair and ears. During the emotion recognition task, the faces were presented on a 17” computer screen (resolution: 800 x 600 pixels) against a gray background. Each trial started with a fixation cross (1 s) and a blank screen (100 ms). Then a face with a neutral, happy or angry expression was presented for 18, 35 or 53 ms, immediately followed by a face with a neutral expression which served as a “mask” (165 ms). The faces were randomly presented in a block-wise fashion, with 12 neutral, happy and angry expressions per block. Prior to each block, participants were informed whether the target expression was a happy or an angry one (4 happy blocks, 4 angry blocks). The neutral expression always served as a distractor. After the presentation of each expression, they had to indicate whether the target

expression had been shown or not (3 s). For statistical purposes, the proportion of correctly recognized expressions in terms of hits and false alarms was determined. On basis of these hit and false alarm rates, indices for recognition accuracy (*d'*) and response bias (*c*) were calculated (Snodgrass & Corwin, 1988). The experiment was run on a Microsoft Windows XP operating system using Matlab 7.7 (MathWorks Inc., Natick, MA, USA).

3.4. Eye-tracking

3.4.1. Data acquisition

In Study I and Study II a remote eye-tracker (ViewPoint PC-60 Head-Fixed; Arrington Research Inc., Scottsdale, AZ, USA) was used to record participants' gaze behavior during face processing. Gaze position was sampled at a rate of 60 Hz with a spatial resolution of 0.15° for tracking resolution and 0.25°-1.0° for gaze position accuracy. Pupil size was also sampled at a rate of 60 Hz with an overall accuracy of 0.03 mm. The recorded data was then analyzed with in-house software written in Matlab 7.13 (MathWorks Inc., Natick, MA, USA).

3.4.2. Data analysis with respect to fixation patterns

In Study I gaze changes were analyzed using GazeAlyze (Berger, Winkels, Lischke, & Hoppner, 2012), a software package written in Matlab 7.13 (MathWorks Inc., Natick, MA, USA)[10]. After smoothing of the data, blinks were interpolated as longs as the blink period did not exceed more than 20% of a trial with correctly recognized expressions. Otherwise, data for this trial was excluded from the analysis. Fixations were coded when gaze remained for at least 100 ms within an area with a size of 1°. Using these criteria, the number and duration of fixations was determined for distinct facial regions, including the face itself (elliptic mask: 411 x 750 pixels), the mouth (rectangular mask: 223 x 87 pixels) and the eyes (rectangular

[10] Initially, the eye-tracking data was analyzed with in-house software written in Matlab 7.7 (MathWorks Inc., Natick MA, USA). The software used algorithms implemented in ILAB (Gitelman, 2002), which were later incorporated into the software package GazeAlyze (Berger, et al., 2012).

mask: 354 x 123 pixels). For statistical purposes, the duration and number of fixations to the face relative to the background, to the eyes relative to the face and to the mouth relative to the face were calculated. In order to investigate OXT effects on gaze behavior over the time course of expression formation, these ratios were determined for fixations occurring during the first 1.6 s of a trial (initial fixation changes) as well as for fixations occurring throughout the whole trial (overall fixation changes).

3.4.3. Data analysis with respect to pupillary responses

In Study II pupillary responses were analyzed using an unpublished software package written in Matlab 7.13 (MathWorks Inc., Natick, MA, USA). After smoothing of the data and blink interpolation, pupil dilation difference scores (baseline correction) were determined for trials with correctly recognized expressions. For each trial, the pupil diameter of the 200 ms preceding the onset of an emotional expression was subtracted from the pupil diameter following the onset of an emotional expression (interval of interest ranging from 800 ms to 3200 ms after expression onset). The difference in pupil diameter was used for statistical purposes.

3.5. Statistical analyses

All statistical analyses were performed using SPSS 20 (IBM SPSS Inc., Chicago, IL, USA)[11]. Most analyses relied on (mixed-design) analyses of variance (ANOVAs), whose significant main and interaction effects were further specified by sub-ANOVAs and *t* tests for dependent and independent samples. Additional analyses, in particular exploratory ones, involved *t* tests for dependent and independent samples, zero-order correlations and multiple

[11] Although different versions of SPSS (IBM SPSS Inc., Chicago, IL, USA) were used for the statistical analyses in the three studies, a re-analysis of the respective data with the latest SPSS version, that is SPSS 20 (IBM SPSS Inc., Chicago, IL, USA), revealed similar results. However, this thesis only provides a summary of the most important results. A detailed description of all results is given in the respective passages of the corresponding publications (see Appendix).

regressions. Whenever appropriate, the degrees of freedom were adjusted to control for violations of the homoscedasticity and sphericity assumption. The significance level for all analyses was set at $p < .05$ (two-sided). In case of (marginally) significant results, Cohen's *f* or Cohen's *d* are reported as effect size estimates (Cohen, 1988).

4. Results

4.1. Participant characteristics

None of the participants reported a significant illness in the interview or scored in the clinical range of the self-report questionnaires. Consequently, there were no differences between participants of the OXT and PLC group in terms of age, general intelligence or psychopathology [Study I and Study II: $F < 2.72$, $p > .05$ for all effects; Study III: $F < 0.09$, $p > .05$ for all effects].

4.2. Substance application

There were some changes in participants' wakefulness [Study I and Study II: $F < 1.55$, $p > .05$ for all main effects of group and group by time interactions, $F(1,45) = 38.94$, $p < .05$, $f = 0.92$ for main effect of time; Study III: $F < 0.54$, $p > .05$ for all group- and time-related main effects and interactions] but not mood [Study I, Study II and Study III: $F < 3.02$, $p > .05$ for all group- and time-related main effects and interactions] or calmness [Study I, Study II and Study III: $F < 1.43$, $p > .05$ for all group- and time-related main effects and interactions] ratings over the time course of substance application. However, these ratings did not differ between participants of the OXT and PLC group, indicating that the observed changes were time- rather than substance-dependent. Accordingly, participants reported, if at all, only subtle side effects (e.g., tiredness, weariness) following substance application.

As expected, there were substantial changes in participants' peripheral OXT levels throughout substance application[12]: While OXT levels were comparable between participants of the OXT and PLC group before substance application, they differed between participants after substance application, with participants of the OXT group displaying higher OXT levels

[12] Although changes in peripheral OXT levels could only be investigated in Study I and Study II, it may be assumed that similar changes occurred in Study III because all studies used the same substance application protocol.

than participants of the PLC group [Study I and Study II: $F < 3.79$, $p > .05$ for all time- and group-related main effects; $F(1,45) = 26.94$, $p < .001$, $f = 0.76$ for group by time interaction].

4.3. Emotion recognition under conditions of unlimited awareness

4.3.1. Emotion recognition

Participants of the OXT group recognized all emotional expressions at lower intensity levels than participants of the PLC group, indicating that OXT generally rather than specifically enhanced participants' recognition sensitivity [Study I and Study II: $F > 4.86$, $p < .05$ for all main effects of group; $F < 1.32$, $p > .05$ for all group by emotion interactions]. There was, however, some evidence suggesting that these effects were more pronounced for fearful [Study I: $t(45) = 2.67$, $p < .05$, $d = 0.59$ for both, male and female faces; Study II: $t(45) = 1.97$, $p < .06$, $d = 0.57$ and $t(45) = 1.83$, $p < .07$, $d = 0.54$ for male and female faces, respectively] and angry [Study I: $t(45) = 2.01$, $p < .05$, $d = 0.78$ for both, male and female faces; Study II: $t(45) = 2.17$, $p < .05$, $d = 0.64$ and $t(45) = 2.73$, $p < .001$, $d = 0.80$ for male and female faces, respectively] expressions than for all other expressions [Study I and Study II: $t < 1.73$, $p > .05$ for all faces with happy and sad expressions]. Besides this, participants' recognition thresholds were generally low for happy expressions, moderate for angry and fearful expressions and high for sad expressions [Study I and Study II: $F > 75.04$, $p < .001$, $f > 1.29$ for all main effects of emotion]. Of note, these effects were essentially the same for male and female faces, indicating that the sex of the faces had no substantial effect on participants' recognition sensitivity [Study II: $F < 1.80$, $p > .05$ for all sex-related main effects and interactions].

Participants of the OXT and PLC group did not differ from one another with regard to the number of correctly recognized expressions, indicating that OXT had no general effect on participants' recognition accuracy [Study I and Study II: $F < 1.54$, $p > .05$ for all main effects of group and group by emotion interactions]. However, there was some evidence suggesting

that OXT specifically enhanced participants' recognition accuracy for fearful [Study I: $t(45) = 2.36$, $p < .05$; $d = 0.69$ for both, male and female faces; Study II: $t(45) = 1.83$, $p < .08$, $d = 0.54$ and $t(37.87) = 2.14$, $p < .05$, $d = 0.64$ for male and female faces, respectively] and sad [Study II: $t(45) = 1.83$, $p < .10$, $d = 0.49$ and $t(45) = 1.95$, $p < .06$, $d = 0.57$ for male and female faces, respectively] expressions as compared to all other expressions [Study I: $t < 0.13$, $p > .05$ for all faces with happy, angry or sad expressions; Study II: $t < 1.58$, $p > .05$ for all faces with happy or angry expressions]. Apart from that, participants' recognition accuracy was generally high for happy expressions, moderate for angry and fearful expressions and low for sad expressions [Study I and Study II: $F > 34.42$, $p < .001$, $f > 0.87$ for all main effects of emotion]. Interestingly, these effects differed for male and female faces, indicating that the sex of the faces had an effect on participants' recognition accuracy [Study II: $F > 4.41$, $p < .001$, $f > 0.31$ for main effect of sex and emotion by sex interaction; $F(1,45) = 0.63$, $p > .05$ for group by sex interaction]. Participants were generally more accurate in recognizing happy expressions in male as compared to female faces and more accurate in recognizing angry, fearful and sad expressions in female as compared to male faces. Moreover, OXT even appeared to differentially affect participants' recognition accuracy for distinct expressions in male and female faces [Study II: $F(2.25,101.25) = 6.93$, $p < .001$, $f = 0.39$ for group by emotion by sex interaction]: There was some, albeit not substantial, evidence suggesting that the aforementioned effects of OXT on participants' recognition accuracy for fearful and sad expressions were more pronounced during the processing of female than male faces [Study II: $t(45) = 1.83$, $p < .08$, $d = 0.54$ and $t(37.87) = 2.14$, $p < .05$, $d = 0.64$ for male and female faces with fearful expressions, $t(45) = 1.83$, $p < .10$, $d = 0.49$ and $t(45) = 1.95$, $p < .06$, $d = 0.57$ for male and female faces with sad expressions, respectively].

4.3.2. Fixation patterns

The duration of fixations that were initially or subsequently directed to a face did not

differ between participants of the OXT and PLC group, neither when considering fixations in general nor when considering expression-specific fixations [Study I: $F < 1.27$, $p > .05$ for all emotion- and group-related main effects and interactions]. Similarly, there were no differences between participants of the OXT and PLC group regarding the duration of fixations that were initially or subsequently directed to distinct facial regions [Study I: $F < 0.84$, $p > .05$ for all group-related main effects and interactions]. However, participants' fixation duration was generally longer for the eye than for the mouth region of a face [Study I: $F > 200.98$, $p < .001$, $f > 2.22$ for all main effects of region], especially during the processing of angry, fearful and sad as compared to happy expressions [Study I: $F > 13.18$, $p < .001$, $f > 0.57$ for all region by emotion interactions and $F < 1.018$, $p > .05$ for all main effects of emotion].

Although OXT had no substantial effect on participants' fixation patterns, there was some, albeit limited, evidence suggesting that OXT mediated the association between participants' fixation patterns and emotion recognition performance. Accordingly, OXT may have specifically enhanced participants' recognition sensitivity for sad expressions by increasing the duration of fixations that were directed to the eye region of these expressions [Study I: $t(144) = 2.14$, $p < .05$; $r(20) = -.52$, $p < .05$ for OXT-mediated association between overall fixation duration and recognition sensitivity for sad expressions; $t(144) < 1.52$, $p > .05$ for all other OXT-mediated associations between fixation duration and recognition sensitivity or recognition accuracy].

4.3.3. Pupillary responses

Participants of the OXT group showed greater pupil dilation during emotion recognition than participants of the PLC group [Study II: $F(1,45) = 4.06$, $p = .05$, $f = 0.29$ for main effect of group], in particular during the processing of male as compared to female faces [Study II: $F > 5.36$, $p < .05$, $f > 0.35$ for main effect of sex and group by sex interaction].

These effects were essentially the same for happy, angry, fearful and sad expressions, indicating that the emotional valence of a particular expression had no substantial effect on participants' pupillary responses [Study II: $F < 1.29$, $p > .05$ for all emotion-related effects and interactions]. Importantly, baseline differences in participants' pupil size did not account for the observed changes in pupil size [Study II: $F < 1.60$, $p > .05$ for all group-, sex- and emotion-related main effects and interactions].

4.4. Emotion cognition under conditions of limited awareness

Participants of the OXT group recognized more expressions, especially positive ones, than participants of the PLC group, indicating that OXT specifically enhanced participants' recognition accuracy for happy expressions [Study III: $F > 4.55$, $p < .05$, $f > .29$ for main effect of group and group by emotion interaction; $F < 2.97$, $p < .05$ for group by time interaction and group by time by emotion interaction]. However, happy expressions were generally better recognized than angry expressions, in particular with increasing presentation times [Study III: $F > 3.75$, $p < .05$, $f > .26$ for main effect of emotion, main effect of time and time by emotion interaction]. Differences in task difficulty may, thus, have mediated the observed effects of OXT on participants' recognition accuracy.

Besides this, participants' recognition accuracy was biased in a conservative rather than liberal direction, particularly during the processing of happy expressions that were presented at longer presentation times [Study III: $F > 8.25$, $p < .05$, $f > 0.39$ for main effect of emotion, main effect of time and time by emotion interaction]. This bias was more pronounced for participants of the OXT than PLC group, albeit equally strong for angry and happy expressions [Study III: $F(2,108) = 5.01$, $p < .05$, $f > 0.39$ for group by time interaction; $F < 2.82$, $p > .05$ for main effect of group, group by emotion interaction and group by time by emotion interaction].

5. Discussion

Previous studies investigating the effects of OXT on social cognition revealed that OXT improves the recognition of and memory for facial expressions under various conditions (Bartz, et al., 2011; Guastella & MacLeod, 2012), probably due to attention- and emotion-related activity changes in the amygdala and superior colliculi (Zink & Meyer-Lindenberg, 2012). The respective findings suggest that various mechanisms, either in concert or alone, contribute to OXT effects on social cognition: OXT may generally facilitate face processing by overtly and/or covertly directing attention to distinct facial features (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008; Leknes, et al., 2012) and OXT may specifically facilitate the processing of positive as compared to negative expressions by changing the affective value of these expressions (Di Simplicio, et al., 2009; Gamer, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010). The aim of the present thesis was to investigate whether OXT in fact improves face processing via the proposed mechanisms. To this end, three studies were performed to examine how OXT affects the recognition of positive and negative expressions under conditions of unlimited (Study I, Study II) and limited (Study III) awareness.

5.1. Emotion recognition under conditions of unlimited awareness

In Study I and Study II, participants' gaze behavior was recorded while they performed an emotion recognition task that involved the presentation of dynamically changing expressions. The studies investigated whether OXT differentially affected participants' recognition sensitivity and recognition accuracy for positive and negative expressions and whether these effects were accompanied by shifts in overt (changes in gaze behavior in terms of fixation patterns) and/or covert (changes in pupil size in terms of pupillary responses) attention

5.1.1. Emotion recognition

In Study I and Study II, OXT enhanced participants' recognition sensitivity for all expressions, irrespective of the expressions' emotional valence, but had no effect at all on participants' recognition accuracy. Importantly, there was no evidence indicating that OXT specifically improved the recognition of positive as compared to negative expressions. On the contrary, there was even some evidence suggesting that OXT improved the recognition of negative rather than positive expressions: OXT effects on emotion recognition were more pronounced during the processing of angry and fearful than happy expressions. However, due to the exploratory nature of the respective analyses, these findings should be regarded as preliminary unless replicated in future studies. Consequently, there was more evidence indicating that OXT generally rather than specifically affected participants' recognition accuracy.

Previous studies, in contrast, reported specific and not general effects of OXT on face processing (Di Simplicio, et al., 2009; Fischer-Shofty, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010). Moreover, most of these studies found that OXT specifically affected the processing of happy instead of angry or fearful expressions (Di Simplicio, et al., 2009; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010). However, these studies used static expressions for the emotion recognition task, whereas dynamic expressions were used in the present studies. Dynamically changing expressions convey more emotion-specific information than static ones (Ambadar, Schooler, & Cohn, 2005), which may explain why participants' recognition accuracy was quite high in these studies. This was most evident during the processing of happy expressions, which were already perfectly recognized at low intensity levels (ceiling effects). OXT may, thus, have had no chance to further improve participants' recognition accuracy for these expressions in the present studies. Although the majority of previous studies demonstrated that OXT specifically improved the

recognition of happy expressions (Di Simplicio, et al., 2009; Marsh, et al., 2010), one study found OXT that specifically improved the recognition of fearful expressions (Fischer-Shofty, et al., 2010), albeit in terms of recognition accuracy and not recognition sensitivity. Interestingly, this was the only study that also used dynamic expressions for the emotion recognition task. However, this study used partial displays of faces that only revealed the eye region during expression formation, whereas full displays of faces were shown in the present studies. The use of partial face displays may have biased emotion recognition towards expressions whose recognition critically depend on the eye region, such as fearful expressions (Adolphs, 2008). This may explain why OXT specifically enhanced participants' recognition accuracy for fearful expressions in this study, while leaving their recognition accuracy for other expressions unaffected (Fischer-Shofty, et al., 2010). However, in the same study, OXT had no effect on participants' recognition sensitivity for fearful expressions, which seems to be at odds with the aforementioned explanation. The absence of a change in participants' recognition sensitivity was most likely due to the rapid unfolding of expressions (ceiling effects). In the present studies, on the contrary, the expressions were unfolding less rapidly, thereby leaving room for OXT to improve participants' recognition sensitivity. Differences in task design may, thus, account for the divergent effects of OXT on participants' recognition sensitivity.

5.1.2. Fixation patterns

In Study I, OXT had no effect at all on participants' gaze changes that initially or subsequently occurred throughout face processing. Accordingly, gaze changes did not appear to account for participants' improvements in emotion recognition following OXT administration. In fact, there was only some evidence suggesting that OXT enhanced participants' recognition sensitivity for sad expressions by directing participants' gaze to the eye region of these expressions. It remains unclear why this effect was limited to sad

expressions, considering that expression-specific effects of OXT on emotion recognition and eye gaze have so far only been reported for fearful (Fischer-Shofty, et al., 2010; Gamer, et al., 2010) and happy (Di Simplicio, et al., 2009; Gamer, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010) expressions. In this regard, it is important to note that this finding emerged in a series of exploratory follow-up analyses, rendering it preliminary unless replicated in future studies.

Although previous studies have never explicitly tested whether OXT effects on emotion recognition are mediated by changes in gaze behavior, they nonetheless demonstrated that OXT enhanced eye gaze under various conditions (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008)[13].The failure to find an effect of OXT on participants' gaze behavior in the present study is, thus, in sharp contrast with previous studies. However, these studies used static expressions, whereas dynamically changing expressions were used in the present study. Besides this, the expressions were much longer presented in the present study than in the other studies. Of note, the distribution of emotion-specific features differs remarkably between static and dynamic expressions (Ambadar, et al., 2005). Normally, the eye region conveys the most important features for emotion recognition (Schyns, et al., 2002; Schyns, et al., 2007, 2009; Smith, et al., 2005). This is particular true for static expressions, but in dynamic expressions such features are also provided by other regions, such as the mouth region (Kennedy & Adolphs, 2010). OXT may, thus, direct gaze to eye region of static rather than dynamic expressions, especially when time is too short to gaze to other regions than those that provide the most relevant information for emotion recognition. This may explain why OXT enhanced eye gaze in previous studies (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008) but not in the present study. Accordingly, other attention-related

[13] Enhanced eye gaze following OXT application has also been reported in a study investigating OXT effects in ASD (Andari et al., 2010), suggesting that OXT may have similar effects on eye gaze in individuals with (Andari, et al., 2010) and without (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008) mental disorders.

processes than those involving gaze changes must have mediated OXT effects on emotion recognition in Study I. Considering that gaze changes indicate shifts in overt rather than covert attention, it may be possible that covert attention shifts accounted for the observed effects.

5.1.3. Pupillary responses

In Study II, OXT increased participants' pupil diameter in response to all emotional expressions, in particular to those expressions that were displayed by male as compared to female faces. Interestingly, pupil dilation was generally greater for female than male faces, indicating that OXT increased pupil size during the processing of male faces rather than decreasing pupil size during the processing of female faces. Notably, changes in pupil size are indicative of shifts in covert attention, implying that OXT generally enhanced participants' attention during face processing, especially for those expressions that usually attract less attention (e.g., happy expressions displayed by male faces).

Similar effects of OXT on pupillary responses have recently been reported in a study investigating OXT effects on implicit and explicit emotion recognition (Leknes, et al., 2012). In this study, OXT also increased participants' pupil size for all expressions, even for those expressions whose emotional valence has been rendered 'invisible' by manipulating the low- and high-frequency spectrum of the expressions. Moreover, these changes in pupil size were related to participants' improvements in recognition accuracy following OXT administration. It may, thus, indeed be possible that shifts in covert rather than overt attention contributed to OXT effects on emotion recognition in Study I.

5.2. Emotion recognition under conditions of limited awareness

In Study III, participants performed an emotion recognition task that involved the presentation of expressions whose emotional valence was subsequently masked by neutral

expressions. The expressions were presented very briefly, ruling out that gaze changes may have occurred during face processing[14]. Like in Study I and Study II, the effects of OXT on participants' recognition accuracy for positive and negative expressions were investigated. However, in contrast to Study I and Study II, it was possible to study these effects in the absence of gaze changes.

OXT enhanced participants' recognition accuracy for all expressions, irrespective of the expressions' emotional valence or presentation time. There was some evidence suggesting that this effect was more evident during the processing of happy than angry expressions, but this was probably due to the fact that happy expressions were generally better recognized than angry expressions, in particular with increasing presentation times (ceiling effects). Taking these differences in task difficulty into account, OXT generally rather than specifically affected participants' recognition accuracy, presumably in a conservative rather than liberal way.

Although it remains inconclusive whether OXT specifically improved the recognition of positive as compared to negative expressions, the present study revealed that OXT had similar effects on emotion recognition as in previous studies (Di Simplicio, et al., 2009; Fischer-Shofty, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010). Previous studies suggested that these effects were due to gaze changes during face processing (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008), but the present study showed that these effects were even present in the absence of gaze changes. Consequently, other attention-related processes than those involving shifts in overt attention must have mediated OXT effects on emotion recognition in Study III.

[14] Fixations generally have a latency of 100-200 ms following stimulus onset and usually have to last at least 100 ms to be further processed in brain areas involved in face processing (Noton & Stark, 1971). Since the emotional expressions were presented for less than 100 ms and were subsequently masked with a neutral expression, it was quite unlikely that fixations actually occurred during expression presentation.

5.3. Mechanisms underlying the effects of oxytocin on emotion recognition

The present studies demonstrated that OXT improved emotion recognition under conditions of unlimited (Study I, Study II) and limited awareness (Study III), irrespective of the expressions' emotional valence (Study I, Study II, Study III). Previous studies, on the contrary, revealed expression-specific effects of OXT on emotion recognition (Di Simplicio, et al., 2009; Fischer-Shofty, et al., 2010; Marsh, et al., 2010). Most of these studies found that OXT improved the processing of positive relative to negative expressions (Di Simplicio, et al., 2009; Marsh, et al., 2010), although specific effects for negative expressions have also been reported (Fischer-Shofty, et al., 2010). In the present studies, however, there was no convincing evidence indicating that OXT differentially affected the processing of positive and negative expressions.

Previous studies suggested that OXT improves emotion recognition by enhancing overt attention for distinct facial features (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008). However, these studies only showed that OXT directs gaze to the eye region, but did not explicitly test whether gaze changes actually lead to improvements in emotion recognition. Study I was explicitly designed to address this issue, but failed to provide substantial evidence indicating that gaze changes accounted for OXT effects on emotion recognition. Moreover, Study III demonstrated that OXT improved emotion recognition even under conditions that precluded gaze changes. Accordingly, it seems possible that OXT effects on emotion recognition do not necessarily have to involve shifts in overt attention. Shifts in covert attention, for example, may also account for OXT effects on emotion recognition as suggested by a recent study examining how OXT affects pupillary responses to emotional expressions (Leknes, et al., 2012). Study II replicated this study by showing that OXT-induced improvements in emotion recognition were accompanied by changes in pupil size. Of note, Study I and Study II were based on data obtained during the same experimental

session, suggesting that OXT affected emotion recognition via covert rather than overt shifts in attention. It may also be possible that shifts in covert attention accounted for OXT effects on emotion recognition in Study III, but this has to remain speculative because pupillary responses were not recorded in this study. Nonetheless, the present studies strongly suggest that OXT improves emotion recognition even in the absence of overt attention shifts, probably due to shifts in covert attention.

Although these conclusions appear to be quite plausible, they should be treated with caution for several reasons. First of all, the sample sizes in all three studies may have been too small to reveal more subtle effects of OXT on emotion recognition and attentional orienting. OXT effects during the processing of positive and negative expressions in male and female faces, for instance, may have become more pronounced in larger samples. Second, the samples of the first two studies were overlapping, indicating that the respective findings were not independent from one another. However, using non-overlapping samples may have limited a thorough investigation of the differential effects of OXT on covert and overt attention shifts during the same emotion recognition task. Third, the samples in all studies consisted of males but not females. The present findings can, therefore, not be generalized to females, which may be problematic considering that OXT appears to exert sex-specific effects on social cognition (Domes, et al., 2010; Lischke, Gamer, et al., 2012a, 2012b). Fourth, changes in pupil size and gaze behavior were only recorded in the first two studies but not in the third study. Although the task design of the latter study may have precluded gaze changes during face processing, it cannot entirely be ruled out that gaze changes did not account for OXT effects on emotion recognition. Similarly, it can only be speculated that changes in pupil size contributed to improvements in emotion recognition. Fifth, the modulatory effect of OXT on brain activity was not assessed in any of the present studies. Consequently, it remains open whether OXT effects on face processing are indeed mediated by attention- and emotion-

related activity changes in the amygdala and superior colliculi (Zink & Meyer-Lindenberg, 2012). Sixth, genetic alterations of the OXT system were also not assessed in the present studies, leaving open whether genetic variants of the OXT-R or CD38 gene influenced OXT effects on emotion recognition and attention allocation (Ebstein, et al., 2012). Taking these limitations into account, it becomes obvious that future studies are needed to further investigate how OXT affects social cognition, preferably at both, the behavioral and neural level.

6. Conclusion

The present studies demonstrated that OXT improves emotion recognition under conditions of unlimited (Study I, Study II) and unlimited (Study III) awareness, presumably by initiating shifts in overt (Study I) and/or covert (Study II, Study III) attention during face processing. Although these findings should be treated with caution unless replicated and extended in future studies, they may nonetheless be relevant for researchers investigating OXT effects on social cognition as well as for clinicians treating individuals with social deficits

6.1. Research implications

The findings of the present studies are broadly consistent with those of previous studies, although it remains inconclusive whether OXT effects on emotion recognition and attention allocation are more pronounced during the processing of positive or negative expressions (Di Simplicio, et al., 2009; Domes, Heinrichs, Glascher, et al., 2007; Domes, Heinrichs, Michel, et al., 2007; Fischer-Shofty, et al., 2010; Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008; Kirsch, et al., 2005; Leknes, et al., 2012; Marsh, et al., 2010). Inconsistencies between the present and previous studies are most likely due to methodical differences in terms of sample characteristics (e.g., gender composition), substance application (e.g., spray dosing) or experimental task (e.g., stimulus presentation), indicating that OXT effects on attention and emotion recognition are highly context- and person-dependent (see Tables 1-3 for a detailed account of the studies' methodical differences). Clearly, further studies are warranted that systematically investigate how situational and individual factors influence attention- and emotion-related effects of OXT (Bartz, et al., 2011). In this respect, it may be particularly interesting to compare OXT effects on attention and emotion recognition between males and females because sex-differences in oxytocinergic

functioning have recently been reported (Domes, et al., 2010; Lischke, Gamer, et al., 2012a, 2012b).

Notwithstanding these inconsistencies, the studies as a whole demonstrated that OXT facilitates emotion recognition (Di Simplicio, et al., 2009; Domes, Heinrichs, Michel, et al., 2007; Fischer-Shofty, et al., 2010; Marsh, et al., 2010) and attention allocation (Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008; Leknes, et al., 2012) on various tasks, regardless whether these tasks require basal or complex processing of emotionally relevant information (Bartz, et al., 2011; Guastella & MacLeod, 2012). Accordingly, OXT affects emotion recognition at early perceptual (e.g., detection of emotional expressions) as well as late conceptual (e.g., evaluation of emotional expressions) stages of face processing, possibly more in the context of positive as compared to negative expressions (Guastella & MacLeod, 2012). However, the effects of OXT over the time course of face processing are not completely understood yet (e.g., Guastella, Carson, Dadds, Mitchell, & Cox, 2009), indicating a need for further studies examining how OXT affects the recognition of positive and negative expressions at different processing stages.

Although the present studies suggested that OXT improves emotion recognition by enhancing attention for salient facial features (Bartz, et al., 2011; Guastella & MacLeod, 2012), the exact mechanisms underlying these effects remain to be determined. Previous studies indicated that these effects are mediated by attention- and emotion-related activity changes in the amygdala and superior colliculi (Domes, Heinrichs, Glascher, et al., 2007; Gamer, et al., 2010; Kirsch, et al., 2005), but it is unresolved whether these activity changes are due to direct or indirect actions of OXT on the corresponding receptors (Zink & Meyer-Lindenberg, 2012). In fact, little is known about the expression and distribution of the OXT-R in the human brain (Meyer-Lindenberg, et al., 2011), leaving open whether OXT acts on specific receptors in the amygdala and superior colliculi. Moreover, even if these receptors are

located in the amygdala and superior colliculi, it is largely unknown how other neuropeptides and neurotransmitter influence OXT actions on these receptors (Meyer-Lindenberg, et al., 2011). Consequently, future studies are needed that further investigate how OXT affects amygdala and superior colliculi activity during face processing. These studies should ideally combine imaging (e.g., OXT-R mapping via positron emission tomography), genetic (e.g., OXT-R genotyping via haplotype analysis) and pharmacologic (e.g. OXT-R blocking via antagonists) methods to fully delineate the mechanisms accounting for OXT effects on social cognition on the neural and behavioral level.

6.2. Clinical implications

The present studies suggest that OXT may generally be useful for treating individuals with marked deficits in social cognition, like, for example, individuals suffering from ASD, SAD or BPD (Meyer-Lindenberg, et al., 2011). However, OXT may be particularly relevant for the treatment of ASD because alterations of the OXT system have been found to be implicated in the disorders' etiology and pathogenesis (Bartz & Hollander, 2008; J. J. Green & Hollander, 2010). For example, individuals with ASD show decreased OXT (Andari, et al., 2010; Modahl et al., 1998) and increased OXT precursor plasma levels (L. Green et al., 2001), indicating abnormalities in OXT synthesis. Accordingly, genetic variants of the CD38 gene, a protein that is involved in the regulation of OXT synthesis (Jin et al., 2007), are related to decreased OXT plasma levels in ASD (Munesue et al., 2010). Moreover, genetic variants of the CD38 (Lerer et al., 2010; Munesue, et al., 2010) and the OXT-R (e.g., Jacob et al., 2007; Lerer et al., 2008; Wermter et al., 2010; Wu et al., 2005; Ylisaukko-oja et al., 2006; Yrigollen et al., 2008) gene are associated with the presence of ASD, suggesting that genetic alterations of OXT synthesis and OXT transmission confer risk for ASD (Ebstein, et al., 2012). Increasing OXT availability by exogenously administering OXT may, therefore, help to ameliorate ASD-related deficits in social cognition, such as difficulties in emotion

recognition (e.g., Ashwin, Chapman, Colle, & Baron-Cohen, 2006; Baron-Cohen, et al., 2001; Clark, Winkielman, & McIntosh, 2008; Humphreys, Minshew, Leonard, & Behrmann, 2007) or abnormalities in gaze behavior (e.g., Corden, Chilvers, & Skuse, 2008; Kirchner, Hatri, Heekeren, & Dziobek, 2010; Kliemann, Dziobek, Hatri, Steimke, & Heekeren, 2010). Interestingly, these deficits are associated with structural and functional alterations of the amygdala and superior colliculi (e.g., Dalton et al., 2005; Dziobek, Bahnemann, Convit, & Heekeren, 2010; Kleinhans et al., 2011; Kliemann, Dziobek, Hatri, Baudewig, & Heekeren, 2012; Nacewicz et al., 2006), which are crucial for OXT effects on social cognition (Meyer-Lindenberg, et al., 2011; Zink & Meyer-Lindenberg, 2012). Administering OXT to individuals with ASD may possibly normalize aberrant amygdala and superior colliculi, thereby facilitating emotion recognition and attentional orienting as suggested by the present studies. Notably, it has recently been shown that OXT administration improved emotion detection (Guastella, et al., 2010) and attentional orienting (Andari, et al., 2010) in individuals with ASD, indicating that OXT may indeed be useful for treating social deficits in ASD (Bartz & Hollander, 2008; J. J. Green & Hollander, 2010). Moreover, these studies suggest that OXT may also be helpful for the treatment of other mental disorders that are marked by deficits in social cognition, such as SAD or BPD (Meyer-Lindenberg, et al., 2011).

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Appendix

The appendix contains the author's version of the manuscripts for Study I, Study II and Study III. These manuscripts have already been submitted for publication. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting and other quality control mechanisms may not be reflected in these manuscripts. A published version of the manuscript pertaining to Study I and Study II can be found in *Psychoneuroendocrinology*, Vol. 37, Issue 4, pp. 475-481 and *Psychoneuroendocrinology*, Vol. 39, Issue 9, pp. 1379-1382, respectively. The manuscript pertaining to Study III is currently under review in *Psychophysiology*.

Appendix A (Study I): Lischke, A., Berger, C., Prehn, K., Heinrichs, M., Herpertz, S. C., & Domes, G. (2012). Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected. *Psychoneuroendocrinology*, *37(4)*, 475-481.

Appendix B (Study II): Prehn, K., Kazzer, P., Lischke, A., Heinrichs, M., Herpertz, S. C., & Domes, G. (under review). Effects of intranasal oxytocin on pupil dilation indicate increased salience of socio-affective stimuli. *Psychophysiology.*

Appendix C (Study III): Schulze, L., Lischke, A., Greif, J., Herpertz, S. C., Heinrichs, M., & Domes, G. (2011). Oxytocin increases recognition of masked emotional faces. *Psychoneuroendocrinology*, *36(9)*, 1378-1382.

Appendix A: Study I

Intranasal oxytocin enhances emotion recognition from dynamic facial expressions and leaves eye-gaze unaffected

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Abstract

Previous studies have shown that oxytocin improves the encoding and recognition of facial expressions, which has been proposed to be mediated by an increased exploration of the eye region during face processing. In the present study, we used eye-tracking to assess visual attention to the eye region while participants performed a dynamic facial emotion recognition task. In a double-blind, placebo-controlled between-subjects design, 47 participants received 24 IU of oxytocin (n = 24) or placebo (n = 23). Although oxytocin administration had no effect on participants' visual scanning of the faces, it generally enhanced recognition performance, as the oxytocin group recognized emotional expressions at lower intensity levels than the placebo group. These findings suggest that oxytocin-induced improvement of facial emotion recognition is independent of modulations in overt visual attention.

Keywords: oxytocin, facial emotion recognition, social cognition, emotion, eye-tracking, visual attention

Introduction

The neuropeptide oxytocin (OXT) is well known for its fundamental role in the regulation of social behavior and social cognition in humans (Heinrichs, von Dawans, & Domes, 2009). Considering that the ability to decode another's facial expression is a prerequisite for social interaction, it is not surprisingly that OXT improves the recognition of emotional expressions in static (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009; Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Guastella et al., 2010; Marsh, Yu, Pine, & Blair, 2010; Schulze et al., 2011) and dynamic (Fischer-Shofty, Shamay-Tsoory, Harari, & Levkovitz, 2010) faces; although it remains inconclusive whether emotion recognition is specifically improved for positive (Di Simplicio, et al., 2009; Marsh, et al., 2010; Schulze, et al., 2011) or negative (Fischer-Shofty, et al., 2010) expressions. Other studies have shown that an enhanced exploration of the eye region is likely to improve emotion recognition because the eye region conveys the most relevant cues for emotion recognition (Schyns, Bonnar, & Gosselin, 2002; Schyns, Petro, & Smith, 2007, 2009; Smith, Cottrell, Gosselin, & Schyns, 2005). Based on this, it seems possible that OXT might improve emotion recognition by directing visual attention to the eye region. Although the proposed mediating effect of visual attention for OXT effects on emotion recognition has not been explicitly tested yet, it has been shown that OXT increases gaze to the eye region of neutral (Andari et al., 2010; Guastella, Mitchell, & Dadds, 2008) and emotional (Gamer, Zurowski, & Buchel, 2010) expressions and that OXT enhances the ability to infer an opponent's mental state from subtle cues around the eye region (Domes, et al., 2007; Guastella, et al., 2010). Regarding the neural mechanisms underlying OXT effects on visual attention during emotion recognition, recent studies have demonstrated that the amygdala and superior colliculi are involved in directing visual attention to the eye region of faces (Adolphs & Spezio, 2006; Gamer & Buchel, 2009). Moreover, OXT effects on visual attention and emotion recognition have been shown to be

mediated by the amygdala and superior colliculi (Gamer, et al., 2010).

Encouraged by these findings, we assessed overt visual attention using eye-tracking during a dynamic emotion recognition task in order to investigate whether visual attention mediates the effects of OXT on emotion recognition. We hypothesized that OXT would generally promote emotion recognition from dynamically changing expressions and that OXT would direct visual attention to the eye region of these expressions. In addition, we expected a positive association between emotion recognition performance and visual attention for the eye region.

Materials and methods

Participants

In a double-blind, placebo-controlled between-subjects design, 47 healthy males [age: $M = 26.09$, $SD = 3.41$] were randomly assigned to receive a nasal spray either containing 24 international units (IU) of OXT ($n = 23$; Syntocinon, Novartis, Basel, Switzerland) or placebo (PLC; containing all ingredients except for the neuropeptide; $n = 24$). Randomization was done by the compounding pharmacist. There were no differences between the groups in age, general intelligence, empathy, alexithmyia or psychopathology [all Fs < 2.72, all ps > .11; see Table S1]. Exclusion criteria for all participants were physical or mental illness, use of medication, substance abuse and smoking. All participants provided written, informed consent and were paid for participation. The study was carried out in accordance with the Declaration of Helsinki and was approved by the ethical committee of the University of Rostock.

Experimental procedure

Following a standardized protocol (Domes et al., 2010), participants self-administered

the nasal spray (three puffs per nostril, each containing 4 IU of OXT or PLC) 45 min before the start of the emotion recognition task (Born et al., 2002). Substance-induced changes in mood, calmness and wakefulness were tracked by administering a multidimensional mood questionnaire (Multidimensionaler Befindlichkeitsbogen, MDBF; Steyer, Schwenkmezger, Notz, & Eid, 1997) directly before substance application and directly before the emotion recognition task. After the experimental session, self-report revealed that participants were not able to discriminate OXT and PLC. In addition, participants did not report any side-effects following substance application.

After arrival at the laboratory, a venous catheter was inserted into the participant's forearm. Following a 30 minute catheter habituation period, blood samples were drawn 5 min before and 45 min after substance application into 5 ml EDTA vacutainer tubes to control for differences in OXT resorption. Immediately after collection, the samples were cooled in ice-chilled water at 4° C, 15 min later centrifuged at 4000 rpm (for 5 min at 4° C) and finally stored in a freezer at -20° C. After completion of the study, the samples were shipped on dry ice at -20° C to the Department of Behavioral and Molecular Neuroendocrinology at the University of Regensburg, Germany, where they were extracted and analyzed using a radioimmunoassay (Landgraf, 1981). The assay detection limit was 0.1 pg/sample, cross-reactivity with other related neuropeptides was < 0.7%, intra-assay precision was 7-10% and inter-assay variability was eliminated by measuring all samples within the same assay.

Experimental task

Forty-five min after substance application, participants performed the emotion recognition task. The task involved the use of computer-manipulated images of faces, whose neutral expression was gradually and continuously changing into an emotional one (see Domes et al., 2008). The images were selected on basis of a validation study from the FACES database (Ebner, Riediger, & Lindenberger, 2010) and consisted of six young male and six

young female individuals, each depicting a neutral, happy, angry, fearful and sad expression.

Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA, USA) and Matlab 7.7 (MathWorks Inc., Natick, MA, USA) were used to convert the images into grayscales and to equalize them in size and cumulative brightness. Thereafter, each image was enclosed within an elliptic mask (411 x 570 pixels) that only revealed the face itself. Winmorph 3.01 (http://www.debugmode.com/winmorph/) was used to morph each emotional expression with a neutral expression in 5% increments, resulting in expressions varying in emotional intensity from 0% (neutral) to 100% (emotional). For each individual face four sets of images (happy, angry, fearful and sad expressions) were obtained, each containing 21 images. Each image of a set of expressions was presented for 800 ms, ranging from 0% to 100% of intensity. Each image was presented on a 20" computer screen (screen size: 30.6 cm x 40.8 cm; resolution: 1024 x 768 pixels) against a gray background at a viewing distance of 55 cm (vertical/horizontal visual angle of approx. 17°/23.5°).

Participants were instructed to press a stop button as soon as they recognized the expression of the face. After stopping the presentation, the face remained visible on screen and participants had to identify the particular expression by making a forced-choice between four possible emotion labels (happy, angry, fearful, sad). No time limit was given for the choice of the emotion label. The intensity level at which the expression was detected and the label that was chosen to identify this expression were used for statistical analyses. Of note, only correctly recognized expressions were considered in the analyses involving data from the emotion recognition task (intensity levels, number of recognized expressions).

Eye-tracking

During the emotion recognition task, participants' eye movements were recorded with a remote infra-red eye-tracker with a chin-rest (Viewpoint PC-60 Head Fixed; Arrington Research Inc., Scottsdale, AZ, USA). Raw data were collected at a 60 Hz sampling rate with a

spatial resolution of approx. 0.15° and 0.25°-1.0° for gaze position accuracy.

Algorithms implemented in ILAB (Gitelman, 2002) were used for the analysis of the eye-tracking data. After smoothing of the raw data with a Gaussian filter, blink and artifact detection was performed. Three participants of the OXT group and one participant of the PLC group had to be excluded from the analysis because of more than 20% lost data. There were no differences between the remaining groups with regard to valid data [all Fs < 1.86, all ps > .15]. Fixations were coded when gaze remained for at least 100 ms within an area with a diameter of 1°. Image-specific templates were used to specify regions covering the whole face (elliptic template: 411 x 570 pixels), the eyes (rectangular template: 354 x 123 pixels) and the mouth (rectangular template: 223 x 87 pixels). For each trial, we calculated the mean duration of fixations to the whole face relative to the screen, to the eye region relative to the whole face and to the mouth region relative to the whole face. In order to investigate the initial allocation of visual attention, we also calculated the mean fixation duration for these regions of interest for the first 1600 ms of each trial.

Statistical analyses

All statistical analyses were performed using SPSS 15 (IBM SPSS Inc., Chicago, IL, USA). OXT effects on mental state and emotion recognition were analyzed with two-way repeated measures ANOVAs, while OXT effects on visual attention were analyzed with two- and three-way repeated measures ANOVAs. For all repeated measures ANOVAs, the Greenhouse-Geisser correction was applied to correct for potential violations of the sphericity assumption. Post-hoc analyses of significant main effects and interactions involved t tests and two-way ANOVAs, respectively. Multiple moderated regression analyses were performed to test for the mediating effect of OXT on the association between emotion recognition performance and visual attention for the eye region. The level of significance for all analyses was set at $p < .05$. In case of significant effects, the effect size measure f was reported

according to Cohen (Cohen, 1988).

Results

Substance application

To test whether participants of the OXT and PLC group differed in peripheral OXT levels (in pg/ml) over the time course of substance application (see Table S2), a two-way repeated measures ANOVA (Group x Time) was run [main effect of time: $F(1,45)= 2.46$, $p = .12$; main effect of group: $F(1,45) = 3.79$, $p = .06$; time by group interaction: $F(1,45) = 26.94$, $p < .001$, $f = 0.76$]. OXT administration resulted in a significant increase in peripheral OXT levels [pre: $M = 24.6$, $SD = 19.0$; post: $M = 39.7$, $SD = 20.1$], whereas PLC administration had no such effect [pre: $M = 27.2$, $SD = 16.6$; post: $M = 22.8$, $SD = 16.8$].

Further two-way repeated measures ANOVA (Group x Time) were performed to test for changes in participants' mental state throughout substance application (see Table S2). Participants' mood [main effect of time: $F(1,45) = 2.49$, $p = .12$; main effect of group: $F(1,45) = 1.78$, $p = .19$; group by time interaction: $F(1,45) = 3.02$, $p = .09$] and calmness [main effect of time: $F(1,45) = 1.31$, $p = .26$; main effect of group: $F(1,45) = 0.32$, $p = .86$; group by time interaction: $F(1,45) = 0.03$, $p = .86$] were unaffected by time and substance application. The trend towards a group by time interaction for participants' mood was due to slightly lower post-application mood in the OXT than PLC group [PLC: $M = 4.5$, $SD = 0.4$; OXT: $M = 4.2$, SD = 0.6; $t(45) = 1.92$; $p = .07$]. Although participants' wakefulness declined throughout substance application, this decline was observed in both groups, ruling out that this was due to OXT administration [main effect of time: $F(1,45) = 38.94$, $p < .001$, $f = 0.92$; main effect of group: $F(1,45) = 1.55$, $p = .22$; group by time interaction: $F(1,45) = 0.09$, $p = .77$].

Emotion recognition

A two-way repeated measures ANOVA (Group x Emotion) demonstrated that OXT administration decreased intensity levels at which the expressions were correctly recognized [PLC: $M = 44.2$, $SD = 8.2$; OXT: $M = 39.1$, $SD = 7.6$; see Figure 1A and Table S1], regardless of the particular type of emotion [main effect of group: $F(1,45) = 4.86$, $p = .03$, $f = 0.32$; group by emotion interaction: $F(1,135) = 1.32$, $p = .27$]. In general, participants' recognition thresholds were low for happy expressions, moderate for angry and fearful expressions and high for sad expressions [main effect emotion: $F(3,135) = 77.30$, $p < .001$, $f = 1.31$]. Exploratory follow-up analyses suggested that OXT specifically lowered participants' recognition thresholds for angry [$t(45) = 2.67$, $p < .01$, $d = 0.76$; see Figure 1A and Table S3] and fearful [$t(45) = 2.01$, $p = .05$, $d = 0.59$; see Figure 1A and Table S3] expressions, while leaving recognition thresholds for the other expressions unaffected [all ts < 1.6, all ps > .12; see Figure 1A and Table S3].

Another two-way repeated measures ANOVA (Group x Emotion) indicated that OXT administration had no effect on participants' recognition accuracy [main effect of group: $F(1,45) = 0.29$, $p = .59$; group by emotion interaction: $F(2.25,101.23) = 1.54$, $\varepsilon = .75$, $p = .22$], which was generally high for happy expressions, moderate for angry and fearful expressions and low for sad expressions [main effect of emotion: $F(2.25,101.23) = 38.46$, $\varepsilon = .75$, $p < .001$, $f = 0.92$]. Exploratory follow-up analyses suggested that OXT specifically enhanced participants' recognition accuracy for fearful [$t(45) = 2.36$, $p = .02$; see Figure 1B and Table S3] but not for the other [all ts < 0.60, all ps > .55; see Figure 1B and Table S3] expressions.

[Insert Table 1 and Figure 1 about here]

Visual attention

Initial allocation of visual attention to facial regions

A two-way repeated measures ANOVA (Group x Emotion) showed that OXT administration had no effect on the duration of fixations that were initially directed to the face [main effect of group: $F(1,41) = 0.16$, $p = .69$; main effect of emotion: $F(2.06,84.36) = 2.21$, $\varepsilon = .69$, $p = .12$; group by emotion interaction: $F(2.06,84.36) = 1.11$, $\varepsilon = .69$, $p = .34$; see Table 1]. A subsequent three-way repeated measures ANOVA (Group x Emotion x Region) revealed that the duration of initial fixations to the eye or mouth region was also unaffected by OXT administration [main effect of group: $F(1,41) = 0.18$, $p = .67$; group by emotion interaction: $F(2.38,97.63) = 1.18$, $\varepsilon = .79$, $p = .32$; group by emotion by region interaction: $F(2.42,91.931) = 1.26$, $\varepsilon = .75$, $p = .29$; see Table 1].

Overall allocation of visual attention to facial regions

A two-way repeated measures ANOVA demonstrated that OXT administration had no effect on the overall duration of fixations that were directed to the face [main effect of group: $F(1,41) = 0.10$, $p = .75$; main effect of emotion: $F(1.96,80.19) = 0.24$, $\varepsilon = .65$, $p =. 78$; group by emotion interaction: $F(1.96,80.19) = 0.78$, $\varepsilon = .65$, $p = .46$; see Table 2]. Another three-way repeated measures ANOVA (Group x Emotion x Region) revealed that the overall duration of fixations to the eye or mouth region was also unaffected by OXT administration [main effect of group: $F(1,41) = 0.16$, $p = .70$; group by emotion interaction: $F(3,123) = 0.76$, $p = .52$; group by emotion by region interaction: $F(3,123) = 0.58$, $p = .63$; see Table 2].

Association between visual attention to the eye region and emotion recognition

Separate multiple regression analyses were performed to test whether OXT improved emotion recognition by directing gaze to the eye region of the different emotional expressions. OXT administration moderated the association between the overall duration of fixations to the eye region of sad expressions and the corresponding intensity levels at which

these expressions were correctly recognized [$t(144) = -2.14$, $p = .04$; see Figure 2]: In the OXT group there was an association between eye gaze and recognition thresholds for sad expressions [$r(23) = -.52$, $p = .02$], whereas such an association was absent in the PLC group [$r(23) = .03$, $p = .90$]. No such moderation was found when considering the number of correctly recognized expressions in these analyses.

[Insert Figure 2 about here]

Discussion

The first aim of the present study focused on the effects of intranasal OXT on emotion recognition from dynamic facial expressions. In the present study, OXT decreased the critical intensity at which participants were able to recognize the emotional expressions. In other words, participants of the OXT group, relative to those of the PLC group, were able to correctly recognize all emotional expressions at lower intensity levels, which seemed to be particularly pronounced during the processing of angry and fearful expressions. This effect was not due to more liberal responding, i.e., participants of the OXT group did not respond earlier during the presentation of the dynamic expressions for the price of lower accuracy. Accuracy in general was quite high in the present study and participants of the OXT group tended to make even fewer errors for the fearful expressions than participants of the PLC group, which is in line with previous studies (Fischer-Shofty, et al., 2010). In contrast to previous studies which suggested specific effects of OXT on the recognition of positive (Di Simplicio, et al., 2009; Marsh, et al., 2010) or negative (Fischer-Shofty, et al., 2010) expressions, the present study supports a more general view: OXT seems to enhance emotion recognition from dynamic facial expressions irrespective of the expressions' emotional valence, although the effect was marginally larger for angry and fearful than any other expression. Differences in task sensitivity might in part explain the divergent results. In

contrast to previous studies that used static emotion recognition tasks (Di Simplicio, et al., 2009; Domes, et al., 2007; Marsh, et al., 2010), the present study used a dynamic emotion recognition task that mainly relied on recognition thresholds rather than on error rates. Hence, differences in item difficulty might have been less relevant in the present as compared to previous studies. For example, previous studies have shown that the same task might differ in the sensitivity to detect subtle effects of OXT in different samples (Domes, et al., 2007; Guastella, et al., 2010). Another possible explanation might be differences in the facial stimuli that have been used in these studies. Fisher-Shofty and colleagues (Fischer-Shofty, et al., 2010), for example, presented the eye region of faces and found a specific effect of OXT on recognition accuracy for fearful expressions. We, in contrast, presented whole faces to investigate the role of visual attention for different regions of the face during emotion recognition and found that OXT had a general effect on recognition thresholds for all expressions in addition to a specific effect on recognition accuracy for fearful expressions. The eye region seems to play a crucial role in the recognition of fear and thus might have been the most sensitive emotional category in the previous study (Fischer-Shofty, et al., 2010). Moreover, since OXT has been found to improve memory for facial expressions (Guastella, Mitchell, & Mathews, 2008; Rimmele, Hediger, Heinrichs, & Klaver, 2009), it could have been possible that OXT enhanced memory for prototypical facial expressions in the present study, thereby enhancing emotion recognition by improving the matching between the presented and previously memorized expressions.

The second aim of the present study was to test whether OXT promotes emotion recognition by enhancing overt visual attention for the eye region of emotional expressions. In all, there was no effect of OXT on the duration of fixations that were directed to the face itself or to distinct regions of the face. The lack of an OXT effect on visual attention contradicts previous studies which suggested that OXT promotes eye gaze during face processing

(Andari, et al., 2010; Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008). Differences in stimulus presentation could explain the disparity of findings. In contrast to previous studies, which presented static faces with neutral (Guastella, Mitchell, & Dadds, 2008) and emotional (Andari, et al., 2010) expressions for normal durations or static faces with emotional expressions for very short durations (Gamer, et al., 2010), we used dynamic faces, which introduce the aspect of increasing emotional intensity over the time course of stimulus presentation. In addition, the presentation time of the facial expressions was on average much longer in the present than in the previous studies (Andari, et al., 2010; Gamer, et al., 2010; Guastella, Mitchell, & Dadds, 2008). In static faces, the eyes might be especially salient because they convey crucial information about an individual's emotional state (Emery, 2000) and attention might be captured by the eyes because they are assumed to be the most informative part of the face (Adolphs, Baron-Cohen, & Tranel, 2002). OXT might be especially involved in the initial allocation of attentional resources during face processing. This interpretation is in line with the above mentioned study by Gamer and colleagues (Gamer, et al., 2010), who showed reflexive saccades towards the eyes even for facial expressions that were only presented for 150 ms. In dynamic faces, the effect of OXT on the initial orienting probably becomes less relevant with increasing presentation times of the faces. OXT might, thus, facilitate eye gaze during the processing of static rather than dynamic faces, in particular when the faces are briefly presented. It should be noted, however, that there was a moderate association between eye gaze and recognition sensitivity during the processing of sad expressions in the OXT group.

In light of the limited sample size, the statistical power is too low to draw definite conclusions. Using more sensitive measures of emotion recognition and/or more fine-grained eye-tracking methods might be a promising approach to investigate the association between visual attention and emotion recognition and its modulation by OXT. Future studies could

additionally investigate whether the changing features of dynamic facial expressions modulate visual attention over time and whether OXT modulates attentional capture by these changing features. Another limitation is the fact that we excluded female participants from the study. Future studies could explicitly investigate the sexual dimorphism of OXT effects in the central nervous system as suggested by previous studies (Carter, 2007; Domes, et al., 2010).

In sum, this is the first study that explicitly investigated the possible role of visual attention for the effect of OXT on facial emotion recognition. Consistent with previous studies showing positive effects of OXT on face processing (Di Simplicio, et al., 2009; Domes, et al., 2007; Fischer-Shofty, et al., 2010; Guastella, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Marsh, et al., 2010; Rimmele, et al., 2009; Schulze, et al., 2011), the present study suggests that OXT promotes emotion recognition from dynamic facial expressions, which resemble a more naturalistic interpersonal situation than static facial expressions. With regard to the role of visual attention as a possible mediating factor, the present findings suggest that the positive effect of OXT on emotion recognition might be independent of modulations of visual attention to specific facial regions, at least during the processing of the dynamic facial expressions.

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Conflict of interest

None declared.

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Contributors

A.L., G.D. and S.C.H. designed the study. A.L. and K.P. collected the data. A.L., C.B. and G.D. analyzed the data. A.L. wrote the manuscript. C.B., G.D., M.H., K.P. and S.C.H contributed to writing, reviewing and editing of the manuscript.

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Tables

Table 1

Visual attention during the initial 1600 ms of a trial

	Placebo (*n* = 24)		Oxytocin (*n* = 20)[a]	
	M	*SD*	*M*	*SD*
Happy expressions				
Face/Screen	.95	.16	.99	.03
Eye/Face	.64	.24	.75	.19
Mouth/Face	.02	.08	.01	.03
Angry expressions				
Face/Screen	1.00	.00	1.00	.00
Eye/Face	.75	.14	.72	.15
Mouth/Face	.01	.03	.00	.00
Sad expressions				
Face/Screen	.94	.18	.96	.08
Eye/Face	.69	.23	.69	.20
Mouth/Face	.01	.06	.01	.03
Fearful expressions				
Face/Screen	1.00	.02	.97	.08
Eye/Face	.66	.20	.66	.18
Mouth/Face	.01	.02	.01	.04

Note. [a]Data from three participants of the oxytocin group and one participant of the placebo group were excluded because of massive blink artifacts.

Table 2

Visual attention during the whole trial

	Placebo (n = 24)		Oxytocin (n = 20)[a]	
	M	*SD*	*M*	*SD*
Happy expressions				
Face/Screen	.96	.14	.97	.04
Eye/Face	.44	.15	.50	.16
Mouth/Face	.26	.12	.23	.14
Angry expressions				
Face/Screen	.96	.08	.99	.03
Eye/Face	.59	.20	.66	.16
Mouth/Face	.13	.10	.09	.07
Sad expressions				
Face/Screen	.96	.08	.97	.08
Eye/Face	.60	.20	.63	.14
Mouth/Face	.12	.08	.10	.08
Fearful expressions				
Face/Screen	.98	.04	.96	.07
Eye/Face	.62	.16	.63	.15
Mouth/Face	.15	.09	.11	.09

Note. [a]Data from three participants of the oxytocin group and one participant of the placebo group were excluded because of massive blink artifacts.

Figures

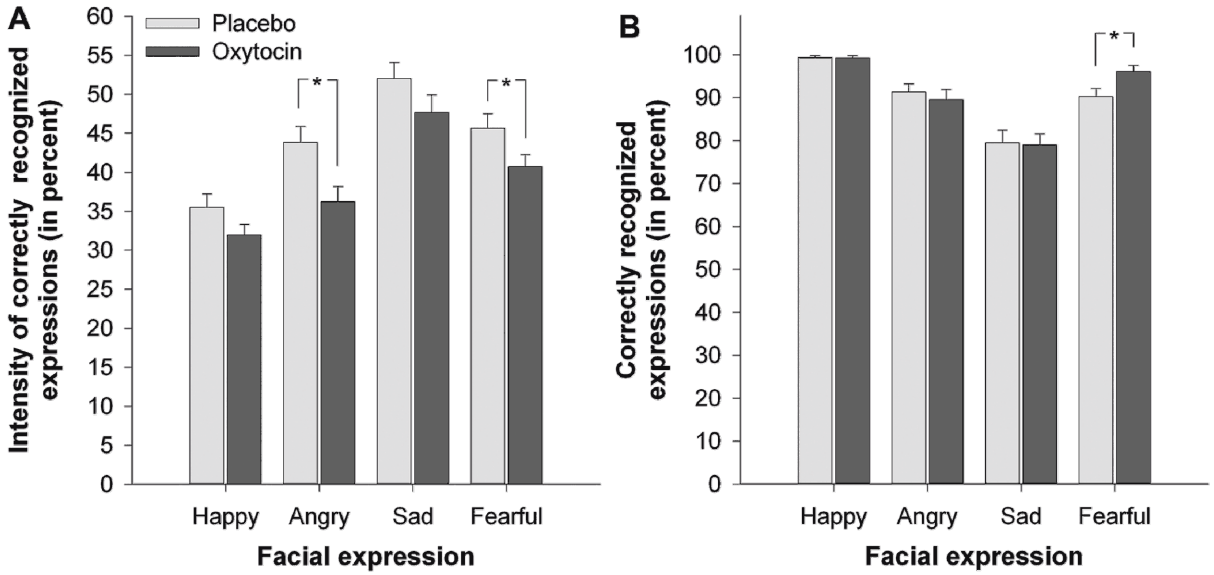

Figure 1. (A) Intensity levels and (B) number of correctly recognized emotional expressions. Asterisks indicate significant post-hoc single comparisons ($p < .05$, two-tailed). Bars represent mean and standard error of the mean in percent.

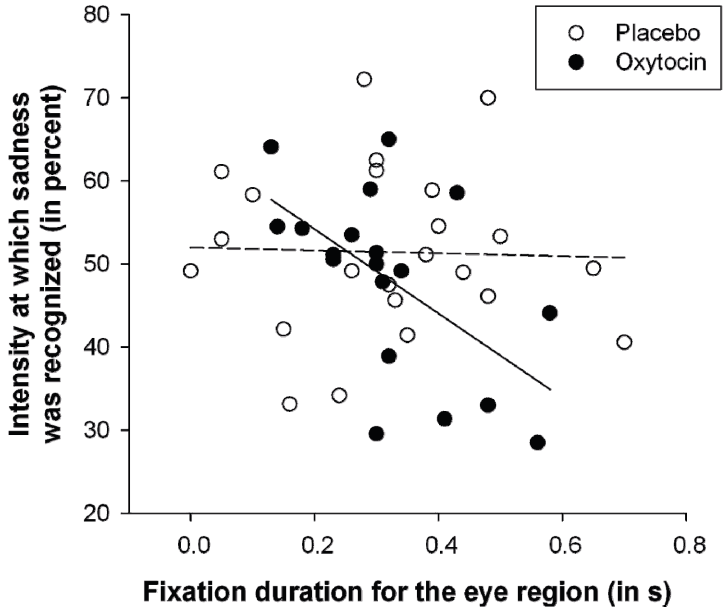

Figure 2. Linear association between overall duration of fixations to the eye region and intensity levels at which sad expressions were correctly recognized. Lines represent linear regression curves for the oxytocin and the placebo group.

Supplemental material

Table S1

Group differences in demographical and psychopathological measures

	Placebo (n = 24)		Oxytocin (n = 23)		Test statistic	
	M	*SD*	*M*	*SD*	*F*(df)	*p*
Age	26.38	3.49	25.78	3.37	$F(1,45) = 0.35$	.56
General intelligence (WST)	33.58	1.41	33.74	1.82	$F(1,45) = 0.11$	.74
General psychopathology (SCL-90-R-GSI)	0.11	0.10	0.21	0.26	$F(1,45) = 2.73$	.11
Anxiety (STAI-T)	31.54	5.38	32.52	6.47	$F(1,45) = 0.32$	.58
Anger (STAXI-T)	15.75	3.34	17.26	4.98	$F(1,45) = 1.50$	.23
Positive affect (PANAS-T)	36.00	3.28	36.13	3.88	$F(1,45) = 0.02$	.90
Negative affect (PANAS-T)	16.08	4.27	16.61	4.50	$F(1,45) = 0.17$	.68
Alexithymia (TAS-20)	39.71	8.30	41.70	10.15	$F(1,45) = 0.54$	.47
Empathy (IRI)	66.83	8.38	65.70	8.40	$F(1,45) = 0.22$	.64

Note. IRI = Interpersonal Reactivity Index (Davis, 1983); PANAS-T = Positive and Negative Affect Scale - Trait Positive and Negative Affect (Watson, Clark, & Tellegen, 1988); SCL-90-R-GSI = Symptom Checklist-90-Revised - Global Severity Index (Franke, 1995); STAI-T = State Trait Anxiety Inventory - Trait Anxiety (Laux, Glanzmann, Schaffner, & Spielberger, 1981); STAXI-T = State Trait Anger Expression Inventory - Trait Anger (Spielberger, 1991); TAS-20 = Twenty-item Toronto Alexithymia Scale (Bagby, Parker, & Taylor, 1994; Bagby, Taylor, & Parker, 1994); WST = Wortschatztest (Schmidt & Metzler, 1992).

Table S2

Group differences in peripheral oxytocin levels and mental state

	Placebo (n = 24)		Oxytocin (n = 23)		Test statistic	
	M	*SD*	*M*	*SD*	*t*(df)	*p*
Peripheral oxytocin levels (pg/ml)						
Pre-application	27.18	16.61	24.63	18.97	*t*(45) = .49	.63
Post-application	22.83	16.91	39.70	20.12	*t*(45) = -4.05	<.001***
Mood (MDBF)						
Pre-application	4.48	0.47	4.42	0.47	*t*(45) = 0.40	.69
Post-application	4.49	0.38	4.21	0.61	*t*(45) = 1.92	.07
Calmness (MDBF)						
Pre-application	4.25	0.65	4.21	0.57	*t*(45) = 0.25	.36
Post-application	4.35	0.60	4.35	0.58	*t*(45) = 0.04	.97
Wakefulness (MDBF)						
Pre-application	3.94	0.72	3.74	0.74	*t*(45) = 0.93	.81
Post-application	3.18	0.84	2.90	0.84	*t*(45) = 1.13	.27

Note. MDBF = Multidimensonaler Befindlichkeitsfragebogen (Steyer, Schwenkmezger, Notz, & Eid, 1997).
***p < .001, two-sided.

Table S3

Group differences in recognition sensitivity and recognition accuracy

	Placebo (n = 24)		Oxytocin (n = 23)	
	M	*SD*	*M*	*SD*
Recognition threshold[s]				
Happy expressions	35.50	8.47	31.92	6.72
Angry expressions	43.81	10.03	36.20	9.45
Sad expressions	51.99	10.20	47.67	10.77
Fearful expressions	45.64	9.22	40.70	7.50
Recognition accuracy[b]				
Happy expressions	11.92	0.28	11.91	0.29
Angry expressions	10.96	1.12	10.74	1.39
Sad expressions	9.54	1.74	9.48	1.50
Fearful expressions	10.83	1.13	11.52	0.85

Note. [a]Intensity levels of correctly recognized expressions (in percent). [b]Number of correctly recognized expressions (in percent).

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Appendix B: Study II

Effects of intranasal oxytocin on pupil dilation indicate increased salience of socio-affective stimuli

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Abstract

To investigate the mechanisms by which oxytocin improves socio-affective processing, we assessed behavioral and pupillometric measures during a dynamic facial emotion recognition task. In a double-blind, between-subjects design, 47 healthy males received either 24 IU intranasal oxytocin or placebo. Participants of the oxytocin group recognized all facial expressions at lower intensity levels than participants of the placebo group. Improved performance in the oxytocin group was associated with greater task-related pupil dilation, indicating increased recruitment of attentional resources. In addition, we found that the female gender-specific stimulus effect diminished in the oxytocin group, in which pupil size was increased for male faces. In sum, our data suggest that improved emotion recognition after oxytocin administration might be due to increased stimulus processing. Oxytocin thereby enhances the salience of stimuli which usually do not recruit much attention.

Keywords: pupillary responses, oxytocin, facial emotion recognition, gender, social cognition

Introduction

The neuropeptide oxytocin (OXT) has been found to play a key role in the regulation of social behavior (Bartz, Zaki, Bolger, & Ochsner, 2011; Guastella & MacLeod, 2012; Heinrichs, von Dawans, & Domes, 2009; Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). OXT is synthesized in the hypothalamus and released into both, the brain and the bloodstream. Apart from its well-known functions in reproduction, such as stimulating uterine contraction during labor and milk ejection, OXT also acts as a neuromodulator with receptors widely distributed in the brain, including the limbic-hypothalamic system, midbrain regions and the brain stem (Landgraf & Neumann, 2004). Since neuropeptides cross the blood-brain barrier after intranasal administration (Born et al., 2002), a number of studies has been conducted in humans demonstrating, for instance, that OXT promotes prosocial behavior, affiliation and trust (e.g., Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), improves social recognition and memory (e.g., Guastella, Mitchell, & Mathews, 2008; Rimmele, Hediger, Heinrichs, & Klaver, 2009; Unkelbach, Guastella, & Forgas, 2008) and attenuates social stress and anxiety (e.g., Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Linnen, Ellenbogen, Cardoso, & Joober, 2012).

As a prerequisite for social cognition, intranasally administered OXT has been consistently found to improve the ability to decode mental and affective state of others' from facial expressions (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009; Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Fischer-Shofty, Shamay-Tsoory, Harari, & Levkovitz, 2010; Lischke, Berger, et al., 2012; Marsh, Yu, Pine, & Blair, 2010; Schulze et al., 2011). It has been argued that improved emotion recognition might be due to an enhanced exploration of the eye region during face processing (Andari et al., 2010; Domes, Heinrichs, Michel, et al., 2007; Guastella et al., 2010; Guastella, Mitchell, & Dadds, 2008). Directly investigating

this hypothesis using eye-tracking and a dynamic facial emotion recognition task, Lischke and colleagues (Lischke, Berger, et al., 2012) recently replicated the effect of enhanced emotion recognition but did not find any OXT-induced alterations in the visual scanning of the faces.

In this study, we aimed to further investigate the mechanisms by which OXT might improve the exploration and recognition of socio-affective stimuli by analyzing pupillometric data during the same dynamic facial emotion recognition task. Pupillometric data, such as pupil diameter, can be used as a sensitive and reliable indicator for cognitive resource consumption. Pupil diameter is controlled by two muscles that are innervated by the sympathetic and parasympathetic branches of the autonomic nervous system, which get their input from brain structures essential for both, affective and cognitive information processing. Increased sympathetic activity increases the activity of the dilator muscle, leading to pupil dilation, whereas decreased parasympathetic activity decreases the activity of the sphincter muscle, which also results in a dilation of the pupil (for a more detailed description of the neural basis of pupillary responses see Hoeks & Ellenbroek, 1993; Steinhauer & Hakerem, 1992). Although increases in pupil diameter can be regulated by either branch of the autonomic nervous system, they are mainly regulated by the parasympathetic branch, whose activity is modulated by the locus coeruleus (Steinhauer, Siegle, Condray, & Pless, 2004).

While *tonic changes* in pupil diameter reflect the sensitivity of the cognitive system in general ("exploration mode"; see Aston-Jones & Cohen, 2005; van der Meer et al., 2010), *phasic changes* have been proven to indicate a stimulus-specific and task-related processing load, with larger pupil dilation reflecting greater processing demands (Beatty, 1982; Loewenfeld, 1993; Steinhauer & Hakerem, 1992). During a digit span recall task, for instance, pupil diameter increases proportionally as a function of the number of digits that have to be maintained in short-term memory – until individuals reach their limit of available cognitive resources (Granholm, Asarnow, Sarkin, & Dykes, 1996; Kahneman & Beatty,

1966). Notably, Just and colleagues (Just, Carpenter, & Miyake, 2003) have demonstrated that pupillary responses reflect an overall aggregate of attentional resource allocation that is not limited to a specific part of the cognitive system. Peak dilation has been found to increase with enhanced processing demand in studies investigating a variety of tasks, comprising language comprehension (e.g., Hyona, Tommola, & Alaja, 1995; Just & Carpenter, 1993), auditory and visual attention (e.g., Karatekin, Couperus, & Marcus, 2004; Kim, Beversdorf, & Heilman, 2000), reasoning and semantic elaboration (e.g., van der Meer, et al., 2010; van der Meer, Friedrich, Nuthmann, Stelzel, & Kuchinke, 2003) or emotional valence identification (e.g., Prehn et al., 2008; Prehn, Heekeren, & van der Meer, 2011; Siegle, Granholm, Ingram, & Matt, 2001; Siegle, Steinhauer, Carter, Ramel, & Thase, 2003). With regard to the processing of visual stimuli with emotional content, very early pupillometric studies have shown an increase in pupil size when people view pleasant and attention-demanding pictures, such as pictures of a baby, a mother holding her child, a partially nude male or female (e.g., Hess & Polt, 1960). Later studies showed that pupil size co-varies with the emotional arousal rather than with the hedonic valence of the pictures (e.g., Bradley, Miccoli, Escrig, & Lang, 2008). Recently, Leknes and colleagues (Leknes et al., 2012) provided first evidence that OXT administration leads to greater stimulus-induced pupil dilation during the identification of subtle and hidden emotional expressions. In that study, participants with lower emotional sensitivity and poorer baseline performance showed greater OXT-induced improvement in addition to larger task-related pupil dilation.

In summary, pupillometric studies support the view that pupil size represents a general index of attentional resource allocation and that pupil size reflects whether stimuli are cognitively engaging or emotionally salient. Following these studies and a first study on the effects of OXT on task-related pupillary responses during face processing, we hypothesized that improved emotion recognition after OXT administration will be accompanied by

increased resource consumption (greater "mental work") as indicated by greater pupil dilation. Given that male and female face stimuli are differently appealing for males, we further expected that OXT will affect pupil dilation differentially during the processing of male as compared to female faces.

Materials and methods

Participants

In a double-blind, placebo-controlled between-subjects design, 47 healthy males were randomly assigned to receive a nasal spray either containing 24 international units (IU)[1] of OXT (n = 23; Syntocinon, Novartis, Basel, Switzerland) or placebo (PLC; containing all ingredients except for the neuropeptide; n = 24). All participants were non-smokers, did not report a current or previous neurological, endocrinological or psychiatric disease and did not take any medication that could have influenced pupillary responses. Participants reported no ophthalmologic problems other than correctable eyesight.

To control for individual differences in general intelligence, empathy, alexithymia and psychopathology, participants completed a test battery including a verbal intelligence test (WST; Schmidt & Metzler, 1992), the Symptom Checklist-90-Revised (SCL-90-R; Franke, 1995), the State Trait Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981), the State Trait Anger Expression Inventory (STAXI; Spielberger, 1991), the Positive and Negative Affect Scales (PANAS; Watson, Clark, & Tellegen, 1988), the Twenty-item Toronto Alexithymia Scale (TAS-20; Bagby, Parker, & Taylor, 1994; Bagby, Taylor, & Parker, 1994) and the Interpersonal Reactivity Index (IRI; Davis, 1983). The psychometric measures revealed no differences between the participant groups in terms of general intelligence, empathy, alexithymia or psychopathology [all Fs < 2.72, all ps > .11]. There

[1] 24 IU is equivalent to approximately 48 μg of the pure neuropeptide.

were also no group differences with regard to participants' age [OXT: $M = 25.78$, $SD = 3.37$; PLC: $M = 26.38$, $SD = 3.49$; $F(1,45) = 0.35$, $p = .56$].

The study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethics committee. All participants gave written informed consent prior to investigation and received payment for participation.

Experimental task

To investigate the mechanisms by which OXT improves the exploration and recognition of socio-affective stimuli, we used a dynamic facial emotion recognition task (Domes et al., 2008; Lischke, Berger, et al., 2012). In this task, participants had to look at male and female faces gradually and continuously changing from neutral to happy, angry, fearful or sad expressions. For this task, we selected four pictures of six young male and six young female faces, showing a neutral, happy, angry, fearful and sad expression, from the FACES database (Ebner, Riediger, & Lindenberger, 2010). The colored pictures were converted into grayscales, equalized in size and cumulative brightness using Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA, USA) and Matlab 7.7 (The MathWorks Inc., Natick, MA, USA). Then, each picture was enclosed within an elliptic mask (411 x 570 pixels) that only showed the face itself. Finally, we used Winmorph 3.01 (http://www.debugmode.com/winmorph/) to transform the neutral expressions into all of the emotional ones in 5% steps. This procedure resulted in 48 sets of pictures [12 (6 male and 6 female) faces x 4 emotional expressions], each containing 21 pictures with increasing emotional intensity from 0% (neutral) to 100% (emotional).

A trial of this task started with a black fixation cross appearing for 1 s on a dark gray background (*baseline phase*). Then, the pictures of a set were presented, starting with 0% intensity and increasing up to 100% in 5% steps (*emotion recognition phase*). Each picture was presented for 800 ms. Participants were instructed to press a stop button as soon as they

recognized the emotional expression the face was beginning to show. Subsequently, participants had to identify the emotional expression by making a forced-choice between four emotion labels (happy, angry, fearful, sad; *forced-choice phase*). No time limit was given for the choice of the emotion label. During a trial, participants were asked to keep their head still, to maintain fixation and to restrict eye blinks, if possible, until the forced-choice phase at the end of the trial.

In total, the task consisted of 48 trials, with six trials for each each of the eight experimental conditions (male happy expression, male angry expression, male fearful expression, male sad expression, female happy expression, female angry expression, female fearful expression, female sad expression). For each condition, we determined the intensity at which the expressions were recognized (recognition sensitivity in terms of intensity levels) and the proportion of correctly recognized expressions (recognition accuracy in terms of error rates)[2].

Experimental procedure

Following a standardized protocol (Domes et al., 2007; Domes, Heinrichs, Michel, et al., 2007; Domes et al., 2010), participants self-administered the nasal spray (three puffs per nostril, each containing 4 IU of OXT or PLC) 45 min before the start of the experiment (Born, et al., 2002). Self-report after the experiment revealed that participants were not able to discriminate whether they had received OXT or PLC. Participants also did not report any side-effects following substance administration.

To control for differences in OXT resorption and as a manipulation check, we inserted a venous catheter into the participant's forearm directly after arrival at the laboratory.

[2] Of note, a report on participants' task performance that did not take the gender of the stimulus material into account has already been provided by Lischke and colleagues (Lischke, Berger, et al., 2012).

Following a 30 min catheter habituation period, blood samples were collected 5 min before and 45 min after substance administration (i.e., directly before the start of the experiment). Immediately after collection, the samples were cooled in ice-chilled water at 4 °C, 15 min later centrifuged at 4000 rpm (for 5 min at 4 °C) and finally stored in a freezer at -20 °C. After completion of the study, the plasma was shipped on dry ice at -20 °C to the Department of Behavioral and Molecular Neuroendocrinology at the University of Regensburg, Germany, where they were extracted and analyzed using a radioimmunoassay (Landgraf, 1981). The assay detection limit was 0.1 pg/sample, and cross-reactivity with other related neuropeptides was < 0.7%. The coefficient of variation for intra-assay precision was 7-10%, whereas inter-assay variation was eliminated by measuring all samples within the same assay.

The experimental session took place in a quiet and slightly dimmed room. Participants were seated comfortably in front of a 20" computer screen (resolution: 1024 x 768 pixels) at a distance of 55 cm. The picture stimuli were presented in a randomized order using the experimental control software Presentation 12.1 (Neurobehavioral Systems, Inc., Albany, CA, USA), running on a Microsoft Windows XP operating system. During task performance, we measured the intensity levels (showing 5% to 100% of the emotion) at which the emotional expressions were correctly recognized as well as the corresponding error rates. To control for differences in attentional resource allocation during task performance, we also measured pupillary responses and gaze behavior. However, only the pupillary data are reported in this study, whereas the gaze data are reported elsewhere (Lischke, Berger, et al., 2012).

Pupillometry

Pupillary responses were continuously recorded using the ViewPoint system (ViewPoint PC-60 Head Fixed; Arrington Research Inc., Scottsdale, AZ, USA), including an infrared light source and a video camera sensitive to infrared light. The ViewPoint system samples pupil size in terms of pixels normalized with respect to the camera window (see

Table 2, Figure 1 and Figure 2). The system was connected with a presentation computer for the transmission of trigger signals that marked the beginning of every trial. The participant's head was placed onto a chin rest above which the camera was positioned, allowing continuous tracking of the participant's right eye during the viewing of the picture sets. Pupil size was recorded at 59.5 Hz (i.e., every 17 ms) and with an overall accuracy of 0.03 mm.

Pupillary responses were only analyzed for correctly answered trials. Cleaning and reduction of the pupil data were conducted with Matlab 7.13 (The MathWorks Inc., Natick, MA, USA) following standard procedures (e.g., Beatty & Lucero-Wagoner, 2000; Granholm, et al., 1996; Prehn, et al., 2008; Prehn, et al., 2011; Verney, Granholm, & Dionisio, 2001). The data were smoothed using an un-weighted 20-point moving average filter. Blinks, defined as large changes in pupil diameter occurring too rapidly to signify actual pupil dilation or constriction, were replaced by linear interpolation. We determined the baseline pupil diameter for each trial by averaging pupil diameter 200 ms before the presentation of the first picture of a set of expressions. This baseline was subtracted from the respective trial (baseline correction; see Table 2 for mean baseline pupil diameter). Finally, stimulus-locked pupillary responses for each trial were averaged for each condition and participant (see Table 2 and Figure 2 for mean task-related pupil diameter).

In our particular experimental task, in which we presented bright faces on a darker background in a slightly dimmed laboratory, we observed an immediate constriction of the pupil at the beginning of each trial; that is, with the presentation of the first picture of a set of expressions. After 800 ms, pupil diameter increased again over the course of the trial; that is, with the presentation of the subsequent pictures of a set of expressions (see Figure 2 and Bradley, et al., 2008). As stated earlier, trials were of variable duration: Each trial ended as soon as the participant recognized the emotional expression and pressed the stop button. On average (across all conditions and both groups), a trial ended after 7.07 s [range: 1.52-17.76 s;

5th percentile: 3.20 s]. To have enough trials for averaging, we only averaged pupillary responses across a time window (interval of interest) of 800 ms to 3200 ms following the presentation of the first picture of a set of expressions, in which at least 95% of trials were included.

When measuring pupillary responses as an index for cognitive and emotional processing, it is important to rule out that changes in pupil diameter are simply due to changes in lighting conditions (e.g., differences in illumination of the room or luminance of the stimuli). Therefore, we kept illumination in the laboratory constant for all sessions and adjusted the cumulative brightness of the pictures to ensure that there were no differences in luminance between male and female faces [$F(1,10) = 0.23$, $p = .64$] or between faces with different emotional expressions [$F(3,30) = 0.10$, $p = .88$].

Statistical analyses

All statistical analyses were performed using SPSS 20 (IBM SPSS Inc., Chicago, IL, USA). In particular, we tested whether OXT administration had an effect on emotion recognition (intensity level at which emotional expressions were correctly identified and the corresponding error rates) and pupillary responses (mean pupil diameter) during task performance. Moreover, we investigated the effects of emotion category (i.e., whether a happy, angry, fearful or sad emotion expression appeared) and gender of the stimulus material (i.e., whether a male or female face was presented) on emotion recognition and pupillary responses. All analyses on intensity levels, error rates and pupil diameters were run with means obtained for each participant and condition. As stated earlier, our design contained eight conditions in total (male happy expression, male angry expression, male fearful expression, male sad expression, female happy expression, female angry expression, female fearful expression, female sad expression) with six trials each. Because error trials were excluded prior to the analyses, mean intensity levels and mean pupil diameters for each

participant and condition were based on approximately 5.5 trials [$M = 5.46$, $SD = 0.31$].

All means were subjected to three-way repeated measures analyses of variance (ANOVAs) with the factors group (OXT, PLC), emotion (happy, angry, fearful, sad) and gender of the stimulus material (male, female). Significant effects were further investigated by two-way repeated measures ANOVAs and two-tailed post-hoc *t* tests. The Greenhouse-Geisser correction was used whenever the assumption of sphericity was violated [$\varepsilon < 1.0$]. The significance level for all analyses was set a $p < .05$. For significant effects, we report partial η^2 and Cohen's *d* as a measure of effect size.

Results

Substance application

To test whether participants of the OXT and PLC group differed in peripheral OXT levels (in pg/ml) over the time course of substance application, a two-way repeated measures ANOVA (Group x Time) was run. This analysis revealed a significant group by time interaction [$F(1,45) = 26.94$, $p < .001$, partial $\eta^2 = .37$], showing a significant increase in peripheral OXT levels in the OXT [pre: $M = 24.6$, $SD = 19.0$; post: $M = 39.7$, $SD = 20.1$] but not in the PLC [pre: $M = 27.2$, $SD = 16.6$; post: $M = 22.8$, $SD = 16.8$] group throughout substance application.

Emotion recognition

Recognition sensitivity

A three-way repeated measures ANOVA (Group x Emotion x Gender) on intensity levels revealed that OXT generally decreased intensity levels at which the emotional expressions were correctly recognized [main effect of group: $F(1,45) = 4.94$, $p = .031$, partial $\eta^2 = .10$], regardless of the emotional valence or the gender of the faces [group by emotion

interaction: $F(3,135) = 1.24$, $p = .30$, partial $\eta^2 = .03$; group by gender interaction: $F(1,45) = 0.01$, $p = .92$, partial $\eta^2 = .00$; group by emotion by gender interaction: $F(3,135) = 0.66$, $p = .58$, partial $\eta^2 = .01$]. There was also a main effect of emotion [$F(3,135) = 75.40$, $p < .001$, partial $\eta^2 = .63$] but neither a main effect of gender [$F(1,45) = 1.80$, $p = .19$, partial $\eta^2 = .04$] nor an emotion by gender interaction [$F(3,135) = 0.69$, $p = .56$, partial $\eta^2 = .02$]. The main effect of emotion indicated that the different emotional expressions were recognized at different levels of intensity: All participants recognized happy expressions at low, angry and fearful expressions at moderate and sad expressions at high intensity levels (see Table 1). Although we did not find a group by gender interaction [$F(1,45) = 0.01$, $p = .92$, partial $\eta^2 = .00$] or a group by emotion by gender interaction [$F(3,135) = 0.66$, $p = .58$, partial $\eta^2 = .01$], we conducted exploratory follow-up analyses to investigate whether OXT differentially affected recognition sensitivity for male and female faces. We found that OXT specifically lowered the recognition threshold for angry expressions in both, male [$t(45) = 2.17$, $p = .035$, $d = 0.64$; see Table 1 and Figure 1A] and female [$t(45) = 2.73$, $p = .009$, $d = 0.80$; see Table 1 and Figure 1A] faces. A similar effect, albeit only on a trend level, was found for fearful expressions in male [$t(45) = 1.97$, $p = .055$, $d = 0.57$; see Table 1 and Figure 1A] and female [$t(45) = 1.83$, $p = .074$, $d = 0.54$; see Table 1 and Figure 1A] faces. However, none of these effects remained significant after correcting for multiple testing, rendering these results preliminary.

Recognition accuracy

Error rates ranged from 0 (male faces with happy expressions in both, the OXT and PLC group; see Table 1) to 0.30 (male faces with sad expressions in the OXT group; see Table 1). A three-way repeated measures ANOVA (Group x Emotion x Gender) on error rates showed no effect of OXT on recognition accuracy [main effect of group: $F(1,45) = 0.15$, $p = .70$, partial $\eta^2 = .00$]. However, we found a main effect of emotion [$F(2.25,101.23) = 34.42$, ε

= .75, $p < .001$, partial $\eta^2 = .43$], a main effect of gender [$F(1,45) = 4.41$, $p = .04$, partial $\eta^2 = .09$] and an emotion by gender interaction [$F(2.25,101.25) = 14.09$, $\varepsilon = .75$, $p < .001$, partial $\eta^2 = .24$], indicating that participants made more errors during the processing of happy expressions in female as compared to male faces and more errors during the processing of angry, fearful and sad expressions in male as compared to female faces (see Table 1). We did not find a group by gender interaction [$F(1,45) = 0.63$, $p = .431$, partial $\eta^2 = .01$] but a group by emotion by gender interaction [$F(2.25,101.25) = 6.93$, $\varepsilon = .75$, $p = .001$, partial $\eta^2 = .13$]. Follow-up analyses revealed that OXT specifically enhanced recognition accuracy for fearful expressions in female [$t(37.87) = 2.14$, $p = .039$, $d = 0.64$; see Table 1] and, at least on a trend level, in male [$t(45) = 1.83$, $p = .075$, $d = 0.54$; see Table 1] faces. A similar trend was found during the processing of sad expressions in female [$t(45) = 1.95$, $p = .057$, $d = 0.57$; see Table 1] and male [$t(45) = 1.83$, $p = .10$, $d = 0.49$; see Table 1] faces. However, these results should be regarded as preliminary because none of these effects remained significant after correcting for multiple testing.

[Insert Table 1 and Figure 1 about here]

Pupillary responses

Baseline-related pupillary responses

To rule out any influence of baseline pupil diameter on pupillary responses, especially between the OXT and PLC group, baseline pupil diameter was also averaged for each experimental condition and participant in an interval of interest (200 ms preceding the presentation of the first picture of a set of expressions; see Table 2) and subjected to a three-way repeated measures ANOVA (Group x Emotion x Gender). We found no main effect of group [$F(1,45) = 0.52$, $p = .473$, partial $\eta^2 = .01$], no group by emotion interaction [$F(2.58,116.01) = 1.60$, $\varepsilon = .86$, $p = .199$, partial $\eta^2 = .03$], no group by gender interaction [$F(1,45) = 0.35$, $p = .556$, partial $\eta^2 = .01$] and no group by emotion by gender interaction

[$F(2.14,96.44) = 0.51$, $\varepsilon = .71$, $p = .617$, partial $\eta^2 = .02$], indicating that baseline pupil diameter was not affected by OXT application. We further found no main effect of emotion [$F(2.58,116.01) = 0.75$, $\varepsilon = .86$, $p = .506$, partial $\eta^2 = .02$], no main effect of gender [$F(1,45) = 0.00$, $p = .973$, partial $\eta^2 = .00$] and no emotion by gender interaction [$F(2.14,96.44) = 0.67$, $\varepsilon = .71$, $p = .526$, partial $\eta^2 = .02$], indicating that neither the emotional valence nor the gender of the faces had an impact on baseline pupil diameter. There was, thus, no evidence for an influence of baseline pupil diameter on task-related pupil dilation.

Task-related pupillary responses

A three-way repeated measures ANOVA (Group x Emotion x Gender) on average task-related pupil diameter in the interval of interest (800-3200 ms following the presentation of the first picture of a set of expressions) showed that OXT increased pupil diameter irrespective of the emotional valence of the faces, at least on a trend level [main effect of group: $F(1,45) = 4.06$, $p = .050$, partial $\eta^2 = .08$; group by emotion interaction: $F(2.47,110.94) = 0.28$, $\varepsilon = .82$, $p = .804$ partial $\eta^2 = .01$; group by emotion by gender interaction: $F(2.33,104.88) = 0.21$, $\varepsilon = .77$, $p = .846$, partial $\eta^2 = .01$]. We also found no main effect of emotion [$F(2.47,110.94) = 1.10$, $\varepsilon = .82$, $p = .352$] and no emotion by gender interaction [$F(2.33,104.88) = 1.29$, $\varepsilon = .77$, $p = .280$, partial $\eta^2 = .03$], indicating that the emotional valence of the faces had in general no effect on pupillary responses. There was, however, a main effect of gender [$F(1,45) = 13.19$, $p = .001$, partial $\eta^2 = .23$], indicating that pupil dilation was generally greater during the processing of female than male faces, as well as a group by gender interaction [$F(1,45) = 5.36$, $p = .025$, partial $\eta^2 = .11$]. Follow-up analyses revealed that OXT specifically increased pupil diameter for male [$t(45) = 2.66$, $p = .011$, $d = 0.78$; see Table 2 and Figure 2B] but not female [$t(45) = -1.10$, $p = .279$; see Table 2 and Figure 2B] faces. Accordingly, there was no significant difference in pupil diameter between male and female faces in the OXT [$t(22) = -1.02$, $p = .320$; see Table 2 and Figure 2B] but in

the PLC [$t(23) = -3.93$, $p = .001$, $d = 0.80$; see Table 2 and Figure 2B] group. Although we found no group by emotion interaction [$F(2.47,110.94) = 0.28$, $\varepsilon = .82$, $p = .84$ partial $\eta^2 = .01$] or group by emotion by gender interaction [$F(2.33,104.88) = 0.21$, $\varepsilon = .77$, $p = .846$, partial $\eta^2 = .01$], we performed exploratory follow-up analyses to further investigate how OXT affected pupillary responses to male and female faces with different emotional expressions. We found that OXT specifically increased pupil diameter during the processing happy [$t(45) = -2.46$, $p = .018$, $d = 0.72$; see Table 2, Figure 1B and Figure 2A] and fearful [$t(45) = -2.07$, $p = .044$, $d = 0.60$; see Table 2, Figure 1B and Figure 2A] expressions in male faces. A similar effect, albeit only on a trend level, was found for angry [$t(45) = -1.78$, $p = .082$, $d = 0.53$; see Table 2, Figure 1B and Figure 2A] and sad [$t(45) = -1.70$, $p = .097$, $d = 0.50$; see Table 2, Figure 1B and Figure 2A] expressions in male faces as well as for happy expressions in female [$t(45) = -1.77$, $p = .084$, $d = 0.52$; see Table 2, Figure 1B and Figure 2A] faces. However, the results of these follow-up analyses should be treated with caution because none of these effects remained significant after correcting for multiple comparisons.

[Insert Table 2 and Figure 2 about here]

Discussion

The aim of the present study was to explore the mechanisms underlying OXT effects on the exploration and recognition of socio-affective stimuli. Forty-seven healthy males received either OXT or PLC before performing a dynamic emotion recognition task. During the emotion recognition task, we measured task performance (intensity levels at which the emotional expressions were correctly recognized and the corresponding error rates) as well as pupillary responses (changes in pupil diameter). The study yielded three main results: First, better emotion recognition in the OXT group was associated with greater pupil dilation (main effect of group). Second, we found a difference in pupil diameter during the processing of

female as compared to male faces (main effect of gender), reflecting male participants' lower interest in male faces. Third, this female gender-specific stimulus presentation effect was diminished in the OXT group, in which pupil size was significantly increased for male faces (group by gender interaction). In the following, we argue that indicators of task performance (improved emotion recognition) in addition to indicators of attentional resource allocation (increased pupil dilation) provide evidence for the view that OXT increases the salience of socio-affective stimuli, especially for stimuli that usually do not recruit much attention.

Indicators of task performance

After OXT administration, participants recognized the emotional expressions at lower intensity levels and made fewer errors, regardless of the specific type of emotional expression (see Table 1). This result has already been presented by Lischke and colleagues (Lischke, Berger, et al., 2012) and is in line with other studies showing improved recognition of emotional and mental states after OXT administration (e.g., Di Simplicio, et al., 2009; Domes, Heinrichs, Michel, et al., 2007; Guastella, et al., 2010; Marsh, et al., 2010; Schulze, et al., 2011). Although we found no effect of the gender of the stimulus material on intensity levels (no gender-related main or interaction effects), the gender of the stimulus material affected error rates during task performance (main effect of gender, gender by emotion interaction, gender by emotion by group interaction), suggesting that male and female faces were differentially processed.

Indicators of attentional resource allocation

Numerous studies have demonstrated that task-related pupil dilation reflect the processing load of a task (Beatty & Lucero-Wagoner, 2000). Thus, pupillary responses "can be used to index the extent of central nervous system processing allocated to a task" (Granholm & Verney, 2004, p. 2). Increased pupillary responses have been observed in the

literature when stimuli are cognitively engaging or emotionally salient.

In our study, pupil size increased in all conditions of the emotion recognition task after an initial constriction caused by the first appearance of a face picture and the corresponding change in lighting conditions (see Figure 2). In line with our hypothesis and consistent with a recent study by Leknes and colleagues (Leknes, et al., 2012), subsequent pupil (re-) dilation was always greater in the OXT than in the PLC group, indicating that improved emotion recognition was associated with greater allocation of attentional resources during task performance (see Figure 1A). Of note, changes in pupil dilation appeared to be most pronounced during the processing of happy expressions. We, thus, suggest that altered attentional resource allocation might also underpin the well-established OXT effects regarding the promotion of prosocial and approach-related behavior.

The gender effect of the stimulus material, showing greater pupil dilation for female than for male faces, is in line with early pupillometric studies reporting greater pupil dilation during the viewing of pleasant and attention-demanding pictures (e.g., Garrett, Harrison, & Kelly, 1989; Hess & Polt, 1960; Hess, Seltzer, & Shlien, 1965; Libby, Lacey, & Lacey, 1973). Hess and Polt (1960), for example, reported that male participants showed greater pupil size in response to a picture of partially nude woman, while female participants were more interested in pictures of a baby and a partially nude man (see also Garrett, et al., 1989; Hess, et al., 1965; Libby, et al., 1973). Although we only used very reduced and simplified stimulus material (grayscales, equalized in size and enclosed within an elliptic mask that only showed the face itself) and no pictures showing erotic scenes or naked individuals, greater pupil dilation for female than for male faces might still indicate male participants' greater interest for female faces. In contrast to the PLC group, pupil dilation in the OXT group did not differ between female and male faces; that is, an increase in attentional resource allocation affected male even more than female faces and was thus reducing the processing advantage of

female faces.

Notably, pupil diameter in the baseline phase did not differ between the groups. Thus, there is no evidence for an altered "exploration mode" of the cognitive system after OXT administration (i.e., no evidence for a general enhanced sensitivity of the cognitive system, see Aston-Jones & Cohen, 2005; van der Meer, et al., 2010). Van der Meer and colleagues (2010), for instance, reported greater pre-experimental baseline pupil diameter in individuals with high fluid intelligence as compared to normal controls (i.e., tonic changes in pupil diameter). In our study, on the contrary, we only observed task-related differences in pupil diameter between the groups (i.e., phasic changes in pupil diameter).

Gamer and Buchel (2012) recently investigated whether OXT modulates sympathetic or parasympathetic activity by measuring skin conductance and heart rate responses during a static emotion recognition task. By showing that OXT differentially enhanced the heart rate response to emotional expressions but had no impact on skin conductance, the authors provided evidence that OXT selectively influences parasympathetic activity. As it has been discussed that changes in pupil diameter are mainly due to altered parasympathetic activity (Steinhauer, et al., 2004), our findings are in line with this view.

Limitations

It should be noted that we only used stimuli with both, an affective and a social content (i.e., faces that were developing an emotional expression) and pupil diameter was generally increased in all conditions in the OXT group (main effect of group). Therefore, it is presently unclear whether OXT also has an impact on pupillary responses during the processing of other kinds of stimuli. To elucidate this question, we recommend to use non-social in addition to socio-affective stimuli in further studies to disentangle affective and social aspects of the stimulus material (e.g., by using pictures of faces with emotional and neutral expressions or pictures of objects).

In our study, the possible impact of the two confounding variables, that is, baseline pupil diameter and luminance of the stimulus material, could be excluded. We also controlled for differences in participants' characteristics (e.g., general intelligence, empathy, alexithymia and psychopathology) and differences in OXT resorption. However, we did not ask our participants about their sexual orientation. Since we argue that differences in pupil diameter are associated with differences in interest for the stimulus material in question, sexual orientation could be a confound, in particular with regard to the reported gender by group interaction. However, the very unlikely case that more homosexual men would have been in the OXT than in the PLC group cannot fully explain the pattern of results obtained. A two-way repeated measure ANOVA with the factors group and emotion, while excluding the factor regarding the gender of the stimulus material, still showed a main effect of group on pupil dilation [$F(1,45) = 4.06$, $p = .050$, partial $\eta^2 = .08$]. Nevertheless, we recommend to rule out this possible confound in further studies when measuring pupillary responses to male and female faces.

It also has to be mentioned that we only investigated the effect of OXT on pupil diameter in a sample of male participants. Since there is considerable evidence that OXT modulates the neural circuitry involved in face processing in males and females differentially, our results might not be generalizable to females. For instance, it has been found that OXT decreases amygdala activity in response to aversive threat-related scenes and faces in male participants (Domes, Heinrichs, Glascher, et al., 2007; Gamer, Zurowski, & Buchel, 2010; Kirsch et al., 2005; Petrovic, Kalisch, Singer, & Dolan, 2008) but increases amygdala reactivity to similar stimulus material in female participants (Domes, et al., 2010; Lischke, Gamer, et al., 2012).

Finally, it has to be acknowledged that the size of our effects is rather small, although comparable with other studies. Therefore, the present findings should be treated with caution

unless replicated in further studies.

Conclusion

In conclusion, the finding of increased pupil dilation, in addition to improved recognition of emotional expressions after OXT administration, provides evidence for OXT-induced enhancement of supplemental attentional resources and increased stimulus processing. Moreover, our findings of diminished differences in pupil dilation between male and female faces after OXT administration demonstrate that OXT increases the salience of socio-affective stimuli in general and, in particular, for those stimuli that usually do not recruit much attention (i.e., by reducing the processing disadvantage of male faces in male participants). Finally, our results are consistent with a recent study by Leknes and colleagues (Leknes, et al., 2012) and provide, in addition, first evidence that OXT promotes the allocation of attentional resources during elaborate conscious processing of emotional expressions in a naturalistic emotion recognition task.

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Conflict of interest

None declared.

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Contributors

A.L., G.D. and S.C.H. designed the study. A.L. and K.P. collected and analyzed the data. K.P. wrote the manuscript. A.L., G.D., M.H., P.K. and S.C.H contributed to writing, reviewing and editing of the manuscript.

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Tables

Table 1

Intensity levels at which the emotional expressions were correctly recognized and the corresponding error rates in the oxytocin and placebo group

	Intensity levels				Error rates			
	Placebo (n = 24)		Oxytocin (n = 23)		Placebo (n = 24)		Oxytocin (n = 23)	
	M	*SEM*	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Happy expressions								
Male	36.53	1.64	32.83	1.45	0.00	0.00	0.00	0.00
Female	34.43	1.99	30.99	1.62	0.01	0.01	0.02	0.01
Angry expressions								
Male	43.56	2.37	36.84	1.96	0.07	0.02	0.05	0.02
Female	44.21	2.21	35.40	2.36	0.10	0.03	0.16	
Sad expressions								
Male	53.23	2.22	47.52	2.46	0.20	0.04	0.3	0.04
Female	52.27	2.52	48.61	2.62	0.18	0.03	0.1	0.03
Fearful expressions								
Male	46.20	1.88	41.06	1.81	0.13	0.03	0.07	0.02
Female	45.22	2.17	40.34	1.52	0.06	0.02	0.02	0.01

Table 2

Baseline and task-related pupil diameter in pixels normalized with respect to the camera window in the oxytocin and placebo group

	Baseline pupil diameter[a]				Task-related pupil diameter[b]			
	Placebo (n = 24)		Oxytocin (n = 23)		Placebo (n = 24)		Oxytocin (n = 23)	
	M	*SEM*	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Happy expressions								
Male	0.21	0.03	0.20	0.06	-0.03	0.01	-0.02	0.01
Female	0.21	0.03	0.20	0.06	-0.03	0.01	-0.02	0.01
Angry expressions								
Male	0.21	0.030	0.20	0.06	-0.03	0.01	-0.02	0.01
Female	0.21	0.028	0.20	0.06	-0.02	0.01	-0.02	0.02
Sad expressions								
Male	0.21	0.03	0.20	0.06	-0.03	0.02	-0.02	0.01
Female	0.21	0.03	0.20	0.06	-0.02	0.01	-0.02	0.01
Fearful expressions								
Male	0.21	0.03	0.20	0.06	-0.03	0.01	-0.02	0.01
Female	0.21	0.03	0.20	0.06	-0.02	0.01	-0.02	0.01

Note. [a]Baseline pupil diameter was averaged across a 200 ms interval before the presentation of the first picture of a set of expressions. [b]Task-related pupil diameter was averaged across an interval of 800 ms to 3200 ms following the presentation of the first picture of a set of expressions.

Figures

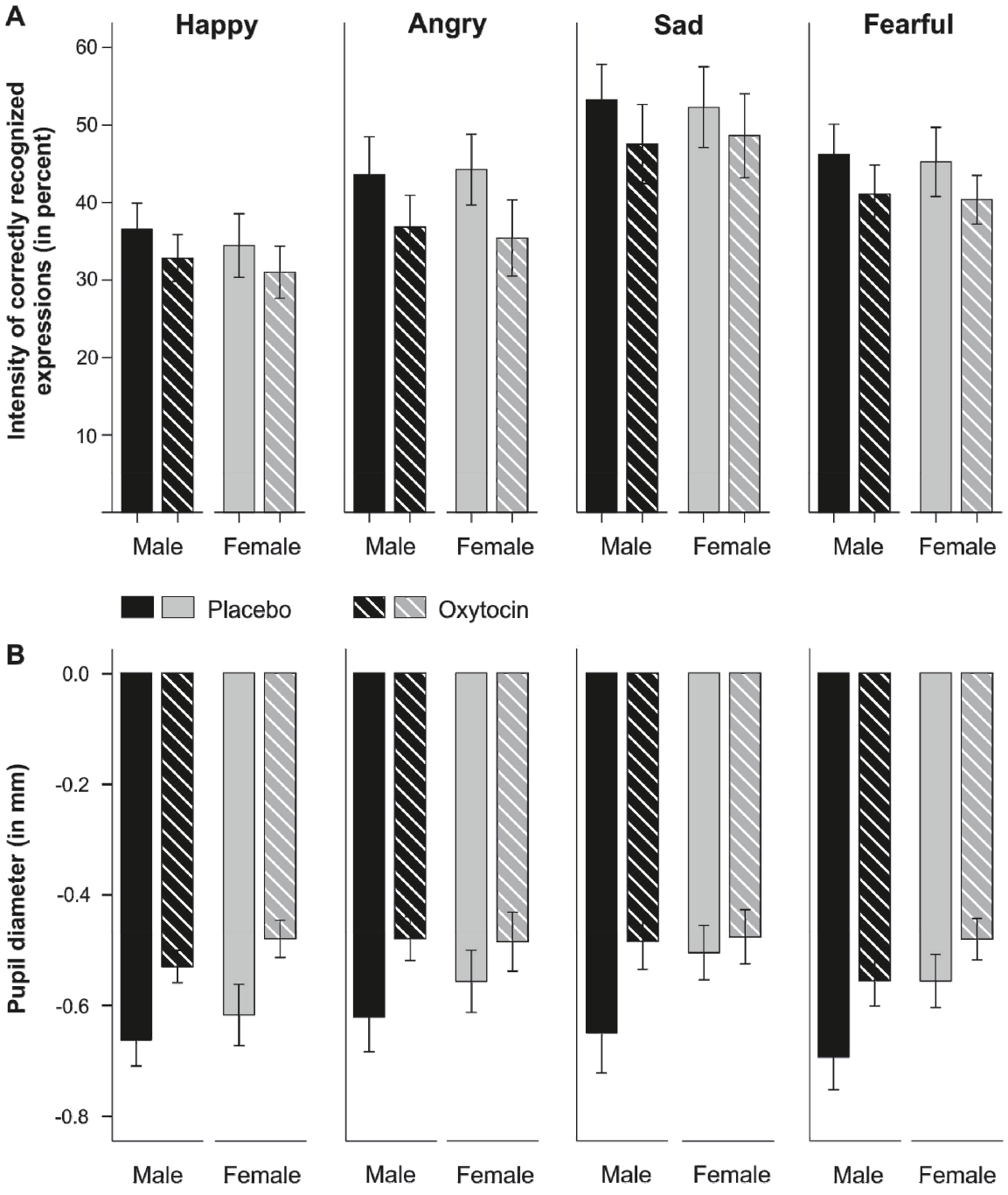

Figure 1. (A) Intensity levels at which the emotional expressions were correctly recognized and (B) task-related pupil diameter averaged across the interval of interest in the oxytocin and placebo group as a function of the emotional expression (happy, angry, sad, fearful) and the gender (male, female) of the faces. Bars represent mean and standard error of the mean in percent or mm.

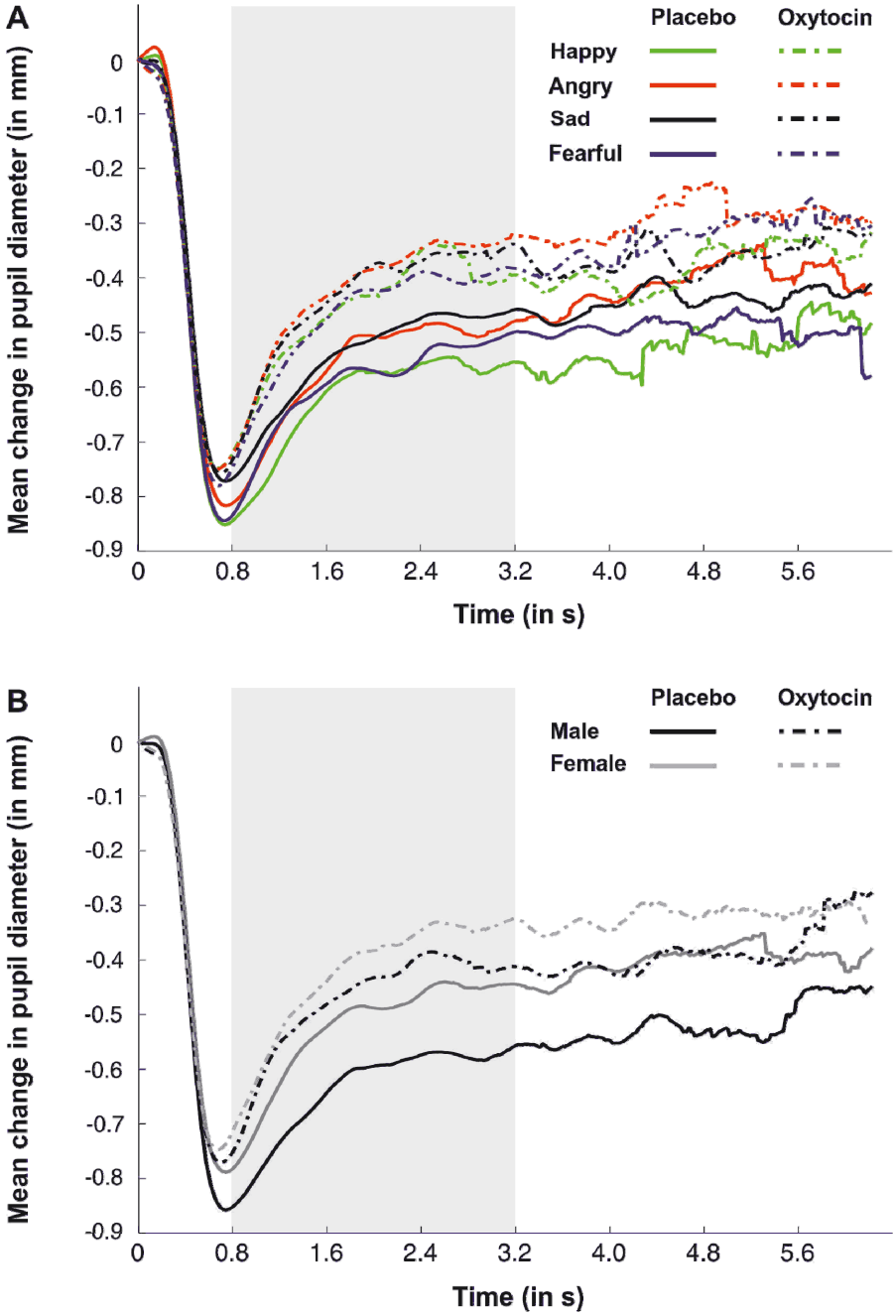

Figure 2. Task-related pupillary responses in the oxytocin and placebo group as (A) a function of the emotional expression (happy, angry, sad, fearful) and (B) the gender (male, female) of the faces. The interval of interest across which the pupillary responses were averaged is highlighted in gray (800-3200 ms following the presentation of the first picture of a set of expressions).

Appendix C: Study III

Oxytocin increases recognition of masked emotional faces

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Abstract

The neuropeptide oxytocin has been shown to improve many aspects of social cognitive functioning, including facial emotion recognition, and to promote social approach behavior. In the present study, we investigated the modulatory effects of oxytocin on the recognition of briefly presented facial expressions. In order to diversify the degree of visual awareness for the facial stimuli, presentation duration was systematically varied. Fifty-six participants were administered intranasal oxytocin or placebo in a double-blind, randomized, between-subjects design. Participants viewed angry and happy target faces or neutral distractors for 18, 35 or 53 ms subsequently masked by neutral faces. Participants had to indicate the presence or absence of the briefly presented target face. Discrimination indices (*d'*) showed that oxytocin generally enhanced detection accuracy of emotional stimuli. This effect was more pronounced for the recognition of happy faces. We provide evidence that a single dose of intranasally administered oxytocin enhances detection of briefly presented emotional stimuli. The possible role of stimulus valence and recognition difficulty is discussed.

Keywords: oxytocin, faces, emotion recognition, attention, peptides

Introduction

The neuropeptide oxytocin (OXT) is essential for bonding and attachment in mammals (Carter, Grippo, Pournajafi-Nazarloo, Ruscio, & Porges, 2008; Donaldson & Young, 2008) and has also been associated with human social behavior (Heinrichs, von Dawans, & Domes, 2009). In recent years, a number of studies focusing on the cognitive and affective effects of OXT have shown that intranasally administered OXT promotes the recognition of emotional states (e.g., Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009; Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Guastella et al., 2010), the recollection of social stimuli (e.g., Rimmele, Hediger, Heinrichs, & Klaver, 2009) and improves the processing of positive social cues and facial expressions in particular (e.g., Di Simplicio, et al., 2009; Gamer & Buchel, 2012; Gamer, Zurowski, & Buchel, 2010; Unkelbach, Guastella, & Forgas, 2008).

Despite these recent advances in characterizing the effects of OXT on the processing of socially relevant stimuli such as emotional facial expressions, it is still unclear whether the reported effects of OXT are entirely due to modulations in evaluation and appraisal of these stimuli (Guastella, Mitchell, & Dadds, 2008) or whether OXT also modulates earlier stages of stimulus processing, such as visual attention and awareness (Guastella, Howard, Dadds, Mitchell, & Carson, 2009). Thus, in the present study, we used short presentation times of emotional stimuli in order to assess the effects of OXT on the recognition of angry and happy facial stimuli under conditions of limited awareness. In order to vary the degree of visual awareness, equidistant increases of presentation durations were used. We expected that intranasally administered OXT would improve the recognition of emotional faces and that the effects would extend even to stimuli presented under conditions of limited awareness.

Materials and methods

Participants

Fifty-six male participants [age: $M = 24.18$; $SD = 3.12$] were assigned to receive either 24 international units (IU) of oxytocin ($n = 28$; Syntocinon, Novartis, Basel, Switzerland) or placebo (PLC; $n = 28$) within a double-blind, randomized, placebo-controlled study design. All participants had normal or corrected-to-normal visual acuity, were free of medication and did not report any history of endocrine, neurological or mental disorder. They were instructed to abstain from caffeine and nicotine on the day of the study. Smokers (more than 5 cigarettes a day) were excluded from participation in the study.

We planned to investigate a sample of 50 participants, with 25 participants in the OXT and 25 participants in the PLC group, to have sufficient power to detect medium-sized differences as determined by G-Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007). Six additional participants were examined to account for presumed technical difficulties and difficulties in substance application.

The study was approved by the ethics committee of the University of Rostock and was carried out at the Department of Psychiatry, University of Rostock, between November 2009 and January 2010.

Experimental procedure

After written informed consent was obtained, participants completed questionnaires on depression (Beck Depression Inventory, BDI; Hautzinger, Bailer, Worall, & Keller, 1995), (trait) anxiety (State Trait Anxiety Inventory, STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981) and were familiarized with the use of the nasal sprays. Participants self-administered 3 puffs of OXT or PLC per nostril (each puff with 4 IU), with the PLC containing all ingredients except for the peptide. Then all participants underwent a training

session to ensure appropriate understanding of the experimental task. Forty-five min after substance application, participants answered a multidimensional mood questionnaire (Mehrdimensionaler Befindlichkeitsbogen, MDBF; Steyer, Schwenkmezger, Notz, & Eid, 1997) and started the experiment.

Randomization of substance allocation procedure was generated by the local compounding pharmacist. This sequence was concealed from all persons involved in recruitment and testing of the participants. Unblinding was done after completion of testing.

Experimental task

The experiment was conducted on a standard computer with a 17" screen with a resolution of 800 x 600 pixels and a refresh rate of 170 Hz (confirmed by a photodiode and an oscilloscope). Each trial started with a fixation cross (1000 ms) and a short blank screen (100 ms). Then, an angry, happy or neutral face was presented for 18, 35 or 53 ms, followed by a "mask" showing a neutral face (see Figure 1). The initial gaze was fixed to the middle of the facial stimulus (between the eyes and the mouth). Participants were explicitly informed that two facial stimuli would always appear in each trial, although they might only perceive one. Facial stimuli (8 males, 8 females) were taken from the Karolinska Directed Emotional Faces database (KDEF; Goeleven, De Raedt, Leyman, & Verschuere, 2008). They were equivalent with regard to luminance [$F(2,45) = .48$, $p = .625$] and recognizability of the expressed emotions [$F(2,45) = .95$, $p = .394$]. Two additional neutral faces (1 male, 1 female) were selected as mask stimuli and presented in a pseudo-randomized order.

The experiment contained 288 trials, divided into 8 blocks with 36 trials each. Prior to each block, an instruction was given regarding the target emotion in the following trials. In each block, 12 angry, happy and neutral facial stimuli were randomly presented with varying durations (18, 35, 53 ms). Following each target-mask pair, participants had 3 s to indicate whether the target emotion was present or absent (4 blocks angry present/absent and 4 blocks

happy present/absent).

[Insert Figure 1 about here]

Statistical analyses

Participants' performance was analyzed based on signal detection theory. Conditional probabilities of hits and false alarms were calculated for each condition and participant. Afterwards, individual discrimination indices [$d' = Z_{hits} - Z_{false\ alarms}$] and response biases [$c = -0.5*(Z_{hits} + Z_{false\ alarms})$] were computed and analyzed using separate mixed-design analyses of variances (ANOVAs) with the within-subjects factors of face valence (angry, happy) and presentation time (18, 35, 53 ms) and the between-subjects factor of group (OXT, PLC). Greenhouse-Geisser corrections were applied if the assumption of sphericity was violated and significant interactions were followed by simple effects analyses. Analyses were performed using SPSS 17 (IBM SPSS Inc., Chicago, IL, USA). The significance level for all tests was $p < .05$.

Results

There were no differences with regard to age [$t(54) = -.16$, $p = .870$], depression [$t(54) = -.05$, $p = .960$] and (trait) anxiety [$t(54) = -.30$, $p = .764$] between the OXT and PLC group. Both groups did not differ on measures of self-reported calmness [$t(54) = .32$, $p = .750$], wakefulness [$t(54) = .45$, $p = .657$] or mood [$t(54) = -.94$, $p = .353$] prior to the start of the experiment.

With respect to recognition accuracy (*d'*), the ANOVA (Group x Valence x Time) revealed a significant main effect of group [$F(1,54) = 7.93$, $p = .007$, partial $\eta^2 = .128$], with participants who were administered OXT showing enhanced recognition performance for emotional faces regardless of valence and presentation time of the target (see Figure 2).

Recognition accuracy increased with longer presentation time of the target stimuli [$F(1.80,97.17) = 74.09$, $p < .001$, partial $\eta^2 = .578$]. In addition, a main effect of valence [$F(1,54) = 150.08$, $p < .001$, partial $\eta^2 = .735$] illustrated that happy faces were generally better recognized than angry faces. A significant interaction of valence and group [$F(1,54) = 4.55$, $p = .037$, partial $\eta^2 = .078$] was followed by simple effects analyses, which showed that the effect of OXT on emotion recognition was more pronounced for happy [$F(1,54) = 8.39$, $p = .005$, partial $\eta^2 = .134$] as compared to angry [$F(1,54) = 3.21$, $p = .079$, partial $\eta^2 = .056$] faces. Furthermore, we found an interaction of valence and presentation time [$F(1.77,19.23) = 3.75$, $p = .032$, partial $\eta^2 = .065$], indicating that recognition accuracy increased more rapidly for happy than for angry faces with longer presentation times. The interaction of group and presentation time was significant at a trend level [$F(1.80,97.17) = 2.97$, $p = .061$, partial $\eta^2 = .052$]. Simple effects analyses of presentation time illustrated a stronger effect of prolonged presentation on recognition accuracy in the OXT [OXT: $F(2,53) = 43.16$, $p < .001$, partial $\eta^2 = .620$] than PLC [$F(2,53) = 18.71$, $p < .001$, partial $\eta^2 = .414$] group. Descriptive results are presented in Table S1.

With respect to response bias (*c*), i.e., how liberal or conservative participants' responses to the stimuli were, the ANOVA (Group x Valence x Time) revealed more conservative responding to longer presentation times of the target stimuli [$F(2,108) = 68.16$, $p < .001$, partial $\eta^2 = .558$] and to happy as compared to angry target stimuli [$F(1,54) = 24.80$, $p < .001$, partial $\eta^2 = .315$]. Simple effects analyses of the significant interaction of valence and presentation time [$F(2,108) = 8.25$, $p < .001$, partial $\eta^2 = .133$] indicated that more conservative responding to happy as compared to angry faces was particularly present at longer presentation times [18 ms: $F(1,54) = 3.13$, $p = .082$, partial $\eta^2 = .055$; 35 ms: $F(1,54) = 31.71$, $p < .001$, partial $\eta^2 = .370$; 53 ms: $F(1,54) = 24.56$, $p < .001$, partial $\eta^2 = .313$]. In addition, the interaction of presentation time and group was significant [$F(2,108) = 5.01$, $p =$

.008, partial η^2 = .085]. Follow-up analyses showed that the effect of time on response behavior was more pronounced in participants who had received OXT [$F(2,53) = 35.48$, $p < .001$, partial $\eta^2 = .572$] as compared to PLC [$F(2,53) = 24.10$, $p < .001$, partial $\eta^2 = .476$]. However, the response bias did not differ significantly between both groups at individual presentation times [all *p*s >.10]. No further main effects or interactions were significant [all *p*s > .05].

[Insert Figure 2 about here]

Discussion

The present study provides evidence that OXT enhances detection of very briefly presented emotional stimuli, suggesting that OXT modulates awareness of socially relevant emotional information in the environment. Thus, OXT presumably modulates even early stages of stimulus processing, which suggests that consistently reported improvements in facial emotion recognition in previous OXT administration studies (e.g., Di Simplicio, et al., 2009; Domes, Heinrichs, Michel, et al., 2007; Guastella, et al., 2010) are not exclusively due to modulations in evaluation and appraisal of the presented stimuli. In addition, it seems that this effect is more pronounced for positive than negative facial stimuli, which is in line with previous reports suggesting a particular role of OXT in the processing of positive social stimuli (e.g., Di Simplicio, et al., 2009; Unkelbach, et al., 2008). However, it should be noted that positive stimuli were more easily recognized in general in the present study. Taking stimulus difficulty into account, by comparing recognition of negative and positive emotions with equal *d'* in the PLC condition (53 ms angry target faces and 18 ms happy target faces), the comparable improvement induced by OXT argues against specific interactions with emotional valence, suggesting instead that task difficulty may have influenced the pattern of results.

Apart from the effective restriction of visual presentation necessary for a detailed evaluation of the stimuli, our design also limited the potential confounding influence of gaze behavior, a factor that was previously found to be modulated by OXT (Guastella, et al., 2008). Due to the short presentation times, modulations of overt visual attention are unlikely to be a crucial mediating factor in the present study. However, OXT-induced modulations of reflexive saccades to the eye region of briefly presented facial stimuli have been reported in a recently published study (Gamer, et al., 2010). Consequently, future studies should involve eye-tracking in order to further investigate how OXT shapes early stages of visual attention.

The most likely neural candidate region for the observed effect of OXT on detection of emotional stimuli seems to be the amygdala, especially in light of its general role in the processing of visual emotional stimuli and studies highlighting the importance of the amygdala in detection of briefly presented emotionally relevant stimuli (for a discussion see Duncan & Barrett, 2007). Recent neuroimaging findings suggest that the impact of OXT on social cognitive behaviour might be a result of its attenuating effects on emotional arousal, reflected by reduction of amygdala activity, at least for negative stimuli and in males (Domes et al., 2007; Domes et al., 2010; Gamer, et al., 2010; Kirsch et al., 2005). The present results may at first seem to be contradictory with these findings. However, enhanced activity for dorsal and lateral subregions of the amygdala for happy faces presented for 150 ms was reported recently (Gamer, et al., 2010), which might be associated with enhanced emotion recognition performance as shown in the present study. Thus, future studies are needed to characterize the time course of OXT effects on the behavioral and neural level, ranging from stimulus detection to detailed stimulus evaluation and subjective emotional responding. In addition, recent results suggest sex-specific modulatory effects of OXT (Domes, et al., 2010). Future studies might thus explicitly investigate, if these distinctions translate to different modulatory effects in the recognition of emotions in male or female facial stimuli. It might be

of further interest to investigate whether the effects of OXT on recognition accuracy are specific to emotional or social stimuli, as OXT might enhance visual processing in general, for instance by increasing sensitivity towards contrast or spatial frequency. Finally, since OXT has been shown to modulate eye gaze even for briefly presented stimuli (Gamer, et al., 2010), it is an interesting question whether visual attention plays a role in the backward-masking paradigm.

To summarize, the present study demonstrates that a single dose of intranasal OXT increases detection accuracy for briefly presented emotional faces. This pattern of results, combined with the results of previous studies, suggests that OXT might influence both, the early stages of stimulus processing and the subsequent conscious evaluation of stimuli on a conceptual level. In addition, the results suggest more pronounced OXT effects on detection of positive stimuli, although future research should address the possible confounding role of valence-specific task difficulty.

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Conflict of interest

None declared.

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Contributors

L.S and G.D. designed the study. A.L. and J.G. collected the data. A.L. and L.S. analyzed the data. L.S. wrote the manuscript. A.L., G.D., M.H. and S.C.H contributed to writing, reviewing and editing of the manuscript.

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Figures

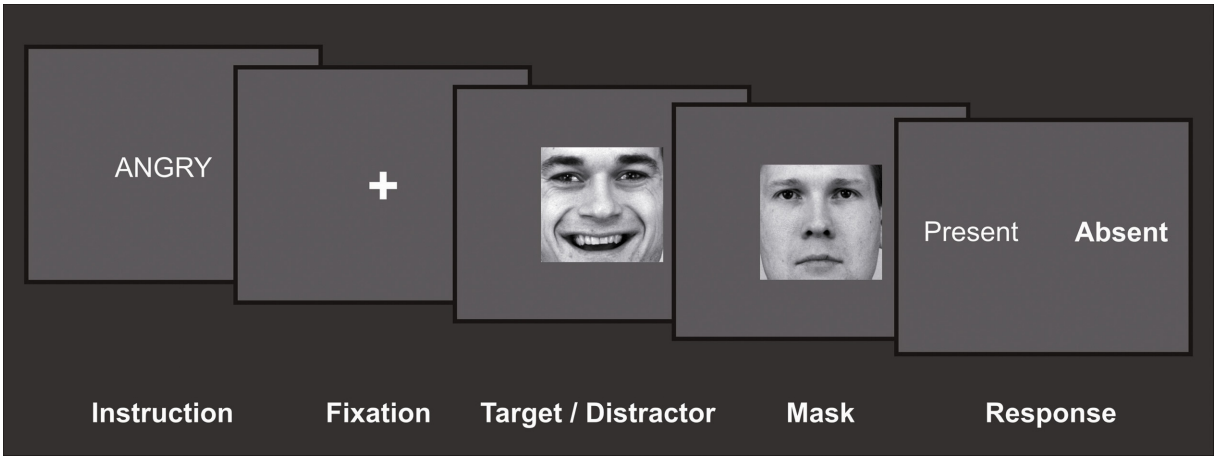

Figure 1. Trial structure of the experiment. Participants viewed an instruction cue prior to each block. A single trial started with a fixation cross of 1000 ms. Afterwards, an angry, happy or neutral face (target/distractor) was presented for 18, 35 or 53 ms and immediately followed by a neutral face (mask) for 165 ms. Participants had 3000 ms to indicate the absence or presence of the instructed emotion.

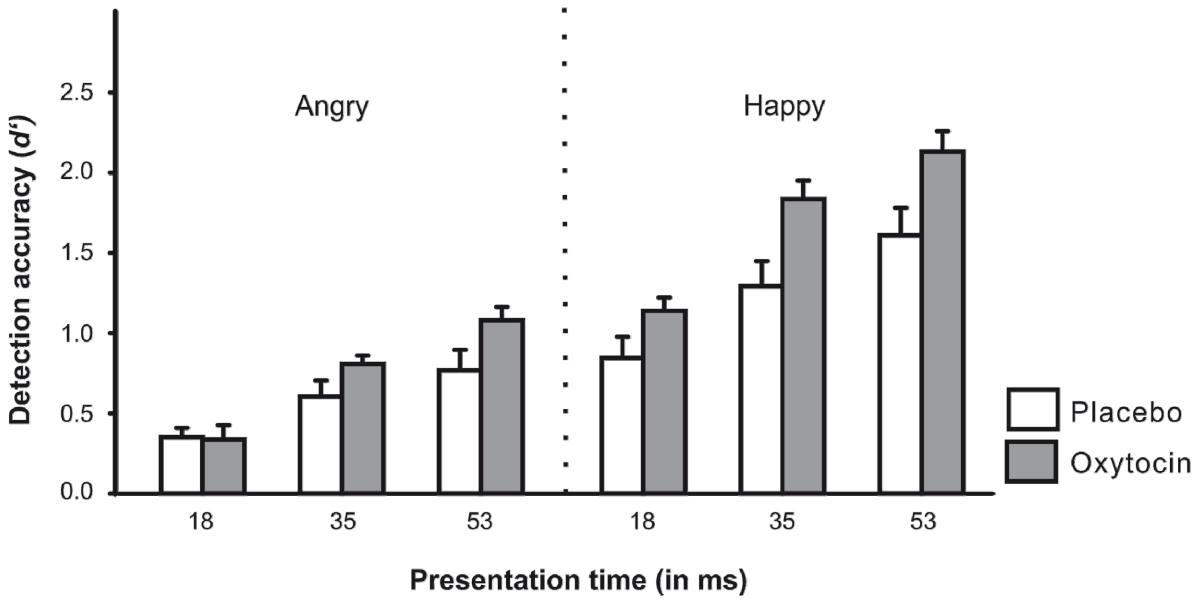

Figure 2. Effects of intranasal oxytocin on recognition accuracy (*d'*) for emotional faces as a function of emotional valence (angry, happy) and presentation time (18, 35, 53 ms). Bars represent mean and standard error of the mean.

Supplementary material

Table 1

Detection accuracy (d') and response bias (c) for the detection of angry and happy faces after oxytocin or placebo administration

	Detection accuracy (*d'*)				Response bias (*c*)			
	Placebo (*n* = 28)		Oxytocin (*n* = 28)		Placebo (*n* = 28)		Oxytocin (*n* = 28)	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Angry faces								
18 ms	0.35	0.31	0.34	0.47	0.13	0.44	0.38	0.39
35 ms	0.61	0.53	0.81	0.28	0.13	0.33	0.13	0.26
53 ms	0.77	0.68	1.08	0.45	-0.05	0.31	0.00	0.31
Happy faces								
18 ms	0.85	0.69	1.14	0.44	0.12	0.46	0.12	0.39
35 ms	1.30	0.82	1.84	0.61	-0.20	0.40	-0.35	0.47
53 ms	1.61	0.90	2.14	0.67	-0.43	0.33	-0.30	0.40