Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans

Matthias Gamer[a,1], Bartosz Zurowski[a,b], and Christian Büchel[a]

[a]Department of Systems Neuroscience, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg, Germany; and [b]Department of Psychiatry and Psychotherapy, University of Lübeck, D-23538 Lübeck, Germany

Edited by Mortimer Mishkin, National Institute for Mental Health, Bethesda, MD, and approved April 5, 2010 (received for review January 25, 2010)

The neuropeptide oxytocin enhances the processing of positive social stimuli and improves the capacity to effectively attend the eye region of conspecifics. To investigate the neural basis of these effects, we combined intranasal oxytocin administration with high-resolution functional magnetic resonance imaging in a unique emotion classification task. Emotional faces were briefly presented while controlling for the initial fixation, and measuring subsequent eye movements. Oxytocin had differential effects on the activity of specific amygdala subregions. On the one hand, it attenuated activation in lateral and dorsal regions of the anterior amygdala for fearful faces but enhanced activity for happy expressions, thus indicating a shift of the processing focus toward positive social stimuli. On the other hand, oxytocin increased the likelihood of reflexive gaze shifts toward the eye region irrespective of the depicted emotional expression. This gazing pattern was related to an increase of activity in the posterior amygdala and an enhanced functional coupling of this region to the superior colliculi. Thus, different behavioral effects of oxytocin seem to be closely related its specific modulatory influence on subregions within the human amygdala.

eyes | facial expression | functional neuroimaging | neuropeptides | social perception

In the past decades, a number of studies have shown that the neuropeptide oxytocin plays an essential role in regulating social behavior in nonhuman mammals. It is crucially involved in initiating maternal and alloparental behavior in rodents (1) and facilitates social approach by reducing anxiety and neuroendocrine stress responses (2). Moreover, it enables social bonding in voles (3) and enhances the saliency of social information, thereby strengthening recognition memory for conspecific animals (4).

Despite these effects of oxytocin on social behavior of rodents and other mammals, this neuropeptide has long been considered a purely female hormone in humans because of its crucial role in initiating parturition as well as milk ejection during lactation. However, in recent years intranasal administration of oxytocin in humans has revealed significant effects of this neuropeptide on a variety of psychosocial functions (for reviews, see refs. 5 and 6). For example, oxytocin increases trust (7) and reduces betrayal aversion (8) in human interactions. It has profound effects on behavioral and endocrine stress responses (9, 10) and enhances memory for social information (11, 12). Moreover, oxytocin increases the human ability to infer an opponent's mental state from subtle cues around the eye region (13), and it improves the ability to identify fearful faces in dynamic displays (14). Because the eye region is a crucial facial feature for classifying fearful expressions (15), these latter findings could be related to an oxytocin-dependent increase in the number and duration of fixations on the eye region, which was shown in a recent study using neutral faces in free-viewing conditions (16).

With respect to the effects of oxytocin on human brain function, neuroimaging studies have consistently revealed that it reduces amygdala activity to unpleasant stimuli (17) and to human faces in general (18). Similar suppressive effects have been reported for trust adaptation in social interactions (8). These findings were interpreted as reflecting the neural mechanism of the behaviorally observed anxiolytic effect of the neuropeptide.

Taken together, these findings suggest that oxytocin increases gaze on the eye region while it decreases amygdala activity when observing human faces. Such findings conflict, however, with a recent study revealing that increased rather than decreased amygdala activation predicted reflexive gaze changes toward the eye region (19). This observation precisely accounts for the clinical findings of reduced spontaneous fixations on the eye region and a failure to identify fearful expressions in a patient with complete bilateral amygdala damage (20, 21). Thus, the behavioral effects of oxytocin in increasing gaze on the eye region (16) seem to be at odds with the reported reduction of amygdala activity in other studies (17, 18). A possible explanation for this discrepancy is the introduction of large, partial-volume effects by the low spatial resolution of standard fMRI acquisition schemes in comparison with the small size of individual amygdala nuclei that may respond differentially to oxytocin treatment. This notion is in agreement with data in voles (22) showing a regionally specific distribution of oxytocin receptors within the amygdala. Moreover, a functional dissociation of amygdala subregions during social conditioning has recently been observed in humans (23). These findings could signify that one amygdala subregion shows an oxytocin-dependent reduction of activity for negative stimuli, possibly reflecting the anxiolytic effect of the neuropeptide, whereas a different subregion is up-regulated by oxytocin, thereby facilitating gaze orienting toward the eye region of emotional faces.

To examine these hypotheses in detail, we carried out a double-blind placebo-controlled study using high-resolution functional magnetic resonance imaging (fMRI). Participants were instructed to classify briefly presented negative (fearful), positive (happy), or neutral facial expressions as quickly and accurately as possible. To examine the effect of oxytocin on gaze-orienting behavior, stimuli were unpredictably shifted either downward or upward on each trial, thus manipulating whether participants initially fixated on the eye or mouth region. Stimuli were briefly presented (150 ms) while measuring brain activity with high-resolution fMRI ($2 \times 2 \times 2\,mm^3$). Furthermore, on-line eye tracking was used to identify reflexive gaze changes that were triggered by these faces but occurred after stimulus offset (19). The pharmacological manipulation was realized in a double-blind between-subjects design with 46 participants either receiving a single intranasal dose of 24 IU oxytocin (Syntocinon spray; Novartis; three puffs per nostril, each with 4 IU oxytocin) or a placebo spray that contained all inactive ingredients except for the neuropeptide (7). This setup allowed us to examine modulatory effects of oxytocin on valence processing as well as on gaze-orienting behavior with high anatomical resolution.

Author contributions: M.G. and C.B. designed research; M.G. and B.Z. performed research; M.G. analyzed data; and M.G., B.Z., and C.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

See Commentary article on page 9033.

[1]To whom correspondence should be addressed. E-mail: m.gamer@uke.uni-hamburg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1000985107/-/DCSupplemental.

Results

Behavioral Data. For each participant and condition, we measured the proportion of correct responses as well as the mean reaction time in the emotion classification task. Overall, participants in both groups were highly accurate in classifying the depicted emotional expressions (placebo: mean ± SD = 96.6 ± 0.8%; oxytocin: mean ± SD = 97.0 ± 0.5%; see Table 1), and none of the experimental factors had a statistically significant influence on classification accuracy.

As can be seen from Fig. 1*A*, response times were longer for fearful faces [main effect emotion: $F(2, 88) = 6.97$, $\varepsilon = 0.86$, $P = 0.003$]. However, we also obtained a significant interaction of emotion and initial fixation [$F(2, 88) = 12.20$, $\varepsilon = 1.00$, $P < 0.001$], indicating that participants were faster in classifying the depicted expression when they initially fixated diagnostically relevant features (i.e., the eye region for fearful faces and the mouth for happy facial expressions) (15). All other effects did not reach statistical significance.

Eye Movement Data. From the acquired eye-tracking data, we calculated whether a fixation change occurred within a period of 1,000 ms following stimulus offset. Subsequently, the proportions of reflexive gaze changes from the eye region toward the mouth and from the mouth region toward the eyes were determined based on whether participants initially fixated on the eyes or mouth, respectively (19).

Across both groups, reflexive gaze changes followed the distribution of diagnostically relevant features in the face [interaction of emotion and initial fixation, $F(2,88) = 13.94$, $\varepsilon = 1.00$, $P < 0.001$]. That is, gaze changes toward the eye region were more likely for fearful and neutral faces than for happy facial expressions that triggered more gaze changes toward the mouth (Fig. 1*B*). In addition, we observed a significant interaction of drug and initial fixation [$F(1,44) = 4.35$, $P = 0.043$], indicating that oxytocin increased the likelihood of gaze changes toward the eyes irrespective of the depicted facial expression.

Imaging Data: Valence Effects. In a number of previous studies it was shown that fearful faces elicit larger amygdala activation than happy faces (24, 25). To examine whether this differential response is altered by oxytocin, we calculated the interaction effect of drug (placebo vs. oxytocin) and facial expression (fearful vs. happy) on the imaging data. In the placebo group, this analysis confirmed previous reports and revealed a significantly enhanced response to fearful faces in the left amygdala. Interestingly, this pattern was reversed under oxytocin (Fig. 2). This effect was specific, because additional analyses contrasting both groups across all facial expressions confirmed that oxytocin had no general suppressing or up-regulating effect on amygdala responses.

Imaging Data: Gaze-Related Effects. As described previously, we observed that oxytocin increased the number of fixation changes toward the eye region. To determine the neural correlate of this effect, we estimated the interaction of drug (placebo vs. oxytocin) and initial fixation (eyes vs. mouth) in the imaging data. This analysis revealed a robust bilateral interaction effect in the superior colliculi that resembled the observed gazing pattern (Fig. 3).

Table 1. Proportion of correct emotion classification in the placebo and the oxytocin group as a function of the depicted facial expression and the initial fixation

	Placebo		Oxytocin	
Emotion	Eyes	Mouth	Eyes	Mouth
Fearful	97.2 (0.8)	97.0 (0.9)	95.5 (1.5)	96.6 (1.0)
Happy	97.1 (1.6)	95.9 (2.5)	98.3 (0.5)	98.1 (0.6)
Neutral	94.4 (1.9)	98.2 (0.5)	96.6 (1.1)	96.9 (0.7)

All figures are percentages. Values in parentheses indicate SEM.

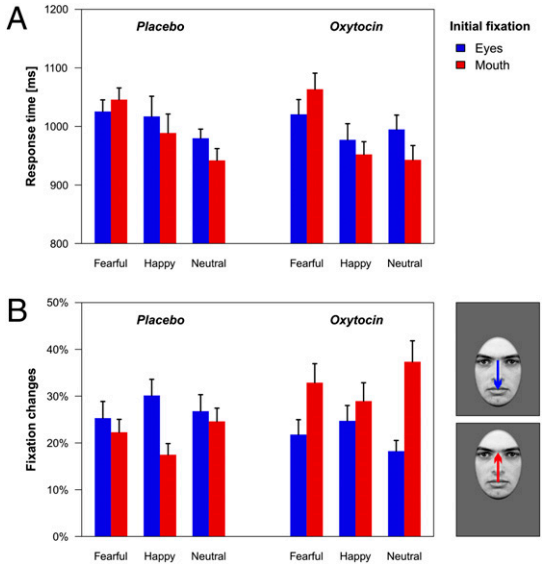

Fig. 1. Behavioral responses and eye movements in the oxytocin and the placebo group as a function of facial expression and initial fixation. (*A*) Response times. (*B*) Proportion of fixation changes targeting the other major facial feature. Error bars indicate SEM.

The superior colliculi can be considered an output structure that is highly relevant for initiating saccades (26). Thus, the aforementioned interaction effect seems to be closely related to the behavioral finding of an increased number of fixation changes toward the eye region in the oxytocin group. To get further insight into how other brain regions might be coupled to the superior colliculi, we classified all stimuli according to the participant's gazing behavior. Subsequently, we contrasted stimuli triggering fixation changes toward the eye region with trials where participants continued fixating on the mouth. This analysis revealed a significant cluster in the right posterior amygdala showing a larger response on trials with fixation changes toward the eye region in the oxytocin as compared with the placebo group (Fig. 4).

To determine whether the enhanced gaze-related response of the amygdala in the oxytocin group is functionally related to the activation of the superior colliculi, we carried out a physio-physiological interaction analysis. First, we extracted the mean time series for each participant from the left superior colliculus ($x = -8$, $y = -30$, $z = -9$), which showed the largest interaction effect of drug and initial fixation. These time series were then used to determine the correlation of this seed region with the whole measured brain volume. Finally, we searched for regions showing an enhanced functional coupling with the superior colliculus in the oxytocin as compared with the placebo group. The analysis revealed a significant cluster in the right posterior amygdala (Fig. 5), which largely overlaps with the gaze-related response in the oxytocin group (Fig. 4). Thus, oxytocin increased the functional coupling of amygdala and superior colliculus.

Discussion

In the placebo as well as the oxytocin group, participants were faster in recognizing fearful expressions when initially looking at the eye region, and identified happy faces with shorter latencies when initially fixating the mouth, which is in agreement with the distribution of diagnostic facial features (15). Correspondingly, gaze changes toward the eye region of fearful faces occurred more frequently than for happy ones, and the opposite pattern was observed for fixation changes toward the mouth (19). Although

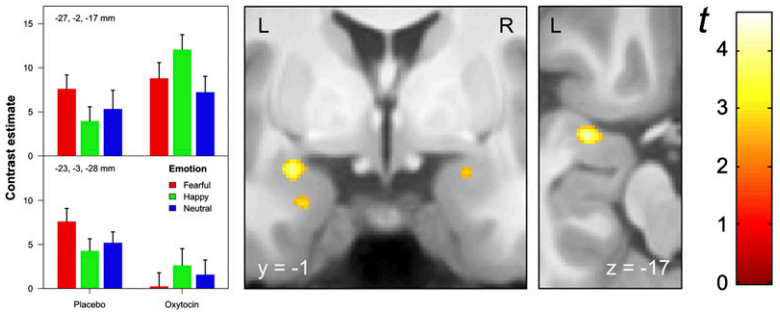

Fig. 2. Amygdala regions showing a significant interaction of group (placebo, oxytocin) and emotional expression (fearful, happy). (*Right*) Statistical map (coronal and axial plane) of the interaction effect revealing two clusters in the left amygdala [dorsal cluster, peak voxel: $x = -27$, $y = -2$, $z = -17$ mm, volume = 121 mm^3, $t(44) = 4.37$, $P = 0.017$, FWE corrected; and ventral cluster, peak voxel: $x = -23$, $y = -3$, $z = -28$ mm, volume = 75 mm^3, $t(44) = 3.88$, $P = 0.058$, FWE corrected]. (*Left*) Contrast estimates of these clusters, with error bars representing SEM. The cluster in the right amygdala did not reach statistical significance [peak voxel: $x = 23$, $y = -1$, $z = -19$ mm, volume = 16 mm^3, $t(44) = 2.74$, $P = 0.004$, uncorrected].

oxytocin did not directly affect the efficiency of processing emotional faces per se, the neuropeptide increased the proportion of fixation changes toward the eyes across all expressions. This resembles a possible mechanism through which oxytocin enhances the human ability to infer the mental state of others from subtle cues around the eyes (13), and improves the detection of fear in faces (14), because both skills critically depend on elaborately scanning the eye region (21).

The effect of oxytocin on gazing behavior was related to an enhanced activation of the right posterior amygdala, most likely the basal nucleus (27), on trials where gaze changes toward the eye region occurred. The anatomical location of this activation closely resembles a recent finding of this region being predictive for the occurrence of reflexive gaze movements toward fearfully widened eyes (19). The current study further demonstrated a functional connection of this amygdala subregion with the superior colliculi, which increased when participants received oxytocin as compared with placebo. Animal research indicates that the superior colliculi are highly relevant for covert shifts of attention as well as for initiating saccades toward behaviorally relevant targets (26). Furthermore, collicular lesions disrupt reflexive attention and eye movements in humans (28). Amygdala lesions, however, impair reflexive gaze orienting toward the eye region (21) and result in an inadequate use of gaze cues pointing to behaviorally relevant locations (29). Thus, both regions, the amygdala and the superior colliculi, are implicated in redirecting attention toward socially relevant locations in the visual field. The current data indicate that specifically the basal nucleus of the amygdala might be crucial for assigning salience to specific facial features in the visual periphery (21). Subsequently, the reflexive orienting of attention

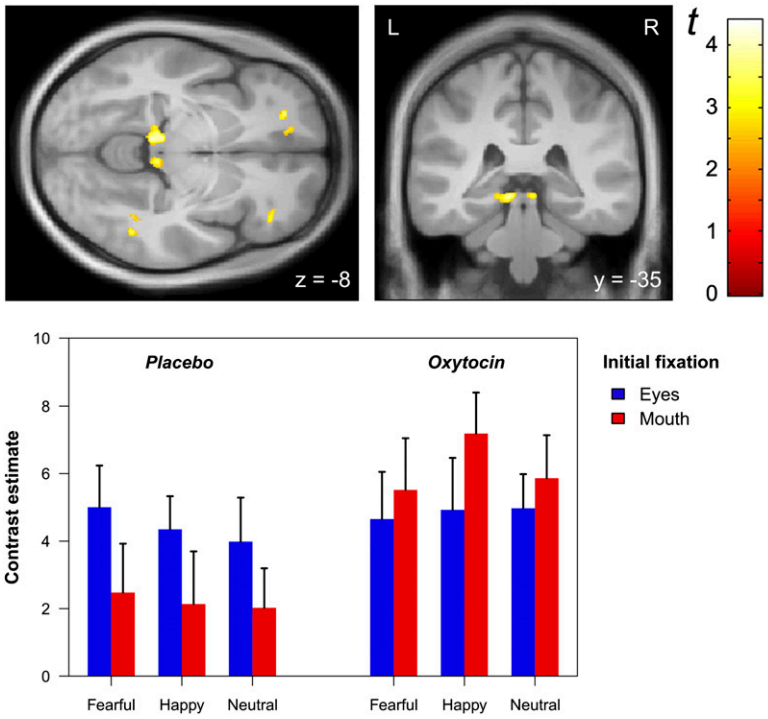

Fig. 3. Regions showing an enhanced response in the oxytocin as compared with the placebo group when participants initially looked at the mouth region. (*Upper*) Statistical map (axial and coronal plane) of this interaction revealing a robust bilateral effect in the superior colliculi [left cluster, peak voxel: $x = -8$, $y = -30$, $z = -9$ mm, volume = 440 mm^3, $t(44) = 4.03$, $P < 0.0001$, uncorrected; right cluster, peak voxel: $x = 6$, $y = -33$, $z = -8$ mm, volume = 116 mm^3, $t(44) = 3.48$, $P < 0.001$, uncorrected]. (*Lower*) Contrast estimates of the peak voxel in the left superior colliculus, with error bars representing SEM.

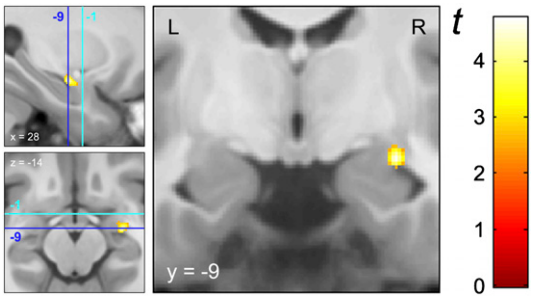

Fig. 4. Amygdala regions reflecting gaze preferences for the eye region on a trial-by-trial basis. (*Right*) Coronal plane shows an enhanced amygdala response in the oxytocin as compared with the placebo group when participants shifted their gaze toward the eye region [peak voxel: $x = 28$, $y = -9$, $z = -14$ mm, volume = 112 mm^3, $t(44) = 4.71$, $P = 0.007$, FWE corrected]. (*Left*) Cluster location on sagittal and axial planes. The lines labeled −1 depict the y position of the valence-related oxytocin effect in the left amygdala that is shown in Fig. 2.

toward these stimuli is enabled by modulating activity in the superior colliculi. Importantly, activity in this circuit seems to be upregulated by oxytocin, thus improving the processing of socially relevant information from the eye region of another person (13).

In addition to the gaze-related effect of oxytocin on amygdala activity, we observed a valence modulation in an anatomically distinct region within the left anterior amygdala, with fearful faces eliciting larger activity than happy expressions in the placebo group. This effect, which is in line with previous studies showing that the amygdala is sensitive to aversive stimuli (24, 25), was found in two different amygdala subregions that correspond to the corticomedial nuclear group as well as to the lateral amygdaloid nucleus (27). However, oxytocin did not only reduce amygdala activity for threatening stimuli in these regions as has been reported (17), but additionally increased responses to happy facial expressions in the present study. Recent accounts on amygdala function have focused on a more general role of this structure in identifying salient stimuli in the environment instead of specifically processing aversive information (30, 31). Animal studies suggest that the lateral and basal nuclei of the amygdala receive highly processed sensory information (32) that enables detection of such biologically relevant stimuli in the environment. The central amygdaloid nucleus (part of the corticomedial nuclear group), with its modulatory influences on sympathetic arousal, was linked to vigilance and attention (33). These subregions of the amygdala might thus be involved in detecting whether a given stimulus is biologically relevant, and allowing the organism to adjust its current level of arousal appropriately.

Typically, negative stimuli have higher behavioral relevance than positive stimuli, which could account for the frequently observed enhanced amygdala activation for aversive stimuli. However, the modulatory effect of oxytocin on amygdala activity in the current experiment might indicate that oxytocin triggers a shift of this processing focus from negative to positive social stimuli to facilitate approach behavior (34). In humans, it was demonstrated that oxytocin reduces misclassifications of positive emotions (35) and enhances the encoding of happy facial expressions (11). Furthermore, it increases trust in human interactions (7) and facilitates the recognition of positive stimuli conceptually associated with sexuality, bonding, and social relationships (36). These behavioral findings indicating an enhanced processing of positive social information while reducing the evaluation of negative stimuli may result from the modulatory effect of oxytocin on amygdala function that was found here.

Recent reviews on oxytocin functions in humans have proposed that this substance might be used to support the treatment of mental disorders that are characterized by marked deficits in social behavior (5, 37). For example, patients with autism spectrum disorder have deficits in social interactions as well as impairments in understanding the mental states of others. Moreover, it was found that these patients show a hypoactivation of the amygdala (38, 39) as well as behavioral difficulties in identifying facial expressions (40). These findings may be related to abnormal fixation patterns and gaze aversion when scanning facial stimuli (41). Our current data suggest that oxytocin might improve these symptoms by enhancing amygdala activity, thereby facilitating gaze shifts toward the eye region. In fact, recent studies showed that a single intranasal dose of oxytocin improved emotion recognition from the eye region in young people with autism spectrum disorders (42), and it increased patients' gazing time on the eye region in free-viewing conditions (43).

Social anxiety disorder is another clinical condition that might benefit from oxytocin treatment. Patients with this disorder show enhanced amygdala reactivity to faces depicting negative emotions (44) and a lower number of fixations on the eye region (45). However, in contrast to autism spectrum disorders, patients with social anxiety disorder typically have a preserved ability to identify facial expressions (46). Thus, their gaze aversion seems to be related more to the enhanced stress and anxiety of being evaluated by others; correspondingly, these patients tend to focus on negative information in social situations (47). As we showed that oxytocin may facilitate a shift of the processing focus from negative to positive social stimuli by suppressing amygdala activity to fearful faces while enhancing it for happy expressions, a supporting ad-

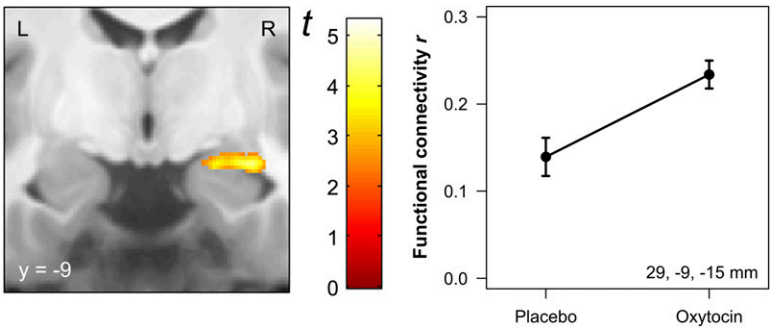

Fig. 5. Amygdala regions showing an enhanced functional coupling with the left superior colliculus in the oxytocin as compared with the placebo group. (*Left*) Significant cluster in the right posterior amygdala on a coronal plane [peak voxel: $x = 29$, $y = -9$, $z = -15$ mm, volume = 179 mm^3, $t(44) = 3.99$, $P = 0.037$, FWE corrected]. (*Right*) Mean correlation coefficients (values were Fisher z-transformed before averaging) between the activity in both regions as a function of group. Error bars indicate SEM.

ministration of this neuropeptide in the treatment of social anxiety might be helpful. First attempts of using oxytocin to improve exposure therapy have been made (48), but future research will have to examine whether positive effects during treatment generalize to real-life conditions.

We have shown that oxytocin has significant effects on anatomically distinct amygdala regions associated with valence processing and gaze orienting. These data complement recent findings from monkeys (49) and humans (23) showing a dissociation of activity within the amygdala, with separate subregions subserving different aspects of face processing. In the current study, oxytocin increased the likelihood of reflexive gaze shifts toward the eye region of faces depicting different emotions. This gaze pattern was related to an increase of activity in the basal amygdaloid nucleus and an enhanced functional coupling of this region to the superior colliculi. Furthermore, oxytocin attenuated responses to fearful expressions while enhancing them for happy faces in the corticomedial part of the amygdala and the lateral amygdaloid nucleus. These specific effects of oxytocin on amygdala function may underlie the diverse modulatory effects of this neuropeptide on human social behavior (5, 6) and may be relevant for novel attempts at using oxytocin for the treatment of mental disorders (50).

Materials and Methods

Subjects. Forty-six healthy, right-handed, male volunteers (age mean ± SD = 25.0 ± 3.7 years) participated in this study that was approved by the ethics committee of the medical faculty of the University of Rostock. Exclusion criteria for participation were a history of neurological or endocrine disease, medication, and drug or alcohol abuse. Furthermore, subjects were instructed to abstain from alcohol and nicotine for 12 h before the experiment. All participants gave written informed consent and were paid for participation.

Experimental Protocol. In a double-blind, placebo-controlled study design, participants were assigned either to the experimental group that received a single intranasal dose of 24 IU oxytocin (Syntocinon spray; Novartis; three puffs per nostril, each with 4 IU oxytocin) or to the control group, which received a placebo spray containing all inactive ingredients except for the neuropeptide. It was previously shown that intranasally applied neuropeptides cross the blood–brain barrier (51) and therefore allow for studying oxytocin effects on brain function (37). Because central nervous oxytocin levels reach a plateau approximately 40 min after substance administration, participants received oxytocin or placebo 45 min before fMRI scanning in the present study. The whole functional neuroimaging session lasted no longer than 45 min.

To control for nonspecific effects of oxytocin, two state questionnaires were completed: (*i*) after arriving at the laboratory, (*ii*) directly before the MRI scanning (~40 min after drug application), and (*iii*) after participants left the MRI scanner. Neither the initial values of these questionnaires nor changes during the experiment differed between the oxytocin and the placebo group (*SI Results*, Fig. S1).

Emotion Classification Paradigm. The experimental task was based on a fully crossed 3 × 2 within-subjects design with the factors emotion and initial fixation. Male and female faces unambiguously depicting neutral, fearful, and happy expressions were selected from several established stimulus sets (*SI Materials and Methods*). A different sample of 72 individual faces (12 males and 12 females for each expression) was randomly selected from this pool of pictures for each participant. These faces were then presented once in a randomized sequence in each of three experimental sessions. A trial started with a fixation cross (2 s) followed by the presentation of the face (150 ms) followed by a blank screen (1,850 ms) and another fixation cross for a randomly chosen period of 2–9 s. To precisely control for the initial fixation, half of the male and female stimuli within each emotional expression were unpredictably shifted either downward or upward on each trial such that the eyes or the mouth appeared at the location of the fixation cross. Participants were instructed to classify the depicted emotional expression as quickly and accurately as possible by pressing the corresponding key on a button box with the right hand. Before scanning, participants were trained on the task using a different set of six faces to familiarize them with the button assignment.

Data Acquisition. Blood oxygenation level-dependent functional images and eye movement data were acquired throughout the experiment. Stimuli were projected onto a translucent screen using a video projector, and subjects viewed this screen via a special mirror that was mounted to the head coil. This dichroic reflector allowed infrared light to pass while reflecting the visible light from the video projector. The 60-Hz MRI-compatible eye-tracking camera (NordicNeuroLab) was hidden behind the mirror and mounted directly in front of the right eye of the participant.

Functional imaging was performed on a 3-Tesla whole-body MR scanner (Trio; Siemens) equipped with a 32-channel head coil. Thirty-five transverse slices (slice thickness, 2 mm; no gap) were acquired in each volume covering the temporal lobe and occipital and orbitofrontal cortex. A T2*-sensitive gradient echo-planar imaging (EPI) sequence was used (repetition time [TR] = 2,240 ms; echo time [TE] = 30 ms; flip angle = 80°; FOV = 208 × 208 mm; in-plane resolution 2 × 2 mm). Additionally, isotropic high-resolution (1 × 1 × 1 mm^3) structural images were recorded using a T1-weighted coronal-oriented MPRAGE sequence with 240 slices.

Data Analysis: Behavior. Details on the eye movement analysis can be found in *SI Materials and Methods*. In short, we determined the proportion of fixation changes (>0.5°) toward the other major facial feature that were triggered by the stimulus but occurred after stimulus offset. That is, when the eyes were presented at the position of the fixation cross, we determined the proportion of downward fixation changes toward the mouth, and when the mouth followed the fixation cross, we calculated the corresponding proportion of upward fixation changes toward the eyes.

The proportion of fixation changes, as well as the behavioral data (proportion of correct emotion classifications and response times), were analyzed with a series of 2 × 3 × 2 ANOVAs using drug (oxytocin vs. placebo) as a group factor, and emotion and initial fixation a within-subjects factors. Where appropriate, the Huynh–Feldt procedure was applied to correct for potential violations of the sphericity assumption. A rejection region of $P < 0.05$ was used for these statistical tests.

Data Analysis: fMRI. Statistical parametric mapping (SPM8, Wellcome Department of Imaging Neuroscience, London) was used for preprocessing and analyzing the imaging data. A narrow 4-mm full-width at half maximum (FWHM) isotropic Gaussian smoothing kernel was used during preprocessing to optimize the detection of small activation foci within the amygdala (for details, see *SI Materials and Methods*).

Three different random-effects analyses were carried out. First, to examine changes in brain activation as a function of the experimental manipulations, we constructed a design matrix for each subject by modeling the onset of the face presentation as separate regressors for all six combinations of emotion (fearful, happy, neutral) and initial fixation (eyes, mouth) in each session. The fixation period between the events served as baseline. Regressors were convolved with the hemodynamic response function, and one contrast was specified to reveal whether oxytocin altered the frequently reported larger amygdala activation for fearful as compared with happy facial expressions (24, 25). A second contrast was computed to compare brain activation between the initial fixations (eyes vs. mouth). Finally, statistical maps for both contrasts were separately subjected to second-level analyses (*t* tests) to compare the responses between the oxytocin and the placebo group.

A second analysis focused on the correlation of brain activity and gazing behavior on a trial-by-trial basis. For this analysis, trials were classified into one of four categories using the acquired eye-tracking data. The first category specified fixation, i.e., no gaze change occurred in a period of 1,000 ms following stimulus offset. Trials with gaze changes toward the other major facial feature represented the second category. The remaining trials were then classified according to whether a gaze change into another direction occurred or whether a given trial could not be analyzed (e.g., due to eye blinks). Table S1 depicts the proportion of trials that fell into each of these four trial categories as a function of the experimental manipulations. This classification was used to specify a design matrix for each subject by modeling the onset of the face presentation as separate regressors for all combinations of emotion, initial fixation, and gazing behavior. These regressors were convolved with the hemodynamic response function. To reveal brain regions mediating the increased likelihood of reflexive gaze changes toward the eye region in the oxytocin group (*Results*), we calculated difference maps for each subject individually, contrasting trials with and without gaze changes toward the eye region. As we did not find an emotion-specific effect of oxytocin on gazing behavior (*Results*), all emotional expressions were pooled in these contrast maps. On the second level, a *t* test was performed to determine differences between the oxytocin and the placebo group.

The third analysis was carried out to test for drug effects on the functional coupling between the superior colliculi and the amygdala. For this aim, we extracted the mean time series for each subject from a seed voxel in the left superior colliculus at $x = -8$, $y = -30$, $z = -9$ (for the identification of this

coordinate, see *Results*). These time series were then used as regressors in subject-specific design matrices, and simple contrast maps were calculated reflecting a correlation of the seed region with the whole measured brain volume. At the second level, a two-sample *t* test was carried out to determine brain regions showing an enhanced coupling with the left superior colliculus in the oxytocin as compared with the placebo group.

Because we were primarily interested in modulatory effects of oxytocin on amygdala activation, we used a small volume correction in predefined bilateral anatomical amygdala regions of interest (52). Anatomical labels for subregions within the amygdala were specified by comparing the location of activation clusters with high-resolution diagrams of the human amygdala as depicted in an anatomical atlas (27). For illustration purposes, only voxels are displayed as statistical maps thresholded at $P < 0.01$, uncorrected, which are overlaid on the mean structural image from all participants. This mean image was generated from the high-resolution T1 images that were normalized using DARTEL flow fields.

ACKNOWLEDGMENTS. We thank G. Müller, K. Müller, and K. Wendt for help with MR scanning. This study was supported by a grant from the Bundesministerium für Bildung und Forschung (BMBF, Network "Social Cognition").

1. Ross HE, Young LJ (2009) Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front Neuroendocrinol* 30:534–547.
2. Bartz JA, Hollander E (2006) The neuroscience of affiliation: Forging links between basic and clinical research on neuropeptides and social behavior. *Horm Behav* 50: 518–528.
3. Hammock EAD, Young LJ (2006) Oxytocin, vasopressin and pair bonding: Implications for autism. *Philos Trans R Soc Lond B Biol Sci* 361:2187–2198.
4. Choleris E, Clipperton-Allen AE, Phan A, Kavaliers M (2009) Neuroendocrinology of social information processing in rats and mice. *Front Neuroendocrinol* 30:442–459.
5. Heinrichs M, von Dawans B, Domes G (2009) Oxytocin, vasopressin, and human social behavior. *Front Neuroendocrinol* 30:548–557.
6. Neumann ID (2008) Brain oxytocin: A key regulator of emotional and social behaviours in both females and males. *J Neuroendocrinol* 20:858–865.
7. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E (2005) Oxytocin increases trust in humans. *Nature* 435:673–676.
8. Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron* 58: 639–650.
9. Heinrichs M, Neumann I, Ehlert U (2002) Lactation and stress: Protective effects of breast-feeding in humans. *Stress* 5:195–203.
10. Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U (2003) Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* 54:1389–1398.
11. Guastella AJ, Mitchell PB, Mathews F (2008) Oxytocin enhances the encoding of positive social memories in humans. *Biol Psychiatry* 64:256–258.
12. Rimmele U, Hediger K, Heinrichs M, Klaver P (2009) Oxytocin makes a face in memory familiar. *J Neurosci* 29:38–42.
13. Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC (2007) Oxytocin improves "mind-reading" in humans. *Biol Psychiatry* 61:731–733.
14. Fischer-Shofty M, Shamay-Tsoory SG, Harari H, Levkovitz Y (2010) The effect of intranasal administration of oxytocin on fear recognition. *Neuropsychologia* 48: 179–184.
15. Smith ML, Cottrell GW, Gosselin F, Schyns PG (2005) Transmitting and decoding facial expressions. *Psychol Sci* 16:184–189.
16. Guastella AJ, Mitchell PB, Dadds MR (2008) Oxytocin increases gaze to the eye region of human faces. *Biol Psychiatry* 63:3–5.
17. Kirsch P, et al. (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* 25:11489–11493.
18. Domes G, et al. (2007) Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biol Psychiatry* 62:1187–1190.
19. Gamer M, Büchel C (2009) Amygdala activation predicts gaze toward fearful eyes. *J Neurosci* 29:9123–9126.
20. Adolphs R, Tranel D, Damasio H, Damasio A (1994) Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature* 372: 669–672.
21. Adolphs R, et al. (2005) A mechanism for impaired fear recognition after amygdala damage. *Nature* 433:68–72.
22. Insel TR, Shapiro LE (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc Natl Acad Sci USA* 89:5981–5985.
23. Davis FC, Johnstone T, Mazzulla EC, Oler JA, Whalen PJ (2010) Regional response differences across the human amygdaloid complex during social conditioning. *Cereb Cortex* 20:612–621.
24. Morris JS, et al. (1998) A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain* 121:47–57.
25. Fusar-Poli P, et al. (2009) Functional atlas of emotional faces processing: A voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *J Psychiatry Neurosci* 34:418–432.
26. Ignashchenkova A, Dicke PW, Haarmeier T, Thier P (2004) Neuron-specific contribution of the superior colliculus to overt and covert shifts of attention. *Nat Neurosci* 7:56–64.
27. Mai JK, Assheuer J, Paxinos G (1997) *Atlas of the Human Brain* (Academic, San Diego).
28. Sereno AB, Briand KA, Amador SC, Szapiel SV (2006) Disruption of reflexive attention and eye movements in an individual with a collicular lesion. *J Clin Exp Neuropsychol* 28:145–166.
29. Akiyama T, et al. (2007) Unilateral amygdala lesions hamper attentional orienting triggered by gaze direction. *Cereb Cortex* 17:2593–2600.
30. Sander D, Grafman J, Zalla T (2003) The human amygdala: An evolved system for relevance detection. *Rev Neurosci* 14:303–316.
31. Costafreda SG, Brammer MJ, David AS, Fu CHY (2008) Predictors of amygdala activation during the processing of emotional stimuli: A meta-analysis of 385 PET and fMRI studies. *Brain Res Rev* 58:57–70.
32. Sah P, Faber ESL, Lopez De Armentia M, Power J (2003) The amygdaloid complex: Anatomy and physiology. *Physiol Rev* 83:803–834.
33. Davis M, Whalen PJ (2001) The amygdala: Vigilance and emotion. *Mol Psychiatry* 6: 13–34.
34. Lim MM, Young LJ (2006) Neuropeptidergic regulation of affiliative behavior and social bonding in animals. *Horm Behav* 50:506–517.
35. Di Simplicio M, Massey-Chase R, Cowen PJ, Harmer CJ (2009) Oxytocin enhances processing of positive versus negative emotional information in healthy male volunteers. *J Psychopharmacol* 23:241–248.
36. Unkelbach C, Guastella AJ, Forgas JP (2008) Oxytocin selectively facilitates recognition of positive sex and relationship words. *Psychol Sci* 19:1092–1094.
37. Heinrichs M, Domes G (2008) Neuropeptides and social behaviour: Effects of oxytocin and vasopressin in humans. *Prog Brain Res* 170:337–350.
38. Critchley HD, et al. (2000) The functional neuroanatomy of social behaviour: Changes in cerebral blood flow when people with autistic disorder process facial expressions. *Brain* 123:2203–2212.
39. Ashwin C, Baron-Cohen S, Wheelwright S, O'Riordan M, Bullmore ET (2007) Differential activation of the amygdala and the 'social brain' during fearful face-processing in Asperger Syndrome. *Neuropsychologia* 45:2–14.
40. Ashwin C, Chapman E, Colle L, Baron-Cohen S (2006) Impaired recognition of negative basic emotions in autism: A test of the amygdala theory. *Soc Neurosci* 1: 349–363.
41. Pelphrey KA, et al. (2002) Visual scanning of faces in autism. *J Autism Dev Disord* 32: 249–261.
42. Guastella AJ, et al. (2010) Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biol Psychiatry* 67:692–694.
43. Andari E, et al. (2010) Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proc Natl Acad Sci USA* 107:4389–4394.
44. Phan KL, Fitzgerald DA, Nathan PJ, Tancer ME (2006) Association between amygdala hyperactivity to harsh faces and severity of social anxiety in generalized social phobia. *Biol Psychiatry* 59:424–429.
45. Horley K, Williams LM, Gonsalvez C, Gordon E (2004) Face to face: Visual scanpath evidence for abnormal processing of facial expressions in social phobia. *Psychiatry Res* 127:43–53.
46. Arrais KC, et al. (2009) Social anxiety disorder women easily recognize fearfull, sad and happy faces: The influence of gender. *J Psychiatr Res*, 10.1016/j.jpsychires.2009.11.003.
47. Amin N, Foa EB, Coles ME (1998) Negative interpretation bias in social phobia. *Behav Res Ther* 36:945–957.
48. Guastella AJ, Howard AL, Dadds MR, Mitchell P, Carson DS (2009) A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. *Psychoneuroendocrinology* 34:917–923.
49. Hoffman KL, Gothard KM, Schmid MC, Logothetis NK (2007) Facial-expression and gaze-selective responses in the monkey amygdala. *Curr Biol* 17:766–772.
50. Heinrichs M, Gaab J (2007) Neuroendocrine mechanisms of stress and social interaction: Implications for mental disorders. *Curr Opin Psychiatry* 20:158–162.
51. Born J, et al. (2002) Sniffing neuropeptides: A transnasal approach to the human brain. *Nat Neurosci* 5:514–516.
52. Tzourio-Mazoyer N, et al. (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15:273–289.