Effects of Intranasal Oxytocin on Thermal Pain in Healthy Men: A Randomized Functional Magnetic Resonance Imaging Study

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ABSTRACT

Objective: Intranasal oxytocin has been shown to affect human social and emotional processing, but its potential to affect pain remains elusive. This randomized, placebo-controlled, double-blind, crossover trial investigated the effect of intranasal oxytocin on the perception and processing of noxious experimental heat in 36 healthy male volunteers.

Methods: Thermal thresholds were determined according to the Quantitative Sensory Testing protocol. A functional magnetic resonance imaging experiment including intensity and unpleasantness ratings of tonic heat was used to investigate the effects of oxytocin within the brain.

Results: Thirty men (aged 18–50 years) were included in the study. Intranasal oxytocin had no significant effect on thermal thresholds, but significantly ($t = -2.06$, $p = .046$) reduced heat intensity ratings during functional magnetic resonance imaging. The effect on intensity ratings was small (−3.46 points on a 100-point visual analog scale [95% confidence interval {CI} = −6.86 to −0.07] and independent of temperature. No effects of oxytocin on stimulus- or temperature-related processing were found at the whole-brain level at a robust statistical threshold. A region of interest analysis indicated that oxytocin caused small but significant decreases in left (−0.045%, 95% CI = −0.087 to −0.003, $t = -2.19$, $p = .037$) and right (−0.051%, 95% CI = −0.088 to −0.014], $t = -2.82$, $p = .008$) amygdala activity across all temperatures.

Conclusions: The present study provides evidence for a significant but subtle inhibitory effect of oxytocin on thermal stimulus ratings and concurrent amygdala activity. Neither of the two effects significantly depended of temperature; therefore, the hypothesis of a pain-specific effect of oxytocin could not be confirmed.

Trial Registration: EUDRA-CT 2009-015115-40

Key words: intranasal oxytocin, experimental pain, functional magnetic resonance imaging, amygdala, human.

INTRODUCTION

Antinociceptive effects of oxytocin have been reported by nearly 30 nonhuman studies, but only a few human studies (1). Only four studies have investigated the effects of oxytocin on human pain using the convenient nasal application route, which has been established as a safe (2) and effective method to increase oxytocin concentrations in the central nervous system (3–5). Singer et al. (6) were the first to test the potential of intranasal oxytocin to alter empathic responses to pain. Although this hypothesis could not be confirmed, they found that oxytocin reduced amygdala reactivity in response to pain in a small subsample. Using an experimental model of placebo analgesia, Kessner et al. (7) showed that intranasal oxytocin enhances the placebo effect. However, no general antinociceptive effect of intranasal oxytocin was evident within their large healthy sample. Mameli et al. (8) explored the potential of oxytocin as an adjunctive analgesic in a small sample of fibromyalgia patients with negative results. Rash and Campbell (9) recently found that intranasal oxytocin reduces behavioral and physiological reactions in response to cold-pressor pain.

Various effects and neural correlates of intranasal oxytocin on the processing of stress and emotion have been identified, yet most neuroimaging studies reported oxytocin

BOLD = blood oxygen level dependent, **CSF** = cerebrospinal fluid, **FWE** = family-wise error, **fMRI** = functional magnetic resonance imaging, **IU** = international units, **MRI** = magnetic resonance imaging, **POMS** = Profile of Mood States, **QST** = Quantitative Sensory Testing, **ROI** = region of interest, **SPM** = statistical parametric mapping, **VAS** = visual analog scale

SDC Supplemental Content

From the Department of Experimental Psychology (Zunhammer, Geis, Greenlee) and Clinic for Psychiatry and Psychotherapy (Zunhammer, Busch, Eichhammer), University of Regensburg, Regensburg, Germany.

Data from the study have been presented as a poster at the Society for Neuroscience meeting in 2013 and at the HBM meeting in 2014.

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effects on the amygdala, a key region for anxiety processing (10,11). For example, intranasal oxytocin was found to attenuate amygdala responses related to threatening scenes (12) and to conditioned fear of faces (13). Interestingly, a recent meta-analysis found 17 neuroimaging studies that report increased amygdala activity in response to experimental pain, which suggests that the amygdala plays a role in acute pain processing (14).

The potential of neuropeptides such as oxytocin as an adjunctive treatment of chronic pain is of considerable interest. Nevertheless, the potential of intranasal oxytocin to affect pain processing is understudied (1). The present trial aimed to investigate the effect of oxytocin on experimental pain perception and processing. The established Quantitative Sensory Testing (QST) protocol was used to test effects of oxytocin on noxious and nonnoxious thermal thresholds. Furthermore, a functional magnetic resonance imaging (fMRI) experiment with tonic heat pain stimulation and visual analog scale (VAS) ratings of heat intensity and unpleasantness was used to measure oxytocin effects on experimental pain perception and processing.

Based on the above-mentioned studies, we hypothesized that intranasal oxytocin should

a) increase noxious thermal pain thresholds;
b) decrease VAS ratings of noxious heat intensity and/or unpleasantness;
c) cause detectable blood oxygen–level dependent (BOLD) signal changes related to thermal stimulus processing on whole brain level; and
d) alter painful stimulus processing in the amygdala.

METHODS

Trial Information

The current study was approved by the ethics committee of the University of Regensburg (Approval No. 11-111-0322) and the responsible federal medical agency. It was registered in a clinical trial registry (EUDRA-CT No. 2009-015115-40) and conforms to the Declaration of Helsinki (15). Written informed consent was obtained from every participant. All measures took place at the magnetic resonance imaging (MRI) facilities of the University of Regensburg at the Bezirksklinikum Regensburg between November 2012 and April 2013.

Study Design

This is a placebo-controlled, double-blind, crossover trial. The random allocation sequence was generated by the Center for Clinical Trials at the University of Regensburg. According to this sequence, the Hospital Pharmacy of the University of Erlangen labeled and numbered the nasal sprays and corresponding emergency wrappers. At study inclusion, participants were assigned sequential participant numbers by author P.E. and hereby allocated to Group A or B at random. Group A received placebo and Group B received oxytocin at Visit 1; Group A received oxytocin and Group B received placebo at Visit 2. Both groups completed an additional training visit (Visit 0) at study inclusion. A period of at least 7 days between all visits was observed to minimize carryover effects.

Participants

Healthy right-handed male volunteers between 18 and 50 years of age were eligible for trial participation. Participants were recruited by advertisement at the University of Regensburg, aiming at a sample size of 36. The sample size was chosen to optimize statistical power within the limits of the devoted resources. Participants received a compensation of 10 Euros per hour. Exclusion criteria were surveyed in a structured interview at inclusion. Exclusion criteria were as follows: allergies against any ingredients of the trial medication; past or present cardiac, major internal, neurological, psychiatric, hormonal, or chronic conditions; acute infections; recent surgery; recent use of illicit drugs, psychotropics, or analgesics; alcohol addiction; and conditions incompatible with fMRI safety. Participants were required to abstain from alcohol and caffeinated beverages at least 24 and 12 hours before visits, respectively.

Procedure

An overview of the experimental procedures is provided in Figure 1. Visits were scheduled between 8 AM and 8 PM, always at the same time of day (±1 hours) within participants.

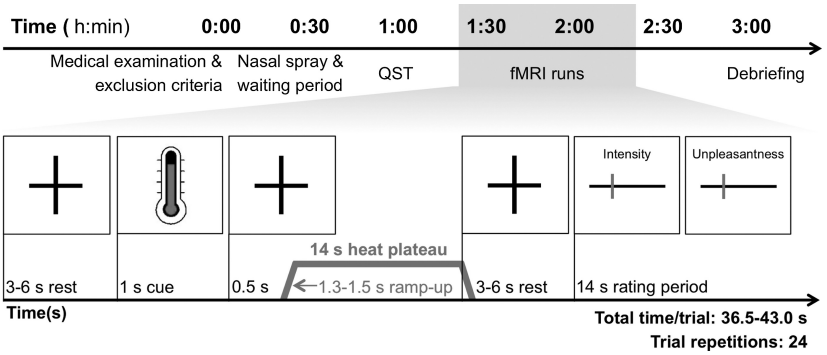

FIGURE 1. Time schedule of experimental procedures (upper row) and schematic overview of a typical trial within the fMRI block design (lower row). The temperature applied during the 14-second heat plateau ranged from 44.7°C to 47.5 ° C in steps of 0.4°C. fMRI = functional magnetic resonance imaging; QST = Quantitative Sensory Testing. Color image is available only in online version (www.psychosomaticmedicine.org).

Each visit started with a medical examination and a reevaluation of inclusion and exclusion criteria. Subsequently, participants received a dose of 32 IU oxytocin, or placebo, applied as four puffs of 0.1 ml per nostril. The dose was chosen according to Singer et al. (6). The oxytocin and the placebo spray only differed in the absence of oxytocin in the placebo. Both sprays had the formulation of Syntocinon Spray (Sigma Tau, Rome, Italy). Participants self-administered the nasal spray under supervision by a physician, according to recommendations by Guastella et al. (16). Testing began after a waiting period of 40 minutes, consistent with the expected peak cerebrospinal fluid (CSF) concentration of nasally applied neuropeptides (4). During the waiting period, participants completed questionnaires and were given standardized instructions for the following testing procedures. Then, thermal thresholding according to the QST protocol (20 minutes) was performed outside the MRI scanner, followed by two fMRI runs. At debriefing, the occurrence and severity of 18 typical side effects were recorded on a 5-point numeric rating scale ranging from 0 (side effect absent) to 4 (severe). In addition, each participant was asked to guess if he received placebo or oxytocin.

Monitoring Mood

The Profile of Mood States (POMS) (17), an adjective rating scale instrument with 65 items, was used to monitor mood changes over the course of the experiment. The POMS total score reflects mood disturbance in general, whereas subscales allow for the monitoring of "tension-anxiety," "depression-dejection," "anger-hostility," "fatigue-inertia," "vigor-activity," and "confusion-bewilderment." The POMS was administered three times: at the beginning of each session, after nasal spray application (near the end of the waiting period), and at the end of each session. At each time point, the participants were asked to rate their "momentary" state.

Quantitative Sensory Testing

Participants were seated in an upright position outside the MRI chamber, with their arms comfortably resting on a cushioned tabletop. Thermal thresholding was performed on the volar surface of the left lower arm, 5 cm proximal from the wrist crease. Thermal stimuli were applied using a Thermosensory Analyzer II (Medoc, Ramat Yishai, Israel) and an MR-safe 30 × 30-mm thermode, kept in place by an elastic strap. All thresholding procedures were performed in the presence of the same experimenter (M.Z.), with no other person or distractors present. Visual, auditory, or social clues indicating the onset of stimulation were precluded by the experimental setup. Written instructions were read aloud to the participant at each session. Cold and warmth detection thresholds, as well as cold and heat pain thresholds were retrieved in this order, according to the established QST protocol (18,19). For each measure, five repetitions were obtained. The first stimulus of each measure was defined as a trial stimulus and discarded from analysis; thresholds were defined as the mean of the last four stimuli.

fMRI—General Information

After QST, participants underwent two separate fMRI experiments. Again, stimulation was applied to the volar surface of the left lower arm. Now the thermode location was 10 cm, or 15 cm proximal from the wrist crease. The thermode was moved in-between the fMRI runs to avoid skin damage. The order of fMRI runs and the order of thermode locations were balanced across participants and medication conditions. Only one of the two fMRI experiments was part of the present study; the other experiment will be reported elsewhere (Zunhammer et al., unpublished observations).

A 3-Tesla Allegra Head Scanner (Siemens, Erlangen, Germany) equipped with a single channel head coil was used for MRI. Functional volumes were obtained with a T2*-weighted echo-planar imaging sequence (repetition time = 2000 milliseconds; echo time = 30 milliseconds; interleaved slicing; flip angle = 90°; 3 × 3 × 3.5-mm voxel size, including a 16% slice gap; field of view = 192 × 192 mm), covering the full brain in 34 horizontal slices coplanar to the anterior and posterior commissure. The first five volumes of each run were discarded to account for T1-saturation effects. In addition a T1-weighted high-resolution structural head volume with 160 sagittal slices was obtained at Visit 1, using a Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (repetition time = 2250 milliseconds, echo time = 2.6 milliseconds, flip angle = 9 °, 1 × 1 × 1-mm voxel size, field of view = 256 × 256 mm). Presentation 14.9 for Windows (Neurobehavioral Systems Inc, Berkeley, CA) was used to display stimuli, retrieve ratings, and log events. Visual stimuli were presented at a resolution of 1024 × 768 pixels and a frame rate of 60 Hz on a screen attached to the head-end of the coil. Participant could see the entire display via a mirror attached to the head coil. The thermal stimulation equipment was the same as for the QST.

MR images were preprocessed and analyzed with statistical parametric mapping (SPM) 8. Volumes were corrected for slice-timing differences using the middle slice as a reference; event onsets were adjusted accordingly. Volume time series were realigned and resliced to the first volume to account for head motion, using SPM's rigid body transformation with fourth-degree B-spline interpolation. Time series were screened for excessive head motion events using ArtRepair (20). Anatomical images were co-registered to the mean realigned functional image, segmented using SPM's Montreal Neurological Institute tissue probability maps, and resampled at 2 × 2 × 2 mm. Realigned functional images were normalized to Montreal Neurological Institute space using the parameters retrieved from the segmentation procedure, preserving signal concentrations ("unmodulated"). All co-registration and segmentation results underwent visual quality control and were corrected if necessary. Images were spatially smoothed with a Gaussian full-width at half maximum (FWHM) kernel of 8 mm to improve signal-to-noise ratio.

fMRI—Experimental Procedure

The fMRI experiment aimed to test if oxytocin induced changes in BOLD activity in response to tonic heat in the brain. Furthermore, it aimed to obtain VAS ratings of heat intensity and/or unpleasantness. For these purposes, a parametric block design (21) with 24 repetitions and a total duration of 14.6 to 17.2 minutes was used. A block outline is provided in Figure 1 (lower row). Block length varied between 36.5 and 43 seconds. Each block started with a visual cue: a red, iconic thermometer was shown onscreen for 1 second to indicate the imminent onset of thermal stimulation, followed by a fixation cross. After 0.5 second, the thermode temperature increased rapidly (10°C/s) from baseline (35°C) to one of eight target temperatures. These

target temperatures ranged from 44.7°C to 47.5°C in steps of 0.4°C and were selected to cover the nonpainful and painful (but tolerable) spectrum in most male participants. Temperatures were selected by interpolating rating data obtained in a previous study (22). Each target temperature was kept at a constant plateau for 14 seconds, before it returned to baseline (10°C/s). The duration of ramp-up and ramp-down varied between 1.3 seconds for 44.7°C and 1.5 seconds for 47.5°C. Over the course of a run, each target temperature was repeated four times in pseudorandomized order. Each of the eight temperatures had to be presented once before being repeated, to achieve an even temperature distribution within runs.

Thermal stimulation was followed by 3 to 6 seconds of rest. Then, a rating phase started: participants were prompted to use a cursor to rate the preceding stimulus on consecutive displays for perceived "intensity" and "unpleasantness." Intensity and unpleasantness were rated on two VASs occupying 70% of the width of the display. The VASs were not ticked or numbered. End points were labeled "no stimulus perceived" and "maximally intense" for the intensity rating and "not unpleasant" to "maximally unpleasant for the unpleasantness rating. Ratings were recorded as integers ranging from 0 to 100, resulting in a 101-point scale. Ratings were entered with a LUMItouch keypad (Photon Control, Vancouver, Canada) held in the right hand. The cursor started at 0 or 100 randomly and could be moved continuously by sustained button press of the index (left) and the middle (right) finger button. Selected ratings could be submitted by pressing the thumb button. Participants were required to submit ratings within 14 seconds; otherwise, the next trial started and the block was discarded. When ratings were submitted in less than 14 seconds, a fixation cross was displayed for the remaining time. Thus, participants had no influence over the pace of the experiment. The next block followed after another resting interval of 3 to 6 seconds at fixation.

Before each run, participants were given standardized instructions. Participants were asked to rate "How hot was the stimulus?" on the intensity scale and "How unpleasant was the stimulus?" on the unpleasantness scale. The difference between stimulus intensity and unpleasantness was illustrated by an analogy according to Price et al. (23). The analogy was adapted by replacing every occurrence of the word "pain" by "heat," to account for the fact that we intended to measure (nonnociceptive) stimulus intensity, not (nociceptive) pain intensity, as in the original protocol. Before the start of the actual fMRI run, two training trials were performed to reduce novelty effects and to reduce sensitization/desensitization effects. Two stimuli with 44.7 and 47.1°C were presented for this purpose.

fMRI—Whole Brain Analysis

The first part of fMRI analysis aimed at identifying brain regions where oxytocin alters the processing of noxious temperatures. Emphasis was put on separating processes related to stimulation in general and pain-related processes. A standard two-step SPM approach was used. In first level analysis, a general linear model was created for each participant to obtain voxel-wise β estimates of within-participant effects. The thermal stimulation period was modeled as a boxcar regressor with blocks of 14 seconds duration, corresponding to the plateau phase of thermal stimulation. Stimulus temperature was added onto the stimulation period as a linear parametric modulator, maintaining SPM's default orthogonalization procedure. The stimulation regressor therefore represented activity related to stimulation per se, whereas the temperature regressor detected activity linearly related to stimulus temperature. Regressors were convolved with SPM's canonical hemodynamic response function. Rating period, the six translational, and the rotational motion parameters were added to the model as nuisance regressors. An autoregressive covariance matrix was used to account for serial correlations. A temporal high-pass filter with a width of 400 seconds was applied. The eye regions and extracerebral tissue were excluded from analysis by using SPM's a priori brain mask. The β contrast maps stimulation > baseline, temperature > baseline, (oxytocin > placebo, stimulation > baseline), (oxytocin > placebo, temperature > baseline), as well as the corresponding reverse contrasts, were obtained for each participant.

To identify activations at group level, a second level analysis was performed. Voxel-wise one-sample t tests were used to test the single-participant contrast maps against the null hypothesis of no effect. The statistical threshold for activation at whole-brain level was defined as $p < .05$, applying SPM 8's family-wise error (FWE) correction for multiple comparisons. Regions were labeled with the Harvard-Oxford atlas for cortical (24) and subcortical regions (25), as included in the FSL analysis software (FMRIBs Software Library, www.fmrib.ox.ac.uk/fsl). Common structural names were added where appropriate. Clusters are overlaid for display on the mean structural image. Graphical artwork was prepared using MRIcron 6/2013 for Mac and arranged in panels using gimp.

fMRI—Region of Interest Analysis of the Amygdala

The second part of fMRI analysis aimed at quantifying the effects of oxytocin on the processing of noxious temperatures within the amygdala. Regions of interest (ROIs) for right and left amygdala were defined anatomically by the tissue probability maps from the subcortical Harvard-Oxford atlas (25), binarized at a threshold of 50%.

The SPM-toolbox marsbar (26) was used to obtain percent signal change estimates for each temperature and medication (placebo versus oxytocin) condition within the ROIs. To estimate peristimulus activity for each temperature separately, the model for fMRI analysis described above had to be adapted: the eight different temperature stimuli were modeled as eight different boxcar regressors, split into 13 time bins of 2-second duration in a finite impulse response model (27). Time bins started at the onset of temperature plateau (0 seconds) and ended 24 seconds poststimulus onset, covering stimulus offset (14 seconds), plus the subsequent resting period (>3 seconds), plus the canonical delay of the hemodynamic response function (6 seconds) (28).

Data Analysis

Statistics were processed with SPSS 21.0.0.0 for Mac. A repeated-measures multivariate analysis of variance was used to test effects of oxytocin on QST thresholds. POMS scores, VAS ratings, and percent signal changes within amygdala ROIs were analyzed using linear-mixed models with maximum likelihood estimation. Sum-of-squares F tests were performed at a two-tailed $\alpha < .05$. For the POMS total score and all subscales, the main and interaction

effects of the factors oxytocin (oxytocin, placebo) and "within-session time points" (before session, before testing, after session) were tested. For the VAS results, the effect of oxytocin was tested, whereas the nonlinear relationship between stimulation temperature and ratings of stimulus intensity and unpleasantness was modeled as a second-order polynomial: mean-centered cofactors temperature and temperature^2, as well as the interaction terms oxytocin * temperature and oxytocin * temperature^2 were included in the model. For amygdala activity, effect of the factor oxytocin was tested including factor time bin, as well as the linear cofactors temperature, intensity ratings, *or* unpleasantness ratings (and the respective interaction terms with oxytocin). Within-participant dependencies were modeled by including individual random intercepts and random effects for all factors, cofactors, and interactions, to obtain a maximal random effects structure (29). A "variance components" covariance matrix was used.

Means are reported ± 1 standard deviation, if not denoted otherwise. Error bars in all figures represent standard errors of the mean corrected for repeated measures (30). Graphs were created using GraphPad Prism 5.0 for Windows.

RESULTS

Sample Description and Medication

Thirty participants successfully completed the study and were eligible for analysis. A detailed participant flow according to the CONSORT criteria is available online, as Supplemental Digital Content 1, http://links.lww.com/PSYMED/A179. No adverse event related to oxytocin occurred. Investigators and participants were blinded until all measures were completed, after which the trial was concluded regularly.

Mean age at study inclusion was 24.9 years (range, 19–30 years). Participants were assigned to Groups A and B in 50.0% of cases. After Visit 1, 77.4% of participants indicated that they believed they received oxytocin, compared with 53.3% after Visit 2. Some participants guessed that they received oxytocin twice, despite being reminded that oxytocin was only given at one of the two visits. Nevertheless, participants guessed the right medication in 50.0% of cases and, as such, did not differ from chance level. The mean side effect score was 3.2 (2.8) for placebo and 2.9 (1.7) for oxytocin, and these values did not differ significantly ($p = .82$) according to a paired t test.

Mood

The POMS total score indicated that participants were generally in good mood before and after the experiment (31). Participants showed significant mood changes over the course of the experiment: they described themselves as significantly more fatigued, confused, and less invigorated after the experiment, compared with the two time points before. Tension, anger, and depression subscales did not change significantly. There was a significant interaction effect of oxytocin * time points for the POMS total score ($F(2,60.3) = 4.34$, $p = .017$) and the confusion subscale ($F(2,60.1) = 5.91$, $p = .005$). Post hoc test indicated that participants showed a nonsignificant tendency toward better mood (-4.36 ± 14.96, $t(27) = 1.54$, $p = .135$) and were significantly less confused (-1.75 ± 2.50, $t(27) = 3.67$, $p = .001$) at the postsession time point, after having received oxytocin, as compared with placebo. Tables with full results for the POMS and its subscales are provided in Supplemental Digital Content 2, http://links.lww.com/PSYMED/A180.

Quantitative Sensory Testing

QST testing started 42 (2) minutes after nasal spray administration, at mean. There were no missing values for the QST procedure. No significant differences between oxytocin and placebo conditions could be found for any thermal thresholding procedure according to repeated-measures multivariate analysis of variance ($F(4,26) = 0.64$, $p = .64$). Results are detailed in Table 1.

fMRI—Data Set

On average, fMRI testing started 76 (6) minutes and ended 130± (10) minutes after nasal spray administration. The last three trials of one session were lost due to a technical failure; however, the remaining data from that participant were retained in analysis. At mean, participants completed their ratings within 7.50± (1.43) seconds. Two trials had to be excluded from analysis because ratings were not submitted within 14 seconds. Overall analysis was based on 1435 trials for 30 participants.

fMRI—Ratings

Mean results for intensity and unpleasantness ratings obtained during fMRI scanning are shown in Figure 2A

TABLE 1. Quantitative Sensory Testing Results

Measure	Placebo	Oxytocin	$F(1,29)$	p
Cold detection threshold	30.98 ± 0.47	30.90 ± 0.41	0.68,	.41
Warmth detection threshold	33.75 ± 0.79	33.99 ± 1.01	2.22	.15
Cold pain threshold	18.37 ± 7.59	18.79 ± 7.68	0.24	.63
Heat pain threshold	44.39 ± 3.15	44.59 ± 2.89	0.29	.59

Effects of oxytocin on thermal detection and pain thresholds were not significant. All means and differences in °C. F statistics were obtained performing a repeated-measures multivariate analysis of variance ($n = 30$, two observations/variable/participant).

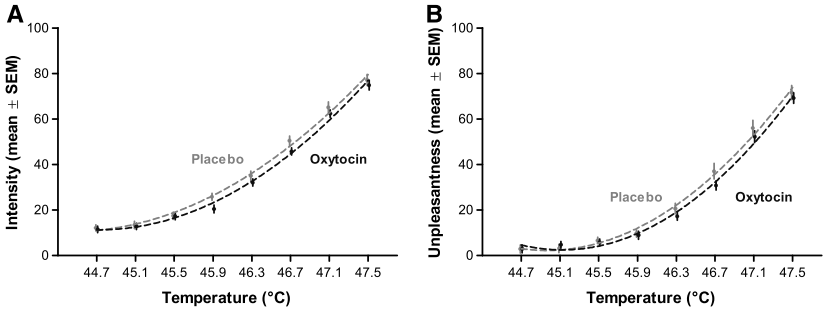

FIGURE 2. A and B, Oxytocin effects on visual analog scale ratings of heat. Parameter estimates indicated that intensity ratings (β = −3.46, 95% CI = −6.86 to −0.07, t = −2.06, p = .046) significantly decreased during oxytocin sessions, compared with placebo sessions. A similar, but nonsignificant trend was apparent for unpleasantness ratings (β = −2.77, 95% CI = −6.97 to −1.42, t = −1.37, p = .18). The mean ratings shown were shifted slightly along the x-axis (±0.02°C) for the sake of illustration. Error bars represent SEM corrected for repeated measures. n = 30, with 48 observations/variable/participant. CI = confidence interval; SEM = standard error of the mean. Color image is available only in online version (www.psychosomaticmedicine.org).

and B, respectively; dashed lines show the curve of best fit. Mixed-model analysis indicated that the factor oxytocin explained a significant amount of variance in intensity (F(1,41.9) = 4.23, p = .046), but not in unpleasantness ratings (F(1,41.2) = 1.87, p = .18). As expected, cofactors temperature and temperature^2 were significant predictors of intensity and unpleasantness ratings (all: p < .001). None of the interaction terms showed a significant effect (p > .16). We therefore conclude that oxytocin decreased intensity ratings after adjusting for repeated measures and the nonlinear effects of temperature.

fMRI—Whole-Brain Analysis

The contrasts temperature > baseline and stimulation > baseline were assessed to confirm that our fMRI paradigm was effectively identifying regions of pain processing. Significant activations were found in the bilateral insula, somatosensory cortex II, cingulate cortex, dorsolateral prefrontal

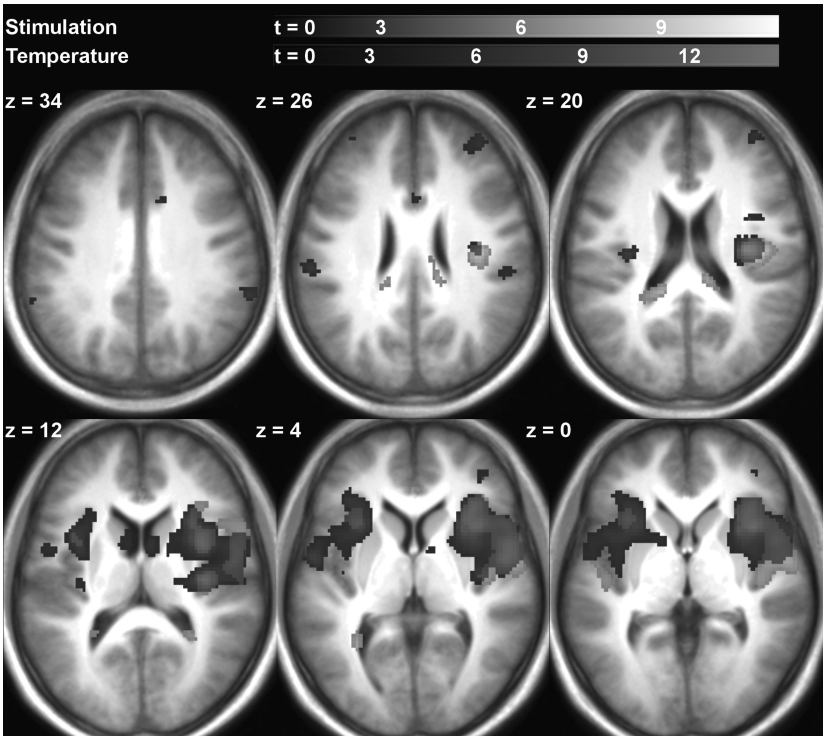

FIGURE 3. Clusters showing increased activity during stimulation period (contrast: stimulation > baseline, gold) and brain regions showing a linear activity increase with heat (contrast: temperature > baseline, red). The threshold of significance was p < .05, corrected for FWE, with a minimum size of 5 voxels. Left hemisphere is shown left. Regions are labeled according to the Harvard-Oxford Atlas. For coordinates and tabular results, see Supplemental Digital Content 3, http://links.lww.com/PSYMED/A181. n = 30 with 48 trials of 14 seconds duration/variable/participant. FWE = family-wise error. Color image is available only in online version (www.psychosomaticmedicine.org).

cortex, and caudate nucleus. Results of this control analysis are provided in Figure 3 and in tabular form in Supplemental Digital Content 3, http://links.lww.com/PSYMED/A181.

The oxytocin > placebo and reverse contrasts were analyzed with respect to the effects of the temperature and the stimulation regressor. No significant effects of oxytocin were found on whole-brain level for any of these comparisons at the statistical threshold of $p < .05$ when applying FWE correction. Using a liberal threshold of $p < .001$ uncorrected for multiple comparisons (minimal cluster size 5 voxels), several clusters were identified, which showed decreased activation during the oxytocin sessions. These preliminary results are provided in Figure 4 and in tabular form in Supplemental Digital Content 4, http://links.lww.com/PSYMED/A182.

fMRI Analysis 2: ROI Analysis of the Amygdala

The percent signal change estimates for right and left amygdala activity are displayed in Figure 5. Main effects and interactions between factors oxytocin, time bin, and (mean centered) temperature were tested using mixed-model analysis. Oxytocin was found to explain a significant amount of variance in BOLD signal in the left ($F(1,30.0) = 4.77$, $p = .037$) and right ($F(1,30.0) = 7.89$, $p = .009$) amygdala. As expected, the time bins explained a significant proportion of variance in both amygdalae (both: $p < .001$). The cofactor temperature did not explain a significant proportion of variance; neither in the left ($F(1,30.1) = 0.66$, $p = .42$) nor in the right ($F(1,32.2) = 1.41$, $p = .24$) amygdala. All interaction terms were nonsignificant. Intensity and unpleasantness ratings did not explain a significant proportion of variance in amygdala activity, when used as a cofactor in mixed-model analysis instead of temperature ($p > .79$ for both variables and hemispheres).

Post Hoc Analyses

Oxytocin-induced differences in intensity ratings (mean change in ratings) did not correlate with oxytocin induced difference POMS total score (mean change in score, Kendall tau-b = −0.170, $p = .21$). Oxytocin-induced differences in amygdala activity (mean change in signal) did not correlate with oxytocin-induced difference in POMS total score (mean change in score, Kendall tau-b = −0.099, $p = .62$). Similarly, there were no significant correlations between mean changes in intensity or unpleasantness ratings and mean changes in amygdala activity (all variables and hemispheres: $p > .79$).

Mean heat intensity (Kendall tau-b = −0.434, $p = .001$) and mean heat unpleasantness (Kendall tau-b = −0.407, $p = .001$) ratings correlated significantly with heat pain

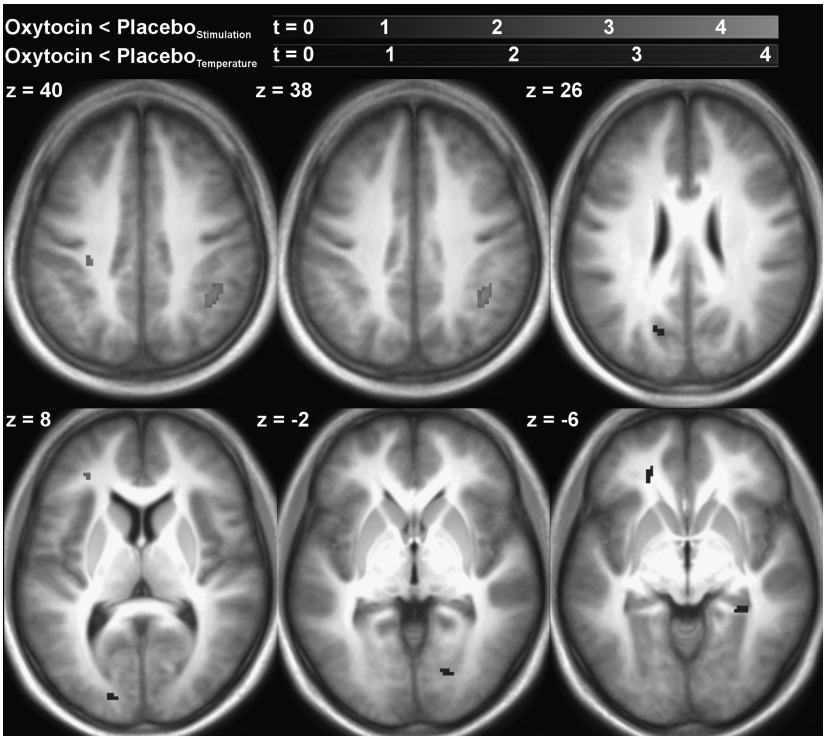

FIGURE 4. Clusters showing decreased stimulus-related activity (green) and decreased temperature related activity (blue) during oxytocin compared with placebo sessions. The statistical threshold was set at $p < .001$, uncorrected for multiple comparisons, with a minimal cluster size of 5 voxels. No significant clusters were found showing increased activation under oxytocin. No significant effects were found when using a threshold of $p < .05$ with correction for multiple comparisons. Left hemisphere is shown left. Regions are labeled according to the Harvard-Oxford Atlas, with tissue probabilities in %. For coordinates and tabular results, see Supplemental Digital Content 4, http://links.lww.com/PSYMED/A182. $n = 30$ with 48 trials of 14 seconds duration/variable/participant. FWE = family-wise error. Color image is available only in online version (www.psychosomaticmedicine.org).

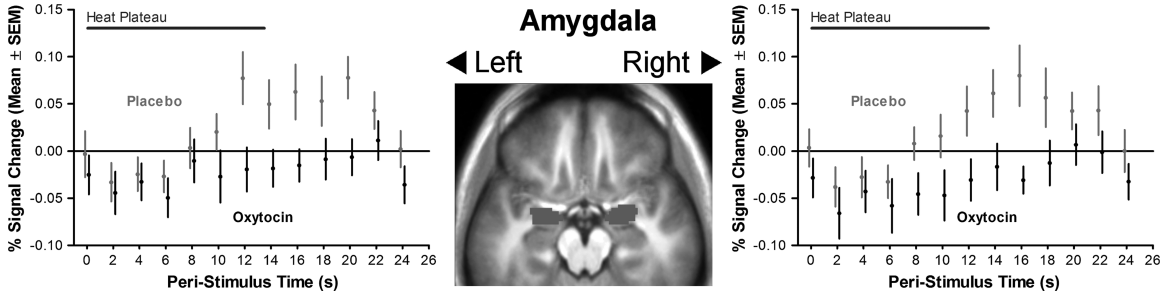

FIGURE 5. Mean time course of bilateral amygdala activity during stimulation and effects of oxytocin. Parameter estimates indicated that left ($\beta = -0.045$, 95% CI = −0.087 to −0.003, $t = -2.19$, $p = .037$) and right ($\beta = -0.051$, 95% CI = −0.088 to −0.014, $t = -2.82$, $p = .008$) amygdala activity was significantly decreased in oxytocin, compared with placebo sessions. Temperature was not found to significantly predict amygdala activity; the depicted mean % signal changes were therefore pooled across temperatures. Error bars represent SEMs corrected for repeated measures. $n = 30$ with 48 trials of 14 seconds duration/variable/participant. CI = confidence interval; SEM = standard error of the mean. Color image is available only in online version (www.psychosomaticmedicine.org).

threshold (data aggregated across sessions), but not with warmth detection threshold, cold detection threshold, and cold pain threshold (all: $p > .11$).

DISCUSSION

The present neuroimaging study is the first to test the effects of intranasal oxytocin on human heat pain processing. Oxytocin did not significantly affect thermal thresholds before fMRI (Hypothesis A), but did significantly reduce participants' heat intensity ratings during fMRI (Hypotheses B). A similar, but nonsignificant trend toward reduced unpleasantness ratings could also be observed. The effects of oxytocin on VAS ratings had a modest (~−4% of the VAS) effect size. No effect of oxytocin on temperature-related cerebral processing was found at whole-brain level using a robust statistical threshold. (Hypotheses C). An ROI analysis showed that oxytocin significantly reduced BOLD responses within the bilateral amygdala (Hypothesis D). Oxytocin-induced changes in mood, subjective heat intensity ratings, and amygdala activity did not correlate significantly.

The Amygdala, Oxytocin, and Pain

By showing that oxytocin reduces the hemodynamic response within the amygdala during heat stimulation (see Fig. 5), our results point to the established effect of intranasal oxytocin on amygdala activity (for review see Ref. (10)). We could therefore consolidate preliminary evidence by Singer et al. (6), who found reduced amygdala reactivity in response to painful stimulation in a sub-group of participants, while studying oxytocin effects on empathy.

Contrary to our expectations, whole-brain analysis was unable to identify the amygdala among the regions showing significant stimulus- or temperature-related activity (see Fig. 3). Owing to its increased power, an ROI analysis could confirm that both amygdalae were active during stimulation and showed a typical hemodynamic response (see Fig. 5). Considering that the canonical delay of the hemodynamic response is approximately 5 seconds (32), the observed amygdala activation peak (12–16 seconds) was related to the second half of the heat stimulus plateau. This time course suggests that amygdala activity was linked to features of heat stimulus perception, rather than threat evaluation. The latter would be expected to occur earlier, corresponding to the stimulus onset, or to the preceding visual cue, that is, at a time when the painfulness of the upcoming stimulus is still uncertain. Nevertheless, the amygdala response and the oxytocin effect were not significantly related to temperature, intensity ratings, or unpleasantness ratings. Therefore, general stimulus-related, rather than pain-specific processes might underlie the observed effects. Our results thus provide additional evidence for an effect of oxytocin on stimulus processing in the amygdala, but do not necessarily support the hypothesis of a specific antinociceptive effect.

Whole-Brain Results and Imaging Paradigm

Our fMRI paradigm detected significant, robust, positive linear associations between noxious temperature and BOLD signal in the insula, somatosensory cortex II, cingulate cortex, dorsolateral prefrontal cortex, and caudate nucleus (see Fig. 3). These results are in agreement with previous results for heat pain (33) and verify that our fMRI paradigm was capable of detecting pain-related cerebral processing.

Despite these positive results, no effect of oxytocin on stimulus- or temperature-related activity could be found with a threshold of $p < .05$, after correction for multiple comparisons with the conservative FWE procedure. Clusters of inhibitory oxytocin effects in the posterior hippocampus, the supramarginal gyrus, as well as several prefrontal, fusiform, and occipital regions (see Fig. 4) could be found with a more liberal statistical threshold of $p < .001$ and a voxel size greater than 5, uncorrected for multiple comparisons. However, we think that strict correction for multiple comparisons is vital for fMRI research (34). Therefore, we consider these results preliminary and recommend that they be interpreted with caution.

Rating Results

Similar to the amygdala results, our behavioral results do not provide unequivocal support of an analgesic effect of oxytocin. The small oxytocin effect on VAS ratings was not found to be temperature dependent. In addition, intensity and, less so, unpleasantness ratings were affected—the opposite would be expected for a clear analgesic effect. We therefore conclude that changes in somatosensory perception and/or changes in other general cognitive states, such as attention, anxiety, or vigilance, may account for the observed oxytocin effects. Our results are in accord with recent findings of Rash and Campbell (9), who found that intranasal oxytocin reduces pain intensity ratings, pain unpleasantness ratings, and descriptive ratings of cold-pressor pain. Although Kessner et al. (7) found no effect of oxytocin on heat pain ratings, our results are compatible with the finding that oxytocin enhances the placebo effect. Our participants guessed to have received oxytocin in 77.4% of cases at Visit 1 and in 53.3% of cases at Visit 2. This high prevalence of placebo beliefs in our sample may have driven the effect.

Social interaction was limited in the present experiment, especially within the MR environment, and no social stimulus material was involved in the present study. These circumstances may have limited the efficacy of oxytocin in comparison to previous studies, as the effects of oxytocin have repeatedly been shown to depend on social context and associated beliefs (35). The discrepancy between our positive results for the VAS ratings and our negative results for the thermal thresholding procedure might be explained by a number of points: the rating procedure involved six times more stimulus repetitions than the thresholding procedure, thus entailing more statistical power. Furthermore, the thresholding and the rating procedures differed in timing, thermode location, stimulus dynamics, stimulus intensity, and response type—all of which may have made a difference. Of note, oxytocin may have exerted an effect on pain perception in the anxiogenic environment of the MR scanner, consistent with the hypothesis of oxytocin as an anxiolytic agent (11).

Oxytocin Effects on Mood

Oxytocin was found to ameliorate negative mood changes occurring over the course of the experimental sessions. Participants described themselves as less confused after the oxytocin session, in comparison to the placebo session. However, these changes in mood were not significantly correlated with changes in intensity ratings or amygdala activity, and therefore may be of limited relevance for the main aim of our study. Of note, the "tension-anxiety" subscale of the POMS, a measure related to stress and anxiety, was not significantly affected by oxytocin.

Limitations

The present study was limited to healthy men. The present results might therefore not generalize to the female population, to patient populations, or to clinical forms of pain, We limited our sample to men because the summary of medical product characteristics for Syntocinon Spray listed uterine contractions as a potential side effect in women (36), which was deemed a potential source of confound for the testing of pain processing. However, a large review on the safety of intranasal oxytocin indicated that female study participants are not reported to experience more side effects than their male counterparts (2). Therefore, our concerns might have been unsubstantiated and we encourage future studies on the effects of intranasal oxytocin on heat pain in women.

Thermal stimulation of the skin was the main method of sensory stimulation used in the present study. The present results may therefore not generalize to other forms of (noxious) somatosensory stimulation. In addition, the stimulus protocol used in our fMRI paradigm involved the same heat stimulus intensities for all participants. Individualized stimulus intensities may have increased the power to detect effects of oxytocin by reducing between-participant variability.

The pathway of intranasal oxytocin into the brain is still unknown. Its peak and retention times in the brain, as well as its bioavailability and metabolism, are incompletely understood. Born et al. (4) found that 40 IU of intranasal vasopressin (a structural sibling of oxytocin) increased concentrations in human CSF 40 and 80 minutes after application; no measurements were obtained beyond 80 minutes, prohibiting the estimation of retention times. A small study assessing oxytocin levels in the human CSF (3) found elevated oxytocin levels 75 minutes after intranasal application; again, no measurements were obtained beyond this time point. Oxytocin concentrations in human saliva have been reported to remain elevated for 7 hours after nasal spray application (37). Oxytocin levels were found to peak between 30 and 60 minutes and return to baseline between 90 and 120 minutes after nasal application in in vivo microdialysates of rat and mouse brain tissues (5). According to these studies, our thresholding procedure (40–60 minutes after application) might have started too early (3) or too late (5), and our fMRI experiments might have taken too long (130 minutes). The present results may therefore be limited to specific time frames, that is, 40 to 60 minutes for the QST and 75 to 130 minutes for the rating and fMRI results. Furthermore, no optimal dose of intranasal oxytocin for eliciting behavioral effects has been determined, yet. Because the present study did not include different dosing conditions, it cannot provide information on a probable dose-response relationship. The present findings are therefore limited to the specific oxytocin dose of 32 IU, which may be too low or even too high (37,38). These issues may have obscured further oxytocin effects from detection and may have resulted in an underestimation of actual effect sizes.

Finally, we want to raise awareness for a limitation underappreciated in human intranasal oxytocin research: oxytocin has repeatedly shown to affect social behavior (39), and there is no reason why this should not apply to experimental

settings, where participants usually interact with experimenters. "Good-participant" behavior might systematically increase under oxytocin, although there is no positive evidence for an oxytocin effect on demand characteristics (40), yet. Such an oxytocin-induced response bias might confound any behavioral measurement in oxytocin studies—even when a double-blind placebo-control is used, as in the present study.

CONCLUSIONS

Our results provide further evidence for a significant but subtle effect of oxytocin on heat stimulus perception and/or appraisal. They further provide evidence for a significant effect of oxytocin on heat stimulus processing in the amygdala. Preliminary evidence for activity decreases in the hippocampus, prefrontal cortex, parietal cortex, and occipital cortex could be obtained. These regions pose potential targets for further investigations on pain-related oxytocin effects. However, the present study could not support the hypothesis of an antinociceptive effect of oxytocin because the oxytocin-induced changes in VAS ratings and amygdala activity were not found to be temperature dependent and oxytocin affected intensity, rather than unpleasantness ratings. Future studies are needed to unravel the effects of intranasal oxytocin on pain-related processes.

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