

The Analgesic Effect of Oxytocin in Humans: A Double-Blind, Placebo-Controlled Cross-Over Study Using Laser-Evoked Potentials

Y. Paloyelis*, C. Krahé*, S. Maltezos†, S. C. Williams*, M. A. Howard* and A. Fotopoulou‡

*Department of Neuroimaging, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK.

†Department of Forensic and Neurodevelopmental Science, Institute of Psychiatry, King's College London, London, UK.

‡Research Department of Clinical, Educational and Health Psychology, University College London, London, UK.

Journal of Neuroendocrinology

Correspondence to: Yannis Paloyelis, Department of Neuroimaging (P089), The Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, De Crespigny Park, London SE5 8AF, UK (e-mail: yannis.paloyelis@kcl.ac.uk).

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Oxytocin is a neuropeptide regulating social-affiliative and reproductive behaviour in mammals. Despite robust preclinical evidence for the antinociceptive effects and mechanisms of action of exogenous oxytocin, human studies have produced mixed results regarding the analgesic role of oxytocin and are yet to show a specific modulation of neural processes involved in pain perception. In the present study, we investigated the analgesic effects of 40 IU of intranasal oxytocin in 13 healthy male volunteers using a double-blind, placebo-controlled, cross-over design and brief radiant heat pulses generated by an infrared laser that selectively activate A δ - and C-fibre nerve endings in the epidermis, at the same time as recording the ensuing laser-evoked potentials (LEPs). We predicted that oxytocin would reduce subjective pain ratings and attenuate the amplitude of the N1, N2 and P2 components. We observed that oxytocin attenuated perceived pain intensity and the local peak amplitude of the N1 and N2 (but not of P2) LEPs, and increased the latency of the N2 component. Importantly, for the first time, the present study reports an association between the analgesic effect of oxytocin (reduction in subjective pain ratings) and the oxytocin-induced modulation of cortical activity after noxious stimulation (attenuation of the N2 LEP). These effects indicate that oxytocin modulates neural processes contributing to pain perception. The present study reports preliminary evidence that is consistent with electrophysiological studies in rodents showing that oxytocin specifically modulates A δ /C-fibre nociceptive afferent signalling at the spinal level and provides further specificity to evidence obtained in humans indicating that oxytocin may be modulating pain experience by modulating activity in the cortical areas involved in pain processing.

Key words: oxytocin, nociception, analgesia, laser-evoked potentials, EEG

doi: 10.1111/jne.12347

Oxytocin is a neuropeptide synthesised in the hypothalamus. It is independently released into the bloodstream, exerting hormonal effects on diverse physiological functions (1), and in the central nervous system, exerting neuromodulatory effects on widely distributed oxytocin and vasopressin receptors (2,3). Oxytocin affects reproductive and social-affiliative behaviours, including neuroprotective (4) and analgesic (5) roles during birth.

Robust preclinical evidence has demonstrated that exogenous oxytocin has antinociceptive effects and has identified plausible underpinning mechanisms (6). One mechanism involves the engagement of GABA-mediated inhibitory circuits at the superficial layers of spinal cord dorsal horn neurones, which express oxytocin

receptors (7) and receive direct projections from hypothalamic oxytocin neurones (8,9), reducing A δ /C-fibre afferent signalling and ascending nociceptive input (10–14). Additional mechanisms involve the engagement of the endogenous opioid (15,16) and cannabinoid (17) systems modulating nociception.

These findings, strengthened by initial evidence from open-label clinical reports indicating that oxytocin produced analgesia in clinical cases (16,18), led to the hypothesis that oxytocin has analgesic effects in humans. However, double-blind, placebo-controlled studies have produced mixed results. In clinical groups, some reports suggested that a single intranasal oxytocin dose provided headache relief (19) and that an i.v. oxytocin infusion decreased colonic

visceral perception in patients with irritable bowel syndrome (20). However, other studies administering intranasal oxytocin daily from 3 to 13 weeks in patients with chronic syndromes did not show any analgesic effects (21,22).

Experimental studies on healthy volunteers using acute noxious electrical, contact heat and cold-pressor stimuli have produced further conflicting results. Most reports have not demonstrated any specific antinociceptive properties for oxytocin (23–25), including a phase 1 open-label trial using intrathecal administration (26). By contrast, Rash and Campbell (27) reported that intranasal oxytocin reduced the perception of pain intensity and unpleasantness. However, because 70% of the sample correctly identified the treatment that they received (possibly as a result of the use of a saline spray as placebo), the observed analgesic effects of oxytocin could have been partially a result of the potentiating effects of oxytocin on placebo analgesia (24).

In the present study, we investigated the effects of 40 IU of intranasal oxytocin on pain perception and its neural correlates using healthy male volunteers and a double-blind, placebo-controlled cross-over design. We used brief radiant heat pulses generated by an infrared laser that selectively activate A δ and C-fibre nerve endings in the epidermis (28). The use of noxious stimuli that specifically engage A δ /C-fibre nociceptive afferent signalling in humans may provide a promising tool for illuminating the antinociceptive properties of oxytocin given evidence from electrophysiological studies in rodents suggesting that oxytocin specifically modulates A δ /C-fibre nociceptive afferent signalling (10,29). We predicted that intranasal oxytocin would reduce subjective pain reports and attenuate the amplitude of the N1, N2 and P2 laser-evoked potentials (LEPs) reflecting the cortical response to nociceptive input (30). Latencies were also examined in an exploratory manner. LEPs reflect temporally distinct cortical processes specifically evoked by the activation of A δ -fibres (31) and are differentially modulated by cognitive (32–34) and pharmacological (35) interventions. Hence, LEPs are ideally suited for investigating the mechanisms underpinning the analgesic effects of oxytocin on pain perception. Given the likely effect of oxytocin on the stress response (36), we also measured salivary cortisol levels to control for stress-related physiological changes contributing to the predicted analgesic effect.

Materials and methods

Participants

Thirteen right-handed, healthy adult male volunteers participated in the present study (mean \pm SD age: 25.69 \pm 4.85 years). Participants did not have a history of medical, neurological or psychiatric problems and were screened for current psychiatric conditions using the Symptom Checklist-90-R (37) and Beck's Depression Inventory II (38). They did not take any prescribed drugs, tested negative on a urine screening test for drugs of abuse, and consumed < 28 units of alcohol per week and < 5 cigarettes per day. Both parents were white European to reduce genetic background variability (39). Day-of-testing lifestyle changes included abstaining from alcohol and heavy exercise for 24 h and not having any beverages or food in the 2 h before the testing session. Data for 18 participants were

excluded for showing very low and inconsistent hand temperatures between testing sessions (< 27 °C) as a result of a central heating fault in the laboratory, with six of these not showing any discernible LEPs at baseline recordings (one participant also scored above the cut-off score of 13 on Beck's Depression Inventory). Although skin temperatures were recorded as a result of their importance for pain perception, the risk of not maintaining consistent skin temperatures within a specific range across active and placebo conditions had not been fully appreciated at the time because, during our pilot studies, the temperature of the laboratory was well controlled. Given the close association between initial skin temperature and the required energy level to reach a given effect, initial skin temperature and consistency across testing conditions in studies investigating the antinociceptive properties of drugs are recognised as important factors that could confound treatment effects (40–46). We aimed to maintain a dataset of at least $N > 11$ [comparable to other LEP studies (47), including studies using LEPs to investigate the analgesic properties of drugs (35): $N = 12$], choosing 27 °C as the cut-off temperature (a temperature as close to the bottom end of a previously reported typical range as possible, between 28.5 and 33.6 °C) (47). This criterion was set post-hoc but before the analysis of results. Participants provided their written informed consent and received compensation for their time. King's College London Research Ethics Committee (PNM/10/11-160) approved the study.

Nociceptive stimulation

We used an infrared neodymium:yttrium-aluminum-perovskite (Nd:YAP; Electronical Engineering, Florence, Italy) laser with a wavelength of 1.34 μ m to generate radiant heat pulses. The pulses had duration of 4 ms, were transmitted via an optic fibre cable, and were focused by a lens to a spot diameter of 6 mm at the target site on the dorsum of the left hand. The spot location was changed after each pulse to avoid nociceptor fatigue and sensitisation (48).

Oxytocin administration

Participants self-administered 40 IU of intranasal oxytocin (Syntocinon; Novartis, Basel, Switzerland) or placebo (same composition as Syntocinon except for oxytocin). They applied one puff containing 4 IU of oxytocin (or placebo) every 30 s, alternating between nostrils. The administration phase lasted approximately 9 min including a 3-min rest at the end. Participants reported no side effects during or immediately after the experimental procedure.

Experimental design

We adopted a double-blind AB/BA (oxytocin-placebo/placebo-oxytocin) cross-over design with baseline measurements before each treatment (49) to test for treatment effects. We obtained baseline measurements before each treatment because they can contain important background information on each participant and increase the precision of the analyses of treatment effects (49). Participants were randomly allocated to a treatment sequence, receiving each treatment on separate visits (mean \pm SD: 13.15 \pm 6.05 days apart) at the same time of the day. Of the 13 participants, seven received oxytocin in the first visit and six in the second visit. Infrared laser stimuli were delivered as part of a battery of tasks and questionnaires, and participants were informed that we investigated the effects of a neuropeptide on brain activity and on a range of mental processes. All participants remained blind with respect to the name of the neuropeptide that they received and also the true purpose of the study until debriefing at the end of the second visit.

Procedures

Participants were seated at a desk facing a computer monitor framed by a screen. The laser equipment and the experimenter were seated behind the screen to prevent visual contact with the participant. Participants rested their left forearm on the desk, extending it through an opening on the screen so that they did not have visual contact with the stimulated hand.

The same testing protocol was followed during each visit. Initially, participants experienced a series of pulses of increasing energy to familiarise them with the equipment, the procedures and the sensations, as well as to determine their individual pain tolerance levels. Participants were asked to focus on the pinprick sensation generated by the activation of the A δ fibres and rate it on an 11-point visual analogue scale (VAS) with anchors (0 = 'no pinprick sensation' to 10 = 'the worst pinprick sensation imaginable'). Participants then received three computer-administered mini-blocks of pulses, each consisting of all laser intensities, in steps of 0.25 J, up to their individually determined tolerance limit, and were asked to provide a VAS rating after each pulse. These were used to determine separately, for each participant, the experimental stimulus intensity corresponding to a clearly perceived pinprick of moderate intensity, and the control stimulus intensity, corresponding to the energy level at the threshold of perception. The same stimulus intensities were used in the oxytocin and placebo visits for each participant. Subsequently, we fitted the electroencephalogram (EEG) cap. Participants then received two blocks of nociceptive stimuli: one immediately before receiving treatment ('baseline') and one approximately 45–50 min after treatment onset ('post-treatment'), when their EEG was being recorded. Each block consisted of 50 experimental and 20 control trials presented in pseudorandom order. Hand skin temperature was recorded with an infrared thermometer at the beginning of each block. Each trial began with a fixation cross (displayed for 6 s), with a laser pulse being delivered midway (at 3 s). Then the word 'Rating' appeared on the screen for 3 s as a cue for participants to give their VAS rating orally using the same anchors as above. Each trial ended with a jittered inter-trial interval (0–5 s; mean trial duration was 11.43 s). The mean \pm SD experimental stimulus intensity was 4.12 ± 0.44 J, range 3.35–4.5 J) and the mean \pm SD control intensity was 1.83 ± 0.40 J, range 1.5–2.5).

Salivary cortisol

Salivary samples were obtained via passive drool to measure the unbound form of cortisol (50) at four time points: two samples were obtained during set-up and averaged to obtain cortisol levels at 'session onset' (Table 1); one

sample at the end of the baseline nociceptive experimental block (before treatment); and a final sample at the end of the post-treatment experimental nociceptive block (approximately 60–65 min after treatment onset). Salivary samples were frozen at -80 °C until assayed. Free cortisol concentration was measured using the Salimetrics Elisa kit (Salimetrics Europe Ltd, Newmarket, UK), which comprises a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary cortisol. The intra-assay coefficient of variation (CV) was between 3.35% and 3.65%, and the inter-assay CV was between 3.75% and 6.41%. The sensitivity of the assay was 0.083 nmol/l.

LEPs

Brief radiant heat pulses generated by an infrared laser selectively activate A δ - and C-fibre skin nociceptors and generate a series of transient, time-locked brain responses (51) that appear as deflections in the EEG and specifically reflect the activation of A δ fibres (52). LEPs comprise a negative-positive deflection, maximal at the scalp vertex (N2–P2 wave, peaking at 200–350 ms when stimulating the hand dorsum), and a preceding smaller negative deflection maximal at the contralateral temporal electrodes (N1 wave, peaking at approximately 160 ms) (48).

EEG recording

EEG data were collected using a 64-channel Neuroscan Quik-cap elasticised cap with passive AgCl electrodes and recorded using SCAN, version 4.3 (Compumedics, Ltd, Charlotte, NC, USA). Data were collected from 64 electrodes positioned on the scalp in accordance with the International 10–20 system. A bipolar electrode on the earlobes was used as the recording reference and additional electrodes on the mastoids were used to serve as reference electrodes for the analyses. The electrooculogram was recorded by placing a bipolar electrode above and below the right eye and two electrodes to the left and right of each eye. Impedances were kept below 5 k Ω . The data were sampled at 5000 Hz.

EEG analysis

EEG data were processed using the MATLAB (R2011a; MathWorks Inc., Natick, MA, USA) open-source toolboxes EEGLAB (53) and ERPLAB (54). We focused our analyses on the experimental trials because the control trials were included to decrease the predictability of the experimental stimuli. EEG data were

Table 1. Raw Mean Visual Analogue Scale (VAS) Ratings, Mean Local Peak Amplitudes and Latencies of the N1, N2 and P2 Laser-Evoked Potentials (LEPs), Salivary Cortisol Levels and Skin Temperatures at the Dorsum of the Left Hand from the Baseline and the Post-Treatment Blocks.

	Session onset		Baseline		Post-treatment	
	Placebo visit, mean \pm SD	Oxytocin visit, mean \pm SD	Placebo visit, mean \pm SD	Oxytocin visit, mean \pm SD	Placebo visit, mean \pm SD	Oxytocin visit, mean \pm SD
N1 local peak amplitude (μ V)	–	–	-9.95 ± 4.31	-9.62 ± 3.93	-9.56 ± 4.02	-8.16 ± 2.88
N1 local peak latency (ms)	–	–	186.14 ± 21.64	192.37 ± 21.18	185.34 ± 18.52	193.80 ± 19.41
N2 local peak amplitude (μ V)	–	–	-12.58 ± 7.10	-12.01 ± 6.76	-11.65 ± 7.39	-9.67 ± 5.45
N2 local peak latency (ms)	–	–	217.79 ± 19.68	218.72 ± 25.53	215.30 ± 21.08	225.41 ± 23.65
P2 local peak amplitude (μ V)	–	–	11.17 ± 5.56	11.29 ± 4.75	10.68 ± 5.22	9.68 ± 3.10
P2 local peak latency (ms)	–	–	333.28 ± 28.31	335.91 ± 36.69	334.70 ± 27.37	339.72 ± 34.36
VAS ratings	–	–	3.83 ± 1.47	3.32 ± 1.29	3.35 ± 1.44	3.12 ± 1.50
Salivary cortisol (nmol/l)	5.56 ± 3.34	5.75 ± 2.78	4.75 ± 3.07	4.14 ± 2.18	3.74 ± 2.44	4.16 ± 2.73
Skin temperature (°C)	–	–	31.09 ± 1.99	30.69 ± 1.80	30.58 ± 1.82	29.79 ± 2.10

down-sampled to 250 Hz and re-referenced using the averaged recordings from the mastoid electrodes before applying a high-pass filter of 0.4 Hz. The EEG was then segmented into -500- to 1000-ms epochs relative to the onset of the stimulus and a 30 Hz low-pass filter was applied. Epochs from the baseline and post-treatment blocks were concatenated and trials with gross artefacts (exceeding 400 μ V) were removed. Ocular artefacts were removed using independent component analyses ('runica' algorithm) in EEGLAB. Prototype blinks and saccades were identified following visual inspection and the CORRMAP utility (55) was used to identify matching artefacts across the datasets for removal. Finally, epochs containing artefacts exceeding $\pm 100 \mu$ V were removed, then epochs were resegmented to -200 to 800 ms and baseline-corrected (-200 to 0 ms). Fewer than 10% of trials were removed from each dataset. The ERPLAB measurement tool was used to measure the local peak amplitude and latency of the LEPs. Average waveforms for each component time-locked to stimulus onset were computed for each block (baseline, post-treatment) and treatment condition (oxytocin, placebo). The peak-to-baseline amplitude and the latency of the N2 (latency window: 100–350 ms) and P2 (latency window: 250–420 ms) components were measured at the Cz electrode using an average reference, and those of the N1 component (latency window: 0–270 ms) were measured at the C4 electrode (contralateral to the stimulated hand), using the Fz electrode as reference.

Statistical analysis

Our primary outcome variables were the mean VAS ratings and the mean local peak amplitudes of the N1, N2 and P2 LEPs for each block. Component latencies and salivary cortisol levels (nmol/l) were secondary outcome variables. We tested for treatment effects (oxytocin versus placebo) using the analysis of covariance (ANCOVA) approach described by Senn (49) and Metcalfe (56) for the analysis of AB/BA cross-over designs with baseline measurements before each treatment. If T = active treatment (intranasal oxytocin), C = control treatment (placebo), X_T and X_C are the corresponding baseline measurements, and Y_T and Y_C the corresponding post-treatment measurements, using the regression command in STATA, version 13 (StataCorp, College Station, TX, USA), we implemented the ANCOVA model: $(Y_{Ti} - Y_{Ci}) = \beta_T + \gamma(X_{Ti} - X_{Ci})$ as described by Senn (49) and Metcalfe (56), separately, for each outcome variable. The intercept term in this model tests for the treatment effect. In the regression model, we also included a binary explanatory variable representing treatment sequence (AB or BA) for each measure where there was a significant period effect (which might reflect a general tendency, irrespective of treatment). We tested for period effects (i.e. changes in measurements across visits, obtained by subtracting post-treatment measurements at the first visit from post-treatment measurements at the second visit) by regressing period differences on treatment sequence. Additionally, we computed baseline-corrected basic estimators of treatment effects by subtracting the corresponding baseline differences weighted by their regression slope. As a control, we tested for treatment effects on skin temperatures using the same approach; we also used paired-sample t-tests to check separately for skin temperature differences between baseline and post-treatment measurements under oxytocin and placebo. Finally, we tested for time effects on salivary cortisol levels (to investigate whether the administration of the nociceptive stimuli increased cortisol levels) using a 3 (Time: session onset, before treatment, post-treatment) by 2 (Treatment: oxytocin, placebo) analysis of variance (ANOVA) model implemented in STATA, version 13 (StataCorp) using the 'regression' command and robust variance estimation ('cluster' option) to correct for data dependence (5) (as a result of the within-subjects factors). We conducted statistical inferences using nonparametric bootstrapping estimation (1000 repetitions), which does not make distributional assumptions on the data (57).

Results

Table 1 presents raw mean VAS ratings, mean local peak amplitudes and latencies of the N1, N2 and P2 LEPs, salivary cortisol levels and skin temperatures at the dorsum of the left hand from the baseline and the post-treatment blocks. Table 2 summarises the results of the regression analyses examining the effects of intranasal oxytocin, compared to placebo, for each outcome variable.

LEPs and VAS scores

Intranasal oxytocin significantly reduced subjective VAS ratings, as well as N1 and N2 local peak amplitudes, and increased N2 local peak latency (Fig. 1 and Tables 1 and 2). Baseline-corrected treatment effect estimators for VAS ratings were significantly correlated with the N2 ($r = -0.57$, $P = 0.042$) but not the N1 ($r = -0.47$, $P = 0.11$) local peak amplitudes, although both correlations were moderate and in the expected direction (i.e. the greater the oxytocin-induced reduction in VAS ratings the greater the oxytocin-induced attenuation of the LEP amplitude). Period effects were significant only for the post-treatment P2 local peak latency ($b = 32.62$, $SE = 11.82$, $Z = 2.76$, $P = 0.019$) and the VAS ratings ($b = -1.55$, $SE = 0.65$, $Z = 2.40$, $P = 0.035$) and hence included in the corresponding regression models (as explained in the Statistical analysis).

Skin temperature

Skin temperatures ranged within expected levels for studies using laser-generated radiant heat stimuli (47). There was no treatment effect on skin temperatures (Table 2). Furthermore, skin temperatures did not differ between treatments across the baseline ($t_{12} = 0.51$, $P = 0.62$) or the post-treatment blocks ($t_{12} = 1.10$, $P = 0.29$).

Salivary cortisol levels

There was no treatment effect on salivary cortisol levels (Table 2). An ANOVA showed a significant effect of Time $\chi^2_2 = 11.14$, $P = 0.004$, with salivary cortisol levels decreasing between session onset and post-treatment block (Table 1). Post-hoc tests showed that salivary cortisol levels were significantly lower at the end of the post-treatment block compared to session onset ($\chi^2_1 = 11.12$, $P < 0.001$) but not compared to the end of the pre-treatment block ($\chi^2_1 = 3.02$, $P = 0.082$); no significant difference was observed between the end of the pre-treatment block and session onset ($\chi^2_1 = 2.01$, $P = 0.16$).

Discussion

In this double-blind, placebo-controlled, cross-over study, we stimulated A δ - and C-fibre skin nociceptors in the dorsum of the hand using infrared laser radiant heat stimuli in healthy male volunteers. We found that a single dose of intranasal oxytocin (40 IU) attenuated both perceived pain intensity and laser-evoked cortical

Table 2. Regression Analyses Showing the Effect of Intranasal Oxytocin (Compared to Placebo) for Each Outcome Variable.

	b	95% CI ^b	SE	Z	P	Estimated treatment effect ^c	d ^d
N1 local peak amplitude	1.65	0.11, 3.20	0.79	2.09	0.036	1.77	0.63
N1 local peak latency	1.06	-5.49, 7.62	3.34	0.32	0.75	3.08	0.26
N2 local peak amplitude	1.79	0.27, 3.32	0.78	2.30	0.021	1.98	0.73
N2 local peak latency	10.03	3.25, 16.81	3.46	2.90	0.004	10.11	0.83
P2 local peak amplitude	-1.07	-2.51, 0.36	0.74	-1.46	0.145	-0.99	-0.38
P2 local peak latency ^e	1.99	-3.70, 7.68	2.90	0.69	0.493	5.02	0.49
VAS ratings ^e	-1.27	-2.25, -0.29	0.50	-2.53	0.011	-0.23	-0.20
Salivary cortisol	0.62	-0.74, 1.98	0.69	0.90	0.37	0.42	0.18
Including initial session recordings	0.52	-0.71, 1.76	0.63	0.83	0.41	0.42	0.20
Skin temperature	-0.51	-1.40, 0.38	0.45	-1.13	0.26	-0.78	-0.46

VAS, visual analogue scale.

^aThe presented statistics correspond to the intercept of the regression line that estimates the effect of interest.

^b95% confidence interval for the b coefficient.

^cEstimated marginal mean for the treatment effect.

^dCohen's d (estimated treatment effect/SD) (90).

^eRegression models included treatment sequence (AB/BA) as a covariate because of the presence of significant period effects, as described in the Statistical analysis and reported in the Results.

responses. Specifically, intranasal oxytocin attenuated the local peak amplitude of the N1 and N2 (but not of P2) LEPs, and increased the latency of the N2 component. Importantly, for the first time, the present study reports an association between the analgesic effect of oxytocin (reduction in subjective pain ratings) and the oxytocin-induced modulation of cortical activity following noxious stimulation (attenuation of the N2 LEP). These effects indicate that oxytocin modulates neural processes contributing to pain perception and are discussed in turn below.

Subjective ratings

We observed a 7% reduction in subjective pain intensity ratings following intranasal oxytocin (compared to placebo). This effect is smaller but comparable to a recent study showing that 40 IU of intranasal oxytocin induced an 11% reduction in intensity ratings following the application of cold-pressor pain on healthy volunteers (27). Our studies differ not only in terms of pain modality, but also in the timing of the intervention following oxytocin treatment. This question has only recently begun to be addressed, with a recent study suggesting changes in regional cerebral blood flow (rCBF) in the resting state peak at 30–42 min from the end of intranasal oxytocin (40 IU) administration (58). Indeed, in the study reporting the larger reduction in pain intensity ratings (27), the intervention commenced at 20 min post-oxytocin treatment and partially overlapped with the temporal window during which the effects of intranasal oxytocin in the brain are maximal, whereas, in the present study, the intervention spanned 45–60 min post treatment. Thus, our study confirms that future research should consider the temporal dynamics of the pharmacodynamic effects of oxytocin in order to illuminate its antinociceptive properties (58).

LEPs and mechanisms mediating the effects of oxytocin on pain

We further observed that intranasal oxytocin specifically modulated the N1 and N2 but not the P2 LEPs. Although the functional significance of LEPs is not yet clearly understood (59), the cortical processes generating LEPs are largely related to the perception of salient changes in the sensory environment (59,60), and contribute to the perception of pain (61,62). The early N1 component, reflecting the activity of cortical generators in the contralateral operculo-insular and primary somatosensory cortices (30,63), is largely driven by the magnitude of the ascending nociceptive input, representing an early stage of sensory processing before the perceptual outcome of the nociceptive input is determined (48,64,65). The later N2 and P2 components reflect cortical generators in the insular and anterior cingulate cortices (30,63), and also relate to the intensity of noxious stimuli, although they largely represent cortical mechanisms that determine the subjective experience of pain (48).

Our observation that intranasal oxytocin reduced the amplitude of the N1 and N2 but not the P2 cortical responses suggests that it affects specific processes contributing to pain experience. First, the reduction in N1 and N2 amplitudes (and increase of N2 latencies) is consistent with effects of oxytocin at spinal levels, affecting the coding of noxious stimulus intensity and the ensuing experience of pain. Indeed, animal studies have shown that oxytocin can specifically reduce A δ /C-fibre afferent signalling and ascending nociceptive input at the level of spinal cord dorsal horn neurones by engaging GABA-mediated inhibitory cellular mechanisms (10–14,29). Additionally, oxytocin may be modulating the pain experience in humans by modulating activity in cortical areas involved in pain processing. In humans, intranasal oxytocin has

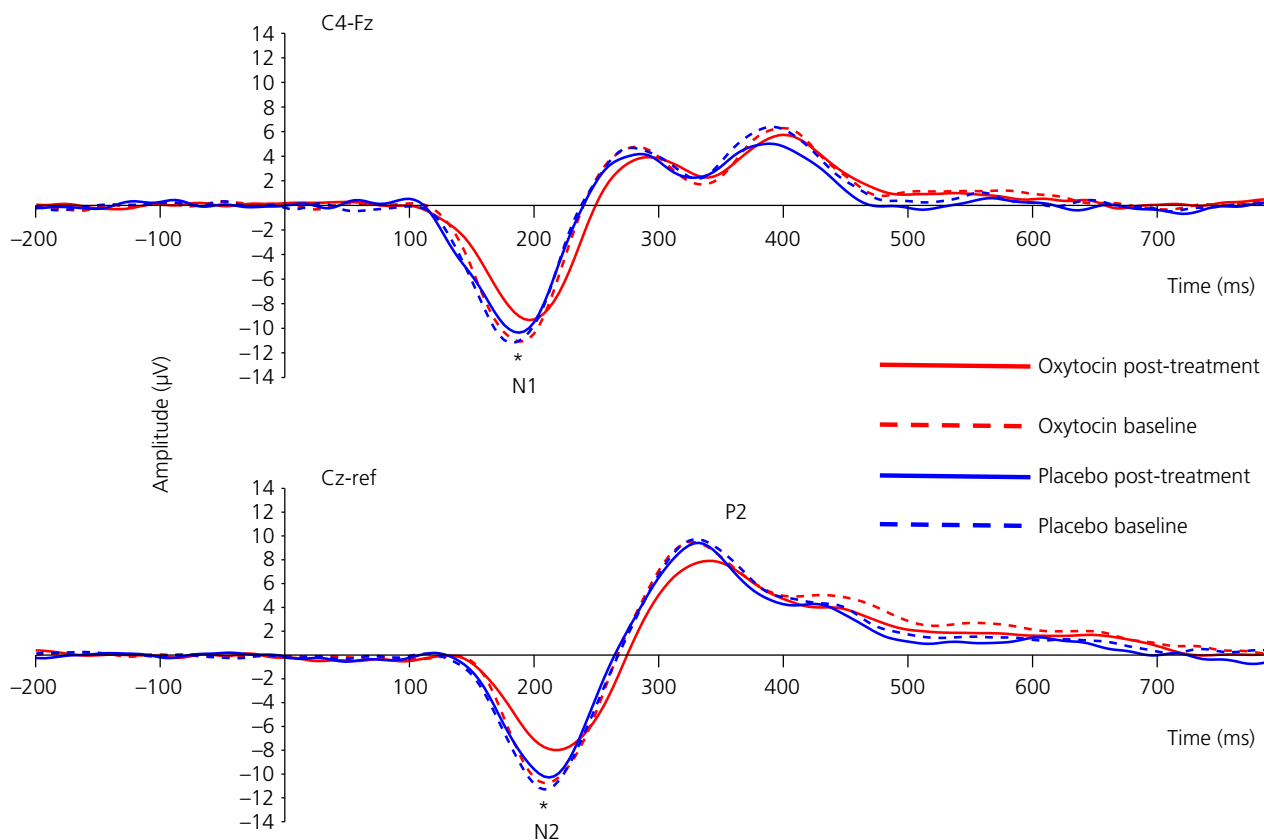


Fig. 1. Grand mean of laser evoked potentials (LEPs) after stimulation of the left-hand dorsum ($N = 13$) at baseline and after receiving treatment (oxytocin or placebo). x-axis: time (ms); y-axis, amplitude (μV). Top: N1 wave recorded at the temporal region contralateral to the stimulated site (C4 versus Fz). Bottom: N2/P2 wave recorded at the vertex (Cz versus average reference). Full waveforms are LEPs obtained after treatment (oxytocin: red; placebo: blue). Dashed waveforms are LEPs obtained before treatment (baseline). There was a significant reduction in N1 local peak amplitude ($P = 0.036$) following oxytocin treatment (compared to placebo and controlling for baseline). There was also a significant reduction in N2 local peak amplitude ($P = 0.021$) and an increase in N2 local peak latency ($P = 0.004$) following oxytocin treatment (compared to placebo and controlling for baseline). There was no significant effect of oxytocin treatment (compared to placebo) on the P2 wave. * $P < 0.05$.

been shown to increase rCBF, and hence neuronal activity, in cortical areas directly implicated in the processing of nociceptive input and the experience of pain, as well as in the descending modulation of nociceptive input, such as the frontoparietal opercula, the insula and the anterior cingulate cortex (58,66,67). Changes in neuronal activation in these cortical areas have been shown to modulate the evoked brain responses to transient nociceptive input (68). For example, the observation of emotionally conflicting information that activates the anterior cingulate cortex modulates the perception of pain and specifically prevents the full expression of the N2 component (reducing its amplitude) (68). Consistent with this and the role of N2 in the experienced pain intensity, we observed that the oxytocin-induced reduction in pain ratings correlated with the oxytocin-induced reduction in N2 amplitude. The lack of intranasal effects of oxytocin on the later P2 component suggests that it may not have influenced the perceived salience of the noxious stimuli. The P2 component has been shown to be modulated specifically by factors reflecting the salience of noxious stimuli (e.g. stimulus probability) (33,59,69) and to reflect multimodal (rather than somatosensory-specific) processes (61).

The intranasal dose administered in the present study has been associated with changes in central function in humans (58), and similar or smaller doses of oxytocin or vasopressin (24–48 IU for oxytocin; 40–80 IU for vasopressin) have been associated with elevations in peptide levels in the cerebrospinal fluid in humans or macaques of no more than approximately 55 pg/ml in actual levels (70–74), or of up to 0.005% of the administered dose (75). It is possible that the observed effects in the present study could be at least partially explained by elevations in central levels of oxytocin, although it is not clear to what extent this elevation reflects endogenous or exogenous oxytocin and, if the latter, what the mechanisms of absorption may be. However, it remains unknown how much oxytocin must enter the brain in primates for a behavioural effect to be observed; in smaller animals, 1 ng has been reported as the lowest i.c.v. dose shown to elicit a behavioural effect (75). Additionally, it is also possible that the antinociceptive effects of intranasal oxytocin observed in the present study are explained by the activation of peripheral receptors, mainly the vasopressin 1A receptor (76). Future studies will need to specifically address the mechanism mediating the antinociceptive effects of

oxytocin in humans; for example, by administering antagonists to block peripheral oxytocin or vasopressin receptors (75).

Impact on the stress response

A further mechanism postulated to potentially mediate intranasal effects of oxytocin on pain sensitivity in humans involves the mitigation of psychological factors such as negative mood, anxiety and the stress response to pain (27). In the present study, we did not observe an effect of oxytocin on salivary cortisol levels. This might be because our participants did not find our pain paradigm (involving a highly controlled stimulus) sufficiently stressful in that there was a reduction in salivary cortisol levels over time irrespective of treatment condition. However, a review of human studies examining the effect of oxytocin on cortisol suggests that oxytocin may exert both anxiogenic and anxiolytic effects, depending on a variety of individual and context variables (77,78), which are not always reflected in changes in cortisol levels (79,80). Furthermore, existing studies have either failed to show a mitigating effect of oxytocin on negative mood (27) or have shown that such an effect was not related to the pain experience (23).

Mediating social effects on pain

The analgesic effects of exogenous oxytocin in humans identify the oxytocin system as a plausible neural mechanism for the transduction of the effects of social support into the neural and physiological changes that modulate the experience of pain in humans (81), and such a hypothesis needs to be addressed in future research. Social factors, such as the supportive presence of others, have been shown to modulate the experience of pain (82). Interaction with other humans, including warm interpersonal contact (83,84) or the perception of trust (85), can result in the release of endogenous oxytocin. Preclinical studies demonstrate that manipulations involving bodily contact such as massage can induce anti-nociceptive effects in rodents similar to those elicited by exogenous oxytocin, by triggering the endogenous oxytocin system (86).

Limitations

A number of limitations characterise the present study. First, we focused on male participants because some degree of sexual dimorphism in the oxytocin system may be expected (87). Hence, our findings cannot be readily extrapolated to women. Second, we used the maximal oxytocin dose safely administered to humans (88) and a high intensity of the noxious stimulus because our aim was to assess the presence of analgesic properties of intranasal oxytocin in humans. Future studies should systematically examine a wider range of dosages and intensities of noxious stimuli, as well as include clinical populations, aiming to characterise the analgesic properties and potential clinical relevance of intranasal oxytocin. Third, future studies should explicitly investigate whether oxytocin influences the salience of noxious stimuli and whether it modulates pain-specific processing or sensory processing in general (23). Fourth, because we did not measure heart

rate variability in the present study, we could not determine whether the analgesic effects of intranasal oxytocin were at least partially mediated by oxytocin-induced modulation of heart rate reactivity to pain (27). Last but not least, although the present study investigated an effect of oxytocin on a phenotype that could be reasonably expected on the basis of animal research, given the small sample size, it is possible that the observed treatment effect is overestimated (89). Our findings will need to be replicated in future studies that are adequately powered to detect even smaller treatment effects.

Conclusions

By selectively stimulating subcutaneous A δ - and C-fibres with an infrared laser and recording the EEG in participants, we provide preliminary evidence indicating that a single intranasal oxytocin dosage (40 IU) attenuated pain intensity ratings and differentially modulated the ensuing cortical LEPs in humans. Our findings are consistent with robust preclinical evidence on the antinociceptive properties of oxytocin and highlight potential neural mechanisms mediating the analgesic effects of oxytocin in humans.

Acknowledgements

We thank our study participants. YP was partly supported by an Economic and Social Research Council fellowship (grant number ES/K009400/1) and partly by a project grant (II/85 069) from the Volkswagen Foundation 'European Platform for Life Sciences, Mind Sciences and Humanities' (to AF). This study was also supported by a project grant from the Institute for the Study of Affective Neuroscience (ISAN)/Hope for Depression Research Foundation (HDRF) (to AF). AF was supported by a European Research Council Starting Investigator Award' (ERC-2012-STG GA313755). MAH and SW are supported by a Medical Research Council Developmental Pathway Funding Scheme award (MR/J005142/1) and the NIHR Biomedical Research Centre for Mental Health at the South London and Maudsley NHS Trust. We also wish to thank Jonathan Bowen, Sana Chhipa, Adam Cunningham, Christina Dardani and Samantha Harrison for their assistance with data collection. The authors report that there are no conflicts of interest.

Received 7 June 2015,
revised 4 December 2015,
accepted 5 December 2015

References

- Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* 2001; **81**: 629–683.
- Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. *Nat Rev Neurosci* 2006; **7**: 126–136.
- Stoop R. Neuromodulation by oxytocin and vasopressin in the central nervous system as a basis for their rapid behavioral effects. *Curr Opin Neurobiol* 2014; **29**: 187–193.
- Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hubner CA, Represa A, et al. Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 2006; **314**: 1788–1792.
- Mazzuca M, Minlebaev M, Shakirzyanova A, Tyzio R, Taccola G, Janackova S, et al. Newborn analgesia mediated by oxytocin during delivery. *Front Cell Neurosci* 2011; **5**: 3.

- 6 Rash JA, Aguirre-Camacho A, Campbell TS. Oxytocin and pain: a systematic review and synthesis of findings. *Clin J Pain* 2014; **30**: 453–462.
- 7 Jo YH, Stoeckel ME, Freund-Mercier MJ, Schlichter R. Oxytocin modulates glutamatergic synaptic transmission between cultured neonatal spinal cord dorsal horn neurons. *J Neurosci* 1998; **18**: 2377–2386.
- 8 Sofroniew MV, Weindl A, Schrell U, Wetzstein R. Immunohistochemistry of vasopressin, oxytocin and neurophysin in the hypothalamus and extrahypothalamic regions of the human and primate brain. *Acta Histochem Suppl* 1981; **24**: 79–95.
- 9 Swanson LW, McKellar S. The distribution of oxytocin- and neurophysin-stained fibers in the spinal cord of the rat and monkey. *J Comp Neurol* 1979; **188**: 87–106.
- 10 Rojas-Piloni G, Lopez-Hidalgo M, Martinez-Lorenzana G, Rodriguez-Jimenez J, Condes-Lara M. GABA-mediated oxytocinergic inhibition in dorsal horn neurons by hypothalamic paraventricular nucleus stimulation. *Brain Res* 2007; **1137**: 69–77.
- 11 Breton JD, Veinante P, Uhl-Bronner S, Vergnano AM, Freund-Mercier MJ, Schlichter R, *et al.* Oxytocin-induced antinociception in the spinal cord is mediated by a subpopulation of glutamatergic neurons in lamina I–II which amplify GABAergic inhibition. *Mol Pain* 2008; **4**: 19.
- 12 Juif PE, Poisbeau P. Neurohormonal effects of oxytocin and vasopressin receptor agonists on spinal pain processing in male rats. *Pain* 2013; **154**: 1449–1456.
- 13 Gong L, Gao F, Li J, Yu X, Ma X, *et al.* Oxytocin-induced membrane hyperpolarization in pain-sensitive dorsal root ganglia neurons mediated by Ca(2+)nNOS/NO/KATP pathway. *Neuroscience* 2015; **289**: 417–428.
- 14 Robinson DA, Wei F, Wang GD, Li P, Kim SJ, Vogt SK, *et al.* Oxytocin mediates stress-induced analgesia in adult mice. *J Physiol* 2002; **540**: 593–606.
- 15 Ge Y, Lundeberg T, Yu LC. Blockade effect of mu and kappa opioid antagonists on the anti-nociception induced by intra-periaqueductal grey injection of oxytocin in rats. *Brain Res* 2002; **927**: 204–207.
- 16 Yang J. Intrathecal administration of oxytocin induces analgesia in low back pain involving the endogenous opiate peptide system. *Spine* 1994; **19**: 867–871.
- 17 Russo R, D'Agostino G, Mattace Raso G, Avagliano C, Cristiano C, Meli R, *et al.* Central administration of oxytocin reduces hyperalgesia in mice: implication for cannabinoid and opioid systems. *Peptides* 2012; **38**: 81–88.
- 18 Madrazo I, Franco-Bourland RE, Leon-Meza VM, Mena I. Intraventricular somatostatin-14, arginine vasopressin, and oxytocin: analgesic effect in a patient with intractable cancer pain. *Appl Neurophysiol* 1987; **50**: 427–431.
- 19 Wang YL, Yuan Y, Yang J, Wang CH, Pan YJ, Lu L, *et al.* The interaction between the oxytocin and pain modulation in headache patients. *Neuropeptides* 2013; **47**: 93–97.
- 20 Louvel D, Delvaux M, Felez A, Fioramonti J, Bueno L, Lazorthes Y, *et al.* Oxytocin increases thresholds of colonic visceral perception in patients with irritable bowel syndrome. *Gut* 1996; **39**: 741–747.
- 21 Mameli S, Pisanu GM, Sardo S, Marchi A, Pili A, Carboni M, *et al.* Oxytocin nasal spray in fibromyalgic patients. *Rheumatol Int* 2014; **34**: 1047–1052.
- 22 Ohlsson B, Truedsson M, Bengtsson M, Torstenson R, Sjolund K, Bjornsson ES, *et al.* Effects of long-term treatment with oxytocin in chronic constipation; a double blind, placebo-controlled pilot trial. *Neurogastroenterol Motil* 2005; **17**: 697–704.
- 23 Zunhammer M, Geis S, Busch V, Greenlee MW, Eichhammer P. Effects of intranasal oxytocin on thermal pain in healthy men: a randomized functional magnetic resonance imaging study. *Psychosom Med* 2015; **77**: 156–166.
- 24 Kessner S, Sprenger C, Wrobel N, Wiech K, Bingel U. Effect of oxytocin on placebo analgesia: a randomized study. *JAMA* 2013; **310**: 1733–1735.
- 25 Singer T, Snozzi R, Bird G, Petrovic P, Silani G, Heinrichs M, *et al.* Effects of oxytocin and prosocial behavior on brain responses to direct and vicariously experienced pain. *Emotion* 2008; **8**: 781–791.
- 26 Eisenach JC, Tong C, Curry R. Phase 1 safety assessment of intrathecal oxytocin. *Anesthesiology* 2015; **122**: 407–413.
- 27 Rash JA, Campbell TS. The effect of intranasal oxytocin administration on acute cold pressor pain: a placebo-controlled, double-blind, within-participants crossover investigation. *Psychosom Med* 2014; **76**: 422–429.
- 28 Bromm B, Treede RD. Nerve fibre discharges, cerebral potentials and sensations induced by CO₂ laser stimulation. *Hum Neurobiol* 1984; **3**: 33–40.
- 29 Hobo S, Hayashida K, Eisenach JC. Oxytocin inhibits the membrane depolarization-induced increase in intracellular calcium in capsaicin sensitive sensory neurons: a peripheral mechanism of analgesic action. *Anesth Analg* 2012; **114**: 442–449.
- 30 Garcia-Larrea L, Frot M, Valeriani M. Brain generators of laser-evoked potentials: from dipoles to functional significance. *Neurophysiol Clin* 2003; **33**: 279–292.
- 31 Treede RD. Neurophysiological studies of pain pathways in peripheral and central nervous system disorders. *J Neurol* 2003; **250**: 1152–1161.
- 32 Bentley DE, Watson A, Treede RD, Barrett G, Youell PD, Kulkarni B, *et al.* Differential effects on the laser evoked potential of selectively attending to pain localisation versus pain unpleasantness. *Clin Neurophysiol* 2004; **115**: 1846–1856.
- 33 Legrain V, Guerit JM, Bruyer R, Plaghki L. Attentional modulation of the nociceptive processing into the human brain: selective spatial attention, probability of stimulus occurrence, and target detection effects on laser evoked potentials. *Pain* 2002; **99**: 21–39.
- 34 Krahé C, Paloyelis Y, Condon H, Jenkinson PM, Williams SC, Fotopoulou A. Attachment style moderates partner presence effects on pain: a laser-evoked potentials study. *Soc Cogn Affect Neurosci* 2015; **10**: 1030–1037.
- 35 Truini A, Panuccio G, Galeotti F, Maluccio MR, Sartucci F, Avoli M, *et al.* Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. *Eur J Pain* 2010; **14**: 222–225.
- 36 Heinrichs M, Baumgartner T, Kirschbaum C, Ehler U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* 2003; **54**: 1389–1398.
- 37 Derogatis LR, Savitz KL. The SCL-90-R and the Brief Symptom Inventory (BSI) in primary care. In: Maruish ME, ed. *Handbook of Psychological Assessment in Primary Care Settings*. 236. Mahwah, NJ: Lawrence Erlbaum Associates, 2000: 297–334.
- 38 Beck AT, Steer RA, Brown GK. *Manual for Beck Depression Inventory II (BDI-II)*. San Antonio, TX: Psychology Corporation, 1996.
- 39 Grewen KM, Light KC, Mechlin B, Girdler SS. Ethnicity is associated with alterations in oxytocin relationships to pain sensitivity in women. *Ethn Health* 2008; **13**: 219–241.
- 40 Arendt-Nielsen L, Bjerring P. Sensory and pain threshold characteristics to laser stimuli. *J Neurol Neurosurg Psychiatry* 1988; **51**: 35–42.
- 41 Dyck PJ, Zimmerman IR, Johnson DM, Gillen D, Hokanson JL, Karnes JL, *et al.* A standard test of heat-pain responses using CASE IV. *J Neurol Sci* 1996; **136**: 54–63.
- 42 Pertovaara A, Morrow TJ, Casey KL. Cutaneous pain and detection thresholds to short CO₂ laser pulses in humans: evidence on afferent mechanisms and the influence of varying stimulus conditions. *Pain* 1988; **34**: 261–269.
- 43 Wu G, Campbell JN, Meyer RA. Effects of baseline skin temperature on pain ratings to suprathreshold temperature-controlled stimuli. *Pain* 2001; **90**: 151–156.
- 44 Hardy JD, Goodell H, Wolff HG. The influence of skin temperature upon the pain threshold as evoked by thermal radiation. *Science* 1951; **114**: 149–150.

- 45 Beattie JW, Woodmansey A. Radiation pain thresholds in relation to skin temperatures. *Ann Rheum Dis* 1955; **14**: 397–403.
- 46 Hall KRL. Relation of skin temperature to pain threshold. *Q J Exp Psychol* 1955; **7**: 74–81.
- 47 Churyukanov M, Plaghki L, Legrain V, Mouraux A. Thermal detection thresholds of Adelta- and C-fibre afferents activated by brief CO₂ laser pulses applied onto the human hairy skin. *PLoS ONE* 2012; **7**: e35817.
- 48 Lee MC, Mouraux A, Iannetti GD. Characterizing the cortical activity through which pain emerges from nociception. *J Neurosci* 2009; **29**: 7909–7916.
- 49 Senn S. *Cross-Over Trials in Clinical Research*, 2nd edn. Chichester: Wiley, 2002.
- 50 Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 1989; **22**: 150–169.
- 51 Carmon A, Mor J, Goldberg J. Evoked cerebral responses to noxious thermal stimuli in humans. *Exp Brain Res* 1976; **25**: 103–107.
- 52 Treede RD, Meyer RA, Raja SN, Campbell JN. Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. *J Physiol* 1995; **483**: 747–758.
- 53 Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* 2004; **134**: 9–21.
- 54 Lopez-Calderon J, Luck SJ. ERPLAB: an open-source toolbox for the analysis of event-related potentials. *Front Hum Neurosci* 2014; **8**: 213.
- 55 Viola FC, Thorne J, Edmonds B, Schneider T, Eichele T, Debener S. Semi-automatic identification of independent components representing EEG artifact. *Clin Neurophysiol* 2009; **120**: 868–877.
- 56 Metcalfe C. The analysis of cross-over trials with baseline measurements. *Stat Med* 2010; **29**: 3211–3218.
- 57 Curran-Everett D. Explorations in statistics: the bootstrap. *Adv Physiol Educ* 2009; **33**: 286–292.
- 58 Paloyelis Y, Doyle OM, Zelaya FO, Maltezos S, Williams SC, Fotopoulou A, et al. A spatiotemporal profile of in vivo cerebral blood flow changes following intranasal oxytocin in humans. *Biol Psychiatry* 2014; DOI: <http://dx.doi.org/10.1016/j.biopsych.2014.10.005> [Epub ahead of print]
- 59 Torta DM, Liang M, Valentini E, Mouraux A, Iannetti GD. Dishabituation of laser-evoked EEG responses: dissecting the effect of certain and uncertain changes in stimulus spatial location. *Exp Brain Res* 2012; **218**: 361–372.
- 60 Mouraux A, Iannetti GD. Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity. *J Neurophysiol* 2009; **101**: 3258–3269.
- 61 Legrain V, Iannetti GD, Plaghki L, Mouraux A. The pain matrix reloaded: a salience detection system for the body. *Prog Neurobiol* 2011; **93**: 111–124.
- 62 Iannetti GD, Mouraux A. From the neuromatrix to the pain matrix (and back). *Exp Brain Res* 2010; **205**: 1–12.
- 63 Perchet C, Godinho F, Mazza S, Frot M, Legrain V, Magnin M, et al. Evoked potentials to nociceptive stimuli delivered by CO₂ or Nd:YAP lasers. *Clin Neurophysiol* 2008; **119**: 2615–2622.
- 64 Garcia-Larrea L, Peyron R, Laurent B, Mauguire F. Association and dissociation between laser-evoked potentials and pain perception. *NeuroReport* 1997; **8**: 3785–3789.
- 65 Iannetti GD, Zambreanu L, Cruccu G, Tracey I. Operculoinsular cortex encodes pain intensity at the earliest stages of cortical processing as indicated by amplitude of laser-evoked potentials in humans. *Neuroscience* 2005; **131**: 199–208.
- 66 Peyron R, Frot M, Schneider F, Garcia-Larrea L, Mertens P, Barral FG, et al. Role of operculoinsular cortices in human pain processing: converging evidence from PET, fMRI, dipole modeling, and intracerebral recordings of evoked potentials. *NeuroImage* 2002; **17**: 1336–1346.
- 67 Treede RD, Kenshalo DR, Gracely RH, Jones AK. The cortical representation of pain. *Pain* 1999; **79**: 105–111.
- 68 Martini M, Valentini E, Aglioti SM. Emotional conflict in a model modulates nociceptive processing in an onlooker: a laser-evoked potentials study. *Exp Brain Res* 2013; **225**: 237–245.
- 69 Franz M, Nickel MM, Ritter A, Miltner WH, Weiss T. Somatosensory spatial attention modulates amplitudes, latency, and latency jitter of laser-evoked brain potentials. *J Neurophysiol* 2015; **113**: 2760–2768.
- 70 Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* 2002; **5**: 514–516.
- 71 Striepens N, Kendrick KM, Hanking V, Landgraf R, Wullner U, Maier W, et al. Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans. *Sci Rep* 2013; **3**: 3440.
- 72 Modi ME, Connor-Stroud F, Landgraf R, Young L, Parr L. Aerosolized oxytocin increases cerebrospinal fluid oxytocin in rhesus macaques. *Psychoneuroendocrinology* 2014; **45**: 49–57.
- 73 Chang SW, Barter JW, Ebitz RB, Watson KK, Platt ML. Inhaled oxytocin amplifies both vicarious reinforcement and self reinforcement in rhesus macaques (*Macaca mulatta*). *Proc Natl Acad Sci USA* 2012; **109**: 959–964.
- 74 Dal Monte O, Noble PL, Turchi J, Cummins A, Averbeck BB. CSF and blood oxytocin concentration changes following intranasal delivery in macaque. *PLoS One* 2014; **9**: e103677.
- 75 Leng G, Ludwig M. Intranasal oxytocin: myths and delusions. *Biol Psychiatry* 2015; May 11. pii: S0006-3223(15)00400-X. doi: 10.1016/j.biopsych.2015.05.003. [Epub ahead of print].
- 76 Schorscher-Petcu A, Sotocinal S, Ciura S, Dupre A, Ritchie J, Sorge RE, et al. Oxytocin-induced analgesia and scratching are mediated by the vasopressin-1A receptor in the mouse. *J Neurosci* 2010; **30**: 8274–8284.
- 77 MacDonald K, MacDonald TM, Brune M, Lamb K, Wilson MP, Golshan S, et al. Oxytocin and psychotherapy: a pilot study of its physiological, behavioral and subjective effects in males with depression. *Psychoneuroendocrinology* 2013; **38**: 2831–2843.
- 78 Weisman O, Zagoory-Sharon O, Feldman R. Oxytocin administration alters HPA reactivity in the context of parent-infant interaction. *Eur Neuropsychopharmacol* 2013; **23**: 1724–1731.
- 79 de Oliveira DC, Chagas MH, Garcia LV, Crippa JA, Zuardi AW. Oxytocin interference in the effects induced by inhalation of 7.5% CO(2) in healthy volunteers. *Hum Psychopharmacol* 2012; **27**: 378–385.
- 80 de Oliveira DC, Zuardi AW, Graeff FG, Queiroz RH, Crippa JA. Anxiolytic-like effect of oxytocin in the simulated public speaking test. *J Psychopharmacol* 2012; **26**: 497–504.
- 81 Uvnäs-Moberg K. Oxytocin may mediate the benefits of positive social interaction and emotions. *Psychoneuroendocrinology* 1998; **23**: 819–835.
- 82 Krahé C, Springer A, Weinman JA, Fotopoulou A. The social modulation of pain: others as predictive signals of salience – a systematic review. *Front Hum Neurosci* 2013; **7**: 386.
- 83 Holt-Lunstad J, Birmingham WA, Light KC. Influence of a 'warm touch' support enhancement intervention among married couples on ambulatory blood pressure, oxytocin, alpha amylase, and cortisol. *Psychosom Med* 2008; **70**: 976–985.
- 84 Grewen KM, Girdler SS, Amico J, Light KC. Effects of partner support on resting oxytocin, cortisol, norepinephrine, and blood pressure before and after warm partner contact. *Psychosom Med* 2005; **67**: 531–538.
- 85 Zak PJ, Kurzban R, Matzner WT. Oxytocin is associated with human trustworthiness. *Horm Behav* 2005; **48**: 522–527.
- 86 Lund I, Ge Y, Yu LC, Uvnäs-Moberg K, Wang J, Yu C, et al. Repeated massage-like stimulation induces long-term effects on nociception: con-

- tribution of oxytocinergic mechanisms. *Eur J Neurosci* 2002; **16**: 330–338.
- 87 Macdonald KS. Sex, receptors, and attachment: a review of individual factors influencing response to oxytocin. *Front Neurosci* 2012; **6**: 194.
- 88 MacDonald E, Dadds MR, Brennan JL, Williams K, Levy F, Cauchi AJ. A review of safety, side-effects and subjective reactions to intranasal oxytocin in human research. *Psychoneuroendocrinology* 2011; **36**: 1114–1126.
- 89 Walum H, Waldman ID, Young LJ. Statistical and methodological considerations for the interpretation of intranasal oxytocin studies. *Biol Psychiatry* 2015; Jun 24. pii: S0006-3223(15)00522-3. doi: 10.1016/j.biopsych.2015.06.016. [Epub ahead of print].
- 90 Egbewale BE, Lewis M, Sim J. Bias, precision and statistical power of analysis of covariance in the analysis of randomized trials with baseline imbalance: a simulation study. *BMC Med Res Methodol* 2014; **14**: 49.