Sniff and mimic - Intranasal oxytocin increases facial mimicry in a sample of men

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Abstract

The neuropeptide oxytocin (OT) has many potential social benefits. For example, intranasal administration of OT appears to trigger caregiving behavior and to improve the recognition of emotional facial expressions. But the mechanism for these effects is not yet clear. Recent findings relating OT to action imitation and to the visual processing of the eye region of faces point to mimicry as a mechanism through which OT improves processing of emotional expression. To test the hypothesis that increased levels of OT in the brain enhance facial mimicry, 60 healthy male participants were administered, in a double-blind between-subjects design, 24 international units (IUs) of OT or placebo (PLA) through nasal spray. Facial mimicry and emotion judgments were recorded in response to movie clips depicting changing facial expressions. As expected, facial mimicry was increased in the OT group, but effects were strongest for angry infant faces. These findings provide further evidence for the importance of OT in social cognitive skills, and suggest that facial mimicry mediates the effects of OT on improved emotion recognition.

Keywords

Facial mimicry; oxytocin; facial expressions; facial feedback; caregiving

1. Introduction

Primates, including humans, are highly social creatures, whose brains have evolved to understand and interact with other individuals (Dunbar, 1998). The face is one of the richest means of (nonverbal) social communication (Ekman and Rosenberg, 2005). Therefore, efficient and accurate recognition and interpretation of facial expressions is critical input into our inferences about and reactions to other people's affective states and behavioral intentions, and the achievement of smooth social interactions and successful goal pursuit in society.

A mechanism suggested to play a key role in the fast and accurate recognition of facial expressions is the simulation of that expression on one's own face (Wood et al., 2016). *Facial mimicry* consists of small changes in the activation of facial muscles of the observer, which match the facial movements perceived on another person's face. Seeing somebody smiling, for example, elicits contractions of the smiling muscles in the perceiver (Dimberg, 1982; Korb et al., 2014), which can be measured using electromyography (EMG) (Dimberg, 1982; Tassinari and Cacioppo, 1992). Information from muscular contractions that comprise facial mimicry reaches somatosensory areas of the brain through facial feedback (Hatfield et al., 1993; Korb et al., 2015), and contributes to the processing of the perceived facial expressions (Barsalou, 2008; Niedenthal et al., 2010). For example, the intensity of smile mimicry predicts judgments of smile authenticity (Korb et al., 2014), and when facial mimicry is blocked recognition of facial expressions becomes slower and poorer (Maringer et al., 2011; Niedenthal et al., 2001; Oberman et al., 2007; Rychlowska et al., 2014; Stel and van Knippenberg, 2008; Wood et al., 2015). When mimicry is inhibited, responses to emotional faces in emotion circuits of the brain, such as the amygdala, are also reduced (Hennenlotter et al., 2009). Facial mimicry is present from early infancy (Field et al., 1982; Meltzoff and Moore, 1977; but see Oostenbroek et al., 2016), is difficult to voluntarily suppress (Korb et al., 2010), and occurs in the absence of conscious perception of the stimulus face (Dimberg et al., 2000; Mathersul et al., 2013; Tamietto et al., 2009).

The important role of facial mimicry for emotion recognition is also illustrated by psychiatric and neurodevelopmental conditions characterized by deficits in social interaction. For example,

anomalies in face processing and difficulties in emotion recognition likely contributing to deficits in empathy and social interaction, are one of the hallmarks of autism spectrum disorders (ASD; Rump et al., 2009; but see Tracy et al., 2011). Some of the difficulties in emotion recognition and social interaction that accompany ASD are likely caused by a lack of or delay in facial mimicry (Beall et al., 2008; McIntosh et al., 2006; Oberman et al., 2009).

Oxytocin (OT) is both a hormone and a neuropeptide that has been implicated in a array of behaviors, including attachment, exploration, and sexuality (Carter et al., 2008; Donaldson and Young, 2008; Meyer-Lindenberg et al., 2011; Strathearn, 2011). For example, OT released into the brain or cerebro-spinal fluid (CSF), either endogenously from the hypothalamus or through exogenous administration, facilitates parental care and pair bonding, as shown most impressively in voles and other rodents (Johnson and Young, 2015; Young et al., 2008), but more recently also in humans (Campbell, 2008; Kim et al., 2014). In addition to its role in mother-infant bonding, recent studies have suggested that OT is also important for fatherhood, and could modulate father-infant interactions (Weisman et al., 2014). Moreover, administration of OT is being tested as a treatment for some of ASD's most debilitating symptoms, such as avoidance of eye contact, and the inability to understand other people's feelings (Anagnostou et al., 2012; Andari et al., 2010; Dadds et al., 2014; Guastella et al., 2015, 2010; Hollander et al., 2007).

In human studies, 24 to 48 international units (IUs) of OT are typically administered through nasal spray, and compared to placebo (PLA). Although the precise mechanisms through which intranasal OT reaches the brain are not well understood (Churchland and Winkielman, 2012; Leng and Ludwig, 2016; Quintana et al., 2015; Veening and Olivier, 2013), OT is believed to reach its maximum effect in the brain after about 45 minutes, and to alter resting regional cerebral blood flow up to at least 78 min after administration (Born et al., 2002; Paloyelis et al., 2014; for an even later peak in CFS see Striepens et al., 2013). Elevated levels of peripheral OT, as measured for example in saliva, can persist for hours after intranasal administration (Van IJzendoorn et al., 2012), although the relationship between central and peripheral OT levels is uncertain (Churchland and Winkielman, 2012). OT is likely to have widespread and long-lasting effects on the brain, especially on brain regions subserving social skills (Bethlehem et al., 2013).

There is currently great interest in the effects of OT on the perception of social stimuli. Research suggests that OT administration robustly improves the recognition of emotional facial expressions in neurotypical (NT) individuals (Bartz et al., 2011; Domes et al., 2007b; Macdonald and Macdonald, 2010; Meyer-Lindenberg et al., 2011; for a review and estimation of the effect size see Van IJzendoorn and Bakermans-Kranenburg, 2012). Moreover, intranasal OT increases positive social behavior of macaque infants and modulates activity in face-responsive brain regions of adult macaques (Liu et al., 2015; Simpson et al., 2014). However, whether OT improves the detection and identification of facial expressions in general (Lischke et al., 2012; Schulze et al., 2011), or of specific emotions, such as happiness (Marsh et al., 2010; Schulze et al., 2011) or fear (Fischer-Shofty et al., 2010), remains an open question in the light of mixed evidence.

Studies carried out in people with ASD, often using multi-dose treatments over periods of several weeks, have also led to mixed results (Anagnostou et al., 2014). Some findings point to improvements in emotion recognition and social functioning, for example on the Reading-the-Mind-in-the-Eyes Test (Baron-Cohen et al., 2001), which requires the recognition of emotional and cognitive states based solely on the part of faces including and surrounding the eyes (Anagnostou et al., 2012; Andari et al., 2010; Guastella et al., 2010; Hollander et al., 2007). Others, however, show no evidence of improvements in emotion recognition or interaction skills, even after weeks of daily OT administration (Dadds et al., 2014; Guastella et al., 2015).

Based on several considerations, we hypothesized that improved emotion recognition after OT administration is due in part to an increase in facial mimicry. First, although its effects on facial mimicry remain unknown, OT was recently shown to facilitate automatic imitation² of finger movements. In a study by De Coster and colleagues (De Coster et al., 2014), forty-eight male volunteers received either OT or PLA, and performed a well-established task, in which index or middle finger movements are made while watching either congruent or incongruent movements on a screen. The incongruency effect, defined by slower responses on incongruent trials (e.g. participant moves index finger while observing middle finger movement) compared to congruent trials (participant performs same movement as on the screen), was bigger in the OT group, compared to the

² For the purpose of the current discussion imitation is used as a synonym of mimicry.

PLA group. According to the authors, these results indicate that OT suppresses control over automatic imitative behavior. Second, OT increases visual processing of the eye-region of the face (Guastella et al., 2008). This is relevant because the eye region carries critical information, and people with ASD make reduced eye contact (Dalton et al., 2005; Rutherford et al., 2007). Eye contact has also been proposed as a trigger for facial mimicry, at least in healthy participants (Neufeld et al., 2015; Niedenthal et al., 2010; Schrammel et al., 2009). Third, testosterone, a steroid hormone that under many aspects has effects opposite of OT on perception and behavior, is associated with decreases in mimicry (Hermans et al., 2006). Finally, at the anatomical level, OT binding sites exist at several areas of the human brainstem, possibly including the facial nuclei (Freeman et al., 2016; Loup et al., 1989).

In summary, the effects of the neuropeptide OT are often described as “prosocial”, and include increasing trust, empathy, bonding, and caregiving (but depending on the context OT may increase aggression, e.g. see Ne’eman et al., 2016). Based on studies in NT individuals and initial clinical trials in individuals with ASD, OT has been implicated in the accuracy of recognition of emotions in faces. However, it remains unclear to date if improved emotion recognition occurs for all or only specific facial expressions. Most importantly, it is unknown if facial mimicry, which contributes to emotion recognition, and is impaired in ASD, is increased through OT administration.

The current study aimed to investigate the hypothesis that OT administration enhances facial mimicry in healthy adult males. In a double-blind, placebo-controlled, between-subjects design, sixty healthy male participants received as nasal spray 24 IUs of either OT or a PLA. The sample was restricted to male participants to reduce complications linked to the female menstrual cycle. A between-subjects design was chosen to prevent habituation to the stimuli, and because it was shown to lead to larger effect sizes in a recent meta-analysis on the effects of OT on emotion recognition (Van IJzendoorn and Bakermans-Kranenburg, 2012). Facial mimicry to dynamic happy and angry facial expressions in adult and infant faces was measured, across two tasks, with facial EMG. To rule out an unspecific motor effect of OT, voluntary facial movements were also assessed with EMG. Changes in mood and empathy were assessed by questionnaire.

Due to its prosocial and anxiolytic effects, OT was expected to lead to increased facial mimicry for both happy and angry facial expressions. Due to OT's role in caregiving behavior, including fatherhood (Weisman et al., 2014), we expected the increase in facial mimicry under OT to be more pronounced for infant faces, compared to adult faces. Based on findings by Lischke et al. (Lischke et al., 2012), who used a similar task and stimuli, we expected speed of emotion recognition to be faster under OT. Emotional facial expressions were also expected to be perceived as more intense under OT. Finally, we explored the effects of OT on questionnaire measures of empathy and mood.

2. Methods

2.1. Participants

Sixty healthy male participants (10 left-handed; average age = 24.85 years, $SD = 4.75$, range 18 to 35 years) were recruited through advertisements on campus and gave written informed consent. The study, which was carried out in compliance with the latest revision of the Code of Ethics of the World Medical Association (Declaration of Helsinki), was approved by the Institutional Review Board at Geneva University (Commission Cantonale d'Ethique de la Recherche) and by the Swiss agency for the authorization and supervision of therapeutic products (Swissmedic). All participants were fluent French speakers with normal or corrected to normal vision. They were excluded if they presented psychiatric disorders, had chronic cardiac, renal, or hormonal diseases, if they were smokers, if they had a cold or any obstruction of the nasal cavities, or if they had taken any medication in the week preceding the experiment. Moreover, participants were asked to abstain from doing sports and drinking coffee or alcohol in the 24 hours before the experiment, and from eating or drinking (other than water) for two hours before the experiment. Two participants were fathers.

2.2. Stimuli

In both the Offset and Intensity tasks, stimuli consisted of short movie clips showing gradual changes in emotional expression displayed by adult and by infant faces. Stimuli were constructed with morphing software (Morpheus Photo Morpher, version 3.17) using happy, angry and neutral

expressions by 12 adult and 12 infant faces (50% male in both age groups). The same adult faces have been shown to induce spontaneous facial mimicry in previous experiments (Halberstadt and Niedenthal, 2001; Korb et al., 2015; Niedenthal et al., 2001). The emotions anger and happiness were chosen because they elicit clearly distinguishable patterns of facial mimicry, from facial muscles that lie far apart from each other. Photos of infant faces were extracted from video recordings of mother-infant interactions (Strathearn et al., 2008). To reduce variation due to differences in the background, a black frame with a central oval-shaped hole was superimposed on the infant faces (for a similar procedure see Korb et al., 2014).

Movie clips in the Offset task showed a full-blown expression of happiness that gradually morphed into an expression of anger, or the reverse. Participants were instructed to indicate the moment at which the first emotional expression was no longer present on the face. In the Intensity task, neutral facial expressions morphed into an expression of happiness or anger. After each movie clip the intensity of expressions was judged. Movie clips, which were shown at a rate of 25 frames per second, lasted 5120 ms in the Offset task and 2000 ms in the Intensity task.

2.3. Procedure

A double-blind, placebo-controlled, between-subjects design was used (see Figure 1). Participants were randomly assigned to two groups before the experiment to receive administration of either OT or PLA. The number of participants who correctly guessed the product they had received (56.7 %), was not greater than chance overall ($\chi^2 = 1.1$, $p = .3$), and was similar across groups (OT: $M = 50$ %, $SD = .5$; PLA: $M = 63$ %, $SD = .5$, $t(58) = 1.03$, $p = .3$). A small but significant age difference was found between groups (OT: $M = 26.1$, $SD = 5.1$; PLA: $M = 23.6$, $SD = 4.1$, $t(58) = 2.1$, $p = .04$). Therefore, Participant Age was included as covariate in all LMM analyses (see below).

Participants gave written informed consent and self-administered a nasal spray (three puffs per nostril, 30 seconds between each puff, one puff containing four IU) containing a single dose of 24 IU of OT (Syntocinon spray, Novartis, Basel, Switzerland) or PLA (identical but for the active compound). Participants then filled out the Empathy Quotient (EQ; Baron-Cohen and Wheelwright,

2004), Interpersonal Reactivity Index (IRI; Davis, 1983), and the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988). Electrodes for EMG were then attached (see below).

In the *Voluntary Short* task, voluntary smiling and frowning were assessed with facial EMG (three trials per expression), to verify correct attachment of the electrodes, and to provide a baseline for participants' voluntary facial expressions. The two main tasks testing for mimicry and emotion recognition, *Offset* and *Intensity*, were completed in counterbalanced order across participants. The *Offset* task began, on average, 56 minutes (range 40 – 88 min) after the nasal spray administration. The average starting time for the *Intensity* task was 54 minutes (range 40 – 70). Both tasks lasted about 20 min. Then, voluntary frowning and smiling were assessed again with 20 trials per expression (*Voluntary Long*), and all questionnaires were completed a second time.

In the *Offset* task (Figure 1B), as described in Korb et al. (2015), participants watched movie clips lasting 5.12 seconds and depicting a facial expression of happiness gradually changing to anger, or the reverse. They indicated with a button press as quickly and accurately as possible the moment at which the initial emotional expression was no longer visible. Responses were made on a response box with the right index finger. Movie clips were shown for their entire length, regardless of participants' responses. Before each movie clip a white fixation cross was shown on black background for an average of 2.5 sec (range 2 to 3 sec). After each clip, if a response had been made, the time of perceived offset in ms from the beginning of the movie clip was shown for one second on a black screen. Otherwise, the text "No response" was shown for the same duration.

After completion of two practice trials, 96 trials were shown in four blocks of 24 trials, and participants were free to rest between blocks. Two blocks contained images of adult faces, and the other two of infant faces. The order of blocks was random across participants. The average duration of the *Offset* task was 20 min ($SD = 1.7$ min). Previous research has demonstrated that the *Offset* task reliably induces facial mimicry of the second half of each stimulus video, and when facial mimicry is blocked, perception of expression offsets is delayed (Korb et al., 2015; Niedenthal et al., 2001).

In the *Intensity* task (Figure 1C and Korb et al., 2015), participants watched two-second-long movie clips depicting a neutral facial expression gradually becoming happy or angry. Before each movie clip a white fixation cross was shown on a black background for an average of 2.5 sec (range 2

to 3 sec). Following each clip participants rated, on separate slides, the intensity of (in this order) perceived happiness and anger on 100-point Likert scales, ranging from “very low intensity” on the far left to “very high intensity” on the far right. Rating sliders were presented with the cursor placed at the midpoint of the scale. Participants changed the position of the cursor by moving the computer mouse to the left or right, and advanced to the next slider or trial by clicking the left mouse button. A total of 96 trials were presented in four blocks of 24 trials. Blocks contained either adult or infant faces, and the order of blocks was random. The average duration of the Intensity task was 19.2 min ($SD = 3.8$). Two practice trials, containing faces not used in the main experiment, were shown first. The Intensity task was inspired by research showing that facial mimicry can reliably be induced by dynamic expressions of happiness and anger created by morphing still images of neutral and emotional expressions into each other (Achaibou et al., 2008; Korb et al., 2015).

In order to investigate if OT affected facial muscular activity in general, voluntary smiling and frowning was measured with EMG right before and after the Intensity and Offset tasks. Specifically, across six (Voluntary Short task) or 40 trials (Voluntary Long task), participants were instructed to voluntary smile or frown with the corresponding words appearing on the screen. Presented in random order, half of the trials required smiling, and the other half frowning. Instructions stressed that participants should begin to produce the expression as strongly and quickly as possible as soon as the written command appeared, to maintain the expression for as long as the command was displayed (3 sec), and to return to a relaxed and neutral face immediately thereafter and in between trials.

2.4. EMG recording

Bipolar EMG was recorded from the left Corrugator Supercilii (CS), Orbicularis Oculi (OC), Zygomaticus Major (ZM), and Masseter (MA) muscles, according to guidelines (Fridlund and Cacioppo, 1986). We used a Biosemi (www.biosemi.com) ActiveTwo amplifier system with Ag/AgCl active electrodes, a sampling rate of 2048 Hz and a bandwidth of DC-1.6 kHz. The common mode sense and right-driven-leg electrodes (serving as ground and reference) were placed below the

hairline on the center of the forehead. Only results for the ZM and CS are reported, since their activation reflects mimicry of happiness and anger, respectively.

2.5. Data analysis

In the Offset task, trials were excluded if no response had been made, or if response time (RT) per participant was lower than $M-2*SD$, or higher than $M+2*SD$. An average of nine trials ($SD = 4.3$) were excluded per participant. The number of trials rejected in the OT ($M = 9.9$, $SD = 5.26$) and the PLA group ($M = 8.2$, $SD = 2.8$) did not differ significantly ($t(58) = 1.6$, $p = .12$, *ns*).

In the Intensity task, trials were excluded if RT on either of the ratings (Happiness, Anger) was lower than $M-2*SD$, or higher than $M+2*SD$, or if emotions had been judged incorrectly, i.e. the highest rating was given on the wrong scale (e.g. a Happiness film clip receiving higher ratings of Anger than Happiness). The remaining data contained an average of 83.3 ($SD = 4.6$) trials per participant.

Only EMG of the CS and ZM was retained for analysis, since these muscles are involved in the mimicry of expressions of happiness and anger, and also distinguish between voluntary smiling and frowning. Data were preprocessed offline in Matlab (version R2012a; www.mathworks.com), using self-made scripts and partially using the EEGLAB toolbox (Delorme & Makeig, 2004). Data were submitted to bipolar montage, bandpass-filtered from 20 to 400 Hz, downsampled to 500 Hz, rectified, smoothed with a 40Hz low-pass filter, and segmented from 500 ms before to 5120 ms (Offset task), 2000 ms (Intensity task), or 3000 ms (Voluntary Short and Voluntary Long tasks) after stimulus onset (SO). For the Offset and Intensity tasks we excluded for each participant trials on which the average amplitude in the baseline period (500 ms preceding SO) of the CS or ZM muscles was lower than $M-2*SD$, or higher than $M+2*SD$ (M = average amplitude over all trials' baselines for the respective muscle).

Artifact rejection based on the EMG occurred independently of that based on RTs. Only trials that survived both RT- and EMG-based rejections were included in analyses of EMG data. An average of 26.6 % and 27.8 % of trials were excluded per participant, respectively, in the Offset and Intensity task. Data from SO onwards were expressed as the percentage of the average of the baseline

(for a similar procedure see de Wied et al., 2006). For statistics, EMG data were averaged over five windows of 1024 ms in the Offset task, two windows of 1000 ms in the Intensity task, and one window of 3000 ms in the Voluntary tasks.

2.6. Statistics

Behavioral and EMG data were preprocessed in Matlab, and analyzed with linear mixed effects models (LMMs) using the lme4 package (Bates et al., 2014) in R (R Development Core Team, 2008). One of the advantages of using LMMs, compared to more traditional analyses like ANOVA, is the ability to include random effects for both participants and stimuli. Doing so reduces Type I errors and makes possible the generalization of findings not only to other samples of participants, but also to other sets of stimuli (Judd et al., 2012). There is currently no universally accepted way to calculate standardized effect sizes for main and interaction effects in LMMs. Here, Cohen's d is provided for main effects of factors including two levels (averaging the raw data for each subject), as well as for t tests and comparisons of interest.

Trials with Adult and Infant faces, and data from the CS and ZM muscles, were analyzed in separate LMMs. F-tests were computed, and degrees of freedom were estimated using the Satterthwaite approximation (anova function of the stats package in R). In all cases, intercepts for Participants and Stimuli were included as random effects, and Participant Age was entered as a covariate, to control for the small but significant difference in age between groups (see above). Main and interaction effects of Participant Age are only reported when significant ($p < .05$) or marginally significant ($p < .1$). Only significant main and interaction effects of the factor Product are reported. Please see supplementary material for full results.

For behavioral analyses, RTs and intensity ratings, respectively for the Offset and the Intensity task, were regressed on the fixed effects Emotion and Product, as well as their interaction. EMG of both tasks was analyzed in the same way over four separate LMMs, one per combination of Muscle (CS, ZM), and Stimulus Age (Adult, Infant). Fixed effects (and their interactions) were Emotion (AngryToHappy and HappyToAngry in the Offset task; Happy and Angry in the Intensity

task), Product (OT or PLA), and Time (five windows in the Offset task; two windows in the Intensity task).

To investigate if OT modulates the amplitude of voluntary facial movements (see Figure A.3), we tested an effect of OT across the two control tasks (Voluntary Short and Voluntary Long) with separate LMMs per muscle (ZM, CS), with the within-subjects factors Expression (Frown, Smile) and Time (Time 1 = Voluntary Short, Time 2 = Voluntary Long), and the between-subjects factor Product (OT, PLA). Due to a technical mistake, voluntary facial movements of one participant were not recorded at Time 1.

Scores of the EQ, the four subscales of the IRI, and the positive and negative subscales of the PANAS, were analyzed in separate LMMs with Time (Time 1, Time 2) and Product (OT, PLA) as fixed effects.

3. Results

3.1. Offset task

An LMM with RTs in the Offset task to adult faces (Figure 2A), produced a marginally significant Emotion X Product X Participant Age interaction ($F(1, 2673.5) = 3.7, p = .05$), reflecting a stronger correlation of Participant Age and RT in the PLA group than in the OT group, and for AngryToHappy than HappyToAngry trials. A similar LMM with RTs to infant faces (Figure 2B) revealed a significant Emotion X Product interaction ($F(1, 2543.82) = 5.7, p = .02$) due to faster RTs in the OT than the PLA group to AngryToHappy stimuli (respective Ms = 3329.7 and 3369.4, SDs = 554.1 and 562.0, $d = .07$), but slower RTs in the OT than PLA group to HappyToAngry stimuli (respective Ms = 3214.9 and 3156.1, SDs = 514.7 and 572.7, $d = .11$).

The hypothesis of increased facial mimicry in the OT group was confirmed for anger (Figure 3A), with a significant Emotion X Product X Time interaction for the CS to infant faces ($F(1, 10209.5.8) = 8.7, p = .003$), and a trend for the same interaction to adult faces ($F(1, 10686.1) = 3.5, p = .06$). Effect sizes (Cohen's d) for the differences between OT and PLA in CS activation to

HappyToAngry infant faces were .23, .39, and .46, respectively for the third, fourth, and fifth time window³. The same differences for adult faces had effect sizes of .36, .44, and .40⁴.

Only a trend for an OT-induced increase of mimicry of happiness in infant faces was found (Figure 3B). The crucial Emotion X Product X Time interaction was not significant for adult faces ($F(1, 10689.1) = .004, p = .9$), but at trend level for infant faces ($F(1, 10206.7) = 3.2, p = .07$). Effect sizes for the differences between OT and PLA to infant faces were .22, .28, and .18, respectively for the third, fourth, and fifth time window. For adult faces they were .24, .23, and .02.

3.2. Intensity task

An LMM on the ratings of perceived intensity to adult faces (Figure 4A) produced a significant interaction of Emotion X Product ($F(1, 2418.2) = 18.2, p < .001$), with higher ratings ($d = .29$) for angry faces in the OT group ($M = 65.4, SD = 19.1$) compared to the PLA group ($M = 60.5, SD = 14.2$), but similar ratings ($d = .04$) across groups for happy faces (respectively for OT and PLA $M = 65.9$ and $65.3, SD = 13.5$ and 13.3).

An LMM on RTs to adult faces (Figure 5A) did not reveal main or interaction effects (all $F < 1$, all $p > .3$). There was a significant Emotion X Product interaction ($F(1, 2609.7) = 11.2, p < .001$) in the same analysis for infant faces (Figure 5B). The interaction was explained by faster ($d = .30$) RTs when rating the intensity of happy infant faces in the OT group ($M = 2086.6, SD = 618.2$) compared to the PLA group ($M = 2303.7, SD = 801.8$), while groups did not differ ($d = .01$) in their RTs for angry faces (respectively for OT and PLA: $M = 2047.8$ and $2051.6, SD = 759.3$ and 807.0).

OT did not modulate facial mimicry in the Intensity task. The crucial Emotion X Product X Time interaction was not significant for neither muscle in response to adult or infant faces (all $F < 2.3$, all $p > .13$).

3.3. Voluntary (control) tasks

For the CS, main or interaction effects involving the factor Product were all far from significant (all $F < .8$, all $p > .37$). For the ZM, significant interactions of Product X Time ($F(1,$

³ Effect sizes are here reported based on actual data, but much bigger effect sizes re obtained when using the model fitted values.

⁴ To further verify how stable these effects are, we removed from each group the participant with the most extreme EMG value (although data was already cleaned at the single-trial level). Effect sizes rose to .39, .55, and .62 for infant faces and to .52, .56, and .60 for adult faces.

2416.5) = 6.5, $p = .01$), and Product X Expression X Time ($F(1, 2416.5) = 6.8$, $p = .009$) emerged. Trends for effects of Product ($F(1, 2416.5) = 3.5$, $p = .06$), and Product X Expression ($F(1, 2416.5) = 2.8$, $p = .09$) were also found. The three-way Product X Expression X Time interaction reflected lower intensity of voluntary smiling at Time 2 vs. Time 1 in the OT group compared to the PLA group (Figure A.3). Additional analyses were carried out to investigate if a general OT-induced reduction of ZM activation could explain the lack of increased mimicry of smiles. Spearman rank correlations were computed between the EMG of the ZM during voluntary smiling at Time 2, and the ZM at times 1 to 5 in response to infant and adult AngryToHappy trials (Figure 3B). These correlations were computed both on the entire sample and on the OT subsample, using the ZM during voluntary smiling at Time 2. Correlation coefficients were all $< .26$, and p values were all $> .16$.

3.4. Questionnaires

For EQ scores a trend for a Time X Product interaction was found ($F(1,58) = 3.54$, $p = .06$) due to greater empathy in the OT ($M = 16.0$, $SD = 7.8$) compared to the PLA group ($M = 14.0$, $SD = 5.4$, $d = .30$) at Time 2, while the two groups did not differ at Time 1 (respectively $M = 15.4$ and 15.3 , $SD = 6.3$ and 6.1 , $d = .02$). The analysis of the positive subscale of the PANAS resulted in a Time X Product interaction ($F(1,58) = 6.7$, $p = .01$) due to a greater decrease in positive mood at Time 2 in the OT group ($M = 44.6$, $SD = 14.3$) compared to the PLA group ($M = 51.0$, $SD = 12.4$, $d = .48$).

4. Discussion

Intranasal OT or PLA were administered, in a double-blind, between-subjects design, to two groups of 30 male participants to investigate, across two tasks with happy and angry dynamic expressions, the effects of OT on facial mimicry and on the perception of facial expressions of emotion. Effects of OT were found on both facial mimicry and emotion perception. However, results differed by type of emotion (happiness, anger) and by age of the stimulus face (adult, infant). In addition, effects of OT on voluntary smiling and frowning, as well as on self-reported mood and empathy, were also assessed.

Facial mimicry was reliably triggered by both emotions across both tasks. Significantly increased facial mimicry in the OT compared to the PLA group was only found for anger to infant faces in the Offset task (Figure 3A). However, smaller increases of facial mimicry also occurred for anger in adult faces (Figure 3A), and for happiness in infant faces (Figure 3B). Mimicry of both anger and happiness were also increased in the Intensity task (Figure 6), but without reaching statistical significance. Therefore, our data converge to show that facial mimicry was increased after OT administration, although effects were most reliable in response to anger compared to happiness, and to infant faces compared to adult faces.

Why did OT particularly boost facial mimicry of anger, and more specifically for infant faces? One possibility is that OT increased participants' willingness to respond to angry faces. Indeed, OT has a general anxiolytic effect (for a discussion about OT's general and specific effects see Churchland and Winkielman, 2012), and moreover can reduce aversion for anger expressions (Evans et al., 2010). In the context of this study, lower aversion to anger faces may have led participants who received OT to be more emotionally engaged with the face overall.

Another possibility is that OT modified the way in which participants visually explored the stimuli. Indeed, other studies have demonstrated that participants who received intranasal OT spend more time fixating the eye region of faces (Guastella et al., 2008). In the eye region anger is easily detected whereas happiness' most distinctive feature, the lifting of the mouth corners, occurs in the lower face region (Ekman and Rosenberg, 2005). Therefore, the here observed greater mimicry of anger may partly be due to the increased tendency in the OT group to look at the eye region. In addition, the relatively larger eyes of infants may also have captured participants attention to the eye region, particularly in the OT group (Glocker et al., 2009). Future studies should include eye tracking to measure whether changes in the pattern of visual exploration of the faces, induced by OT, accompany increases in facial mimicry.

Why did OT mainly increase facial mimicry to emotion expressions in infants? Possibly, OT boosted mechanisms associated with caregiving behavior in male participants, thus augmenting the significance of expressions in infants. Maternal love is accompanied by activation in areas of the OT system (Bartels and Zeki, 2004; Strathearn, 2011). But also in male participants OT administration

can trigger and reinforce feelings of bonding and caregiving towards infants (Feldman, 2012; Naber et al., 2013; Weisman et al., 2014). This hypothesis is intriguing with respect to the fact that, with the exception of two participants, the entire sample tested here consisted of childless men. Previous studies on OT and caregiving have typically tested either females or fathers. Our data are therefore the first to indicate that a small dose of intranasal OT may elicit changes in spontaneous facial mimicry to infant faces even in childless males.

Behavioral data suggest distinct effects of OT on the perception of facial expressions in infant and adult faces. When watching infant faces, participants in the OT group were particularly attentive to expressions of happiness, as shown by faster RTs to AngryToHappy and slower RTs to HappyToAngry trials in the Offset task (Figure 2B), and by faster RTs for happy faces in the Intensity task (Figure 5B). In contrast, OT made participants pay more attention to anger in adult faces, as suggested by non-significantly slower RTs to AngryToHappy trials in the Offset task (Figure 2A), and by higher perceived intensity of anger in the Intensity task (Figure 4A).

Why did participants in the OT group focus on happiness in the infant faces and on anger in adult faces, as suggested by their behavioral responses across both the Offset and the Intensity tasks? Part of the answer might lie in the fact that anger and happiness can have different meanings in adults and infants. In fact, emotions in infants are less distinctive than in adults, and emotions of anger and sadness, for example, are often confounded by adult observers (Oster, 2005; Sullivan and Lewis, 2003). The ambivalence of anger expressions in infant faces, which can also be interpreted as expressing sadness or distress, might have contributed to the here reported behavioral effects. Administration of OT might have further accentuated the perceived ambivalence of infants' anger expressions, by increasing participants' attention to the infant faces. Alternatively, based on the saliency hypothesis of OT (Shamay-Tsoory et al., 2009), happiness in infants, and anger in adults, might have become more relevant after OT administration. Especially if OT increased feelings of caregiving, as speculated above, anger in adult faces could have enhanced aggressive responses in participants (Ne'eman et al., 2016). The saliency hypothesis could also help explain why facial mimicry of anger was more increased under OT. If mimicry can be modulated by attention and by perceived stimulus relevance (Cannon et al., 2009; Seibt et al., 2015), then distressed infants in need

of care and protection on the one hand, and angry adults perceived as aggressors on the other hand, might both become highly salient under OT, and thus be mimicked more. On the basis of the literature and our current results it can be assumed that anger in adult faces and anger/distress in infants could evoke different emotional responses (fight off aggressor vs. provide protection and care), and that OT administration might intensify these age-related differences.

What are the neural mechanisms through which OT might increase facial mimicry? The neural circuitry underlying the motor output of facial mimicry remains unclear to date, but might include both cortical (premotor and primary motor cortices, motor areas of the ACC, IFG, parietal areas linked to the MNS), and subcortical areas like the basal ganglia (Korb et al., 2015; Korb and Sander, 2009; Likowski et al., 2012; Rinn, 1984). Emotional “coloring” of motor commands to the face can also occur at the subcortical level, for example via the reticular formation. Much uncertainty remains also about the brain areas that can be reached with intranasal administration of OT (Churchland and Winkielman, 2012; Quintana et al., 2015; Veening and Olivier, 2013). Subcortical areas involved in facial mimicry are the likely targets of intranasal OT, due to their greater phylogenetic age, to their anatomical proximity with the nasal cavities, and to their role for various emotional reactions. In line with this, neurons with OT receptors have been observed in the brainstem motor nuclei of the facial nerves, which innervate the facial muscles (Loup et al., 1989), and in the spinal trigeminal nucleus (Freeman et al., 2016), which receives sensory information from the face and oral cavity.

Modulation of facial mimicry might also occur indirectly, via OT’s effects on emotion hubs, such as the amygdala. Indeed, several studies have shown that oxytocin can change neural activity in the amygdala, and amygdala-dependent social learning (Domes et al., 2007a; Gamer et al., 2010; Hurlemann et al., 2010). Moreover, OT’s effects on amygdalar response to facial expressions can vary by personal relevance. For example, Strathearn and Kim (2013) found greater amygdala response in first-time mothers to sad than happy expressions of unknown baby faces, but the reverse pattern for pictures of their own baby. By modulating amygdala activation to facial expressions, OT could impact early stages of visual processing, either through a direct subcortical route or through a fast feedforward pathway including the visual cortices (Vuilleumier, 2005), which can provide the type of fast and

automatic stimulus perception required for facial mimicry. Interestingly, in macaque monkeys responses to fearful and aggressive faces in face-responsive regions of the visual cortex, and functional coupling of the same areas with the amygdala, were decreased after OT administration (Liu et al., 2015). In newborn macaques intranasal oxytocin also increased positive social behavior, including time spent close to human caregivers (Simpson et al., 2014). Although these findings are of great importance to understand how OT modulates face processing in the primate brain, they do inform us on the effects of OT on facial mimicry. In relation to this, it needs to be emphasized that, in humans, facial mimicry can occur without conscious stimulus perception, and even in the absence of a functioning visual cortex (Dimberg et al., 2000; Tamietto et al., 2009). To further disentangle the role of the amygdala and various somatomotor areas in human facial mimicry, future studies should attempt to study the modulation of facial mimicry through OT, while monitoring facial movement with EMG and neural activity with brain imaging techniques.

Intranasal OT administration likely also led to increased levels of OT in the blood, which could have indirectly influenced participants' responses to the facial expressions. Indeed, although the amount of OT (24 IU) administered in this study exceeds the estimated oxytocin content in the pituitary gland (Heller and Zaimis, 1949), it has been shown that only a fraction of intranasal OT can be measured in the cerebral spinal fluid (CSF), suggesting that its concentration in the brain only increases by a small amount (Leng and Ludwig, 2016). In contrast, OT concentrations in the blood increase sharply after intranasal administration. Thus, the possibility remains that the resulting afferent feedback of OT from the periphery contributed to the observed effects by stimulating endogenous OT release within in the brain (Striepens et al., 2013). Although OT in the periphery cannot, in principle, affect neural activity of the brain directly, it might cause autonomic changes (e.g. in heart rate), which themselves can influence OT levels in the brain. This hypothesis was clearly beyond the scope and possibilities of this study, but should be investigated in future experiments.

Both voluntary facial expressions, and self-reported measures of empathy and mood, were measured shortly after administration of the nasal spray to provide a baseline (Time 1), and 81 min after administration to measure OT-induced changes (Time 2). Amplitude of voluntary frowning did not differ between Time 1 and Time 2 in neither participant group, proving that OT did not change

overall facial muscle activation (Figure A.3). This suggests that effects of OT are limited to automatic facial reactions, and do not impact voluntary facial movements. However, EMG of the ZM during voluntary smiling was significantly reduced at Time 2 in the OT group. Since voluntary facial expressions at Time 2 were recorded after completion of the Offset and Intensity task, one cannot exclude that the observed decrease of voluntary smiling in the OT group was due to a change in mood, itself triggered by the greater mimicry of anger in particular. Indeed, assuming a specific facial or bodily posture can change a person's mood and autonomic nervous system activity (Ekman et al., 1983; Flack, 2006), and therefore greater mimicry of anger might have impacted participants' mood in the OT group, reducing their later motivation to engage in voluntary smiling. In line with this, OT led to a significant decrease in positive mood, as measured with the PANAS (Figure A.4). In addition, OT non-significantly ($p = .06$) increased empathy, as measured with the EQ.

This study is not without limitations, including the absence of eye-tracking measures and the use of a males-only sample. The choice of a male sample was made for practical reasons, but effects of OT on facial expression recognition may differ between sexes (Campbell et al., 2014). Intranasal administration of a different anxiolytic could have helped to better disentangle OT's general anxiolytic effects from its more specific effects on facial mimicry and face processing. For example, administration of the neuropeptide arginine vasopressine (AVP) as well as of moderate doses of alcohol, have been reported to partly induce similar effects as OT (Mitchell et al., 2015; Thompson et al., 2004). Finally, although our sample was 22% larger than the median sample size of previous studies, a recent review suggests that much larger groups of participants may be needed to obtain adequate statistical power (Walum et al., 2016). These results therefore will need to be confirmed and replicated in a bigger sample.

5. Conclusions

The present study demonstrated that the intranasal administration of OT to healthy male participants can increase facial mimicry, in particular for anger expressions in infant faces. Because facial mimicry contributes to emotion recognition and is reduced in ASD, these findings suggest that OT administration to healthy male participants, and possibly also to individuals with ASD, can

increase facial mimicry and improve emotion recognition. Results also indicate that effects of OT might differ depending on the stimulus, with greater effects of OT on facial mimicry of anger compared to happiness, and for infants compared to adult faces. Caution is however warranted in the interpretation and generalization of these results, which require replication in a larger sample.

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A

- Informed consent
- 24 IU OT or PLA
- Questionnaires
- Electrode positioning
- Voluntary Short

B

OFFSET TASK

C

INTENSITY TASK

D

- Voluntary Long
- Questionnaires

A

Adult faces

B

Infant faces

A

Adult faces

B

Infant faces