Intranasal Oxytocin Decreases Fear Generalization in Males, but Does Not Modulate Discrimination Threshold

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Abstract

Background. A previously acquired fear response often spreads to perceptually or conceptually close stimuli or contexts. This process, known as fear generalization facilitates the avoidance of danger and dysregulations in this process play an important role in anxiety disorders. Oxytocin (OT) has been shown to modulate fear learning, yet effects on fear generalization remain unknown.

Methods. We employed a randomized, placebo-controlled, double-blind, between-subject design during which healthy male participants received either intranasal OT or Placebo (PLC) following fear acquisition and before fear generalization with concomitant acquisition of skin conductance responses (SCR). 24h-72h before the fear learning and immediately after the fear generalization task participants additionally complete a Discrimination threshold task.

Results. Relative to PLC, OT significantly reduced perceived risk and SCR responses towards the CS+ and GS1 (the generalization stimulus that is most similar to CS+) during fear generalization, whereas the discrimination threshold was not affected.

Conclusions. Together the results suggest the OT can attenuate fear generalization in the absence of effects on discrimination threshold. This study provides the first evidence for effects of OT on fear generalization in humans and suggests that OT may have therapeutic potential in anxiety disorders characterized by dysregulated fear generalization.

Keywords: *Oxytocin, Fear generalization, Discrimination threshold, Skin conductance responses (SCR)*

Introduction

Fear generalization refers to the expression of a fear response to a neutral stimulus (light, tone, or smell) that is similar to a previously conditioned fear-associated stimulus. High perceptual similarity between the fear-associated stimulus and the neutral event promotes the generalization of fear (Pavlov, 1927; Desiderato & Wassarman, 1967; Dunsmoor & Paz, 2015). The generalization of fear represents an adaptive mechanism that promotes survival by facilitating defensive responses towards a potential danger. Maladaptive dysregulations in this mechanism have been increasingly recognized as important contributor to the development and maintenance of exaggerated fear and anxiety and represent a key diagnostic feature of a range of debilitating psychiatric disorders (Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015), particularly generalized anxiety disorder (GAD) (Lissek et al., 2014; Greenberg, Carlson, Cha, Hajcak, & Mujica‐Parodi, 2013), panic disorder (Lissek et al., 2009), post-traumatic stress disorder (Kaczkurkin et al., 2017), and social anxiety disorder (Ahrens et al., 2016). Thus, understanding the neurobiological basis of fear generalization and identifying effective behavioral and pharmacological interventions that inhibit fear generalization is of significant translational and clinical interest. Moreover, fear conditioning and generalization is an evolutionary highly preserved mechanism that can be examined by means of the Pavlovian fear-conditioning paradigm across species thus promoting translational determination of aversive learning mechanisms across species and facilitating the translation from basic research to clinical application (Bowers & Ressler, 2015).

The evolutionary highly conserved hypothalamic neuropeptide oxytocin (OT) has been increasingly studied as potential treatment for enhancing the regulation of fear and anxiety (Kendrick, Guastella, & Becker, 2017; Neumann & Slattery, 2016). Previous studies combining the intranasal administration of OT with functional MRI in humans have demonstrated repeatedly an attenuation of amygdala reactivity towards threatening social cues. Several previous studies reported that a single dose of intranasal OT induced robust downregulation of amygdala responses to threatening faces (Domes et al., 2007; Grace, Rossell, Heinrichs, Kordsachia, & Labuschagne, 2018; Kirsch et al., 2005; Petrovic, Kalisch, Singer, & Dolan, 2008). Even in the patients with generalized social anxiety disorder, such that Labuschagne et al. (2010) found that OT attenuated hyper-reactivity of the amygdala towards fearful faces in patients with this disorder. Furthermore, a dose-responses study (Spengler et al., 2017) demonstrated pronounced effects of a single dosage of 24IU OT on attenuating amygdala threat reactivity to ambiguous fearful faces (35% emotional intensity). Together, results from these previous studies indicate that OT can effectively attenuate amygdala threat-reactivity and shift the perception of ambiguous fearful faces towards neutral.

Based on convergent evidence for a role of OT in the regulation of fear and amygdala-threat responses, initial studies have employed Pavlovian fear conditioning

and extinction paradigms to examine effects on aversive learning, yet effects on fear generalization have not been examined. OT is produced primarily in hypothalamic brain regions and released via the pituitary in the periphery and various brain regions (Donaldson & Young, 2008), especially amygdala, hippocampus and medial prefrontal cortex (mPFC). These regions highly overlap with the fear generalization network (Dunsmoor, Prince, Murty, Kragel, & LaBar, 2011; Greenberg et al., 2013a). Taken together, it is reasonable to assume that OT may be able to reach fear-associated brain areas to inhibit fear generalization. OT may exert its effects on fear generalization via different mechanisms. One possible path is that OT promotes the extinction of fear generalization, such that is decreases the behavioral and physiological responses to fear generalization stimuli. Previous studies explored the effect of OT on fear extinction in both human and animal studies. In healthy male humans, OT administered following threat conditioning and prior to immediate extinction training facilitated extinction in the context of increased activation of the prefrontal cortex and attenuated amygdala activity (Eckstein et al. 2015). In rodents, OT effects on fear extinction are highly regional specific and depend on the time of the administration (before or after the fear conditioning) (Lahoud & Maroun, 2013). More specifically, OT acted as an enhancer of the fear response during extinction following local infusions in the dorsal raphe nucleus or intracerebroventricular regions (Kovács, Bohus, Versteeg, De Kloet, & De Wied, 1979; Toth, Neumann, & Slattery, 2012), whereas OT infusion into the central amygdala or the dorsolateral septum facilitated the extinction of fear (Viviani & Stoop, 2008; Zoicas, Slattery, & Neumann, 2014). A second possible pathway via which OT may modulate fear generalization is through reducing the discrimination threshold. Holt et al (2014) tested discrimination thresholds of human faces and non-social control stimuli before participants underwent a Pavlovian fear conditioning procedure with concomitant acquisition of the psychophysiological fear response as assessed by skin conductance responses (SCR). Results have indicated that in humans the SCR – an autonomic threat detection index – is highly sensitive to small perceptual difference between stimuli. Furthermore, Tuominen et al. (2019) also provided neural evidence that fear generalization responses were influences by perceptual discrimination thresholds. They found that when the stimuli were under the discrimination thresholds, specific regions of the anterior insula and superior frontal cortex were activated. Importantly, a recent animal study by Ferretti et al. (2019) demonstrated that oxytocinergic projections from the paraventricular nucleus of the hypothalamus (PVN) to the central amygdala (CeA) are crucial for the discrimination of both positively and negatively valence emotional stimuli.

Against this background the present study aimed at examining the effects of OT on fear generalization and the underlying pathways. To this end 24 IU of OT were intranasally administered to healthy male participants following fear acquisition to determine effects on subsequent fear generalization. Considering that changes in the discrimination threshold may modulate fear generalization, discrimination thresholds

were assessed before and after the experiment by means of the previously validated Just Notice Differences (JNDs) procedure (Holt et al., 2014; Tuominen et al., 2019). Additionally, OT has been proposed to specifically modulate the salience of social stimuli (Shamay-Tsoory & Abu-Akel, 2016), whereas an increasing number of studies reported effects on fear learning and salience processing independent of social context (Eckstein et al., 2015; Yao et al., 2018a). To additionally examine whether OT effects on fear generalization are limited to social stimuli we therefore included both social (face morphs between two different female faces) and non-social (circle with different size) fear generalization stimuli. We hypothesized that OT would reduce shock expectations during fear generalization and the psychophysiological fear response (SCR) thus facilitating the extinction of fear generalization. Furthermore, given that a review by the Shahrestani, Kemp, and Guastella (2013) indicated the intranasal OT promoted emotion recognition of facial stimuli, we expected that OT could alternatively modulate fear generalization by reducing the discrimination threshold relative to the PLC group.

Methods

Participants

Male university students with age ranging from 18 to 25 years were recruited via advertisements and flyers on the campus. To account for previously reported sex-differences of OT on salience and social threat processing as well as brain regions involved in threat generalization (Ma et al., 2018; Luo et al., 2017), only male participants were included in this study. All volunteers were pre-screened in telephone interviews and were excluded if they: (1) had no normal or corrected to normal vision; (2) were previously diagnosed with a neuropsychological disorder; (3) had rhinitis or common cold; (4) were using medication or underwent therapy; (5) reported substance abuse. According to the aforementioned criteria, eight volunteers were excluded (7 reported rhinitis or common cold and one declined participation in the experiment). In total, sixty-three eligible university students underwent the experiment following randomization into either an experimental group (oxytocin treatment) or control (placebo treatment) group. Notably, data from one participant in the control group was removed because of a technical failure during SCR acquisition. Participants received compensation of 100 RMB. Each participant provided written informed consent and all the study protocol contributing to this work complied with the ethical standards of the local ethical council of Shenzhen University and with the Helsinki Declaration of 1975, as revised in 2008.

Design

A randomized, placebo-controlled double-blind between-subject trial was conducted during which sixty-three eligible males were randomly assigned to either an experimental group (n = 30) or control group (n = 33, see Figure 1). We adopted posteriori power calculation after the data analysis and found the power was sufficient to support the hypothesis (See more details in supplementary materials).

Randomization was conducted via a computer-based random number generator. Participants in the experimental group received 24 International Units (IU) OT via intranasal administration of Syntocinon-Spray (ProSpec, Israel). Participants were asked to administer 3 puffs per nostril with 4 IU per puff. Participants were asked to wait for one minute between the puffs to ensure that OT was fully absorbed once each puff was completed. In case the Syntocinon-Spray came out of the nostril cavity or was swallow, additional puffs would be added. Participants in the placebo-controlled group (PLC) were delivered intranasally with equivalent volumes of 0.9% NaCl per nostril. To ensure double-blinding of the experimenter and participants the bottom of bottles for OT and PLC were labeled with blue and dark color, respectively, by a research assistant who was not involved in the administration. Another research assistant blinded for the color coding was specifically responsible for distribution and recording the color code of the bottle for each participant.

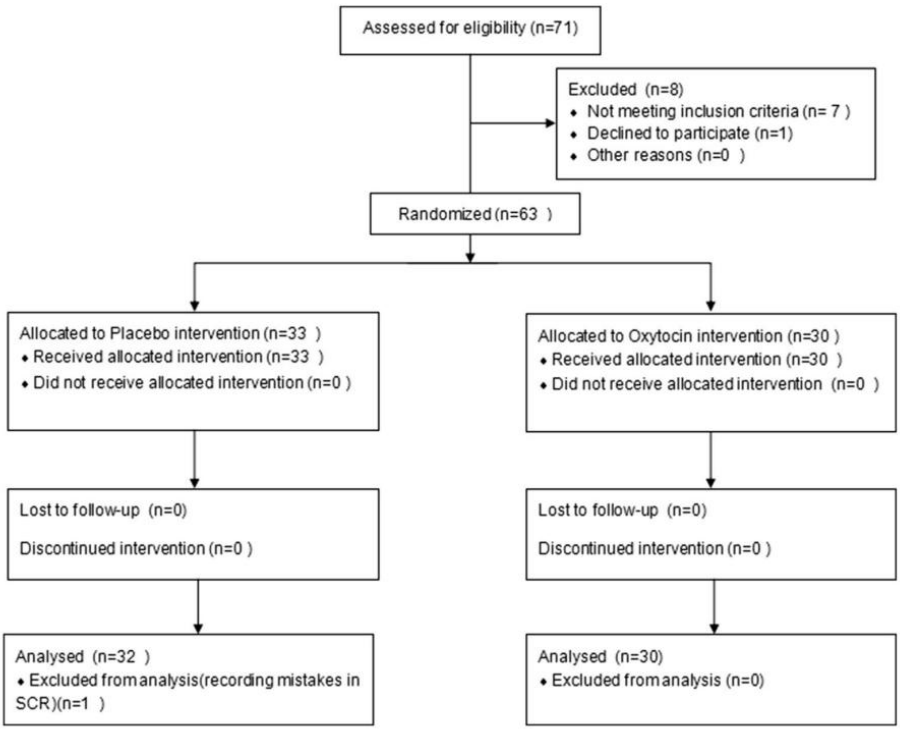

Figure 1. Flowchart of the study: we recruited 71 male participants in this study by advertisements and flyers on the campus. Eight participants were excluded (seven reported rhinitis or common cold and one declined participation in the experiment). Consequently, 63 participants were arranged randomly into two treatment groups: oxytocin administration (n = 30) and placebo administration (n = 33). After data collection, one participant in the placebo group was excluded due to technical failure during SCR recording.

Procedure

Participants were asked to visit the lab two times on different days throughout this research project (see supplementary figure 1). During the first visit, subjects were required to perform the Forced Choice Discrimination Task (FCDT). To minimize the

practice effect of FCDT, participants were arranged to come back to the lab after approximately 24 hours. For the second visit, all participants initially completed three questionnaires (the Beck Depression Inventory II, BDI-2; State-Trait Anxiety Inventory, STAI; Liebowitz Social Anxiety Scale, LSAS). Given that variations in depressive and anxiety symptom load have been associated with fear generalization we decided to control for between-group differences. Next, participants underwent the fear learning task that lasted approximately 15 minutes. Once the fear learning stage was completed participants were administered either OT or PLC and waited for 40 minutes to maximize the treatment effect of OT on the subsequent fear generalization task that lasted approximately 20 minutes. Finally, all participants were retested with the FCDT.

Stimuli

Social stimuli included two pictures of neutral female faces selected from the Chinese Affective Face System (CAFS, Gong, Huang, Wang, & Luo, 2011) which served as threat (CS+) and safety cues (CS−). A face-morphing software (Squirlz morph Version 2.1; Xiberpix, Solihull, UK) was used to create four stimuli for generalization (GS) by morphing the two faces in 20% steps (Schiele et al., 2016), with the GS most similar to the CS+ referred to as GS1 and the GS most similar to the CS− as GS4 (see Supplementary figure 2). Non-social stimuli included 6 circles of varying sizes. Conditioned and generalization stimuli were circles of different sizes. The smallest ring was 2 inches. Circles increased successively in size by 20%. The face stimuli were the same as the circle stimuli, half of the participants receive the face A as CS+, while the other half received the face B as the CS+. The task was present using the E-prime 2 software and a 22-in Lenovo monitor with 60Hz resolution.

Conditioned Generalization Paradigm

The conditioned generalization paradigm used in our research was a modified version compared with that used in a previous study (Lissek et al., 2008; 2014, see supplementary figure 2). The conditioned stimuli included 10 circles of varying sizes, while the unconditioned stimulus was a mild electric shock (50 ms) delivered to the right wrist. The latter was produced by a multichannel electrical stimulator (type: SXC-4A, Sanxia Technique Inc., China) and was delivered through a pair of Ag/AgCl surface electrodes. Before the experiment, we tested the participants' pain threshold and adjusted the potency of the electric shock to a level that the participants described as "highly uncomfortable but not painful." The paradigm consisted of two different phases: learning, and generalization. The learning phase linked the conditioned fear response (shock) to the conditioned stimuli. The largest circle was the conditioned fear cue (CS+), which was paired with an uncomfortable electric stimulation. This phase presented the CS+ 12 times, of which nine included an electric stimulation (reinforcement rate, 75%). The smallest circle was the conditioned safety stimulus (CS-) and was never paired with an electric shock across its 12 presentations. The participants were required to rate their perceived likelihood of receiving an electric

shock once the CS+ or CS- was presented on a 9-point scale: 1 indicated no risk; 5, moderate risk; and 9, high risk. The assignments of the large and small circles to the CS+ and CS- were counter-balanced across subjects. Each stimulus was presented for 6 s, followed by a 50-ms electric shock in the case of a CS+. The inter-trial-interval (ITI) consisted of a fixation cross presented for a random time frame of 8-12 s (Guhn et al., 2014). Each type of the circle and face was presented in 12 trials, respectively (total, 72 trials). The sequence of the stimuli was pseudo-randomized such that maximally three same type of stimuli followed each other. To avoid fear extinction, the reinforcement rate of CS+ was 50% in the generalization phase. The participants' task was the same as that in the learning phase. When the participants had finished 20 trials, they were permitted to take a break. The same assignment of the largest and smallest circle to the CS+ and CS- in the learning phase was applied to the generalization phase; thus, half of the participants received the largest circle as the CS+, while the other half received the smallest circle as the CS+.

Three questions were sequentially presented to the participants immediately following both fear learning and generalization tasks: (1) valence rating: how much pleasure do you feel when this stimulus was presented? (1 very unhappy to 9 very happy). (2) arousal rating: how much arousal do you feel when this stimulus was presented? (1 very calm to 9 very excited). We added the valence and arousal rating is to test whether the OT can reduce fear generalization by modulating the subjective emotional rating. (3) attractiveness rating: how much attractiveness do you feel when this stimulus was presented? (1 no attractive to 9 very attractive). We defined the attractiveness in our study is that an attractive stimuli (face and circle) attract the participant, and the participant can make a judgement about them, tends to look at them. The three questions were answered on a 9-point Likert scale.

Discrimination Thresholds - Forced Choice Task

The Forced Choice Task was used to measure perceived discrimination threshold as reflected by the Just Noticeable Difference (JND) in psychology (Holt et al., 2014, Tuominen et al., 2019). JNDs were calculated by testing the accuracy of distinguishing the changed stimulus (morphs from Face A and Face B, or different size squares between square A and square B) from the initial stimulus by using a 2 alternative forced choice (2-AFC) discrimination task (see supplementary figure 3). This 2-AFC task consisted of three runs of 80 trials each. A stimulus was shown for 500 ms. After a 500 ms inter-trial interval, subjects were presented with the stimulus A and a morph stimulus side by side and asked to select which stimulus they had previously seen by pressing the left or right key, the response time was unlimited. The position of the stimulus A and the morph stimulus were pseudo-randomized across trials. The morph stimuli used in this task were 6, 12, 24, 48, and 100 physical steps from the stimulus A. Responses were followed by a 1 s inter-trial interval. A Weibull function was fitted to the responses: $y = 1-e^{(-(x/a)^{b})}$, in which y was the proportion of correct responses, x was the morph level, and a, and b were parameters for scale

and shape, respectively. The JND corresponded to the morph level at which the subject achieved 75% accuracy on the 2-AFC task (Figure 2A) (Clementz et al., 2007; Holt et al., 2014; Parkes, Lund, Angelucci, Solomon, & Morgan, 2001; Tuominen et al., 2019). An example of this morph continuum is shown in Figure 3A.

SCR recording

SCR was measured using an 8-Slot BioNex (Mindware, Model 50-3711-08) (https://www.mindwaretech.com/product_detail.asp?ItemID=1512) device. We recorded the SCR data with BioLab Acquisition Software 3.2.1 with a 1000 Hz sample rate in our experiment by Electrodermal activity (EDA) channel. Before the experiment, we asked the participants to wash their hands with clean warm water without using alcohol or liquid soap with alcohol. Then the Ag/AgCl electrodes with 0.5% Chloride Wet Gel were placed on the thenar and hypothenar eminence of the participants' left palms. The date was calculated by the Basic Signal Analysis (BAS) 3.2.4 software. We calculated the difference of the mean value of 3s before the stimulus onset as baseline and the maximum values within 6s after the stimulus onset. If a trial lacked an SCR peak, it was recorded as a zero-response trial; specifically, if the max-baseline amplitude for any trial was below 0.02 μs, the trial was scored as a zero-response trial (Boucsein et al., 2012).

Data analysis

Behavioral data and SCR data were analyzed using SPSS 21.0. Behavioral data and SCR were examined by means of repeated-measures ANOVAs. The ratings of valence, arousal and attractiveness of the stimuli after the fear learning and generalization were also examined with repeated-measures ANOVAs. We calculated the partial eta-square as a measure of the effect size and accessed the assumption of sphericity with Mauchly's test. Besides, non-sphericity was correct by Greenhouse-Geisser correction and Bonferroni correction was adopted when pairwise comparisons were conducted. Significance threshold was set to $p < 0.05$ with two-tailed tests.

Results

Demographics Data

No significant between-group differences were observed on all neuropsychological indices (anxiety trait, anxiety state, depression, social anxiety and avoidance) and mean age (See Table 1).

Table 1. Baseline data on age, anxiety, depression, and social anxiety in two groups

	Oxytocin group	Placebo group		
	M* ± *SD	***M* ± *SD***	***T***	***p***
Age(year)	19.96±1.65	19.75±1.13	0.61	0.55
STAI_Trait[†]	39.33±7.64	38.15±10.32	0.51	0.61
STAI_State	35.20±7.52	35.62±9.28	-0.20	0.84
BDI_II[‡]	7.43±6.95	7.59±5.91	-0.10	0.92
LSAS_FA[§]	21.93±9.92	21.88±9.09	0.02	0.98
LSAS_A[¶]	24.90±11.41	23.78±7.07	0.46	0.65

[†]State-Trait Anxiety Inventory [‡]Beck depression inventory-II [§] LSAS_FA: Liebowitz Social Anxiety Scale_Fear/Anxiety subscale [¶] LSAS_A: Liebowitz Social Anxiety Scale_ Avoidance subscale. M = mean, SD = standard deviation.

Perceived risk score

Fear learning

During the fear acquisition phase a significant main effect of stimuli was observed ($F(1, 60) = 405.344$, $p < 0.001$, $\eta_p^2 = 0.871$). The perceived risk of the CS+ was significantly higher than that of CS-. In addition, a significant main effect of stimulus material was also observed ($F(1, 60) = 11.222$, $p = 0.001$, $\eta_p^2 = 0.158$); participants perceived the facial CS with higher risk relative to the non-social (circle) CS. However, we did not observed significant results in terms of a main effect of group ($F(1, 60) = 0.000$, $p = 0.988$, $\eta_p^2 = 0.000$), group x stimuli interaction effect ($F(1, 60) = 0.382$, $p = 0.539$, $\eta_p^2 = 006$), group x material interaction effect ($F(1, 60) = 2.434$, $p = 0.124$, $\eta_p^2 = 0.039$), and material x stimuli interaction effect ($F(1, 60) = 0.511$, $p = 0.477$, $\eta_p^2 = 0.008$). Likewise the stimuli x material x group interaction effect was not significant ($F(1, 60) = 0.431$, $p = 0.514$, $\eta_p^2 = 0.007$). The results of the fear learning task are displayed in Figure 2A. Collectively, the findings suggest a successful acquisition of the fear response in both groups.

Fear generalization

For the fear generalization a significant main effect of stimuli was observed ($F(2.6, 156.5) = 265.753$, $p < 0.001$, $\eta_p^2 = 0.816$). Results from post-hoc test indicated that perceived risk of CS+ was higher than that of other GS and CS- ($p < 0.001$): GS1 > GS2-4, CS- ($p < 0.001$); GS3 > GS4, CS- ($p < 0.001$); GS4 > CS- ($p = 0.073$).

Moreover a significant main effect of group was observed ($F(1, 60) = 6.819$, $p = 0.011$, $\eta_p^2 = 0.102$), suggesting that that OT group showed a considerably lower perceived risk relative to PLC group. Moreover, a group x stimuli interaction effect was also significant ($F(2.6, 156.5) = 4.301$, $p = 0.009$, $\eta_p^2 = 0.067$) and results from simple effect analyses indicated significantly higher perceived risk of CS+ ($F(1, 60) = 14.197$, $p < 0.001$, $\eta_p^2 = 0.191$) and GS1 ($F(1, 60) = 5.427$, $p = 0.023$, $\eta_p^2 = 0.083$) in the PLC group as compared to the OT group. However, we did not observe significant results in terms of a main effect of materials ($F(1, 60) = 2.809$, $p = 0.099$, $\eta_p^2 = 0.045$), material x group interaction effect ($F(1, 60) = 1.759$, $p = 0.190$, $\eta_p^2 = 0.028$, material x generalization stimuli interaction effect ($F(3.0, 183.9) = 1.560$, $p = 0.200$, $\eta_p^2 = 0.025$), material x generalization stimuli x group interaction ($F(3.1, 183.9) = 0.990$, $p = 0.400$, $\eta_p^2 = 0.016$). Results of the fear generalization task are displayed in Figure 5B.

To determine the effect of OT on extinction of fear generalization between the two groups, the presentation of each generalization stimulus was segmented into three parts (Block1, Block2, Block3) according to the phase of the extinction procedure based on time frame. We found a significant main effect of time in the perceived risk of CS+ ($F(2, 120) = 8.894$, $p < 0.001$, $\eta_p^2 = 0.129$). Pairwise comparison showed that the perceived risk of Block1 was much higher than those of Block2 ($p = 0.053$) and Block3 ($p = 0.001$). A significant time x group interaction effect was observed on CS+ ($F(2, 120) = 3.148$, $p = 0.046$, $\eta_p^2 = 0.050$). Results from simple effect analyses indicated that OT group rated the perceived risk of the CS+ lower as compared to PLC group in Block1 ($F(1, 60) = 6.390$, $p = 0.014$, $\eta_p^2 = 0.096$), Block2 ($F(1, 60) = 7.978$, $p = 0.006$, $\eta_p^2 = 0.117$) and Block3 ($F(1, 60) = 16.601$, $p < 0.001$, $\eta_p^2 = 0.217$). (Figure 2C) In the GS1, a significant main effect of time ($F(2, 120) = 21.923$, $p < 0.001$, $\eta_p^2 = 0.259$) was observed. The perceived risk of Block1 (6.155, 0.196) was significantly higher than that of Block2 (4.945, 0.243, $p < 0.001$) and Block3 (4.808, 0.241, p<0.001). A main effect of group was significant ($F(1, 60) = 5.727$, $p = 0.020$, $\eta_p^2 = 0.087$). However, an interaction effect of time and group was not significant ($F(2, 120) = 0.732$, $p = 0.483$, $\eta_p^2 = 0.012$). Although the interaction effect was not significant, we exploratory calculated the simple effect and found that OT group rated the perceived risk of the GS1 lower as compared to PLC group in Block2 ($F(1, 60) = 3.350$, $p = 0.072$, $\eta_p^2 = 0.053$) and Block3 ($F(1, 60) = 5.826$, $p = 0.019$, $\eta_p^2 = 0.089$). Results for extinction of fear generalization are displayed in Figure 2D.

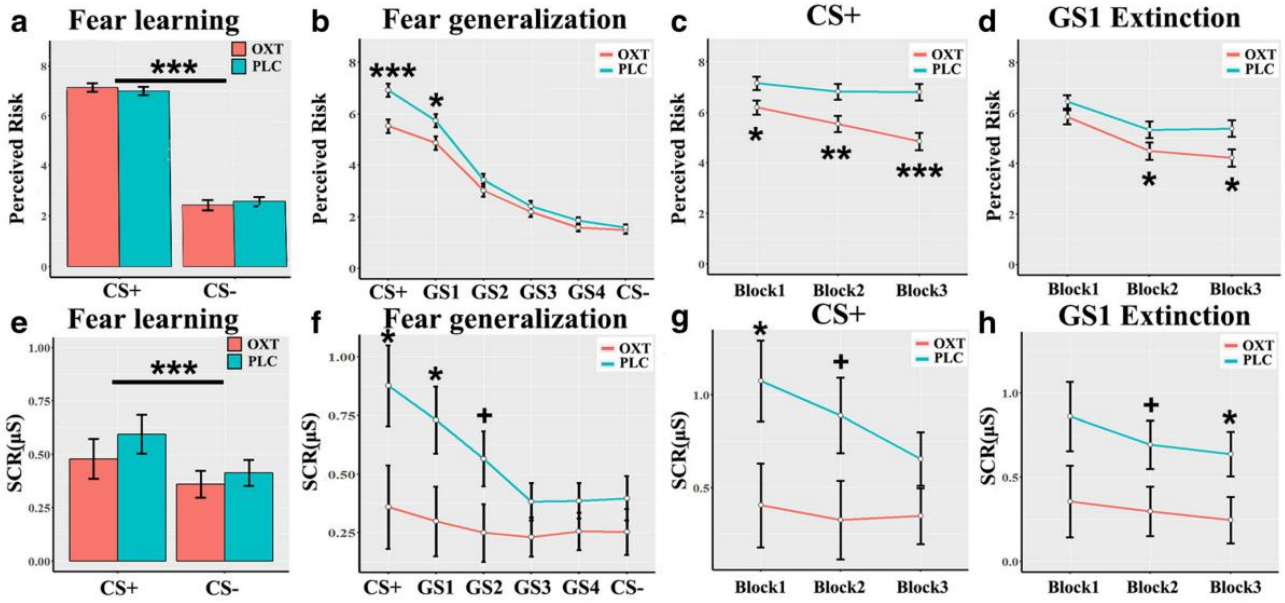

Figure 2. Threat rating and SCR during fear learning and generalization: A: The CS+ showed a significantly larger perceived risk than CS- ($p < 0.001$) in the fear learning stage for both, face and circle materials. No significant differences in perceived risk were observed between the oxytocin and placebo group. B: The participants in the placebo group exhibited a higher perceived risk compared to the participants in the oxytocin group for the CS+ ($p < 0.001$)and GS1 ($p = 0.023$) in fear generalization phase for both face and circle materials. C: During extinction of fear generalization, the placebo group reported a higher perceived risk for the CS+ in block1 ($p = 0.014$), block2 ($p = 0.006$), block3 ($p < 0.001$) compared to the oxytocin group. D: During extinction of fear generalization, the placebo group rated the perceived risk of the GS1 in block2 ($p = 0.072$), block3 ($p = 0.019$) higher than compared to the oxytocin group. E: The SCR of CS+ was larger than that of CS- in fear learning ($p < 0.001$) for both face and circle. And there was no significant difference between the oxytocin and placebo groups. F: The SCR of CS+ ($p = 0.041$), GS1 ($p = 0.041$) in placebo group was significantly higher than that in oxytocin group, and GS2 showed a marginal significant difference ($p = 0.067$) for both face and circle materials. G: During extinction of fear generalization, the SCR of CS+ in block1 ($p = 0.011$), block2 ($p = 0.059$) was higher in the placebo group as compared to the oxytocin group. H: During extinction of fear generalization, the placebo group exhibited a higher SCR for GS1 in block2 ($p = 0.059$) and block3 ($p = 0.045$) compared to the oxytocin group.

SCR data during fear learning

A significant main effect of stimuli ($F(1, 60) = 14.529$, $p < 0.001$, $\eta_p^2 = 0.195$) was observed, but no main effect of group ($F(1, 60) = 0.679$, $p = 0.413$, $\eta_p^2 = 0.011$) or stimuli x group interaction effect ($F(1, 60) = 0.626$, $p = 0.432$, $\eta_p^2 = 0.010$). With regard to the SCRs, results of stimuli main effect indicated that CS+ was significantly higher than CS-. However, no significant results for material ($F(1, 60) = 1.506$, $p = 0.225$, $\eta_p^2 = 0.024$), material x group interaction ($F(1, 60) = 0.011$, $p = 0.918$, $\eta_p^2 = 0.000$), material x stimuli interaction ($F(1, 60) = 1.019$, $p = 0.317$, $\eta_p^2 = 0.017$), and material x stimuli x group interaction ($F(1, 60) = 0.017$, $p = 0.898$, $\eta_p^2 = 0.000$) were observed (see Figure 2E).

SCR data during fear generalization

Examination of the SCR data during fear generalization revealed a marginal significant effects of group ($F(1, 60) = 3.276$, $p = 0.075$, $\eta_p^2 = 0.052$). The SCR in OT group was considerably lower than that in placebo group. An interaction effect of generalization stimulus and group was significant ($F(2.1, 127) = 4.412$, $p = 0.013$, $\eta_p^2 = 0.069$). Results from simple effect analyses indicated that SCR of CS+ and GS1 in the OT group were lower as compared to the placebo group (CS+: $F(1, 60) = 4.351$, $p = 0.041$, $\eta_p^2 = 0.068$; GS1: $F(1, 60) = 4.380$, $p = 0.041$, $\eta_p^2 = 0.068$). SCR of GS3 was marginally significant different between the two groups ($F(1, 60) = 3.490$, $p = 0.067$, $\eta_p^2 = 0.055$), with the OT group exhibiting lower score as compared to PLC. However, no significant effect of materials ($F(1, 60) = 0.099$, $p = 0.754$, $\eta_p^2 = 0.002$), material x group interaction ($F(1, 60) = 0.033$, $p = 0.857$, $\eta_p^2 = 0.001$), material x generalization stimulus interaction ($F(3.4, 205) = 0.990$, $p = 0.424$, $\eta_p^2 = 0.016$), and material x generalization stimuli x group interaction ($F(3.4, 205) = 1.300$, $p = 0.274$, $\eta_p^2 = 0.021$) was observed (see Figure 2F).

To determine treatment effects on extinction of fear generalization each generalization stimulus was segmented into three parts (Block1, Block2, Block3) based on the presentation time frame. The main effect of time was marginal significant on CS+ ($F(1.7, 101) = 2.667$, $p = 0.084$, $\eta_p^2 = 0.043$). A significant main effect of group on the CS+ ($F(1, 60) = 4.276$, $p = 0.043$, $\eta_p^2 = 0.067$) was also observed. Although the time x stimuli interaction effect was not significant ($F(1.7, 101) = 1.623$, $p = 0.202$, $\eta_p^2 = 0.026$)), results of an exploratory simple effect analysis indicated that CS+ in OT showed a lower SCR than that in the placebo group in Block1 ($F(1, 60) = 6.910$, $p = 0.011$, $\eta_p^2 = 0.102$), and a marginal significant lower SCR than that in PLC in Block2 ($F(1, 60) = 3.707$, $p = 0.059$, $\eta_p^2 = 0.058$) (Figure 2G). In GS1, the main effect of group was significant ($F(1, 60) = 4.380$, $p = 0.41$, $\eta_p^2 = 0.068$). But we did not find the significant main effect of time ($F(1.7, 100) = 1.452$, $p = 0.239$, $\eta_p^2 = 0.024$). Although the time x group interaction effect of SCR in GS1 was non-significant ($F(1.7, 100) = 0.207$, $p = 0.772$, $\eta_p^2 = 0.003$), results of simple effect analysis revealed that GS1 in OT showed a lower SCR than that PLC in Block3 ($F(1, 60) = 4.182$, $p = 0.045$, $\eta_p^2 = 0.65$), a marginal significant lower SCR than that in PLC in Block2 ($F(1, 60) = 3.711$, $p = 0.059$, $\eta_p^2 = 0.058$) (see Figure 2H).

Discrimination threshold

The FCDT was conducted before and after the experiment to explore whether OT influenced discrimination thresholds. We adopted 2 materials (face, square) x 2time (pretest, posttest) x 2group (OT, PLC) repeated-measures ANOVAs. The results suggested that the main effect of the material was significant ($F(1, 60) = 27.825$, $p < 0.001$, $\eta_p^2 = 0.317$) suggesting that the JND for face stimuli (21.6, 2.008) was considerably higher than for the non-social (circle) stimuli (10.909, 0.828). The main effect of time was also significant ($F(1, 60) = 5.988$, $p = 0.017$, $\eta_p^2 = 0.091$). The JND of the Posttest (14.842, 1.201) was much lower than that of the pretest (17.688, 1.375).

However, neither the main effect of the group ($F(1, 60) = 0.375$, $p = 0.543$, $\eta_p^2 = 0.006$) nor the group x time interaction effect ($F(1, 61) = 0.004$, $p = 0.952$, $\eta_p^2 = 0.000$) reached significance. Moreover the analysis revealed a non-significant material x group interaction effect ($F(1, 60) = 0.079$, $p = 0.780$, $\eta_p^2 = 0.001$), non-significant material x time ($F(1, 60) = 0.977$, $p = 0.327$, $\eta_p^2 = 0.0106$), and a non-significant material x time x group interaction effect ($F(1, 60) = 0.240$, $p = 0.626$, $\eta_p^2 = 0.004$) for the JND (see Figure 3B).

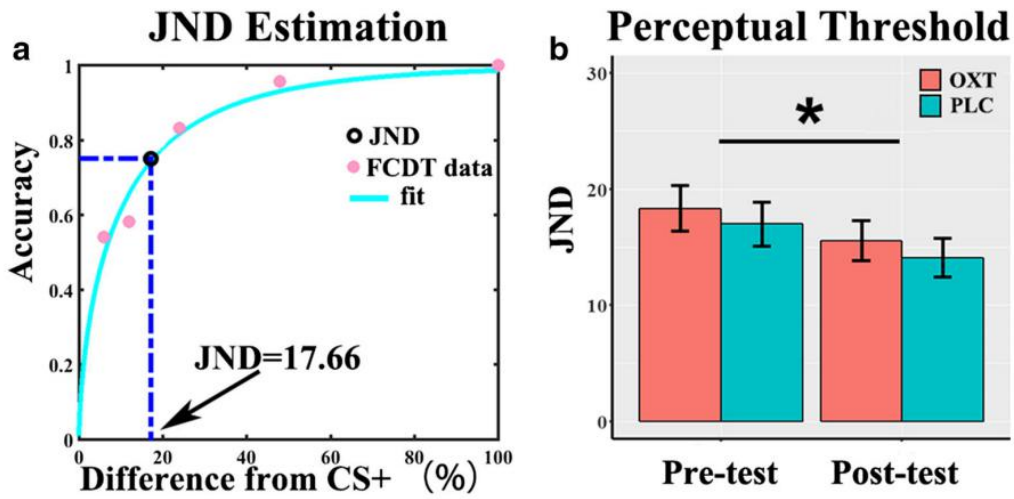

Figure 3. Perceptual threshold: A: A example of JND estimation. The pink dots represent the accuracy of the different type of stimuli in the FCDT. We fitted the data with Weibull function and choose 75% accuracy as the JND value. For example, this participant's JND is 17.66, which means that when a stimulus was <17.66% different from stimulus A, the participant cannot distinguish the stimuli (black circle represented JND). B: The perceptual threshold of the pre-test in both face and square materials were much higher than that of the post-test ($p = 0.017$). There was no significant difference between the oxytocin and placebo group ($p = 0.543$).

Emotional ratings of the generalization stimuli

Valence rating

For valence rating after fear learning, a significant main effect of stimuli was observed ($F(1, 60) = 97.211$, $p < 0.001$, $\eta_p^2 = 0.618$). The valence of the CS+ was significantly lower than that of the CS-. A significant main effect of material was also observed ($F(1, 60) = 7.728$, $p = 0.007$, $\eta_p^2 = 0.114$). The valence of the facial stimuli was much lower than that of the circle stimuli. Furthermore, a non-significant main effect of group ($F(1, 60) = 0.479$, $p = 0.492$, $\eta_p^2 = 0.008$), non-significant stimuli x group interaction effect ($F(1, 60) = 0.967$, $p = 0.329$, $\eta_p^2 = 0.016$), non-significant material x stimuli interaction effect ($F(1, 60) = 0.304$, $p = 0.583$, $\eta_p^2 = 0.005$), and non-significant the material x group x stimuli interaction effect ($F(1, 60) = 1.600$, $p = 0.211$, $\eta_p^2 = 0.026$) were observed in the valence rating following the fear learning phase. The results of valence score after the fear learning are displayed in the Figure 4A.

For the valence rating after fear generalization a significant main effect of the stimuli was observed ($F(2.3,135) = 50.368$, $p < 0.001$, $\eta_p^2 = 0.456$). The interaction effect of the material x stimuli was significant ($F(4.2, 251) = 5.136$, $p < 0.001$, $\eta_p^2 = 0.079$); results of simple effect showed that the valence scores of CS+ ($F(1, 60) = 12.824$, $p = 0.001$, $\eta_p^2 = 0.177$) and GS1($F(1, 60) = 9.326$, $p = 0.003$, $\eta_p^2 = 0.135$) in the facial stimuli were much lower than that in the circle stimuli. However, the main effect of the group ($F(1, 61) = 0.279$, $p = 0.599$, $\eta_p^2 = 0.005$), the interaction effect of material x group ($F(1, 60) = 0.989$, $p = 0.324$, $\eta_p^2 = 0.016$), and the material x stimuli x group interaction effect ($F(4.2, 251) = 1.806$, $p = 0.125$, $\eta_p^2 = 0.029$) did not reach statistical significance (see Figure 4B).

Arousal rating

For the arousal rating after fear learning, the main effect of the stimuli was significant ($F(1, 60) = 46.665$, $p < 0.001$, $\eta_p^2 = 0.437$); indicating that the arousal of CS+ was higher than the arousal of CS-. A non-significant main effect of group was observed ($F(1, 60) = 1.079$, $p = 0.303$, $\eta_p^2 = 0.018$). Likewise, a non-significant group x stimuli interaction effect ($F(1, 60) = 0.081$, $p = 0.777$, $\eta_p^2 = 0.001$), a non-significant group x material interaction effect ($F(1, 60) = 0.060$, $p = 0.808$, $\eta_p^2 = 0.001$), and a non-significant group x stimuli x material interaction effect ($F(1, 60) = 1.156$, $p = 0.287$, $\eta_p^2 = 0.019$) were observed. Besides, the main effect of material ($F(1, 60) = 2.193$, $p = 0.144$, $\eta_p^2 = 0.035$) and the interaction effect of the material and stimuli ($F(1, 60) = 0.943$, $p = 0.335$, $\eta_p^2 = 0.015$) were not significant (see Figure 4C).

For the arousal rating after fear generalization, a significant main effect of the stimuli ($F(2.8, 169) = 35.973$, $p < 0.001$, $\eta_p^2 = 0.375$) was observed. The main effect of the material was also significant ($F(1, 60) = 11.566$, $p = 0.001$, $\eta_p^2 = 0.162$). The facial stimuli showed higher arousal than the circle stimuli. However, the main effect of the group ($F(1, 60) = 0.221$, $p = 0.640$, $\eta_p^2 = 0.004$), the group x stimuli interaction effect ($F(2.8, 169) = 0.633$, $p = 0.675$, $\eta_p^2 = 0.010$), the group x material interaction effect ($F(1, 60) = 0.280$, $p = 0.599$, $\eta_p^2 = 0.005$), and the group x material x stimuli interaction effect ($F(4.1, 245) = 0.470$, $p = 0.761$, $\eta_p^2 = 0.008$) were all non-significant (see Figure 4D).

Rating of Attractiveness

For the attractiveness rating after the fear learning, the main effect of the stimuli was significant ($F(1, 60) = 16.222$, $p < 0.001$, $\eta_p^2 = 0.213$); the attractiveness score of the CS- was higher than that of the CS+. A significant main effect of material ($F(1, 60) = 6.307$, $p = 0.015$, $\eta_p^2 = 0.095$) was found suggesting that the facial stimuli rated higher than the non-social (circle) stimuli. However, a non-significant main effect of the group ($F(1, 60) = 0.386$, $p = 0.537$, $\eta_p^2 = 0.006$), non-significant group x stimuli interaction effect ($F(1, 60) = 0.088$, $p = 0.768$, $\eta_p^2 = 0.001$), non-significant material x group interaction effect ($F(1, 60) = 1.431$, $p = 0.236$, $\eta_p^2 = 0.023$), non-significant material x stimuli interaction effect ($F(1, 60) = 0.696$, $p = 0.408$, $\eta_p^2 = 0.011$), and

non-significant material x stimuli x group interaction effect ($F(1, 60) = 1.599$, $p = 0.211$, $\eta_p^2 = 0.026$) were observed for the attractiveness ratings (see Figure 4E).

For the attractiveness rating after the fear generalization, the main effect of the stimuli was significant ($F(2, 124) = 6.097$, $p = 0.003$, $\eta_p^2 = 0.092$); GS1 was less attractive than GS4 ($p < 0.05$). We also found a significant interaction effect of material x stimuli ($F(3.9, 233) = 2.889$, $p = 0.024$, $\eta_p^2 = 0.046$); results of simple effect indicated that GS2 ($F(1, 60) = 4.128$, $p = 0.047$, $\eta_p^2 = 0.064$), GS3 ($F(1, 60) = 11.769$, $p = 0.001$, $\eta_p^2 = 0.164$) and GS4 ($F(1, 60) = 6.203$, $p = 0.016$, $\eta_p^2 = 0.094$) of the facial stimuli were rated as more attractive than circle stimuli. However, the main effect of material ($F(1, 60) = 0$, $p = 0.985$, $\eta_p^2 = 0.000$), the group x stimuli interaction effect ($F(2.1, 124) = 0.747$, $p = 0.480$, $\eta_p^2 = 0.012$), and the group x stimuli x material interaction effect ($F(3.9, 233) = 1.482$, $p = 0.210$, $\eta_p^2 = 0.024$) failed to reach statistical significance (see Figure 4F).

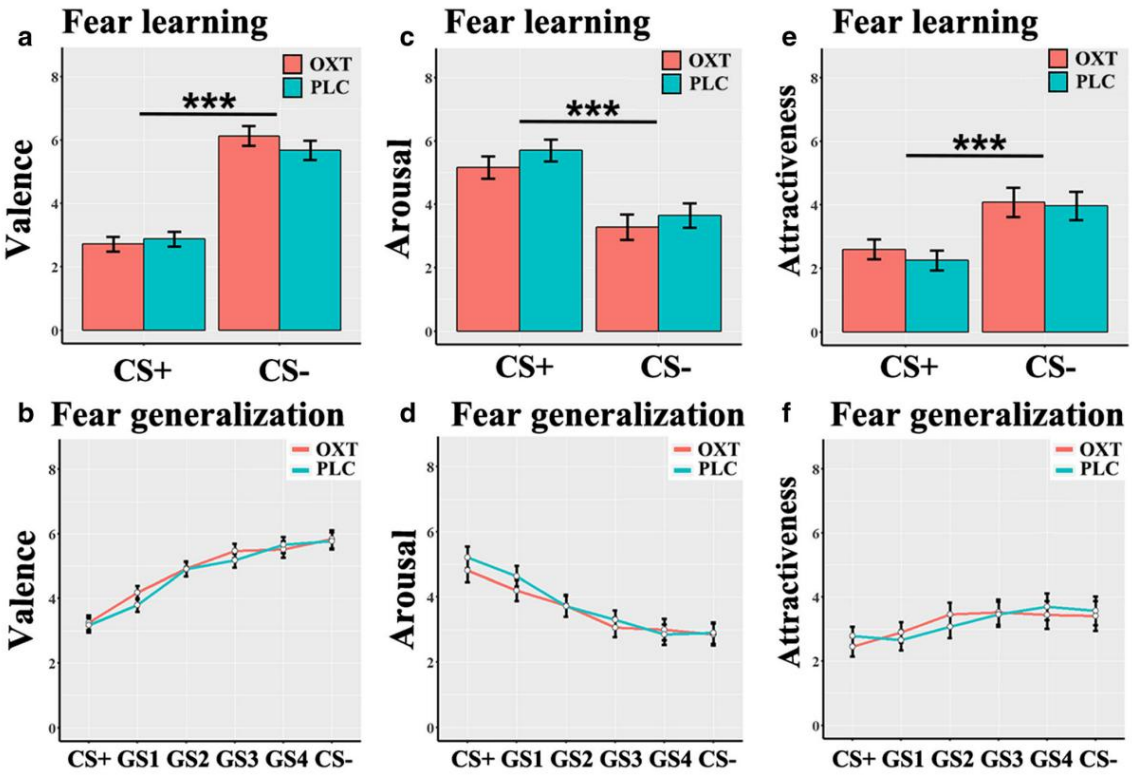

Figure 4. Results of the affective rating and attractiveness score after the fear generalization: A: The pleasure of CS+ for both face and circle materials was lower than that of CS- after fear conditioning ($p < 0.001$). No significant group effect was found. B: The main effect of stimulus was significant ($p < 0.001$) after fear generalization, but no difference were found between the oxytocin and placebo group. C: The arousal of CS+ in both face and square materials were larger than that of CS- after fear learning ($p < 0.001$). There was no significant difference between groups. D: After fear generalization, the main effect of generalization stimuli was significant ($p < 0.001$), but again no difference was found between

treatment groups. E: After fear learning, the participants rated the CS- more attractive than CS+ ($p < 0.001$). F: The main effect of attractiveness was significant ($p = 0.003$), but there was no significant difference between groups.

Discussion

The present experiment revealed the first evidence that intranasal OT has the potential to modulate fear generalization in the healthy humans. More specifically, the results of the present study suggest that during a fear generalization paradigm, intranasal OT reduced the perceived risk of CS+ and GS1 accompanied by an attenuated SCR response to both, CS+ and GS1 in the OT relative to the PLC group. Moreover, we additionally found that during the fear generalization task OT facilitated the decrease of the perceived risk of CS+ during time. Notably, the interaction of materials×group or materials × group × stimuli was not significant, suggesting that OT did not differentially modulate processing of different materials, and the observed effects of OT generalize across social and non-social contexts of fear generalization. Additionally, the discrimination threshold assessed after fear generalization was not affected by OT, suggesting that effects of OT on the discrimination threshold may not have contributed to its effects on fear generalization. Finally, no effects of OT were observed on the perceived pleasantness, arousal and attractiveness of the stimuli after fear generalization, which argues against unspecific effects of OT on stimulus perception.

We found that OT attenuated the behavioral and physiological response to CS+ and GS1 during fear generalization. These findings indicate that OT does not only reduce the response to conditioned fear stimuli but may also have the ability to modulate fear generalization. Fear learning and generalization is neurally mediated by limbic-prefrontal circuits, including the amygdala and (medial) prefrontal regions (Lopresto, Schipper, & Homberg, 2016). Previous studies reported modulatory effects of intranasal OT on these circuits, such that several previous studies observed that OT attenuated amygdala reactivity in response to threatening social stimuli, including fearful faces (Kirsch et al., 2005). Moreover, OT has been shown to attenuate amygdala threat reactivity in patients with exaggerated amygdala responsivity such that it attenuated elevated amygdala responses to fearful faces in generalized anxiety disorders (Labuschagne et al., 2010). Previous studies furthermore reported effects on ambiguous threatening faces, such that Quintana et al. (2015) reported decreased anger ratings for ambiguous faces and Spengler et al. (2017) reported that ambiguous fearful stimuli (35% emotional intensity) were judged more neutral in the context of decreased amygdala reactivity following OT. In addition, previous studies reported that OT does not only attenuate amygdala reactivity but also strengthens the connectivity of the amygdala with medial prefrontal regions engaged in emotion regulation and suppression of a previously learned fear responses (e.g. Eckstein et al., 2017).

With respect to the different pathways that may explain the effects of OT on fear generalization, we found that OT did not modulate extinction of fear generalization, but promoted the decrease of the perceived risk of CS+ in the fear generalization. In the present study the OT significantly modulated the reduction during time on the perceived risk of CS+ rather than SCR of CS+. This might be due to the different time-courses of the two fear indices and the different brain systems mediating these processes. For instance, previous studies reported that SCR responses towards threat-associated stimuli precede the conscious awareness of threat-contingency (Knight et al., 2003). Moreover, threat effects on autonomous responses including the SCR are mediated by limbic and brainstem regions (Becker et al., 2012; Mangina & Beuzeron-Mangina, 1996) while risk assessments and threat anticipation additionally require the engagement of prefrontal regions (Kirlic et al., 2017; Nitschke, Sarinopoulos, Mackiewicz, Schaefer, & Davidson, 2006). A previous study examining the effects of OT on extinction revealed a general reduction of amygdala activation, yet a threat-stimulus specific enhancement of prefrontal activation following OT (Eckstein et al., 2015) during threat extinction, suggesting differential effects on brain systems engaged in autonomous threat reactivity and risk evaluation. Moreover, it should be pointed out that in our fear generalization task, CS+ was also paired with shock with a 50% chance, whereas fear extinction procedures in other studies did not pair the CS+ with a shock during extinction. Therefore, CS+ in fear generalization phase was not the standard extinction. This reduction effect with time might due to the decrease of reinforcement rate (75% in fear learning and 50% in fear generalization). In brief, we found the main effect of time that the perceived risk of CS+ reduced with time and OT promoted this reduction effect in fear generalization phase.

Our results showed that OT did not affect discrimination thresholds. This argues against the second potential pathway via which OT may affect fear generalization, specifically that OT may decreased the discrimination threshold of the generalized stimuli and in turn attenuates fear generalization. Although several previous studies reported that OT enhanced emotion recognition in faces (Shahrestani et al., 2013) most of these studies examined motion recognition using un-ambiguous emotional faces and did not sensitively asses the threshold of discrimination using ambiguous faces. Moreover, Domes et al (2013) demonstrated that intranasal OT did not have an impact on discrimination performance in both healthy adults and individuals with autism spectrum disorder (ASD) when facial and non-social stimuli (house) were used. Together, despite the lack of apparent OT effects on the discrimination threshold we cannot fully exclude that the combination with the fear generalization task may have influenced the sensitivity to determine OT effects on the discrimination threshold and future studies are needed to confirm the lack of OT effects on the discrimination threshold.

Furthermore, the present design does not allow us to exclude alternative pathways by which OT may have affected fear generalization. For instance, ambiguity-based uncertainty played a critical role in fear generalization and might be also influenced by OT (Onat & Büchel, 2015). Several previous studies reported effects of OT on insula activation in healthy subjects, including modulated insular responses to potentially threatening stimuli (Striepens et al., 2012) and salient cues in the environment independent of social context (Yao et al., 2018a; Yao et al., 2018b). Our results did not test the value of the accurate uncertainty, but the fearful response to fear generalization was inhibited by OT. We inferred that OT might also be able to decrease the feeling of uncertainty during fear generalization. In line with these results, Spengler et al. (2017) reported that intranasal OT made the ambiguous fearful stimuli (35% emotional intensity) more neutral, which might inhibit the ambiguity-based uncertainty. However, some other researchers have found that OT enhanced startle reflex responses in the unpredictable threat condition in the conditioned fear learning task (Grillon et al., 2013). Because we did not directly assess levels of uncertainty in our study, it remains more evidence to clarify the details.

At the end of the fear learning and generalization, we tested the valence, arousal and attractiveness of each stimulus. We found that no effect of OT on the ratings of valence, arousal and attractiveness were observed, arguing against the notion that unspecific effects of OT on stimulus perception may have affected effects on fear generalization. Alternatively, the lack of effects might be due to the fear generalization paradigm that contained several trials and took nearly 20 minutes. The emotional rating was after repeated presentation of the stimuli during the fear generalization task. Although the reinforcement rate of CS+ in the fear generalization was 50%, we still found the reduction of perceived risk of CS+ with time in fear generalization stage. We inferred that no OT effect in the emotional rating after the fear generalization was due to the this decrease with time. Of course, another possiblility was due to the distinguishing of physiological reactions and subjective emotional feeling after the fear generalization, which required more evidence. Besides, in this experiment, we found no significant effect between the social stimuli and non-social stimuli. Although previous studies reported that oxytocin specifically modulated processing of social information (face stimulus,) (Gorka et al., 2015; Xu et al., 2019), studies in animals and healthy humans also reported that OT affected basal emotional processes, including fear extinction, independent of social context (Eckstein et al., 2015; Onaka et al., 2012; Yao et al., 2018a; Yoshida et al., 2009). Therefore, our results indicated that OT modulates fear generalization function in healthy human across social and non-social stimuli.

Our research provides the first preclinical evidence for a potential of OT to modulate fear generalization in humans. Our data resonates with previous findings in animals and humans suggesting a role of OT on threat and stress-related processes including

fear-related learning. Overgeneralization is not only the important symptom of GAD (Lissek et al., 2014; Greenberg et al., 2013b), but also fear memory-related disorders, such as panic disorder (Lissek et al., 2009) and Post-Traumatic Stress Disorder (Kaczkurkin et al., 2017). Therefore, the present results suggest a potential therapeutic application of OT in disorders associated with dysregulated fear generalization.

The present study has some limitations. First of all, we recorded only behavioral data and physiological data without any functional neuroimaging data and therefore the neural mechanism underlying the effects of OT on fear generalization in human remain to be determined. Furthermore, future research employing clinical trial designs are needed to determine the therapeutic potential effect of intranasal oxytocin in anxiety disorders. More specifically, overgeneralization showed an abnormal gradient in fear generalization, especially less degradation of fear response to GS, which played an important role in many anxiety disorder (Laufer, Israeli, & Paz, 2016; Lissek et al., 2009; Lissek et al., 2014). OT may be able to recover the over intensity of fear response to GS by inhibiting the activity of amygdala. In addition, in this experiment, in order to rule out the extra variable evoked by female menstrual cycle and account for previously reported sex-differences in the effects of OT, we only recruited male adults. Accumulating evidence suggests that OT may exert different or even opposing effects in men and women during early social threat perception (Luo et al., 2017) as well as social evaluation and interaction (Ma et al., 2018; Gao et al., 2016). Besides, the present study was not pre-registered. Pre-registration is a good method to improve the quality of the study by controlling for publication bias and selective reporting and future studies can use this method.

Conclusion

The present study found that OT decreases fear generalization but did not affect the discriminate threshold. This research helps to elucidate novel evidence on the effects of OT on fear generalization and suggest that OT may have beneficial effects in disorders with dysregulated fear generalization and exaggerated fear reactivity.

Acknowledgments

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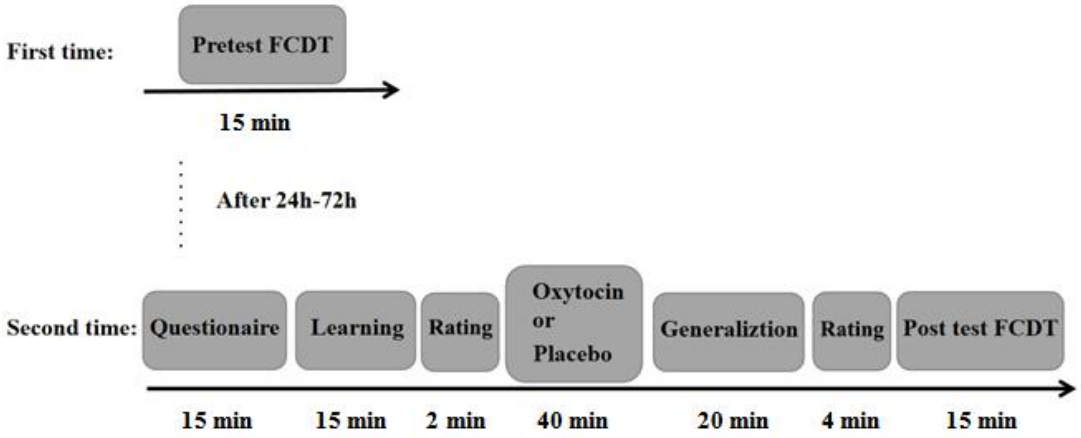
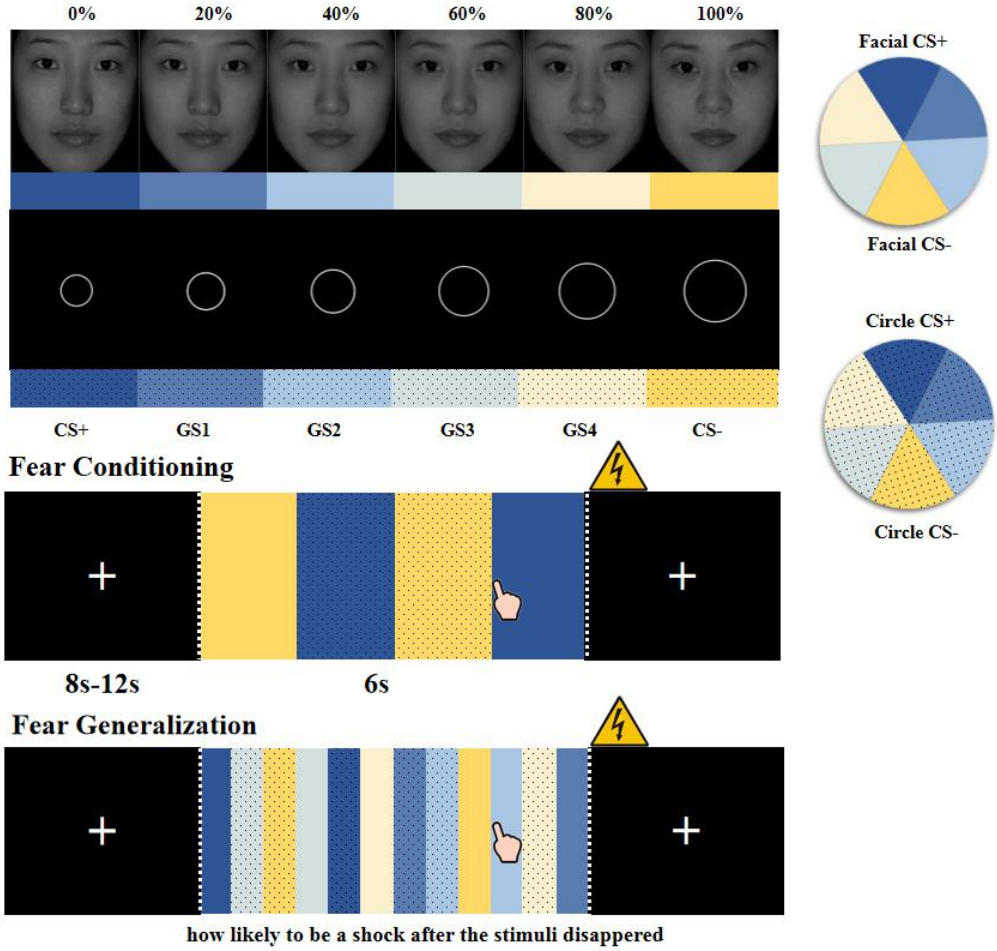
Supplementary Materials

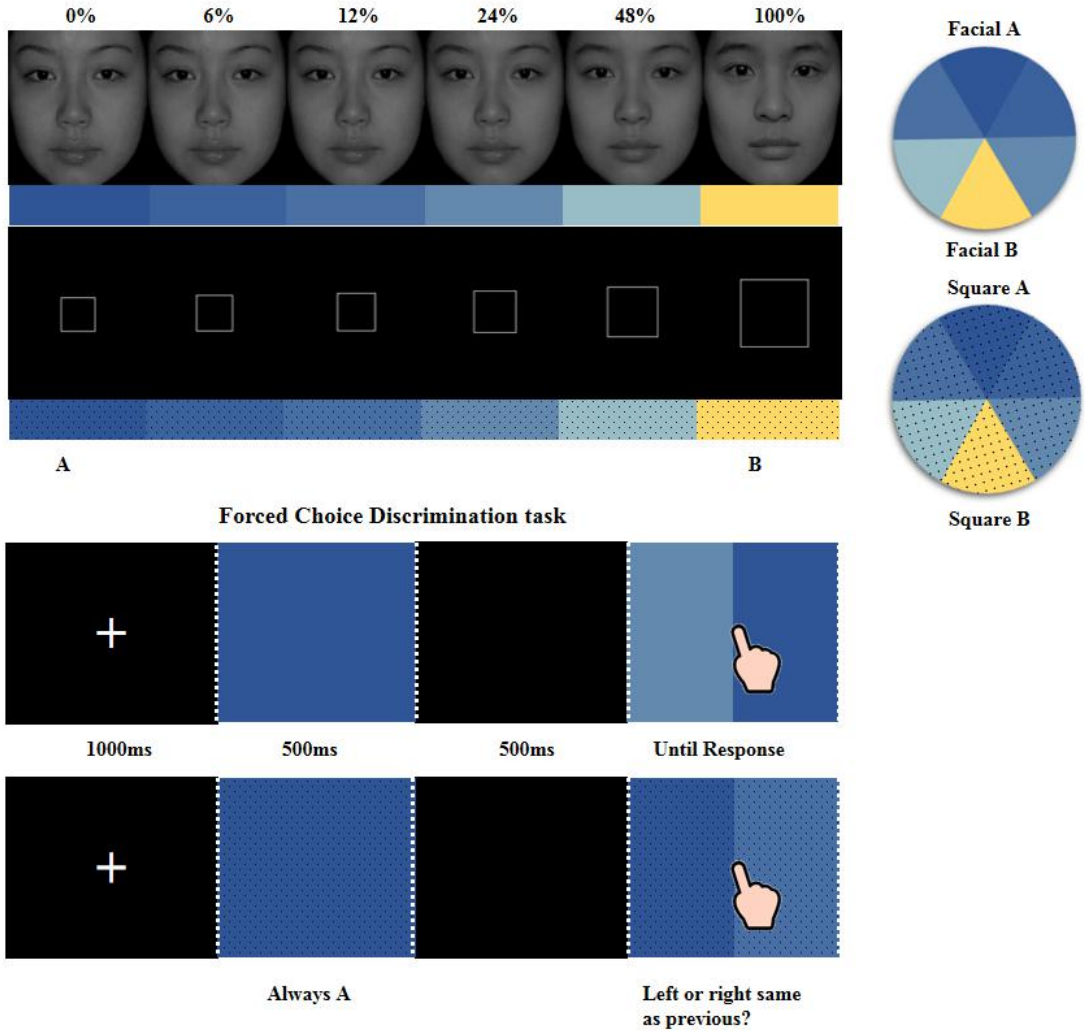


Supplementary figure 1. Experimental procedures: The participants came the lab twice. During the first visit they underwent a 15min Forced Choice Discrimination Task (FCDT). In order to decrease the effect of FCDT on fear learning and generalization, they were asked to come back after an interval of 24h-72h. During the second visit participants completed 15min questionnaires and next underwent the fear learning task. After the learning task, the intranasal treatment (oxytocin or placebo) was administered. 40min after treatment subjects performed the fear generalization task followed by the FCDT.



Supplementary figure 2. Materials and procedure in fear learning and generalization task.1

Materials: The social materials were 2 faces (CS+ and CS-) and 4 facial morphs (GS1-4) with the GS most similar to the CS+ referred to as GS1 and the GS most similar to the CS− referred to as GS4. The different color gradient from blue to yellow represented the facial CS+ to CS-. The non-social stimuli included 6 circles of varying sizes. Conditioned and generalization stimuli were circles of different sizes. The smallest ring was 2 inches. Circles increased successively in size by 20%. The different color gradient with black dot from blue to yellow represented the circle CS+ to CS-. Procedure: In the fear conditioning stage, a 8s-12s fixation cross was shown followed by a 6s conditioned stimulus(Facial CS+ or CS-, circle CS+ or CS-). During the presentation of the conditioned stimulus, the participants were asked to indicate ”how likely is the shock after the stimulus disappeared” indicated on 1-9 rating scale (1:no chance-9:very likely). When the CS+ disappeared, there was 75% chance of a 50ms shock. During fear generalization, participants were presented a 8s-12s fixation cross, followed by the conditioned stimulus or generalization stimuli presented for 6seconds. The participants were asked to answer the same question as in the fear learning stage. In 50% of the trials the CS+ was followed by a 50ms shock.



Supplementary figure 3. Materials and procedure in the Forced Choice Discrimination Task: Materials: The facial materials in the FCDT were two neutral female faces (Face A, B) selected from CAFS and their facial morphs. The facial morphs were created by using face A and face B. For example, 6% facial morph represents a combination of 6% face B and 94% face A. The

colorful rectangle below represented different stimuli (blue represented face A, yellow represented face B). The nonsocial stimuli were squares with gradually changing sizes. The diameter of the smallest square was 2 inch. The blue dot below represents square A and the dot yellow represented square B. Procedure: An 1000ms fixation cross was displayed on the screen, followed by the stimuli A (face A or square A). A 500ms black blank screen followed the stimulus A and was followed by stimulus A and another stimulus (6%, 12%, 24%, 48%, stimulus B) randomly presented on both sides of the screen. Next the participants selected the one was the same as previous figure via button press (left one press “F”, and right one press ”J”). After the response the next trial started.

A Posteriori Power calculation

we calculated the power of the interaction effect between fear generalization stimuli and group for both behavioral and SCR results. We adopted the post hoc function in G-power version 3.1.9.4 (Faul et al., 2009) and we found the power of interaction effect between stimuli and group in perceived risk was 0.8446 (correlation among repetition measures = 0.43, Effect size f = 0.2679). The power in SCR results was 0.9999 (correlation among repetition measures = 0.87, Effect size f = 0.2722). In addition, we also calculated the Bayesian factors for the interaction effect based on the current sample size and effect size (Wagenmakers et al., 2018). We found the BF10 of interaction effect between stimuli and group in perceived risk was 22.67 and in SCR was 200.08. The posteriori power calculation indicated that the power of our main results were sufficient.