JYU DISSERTATIONS 493

Haoran Dou

Effects of Social Exclusion, Observational Learning, and Oxytocin on Fear Learning and Generalization

Evidence from Behavioral and Brain Activity Studies

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF EDUCATION AND PSYCHOLOGY

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ABSTRACT

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In fear learning, a neutral stimulus is associated to a threat. In fear generalization, fear responses are transferred to stimuli resembling threat stimuli. Here, I investigate how social exclusion, observational fear learning, and oxytocin—a hormone produced in social bonding—affect fear learning and generalization. An associative learning paradigm in which participants were learning associations between conditioned stimuli (i.e., geometric figures or pictures of faces) and unconditioned stimuli (i.e., electric shock) was applied together with a fear generalization procedure. **Study I** explored whether social exclusion, which was experimentally induced before fear learning, influences fear learning and generalization. Behavioral responses showed that social exclusion reduced fear learning and increased generalization. Brain functional near-infrared spectroscopy measurements indicated that the medial prefrontal cortex mediated the relationship between social exclusion and fear learning. **Study II** examined whether observational fear learning affects fear generalization differently than direct learning. The behavioral findings indicated that compared to direct learning, observational learning reduced fear learning and increased fear responses to generalization stimuli resembling the safety stimulus without having ever been paired to electric shock. Brain event-related potential results indicated that fear discrimination (reflected by the P1 component) and motivated attention (reflected by the late positive potential component) to generalization stimuli were reduced when learning occurred by observing another person compared to situations where participants learned by themselves. **Study III** investigated how oxytocin administrated after fear learning affects the fear responses to the generalization stimuli. The results showed that oxytocin compared to placebo attenuated generalization. Overall, social exclusion and observational learning increased fear generalization, while oxytocin had the opposite effect. These results are relevant in understanding social and hormonal factors underlying excessive fear generalization and they can be utilized in future studies aiming to overcome problems related to overgeneralized fear.

Keywords: Fear generalization, social exclusion, oxytocin, observational fear learning

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Dou, Haoran
Sosiaalisen syrjinnän, sosiaalisen oppimisen ja oksitosiinin vaikutukset pelon oppimiseen ja yleistymiseen: Todisteita käyttäytymis- ja aivoaktiivisuustutkimuksista
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Pelon oppimisessa neutraali ärsyke opitaan yhdistämään uhkaan. Pelon yleistymisessä taas pelkoreaktiot siirtyvät ärsykkeisiin, jotka muistuttavat uhkaavia ärsykkeitä. Tutkin kuinka sosiaalinen syrjintä, sosiaalinen pelon oppiminen ja oksitosiini - hormooni, jota erittyy sosiaalisten siteiden muodostuessa - vaikuttavat pelon oppimiseen ja yleistymiseen. Tutkimuksissa käytettiin assosiatiivisen oppimisen koeasetelmaa, jossa tutkittavat opettelivat assosiaatioita ehdollisen ärsykkeen (geometrinen kuvio tai kasvokuva) ja ehdottoman ärsykkeen (sähköisku) välillä. Lisäksi käytettiin pelon yleistymisen tutkimusasetelmaa. **Osatutkimus I** selvitti kuinka ennen pelon oppimista kokeellisesti tuotettu sosiaalinen syrjintä vaikuttaa pelon oppimiseen ja yleistymiseen. Käyttäytymisvasteet osoittivat, että sosiaalinen syrjintä heikensi pelon oppimista ja tehosti pelkoreaktioita yleistymisärsykkeisiin. Aivojen toiminnalliset lähi-infrapunaspektroskopiamittaukset osoittivat, että mediaalinen prefrontaalinen aivokuori välitti sosiaalisen syrjinnän vaikutusta pelon oppimiseen. **Osatutkimus II** selvitti vaikuttaako sosiaalinen pelon oppiminen pelon yleistymiseen eri tavalla kuin suora oppiminen. Käyttäytymisvastetulokset osoittivat, että suoraan oppimiseen verrattuna sosiaalinen pelon oppiminen heikensi pelon oppimista ja lisäsi pelkoreaktioita yleistymisärsykkeisiin, jotka olivat samankaltaisia kuin turvallinen ärsyke, jota ei ollut aiemmin yhdistetty sähköiskuun. Aivojen sähköiset jännitevasteet osoittivat, että pelon erottelu ja motivoitunut tarkkaavuus ärsykkeisiin olivat vähentyneet kun pelon oppiminen tapahtui toista ihmistä havainnoimalla verrattuna itse opittuun pelkoon. **Osatutkimus III** selvitti kuinka pelon oppimisen jälkeen annosteltu oksitosiini vaikuttaa yleistymisärsykkeisiin liittyviin pelkoreaktioihin. Tulokset osoittivat, että oksitosiini vaimensi pelon yleistymistä verrattuna plaseboon. Kokonaisuudessaan tutkimuksen tulokset osoittivat, että sosiaalinen syrjintä ja sosiaalinen oppiminen lisäävät pelon yleistymistä ja oksitosiinilla on päinvastainen vaikutus. Nämä tulokset lisäävät ymmärrystä sosiaalisten ja hormonaalisten tekijöiden vaikutuksesta liialliseen pelkojen yleistymiseen ja niitä voidaan hyödyntään tulevissa tutkimuksissa, joissa pyritään löytämään menetelmiä liiallisen pelon yleistymisen voittamiseksi.

Avainsanat: pelon yleistyminen, sosiaalinen hyljeksintä, oksitosiini, sosiaalinen pelon oppiminen

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With the help of suggestions and comments from the coauthors, the author contributed to all the studies as follows: the author formulated the research questions, designed the experiments, collected and analyzed the data, and wrote the manuscripts.

FIGURES

TABLE

CONTENTS

1 INTRODUCTION

According to Darwin's theory of evolution, learning is regarded as an individual organism's adaptation to its environment (Darwin, 1859; Skinner, 1984). There are many threats in natural environments, but they are usually indicated by subtle cues. Thus, learning associations between environmental cues and threats is crucial for survival. For instance, acquiring the associations of faint sounds or odors with fierce predators is beneficial to avoid confronting those predators (Öhman & Mineka, 2001).

This establishment of the relationship between two stimuli (e.g., faint sounds and approaching predator) is known as associative learning (Pavlov, 1927). Learning the associations between innocuous stimuli and aversive stimuli is termed fear learning (Watson & Rayner, 1920). After fear learning, fear responses are limited not only to the specific stimuli that have already established associations to aversive stimuli, but also to stimuli that resemble the aversive stimuli. This phenomenon that fear responses develop to stimuli that are similar to previously experienced events is regarded as fear generalization (Hovland, 1937). Fear generalization provides adaptive functions that allow animals and humans to produce appropriate defensive responses to novel stimuli that can be harmful. However, if fear generalization is unrestrained, fear responses can transmit too extensively and lead to extensive fear and even to some anxiety disorders (Lissek et al., 2008).

The clinical significance of fear learning and generalization has received a large amount of attention (for reviews, see Duits et al., 2015; Dymond et al., 2015). Fear learning in anxiety disorders has been studied with laboratory measures for nearly a century (Davey, 1992; Duits et al., 2015; Lissek et al., 2005; Pavlov, 1927; Watson & Rayner, 1920). According to meta-analyses investigating studies conducted between the 1920s to 2015 on fear learning in anxiety disorders, anxiety patients exhibit enhanced fear responses to safety stimuli (stimuli that are never paired with aversive stimuli) in fear learning (Duits et al., 2015; Lissek et al., 2005). The implications of fear generalization in anxiety disorders were mostly overlooked from the 1920s to the 2000s, but the clinical significance of fear generalization has drawn renewed attention over the last 15 years (Dymond et al., 2015; Lissek et al., 2008). Namely, the

overgeneralization of fear is a common finding in disorders where anxiety plays a key role, particularly in generalized anxiety disorder (Lissek et al., 2014; Greenberg et al., 2013b), panic disorder (Lissek et al., 2009), and post-traumatic stress disorder (Kaczkurkin et al., 2017).

Due to the clinical significance of fear learning and generalization, it is meaningful to explore the factors influencing them. In the last century, researchers focused on the establishment of basic laws in fear learning and generalization (Hovland, 1937; Kehoe & Gormezano, 1980; Lashley & Wade; 1946; Pavlov, 1927; Schlosberg, 1937; Slivinske & Hall, 1960). Recently, researchers have begun to consider the effects of social aspects on fear learning and generalization. For instance, social factors, such as close social relationships (see the review, Gunnar & Sullivan, 2017), threatening social contexts (Karos et al., 2015), and social groups from different races (Olsson et al., 2005), have received increasing attention in research. However, our knowledge is still limited regarding the influences of some social factors and hormonal factors related to social behavior. To this end, this thesis focuses on the effects of social exclusion, observational learning, and oxytocin on fear learning and generalization.

1.1 Concepts of fear learning and generalization

Ivan Pavlov put forward the concept of "conditioned reflex" based on a series of studies on dogs in 1927. A method to induce associative learning therefore carries his name: Pavlovian conditioning. After a century, the behavioral and neural mechanisms of Pavlovian conditioning have been explored in various species (Haaker et al., 2019; Kalin et al., 1996; MacQueen et al., 1989; Shelton et al., 1996).

Fear learning is a special kind of classical Pavlovian conditioning and has received much attention in research conducted on humans (LeDoux, 2000). One early and influential study by Watson and Rayner (1920) applied a case study to investigate fear conditioning. Specifically, they conducted a fear conditioning experiment with a healthy nine-month-old infant named Albert B. When Albert saw a white rat for the first time, he was curious about it. Then, conditioning was performed by making a loud noise every time after the rat was given to Albert. He was naturally frightened because of the noise. After the conditioning, Albert showed an avoidance behavior when the experimenter presented him with the white rat without the loud noise. In terms of conditioning, the conditioned stimulus (CS) is neutral (in Albert's case, a white rat), and the unconditioned stimulus (US; in Albert's case, a loud noise) naturally elicits a response. The response to a US is regarded as an unconditioned response (UR), and the learned association leads to a conditioned response (CR) to CS. Alternatively, the CR disappears gradually when the CS is no longer paired with the US, which is termed fear extinction (Pavlov, 1927).

Fear responses to a CS can transfer to other stimuli. For example, Watson and Rayner (1920) found that the CR to the rat could transfer to other animals or objects with fur, such as rabbits or even to fur coats. This phenomenon, the transfer of the conditioned response of a specific stimulus to another similar stimulus, is regarded as "stimulus generalization" (Pavlov, 1927). Fear generalization is a special kind of stimulus generalization and the US of fear generalization is aversive stimuli (e.g., shock, white noises) (e.g., Dymond et al., 2015) rather than appetite stimuli (e.g., food, monetary reward) (e.g., Ayres & Cihak, 2010; Hearst 1962; Laufer et al., 2016; Zhang et al., 2014). Pavlov (1927) also noted the stimulus generalization phenomenon in his study.

Hovland (1937) conducted the first experiment of fear generalization in humans. There were two phases (the acquisition phase and the generalization phase) in Hovland's experiment. In the acquisition phase, a tone (CS+) was paired with a weak shock to the participant's wrist several times. In the generalization phase, there were four tones, one of which was used as CS+ in fear learning and the other three tones were similar, but not identical, to the CS+ with increasing extent (generalization stimuli, [GSs]). Hovland found that fear responses to the generalization stimuli increased along with the stimulus's similarity to the CS+, which is termed "a generalization gradient."

1.2 Discrimination and stimulus generalization

Since the discovery of stimulus generalization by Pavlov (1927), there has been a long-term debate on why stimulus generalization occurs (Guttman & Kalish, 1956; Hovland, 1937; Lashley & Wade; 1946; Slivinske & Hall, 1960; Struyf et al., 2015). One view is that generalization is based on the stimulus similarity and that generalization stimuli evoke a generalization gradient (Pavlov, 1927). This stimulus similarity perspective was supported by Hovland (1937) and he tested participants' discrimination threshold (the smallest difference in the stimuli a person can successfully detect; just noticeable difference) for the generalization stimuli and found that the participants were able to differentiate the generalization stimuli (Hovland, 1937). The discriminated generalization stimuli still evoked a generalization gradient based on the stimulus similarity, which supports the stimulus similarity perspective. This view has also been supported by evidence from an animal study (Guttman & Kalish, 1956). Similarly, evidence from Roesmann et al. (2020) supported the stimulus similarity perspective that generalization was not a mere failure of discrimination. They adopted an implicit fear generalization task in which faces were applied as stimuli. The faces applied as GSs were similar to the CS+ in their facial features, but the faces of the GSs and CS+ were different in their identities. Roesmann et al. (2020) found that the participants showed fear responses to the GSs even if they were able to discriminate between them.

However, the stimulus similarity perspective was criticized by Lashley and Wade (1946). They hold the view that "Stimulus generalization is

generalization only in the sense of failure to note distinguishing characteristics of the stimuli or to associate them with the conditioned reaction" (Lashley & Wade, 1946, p. 81). In line with this view, Slivinske and Hall (1960) tested the discrimination of the stimuli used in Hovland (1937) and found contradictorily to the original study that participants were not able to differentiate the generalization stimuli. Moreover, this view was also supported by physiological findings in the study by Holt et al. (2014). They tested the discrimination threshold through testing the just noticeable differences (JNDs) of the generalization stimuli before and after fear learning and generalization. They found that participants did not show increased skin conductance response (SCR; an effective index of sympathetic and parasympathetic activity) to the generalization stimuli that were over the discrimination thresholds. These findings were repeated by Tuominen et al. (2019). Although most of the previous evidence has supported the stimulus similarity perspective, the debate on stimulus generalization and discrimination is still ongoing.

1.3 Elements of fear learning and generalization in humans

1.3.1 Fear acquisition and generalization phases

In an acquisition phase of a laboratory experiment, participants acquire conditioned fear responses when a neutral stimulus is paired with an aversive stimulus several times. This is regarded as fear acquisition training or reinforcement (for a review, see Lonsdorf et al., 2017). As a result of fear acquisition training, the participants develop CR to CS, indicating fear learning. The reinforcement rate, the probability of the CS being paired with the US, is usually less than 100% (i.e., partial reinforcement) with the purpose of establishing an unpredictable context wherein the extinction effect is reduced (Weinstock, 1954).

There are two main types of conditioning procedures: simple conditioning and differential conditioning. The simple conditioning procedure was adopted in some early fear conditioning studies (e.g., Pavlov, 1927; Watson & Rayner; 1920). In the procedure, a neutral stimulus is paired with an aversive stimulus. In the differential conditioning procedure, there are two different neutral stimuli. One is paired with a US and is labeled as the CS+, while the other is never paired with a US and is labeled as the CS-. Previous studies have found that the simple conditioning procedure is inefficient to evoke conditioned responses to the conditioned stimuli compared with the differential conditioning procedure (Hovland, 1937), which was repeated by the following studies (Guttman & Kalish, 1956; Lee et al., 2018). Moreover, Lissek et al. (2005) recommended that it is better to use the differential conditioning procedure instead of the simple conditioning procedure because the differential conditioning procedure is able to measure the fear responses to the CS-. Moreover, the differential conditioning procedure allows researchers to compare the fear excitability to the CS+ and the fear inhibition to the CS- (Dvir et al., 2019). When

the CS+ is presented to the participants, the fear responses are excited compared with the baseline (such as the responses to a fixation point) because the participants have learned the CS+ (paired with an aversive US) is a threatening stimulus. When the CS- is presented to the participants, the fear responses are inhibited compared with the baseline because the participants have learned the CS- (never paired with an aversive US) is a safety stimulus (Lissek et al., 2005). The differential learning can be calculated as the CR to the CS+ minus the CR to the CS- (CS+ minus CS-) (Duit et al., 2015). Perhaps for this reason, in the research in humans, most of the fear conditioning studies have used the differential conditioning procedure, while the simple conditioning procedure is frequently adopted in animal studies (for a review, see Lonsdorf et al., 2017).

Studies on generalization have investigated whether conditioned responses to the CS+ transform to the GS, which would indicate fear generalization. In generalization, the GSs, including the CSs applied in the acquisition (CS+, and possibly CS-) are presented to participants. The fear responses to the generalization stimuli form a generalization gradient (Lissek et al., 2008). The generalization gradient is often based on perceptual similarity (Hovland, 1937, Lissek et al., 2008). Recently, a generalization gradient based on non-perceptual similarities (such as conceptual similarities) has also been found in some studies (see the review by Dunsmoor & Murphy, 2015). For example, Dunsmoor et al. (2011b) found that conceptually related stimulus pairs (e.g. a spider and a spider web) transfer fear responses more than conceptually unrelated stimulus pairs (e.g. a spider and a trash can). Since fear generalization based on perceptual similarity has been documented in a large number of studies (for a review, see Dymond et al., 2015), I focus on the generalization based on perceptual similarity in this thesis.

1.3.2 Materials of fear learning and generalization studies

In fear learning, various stimuli from simple figures geometric figures (Lissek et al., 2010), lights (Pine et al., 2001), and tones (Staib & Bach, 2018), to complex stimuli, such as faces (Holt et al., 2014), virtual reality contexts (Baas et al., 2004), and films (Kunze et al., 2015) have been applied. Similarly, in fear generalization, generalization stimuli are selected so that the same stimuli as those applied in the acquisition phase are presented among one or more other stimuli differing from the original ones, such as geometric figures with increasing diameters (Lissek et al., 2008), and figures with gradually changing colors (Gumbert et al., 2000; Shepard & Chang, 1963), to complex stimuli, such as facial stimuli with gradually changing identities (Ahrens et al., 2016; Onat & Büchel, 2015). Conditioned stimuli have been applied in different sensory modalities, such as in visual (Holt et al., 2014), auditory (Yang et al., 2008), or olfactory (Gottfried & Dolan, 2004) modalities. Here, visual stimuli are used as conditioned stimuli and generalization stimuli.

In addition to the conditioned stimuli used in fear learning and generalization, unconditioned stimuli used in fear learning and generalization will also be introduced here. One of the most–used US in humans is a mild electric shock

to the wrist applied with an intensity of "very annoying but not painful" (e.g., Lipp et al., 2014). White noise is also used as one of the USs in fear learning in humans with an intensity of approximately 90–110 dB (see the review, Grillon & Baas, 2003). Moreover, recently, some other aversive stimuli have also been adopted as the US in fear learning, such as pictures of screaming faces (Glenn et al., 2012), air blasts (Reichenberger et al., 2019), threatening film clips (Doronbekov et al., 2005), and aversive imagery (Mueller et al., 2019). According to the evidence in Glenn et al. (2012), an electric shock is more aversive than a screaming face in fear learning. The electric shock and white noise can both evoke aversive responses (Lissek et al., 2005), but the common usages of the shock and the white noise are different. For example, an electric shock can create a threatening context (Blanch et al., 2014; Weymar et al., 2013), whereas white noise focuses on inducing an acoustic startle reflex (Sperl et al., 2016). Considering that the electric shock is efficient to evoke an aversive response and create a threatening context (Schmitz & Grillon, 2012), the electric shock is applied as the US in fear learning and generalization experiments in this dissertation.

1.3.3 Outcome measures of fear learning and generalization

Different outcome measures have been used to investigate fear learning and generalization. Researchers usually adopt the skin conductance response (SCR) as one kind of conditioned response (Knight et al., 2006). The SCR, an enhancement of the electrical conductivity in the palm due to an improvement in eccrine sweat gland activity, is an effective method for measuring the activities of the autonomic nervous system (ANS; Mackersie & Calderon-Moultrie, 2016). Moreover, fear-potentiated startle has also been widely used as an outcome measure for fear learning and generalization (Grillon & Davis, 1997). A fear-potentiated startle reflects an increased eyeblink muscle response due to the acoustic startle reflex upon hearing a loud noise. Heart rate and pupil size have been adopted in some fear learning and generalization studies (Leuchs et al., 2017; Roy et al., 2013; Wang et al., 2021).

In addition to physiological responses, subjective behaviors, such as the US-expectancy ratings (Boddez et al., 2013), avoidance behaviors (Delgado et al., 2009), and affective ratings (Blechert et al., 2007) have been used as outcome measures for reactions to the CS. US-expectancy ratings and SCR are usually applied when the US is an electric shock (e.g., Lissek et al., 2008, 2010; Holt et al., 2014; Tuominen et al., 2019). Considering that the US is an electric shock in this dissertation, I adopted the US-expectancy ratings and SCR as the outcome measures for fear learning and generalization.

1.4 Neural basis of fear learning and generalization

In recent years, the neural mechanisms of fear learning and generalization have been investigated in many species (LeDoux, 2000). Both animal and human

studies indicate that the neural circuitry of fear learning comprises the amygdala, hippocampus, and prefrontal cortex as the key brain areas (Herry et al., 2008). The methods used on animals include electrical and chemical stimulations, in vivo single-unit recordings, permanent lesions, and targeted pharmacological inactivation of the brain (Kim & Jung, 2006). Through these methods, animal studies have defined the neural circuitry critical for fear learning. Specifically, the perceptual stimuli are transmitted from the visual area to the lateral amygdala. Through the effect of long-term potentiation, CS gradually formulates associations with US in the basal and lateral amygdala (Grewe et al., 2017; Herry et al., 2008; LeDoux, 2000; Maren, 2003). Due to these associations, the CS signals are also able to activate the central amygdala. The signals from the central amygdala are projected to the hypothalamus, the brain stem, and other brain areas related to specific fear CRs (Kim & Jung, 2006; Yehuda & LeDoux, 2007). The PFC also activates during fear learning: the connectivity between the medial prefrontal cortex (mPFC) and the amygdala is related to the differentiation between the CS+ and the CS- (Likhtik et al., 2014).

In fear generalization, the visual signals related to the perception of GS are transmitted from the retina to the thalamus and further projected to the visual cortex. The hippocampus receives the signals from the visual cortex and matches the visual signals of the GS to the visual signals of the CS+ that are already stored (Gluck & Myers, 1993). On one hand, if the visual signals of the GS match with the visual signals of the previously learned CS+, the amygdala and the insula activate and produce fear responses (Lissek et al., 2014). On the other hand, if the visual signals of the GS do not match the visual signals of the previously learned CS+, the signals are projected to the ventromedial prefrontal cortex, which inhibits fear responses (Greenberg et al., 2013a, 2013b).

Fear learning and generalization do not rely only on the amygdala, the hippocampus, and the prefrontal cortex but also on other brain areas. For instance, the feedback of the amygdala is projected to the visual area in the occipital lobe through the ventral pathway (Amaral et al., 2003). The signals of the visual area are enhanced in fear learning, and a generalized threat strengthens the visuocortical activities (Lithari et al., 2016; Stegmann et al., 2020). The signals of fear are projected from the amygdala to the area related to the expression of fear (e.g., the anterior cingulate cortex [ACC], the parietal lobe, and the insula), as shown by experiments where conditioned threat stimuli and generalized threats increase the activations of the insula, the ACC, and the parietal lobe (Knight et al., 2010).

To record the neural activities associated to fear learning and generalization in humans, several non-invasive neuroimaging methods have been applied, such as functional magnetic resonance imaging (fMRI), magnetoencephalogram (MEG), electroencephalogram (EEG), and functional near-infrared spectroscopy (fNIRS) (Lissek et al., 2014; Nelson et al., 2015; Roesmann et al., 2020; Yoshiike et al., 2018). Among these methods, MEG, fNIRS, and EEG show higher temporal resolutions and a more silent recording environment compared with fMRI. Moreover, EEG and fNIRS can be portable and are economical and easy to obtain compared with MEG (Pinti et al., 2020;

Quaresima & Ferrari, 2019). In contrast to EEG and MEG, fMRI can provide higher spatial resolution and images of subcortical brain structures.

The dissertation aims to examine the cortical responses related to fear learning and generalization. The fNIRS and EEG are sensitive to recording the cortical responses (Nishiyori, 2016; Pinti et al., 2020; Srebro, 1985), and were therefore selected for **Study I** and **Study II,** respectively (**Study III** was a behavioral study).

The methods of fNIRS and EEG are briefly introduced next. As for fNIRS, it can be used to measure the hemodynamic responses evoked by neural activities. This method adopts small light source sensors to transmit the near-infrared light to the cortex via the scalp (several centimeters deep), and other small light-detecting sensors receive the near-infrared light that has already passed through the cortex. The hemoglobin characterizes a low absorption of the near-infrared light when the wavelength is beyond 650 nm, which allows researchers to test the concentrations of oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb) in the brain through the spectroscopy method (Boas et al., 2014). EEG is a method by which to record the electrical activities of the brain. The communication between neurons relies on electrical activities. The excitatory and inhibitory postsynaptic potentials of neurons converge and produce the EEG signals, which can be recorded from the scalp. Event-related potentials (ERPs) are based on the EEG method and reflect the electrical potentials that can be revealed by averaging tens or hundreds of the EEG responses to a specific event (Luck, 2012).

1.5 Social factors and hormonal factors related to social interaction in fear learning and generalization

Human beings are social animals. In daily life, fear learning and generalization often occur in a social environment, which reminds us that fear learning and generalization might also be influenced by social factors. In line with this view, recent studies have found that some social factors could affect fear learning (Gunnar & Sullivan, 2017; Karos et al., 2015; Olsson et al., 2005). For instance, social groups also affect fear learning (Koenig et al., 2017; Olsson et al., 2005). For example, Olsson et al. (2005) found that compared with another racial group, participants established stronger associations between the aversive stimuli and the individuals from the same race group. Moreover, a previous study reported that threatening social content can modulate fear learning (Karos et al., 2015). The researchers manipulated the social contents by placing an angry or a happy face as the background in fear learning and found that the threatening social context (an angry face) increased the differentiation of the threat stimuli and the safety stimuli (Karos et al., 2015). In addition to social groups and social context, Gunnar and Sullivan proposed that close social relationships play an important role in fear learning, especially in the early years of human life (for a review, see Gunnar & Sullivan, 2017). In line with this, an

animal study reported that the appearance of the rat's mother helped the rat to discriminate between the threat stimuli and the safety stimuli in a differential conditioning procedure (van Rooij et al., 2016). Furthermore, a human study also found that the participant's social support figures (e.g. the photos of their parents) reduced the conditioned fear response in fear learning in adults (Hornstein et al., 2016). However, previous studies on social relationships have focused on the effect of positive social relationships (such as one's relationships with family and friends) on fear learning (Ferreira et al., 2019; Hornstein et al., 2016; Hornstein & Eisenberger et al., 2017; Morozov & Ito, 2019; Toumbelekis et al., 2019), but few studies have explored how negative social relationships (such as social exclusion) influence fear learning and generalization. Moreover, oxytocin, a hormonal factor related to social interaction, plays an important role in social relationships (Bartz et al., 2011; Feldman, 2012; Uvnäs-Moberg, 1998). Positive social relationships have been reported to be mediated by the oxytocin system in previous studies (Chen et al., 2011; Kreuder et al., 2019; Olff, 2012). Although oxytocin has been found to facilitate fear learning (Eckstein et al., 2016), few studies explore how oxytocin affects fear generalization. Social relationships and oxytocin have been found to affect another social factor, observational fear learning (Dore et al., 2013; Kruppa et al., 2019; Xu et al., 2019; Yusufishaq & Rosenkranz, 2013; Zayia et al., 2021). Observational fear learning refers to humans learning fear experiences from others in their society, which has been researched by previous studies (Debiec & Sullivan, 2014; Haaker et al., 2017; Kim et al., 2011; Olsson et al., 2007). Previous studies have also focused on the effect of direct fear learning on fear generalization (Lissek et al., 2008, 2010; Holt et al., 2014; Tuominen et al., 2019); however, few studies explore how observational learning affects generalization.

Next, I will introduce the relationships between three factors—social exclusion, observational learning, and oxytocin—and fear learning and generalization in more details.

1.5.1 Social exclusion, fear learning and generalization

Social exclusion is broadly defined as "the experience of being kept apart from others physically or emotionally" (Wesselmann et al., 2016, p. 5). Social exclusion usually leads to several negative outcomes, such as self-reported distress (Blackhart et al., 2007; Eisenberger et al., 2003); anxiety (Heeren et al., 2017); stress (Beekman et al., 2016; Blackhart et al., 2007); and impairments in cognition (Baumeister et al., 2002), memory (Fuhrmann et al., 2018; Xu et al., 2018), and cognitive control (Themanson et al., 2014). For instance, Blackhart et al. (2007) reported that experimentally induced social exclusion increased self-reported distress and salivary cortisol, reflecting physiological stress in the social exclusion group relative to the social inclusion and control groups.

Although it is unclear how experimentally evoked social exclusion influences fear learning at the behavioral level, it is known that brain regions activated during social exclusion overlap with the brain regions that respond to the threats during fear learning. For instance, the insula and the anterior

cingulate cortex participate in both (Goodman et al., 2018; Johansen et al., 2010). To investigate fear generalization, Onat and Büchel (2015) applied a generalization gradient task with the facial fear GSs and recorded the fMRI data during the task. The results showed that the facial GS activated the anterior insula, the anterior cingulate cortex (ACC), and the hippocampus. To study the brain activations associated with social exclusion, Eisenberger et al. (2003) applied a classical social exclusion paradigm, the Cyberball paradigm (an online experimental paradigm to evoke feelings of social exclusion) and recorded the fMRI data. They found that the activation of the ACC area was significantly larger in the social exclusion condition than in the control condition. The self-distress scores after social exclusion were positively correlated with the activations of the ACC. The anterior insula showed larger activations during the social exclusion condition compared with the social inclusion condition (DeWall et al., 2010). Together, considering that the brain areas relevant for social exclusion and fear generalization seem to overlap, it is relevant to study whether social exclusion can modulate fear generalization by affecting the neural activities in the anterior insula and the dorsal ACC. In addition to the anterior insula and the ACC, the role of the prefrontal cortex in fear learning and social exclusion has been examined by previous studies (Gradin et al., 2012; Moor et al., 2012). For instance, Eisenberger et al. (2003) provided evidence that participants in the social exclusion group evoked stronger activities in the ventrolateral prefrontal cortex (VLPFC). Chester and Dewall (2014) expanded this finding, concluding that the insufficient recruitment of the VLPFC explains how social exclusion disturbs the regulation of negative emotions. Moreover, mPFC is activated during social exclusion (Gradin et al., 2012; Moor et al., 2012). Sebastian et al. (2011) also adopted the Cyberball paradigm and reported that social exclusion activates the bilateral mPFC and the VLPFC. The significance of the mPFC is further pointed out in a study by Gradin et al. (2012), who found that schizophrenia patients exhibited weaker activations in the mPFC than did the healthy control group during social exclusion. Taken together, these studies indicate that social exclusion disturbs the function of the prefrontal cortex. Additionally, the prefrontal cortex is also involved in the neural circuitry of fear learning and generalization, which has been suggested in both animal and human research (Likhtik & Paz, 2015; Perusini & Fanselow, 2015). For instance, Motzkin et al. (2015) recruited patients with an mPFC dysfunction and recorded the fMRI data, finding that, relative to the healthy controls, the patients showed stronger amygdala activations to threatening stimuli. As for fear generalization, similar findings showed that the patients with general anxiety exhibited significantly higher shock expectancy of the GS relative to the healthy controls, with a decreased signal in the vmPFC (Greenberg et al., 2013a). In summary, social exclusion might modify the function of the mPFC and influence fear learning and generalization.

To measure the activity of mPFC, the fNIRS method has been adopted in this study, similar to previous studies measuring the activity of mPFC in fear learning (Guhn et al., 2012, 2014; Ma et al., 2013; Yoshiike et al., 2018). For

example, Ma et al. (2013) found that the stimuli paired with a negative facial expression evoked larger activations of the prefrontal cortex compared with the stimuli paired with a neutral facial expression. In their fNIRS study of fear learning, Guhn et al. (2012) have reported that the CS+ elicited a larger mPFC activation compared with the CS-. The prefrontal cortex activities recorded by fNIRS in fear learning were reported by Yoshiike et al. (2018). However, no previous study has investigated the mPFC activity during fear generalization.

1.5.2 Observational learning and fear generalization

Observational fear learning, acquiring the association between cues and threats by observing others' fear responses, has been studied in different sensory modalities, such as visual, auditory, and olfactory (Debiec & Sullivan, 2014; Haaker et al., 2017; Kim et al., 2010). Different species, such as humans, rats, and monkeys, are capable of observational learning (Debiec & Olsson, 2017). Moreover, human needs (e.g., hunger, thirsty, and threat avoidance) guide the information selection and selective response to the stimuli that contain motivational significance (e.g., food, water, and threat cues), which makes individuals pay sustained attention to the affective stimuli compared with the emotionally neutral stimuli. The attention that is captured by emotional stimuli with motivational significance, is called "motivated attention" (Lang et al., 1997). In fear learning, previous studies have reported that the CS+ attracts more motivated attention compared with the CS- (Keil et al., 2012; Nelson et al., 2015). Similarly, in fear generalization, the generalization stimuli resembling threatening stimuli capture more motivated attention compared with the generalization stimuli resembling safety stimuli (Roesmann et al., 2020).

To record the rapid reactions of the brain to threatening stimuli in fear learning and generalization, ERPs have been recorded in previous studies (Baas et al., 2002; Bublatzky & Schupp, 2012; Nelson et al., 2015; Pizzagalli et al., 2003). In the early components of ERPs, the P1 component, peaking approximately at 120 ms post-stimulus on occipital sites, has been observed to differentiate between CS+ and CS- in fear learning (Bublatzky & Schupp, 2012; Pizzagalli et al., 2003). Specifically, Pizzagalli et al. (2003) found that facial CS+ evokes higher P1 amplitudes compared with facial CS-. Although the P1 has not been reported in a fear generalization task, one MEG study reported an enhancement to GS+ (GS resembling CS+) compared with GS- (GS resembling CS-) on occipital sites around 120ms (Roesmann et al., 2020). The results of P1 in fear learning and generalization are mixed, however. Some other studies did not find enhancements of the P1 component to the CS+ or GS+ (Ferreira et al., 2019; Lei et al., 2021; Nelson et al., 2015). In general, the P1 component reflects early visual cortex responses and seems to be influenced by threat-driven attention via a subcortical pathway involving the amygdala (Linke et al., 1999). Rotshtein et al. (2010) reported that patients with amygdala dysfunction showed a decreased P1 amplitude for the fear expression in the occipital area. The P1 component has also been found to be relevant to early threat discrimination (Forscher et al., 2016; Linton & Levita, 2021; Meynadasy et al.,

2019; Thorpe, 2009). Thus, the larger P1 amplitudes evoked by CS+ than by CS- in fear learning may indicate enhanced stimulus-driven attention to the threatening stimulus for threat discrimination.

In the late components of ERPs, the late positive potential (LPP) component is a positive polarity component at the parieto-central regions, with a long duration that begins around 300 ms post-stimulus and lasts for even seconds (Cuthbert et al., 2000). The LPP component has been investigated in some studies of fear learning and generalization (Nelson et al., 2015; Panitz et al., 2015; Roesmann et al., 2020). In the fear learning studies, CS+ evokes larger LPP amplitudes compared with CS- (Baas et al., 2002; Bublatzky & Schupp, 2012). Studies on LPP in the fear generalization phase are still scarce, but there is some evidence that LPP reflects motivated attention in fear generalization (Roesmann et al., 2020). For instance, Nelson et al. (2015) reported that the more the GS is similar to the CS+, the larger the LPP amplitudes evoked by the GSs. The greater LPP amplitudes of CS+ or GS+ (the GS that resembles CS+) have been considered to reflect motivated attention (Lang et al., 1997; Schupp et al., 2005). When the motivated attention was captured by the CS+ and the GS+, attentional resources were allocated to these threatening stimuli for a long time period (Ferreira et al., 2019). In direct fear learning and generalization, enhancements in early threat discrimination and late motivated attention to the CS+ and GS+ have been found (Bublatzky & Schupp, 2012; Nelson et al., 2015; Roesmann et al., 2020). However, it is still unknown whether or not the CS+ and GS+ in observational learning evoke larger P1 (reflecting threat discrimination) and LPP (reflecting motivated attention) amplitudes compared with CS- and GS-.

Previous research suggests that observational fear learning contributes to the development of anxiety (De Rosnay et al., 2006; Mineka & Zinbarg, 2006). For example, De Rosnay (2006) has found that when an infant observes its mother's interaction with strangers, the anxiety experienced in regard to strangers is transmitted from the mother to the infant. Although fear generalization based on direct learning is related to social anxiety (Ahrens et al., 2016), the relationship between social anxiety and fear generalization after observational learning remains unclear.

1.5.3 Oxytocin and fear generalization

An evolutionarily conserved hypothalamic neuropeptide, oxytocin was originally regarded as a neurohypophyseal hormone with a special role in milk ejection and mammalian parturition. Over the last 20 years, the role of oxytocin has been recognized in social cognition, social affiliation, and pro–social behaviors (Bartz et al., 2011; Feldman, 2012; Uvnäs–Moberg, 1998). Accumulating evidence has suggested that social affiliations, including parent–infant social bonding (Gordon et al., 2008; Levine et al., 2007) and romantic attachment (Algoe et al., 2017; Schneiderman et al., 2012), increase oxytocin levels in humans. A distinct feature of many social mammals (e.g., rats and humans) is their effective recovery from negative events when they stay

together, which is called the "social buffering" effect (Davitz & Mason, 1955; Hennessy et al., 2000; Kikusui et al., 2006; Mendoza et al., 1978). Oxytocin closely connects with the social buffering effect (Crockford et al., 2017; Winslow et al., 2003). For instance, Smith and Wang (2014) put microtus ochrogasters under stress and made them recover independently or with others. Their results indicate that paraventricular nucleus oxytocin plays a mediation role in the social buffering effect on stress, which is regarded as a potential treatment target of stress–related disorders. In humans, Seltzer et al. (2010) recruited mother–daughter dyads for their study, wherein the daughters were exposed to social stress and had to recover with their mothers or alone. The results showed that the daughters who recovered with their mothers exhibited higher oxytocin levels and that their cortisol levels returned to the baseline level most quickly, relative to the condition of independent recovery.

The intranasal administration of oxytocin has also been reported to be a potential intervention for decreasing fear and anxiety (Neumann & Landgraf, 2012; Neumann & Slattery, 2016). Specifically, intranasal oxytocin in humans reduced the amygdala activities of threats. More specifically, previous studies have found that intranasal oxytocin induces the stable downregulation of amygdala reactions to fear expressions (Domes et al., 2007; Kirsch et al., 2005). This finding has been replicated in generalized social anxiety disorder studies. For instance, Labuschagne et al. (2010) found that oxytocin decreases the activities of the amygdala toward threatening faces in generalized social anxiety patients. Moreover, Spengler et al. (2017) provided evidence that oxytocin exhibits the effects of decreasing amygdala reactions to ambiguous fear expressions.

Based on consistent evidence that oxytocin attenuates fear-related amygdala responses, initial research has employed Pavlovian fear learning to explore the effects on aversive learning (Eckstein et al., 2016). However, the influences of oxytocin on fear generalization remain unclear. One potential pathway for oxytocin to exhibit its influences on generalization is by decreasing the discrimination threshold of the generalization stimulus. Holt et al. (2014) examined the just noticeable difference of the GSs before the participants completed the fear learning and generalization task by recording their SCR. The results indicated that the discrimination thresholds modulate the fear responses (enhanced SCRs) to the generalization stimuli.

1.6 Aims of the research

This dissertation aims to investigate the effects of social exclusion and observational learning on fear learning and generalization, and effect of oxytocin, a hormone related to social relationships, on fear generalization. To achieve these aims, three studies were conducted for this dissertation.

Study I examined the effects of social exclusion on fear learning and generalization by recording mPFC activities with the fNIRS method, SCR

activities, and shock expectancy ratings. Social exclusion was evoked by a computer-controlled game (Cyberball paradigm, Williams & Jarvis, 2006). Although previous studies have found that being excluded by social groups has many negative psychological outcomes (Blackhart et al., 2007; DeWall & Bushman, 2011; Eisenberger et al., 2003; Themanson et al., 2014; Wesselmann et al., 2016), it remains unclear whether social exclusion influences fear learning and generalization. Based on the negative effects of social exclusion on cognitive functions (Blackhart et al., 2007; Eisenberger et al., 2003; Themanson et al., 2014), social exclusion was expected to impair fear learning and increase fear generalization. Because previous studies have reported that social exclusion affects the function of the mPFC (Gradin et al., 2012; Moor et al., 2012), social exclusion was expected to decrease the mPFC activity during fear learning and generalization.

Study II examined the effect of observational learning on fear learning and generalization. Participants learned the CS–US associations in pairs (each participant took turns performing observational learning and direct learning) and performed a generalization phase independently and after both learning types. EEG was recorded in the acquisition and the generalization phases. According to a previous study (Egorova et al., 2015), observational learning reduces the differentiation of CS+ and CS- compared with direct learning. Therefore, I expected that in fear learning, observational learning decreases the differentiation of CS+ and CS- at the behavioral level. I also expected that direct learning evokes larger ERPs (P1, LPP) to the CS+ compared with observational learning. In fear generalization, I expected that observational learning increases behavioral responses and decreases the ERPs responses (P1, LPP) to the GS compared with direct learning. Furthermore, observational learning has been found to facilitate the development of social anxiety (De Rosnay et al., 2006; Mineka & Zinbarg, 2006), and social anxiety to facilitate increased fear generalization (Ahrens et al., 2016). Thus, I expected social anxiety to be correlated with fear generalization after observational learning. Moreover, pain sensitivity is defined as the "proneness to react to standardized experimental or pathological stimuli"(Ravn et al., 2012) and could be modulated by certa negative emotional states, such as stress, pain-related fear, and anxiety (Bailey et al., 2010; Bement et al., 2010; Caceres & Burns, 1997; Kirwilliam & Derbyshire, 2008). Based on the previous findings that social anxiety was positively correlated with pain sensitivity (Asmundson & Carleton, 2005) and observational learning increased pain sensitivity (Vögtle et al., 2013), I expected to find that pain sensitivity mediates the association between social anxiety and fear generalization after observational learning.

Study III investigated the influence of intranasal oxytocin on fear generalization and whether the discrimination threshold mediates the relationship between oxytocin and fear generalization by recording behavioral and SCR responses. Faces and circles were adopted as stimuli in fear learning and generalization tasks. Previous studies have reported that oxytocin facilitates fear learning, extinction, and fear discrimination (Eckstein et al., 2015, 2016; Hu et al., 2019; Olivera-Pasilio & Dabrowska, 2020); thus, oxytocin was

expected to reduce the behavioral and physiological responses to fear generalization by decreasing the perception threshold. According to previous studies, oxytocin selectively reduced the fear responses to socially relevant stimuli (Petrovic et al., 2008). Thus, I expected to find that oxytocin reduces fear responses more to social (i.e., faces) than non-social stimuli (i.e., circles).

2 METHOD

2.1 Participants

In **Study I**, 44 healthy adults from Shenzhen University, aged 18–25 years, participated in the experiment. Twenty-three (12 females) performed the exclusion group task, and 21 (9 females) completed the inclusion group task.

In **Study II**, 62 eligible healthy adults (28 males and 34 females), 18–23 years old (average age: 20.3 ± 1.3 standard deviation), participated in the experiment. The participants were recruited from Shenzhen University via posters and social media.

In **Study III**, 63 eligible healthy adults, aged 18–25 years, were recruited. Thirty were administered intranasal oxytocin, and 32 were administered normal saline.

In all three studies, the inclusion criteria were as follows: no hearing impairment, naive to the fear conditioning paradigm, and normal or corrected-to-normal vision. The exclusion criteria for all experiments were as follows: a history of head injuries, neurological and psychiatric disorders (e.g., schizophrenia), and other affective problems (e.g., anxiety, depression). The differences in the groups' scores on the questionnaires that measured items from the State-Trait Anxiety Inventory (STAI), Liebowitz Social Anxiety Scale (LSAS: fear, avoidance, measuring social anxiety symptoms), Rejection Sensitivity Questionnaire (RSQ), Beck Depression Inventory-II (BDI-II), and Connor–Davidson Resilience Scale (CD-RISC) in **Studies I** and **III** were not significant (Table 1).

TABLE 1 Questionnaire results in **Study I** and **Study III**.

	Study I			Study III		
	Exclusion	Inclusion		Oxytocin	Placebo	
	Mean* ± *SD	**Mean ± SD**	***T* *(p)***	***Mean* ± *SD***	***M*ean ± *SD***	***T* *(p)***
Trait Anxiety Inventory	43.22 ± 7.34	42.71 ± 6.67	0.24 0.81	39.33 ± 7.64	38.15 ± 10.32	0.51 0.61
State Anxiety Inventory	35.47 ± 7.26	36.48 ± 8.96	0.41 0.69	35.20 ± 7.52	35.62 ± 9.28	0.20 0.84
Beck Depression Inventory–II	8.61 ± 6.76	9.29 ± 7.85	0.31 0.76	7.43 ± 6.95	7.59 ± 5.91	0.10 0.92
Rejection Sensitivity Questionnaire	63.43 ± 10.00	62.13 ± 11.30	0.40 0.69			
The Connor–Davidson Resilience Scale	58.48 ± 10.19	62.92 ± 10.78	1.41 0.17			
Liebowitz Social Anxiety Scale–Fear				21.93 ± 9.92	21.88 ± 9.09	0.02 0.98
Liebowitz Social Anxiety Scale–Avoidance				24.90 ± 11.41	23.78 ± 7.07	0.46 0.65

SD = standard deviation, Exclusion/Inclusion = social exclusion/inclusion group in the Cyberball task in **Study I**, Oxytocin/Placebo = oxytocin/placebo group in **Study III**.

2.2 Ethical aspects

All the studies in this dissertation were performed in line with the Declaration of Helsinki (2008). All experiments in this dissertation were approved by the Ethical Committee at Shenzhen University. Before starting all experiments, written informed consent for each experiment was acquired from each participant. Moreover, each participant volunteered to take part in the experiment and were informed of the right to withdraw from it at any time without consequences. All the data in the dissertation were recorded, analyzed,

and stored in the secure server belonging to Shenzhen University. The electric shock administered in all experiments was safe and non-harmful to the participants. The electric shock sensitivity of each participant was tested with a subjective feeling of "highly uncomfortable but not painful" (Haaker et al., 2013). All the shock intensities of the participants had a maximum of eight mA. If the shock intensity reached the maximum, the participants who felt uncomfortable with its intensity were not allowed to participate for safety reasons.

2.3 Stimuli

Study I adopted 10 gradually increased sizes of circles in fear learning and generalization (Figure 1). The smallest geometric figure measured 5.08 cm. The largest and the smallest circles were regarded as CS+ and CS-, respectively, and the other eight circles (gradually increased by 15% increments) were divided into four GSs, meaning that each GS included two circles. The maximum-sized circle was paired with a shock, and the minimum-sized circle was not paired with a shock. Half of the participants learned the rule that the maximum-sized circle was paired with a shock and the minimum-sized circle was not paired with a shock. The other half learned the rule that the minimum-sized circle was paired with a shock and the maximum-sized circle was not paired with a shock.

Study II used four types of geometric figures (blue circle, yellow triangle, purple square, and green rhombus). The smallest geometric figure was 5.08 cm in diameter, with a step-wise increase of 14.29%. The geometric figures in fear learning and generalization had eight different sizes (CS+, GS1-6, and CS-). Half of the participants learned the rule that the largest circle was paired with a shock, and the other half learned the rule that the smallest circle was paired with a shock. The rule that the smallest geometric figure was paired with a shock or the largest geometric figure was paired with a shock in the first two blocks differed from the rule in the second two blocks.

Study III adopted faces as social stimuli and geometric figures as non-social stimuli. Specifically, for the social stimuli, two faces with different identities were chosen from the Chinese Affective Face System (CAFS) (Gong et al., 2011). The two female faces had neutral expressions and were regarded as CS+ and CS- in fear learning. The GSs included four gradually changing faces (GS1–GS4), created by morphing the two chosen faces with a 20% increasing step each time (Schiele et al., 2016). GS1 was the most similar to CS+, and GS4 was the most similar to CS-. In the non-social stimuli, the study adopted six circles that gradually increased in size and were used in fear learning and generalization as CS+, GS1-4, and CS-. The minimum-sized circle measured 2 in, with an increasing step of 20%. Half of the participants learned the rule that one face and the largest circle were paired with a shock, and the other half learned the rule that the other face and the smallest circle were paired with a shock. The stimuli used in the forced-choice discrimination task (FCDT;

a task for testing the individual's discrimination threshold) were also social and non-social stimuli. The social stimuli were two other female faces chosen from the CAFS and morphed with 0%, 6%, 12%, 24%, 48%, and 100% similarity with one of the faces. The non-social stimuli were six squares, whose minimum diameter was 2 in and increased by 0%, 6%, 12%, 24%, 48%, and 100%.

2.4 Procedure

In **Studies I and III**, each participant was brought into a quiet room to complete the task individually. In **Study II,** the participants completed the fear learning task in pairs and performed the fear generalization task alone. The distance between the participants and the screen was 60 cm in **Study I** and **Study III**. In **Study II,** the distance between the participants and the screen was 80 cm. The participants were requested to refrain from moving a lot or talking during the formal experiments and to pay attention to the task. When the participants came to the laboratory, they first underwent the shock sensitivity test, where they were administered a random series of electric shocks from low to high intensity and verbally reported their discomfort level using a Likert scale (from 1 = no feeling to 10 = too unpleasant to bear). The final shock intensity that was recorded for each participant was based on the subjective reports that the shocks were "highly uncomfortable but not painful"; the subjective rating average was around 6 (Haaker et al., 2013; Lei et al., 2019). In all studies, the participants completed this shock sensitivity test before the formal experiment.

In **Study I**, the participants completed questionnaires (Table 1). Then, they completed the habituation phase in which the CSs were presented to them without any task. Next, each participant completed the Positive and Negative Affect Schedule (PANAS) scale before the Cyberball task. Cyberball 4.0, a computer video game on the computer that was developed by Williams et al. (2012), was adopted for evoking social exclusion and inclusion.

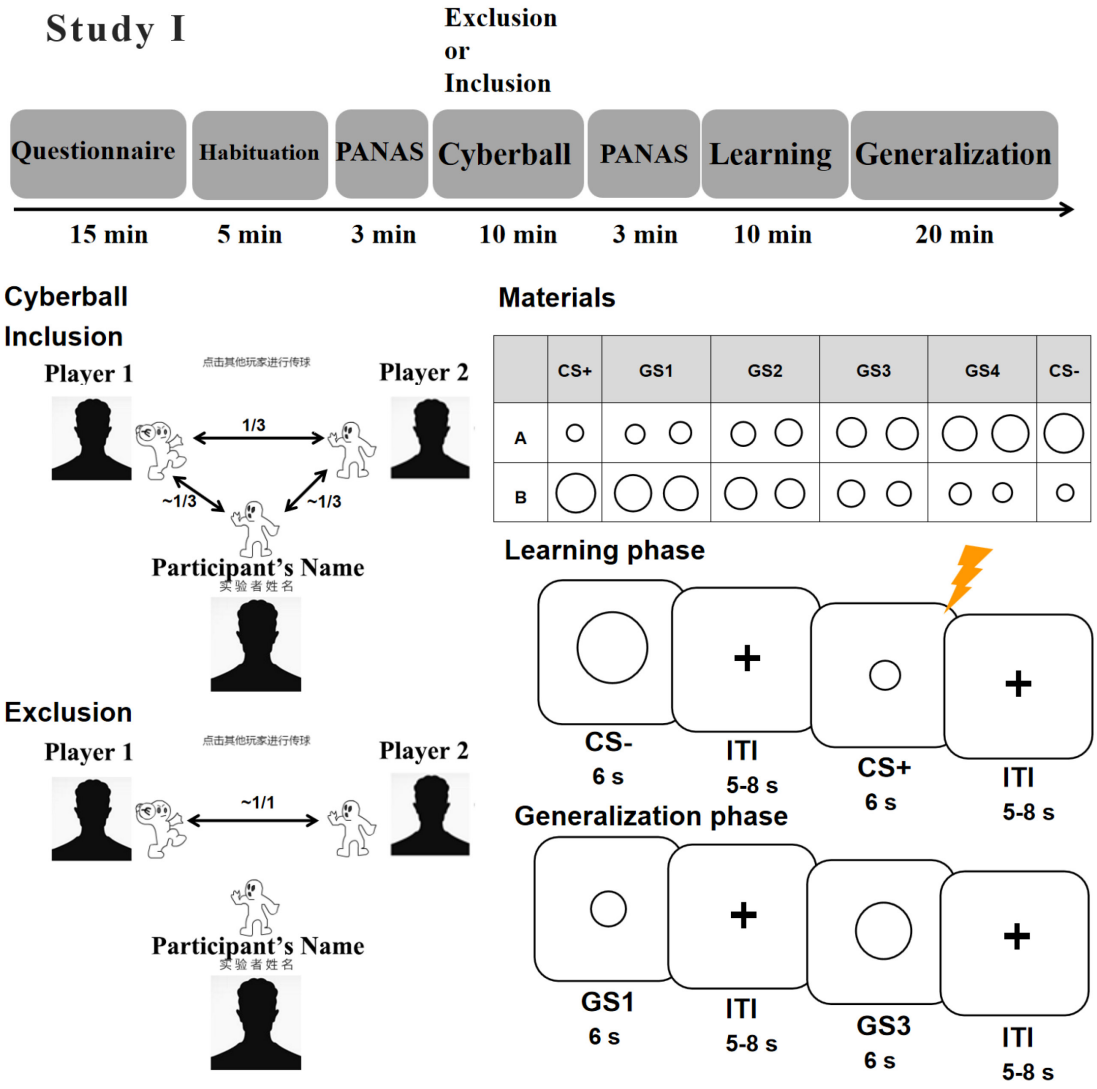

FIGURE 1 Flow chart and procedure in **Study I**. In the Cyberball task, 1/3 or 1/1 indicates the percentage of probability of passing the ball. In the learning and generalization tasks, CS+ indicates the conditioned stimuli (CS) that is paired with a shock, and CS- indicates the CS that is not paired with a shock. The yellow lightning icon indicates an electric shock. GS = generalization stimulus, ITI = inter-trial interval (interval between trials). s = second. min = minute. The figure is adapted from Dou et al. (2020).

The participants were randomly assigned to either the social exclusion or the social inclusion group. In the inclusion group, the probabilities of the computer-controlled player passing the ball to the participant or to another virtual player were equal. The participants in the social inclusion group had a 33% chance of receiving the ball. The participants in the social exclusion group only received the ball from the virtual player the first time, indicating their nearly zero chance of receiving the ball. After the Cyberball task, the participants completed the PANAS scale again.

Then, the participants performed the tasks based on the Pavlovian fear learning and generalization paradigm, including three stages: habituation, learning, and generalization. In the habituation stage, CS+ and CS- (12 trials)

were presented to the participants with no shock. This task had already been completed by the participants previously. In the learning stage, 12 trials of CS+ were presented to the participants, nine of them paired with a shock. The shock was immediately followed by the offset of the CS+. There were also 12 trials of CS-. In the fear generalization stage, six stimuli (including CS+, GS1-4, and CS-) were presented to the participants in a pseudo-random sequence, and each stimulus had 12 trials. There was a 50% chance of receiving a shock after CS+ in the generalization phase. The participants' task in fear learning and generalization was to rate their shock expectancy—from CS+ or CS- when the CS showed up—using a three-point Likert scale (0–2 = ranging from no shock to certain shock). In all stages, the stimuli had a 6-s duration each, and a 50-ms shock was followed by the stimuli in CS+. Moreover, the inter-trial interval (ITI) in **Study I** lasted from 5 s to 8 s, with a fixation in the center of the screen (Guhn et al., 2014).

In **Study II,** the observational learning task and the conditioned fear generalization task were adapted from those used in previous research (Lissek et al., 2008; Pärnamets et al., 2020). When the participants came to the laboratory, they were asked to fill in the STAI, the BDI-II, the Pain Sensitivity Questionnaire (PSQ), and the LSAS.

There were four blocks in the experiment. In each block, the participants completed one fear learning stage and one fear generalization phase. During the fear learning stage, one minimum-sized geometric figure (CS+) was presented on the screen for 3 s, followed by a 50-ms shock (with a 75% chance of being administered). One maximum-sized geometric figure was also presented to the participants for 3 s but was not followed by a shock (CS-). A random 4–6-s ITI was presented to the participants. A pair of participants with the same gender completed the task together. One was assigned as a "demonstrator" and the other as an "observer." The demonstrator learned the association between CS+ and the US on his/her own, while the observer learned the association by observing the responses of the demonstrator. The pair of participants exchanged roles at the beginning of the third block. In each block, there were 15 trials for each CS. The observer answered the following four questions when the acquisition phase ended: 1. "Did the demonstrator help you learn the rules?" (9-point Likert scale, ranging from not at all to totally agree). 2. "Did you sympathize with the demonstrator?" (1–9 = not at all to totally sympathize). 3. "Did you perceive the pain of the demonstrator?" (1–9 = not at all to totally perceive). 4. "To what extent are you similar to the demonstrator?" (1–9 = not at all to totally similar).

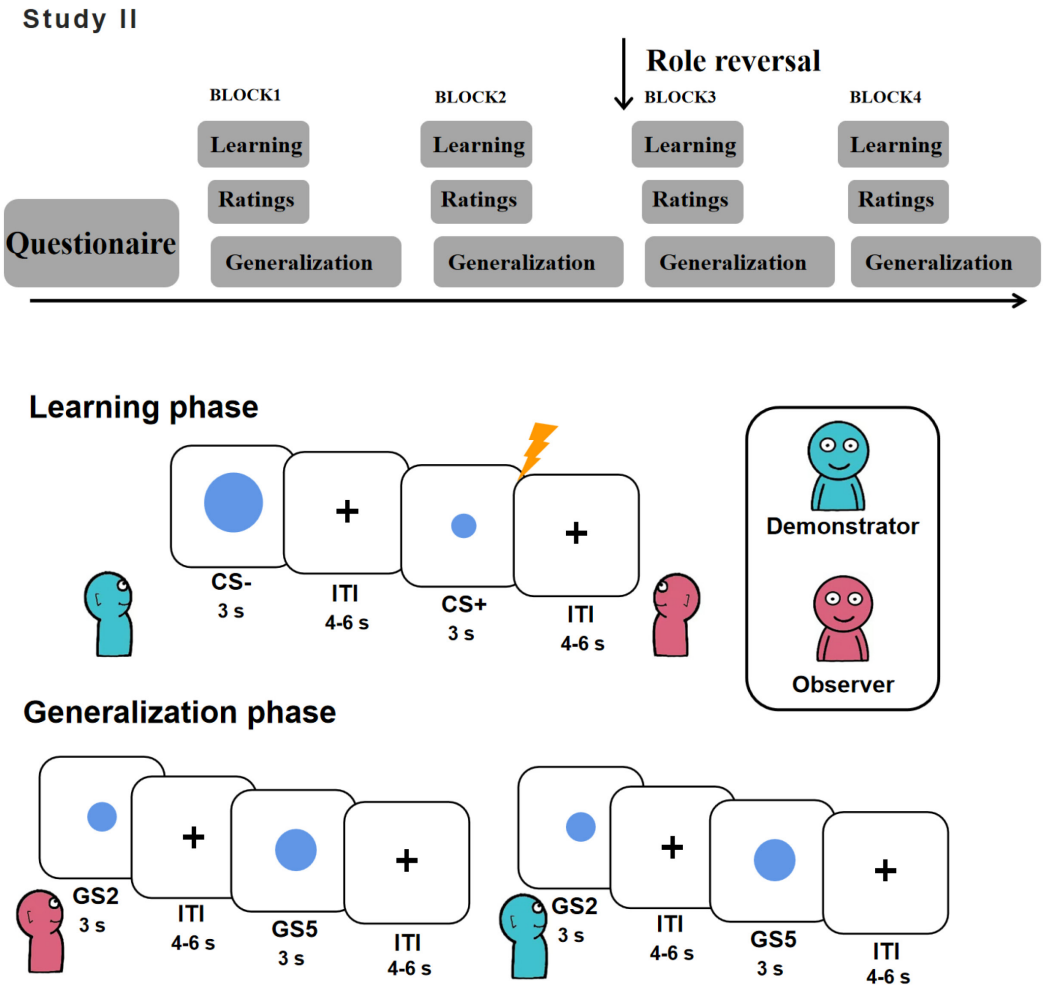

FIGURE 2 Flow chart and procedure in **Study II**. The flow chart shows each task in **Study II**. The observer and the demonstrator learn the rules together and complete the fear generalization task independently. The blue cartoon figure represents the demonstrator, and the red cartoon figure represents the observer. GS = generalization stimulus, CS+ = conditioned stimulus paired with a shock, CS- = conditioned stimulus never paired with a shock, ITI = inter-trial interval. s = second. The figure is adapted from Dou et al. (submitted manuscript).

In fear generalization, the participants completed the task in different rooms without seeing each other. The GSs (GS1-GS6), CS were shown on the screen for three seconds. Only at the end of the trial of CS+, the CS+ was paired with a shock. The ITI was the same as that for fear learning. Each CS contained 10 trials, and each GS included five trials in each block. Finally, the participants subjectively reported whether they had learned the roles by observing others. The participants who answered "Yes" were included in the subsequent data analysis. One dyad with some EEG recording problems and another dyad reporting that they could not learn the roles were excluded from the data analysis.

In **Study III,** participants came to the lab twice. The first time, all the participants completed a perception forced choice discrimination task (FCDT) (Holt et al., 2014). When the participants came to the lab for the second time, they filled out several questionnaires before the formal experiment (Table 1). After that, participants completed the fear learning task. The fear learning task, revised from Lissek et al. (2008), which were the facial CS+, the facial CS-, the circle CS+, and the circle CS-. Each stimulus was presented to the participants 12 times. The CS- stimuli were never paired with shocks, and nine trials of the CS+ were followed by a shock in 12 trials. Participants were asked to judge how likely the stimuli would be followed by a shock on a nine-point Likert scale (one to nine = no risk to high risk). The duration of all the stimuli was six seconds. After that, a fixation cross was presented to the participants with a random duration from eight to 12 seconds. The participants received the stimuli with a pseudo-randomized sequence such that the number of the same stimuli followed by each other was lower than three.

After the fear learning task, the participants were asked to complete emotional ratings, such as the arousal, valence, and attractiveness of each stimulus. Then the participants were randomly arranged into the oxytocin group or placebo group. The participants in the oxytocin group were administered 24 international units of intranasal oxytocin and the participants in the placebo group were administered the same dose of normal saline. After 40 minutes, the participants performed the fear generalization task. In the fear generalization stage, the procedure was the same as that in the fear learning stage except that eight different types of generalization stimuli, such as the facial GS1-4 and the circle GS1-4, were presented to participants. Moreover, the CS+ stimuli also had a 50% chance of being followed by an electric shock. After every 20 trials, all the participants were able to have a rest. The participants performed the emotional ratings according to the stimuli shown in the fear generalization stage followed by the fear generalization task.

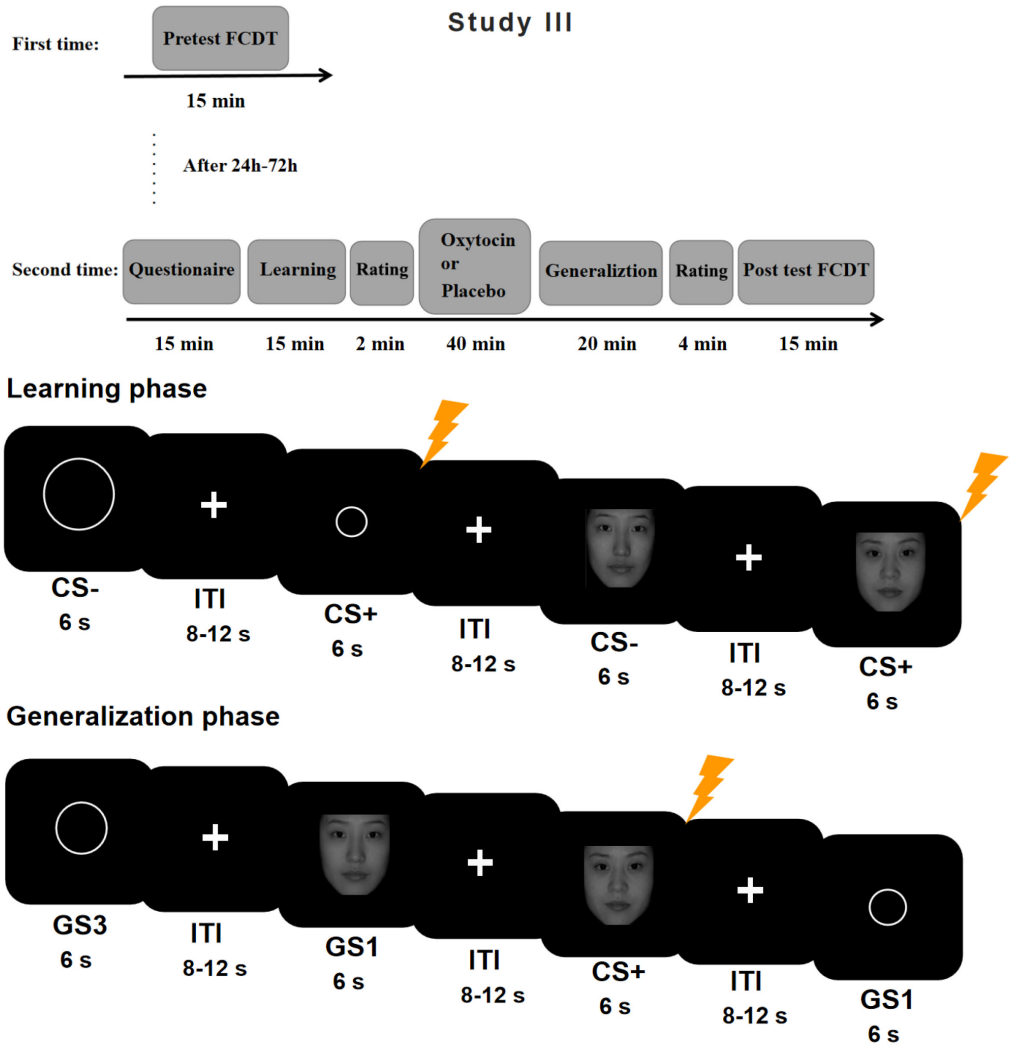

FIGURE 3 Flow chart and procedure in **Study III**. FCDT = forced-choice discrimination task, CS+ = conditioned stimulus paired with a shock, CS- = conditioned stimulus not paired with a shock, ITI = inter-trial interval. The flow chart shows the duration of each task. The procedures for learning and generalization are presented in the figure. s = second. m = minute. h = hour. This figure is adapted from Dou et al. (2021).

After the emotional ratings in the fear generalization stage, the participants completed the post-test FCDT. The pre-test and the post-test FCDT were the same for measuring the just noticed difference (JND) of the social and the non-social stimuli. The procedure for the FCDT was as follows: the participants were presented with a stimulus on the screen for 500 ms, followed by a fixation cross with a 500-ms duration. The participants then saw two stimuli (origin stimulus and morph stimulus) on the screen and judged which stimulus was previously presented by pressing a key on the keyboard. The origin and the morph stimuli were randomly placed in the right or the left position. A 1,000-ms fixation cross was shown until the participants responded.

2.5 Analysis of behavioral data

2.5.1 Shock expectancy and emotional ratings of the stimuli

In all studies, the shock expectancy ratings were calculated as an effective index for measuring whether the participant had learned the CS–US contingency. The mean values of the shock expectancy ratings were calculated for each condition. In **Study II**, the shock expectancy ratings for the GSs (GS1–GS6) were fitted with a linear model (y = p1 * x + p2, x = the size of each stimulus, y = the shock expectancy ratings for each stimulus, p1 = slope, p2 = intercept, using the Curve Fitting Tool in Matlab version 2013b), and the slope of the function represented the fear generalization gradient. The slopes of the two learning types (direct learning versus observational learning) were compared using a paired t-test.

In **Study III**, the participants completed the emotional ratings for the stimuli after the fear learning and the generalization stages. These included ratings on the valence (unhappy–happy), arousal (calm–excited), and attractiveness (unattractive–attractive) of the stimuli. The participants used a nine-point Likert scale in their ratings.

2.5.2 Discrimination threshold

The accuracy (the proportion of correct responses) of the FCDT for each stimulus was calculated. Next, the accuracy values of the morphs, including faces and squares, were fitted with the Weibull function (y = 1− e^(−(x/a)^ b), x = the morph level, y = the accuracy of each stimulus, a and b = the parameters for scale and shape, respectively, Matlab version 2013b). After fitting the curve, the JND for each participant was the morph level with 75% accuracy of the curve (Figure 10; Tuominen et al., 2019).

2.6 SCR recordings and analyses

Study I adopted the BIOPAC MP150 system (CA, USA) to record SCR. SCR is a method to record the conductor changes of skin when stimuli occur reflecting the activities of the autonomic nervous system. To remove artefacts, the SCR data were high-pass fltered at 0.0159 Hz (Staib et al., 2015). According to a previous experiment (Boucsein et al., 2012), the zero-response trials, where the peak value was lower than 0.02 μs 1–4 s after the stimulus onset, were also removed. The activation of SCR for each trial was calculated by subtracting the minimum value from the maximum value 1–4 s after the stimulus onset. Two participants' data were excluded from the whole analysis due to a recording mistake. Finally, 42 participants' data were analyzed and reported.

In **Study III**, the Mindware (OH, USA) system was used to record the SCR data. The activations of SCR were calculated via the following method: the maximum values 0–6 s after the stimulus onset minus the average value 3 s

before the stimulus onset. Similarly, zero-response trials, where the activations were lower than 0.02 μs, were removed (Boucsein et al., 2012).

2.7 Recordings and analysis of brain activities

2.7.1 fNIRS recordings and analyses

In **Study I**, the functional near-infrared spectroscopy (fNIRS) data were measured using the NIRScout 1624 system (LA, USA). To reduce the longitudinal data drifts and system noises, a 0.01–0.2 Hz bandpass filter was applied to the raw data (Gervain et al., 2011, Piper et al., 2014). Based on the Beer-Lambert law, the NIRS signals were then calculated and transformed into oxy-Hb and deoxy-Hb concentrations. To minimize the effect of individual differences in the absolute signal value, the Z-score normalization method was used in fear learning and generalization (Yang et al., 2016). Specifically, the Z-score transformation was calculated. Lastly, the mean values 0.5–5 s after the stimulus onset were calculated for the activation. The region of interest was the mPFC; therefore, the activity from the channels around the mPFC according to the 10–20 location system (FP1/FP2; Koessler et al., 2009) and the significant activation channels in the CS+ or the CS- conditions were combined. Two channels around FP1 (left mPFC) were finally chosen for the subsequent analysis.

2.7.2 Electrophysiological recordings and analyses

In **Study II**, EEG activity was recorded by using a 64-channel wireless EEG recording system (NeuSen.W64, Changzhou, China). A 30-Hz low-pass filter and a 0.1-Hz high-pass filter were adopted in the preprocessing of the EEG data. The independent component algorithm (ICA) method was used to detect and remove the ocular artifacts. Each epoch began 500 ms (baseline) prior to the onset of the stimulus; each lasted for 1,500 ms and was corrected by the baseline. According to previous results (Luo et al., 2013), the parietal-occipital area (POz, PO3, PO4) and the occipital area (Oz, O1, O2) for the P1 component were selected, with a time window of 100–150 ms. Moreover, the parietal (Pz, P1, P2) and the parietal-occipital (POz, PO3, PO4) areas were selected in previous studies (Auerbach et al., 2015; Carolan et al., 2014), with a time window of 300–750 ms.

2.8 Statistical analyses

Study I adopted a repeated-measure Analysis of Variance (ANOVA) to analyze the data on shock expectancy ratings, SCR, and fNIRS in the fear learning stage. The two variables were the between-subject variable group (exclusion,

inclusion) and the within-subject variable stimulus (CS+, CS-). The differences between CS+ and CS- in the shock expectancy ratings by the exclusion and the inclusion groups were tested with independent sample t-tests. A repeated-measure ANOVA was used to analyze the shock expectancy ratings, SCR, and fNIRS data in the generalization stage. Group (including exclusion and inclusion groups) was a between-subject variable, and GS (including CS+, GS1–4, and CS-) was a within-subject variable. Furthermore, the slope of the linear fit of these values for each participant was calculated. Independent-sample t-tests were performed to identify the differences in slope between the inclusion and the exclusion groups.

To examine the mediation effect of the mPFC activities on the associations between social exclusion and shock expectancy ratings in fear learning and generalization, the bootstrap method (number of iteration = 5,000) was adopted.

Study II adopted a repeated-measure ANOVA to analyze the shock expectancy ratings, P1, and LPP in the fear learning stage. The learning type (direct learning, observational learning) and the stimulus (CS+, CS-) were within-subject variables. A repeated-measure ANOVA was applied to analyze the shock expectancy ratings, P1, and LPP data. To increase the number of trials for each generalization condition, GS1–GS3 resembling the CS+ were averaged as GSs similar to CS+ (GS+), and GS4–GS6 resembling the CS- were averaged as GSs similar to CS- (GS-). The within-subject variables were the learning type (direct learning, observational learning) and the stimulus (GS+, GS-). The linear regression values among the behavioral data, EEG results, and questionnaires were calculated. Similarly, the mediation effect of pain sensitivity on the association between social anxiety and the generalization gradient in observational learning was calculated with a bootstrap method (sample size = 5,000) (IBM; Hayes, 2013).

Study III adopted a repeated-measure ANOVA to analyze the shock expectancy ratings, SCR, and emotional ratings data in fear learning. The three variables were the group, the stimulus, and the material. The group, including two factors (oxytocin and placebo), was a between-subject variable. The stimulus (including CS+ and CS-) was a within-subject variable. The material (including social and non-social stimuli) was a within-subject variable. In the generalization, a repeated-measure ANOVA was used to analyze the data on shock expectancy ratings, SCR, and emotional ratings. The group variable, a between-subject variable, had two factors (oxytocin and placebo groups). The within-subject variables were stimulus (CS+, GS1–4, CS-) and type (face, circle). The discrimination threshold data were also analyzed with a repeated-measure ANOVA. The within-subject variables were time (pre-test, post-test) and material (face, circle). The group variable was a between-subject variable with two factors (oxytocin and placebo groups).

In all studies, when using a repeated-measure ANOVA, Mauchly's test was performed, and if the assumption of sphericity was violated, the Greenhouse–Geisser correction was applied. During all the follow-up pairwise comparisons, the p values were corrected by using the Bonferroni correction method. The alpha value in all studies was 0.05.

3 RESULTS

3.1 Study I: The influence of social exclusion on fear conditioning and generalization

Study I adopted the Cyberball paradigm to evoke the participants' social exclusion and the classic Pavlovian fear conditioning and generalization paradigm by recording the shock expectancy ratings, SCR, and mPFC activities. The aim of Study I is to investigate the effect of the evoked social exclusion experience on fear learning and generalization. The shock expectancy ratings in fear learning showed a significant main effect of the stimulus ($\eta_p^2 = 0.876$) (CS+ versus CS-) and an interaction effect of the stimulus by group ($\eta_p^2 = 0.211$) (inclusion group versus exclusion group). However, the main effect of the group was not significant ($\eta_p^2 = 0.031$). Follow-up pairwise comparisons demonstrated that the shock expectancy ratings for CS+ by the exclusion group were lower than those by the inclusion group, while the shock expectancy ratings for CS- by the exclusion group were significantly higher than those by the inclusion group (Figure 4A). The independent sample t-test on the shock expectancy ratings for CS+ versus CS- by the exclusion and the inclusion groups showed that the exclusion group had a significantly lower shock expectancy rating difference compared with the inclusion group. These results indicated that the differentiation of CS+ and CS- was reduced in the social exclusion group compared with the social inclusion group. In the shock expectancy ratings in fear generalization, the main effect of GSs (CS+, GS1–GS4, CS-) was significant ($\eta_p^2 = 0.739$), and the main effect of the group was also significant ($\eta_p^2 = 0.149$). However, the interaction effect was not significant ($\eta_p^2 = 0.046$). Follow-up pairwise comparisons showed that the shock expectancy ratings by the exclusion group were significantly higher than those by the inclusion group (Figure 4B). This finding indicated that the social exclusion group reported higher shock expectancy ratings for the GSs compared with the social inclusion group.

The repeated-measure ANOVA for SCR in the acquisition phase indicated that the main effect of the stimulus ($\eta_p^2 = 0.110$) (CS+ > CS-) and that of group was significant ($\eta_p^2 = 0.096$) (exclusion > inclusion), while the interaction effect between the stimuli and the group was not significant ($\eta_p^2 = 0.027$) (Figure 4C). This finding indicated that social exclusion evoked enhanced SCRs of CSs compared with social inclusion. In the fear generalization phase, the SCR results showed a significant main effect of both the group ($\eta_p^2 = 0.094$) (exclusion > inclusion) and the stimuli ($\eta_p^2 = 0.184$) (CS+ > CS-). However, the interaction effect between the group and the stimulus was not significant ($\eta_p^2 = 0.017$) (Figure 4D). This result indicated that social exclusion enhanced the SCRs of the GSs.

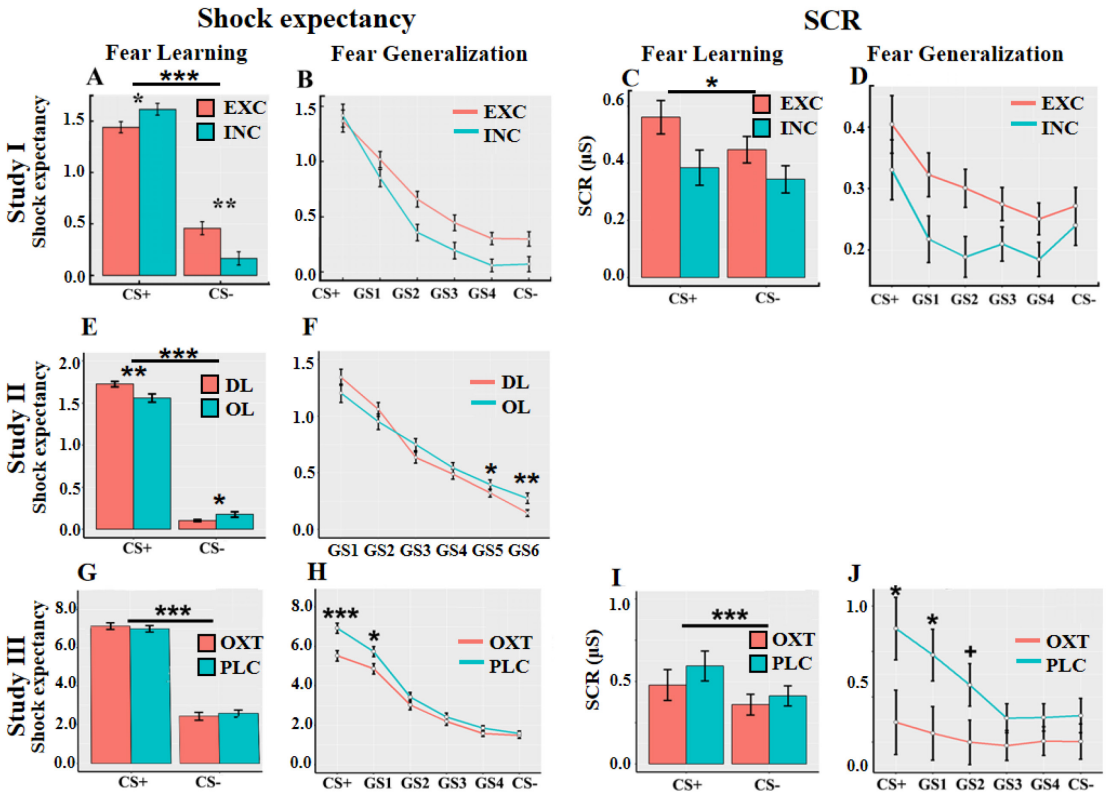

FIGURE 4 The results of the shock expectancy ratings and SCR in fear learning and generalization in all studies. A) The shock expectancy ratings of CS+ in the inclusion group were higher than those in the exclusion group. The shock expectancy ratings of CS- in the inclusion group were lower than those in the exclusion group (**Study I**). EXC = exclusion group. INC = inclusion group. B) The shock expectancy ratings of GSs in the exclusion group were higher than those in the inclusion group (**Study I**). OL = observational learning. DL = direct learning. C) The SCRs of CSs in the exclusion group were larger than those in the inclusion group(**Study I**). D) The SCRs of GSs in the exclusion group were larger than those in the inclusion group (**Study I**). E) The shock expectancy ratings of CS+ in direct learning were higher than those in observational learning. The shock expectancy ratings

of CS- in direct learning were lower than those in observational learning (**Study II**). F) The shock expectancy ratings of GS5 and GS6 after observational learning were higher than those after direct learning (**Study II**), G, I) The shock expectancy and SCRs of CS+ were larger than those of CS- in both groups (oxytocin, placebo) (**Study III**). OXT = oxytocin. PLC = placebo. H,J) The shock expectancy and SCRs of CS+ and GS1 in the oxytocin group were lower than those in the placebo group (**Study III**). SCR = skin conductance response. GS = generalization stimulus. CS+ = conditioned stimulus paired with a shock. CS- = conditioned stimulus not paired with a shock. The error bar represents the standard error (SE) of the mean. ***p < 0.001, **p < 0.01, *p < 0.05, ^{+}p < 0.1. The figure is adapted from Dou et al. (2020).

The fNIRS results (the oxy-HB levels of the left mPFC) in the acquisition phase showed a significant stimulus x group interaction (η_p^2 = 0.113), while the main effect of the stimulus (η_p^2 = 0.059) and that of the group (η_p^2 = 0.024) were non-significant. Follow-up pairwise comparisons showed that the oxy-HB in the mPFC of CS+ was significantly higher than that of CS- in the inclusion group (Figure 5A, B), while there was no significant difference between the oxy-HB activities of CS+ and CS- in the exclusion group (Figure 5A, C). Furthermore, the oxy-HB of CS+ in the inclusion group was significantly larger than that in the exclusion group (Figure 5A). In fear generalization, although there were no significant results in the repeated-measure ANOVA, the slope of the linear fit of the oxy-HB activities in the left mPFC showed that the slope of the inclusion group (β = -0.505, SD = 0.82) (Figure 5D, F) was steeper than that of the exclusion group (β = 0.125, SD = 0.625) (Figure 5E, F). There were no significant results for deoxy-HB in the left mPFC in fear learning and generalization.

The oxy-HB value in the left mPFC of CS+ in fear learning showed a significant mediation effect on the relationship between social exclusion and shock expectancy ratings for CS+ in fear learning (indirect effect = 0.053; confidence interval [CI 95%]: [0.005, 0.153]) (Figure 5G).

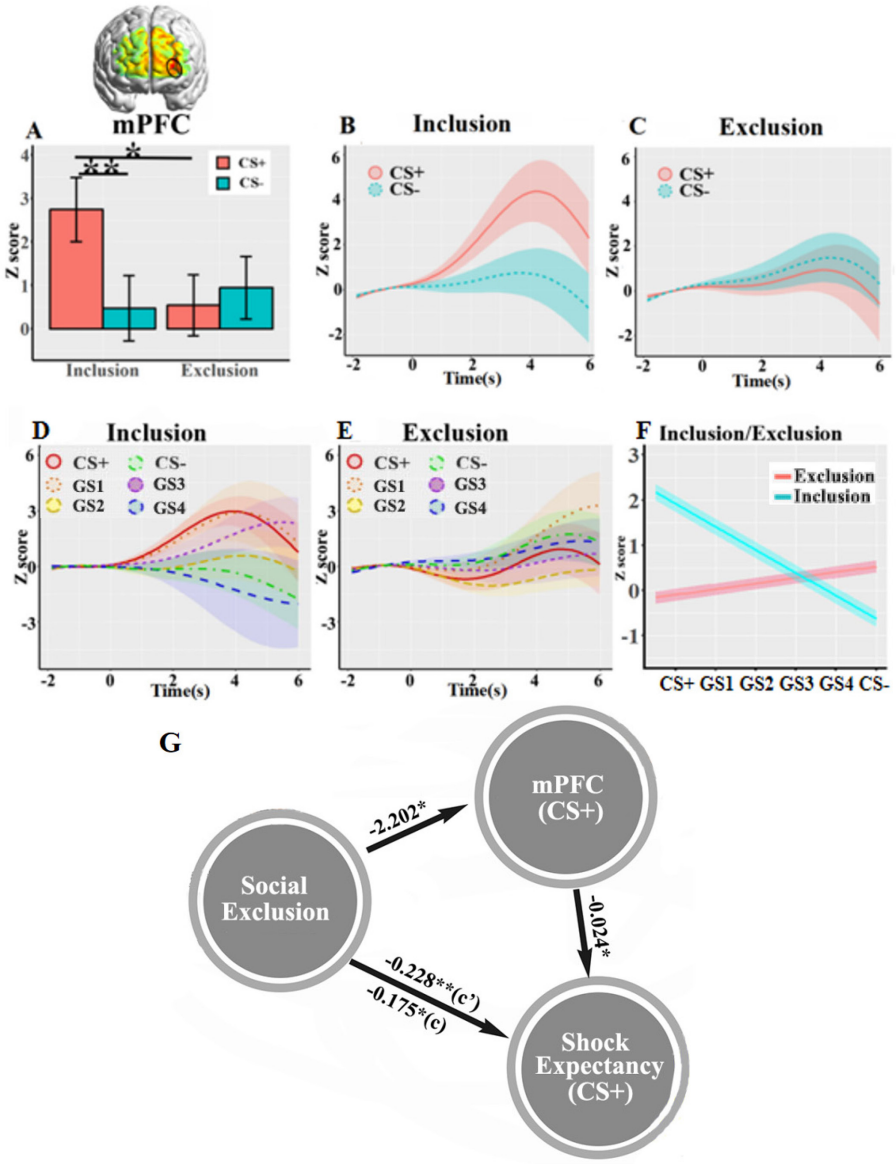

FIGURE 5 The results of the effect of the oxygenated hemoglobin concentration in the left medial prefrontal cortex (mPFC) in fear learning and generalization in **Study I**. A) Activity of mPFC in fear learning. The mPFC activity of CS+ was larger than that of CS- in the inclusion group, but the mPFC activity of CS+ showed no differences from that of CS- in the exclusion group. The mPFC activity of CS+ in the inclusion group was larger than that in the exclusion group. B) and C) Time sequence of mPFC activity for CS+ versus CS- in inclusion and exclusion groups. D) and E) Time sequence of the z-score of mPFC in CS+, GS1–4, and CS- in the inclusion and the exclusion groups. F) Slopes of the linear model fit in the inclusion and the exclusion groups. The fear generalization gradient (the more similar to CS+, the larger the mPFC activities) in the inclusion group was steeper than that in the exclusion group. G) Mediation effect of mPFC of CS+ in fear learning on the association of social exclusion and shock expectancy ratings of CS+. GS = generalization stimulus. CS+ = conditioned stimulus paired with a shock. CS- = conditioned stimulus not paired with a shock. c = total effect, c' = direct effect. $^{**}p < 0.01$, $^{*}p < 0.05$. The figure is adapted from Dou et al. (2020).

3.2 Study II: The influence of observational learning on fear generalization

By adopting an observational fear learning paradigm with two real persons recording behavioral and EEG data, **Study II** investigated how observational fear learning influenced fear generalization. For the shock expectancy ratings in fear learning, the main effect of the stimulus was significant (η_p^2 = 0.908) (CS+ > CS-). The shock expectancy ratings of CS+ were larger than those of CS-. The interaction effect of the learning type and the stimulus was also significant (η_p^2 = 0.182). Follow-up pairwise comparisons showed that the shock expectancy ratings of CS+ in direct learning were significantly larger than those of CS+ in observational learning, while the shock expectancy ratings of CS- in direct learning were significantly lower than those of CS- in observational learning (Figure 4E). However, the main effect of the learning type was not significant (η_p^2 = 0.031). In the P1 results, the main effect of the stimulus was significant (η_p^2 = 0.094), and CS+ evoked a significantly larger P1 amplitude than CS-. The main effect of the learning type was not significant. Moreover, the interaction effect of the stimulus and the learning type was not significant. Similar findings were shown in the LPP results. The main effect of the stimulus was significant (η_p^2 = 0.291). The LPP amplitudes of CS+ were significantly larger compared with those of CS-. The main effect of the learning type was not significant. The interaction effect of the stimulus and the learning type was also non-significant. These findings indicated that observational and direct learning showed similar P1 and LPP enhancement effects to the CS+.

In fear generalization, to reduce the number of generalization stimulus comparisons, GS1–GS3 were combined as GS+ and GS4–GS6 were combined as GS-. The repeated-measure ANOVA of shock expectancy ratings in fear generalization showed that the main effect of the stimulus (GS+ versus GS-) was significant (η_p^2 = 0.770) (GS+ > GS-) (Figure 6A). A significant interaction effect between the learning type and the stimulus was observed (η_p^2 = 0.069), and the pairwise comparison indicated that the shock expectancy ratings for GS- in the observational learning condition were significantly larger than those in the direct learning condition (Figure 6A). However, no significant main effect of the learning type was found. The independent sample t-test on the shock expectancy ratings for GS+ versus GS- by observational learning and direct learning showed that the GSs after observational learning had a significantly smaller shock expectancy rating difference (GS+ vs. GS-) compared with the GSs after direct learning (Figure 6B).

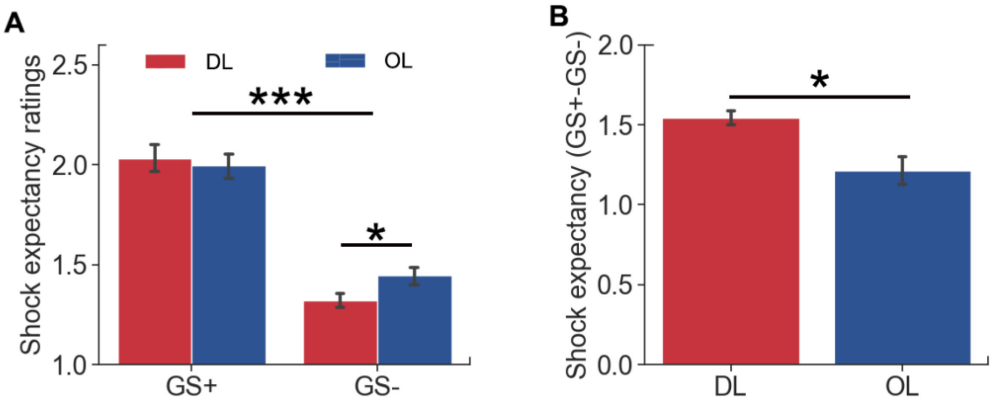

FIGURE 6 The shock expectancy ratings of the GS+ and GS- in **Study II**. A) Bar plot for shock expectancy ratings of the GS+ and GS-. The shock expectancy ratings of GS+ were higher than those of the GS-. The shock expectancy ratings of GS- after observational learning were higher than those after direct learning. B) Bar plot for the differences of shock expectancy ratings between GS+ and GS-. The differences of shock expectancy ratings between GS+ and GS- after direct learning were higher than those after observational learning. The red bar represents the direct learning condition, and the blue bar represents the observational learning condition. GS+ = generalization stimuli resembling conditioned stimuli paired with a shock. GS- = generalization stimuli resembling conditioned stimuli not paired with a shock. OL = observational learning. DL = direct learning. $^{**}p < 0.01$, $^{*}p < 0.05$.

To test the relationship between the acquisition phase and the generalization phase in the observational learning condition, Pearson's correlations were calculated among the shock expectancy ratings of the GS-, the CS- and the differences of CS+ and CS-. The results in the observational learning condition showed that the shock expectancy ratings of the GS- positively correlated with the shock expectancy ratings of the CS- but negatively correlated with the shock expectancy rating differences between CS+ and CS-. These results indicated that the increased shock expectancy ratings of GS- after observational learning are related to the increased shock expectancy ratings of the CS- and decreased differentiation of CS+ and CS- in observational fear learning.

In the EEG results of the P1 component, GS+ in the direct learning condition evoked significantly larger P1 amplitudes than GS- did. However, there were no significant differences between GS+ and GS- in the observational learning condition. Furthermore, GS+ in the direct learning condition evoked larger P1 amplitudes compared with GS+ in the observational learning condition (Figure 7A, B, C). For the LPP component, GS+ in the direct learning condition evoked significantly larger LPP amplitudes than GS- did. However, there were no significant differences between GS+ and GS- in the observational learning condition. Furthermore, GS+ in the direct learning condition evoked larger LPP amplitudes compared with GS+ in the observational learning condition (Figure 7A, D, E).

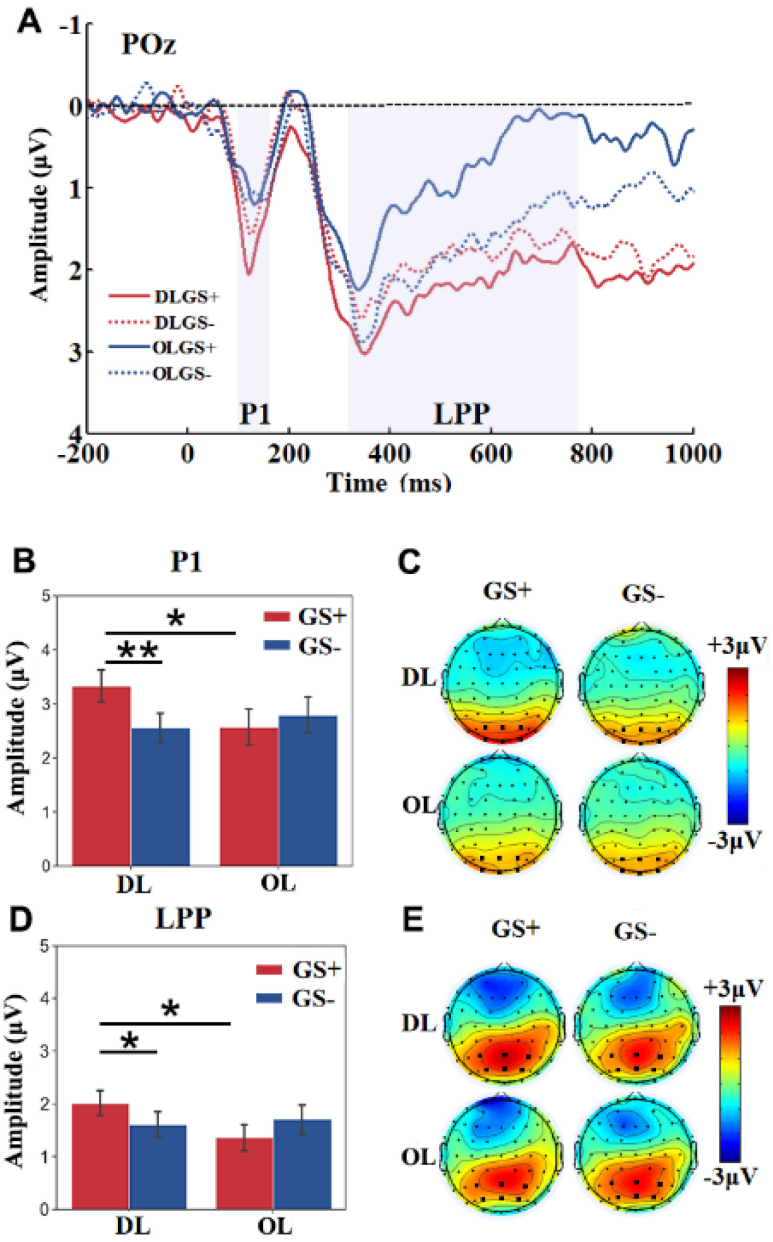

FIGURE 7 The ERP results of the P1 and LPP components in **Study II**. A) Waveforms of P1 and LPP at the POz electrode. The red line represents the direct learning condition, and the blue line represents the observational learning condition. The solid line represents GS+, and the dotted line represents GS-. C) and E) Topographic maps of the P1 and LPP components; the black dots mark the region of interest. B) and D) Bar plot of P1 and LPP amplitudes. The GS+ in direct learning evoked larger P1 and LPP amplitudes than the GS+ did after observational learning. The GS+ evoked larger P1 and LPP amplitudes than the GS- did after observational learning, but the GS+ showed no P1 and LPP differences with the GS- after observational learning. The error bar represents the standard error. LPP = late positive potential. GS+ = generalization stimuli resembling conditioned stimuli paired with a shock. GS- = generalization stimuli resembling conditioned stimuli not paired with a shock. OL = observational learning. DL = direct learning. $^{**}p < 0.01$, $^{*}p < 0.05$.

To explore a possible extinction effect of generalization stimuli in fear generalization, the experiment was divided into early and late phases based on the trial number (the first half of the trials were in the early phase, and the remaining half of the trials were in the late phase). Additionally, a repeated-measure ANOVA was adopted in the shock expectancy ratings. The within-subject variables were time (early-phase, late-phase), stimulus (GS+, GS-), and learning type (direct learning, observational learning). The results showed that the main effect of time and the interaction effects of time with other variables were not significant, which indicated that the extinction effect of the generalization stimuli was not obvious in the generalization phase.

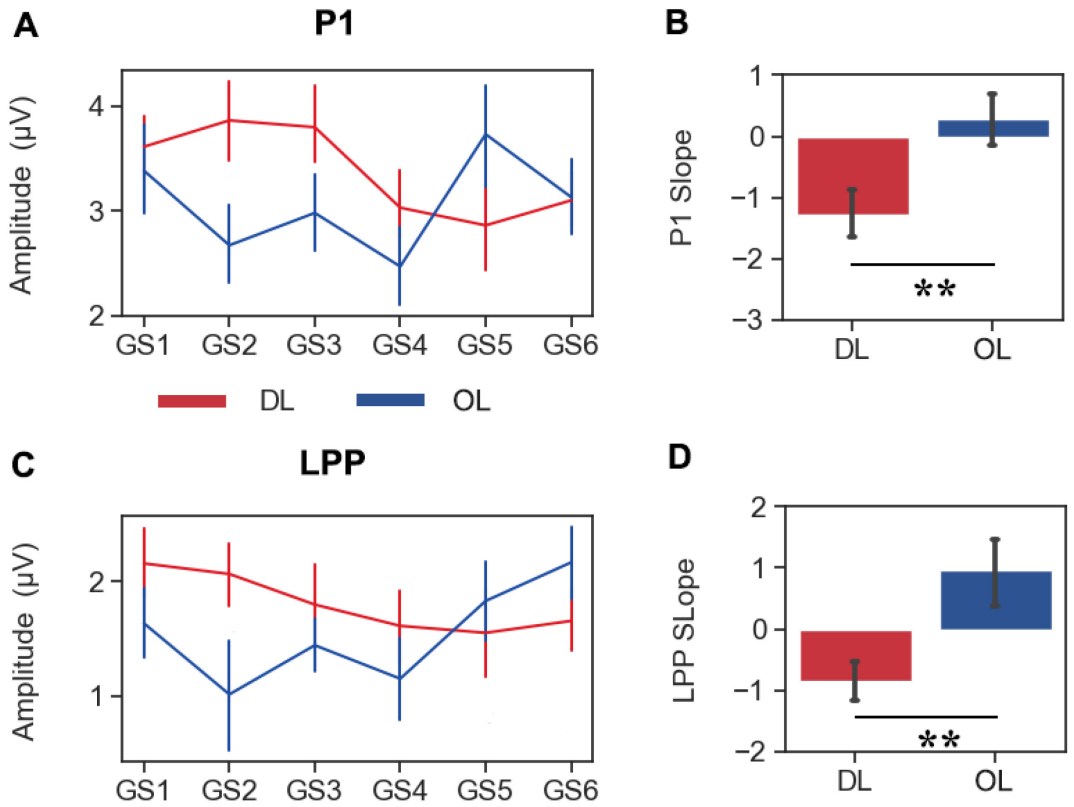

FIGURE 8 The ERP slope results in the generalization in **Study II**. A) Line plot of the P1 component in fear generalization, red line = direct learning, blue line = observational learning (GS1 is the most similar to CS+, CS6 is the most similar to CS-). B) Fear generalization slopes of the linear model fitting of P1 components. The fear generalization gradient of the P1 amplitudes after direct learning was steeper than that after observational learning (the more negative the slope is, the steeper the fear generalization gradient is). C) Amplitudes in LPP components. D) Fear generalization slopes of the linear model fitting of the LPP components. The fear generalization gradient of the LPP amplitudes after direct learning was steeper than that after observational learning. GS = generalization stimulus. LPP = late positive potential. DL = direct learning, OL = observational learning. $^{**}p < 0.01$, $^{*}p < 0.05$.

The results of the linear model fit in the behavioral data indicated that the slope of the shock expectancy ratings in the direct learning condition was significantly steeper than that in the observational learning condition. The

results of the linear model fit in the EEG data suggested that the slope of the P1 component in the direct learning condition was significantly steeper than that in the observational learning condition (Figure 8B). Consistent with the trend of P1, the slopes of LPP in the direct learning condition were significantly steeper than those in the observational learning condition (Figure 8D).

The relationship between fear generalization and anxiety was also found in **Study II**. The trait anxiety scores were positively correlated with the generalization slope of the shock expectancy ratings in the direct learning condition (Figure 9B), while the trait anxiety scores did not correlate with the generalization slope of the shock expectancy ratings in the observational learning condition. More interestingly, the social anxiety score (LSAS_fear: fear subscale of LSAS) was positively correlated with the generalization slope of the shock expectancy ratings in the observational learning condition (Figure 9A), while there was no significant correlation between the social anxiety scores and the generalization slope in the direct learning condition. The mediating effect analysis suggested that the pain sensitivity score mediated the effect of social anxiety on the fear generalization slope of the shock expectancy ratings (indirect effect = 0.0142; CI 95%: [0.0032, 0.0306]) (Figure 9C).

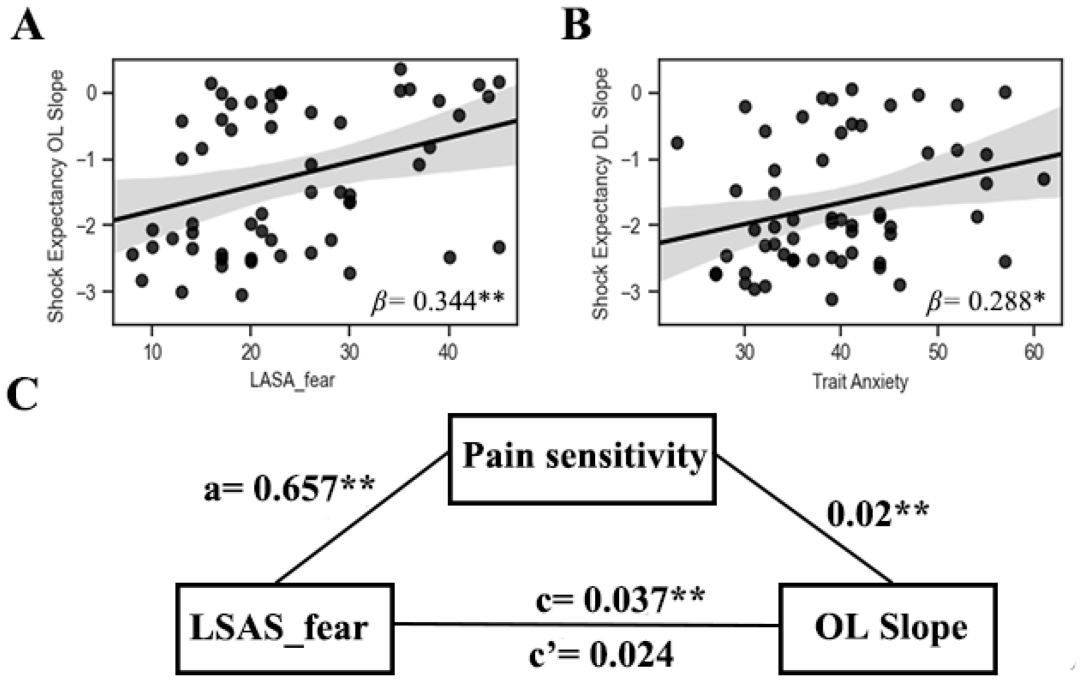

FIGURE 9 Regression model of fear generalization slope and anxiety in **Study II**. A) Social anxiety (LSAS_fear: fear subscale of Liebowitz Social Anxiety Scale) predicted the fear generalization slope of the shock expectancy ratings in the observational learning condition. B) Trait anxiety scores predicted the generalization slope of the shock expectancy ratings in the direct learning condition. β = regression coefficient. C) Pain sensitivity mediated the association between social anxiety scores and the generalization slope of shock expectancy ratings in the observational learning condition. DL = direct learning. OL = observational learning. c = total effect, c' = direct effect. $^{**}p < 0.01$, $^{*}p < 0.05$.

3.3 Study III: The impact of oxytocin on fear generalization

By comparing the behavioral and physiological responses of male adults with the Pavlovian fear conditioning paradigm, **Study III** examined whether intranasal oxytocin influenced fear generalization and the perceptual discrimination threshold. In fear learning, the repeated-measure ANOVA results of the shock expectancy ratings in fear learning showed that the main effect of the stimulus (CS+ versus CS-) was significant ($\eta_p^2 = 0.871$) (CS+ > CS-) (Figure 4G); the main effect of the material (face versus circle) was also significant ($\eta_p^2 = 0.158$) (face > circle). However, the main effect of the group ($\eta_p^2 = 0.000$) (oxytocin versus placebo), the interaction effect between the group and the stimuli ($\eta_p^2 = 0.006$), the interaction effect between the group and the material ($\eta_p^2 = 0.039$), and the interaction effect among the group, the material, and the stimulus ($\eta_p^2 = 0.007$) were not significant. Furthermore, the SCRs in fear learning showed similar results compared with the shock expectancy ratings. Specifically, there was a significant main effect of the stimulus ($\eta_p^2 = 0.195$) (CS+ > CS-) (Figure 4I), while there were no other significant results of the main effects or the interaction effects among the stimulus, the material, and the group variables. After fear learning, the valence, arousal, and attractiveness ratings showed a significant main effect of the stimulus (valence: CS+ < CS-; arousal: CS+ > CS+; attractiveness: CS+ < CS-) and the material (valence: face < circle; arousal: face > circle; attractiveness: face > circle), while there were no significant main effects or interaction effects among the stimulus, the material, and the group variables. Taken together, the results indicated that the manipulation of fear conditioning was successful, and the baseline values between the oxytocin and the placebo groups before the administration of intranasal oxytocin were not significantly different.

In fear generalization, the repeated-measure ANOVA of the shock expectancy ratings showed that the main effect of the stimulus (CS+, GS1, GS2, GS3, GS4, CS-) was significant ($\eta_p^2 = 0.816$). The pairwise comparisons indicated that the shock expectancy ratings of the CS+ were significantly larger than those for the other stimuli. The shock expectancy ratings of the GS1 were significantly larger than those of the GS2–GS4 and CS-. The shock expectancy ratings of GS2 were significantly larger than those of the GS3, GS4, and CS-, which indicated that the participants produced more shock expectancy to the GS2 compared with the CS-. The shock expectancy ratings of GS3 were significantly larger than those of the GS4 and CS-. The main effect of the group was also significant ($\eta_p^2 = 0.102$) (oxytocin < placebo). A significant interaction effect of the group and the stimulus was observed ($\eta_p^2 = 0.067$). Follow-up pairwise comparisons indicated that the shock expectancy ratings for CS+ and GS1 in the oxytocin group were significantly lower than those in the placebo group (Figure 4H). However, no other significant main effects or interaction effects among the material, the group, and the stimulus were observed. Taken together, oxytocin reduced the shock expectancy ratings to the CS+ and GS1 in fear generalization.

The SCR results in fear generalization were consistent with the results of the shock expectancy ratings. Specifically, the main effect of the stimulus in SCR was significant. The pairwise comparisons indicated that the SCR of CS+ was significantly larger than that of GS2, GS3, GS4, and CS-. The SCR of GS1 was significantly larger than that of GS1 and CS-. The SCRs of the GS2 had no significant differences between the SCRs of GS3, GS4, and CS-, which indicated that the GS2 did not evoke larger SCRs compared to the CS-. The main effect of the group was marginally significant (η_p^2 = 0.052) (oxytocin < placebo). A significant interaction effect between the group and the stimulus was observed (η_p^2 = 0.069). The follow-up comparisons showed that the SCRs to CS+ and GS1 in the oxytocin group were significantly smaller than those in the placebo group (Figure 4J). The SCR to GS2 in the oxytocin group was marginally significantly smaller than that in the placebo group. However, there were no other significant main effects or interaction effects among the material, the group, and the stimulus on SCR. The emotional ratings (valence and arousal) and attractiveness after fear generalization only showed a significant main effect of GS; there was no significant main effect of the group or an interaction effect between the group and the stimulus (Figure 10C, D, E). Taken together, oxytocin reduced the SCRs to the CS+ and GS1 in fear generalization.

The repeated-measure ANOVA of the discrimination threshold suggested that the main effect of the material (face versus circle) was significant (JND: face > circle); a significant main effect of time (pre-test, post-test) was observed as well (JND: pre-test > post-test) (Figure 10B). The main effect of the group, the interaction effect of the group and time, and the interaction among the group, time, and the material were not significant. The maximum value of the JND in each condition of all participants was 34.81%. Taken together, oxytocin did not modulate the discrimination threshold.

To explore the extinction effect of GS1, the repeated-measure ANOVA was applied for the shock expectancy ratings and SCR. The within-subject variable was time (Block1, Block2, Block3) and the between-subject variable was group (oxytocin, placebo). The shock expectancy ratings showed that the main effect of time was significant (Block1 > Block2, Block3). The main effect of group was significant (oxytocin< placebo). The interaction effect of Time and group was not significant. For SCRs, the main effect of group was significant (oxytocin< placebo). The main effect of time was not significant and the interaction effect of time and group was non-significant. Taken together, the oxytocin did not modulate the extinction effect of GS1 neither as indexed by shock expectancy ratings nor SCRs.

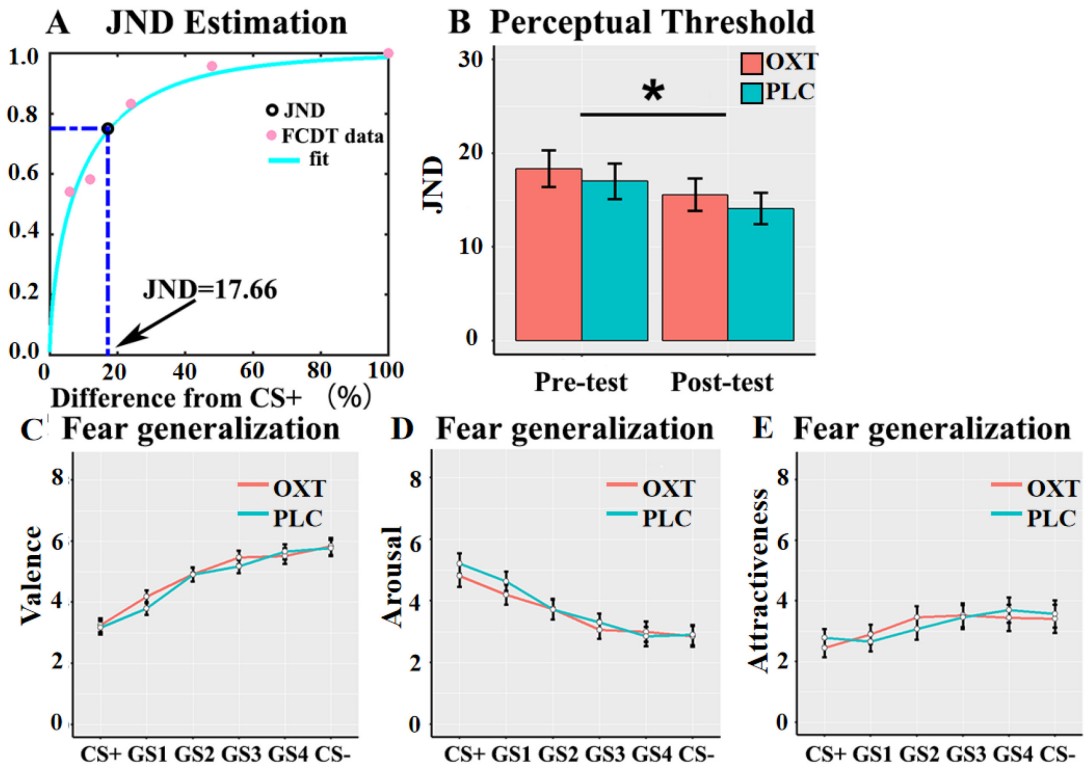

FIGURE 10 Discrimination threshold and emotional and attractiveness ratings in **Study III**. A) Example of the estimation of just noticeable difference in one participant. B) Perceptual threshold between the oxytocin and placebo groups during pre-test and post-test. There is no effect of oxytocin on the perceptual threshold. C) and D) Line plot of the emotional ratings in the fear generalization of the oxytocin and placebo groups. E) Line plot of the attractiveness rating of the oxytocin and placebo groups in fear generalization. There were no effects of oxytocin on the emotional ratings and attractiveness ratings. JND = just noticeable difference. FCDT = forced-choice discrimination task. OXT = oxytocin. PLC = placebo. GS = generalization stimulus. CS+ = conditioned stimulus paired with a shock. CS- = conditioned stimulus not paired with a shock. *$p < 0.05$. The figure is adapted from Dou et al. (2021).

4 DISCUSSION

This dissertation has investigated how two social factors, social exclusion (**Study I**) and observational learning (**Study II**), affect fear learning and generalization, and how oxytocin, a hormone related to social relationships, affects fear generalization (**Study III**).

Study I investigated the effects of an experimentally evoked social exclusion on behavioral responses, skin conductance responses (SCRs), and oxygenated hemoglobin concentration in the medial prefrontal cortex (mPFC) in the fear learning and generalization task. In fear learning, social exclusion impaired differential learning (CS+ minus CS-) as indexed by the shock expectancy ratings and the mPFC activities and enhanced SCRs to CSs compared to social inclusion. In fear generalization, the GSs evoked higher shock expectancy ratings and higher SCRs in the social exclusion group compared with the social inclusion group. Social exclusion impaired the function of mPFC in fear generalization compared with social inclusion. **Study I** provided the first evidence of the influence of social exclusion on fear learning and generalization and the underlying neural correlates.

Study II examined the effects of direct and observational learning on the shock expectancy ratings and ERP components elicited in fear learning and generalization. In fear learning, the shock expectancy results indicated that observational learning reduced differential fear learning (CS+ minus CS-) compared with direct learning. The EEG results indicated that the CS+ elicited larger P1 and LPP amplitudes than the CS- in both direct and observational learning. In fear generalization, observational learning led to increased shock expectancy ratings for the GS resembling the safety stimulus compared with direct learning. The GS resembling the threatening stimulus evoked larger P1 and LPP amplitudes than the GS resembling the safety stimulus after direct learning but not after observational learning. **Study II** uncovers the behavioral and neural responses to fear generalization when the fear experiences are learned from observation.

Study III investigated the effects of intranasal oxytocin administered to participants after fear learning on the behavioral and physiological responses recorded during fear generalization. The participants in the oxytocin group

showed reduced fear responses, as reflected by their decreased shock expectancy ratings and attenuated SCRs in fear generalization compared with the participants in the control group. However, the just noticeable difference indexing the discrimination threshold in the oxytocin groups showed no difference from that in the placebo group. The results of **Study III** contribute to a better understanding of intranasal oxytocin's effect on fear generalization.

Next, I will discuss the findings of each study in detail.

4.1 Social exclusion impairs fear learning and increases fear generalization

In **Study I**, in agreement with the hypothesis, social exclusion impaired fear learning. Specifically, participants in the social exclusion group showed lower shock expectancy ratings to the CS+ but higher shock expectancy ratings to the CS- compared with the participants in the social inclusion group. Moreover, the shock expectancy rating differences between the CS+ and CS- in the social exclusion group were lower than those in the social inclusion group indicating that social exclusion reduces differential learning (reflected by the differences between responses to CS+ versus CS-). Previous studies have provided evidence that social exclusion can impair cognitive performance and working memory (Baumeister et al., 2002; Cacioppo & Hawkley, 2009) and that both of them can contribute to fear learning (Carter et al., 2003; Kaufman et al., 2009). For cognitive performance, previous studies found that social exclusion reduced participants' intelligence quotient (IQ; Baumeister et al., 2002; Twenge & Baumeister, 2005), and IQ has been reported to affect fear learning (Kaufman et al., 2009). Working memory is termed as "systems that are assumed to be necessary in order to keep things in mind while performing complex tasks such as reasoning, comprehension and learning" (Baddeley, 2010). Previous studies have found that social exclusion reduced working memory performances (Fuhrmann et al., 2018; Xu et al., 2018) and that reduced working memory performances, in turn, impaired fear learning (Carter, 2003). Thus, it is possible, that our result showing that social exclusion reduces fear learning might be due to decreased cognitive performance and/or impaired working memory function in the socially excluded participant group.

The skin conduction response results of fear learning in **Study I** showed that social exclusion increased the SCRs to the CS+ and the CS- compared with social inclusion. A previous study reported that social exclusion showed higher skin conductance levels (a slow tonic modulation of sympathetic arousal) compared with social inclusion (Kelly et al., 2012). Thus, it is possible that the increased SCRs to the CSs in **Study I** might be due to the increased skin conductance levels after social exclusion.

In addition to the behavioral results, the results of mPFC in fear learning showed that the CS+ of the social inclusion group evoked an enhanced activa-

tion in the left mPFC compared with CS-, while no such difference was observed in the social exclusion group. The findings in the social inclusion group in **Study I** were in line with a previous study in which the researchers found that CS+ in fear learning enhanced the oxy-Hb concentration in the mPFC (Guhn et al., 2012). In **Study I**, the mPFC activity for the CS+ and the CS- did not differ in the social exclusion group, which indicated that social exclusion might impair differential learning (CS+ minus CS-). Previous studies have also reported that the mPFC lesion evoked reduced differentiation between the CS+ and CS- in fear learning (Frysztak & Neafsey, 1994; Lee & Choi, 2012; Powell et al., 1994). Furthermore, the mediation effect analysis showed that the mPFC activity partially mediated the influence of social exclusion on the shock expectancy ratings of the CS+ in fear learning. The fact that the mediation effect was partial reflected that the effect of social exclusion on the shock expectancy ratings of the CS+ is also influenced by some factors such as the activity of other brain regions or non-brain-related factors.

In fear generalization, the participants in the social exclusion group perceived more shock risk from the GSs than the participants in the social inclusion group. This result was in line with previous findings showing that individuals suffering from social exclusion are more likely to interpret ambiguous situations as threats than those individuals who are not excluded (Zadro et al., 2006). Similar to the shock expectancy results, social exclusion enhanced the SCRs to the GSs compared with social inclusion. Social exclusion has been reported to increase stress in previous studies (Beekman et al., 2016; Blackhart et al., 2007). The findings of the SCRs in **Study I** were in line with the effects of stress on fear generalization (Kausche et al., 2021b), that is, the stress group demonstrated higher SCRs compared with the control group. Furthermore, the slope of fear generalization in the mPFC of the inclusion group was significantly steeper than that of the exclusion group. Specifically, in the inclusion group, the more similar to the CS+ the GSs were, the greater mPFC activities the GSs evoked, while this effect was absent in the social exclusion group (**Study I**). This finding is in line with the results of a study investigating the effect of stress on fear generalization (Kausche et al., 2021a). The researchers found that the stress group showed a more gradual generalization gradient of vmPFC than the control group (Kausche et al., 2021a). It is possible that the effects of social exclusion on the generalization gradient of the mPFC were due to stress.

Another potential mechanism for impaired fear learning in the social exclusion group is increased anxiety. Even though I did not measure anxiety directly, the results of the positive and negative affect schedule (PANAS) scale showed that the worry and nervousness ratings increased after social exclusion (but not after social inclusion). Considering that the concepts of worry and nervousness are closely related to anxiety (Borkovec et al., 1982; Hoyer et al., 2001; Mathews, 1990), the increased anxiety state might have led to an increase in the fear responses to the CS-. This is supported by a study that investigated how anxiety influenced fear learning (Liao & Craske, 2013). The authors found that state anxiety increased the fear responses to the safety stimuli in fear learn-

ing. State anxiety evoked by social exclusion might also influence the generalization phase. In fear generalization, the participants in the social exclusion group perceived more shock risk and showed larger SCRs to the GSs compared to the social inclusion group. This result is similar to a previous study (Xu et al., 2016). The researchers reported that the experimentally evoked state anxiety increased shock expectancy ratings and SCRs to the generalization stimuli (Xu et al., 2016). Furthermore, the slope of fear generalization in the mPFC of the inclusion group was significantly steeper than that of the exclusion group (**Study I**). This result is similar to findings in anxiety disorders; that is, patients with generalized anxiety disorder showed a decreased slope of fear generalization gradient in the vmPFC region compared with healthy controls (Cha et al., 2014).

4.2 Observational learning reduces fear learning and increases fear responses to safety generalization stimuli

In **Study II**, the behavioral results of fear learning showed that observational learning exhibited lower shock expectancy ratings to the CS+ and higher shock expectancy ratings to the CS- compared with direct learning. The shock expectancy rating differences between the CS+ and the CS- in observational learning were smaller than those in direct learning, indicating that observational learning reduced differential learning (the CS+ minus the CS-) compared with direct learning. In line with these findings, one previous study (Egorova et al., 2015) also found that observational learning evoked a smaller difference of SCRs between objects paired with high- or low-intensity threats compared with direct learning. Here, in fear generalization, the finding that after observational learning, the participants perceived more shock expectancy for the GS- relative to direct learning support my hypothesis. Previous studies have shown that the differentiation of the CS+ and CS- in fear learning affects subsequent fear generalization (Dunsmoor & LaBar, 2013; Hanson, 1957; Struyf et al., 2015). Also, in **Study II**, observational learning might have increased the shock expectancy to GS- in fear generalization due to reduced differential learning. This is because I found that the smaller the shock expectancy differences between CS+ and CS- in observational learning were, the higher the shock expectancy ratings of the GS- in fear generalization participants had.

The results of the early ERPs showed that the CS+ in both direct learning and observational learning evoked larger P1 than the CS-. These results are consistent with previous findings of direct fear learning (Bublatzky & Schupp, 2012; Dolan et al., 2006; Linton & Levita, 2021; Pizzagalli et al., 2003). For instance, Pizzagalli et al. (2003) adopted facial stimuli as CSs and found P1 enhancements from the threat CSs. The P1 component has been reported to reflect early threat discrimination (Forscher et al., 2016; Linke et al., 1999; Meynadasy et al., 2019; Sperl et al., 2021; Thorpe, 2009; Wieser & Keil, 2020). Thus, the findings of the P1 component in fear learning of **Study II** showed no

differences between the learning types in the early threat discrimination. In the generalization phase of **Study II**, the GS+ evoked higher P1 amplitudes than the GS- after direct learning, indicating that generalization stimuli resembling threatening stimuli are successfully discriminated from generalization stimuli resembling the safety stimuli in the early time window (100–150 ms). Only one previous MEG study has reported early electrophysiological responses of GSs (Roesmann et al., 2020). The authors found that GS+ evoked a larger P1 component compared with the GS- in the occipito-temporal region with a time window of 107–147 ms. Moreover, the results in **Study II** showed that the enhancement of P1 to the GS+ was not found after observational learning compared with the GS-, indicating that GS+ might not be discriminated from GS- after observational learning in the early time window (100–150 ms). Additionally, the slope analysis results of the P1 component in **Study II** showed that after direct learning, the more similar to the CS+ the GS is, the higher P1 amplitude it evoked, but this trend was absent after observational learning. The slope analysis results of the P1 component in fear generalization after direct learning were in line with previous findings (Frenkel & Bar-Haim, 2011; Meynadasy et al., 2019). For instance, Meynadasy et al. (2019) adopted five morphed (2–45%) ambiguously emotional faces showing expressions ranging from neutral to fearful and found that regarding the morphs with 21% or higher similarity, the more fearful the morph was, the larger P1 amplitude it evoked. Of course, the slope analysis results of P1 needed to be interpreted cautiously. This is because the trial number of each generalization stimulus (GS1–GS6) in **Study II** was only 10, which might influence the accuracy of the ERPs.

Regarding the results of the late ERPs, the LPP amplitudes in fear learning were enhanced to the CS+ compared with the CS- in both direct and observational learning. The same has been found for direct fear learning in several previous studies (Dolan et al., 2006; Panitz et al., 2015; Pizzagalli et al., 2003). For instance, Panitz et al. (2015) adopted facial stimuli as CSs and found that the CS+ evoked larger LPP amplitudes compared with the CS-. In fear learning, previous studies have provided evidence that LPP reflects motivated attention (Ferreira et al., 2019; Schupp et al., 2005), which is also a plausible interpretation here. In fear generalization, I found an LPP enhancement to the GS+ compared with the GS- after direct learning, which is in line with previous findings showing that the GS+ evoked a larger LPP component than the GS- (Nelson et al., 2015; Roesmann et al., 2020). The LPP in the fear generalization task also reflects motivated attention toward GS stimuli (Nelson et al., 2015; Roesmann et al., 2020). Specifically, GS+, which is similar to threat stimuli, might be endowed with motivational significance in a strategy called "better safe than sorry" (it is better to generalize fear to a range of stimuli in order to avoid a real threat) (Forbes et al., 2011). Attentional resources might be allocated to the GS+ with motivated significance for a long time (Lang et al., 1997; Schupp et al., 2005). However, here, the effect of LPP enhancement from the GS+ was not found after observational learning, indicating that the motivated attention might not be attracted by the GS+ in fear generalization

after observational learning. This finding is also supported by the slope analysis results of the LPP. The slope analysis showed that after direct learning, the more similar to the CS+ the GS is, the higher the LPP amplitude it evoked, but this trend was absent after observational learning. In general, the ERP findings for both the early and late stages (P1 and LPP, respectively) of the fear learning and generalization in the parieto-occipital regions are in accordance with the multiple-wave model (Pessoa & Adolphs, 2010, 2011), which states that the visual information in the parieto-occipital regions is activated several times in order to evaluate the biological significance of the visual threat stimuli.

The relationship between anxiety and fear generalization was also investigated in **Study II**. The social anxiety scores (fear subscale of the Liebowitz social anxiety scale) predicted the fear generalization slope of the shock expectancy ratings after observational learning. The higher social anxiety scores the participant had, the more easily fear responses transformed to generalization stimuli that were similar to CS- after observational learning. There might be at least two explanations for this finding. One is that the participants with higher social anxiety felt more state anxiety in the real social context the observational learning exposed them to than those with less social anxiety (Shimizu et al., 2011), and the increased anxiousness increases fear responses to CS-. Although the anxious state was not measured in **Study II**, a previous study found that the anxious state reduced fear inhibition to safety stimuli in fear learning (Liao & Craske, 2013). The increased fear responses to the CS- evoked a more gradual generalization gradient, which increased fear to the GS- (Duit et al., 2015). However, there is another way to interpret the relationships between social anxiety and fear generalization after observational learning. In **Study II**, the pain sensitivity scores (tested through the pain sensitivity questionnaire) mediated the relationship between social anxiety and fear generalization slope after observational learning. This mediation effect of pain sensitivity might be interpreted as follows. First, the results of **Study II** showed that the social anxiety scores positively correlated with the pain sensitivity scores. In line with this finding, previous studies have also found that anxiety has a significant influence on pain sensitivity (Failla et al., 2020; Kleiman et al., 2011; Vassend et al., 2011; Von Graffenried et al., 1978). Moreover, Asmundson and Carleton (2005) also found that injury sensitivity (testing the fear of injury) was positively correlated with social anxiety symptoms. Second, in **Study II**, higher pain sensitivity predicted higher fear responses to the generalization stimuli resembling safety stimuli after observational learning. The participant with higher pain sensitivity might have increased fear of pain when they are observing other participants who receive electric shocks in observational learning. Although the fear of pain in observational learning was not tested, a previous study reported that pain sensitivity positively correlated with fear of pain (Horn et al., 2014). Moreover, shock expectancy ratings are subjective ratings of threat anticipation (Weike et al., 2007), which is closely related to fear of pain (Yang et al., 2016). Therefore, it can be expected that increased fear of pain during observational learning can enhance the shock expectancy to the generalization stimuli resembling the

safety stimuli. Future studies are needed to clarify the details of the mediation effect of pain sensitivity on how social anxiety influences fear generalization after observational learning.

In the generalization phase of **Study II**, only the last trial of the CS+ was paired with a shock. The aim of this design was to reduce the effect of direct learning experience on fear generalization (see also Pärnamets et al., 2020). However, this manipulation that a shock was only paired with the last trial of the CS+ might have allowed the GSs to produce fear extinction. Thus, the generalization differences between observational learning and direct learning might be due to the different extinction effects between observational learning and direct learning. In order to exclude the possibility that observational learning affects generalization by modulating fear extinction in the generalization phase, the extinction effect on the shock expectancy ratings in the generalization phases were calculated in **Study II**. The shock expectancy results showed that the extinction effect was not different between the generalization stimuli after direct learning and observational learning. Thus, the difference between the generalization stimuli after direct and observational learning might not be due to the difference of extinction effect from direct and observational learning. Of note, the CSs (CS+ and CS-) in fear generalization were analyzed in **Study I**, however, the CSs in the generalization phase of **Study II** were not applied in the final data analysis. This is because the number of the trials of the CSs is larger than the number of the other GSs in fear generalization in **Study II**. The larger number of the CSs than the GSs might make the CSs produce more extinction effects compared with the GSs, which finally influences the analysis for the shock expectancy and ERPs in **Study II**. Future studies are recommended to avoid this issue (different trial numbers between each CS and each GS).

4.3 Oxytocin reduces fear generalization

In **Study III**, the most important result was that a dose (24 IU) of oxytocin reduced the shock expectancy and SCRs to the GSs. This finding was expected because oxytocin has been found to inhibit fear responses (Viviani et al., 2011), and the participants who were administered oxytocin perceived the ambiguously fearful stimuli as more neutral (Spengler et al., 2017).

Previous research found that Pavlovian fear conditioning and generalization were mediated by the prefrontal-limbic circuit (Dunsmoor et al., 2011a). Kirsh et al. (2005) found that intranasal oxytocin could modulate the prefrontal-limbic circuits by decreasing the amygdala reactivity to fearful faces. In agreement with the effect of oxytocin on fear generalization, Quintana et al. (2015) also reported that intranasal oxytocin reduced the anger ratings of ambiguous faces. Eckstein et al. (2017) reported that oxytocin decreased amygdala activity and enhanced the functional connectivity between the amygdala and the mPFC. The potential functions of the mPFC in fear learning and generalization are related to fear inhibition and fear discrimination

(Likhtik et al., 2014; Motzkin et al., 2015; Pollak et al., 2010). Based on this, I further tested whether the discrimination thresholds mediated the association of oxytocin with fear generalization. The results of the discrimination thresholds in the post-test showed no significant difference between the oxytocin and the placebo groups, indicating that oxytocin did not modulate the discrimination thresholds. This result is inconsistent with the hypothesis that oxytocin would reduce the discrimination thresholds. Some previous studies have found that oxytocin increased fear discrimination (Olivera-Pasilio & Dabrowska, 2020) or facilitated differential learning (Eckstein et al., 2015). However, Domes et al. (2013) adopted a discrimination task and administered oxytocin or saline to participants. They found no difference in the discrimination ability between the oxytocin and the placebo groups, which is similar to our finding. The inconsistent results of the oxytocin effects on differential learning and discrimination thresholds might be due to the different experiment contexts (Struyf et al., 2015). Specifically, the differentiation of the CS+ and CS- is tested in a threatening context (aversive stimuli presented), but perceptual discrimination is tested in a safety context (no aversive stimuli presented). Oxytocin is sensitive to threatening context (Lischke et al., 2012), which might modulate differential learning and discrimination thresholds differently. Furthermore, previous studies have examined the relationships between fear generalization and discrimination thresholds (Holt et al., 2014; Tuominen et al., 2019). For example, Holt et al. (2014) found that fear generalization (as indexed by SCR) occurred only when the stimuli were presented under the discrimination thresholds. Thus, the potential pathway by which oxytocin would inhibit fear transmission to other objects by reducing the discrimination threshold seems not to be feasible.

In addition to the discrimination thresholds, some other interpretations of how oxytocin reduced the fear responses to the generalization stimuli, have also been ruled out in previous studies. First, oxytocin facilitates the extinction of fear responses to conditioned stimuli (Eckstein et al., 2015; Hu et al., 2019). For instance, Eckstein et al. (2015) reported that oxytocin decreased the SCRs and amygdala activities to the conditioned stimuli in the late stage of fear extinction. Oxytocin might reduce the fear responses to generalization stimuli by increasing fear extinction. However, in **Study III**, the results of GS1 in both shock expectancy ratings and SCRs showed that the extinction effect and the interaction effect between extinction and group were not significant, which indicated that there was no extinction effect for GS1. Thus, the potential pathway by which oxytocin reduced fear generalization through facilitating extinction might be ruled out. Second, oxytocin has been found to increase the trustworthiness and attractiveness of unfamiliar faces (Striepens et al., 2014; Theodoridou et al., 2009). The materials used in **Study III** included female faces. The male participant in the oxytocin group might reduce the fear responses to generalization stimuli by finding the female faces more attractive. However, the results showed that oxytocin did not influence the attractiveness ratings of the facial stimuli. Thus, the interpretation that oxytocin affected fear generalization by modulating attractiveness is not plausible.

Although the shock expectancy for GSs was larger for the control group than the oxytocin group, the valence and arousal ratings of GSs after fear generalization did not show any differences between the oxytocin and the placebo groups. A possible reason for the inconsistent results between these measures is that SCRs reflect the activities of the autonomous nervous system (Mackersie & Calderon-Moultrie, 2016), while the valence and arousal ratings of the stimuli are based on conscious ratings of the GSs. This finding indicates that oxytocin might not modulate the valence and arousal ratings of the GSs. Another possible reason is that the valence and arousal ratings were completed after the generalization phase, resulting in the emotional experience no longer being the most intensive. Lastly, **Study III** found no significant difference between the social and non-social GSs in both the oxytocin and placebo groups. According to previous studies, whether oxytocin specifically influences generalization when social materials are applied is still unclear. Some researchers found that the oxytocin modulated the social and non-social materials differently (Gorka et al., 2015; Petrovic et al., 2008; Xu et al., 2019), but many other researchers have found that oxytocin shows a similar effect between the social and non-social materials on emotional processing, such as fear extinction (Eckstein et al., 2015; Harari-Dahan & Bernstein, 2014; Onaka et al., 2012; Yao et al., 2018; Yoshida et al., 2009).

4.4 General discussion

In this dissertation, I found that social exclusion (**Study I**) and observational learning (**Study II**) modulate fear acquisition. The reduced differential learning in the acquisition phase influenced the following generalization phases in **Studies I** and **II**. These findings indicate that the generalization was based on the experiences established in the acquisition phase. These findings were consistent with the previous animal and human studies finding that the differentiation of CS+ and CS- in the acquisition phase modulates the subsequent generalization gradients (Dunsmoor & LaBar, 2013; Hanson, 1957). Could the oxytocin affect the generalization phase without modulating the acquisition phase? Here, in **Study III**, oxytocin was administrated to the participants after fear acquisition. Fear acquisition showed no differences between the oxytocin and placebo groups at baseline, but oxytocin modulated fear generalization, which indicates that oxytocin can influence fear generalization without modulating fear acquisition.

The debate over whether stimulus generalization is independent of the perceptual discrimination ability of the generalization stimuli has been argued for nearly a century (for a review, see Struyf et al., 2015). Some findings support the stimulus similarity perspective that the responses to generalization stimuli produce a generalization gradient based on the stimulus similarity, and that generalization is independent of discrimination (Pavlov, 1927; Roesmann et al., 2020). However, some other researchers consider generalization as a byproduct

of a failure of discrimination (Lashley & Wade; 1946; Slivinske & Hall, 1960). In all experiments of this dissertation, the shock expectancy ratings for the generalization stimuli showed generalization gradients in which the more similar they were to CS+, the more shock risk participants expected. Furthermore, in **Study III**, the perceptual discrimination threshold test was conducted before and after the fear learning and generalization. The difference in the GS2 to the original CS+ was 40% and the difference in the GS3 to the original CS+ was 60%. The maximum value of the discrimination threshold among all the conditions and participants was 34.81%, which suggests that the participants might have been able to discriminate between the CS+ and the GS2 (the CS+ and the GS3). The shock expectancy results in **Study III** showed that the GS2 evoked larger shock expectancy ratings than the CS- and that the GS3 evoked larger shock expectancy ratings than the CS- did. The shock expectancy ratings support that generalization is independent of discrimination. However, the SCR results in **Study III** showed that the SCRs to the GS2 showed no difference from the SCRs to the CS- and that the SCRs to the GS3 showed no difference from the SCRs to the CS-, which supports that generalization is a byproduct of a failure of discrimination. The results showed that the shock expectancy ratings were more sensitive to fear generalization compared with the SCR, consistent with the findings of previous studies (Holt et al., 2014; Tuominen et al., 2019). Therefore, the findings in this dissertation generally support the stimulus similarity perspective.

The inconsistent results of the subjective ratings and the SCRs to fear generalization were found in **Studies I** and **III.** In **Study I**, social exclusion evoked larger SCRs but lower shock expectancy ratings to the CS+ in fear learning compared with social inclusion. In **Study II**, the results of shock expectancy indicated that observational learning reduced differential learning (CS+ minus CS-) compared with direct learning, but the ERP results (P1 and LPP) indicated that observational learning showed no difference in differential learning compared with direct learning. In **Study III**, oxytocin reduced the SCR and shock expectancy ratings to the generalization stimuli, but did not affect the subjective affective ratings to the generalization stimuli. These seemingly inconsistent patterns of results might be due to the different brain systems affecting of the outcome measures. The SCRs reflecting the autonomic nervous system activity are enhanced by CS+ and are mediated by the limbic and brainstem regions (Becker et al. 2012). The shock expectancy ratings mainly include risk assessments and threat predictions and are mediated by certain prefrontal regions, such as orbitofrontal cortex (Kirlic et al. 2017; Nitschke et al. 2006). The subjective affective ratings (valence and arousal) are related to affective evaluation involving the dorsolateral and the ventrolateral prefrontal cortex (Dolcos et al., 2004). The ERPs of the P1 and LPP components are involved in the early visual and attentional responses to the CS+ and GS+ in the parieto-occipital cortex (Bublatzky & Schupp, 2012; Nelson et al., 2015). More research is needed to investigate why the different measures show somewhat different patterns of results for fear learning and generalization.

Fear learning has been regarded as an effective laboratory model to study the pathogenesis of anxiety disorders for nearly a century (Duits et al., 2015; Gorman et al., 2000; Watson & Rayner, 1920). According to the findings of two meta-analysis studies in anxiety disorders, the most robust finding is that anxiety patients show a failure of inhibiting fear responses to safety stimuli (CS-) in fear learning compared with healthy participants (Duits et al., 2015; Lissek et al., 2005). Recently, increasing evidence has supported that the failure of inhibiting fear responses to safety stimuli in anxiety disorder is due to excessive fear generalization (Duits et al., 2015; Gazendam et al., 2013). Anxiety disorder patients transform more threat from the CS+ to GS compared to the healthy controls, which is regarded as overgeneralization. Overgeneralization is a key diagnostic feature of a range of anxiety-related disorders, particularly generalized anxiety disorder (Lissek et al., 2014; Greenberg et al., 2013) and panic disorder (Lissek et al., 2009). Considering that excessive fear generalization has been regarded as a key diagnostic feature in anxiety disorders (for a review, see Dunsmoor & Paz, 2015), a method that can decrease the fear responses to generalization stimuli might be beneficial for the intervention of anxiety disorders. In **Study III**, the oxytocin decreased fear responses to generalization stimuli, which might provide two potential possibilities for the intervention of anxiety disorders. First, intranasal oxytocin might have a therapeutic effect on the disorders associated with excessive fear generalization. Previous findings have shown, indeed, that intranasal oxytocin could reduce the anxiety symptoms (e.g., overgeneralization) in patients with an anxiety disorder (for a review, see Naja & Aoun, 2017). Second, previous studies have shown that oxytocin can also be released with close interactions (e.g., spouse, parent-infant) (Scatliffe et al., 2019; Scheele et al., 2012). Therefore, anxiety symptoms (e.g., overgeneralization) in the anxiety disorders might also be reduced if possibilities for close interactions are improved, but further research is needed on this topic.

There are some limitations in this dissertation. First, the amygdala and the hippocampus are important brain regions in fear learning and generalization (Phillips & LeDoux, 1992). However, the fNIRS and EEG methods applied in this dissertation cannot record signals from these areas very well. Future studies could adopt other neuroimaging methods to record the signals from the subcortical structures in fear learning and generalization, such as fMRI. Second, the age ranges of the participants in all three studies were circumscribed as most of the participants (18-25 years old) were young adults who were undergraduate or graduate students. Therefore, the results cannot necessarily be generalized to all age groups. Moreover, **Study III** recruited only male participants. The accumulating evidence suggests that the effect of oxytocin on humans shows a gender difference during social threat perception and interaction (Gao et al., 2016; Luo et al., 2017). The results of **Study III** cannot necessarily be generalized to both genders. Third, in **Studies I** and **II**, the discrimination threshold was not tested. Due to the lack of a discrimination threshold test, it is unclear whether or not the participants were able to discriminate the generalization stimuli and the threatening conditioned stimuli. Besides, the stimulus used in the discrimination threshold in **Study III** was not the same as that used in

the fear learning and generalization experiment. Specifically, in the discrimination threshold task, the squares and female faces were adopted; however, the circles and female faces (different identities from the discrimination threshold task) were applied in fear learning and generalization. Although the diameter of the largest and smallest circle and square are the same, the different shapes and faces with different identities might have affected the judgments as to whether the generalization stimuli could be discriminated or not. Future studies are recommended to have the same stimuli between the discrimination threshold task and the fear generalization task.

Additionally, some suggestions for the future studies are offered. First, in addition to the social and the social-related hormonal factors examined in the three studies, other social factors might also influence fear generalization. For instance, by using a longitudinal design, Sheridan et al. (2018) investigated the sensitivity to reward learning among children who grew up in institutions. Their findings showed that compared with parent-raised children, institution-raised children exhibited lower accuracy in a reward-associative learning task. Therefore, early parental deprivation may also modulate aversive associative learning and generalization. However, little research has explored the effect of early parental deprivation on fear learning and generalization. Second, loneliness is known to be related to both adverse mental and physical outcomes (Cacioppo et al., 2002; Jones et al., 1990) and is related to the outcomes of social exclusion (Leary, 1990). Future research could investigate fear learning and generalization in lonely participants. Third, positive social interactions with parents and friends provide plenty of social support. The social buffer effect (Davitz & Mason, 1955; Hennessy et al., 2000; Kikusui et al., 2006; Mendoza et al., 1978) has been found to reduce stress and fear response, indicating that social support may reduce over-generalization. Future studies could investigate whether social support could reduce fear generalization. Lastly, in addition to relying on perceptual similarity, fear generalization has been found to be based on some other factors. For instance, some researchers hold the view that fear generalization is a byproduct of perceptual errors (incorrect stimulus perception) (for a review, see Zaman et al., 2021a; Zaman et al., 2021b). Specifically, if the participant perceives a stimulus as the CS, the participant will produce increased fear responses; however, if the participant perceives the exact same stimulus as a different stimulus from the CS, the participant will produce decreased fear responses (Struyf et al., 2017). In this dissertation, none of the three studies tested the perceptual errors of each generalization stimulus, resulting in a lack of evidence in regard to the view that fear generalization is a byproduct of perceptual errors. Future studies could explore the association between percetual errors and generalization.

YHTEENVETO (SUMMARY)

Sosiaalisen syrjinnän, sosiaalisen oppimisen ja oksitosiinin vaikutukset pelon oppimiseen ja yleistymiseen: Todisteita käyttäytymis- ja aivoaktiivisuustutkimuksista

Neutraalin ärsykkeen yhdistämistä uhkaavaan ärsykkeeseen sanotaan pelon ehdollistumiseksi. Ihmiset tuottavat pelkoreaktiota myös ärsykkeisiin, jotka ovat samankaltaisia kuin ärsykkeet, jotka ovat aiemmin yhdistyneet uhkaan. Tätä kutsutaan pelon yleistymiseksi. Esimerkiksi koiran hyökkäyksen jälkeen ihminen voi pelätä kilttejäkin koiria tai muitakin eläimiä, joilla on terävät hampaat ja kynnet. Oikeasuhteinen pelon yleistyminen on tärkeää yksilön joustavalle käyttäytymiselle, kun taas yliyleistyminen liittyy joihinkin ahdistuneisuushäiriöihin.

Tämä väitöskirjatutkimus selvitti sosiaalisen syrjinnän, sosiaalisen pelon oppimisen ja oksitosiinin vaikutuksia pelon oppimiseen ja yleistymiseen. Käyttäytymisvasteita, fysiologisia vasteita ja aivoaktiivisuutta pelon oppimisen ja yleistymisen aikana mitattiin käyttämällä sähköiskun ennakointia (**Osatutkimukset I, II, III**), ihon sähkönjohtavuutta (**Osatutkimukset I, III**), aivosähkökäyrää (**Osatutkimus II**) ja toiminnallisen lähi-infrapunaspektroskopian (**Osatutkimus I)** menetelmiä. Sähköiskun ennakointi on tutkittavan subjektiivinen arvio siitä, kuinka todennäköisenä hän pitää sähköiskun liittymistä kuhunkin yleistymistä mittaavaan ärsykekuvaan. Ihon sähkönjohtavuus on epäsuora sympaattisen hermoston aktiivisuuden mittari, joka ilmentää emotionaalista tilaa. Aivosähkökäyrämittaus ja toiminnallinen lähi-infrapunaspektroskopia ovat kajoamattomia, edullisia ja liikuteltavia aivoaktiivisuuden mittareita.

Osatutkimus I selvitti kuinka kokeellisesti tuotettu sosiaalinen syrjintä vaikuttaa pelon oppimiseen ja yleistymiseen. 44 vapaaehtoista tutkittavaa jaettiin satunnaisesti sosiaalisen hyväksymisen ja syrjinnän ryhmiin. Sosiaalinen hyväksyminen ja syrjintä tuotettiin tietokonepelin avulla. Tutkittava pelasi peliä kahta muuta tietokoneohjattua pelaajaa vastaan. Hyväksymisen ryhmässä tutkittava otettiin jatkuvasti mukaan pallonheittelypeliin, kun taas sosiaalisen syrjinnän ryhmässä tutkittavalle ei heitetty palloa enää yhden heittokerran jälkeen. Pelaamisen jälkeen tutkittavat osallistuivat pelon oppimisen ja yleistymisen kokeisiin. Pelon oppimisen vaiheessa, heidän tuli oppia ehdollisen ärsykkeen (ympyrä) ja ehdottoman ärsykkeen (sähköisku) välinen yhteys. Tulokset osoittivat, että sosiaalisen syrjinnän ryhmässä sähköiskun ennakoinnin ero ehdollisiin ärsykkeisiin, joihin liittyi ja joihin ei liittynyt sähköiskua, pienentyi verrattuna sosiaalisen hyväksynnän ryhmään. Ihon sähkönjohtavuus ehdolliseen ärsykkeeseen oli suurempi sosiaalisen syrjinnän ryhmässä. Pelon yleistymisen koetilanteessa sosiaalisen syrjinnän ryhmän arviot sähköiskun ennakoinnista osoittivat suurempaa pelon yleistymistä kuin sosiaalisen hyväksymisen ryhmän arviot. Lähi-infrapunaspektroskopian tulokset osoittivat, että mediaalinen prefrontaalinen aivokuori välittää sosiaalisen syrjinnän vaikutusta pelon oppimiseen.

Osatutkimus II selvitti, onko pelon yleistyminen erilaista silloin kun pelko opitaan itse (suora oppiminen) verrattuna sosiaaliseen oppimiseen, jossa pelko opitaan toista ihmistä havainnoimalla (sosiaalinen oppiminen). 58 tervettä aikuista osallistui tutkimukseen vapaaehtoisina. Käyttäytymisvasteita ja aivosähkökäyrää mitattiin pelon oppimisen ja yleistymisen kokeiden aikana. Tutkittavat osallistuivat kokeisiin pareittain. Toisella parista suoran oppimisen tilanne oli ensin ja sosiaalisen oppimisen tilanne toisena. Toilla parista tämä järjestys oli päinvastainen. Kunkin oppimisvaiheen jälkeen he tekivät pelon yleistymistä mittaavan tehtävän itsenäisesti. Tulokset osoittivat, että suoraan oppimiseen verrattuna sosiaalinen pelon oppiminen heikensi pelon oppimista ja lisäsi sähköshokin ennakoimista turvallisiin yleistymisärsykkeisiin. EEG tulokset osoittivat, että varhainen pelon sensorinen erottelu (P1 vaste) ja myöhempi motivoitunut tarkkaavuus (myöhäinen positiivinen potentiaali) yleistymisärsykkeisiin oli vähentynyt sosiaalisessa oppimisessa verrattuna suoran oppimisen tilanteeseen.

Osatutkimus III selvitti kuinka oksytosiini vaikuttaa pelon yleistymiseen. Oksitosiini on hormoni, joka on merkittävässä roolissa sosiaalisissa suhteissa ja se vähentää stressiä ja ahdistusta. 63 tervettä miespuolista tutkittavaa osallistui tutkimukseen ja heidät jaettiin satunnaisesti kahteen ryhmään. Molemmat ryhmät osallistuivat ensin pelon oppimisen kokeeseen. Tämän jälkeen toinen ryhmä sai oksitosiinia ja toinen saman määrän suolaliuosta (plasebo). Tämän jälkeen molemmat ryhmät tekivät pelon yleistymistä mittaavan tehtävän, jonka aikana heidän käyttäytymisvasteitaan ja ihon sähkönjohtavuuttaan mitattiin. Tulokset osoittivat, että verrattuna plaseboon oksytosiini vähensi sähköiskun ennakointia ja ihon sähkönjohtavuutta yleistymisärsykkeisiin. Nämä tulokset viittaavat siihen, että oksytosiini vähentää pelon yleistymistä.

Tämän väitöskirjan tutkimukset yhdessä osoittavat, että sosiaalinen syrjintä ja sosiaalinen pelon oppiminen tehostavat pelon yleistymistä (**Osatutkimukset I ja II**), kun taas oksytosiini vähentää sitä (**Osatutkimus III**). Kaikki tutkimukset osoittivat pelkoreaktioiden kasvun yleistymisärsykkeiden samankaltaisuuden funktiona, mikä viittaa siihen, että pelon yleistyminen perustuu havainnon samankaltaisuuteen. Koska pelon yliyleistyminen ja ahdistuneisuushäiriöt ovat yhteydessä toisiinsa, sosiaalinen syrjintä ja sosiaalinen pelon oppiminen voivat lisätä herkkyyttä ahdistuneisuushäiriöihin lisäämällä pelon yleistymistä. Sen sijaan oksytosiini saattaisi tulevaisuudessa tarjota mahdollisen intervention joihinkin ahdistuneisuushäiriöihin, joissa havaitaan pelkojen yliyleistymistä. Tutkimukseni tulokset tarjoavat perustan uusille tutkimuksille, jotka voisivat selvittää muiden sosiaalisten tekijöiden kuten yksinäisyyden ja sosiaalisen tuen merkitystä pelon yleistymiseen.

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ORIGINAL PAPERS

I

SOCIAL EXCLUSION INFLUENCES CONDITIONED FEAR ACQUISITION AND GENERALIZATION: A MEDIATING EFFECT FROM THE MEDIAL PREFRONTAL CORTEX

by

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Social exclusion influences conditioned fear acquisition and generalization: A mediating effect from the medial prefrontal cortex

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ABSTRACT

Fear acquisition and generalization play key roles in promoting the survival of mammals and contribute to anxiety disorders. While previous research has provided much evidence for the repercussions of social exclusion on mental health, how social exclusion affects fear acquisition and generalization has received scant attention. In our study, participants were divided into two groups according to two Cyberball paradigm conditions (exclusion/inclusion). Both groups underwent a Pavlovian conditioning paradigm, functional near-infrared spectroscopy (fNIRS), and skin conductance response (SCR) assessments. We aimed to determine the effects of social exclusion on fear acquisition and generalization and whether modulation of the medial prefrontal cortex (mPFC) mediates this relationship. Our results showed that socially excluded participants featured significantly higher and lower shock risk scores to safety stimuli (conditioned stimulus, CS-) and threatening stimuli (CS+), respectively, than did socially included subjects during fear acquisition. The exclusion group had increased skin conductance responses (SCRs) to CS and exhibited heightened shock risk and increased SCRs to generalized stimuli compared with the inclusion group. The fNIRS results demonstrated that the CS + evoked larger oxy-Hb changes in the mPFC in the inclusion group than in the exclusion group during fear acquisition. Furthermore, the oxy-Hb of left mPFC of CS + mediated the effect on the association between social exclusion and perceived risk of CS+ in the fear acquisition. Our results indicate that social exclusion impairs fear acquisition and generalization via the mediation of the mPFC and that social exclusion increases susceptibility to anxiety disorders through bias processing of fear discrimination in fear acquisition and generalization. By studying the role of social relationship in fear acquisition and generalization, our research provides new insights into the pathological mechanisms of anxiety disorder.

1. Introduction

Learning and discriminating threatening stimuli in complex natural environments are crucial to an individuals' survival (Öhman and Mineka, 2001; Onat and Büchel, 2015). Pavlov's classic conditioning of fear learning effectively models many disorders characterized by aberrant fear learning and generalization. The conditioning connects an aversive or threatening stimulus, referred to as the unconditioned stimulus (US), and a neutral object, referred to as a conditioned stimulus (CS). After conditioning, the CS, US, and even the generalization stimulus (GS), which is similar to CS, elicit similar fear responses (Linnman et al., 2011; Pavlov, 2010; Redondo and Marcos, 2003). This process can be summarized by the adage, "once bitten, twice shy." If the CS is too closely associated with the US due to excesses in the intensity of the US or frequency of the connections, it will generalize to another safety object; this aberrant fear generalization, called overgeneralization, is regarded as a biomarker in some anxiety disorders (Dymond et al., 2015; Lissek et al., 2010). Social relationships help human beings, who are social animals, to defend against threatening events throughout their lives (Cohen, 2004). However, the severance of such relationships (e.g., social exclusion) induces suffering and threatens the following behavioral responses and psychological state (Leary, 1990). More specifically, Baumeister and Tice

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(1990) proposed the "exclusion theory" of anxiety showing that the fear of being excluded from social relationships was deeply rooted and had innated based would arise social anxiety. Therefore, social exclusion may disturb fear learning and generalization. Although previous research has explored fear learning and generalization by studying features of fear memory (Xu and Südhof, 2013) or individual psychological states (e.g., anxiety) (Dunsmoor and Paz, 2015), few studies have been conducted on how the strength of an individual's social relationships inform the effect of social exclusion on fear acquisition and generalization.

Behavioral research has shown that social exclusion induces suffering and impairs cognitive control among individuals who experience it (Themanson et al., 2014); to prevent any consequent decline of their mental health, it is vital to determine whether social exclusion affects the fear acquisition and generalization. Prior research has provided some insight into this question: A young adult's risk of developing a social anxiety disorder can be predicted from his or her reaction to social exclusion (Gutz et al., 2015; Gutz et al., 2016; Heeren et al., 2017; Levinson et al., 2013), and psychopathological disorders, such as depression, post-traumatic stress disorder, and anxiety (Foa et al., 1992; Marsch et al., 2007; McDevitt et al., 2011; Pollak et al., 2010), have been found to compromise a patient's conditioned fear acquisition and generalization. Specifically, individuals with anxiety disorders cannot reliably discriminate threatening stimuli from safe stimuli relative to healthy controls (Cha et al., 2014; Klemenhagen et al., 2012; Lissek et al., 2005, 2009; Lissek et al., 2013). Social exclusion may impair normal conditioned fear acquisition and generalization, which may further induce the onset of an anxiety disorder. Supporting this hypothesis, Olsson et al. (2013) found that a high-rejection sensitivity group demonstrated an increased skin conductance response (SCR) during fear learning and extinction relative to a low-rejection sensitivity group. However, no direct evidence proves this hypothesis. It remains unclear how social exclusion affects fear acquisition and generalization and its cognitive neural mechanism.

Prior research has begun to elucidate the neural correlates for social exclusion and fear acquisition. Interestingly, functional magnetic resonance imaging studies have indicated that neural activity associated with social exclusion and fear acquisition overlap in threat-processing areas, such as the dorsal anterior cingulate cortex, and the insula (DeWall et al., 2010; Eisenberger et al., 2003; Krabbe et al., 2018; Sotres-Bayon and Quirk, 2012). For example, Onat and Büchel (2015) used a new fear-tuning paradigm to explore the neural basis of human fear generalization and thereby found activations in the posterior and anterior cingulate cortices, subcallosal cortex, anterior insula, and hippocampus. Similarly, Eisenberger et al. (2003) recorded fMRI data of individuals undergoing the Cyberball paradigm: a participant plays a virtual ball-tossing game with two other computer-controlled players, who finally exclude the participant from the game. The researchers found that participants exhibited a larger activation in the ACC during the social exclusion condition than during the social inclusion condition. After the game ended, participants were asked to report feelings of self-distress, and reported self-distress was found to positively correlate with the activity of the ACC. Results from other investigations have also provided evidence that the anterior insula is related to neural responses to exclusion (DeWall et al., 2010). Taken together, social exclusion may influence fear acquisition and generalization by modulating the activity of threat-processing areas.

Aside from threat-processing areas, the prefrontal cortex (PFC) is integral to both conditioned-fear and social-exclusion processing. On the one hand, several investigations have linked the prefrontal cortex (PFC) to social exclusion (Bolling et al., 2011; Moor et al., 2012; Gradin et al., 2012; Vijayakumar et al., 2017). An earlier investigation demonstrated that social exclusion activated the ventrolateral PFC (Eisenberger et al., 2007), an area associated with emotion regulation; this result was supported by Chester and Dewall (2014) who found that social exclusion impairs self-regulation through the recruitment of the ventrolateral PFC. Adopting the Cyberball paradigm to explore the neural mechanisms underlying responses to social exclusion among young adults, Sebastian et al. (2011) demonstrated that the bilateral medial PFC (mPFC) was involved in social exclusion processing. More recent research has confirmed a connection between the medial PFC and social exclusion (Bolling et al., 2011; Moor et al., 2012; Gradin et al., 2012; Vijayakumar et al., 2017). The significance of the medial PFC is further highlighted by the finding that patients with Schizophrenia exhibited significantly less activity in this region than healthy controls (Gradin et al., 2012). Prior research therefore indicates that social exclusion alters the normative neural activity of the PFC. On the other hand, the PFC also plays a pivotal role in conditioned fear learning and generalization (Perusini and Fanselow, 2015). Research on animals and humans have provided evidence that the PFC modulates the processing of threatening stimuli during acquisition and generalization (Likhtik and Paz, 2015). For example, the theta synchrony between the mPFC and amygdala in mice was associated with better discrimination between CS+ and CS- (Likhtik et al., 2014). Furthermore, Motzkin et al. (2015) found that patients with dysfunction of the mPFC exhibited significantly increased activation in the amygdala when confronted with threatening stimuli compared with healthy controls. In fear generalization, patients with general anxiety disorder exhibited a deficient activation of the ventromedial PFC relative to healthy controls when presented with generalized stimuli, causing a larger rating of shock likelihood relative to controls (Greenberg et al., 2013). Considered together, past investigations indicate that social exclusion inhibits mPFC activity following fear acquisition and generalization.

Enhancing psychological resilience may help to address the repercussions of social exclusion on fear acquisition and generalization and prevent anxiety disorders; indeed, psychological resilience has an important effect on human mental health (Davydov et al., 2010). For example, after controlling for age and sex, Hjemdal et al. (2011) found that higher resilience scores predicted lower scores for depression, anxiety, stress and obsessive-compulsive symptoms. Another study reported that psychological resilience correlated with self-regulation among youth at risk of social exclusion (Artuch-Garde et al., 2017); this finding may indicate the individuals with higher psychological resilience are able to self-regulate after social exclusion – i.e., psychological resilience may protect against the negative effects of social exclusion.

In the current research, we employed the Cyberball paradigm—a virtual ball-tossing game that is effective to consistently induce feelings of social exclusion (Williams and Jarvis, 2006). In addition, we used a classic fear generalization paradigm based on perceptual generalization of shapes, sizes or colors (Dunsmoor and Murphy, 2015). Based on previous findings, we hypothesized that social exclusion induced by the Cyberball paradigm would influence fear acquisition and generalization by inhibiting the function of the mPFC (Olsson et al., 2013; Sebastian et al., 2011; Likhtik and Paz, 2015). Specifically, we speculated that in relation to control groups, individuals experiencing social exclusion may demonstrate impaired fear acquisition and generalization and an increased SCR to CS and GS and their mPFC activity may be prohibited. Moreover, traits of psychological resilience may overcome the negative effects of social exclusion on fear acquisition. Our research aims to enhance the understanding of anxiety disorders, by clarifying the effect of social relationship on fear acquisition and generalization and its cognitive neural mechanisms.

2. Methods

2.1. Participants

A total of 44 healthy adults (18–25 years of age) were recruited from Shenzhen university; 23 of the participants comprised the exclusion group (12 women), while 21 were included in the inclusion group (nine women). To ensure that the effect size of the experiment was appropriate, we calculated power with a post hoc function in Gpower (Faul et al., 2009). We found that the power of the interaction effect of fear acquisition was 0.9674 ($\alpha = 0.05$, effect size $f = 0.5060$) and the power of the

group effect of fear generalization was 0.9673 ($\alpha = 0.05$, effect size $f = 0.4184$). All the participants were right-handed with normal or corrected-to-normal vision and did not have any history of psychiatric or neurological disease. Each participant provided written informed consent, and the experimental procedure was approved by the ethical council of Shenzhen University. The participants received 70 RMB (approximately 12 USD) for their participation. To ensure that the two groups did not differ in terms of anxiety, depression, or personalities of the participants, the following questionnaires were administered to the participants before the formal experiment: the State-Trait Anxiety Inventory (Laux et al., 1981), the Beck depression inventory-II(Beck et al., 1996), the rejection sensitivity questionnaire (Downey and Feldman, 1996), and the Connor-Davidson resilience scale(CD-RISC)(Connor and Davidson, 2003). As evaluated by an independent *t*-test, no significant differences between the results of the two groups were found (Table 1).

2.2. Design

Our experiment used acquisition and generalization stimuli as the within-subject factor and social relationship as the between-subject factor. The former featured six conditions: CS- and CS + as the acquisition stimuli (circles of two different sizes); and GS1 GS2, GS3, and GS4 (circles whose size gradually varied between those of the CS- and CS+) as the generalization stimuli. We included two social-relationship conditions: exclusion and inclusion. Outcome measures included the mean perceived-risk of the stimuli as behavioral data, the SCRs as physiological data, as well as changes in oxy-Hb and deoxy-Hb concentrations in the PFC as indicated by fNIRS data. The experiment was performed in a quiet room, with a distance of 60 cm between the participants and the screen. The participants completed the questionnaires at the beginning of the experiment. We then allocated participants so that each group featured a balanced distribution of participant characteristics: sex, age, anxiety, depression, rejection sensitivity and psychological resilience.

2.3. Procedure

2.3.1. Cyberball

The present study used the Cyberball 4.0 computer program developed by Williams et al. (2012). Cyberball is a classic, effective paradigm for modelling social exclusion (Williams and Jarvis, 2006); it has been proven to manipulate social exclusion and consistently elicit social pain (DeWall et al., 2010; Eisenberger , 2012).

The participants were informed that they would play a virtual ball-tossing game on the internet with other players (Fig. 1). Before the experiment, we obtained one photo from each participant. For the image, the participants were free to display any facial expression. The virtual ball-tossing game included two other players controlled by the computer: one woman and one man whose photos featured neutral facial expressions. The names and photos of the three players were presented throughout the game. The purpose of these manipulations was to enhance the game's verisimilitude (Fung and Alden, 2017); when the participants did not receive the ball, they would attribute the rejection to the names or appearances of the virtual players.

Table 1
Evaluation of anxiety, depression, and personality using questionnaires.

	Exclusion group	Inclusion group	
	M ± *SD*	T	p
STAI_Trait[a]	43.22 ± 7.34	−0.237	0.814
STAI_State	35.47 ± 7.26	0.407	0.686
BDI-II[b]	8.61 ± 6.76	0.307	0.760
RSQ[c]	63.43 ± 10.00	0.402	0.690
CD-RISC[d]	58.48 ± 10.19	1.411	0.166

[a] State-Trait Anxiety Inventory.
[b] Beck depression inventory-II.
[c] Rejection sensitivity questionnaire.
[d] The Connor-Davidson resilience scale.

Fig. 1. Cyberball paradigm. Player 1 and Player 2 were pseudo-players controlled by the computer. They were provided with photos and names to enhance the credibility of the game.

The ball-tossing game consisted of 30 ball tosses. Once the ball was received, the virtual players passed the ball within a random delay of 0–4 s. In the inclusion group, the pseudo player would toss the ball to the participant and the other virtual player with the same probability. In the exclusion group, however, the participants only received the ball once, at the beginning of the experiment. We randomly assigned the participants into the exclusion and inclusion groups. The participants in both groups were required to complete the Positive and Negative Affect Schedule (PANAS) scale before and after the Cyberball paradigm. After completion of the PANAS scale. The participants then underwent a fear acquisition and generalization task.

2.3.2. Conditioned generalization paradigm

The conditioned generalization paradigm used in our research was identical to that employed in a previous study (Lissek et al., 2008, 2014). The conditioned stimuli included 10 circles of varying sizes (Fig. 2), while the US 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, the participants received a series of electric stimuli and were asked to rate the intensity of each on a verbal analog scale: 1 corresponded to not unpleasant/painful/annoying, while 10 indicated very unpleasant/painful/annoying. The shock was calibrated specifically for each participant to determine the degree that the participants considered highly uncomfortable but not painful: a score of 7 out of 10 (Haaker et al., 2013; Lei et al., 2019). The paradigm consisted of three different phases: pre-acquisition, acquisition, and generalization. The pre-acquisition phase contained six CS+ (conditioned stimulus) and six CS-. None of these circles were accompanied by the electric shock (US). The acquisition phase linked the conditioned fear response (shock) to the conditioned stimuli. Either the largest or smallest circle was used as the conditioned fear cue (CS+), which was matched to the US. This phase presented the CS+ 12 times, nine of which featured an electric shock (reinforcement rate, 75%). The US was presented at CS + offset. In the case that the largest circle was used as the CS+, we assigned smallest as the conditioned safety stimulus (CS-), which was subsequently never paired with an electric shock in any of its 12 presentations; if the smallest circle was used as the CS+, the largest was used as the CS-. The participants were tasked with rating the perceived likelihood of receiving an electric shock once the CS + or CS- was presented on a 3-point scale: 1

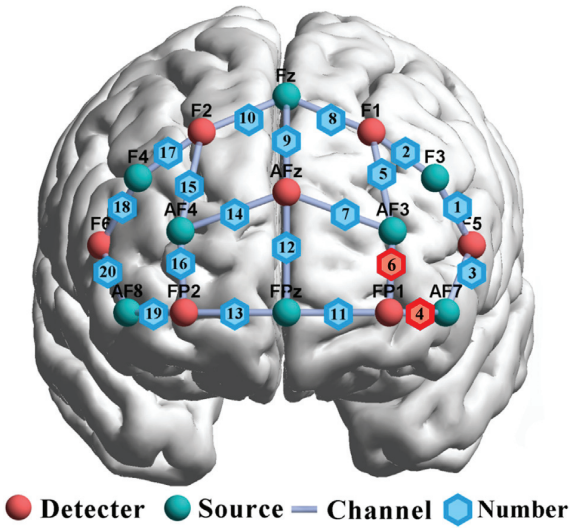

Fig. 2. Region of interest (ROI) in the PFC. Red and green balls indicate detectors and sources, respectively, while the branches represent channels. The red channels indicate the ROI (region of interest) of left mPFC.

indicated no risk; 2, moderate risk; and 3, 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+. Whether the instruction contained the information of the CS-US association or not significantly affected the participants' reactions in the following fear learning task (Duits et al., 2017). In this study, the instruction was not specified which circle was associated with the US. Specifically, we gave the instruction to the participants: "The shocks will only be administered following a circle, and you need to learn which circle will be associated to the shock on your own". The inter-trial-interval (ITI) consisted of a fixation cross presented for a random time frame of 5–8 s (Guhn et al., 2014). The generalization phase featured six types of circles: CS-, GS1, GS2, GS3, GS4, and CS+. The GS consisted of two contiguous circles, while the CS featured one circle. Conditioned and generalization stimuli were circles of different sizes (10 different sizes). The smallest circle was 2 in. Circles increased successively in size by 15% increments: 2.00 (CS+), 2.30 (GS1), 2.60 (GS1), 2.90 (GS2), 3.20 (GS2), 3.50 (GS3), 3.80 (GS3), 4.10 (GS4), 4.40 (GS4), 4.70 (CS-)(in). Each type of circle was presented in 12 and six conditioning and generalization trials, respectively (total, 72 trials). The sequence of the stimuli was pseudo-random: at most, two circles of the same size followed one another. To avoid fear extinction, the reinforcement rate of CS+ was set to 50% in the generalization phase. The participants' task was the same as that in the acquisition phase. When the participants had finished 20 trials, they were permitted a break. The same assignments of the largest and smallest circles to the CS+ and CS- in the acquisition phase were 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+.

2.3.3. SCR recording

SCR was measured using a BIOPAC MP150 system with EDA Isotonic Gel Electrodes. We recorded and analyzed the SCR data with AcqKnowledge 5.0 software (http://www.biopac.com/product/acqknowledge-software, RRID: SCR_014279). The sample rate in our experiment was 1000 Hz. The Ag/AgCl electrodes with gel were placed on the middle and index fingers of the participants' left hands. Before the experiment, we tested the participants' SCR sensitivity. To exclude the SCR non-responders, we asked the participants to breathe deeply to induce an increase of SCR. The participants whose SCR increases were lower than 0.02 μs at the beginning of the experiment were excluded (Boucsein et al., 2012; Hornstein et al., 2016). Finally, four participants could not pass the sensitivity test. To remove artifacts during SCR recording, a 0.0159 Hz high-pass filter was applied (Matthias et al., 2015). Trials with recording artifacts during fear acquisition and generalization were removed from further analyses (n = 82, 2%). If any trial lacked an SCR peak (no rise in SCR during the 1- to 4-s stimulus window), it was recorded as a zero-response trial; specifically, if the max-min amplitude for any trial was below 0.02 μs, the trial was scored as a zero-response trial (Boucsein et al., 2012). We calculated the difference between maximum and minimum values with a time window of 1–4 s after the stimulus onset. On account of recording mistakes, the data of two participants in the inclusion group were excluded; forty-two valid data were thus analyzed in our experiment.

2.3.4. Functional near-infrared spectroscopy (fNIRS)

We adopted the NIRScout 1624 system (NIRx Medical Technologies, LLC. LA, USA) to record NIRS data. We selected the PFC as the region of interest (ROI), which consisted of eight sources and seven detectors with 760 nm and 830 nm wavelengths, respectively (Fig. 2). The locations of the sources and detectors were placed based on the 10-20 system (Jasper, 1958). A total of 20 channels were recorded with an average distance of 3 cm from one another. The sample rate of the data was 7.81 Hz.

Regarding artifact rejection, we used the preprocessing method included in the Nirs-lab software (http://nirx.net/nirslab-1/) (Burns et al., 2018; van der Kant et al., 2018; Xu et al., 2014; Zhang et al., 2018). There are two forms of movement artifacts in the NIRS data: transient spikes and abrupt discontinuities. The former were identified and removed and the contaminated data was then replaced by linear interpolation. Discontinuities were automatically detected and corrected by Nirs-LAB when the "jump" exceeded within SD of the variance of the rest of the data. The fNIRS data were converted into oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) concentrations using the Beer–Lambert law to calculate relative changes in their concentrations during fear acquisition and generalization stages. The raw data were digitally bandpass-filtered (0.01 Hz–0.2 Hz) to remove longitudinal signal drifts (Gervain et al., 2011; Piper et al., 2014) and noise from the Nirs system. We normalized signals as Z-scores, because the absolute concentration values significantly differed among participants (Yang et al., 2016). We obtained the Z-scores (z) by calculating the differences between the mean of the baseline (μ2) and the concentration value (μ1) divided by the SD during the baseline period (σ): $z=(\mu1-\mu2)/\sigma$. We extracted time series of concentration from 2 s before stimuli onset to 6 s after stimuli offset with a baseline from −2 s to 0 s. The Z-scores were calculated as the difference between the mean of the baseline (-2–0 s) and the concentration (-2–6 s) values divided by the SD during the baseline period. Finally, we averaged 0.5s–5 s for the final analysis.

2.4. Statistical analysis

In the acquisition stage, a 2 × 2 (social relationship [exclusion/inclusion] x acquisition stimulus [CS+, CS-]) analysis of variance (ANOVA) with repeated measures of perceived risk and SCR was performed. In the generalization stage, we adopted a 2 × 6 (social relationship [exclusion group, inclusion group] x generalization stimulus [CS+, GS1,GS2,GS3,GS4,CS-]) ANOVA with repeated measures of perceived risk and SCR. A linear-regression analysis was performed to test whether the resilience scores could predict behavioral data obtained in the fear acquisition stage. When Mauchly's test indicated that the assumption of sphericity had been violated, Greenhouse–Geisser correction was used to correct the degree of freedom. Bonferroni correction was further applied to adjust the p-value for all pairwise comparisons. The alpha level was set to 0.05 in our statistical analysis.

We selected the channels in which we detected significant increases in oxyHb in response to CS + or CS- during both the fear acquisition and generalization stages ($t(22) > 2.0$ and $p < 0.05$ for all, FDR corrected). The medial prefrontal cortex was selected as the region of interest; this region

corresponds approximately to the FP1/FP2 channels in the 10-20 system (Koessler et al., 2009). After screening the channels, channels 6 and 4 (adjacent to FP1: the left mPFC) were selected for further analysis (Fig. 2). The results obtained from other activated channels are reported in the supplementary materials(see Supplementary Fig. 1). We then performed a 2 × 2 (social relationship × acquisition stimulus) ANOVA and a 2 × 6 (social relationship × generalization stimulus) ANOVA with repeated measures to analyze the fNIRS data obtained during fear acquisition and generalization stages in the mPFC, respectively. Besides, the mean values obtained from the left mPFC region following the presentation of each of the six stimuli were plotted as a six-point gradient in fear generalization. Finally, we calculated the slope of the linear fit of these values for each participant (Cha et al., 2014). The independent-sample *t*-test was adopted to identify the differences in slope between the inclusion and the exclusion groups.

At last, to test our expectation that the mPFC would mediate the effect of social exclusion on the fear acquisition and generalization, we utilized the Bootstrap method proposed by Hayes (2017). Data analysis was done using the PROCESS plug-in of SPSS statistics software (IBM, Hayes, 2017). The sample size of the Bootstrap analysis was 5000. According to the previous research(Legate et al., 2013), we defined the social exclusion in our study as a dummy variable(social exclusion condition coded 1 and the social inclusion condition coded 0).

3. Results

3.1. Behavioral results

With respect to the data from the fear acquisition phase, a 2 × 2 (social relationship × acquisition stimulus) analysis of variance (ANOVA) with repeated measures of perceived risk showed that the interaction between the two factors was significant ($F(1, 42) = 11.233$, $p = 0.002$, $\eta^2_p = 0.211$). The main effect of stimulus was also significant; the perceived risk of CS+ was significantly higher than that of CS- ($F(1, 42) = 296.512$, $p < 0.001$, $\eta^2_p = 0.876$). However, the main effect of group was nonsignificant ($F(1, 42) = 1.353$, $p = 0.251$, $\eta^2_p = 0.031$). The simple effect analysis showed that participants in the exclusion group perceived more risk than those in the inclusion group under the safe condition (CS-) ($F(1, 42) = 10.474$, $p = 0.002$, $\eta^2_p = 0.200$). Under the threat condition (CS+), the participants in the exclusion group perceived less risk than those in the inclusion group ($F(1, 42) = 5.262$, $p = 0.027$, $\eta^2_p = 0.111$) (Fig. 3A).

A two-way repeated ANOVA was applied to analyze the data obtained during the fear generalization phase. We found that the main effects of stimulus ($F(5, 210) = 118.831$, $p < 0.001$, $\eta^2_p = 0.739$) and group ($F(1, 42) = 7.367$, $p < 0.01$, $\eta^2_p = 0.149$) were significant. The interaction between the stimulus and group was nonsignificant ($F(5, 210) = 2.009$, $p = 0.079$, $\eta^2_p = 0.046$) (Fig. 3B). Although the interaction was nonsignificant, pairwise comparisons revealed that the participants in the exclusion group perceived significantly more threat to GS2($F(1, 42) = 8.693$, $p = 0.005$, $\eta^2_p = 0.171$), GS3($F(1, 42) = 6.019$, $p = 0.018$, $\eta^2_p = 0.125$), GS4($F(1, 42) = 9.395$, $p = 0.004$, $\eta^2_p = 0.183$), and CS-($F(1, 42) = 5.707$, $p = 0.021$, $\eta^2_p = 0.120$) than did the participants in the inclusion group. There was no statistically significantly difference in the perceived threat of CS+ and GS1 between the two groups(CS+: $F(1, 42) = 0.121$, $p = 0.730$, $\eta^2_p = 0.003$; GS1:$F(1, 42) = 2.439$, $p = 0.126$, $\eta^2_p = 0.055$).

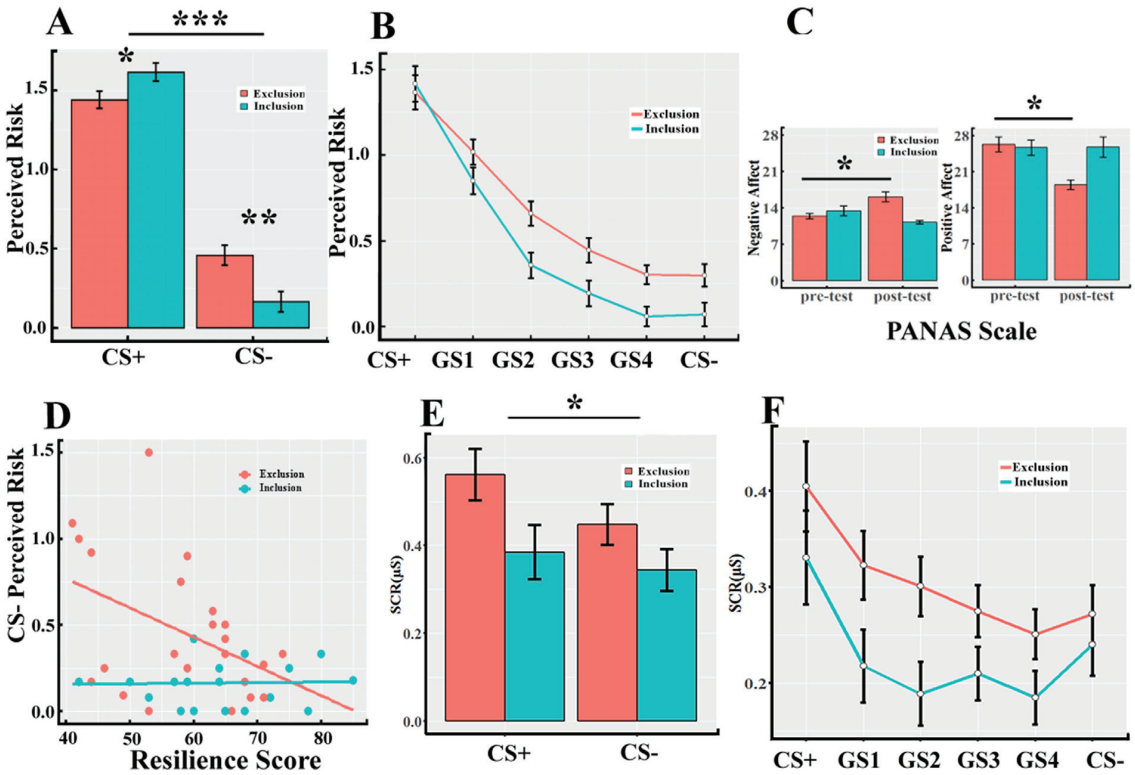

Fig. 3. A, shock-risk ranking during fear acquisition. The social exclusion group showed a larger risk to CS- and a smaller risk to CS + compared to the social inclusion group. B, shock-risk ranking during fear generalization. The social exclusion group felt a greater risk compared with social inclusion group. C, negative and positive affects of the PANAS scale. After social exclusion, negative affect increased, and positive affect decreased, indicating that the social exclusion was effective. D, linear regression of resilience and perceived risk (CS- in fear acquisition); the larger the resilience, the lower the perceived risk when experiencing exclusion. E, SCR during fear acquisition. F, SCR during fear generalization. The social exclusion group evoked a larger SCR response relative to social inclusion group. "*", $p < 0.05$; "**", $p < 0.01$. mmho:milliohm

We also implemented the PANAS scale to test the validity of the Cyberball paradigm. In the negative affect, the interaction effect of time and group was significant ($F(1, 42) = 25.51$, $p < 0.001$, $\eta^2_p = 0.378$). Simple effect analysis showed that the exclusion group felt more negative affect after the Cyberball paradigm ($p < 0.001$); in the inclusion group, negative emotions of the participants reduced after completion of the test relative to before the test ($p = 0.013$). In the positive affect, the interaction effect was also significant ($F(1, 42) = 13.28$, $p < 0.001$, $\eta^2_p = 0.24$). Specifically, the simple effect analysis showed that the exclusion group had less positive affect when they experienced the Cyberball paradigm ($p < 0.001$). However, there was no difference between the pre-test and post-test in the inclusion group ($p = 0.952$). These results indicate that the Cyberball paradigm was effective (Fig. 3C).

Interestingly, we also found that resilience scores of the exclusion group could predict the risk ranking of safety stimuli during fear acquisition (Beta = −0.435, T = −2.214, p = 0.038). This was not the case for the inclusion group (Beta = 0.029, $T = 0.125$, p = 0.902) (Fig. 3D). Further, the resilience scores showed a significant negative correlation with trait anxiety score ($r = -0.571$, $p < 0.001$). The trait anxiety score also had a positive correlation with the perceived risk of CS-($r = 0.344$, p = 0.022).

3.2. SCR results

A two-way repeated ANOVA was adopted to analyze data from the fear acquisition phase. The interaction effect was nonsignificant ($F(1, 40) = 1.099$, $p = 0.301$, $\eta^2_p = 0.027$), while the main effect of group was significant ($F(1, 40) = 4.231$, $p = 0.046$, $\eta^2_p = 0.096$). SCR of the exclusion group was higher than that of the inclusion group. The main effect of stimulus was significant (CS+ > CS-, $F(1, 40) = 4.924$, $p = 0.032$, $\eta^2_p = 0.110$) (Fig. 3E).

Concerning the data from the fear generalization phase, we adopted a two-way repeated ANOVA for analysis and found the interaction effect to be nonsignificant ($F(5, 200) = 0.681$, $p = 0.638$, $\eta^2_p = 0.017$). However, we found a significant main effect of group ($F(1, 40) = 4.142$, $p = 0.043$, $\eta^2_p = 0.094$) and stimulus (CS+ > CS-, $F(5, 200) = 9.033$, $p < 0.001$, $\eta^2_p = 0.184$) (Fig. 3F). The exclusion group elicited a larger SCR relative to that elicited by the inclusion group.

3.3. fNIRS results

A two-way repeated ANOVA was adopted to analyze data from the left mPFC region during the fear acquisition phase. The interaction effect was significant ($F(1, 42) = 5.364$, $p = 0.026$, $\eta^2_p = 0.113$) (Fig. 4A). The main effects of group and stimulus were nonsignificant ($F(1, 42) = 1.030$, $p = 0.316$, $\eta^2_p = 0.024$; $F(1, 42) = 2.618$, $p = 0.113$, $\eta^2_p = 0.059$; respectively). We then conducted a simple analysis of the interaction effect. In the inclusion group, CS + elicited a heightened oxy-Hb reaction relative to that elicited by CS- ($F(1, 42) = 7.402$, $p = 0.009$, $\eta^2_p = 0.150$)(Fig. 4B). In the exclusion group, no significant difference was found between oxy-Hb activity in response to CS+ and CS- during fear acquisition (Fig. 4C). Moreover, the CS+ in the inclusion group elicited an increased oxy-Hb reaction relative to that prompted by the CS+ in the exclusion group ($F(1, 42) = 5.364$, $p = 0.026$, $\eta^2_p = 0.113$). The oxy-Hb activation elicited by CS- were not significantly different between the inclusion and the exclusion groups. The ANOVA results of the deoxy-Hb from the left mPFC during fear acquisition were not significant(See Supplementary Materials 1.3).

A two-way repeated ANOVA was adopted to analyze brain data obtained during fear generalization. Neither the interaction nor main effects of group or stimulus were significant ($F(1, 42) = 1.609$, $p = 0.159$, $\eta^2_p = 0.037$; $F(1, 42) = 0.608$, $p = 0.440$, $\eta^2_p = 0.014$; $F(1, 42) = 1.268$, $p = 0.279$, $\eta^2_p = 0.029$; respectively). To provide more details, the paired comparison of the interaction effect was also calculated(see Supplementary Fig. 2). However, the time sequence graph showed that left mPFC reflected a trend in the inclusion group (Fig. 5A and B): the more similar a stimulus was to CS+, the more activation in mPFC in inclusion group. This trend was less evident in the exclusion group (Fig. 5C). More intuitively, we calculated the slopes of the linear fit of the oxy-Hb values corresponding to the left mPFC in the fear generalization of the exclusion and inclusion groups. We found that the slope of the inclusion group(-Beta = −0.505, SD = 0.82) was steeper than that of the exclusion group(Beta = 0.125, SD = 0.625)($t = -2.888$, $p = 0.006$)(Fig. 5D). Besides, the ANOVA results of the deoxy-Hb from the left mPFC in the fear generalization were also not significant(See Supplementary Materials1.3).

3.4. Mediation analyses results

We found that the oxy-Hb of left mPFC in the threatening stimuli(CS+) exerted significant indirect effects(the CI did not contain 0) on the association between the social exclusion and the perceived risk of CS+ in the fear acquisition (indirect effect = 0.053; 95% bootstrapped confidence interval, CI: [0.005, 0.153]), with results suggesting a partial mediation(see Fig. 6). Other models were not significant in the fear generalization. Besides, if the dependent variable was changed to SCR, the model would either not significant.

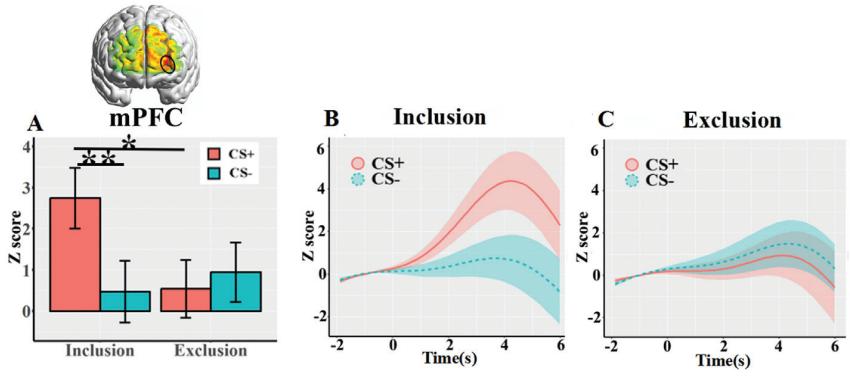

Fig. 4. fNIRS results obtained during fear acquisition. A, bar graph of oxy-Hb in the left mPFC during fear acquisition between 0.5 and 5 s. The topographic map showed the location activated by CS+ in the left mPFC in the inclusion group. B, time sequence of oxy-Hb of inclusion group during fear acquisition in the left mPFC. The ribbons of the lines were the standard error. C, time sequence of oxy-Hb of exclusion group during fear acquisition in the left mPFC.

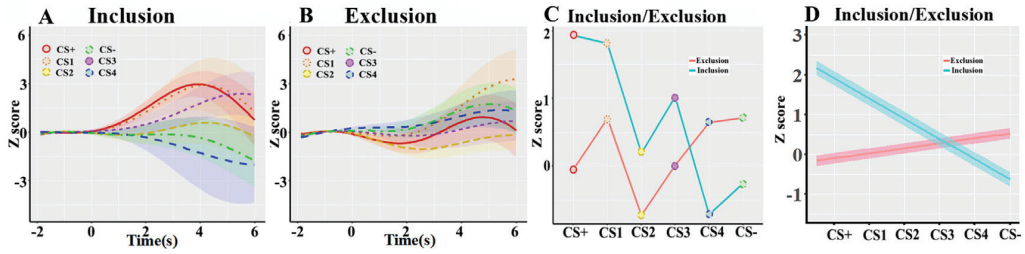

Fig. 5. fNIRS results obtained during fear generalization. A, time sequence of oxy-Hb concentration of the left mPFC in inclusion group during fear generalization; B, time sequence of oxy-Hb concentration of the left mPFC in exclusion group during fear generalization; C, a line graph of oxy-Hb in left mPFC during fear generalization. D, the slope graph of the z-score of the oxy-Hb concentration in the left mPFC on the social exclusion group and inclusion group in the fear generalization. The ribbons of the lines were ±1 se.

4. Discussion

The present study used Cyberball, Pavlovian fear conditioning, and fNIRS to examine whether social exclusion influences fear acquisition and generalization and whether the mPFC could mediate this effect. Our findings support our hypothesis by showing that social exclusion impaired fear acquisition and enhanced fear of safety stimuli during generalization, and this effect is mediated by the activation of the medial PFC in fear acquisition. More specifically, our behavioral data showed that the social exclusion group perceived a higher shock risk to safety stimuli(CS-), but a lower shock risk to threat stimuli(CS+) relative to the social inclusion group; this result may reflect the fear discrimination function has been impaired. In consistent with our study, the anxiety-prone individuals showed a impaired fear discrimination via US expectancy in the fear acquisition compared with the healthy individuals (Dibbets et al., 2015). Moreover, Duits et al. (2015) reported a meta-analysis of conditioned fear learning on anxiety disorders showed the patients with an anxiety disorder showed increased fear responding to conditioned safety cues. Our SCR data obtained during fear acquisition showed that both the CS+ and CS- evoked a larger response than in the exclusion group compared with the inclusion group. These results agree with the findings of previous research. Recruiting participants with high- and low-rejection sensitivities, Olsson et al. (2013) implemented a fear acquisition paradigm with geometric figures and found that the high-rejection group exhibited larger fear responses, as measured via SCR, to safety stimuli relative to the low-rejection group. However, CS + induced larger SCR responses in the exclusion group, but a smaller perceived risk compared with CS+ in the inclusion group. We surmised that the inconsistency regarding the risk ranking and SCR responses to threat stimuli (CS+) may occur due to the different effects of social exclusion: it may alter risk ranking by disrupting fear discrimination ability and affect SCR by enhancing the activation of the sympathetic nervous system.

Our fNIRS data indicate that social exclusion inhibits the function of mPFC during fear acquisition where CS + induced mPFC activation more potently in the inclusion group than the exclusion group. Moreover, CS + evoked greater activation of the mPFC in the inclusion group than did the CS-, an effect absent in the exclusion group. Guhn et al. (2012) also found that CS + induced an increase in oxy-Hb concentration in the mPFC during fear acquisition; however, their observed time courses of fear extinction differed from that found by the present study: 10 s after the CS + onset rather than 1–5 s after the CS+. Their use of complex faces rather than simple circles as stimuli as well and their observation of right-hemisphere activation rather than left likely account for the variance in findings. Previous research has provided evidence supporting the critical role of the mPFC in fear inhibition (Nili et al., 2010; Motzkin et al., 2015) and fear discrimination (Pollak et al., 2010; Stevens et al., 2013; Likhtik et al., 2014). Our data provide support for the hypothesis

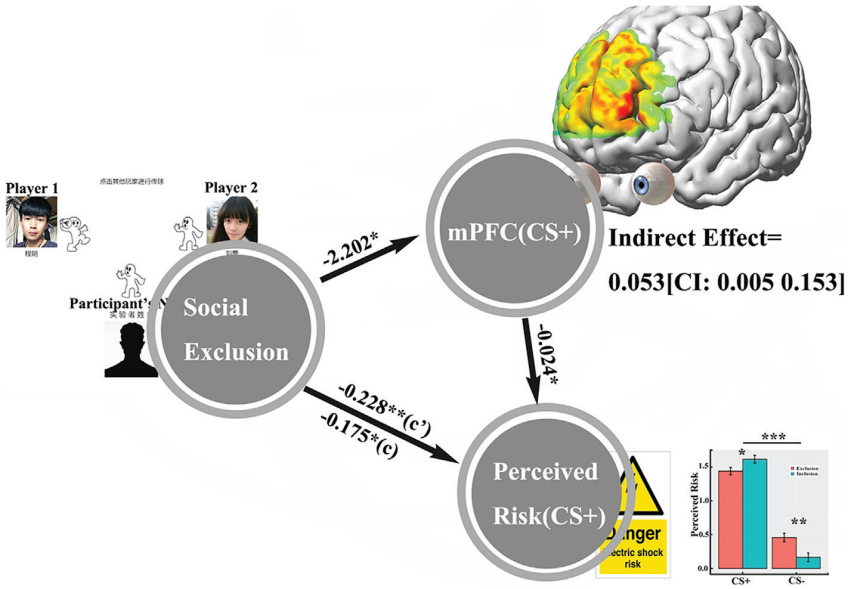

Fig. 6. Mediation analysis to explore the left mPFC influencing the relationship between social exclusion and fear acquisition. The oxy-Hb of left mPFC of CS + mediated the effect on the association between social exclusion and perceived risk of CS+ in the fear acquisition. "*", $p < 0.05$; "**", $p < 0.01$.

that the mPFC influences fear discrimination in the fear acquisition. Moreover, the mediation analysis also showed that mPFC mediated the relationship between the social exclusion and perceived risk of threatening stimulus in the fear acquisition. And the effect is partial mediation effect; therefore we inferred that social exclusion may also affect threat-related regions directly, such as the dorsal anterior cingulate and the anterior insula (Eisenberger et al., 2003; Masten et al., 2009); the altered threat-related brain regions could then disturb conditioned fear acquisition and generalization.

With respect to fear generalization, our results showed that the exclusion group perceived more shock risk to generalization stimuli and safety stimuli (CS-) relative to the inclusion group, suggesting that the ability to inhibit conditioned fear was impaired after social rejection. In agreement with the behavioral data, we found that larger SCR responses were evoked in the exclusion group than in the inclusion group. The consistency of our results provides evidence for the role of the sympathetic nervous system in responses to social exclusion. The findings of prior investigations agree with our results; patients with anxiety disorders, such as generalized anxiety disorder(GAD) and social anxiety disorder showed a much higher shock risk to generalized stimuli relative to healthy controls (Lissek et al., 2014; Ahrens et al., 2016). Zadro et al. (2006) associated participants excluded from the Cyberball task with a larger likelihood of interpreting ambiguous situations in a threatening. The present study further found the fear generalization gradient in the left mPFC of the inclusion group to be steeper than that of the exclusion group. Specifically, we found a trend in the data obtained from the social inclusion group during fear generalization: the higher the oxy-Hb concentration in the mPFC, the more similar the stimulus was to CS+. However, this trend was absent in the social exclusion group. The patients with GAD achieved consistent results; subjecting participants to a fear generalization task and concurrent fMRI, Cha et al. (2014) associated GAD patients with a decrease in the slope of the linear fit of the vmPFC data relative to data obtained from healthy controls, indicating that patients showed less discriminating vmPFC activity. Another study found that the discrimination of conditioned stimuli by the visual cortex was impaired by social anxiety (Ahrens et al., 2014). Additionally, Levinson et al. (2013) found that the extent to which a young adult's reactions to social exclusion could be used to predict their social anxiety symptoms. More recent research has found further support for the correlation between social exclusion and social anxiety disorder (SAD) (Gutz et al., 2016; Gutz et al., 2015; Heeren et al., 2017). We therefore inferred that social exclusion impairs conditioned fear acquisition and generalization via the mediation of the mPFC, and may finally increase the susceptibility of some anxiety disorder.

From a theoretical point of view, previous research emphasized has provided support for an SAD model advanced by Clark and Wells (1995): patients with social phobia experience a delayed recovery from social-negative experience and consequent post-event rumination on the negative event. Moreover, participants with high social anxiety would have a longer time to recover from social exclusion than would those with low social anxiety (Zadro et al., 2006), and rumination reportedly suppresses recovery from social exclusion (Wesselmann et al., 2013). Other investigations have found that individuals with SAD were more likely to blame themselves after being socially excluded than were those without SAD; i.e., social exclusion activated the negative self-belief characteristic of SAD disorders (Gutz et al., 2016). In our study, rumination after social exclusion from the Cyberball task may include appraisal processing of others because the participants provided their name and photo and may thus ascribe the social exclusion to their degree of perceived attractiveness. A recent fMRI study also suggested that patients with SAD exhibit abnormal inferior temporal gyrus (IFG) activity in response to the Cyberball paradigm (Heeren et al., 2017); the IFG is a neural area related to self-related (Morin and Michaud, 2007) and inner-language processing (Shergill et al., 2002). Although post-event rumination data were not collected after the Cyberball task, we found that the individuals in the exclusion group showed more negative emotions and diminished positive emotions relative to those in the inclusion group. These findings were consistent with previous research; for example, more participants in the exclusion group reported negative psychological states (more thwarted psychological-need states and more negative moods) than participants in the inclusion group (Zwolinski, 2012). Therefore, we consider negative psychological states, such as rumination or other negative emotions (frustration, worry, nervousness, anger), were evoked by social exclusion from the Cyberball task. This finding also agrees with the previous findings: negative emotions, such as worry, may also increase fear responses to CS+ and CS- during fear acquisition (Gazendam and Kindt, 2012). Our findings support and expand the Clark and Wells model. Bolstering evidence for the model, we found post-exclusion abnormal behavioral reactions to fear acquisition and generalization, as well as a delayed recovery of mPFC activity where the social exclusion group showed less activation in the mPFC compared to social inclusion group. Concerning our expansion of the SAD model, as fear acquisition and generalization are significant components of several anxiety disorders - perhaps more impactful than social anxiety (Lissek et al., 2005, 2009, 2014) - social exclusion may have a more generalized negative effect on the development of anxiety disorders.

Interestingly, our data indicated that the psychological resilience traits of the participants in the exclusion group helped them to overcome the negative effect of social exclusion and fear inhibition. Further, resilience traits were negatively correlated with anxiety traits. This observation may be explained by the strong correlation between such resilience and human health (Davydov et al., 2010), as well as by the observation that high-resilience individuals with sufficient psychological resources deal with negative events more rationally (Peng et al., 2012). This finding indicates that improving resilience might be regarded as an effective clinical intervention for preventing the development of anxiety disorders.

Our research is subject to the limitations of a small-to-medium sample size and circumscribed age range. The random assignment of the ITI in the fear acquisition stage to around 5–8 s is another potential limitation, as this duration may have been too short to allow SCRs to return completely to baseline after a shock was delivered. The short ITI may thus have diminished the accuracy of SCR during fear acquisition. Moreover, participants may be more sensitive to social stimuli than to simple perceptual stimuli after social exclusion. Future research should consider changing our fear acquisition and generalization stimuli to social stimuli (such as faces, see Öhman and Mineka, 2001) and explore the same question considered by the present study in patients with anxiety disorders, such as SAD. There was another limitation in the research that although the slope of mPFC in the fear generalization between the exclusion group and the inclusion group was significant different, we found no significant ANOVA results in the mPFC in the fear generalization. That possibly because the social exclusion was not directly affect fear generalization but though fear acquisition, which may decrease the effect of social exclusion on the mPFC. The further study could explore the direct effect from social exclusion to fear generalization through testing the activity of mPFC when the social exclusion task was between the fear acquisition and fear generalization. Furthermore, fNIRS is an optical technology with a low spatial resolution of approximately 3 cm with the light emitters/detectors placed according to the 10-20 system, which may have been inappropriate for obtaining data from our selected region of interest. Thus, future research should employ other high spatial resolution methods, such as high-field fMRI or PET, to both validate and expand upon the findings of the present study.

5. Conclusions

The present study found that social exclusion impairs fear acquisition and generalization, and this effect is mediated by the activation of the medial PFC. This research helps to elucidate the neural and the

psychological mechanisms of the effect of social exclusion on anxiety disorders.

CRediT authorship contribution statement

Dou Haoran: Conceptualization, Methodology, Software, Writing - original draft, Writing - review & editing. **Lei Yi:** Conceptualization, Supervision, Writing - review & editing. **Cheng Xiaojun:** Data curation. **Wang Jinxia:** Visualization. **Leppänen H.T. Paavo:** Writing - review & editing.

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Appendix A. Supplementary data

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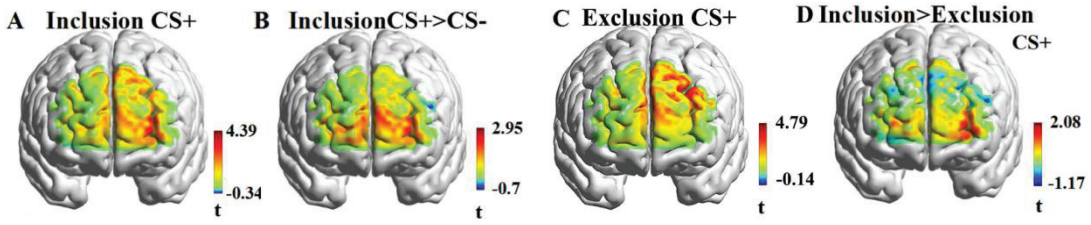
Supplementary materials

Results

1.1 Other significantly activated channels

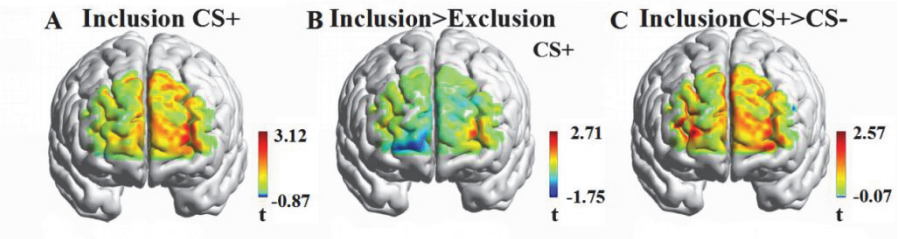
During the fear acquisition stage, threat stimuli (CS+) presented to the inclusion group during the fear acquisition phase activated several channels in the prefrontal cortex (PFC) (CH2, $T = 2.69$, $p = 0.014$; CH4, $T = 2.71$, $p = 0.013$; CH5, $T = 2.97$, $p = 0.0075$; CH6, $T = 4.39$, $p = 0.00028$; CH8, $T = 2.95$, $p = 0.0078$; CH12, $T = 3.14$, $p = 0.0051$; CH17, $T = 2.69$, $p = 0.014$) (Supplementary Figure 1A). In the inclusion group, we found these channels showed a significantly higher activation in response to CS+ than to CS- (CH6, $T = 2.70$, $p = 0.01$; CH12, $T = 2.95$, $p = 0.005$; CH13 $T = 2.04$, $p = 0.047$) (Supplementary Figure 1B). The presentation of threat stimuli (CS+) to the exclusion group during fear acquisition activated channels in the PFC (CH1, $T = 2.67$, $p = 0.014$; CH2, $T = 4.09$, $p = 0.0005$; CH5, $T = 4.79$, $p = 0.000008$; CH7, $T = 3.15$, $p = 0.0046$; CH8, $T = 3.17$, $p = 0.0044$; CH9, $T = 3.09$, $p = 0.0053$; CH12, $T = 3.00$, $p = 0.0065$) (Supplementary Figure 1C). In the exclusion group, we found no channels that showed significant CS+ > CS-. Safety stimuli (CS+) of the two groups was not survive after the FDR correction.



Supplementary Figure 1.

In addition, we calculated the difference in the oxy-HB changes induced by CS+ between the exclusion and the inclusion groups; we found that CS+ induced a greater difference in the inclusion group than in exclusion group (CH 4, $T = 2.08$, $p = 0.043$; CH 6, $T = 2.07$, $p = 0.044$). We then calculated differences in oxy-Hb changes induced by CS- between the exclusion and the inclusion group, but no significant difference between the two groups was found (Supplementary Figure 1D).

During fear generalization, consistent with the results obtained during fear acquisition, we found that the CS+ activated the following channels in the inclusion group during the generalization stage: CH 2, $T = 3.12$, $p = 0.0053$; CH 4, $T = 3.11$, $p = 0.0055$; CH 6, $T = 2.99$, $p = 0.0072$; CH 16, $T = 3.01$, $p = 0.0069$ (Supplementary Figure 2A). However, no channels activated by CS+ could survive following FDR correction in the exclusion group. Safety stimuli (CS+) in the fear generalization stage did not survive in the two groups after the FDR correction.



Supplementary Figure 2.

1.2 The simple effect of interaction effect in fear generalization.

We then tested for differences in the activation of the left mPFC elicited by the CS+ in the exclusion and inclusion groups during fear generalization by using an independent sample t-test; CS+ in the left mPFC of participants in the inclusion group showed significantly higher activation than in those of the participants in the exclusion group (t = 2.08, p = 0.043, Supplementary Figure 2B). The difference between the CS+ and CS- was also significant (t = 2.57, p = 0.0183, paired sample t, Supplementary Figure 2C).

1.3 The ANOVA result of the deoxy-Hb in the fear learning and generalization.

We also calculated changes in deoxy-Hb concentrations in the mPFC during fear learning and generalization. We found no significant interaction effect between stimulus and group during fear acquisition (F(1, 42) = 0.767, p = 0.386, η_p^2 = 0.018). The main effects of group and stimulus were nonsignificant (F(1, 42) = 0.331, p = 0.586, η_p^2 = 0.008; F(1, 42) = 0.797, p = 0.377, η^2_p = 0.019; respectively). In addition, there was no significant interaction effect between the stimulus and group during fear generalization (F(5, 210) = 0.201, p = 0.962, η_p^2 = 0.005). The main effects of group and stimulus during fear generalization were also nonsignificant (F(1, 42) = 1.562, p = 0.218, η_p^2 = 0.036); F(5, 210) = 0.688, p = 0.633, η_p^2 = 0.016; respectively).

II

DIFFERENT BEHAVIOURAL AND NEURAL ACTIVITY ASSOCIATED WITH FEAR GENERALISATION AFTER OBSERVATIONAL AND DIRECT LEARNING IN HUMANS

by

Dou Haoran, Lei Yi, Pan Yafeng, Li Hong, & Astikainen Piia, 2022

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III

INTRANASAL OXYTOCIN DECREASES FEAR GENERALIZATION IN MALES, BUT DOES NOT MODULATE DISCRIMINATION THRESHOLD

by

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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.

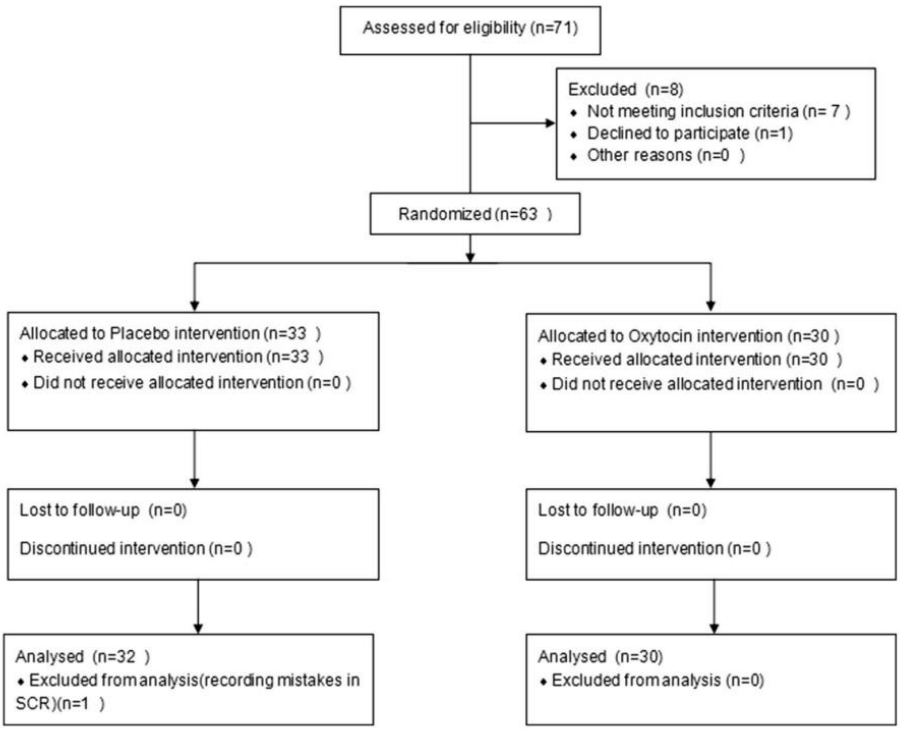

Figure 1. Flowchart of the study: we recruited 71 male participants in this study by advertisements and flyers on the campus. Eight participants were excluded (seven reported rhinitis or common cold and one declined participation in the experiment). Consequently, 63 participants were arranged randomly 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.

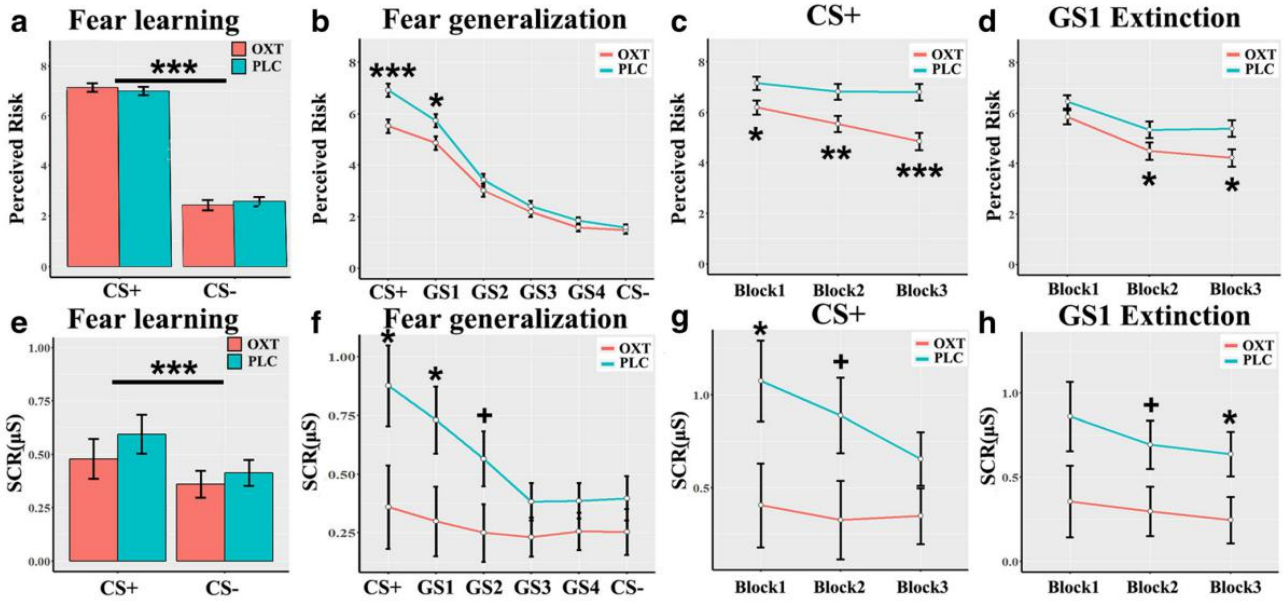

Figure 2. Threat rating and SCR during fear learning and generalization: A: The CS+ showed a significantly larger perceived risk than CS- ($p < 0.001$) in the fear learning stage for both, face and circle materials. No significant differences in perceived risk were observed between the oxytocin and placebo 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).

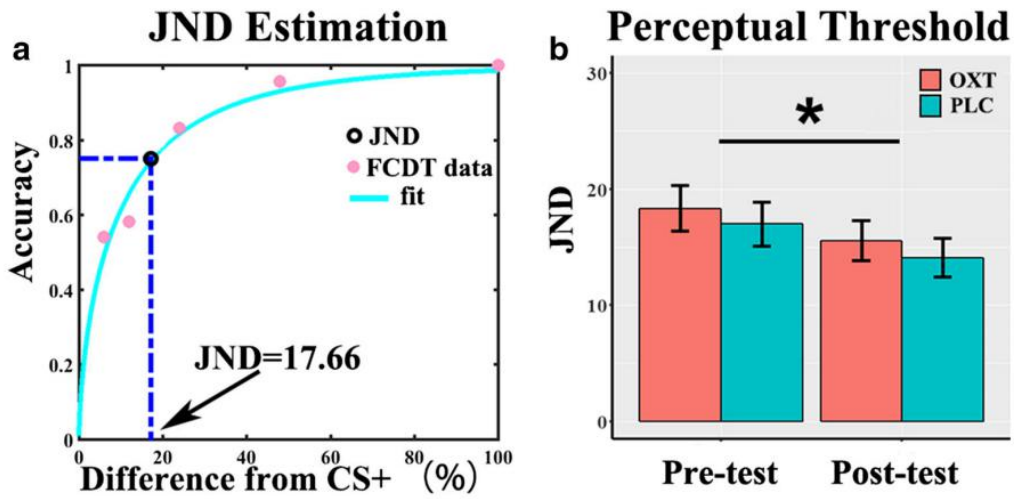

Figure 3. Perceptual threshold: A: A example of JND estimation. The pink dots represent the accuracy of the different type of stimuli in the FCDT. We fitted the data with Weibull function and choose 75% accuracy as the JND value. For example, this participant's JND is 17.66, which means that when a stimulus 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).

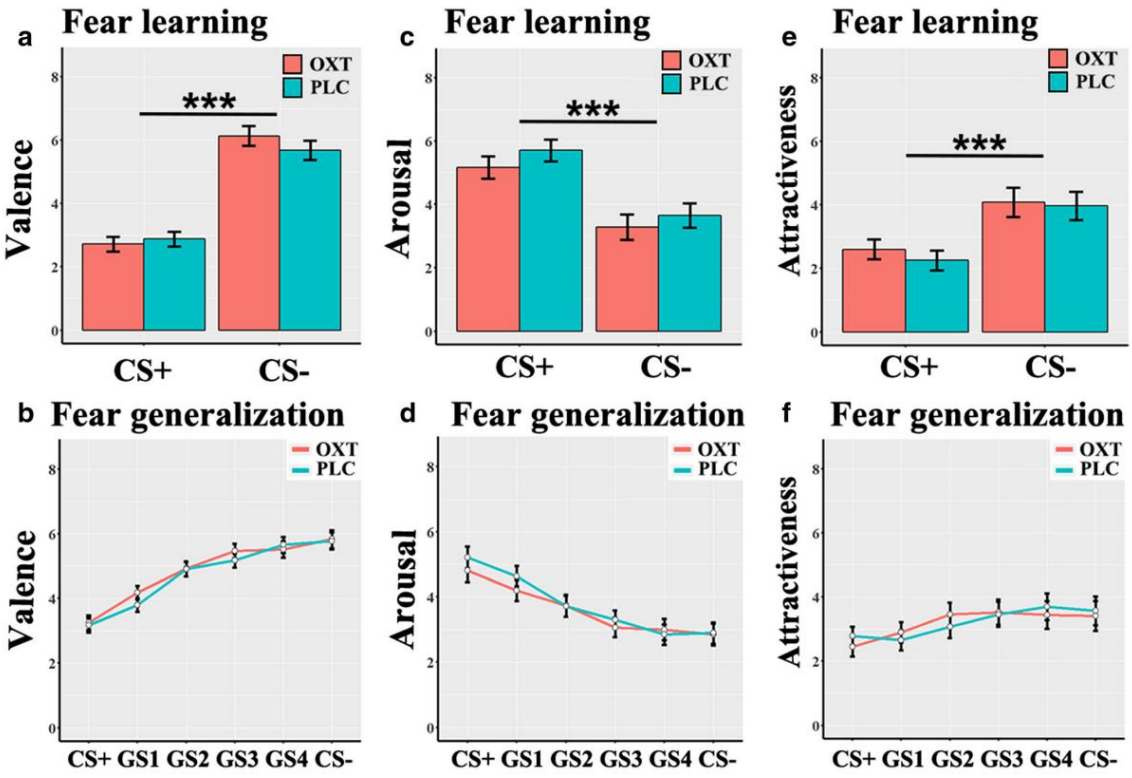

Figure 4. Results of the affective rating and attractiveness score after the fear generalization: A: The pleasure of CS+ for both face and circle materials was lower than that of CS- after fear conditioning ($p < 0.001$). No significant group effect was found. B: The main effect of stimulus was significant ($p < 0.001$) 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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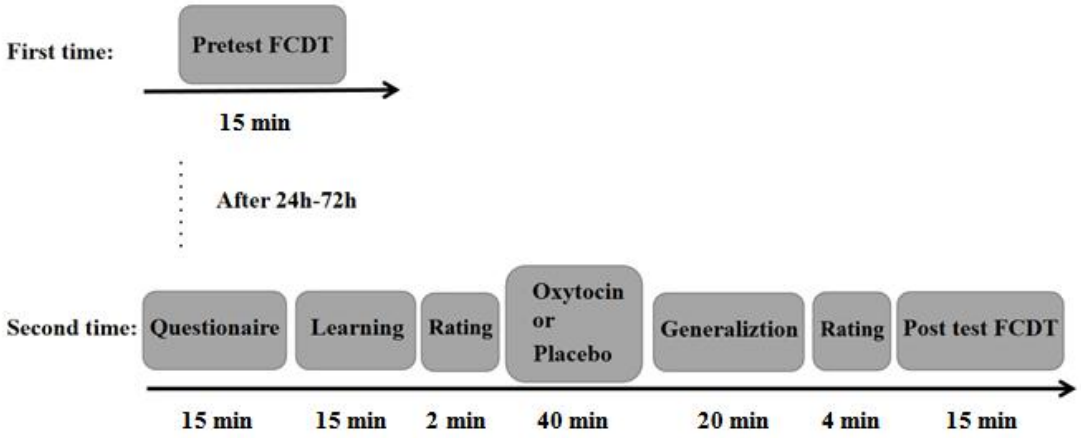
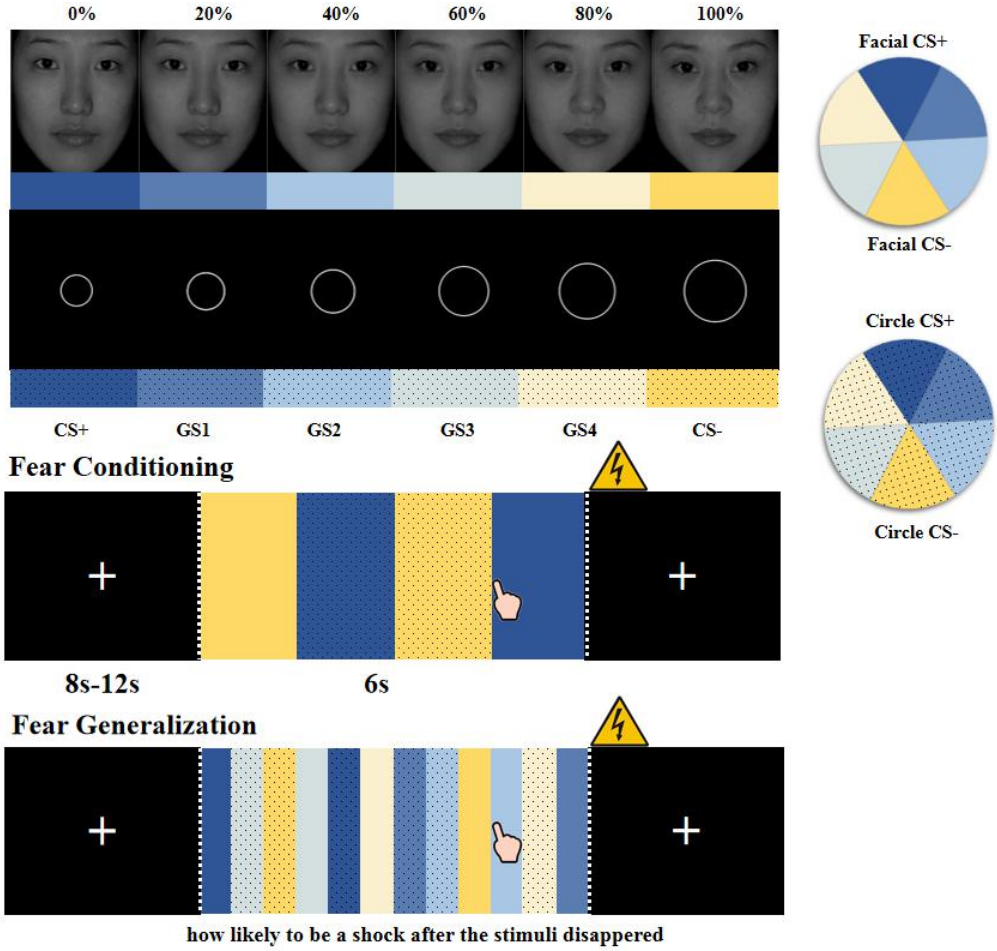
Supplementary Materials

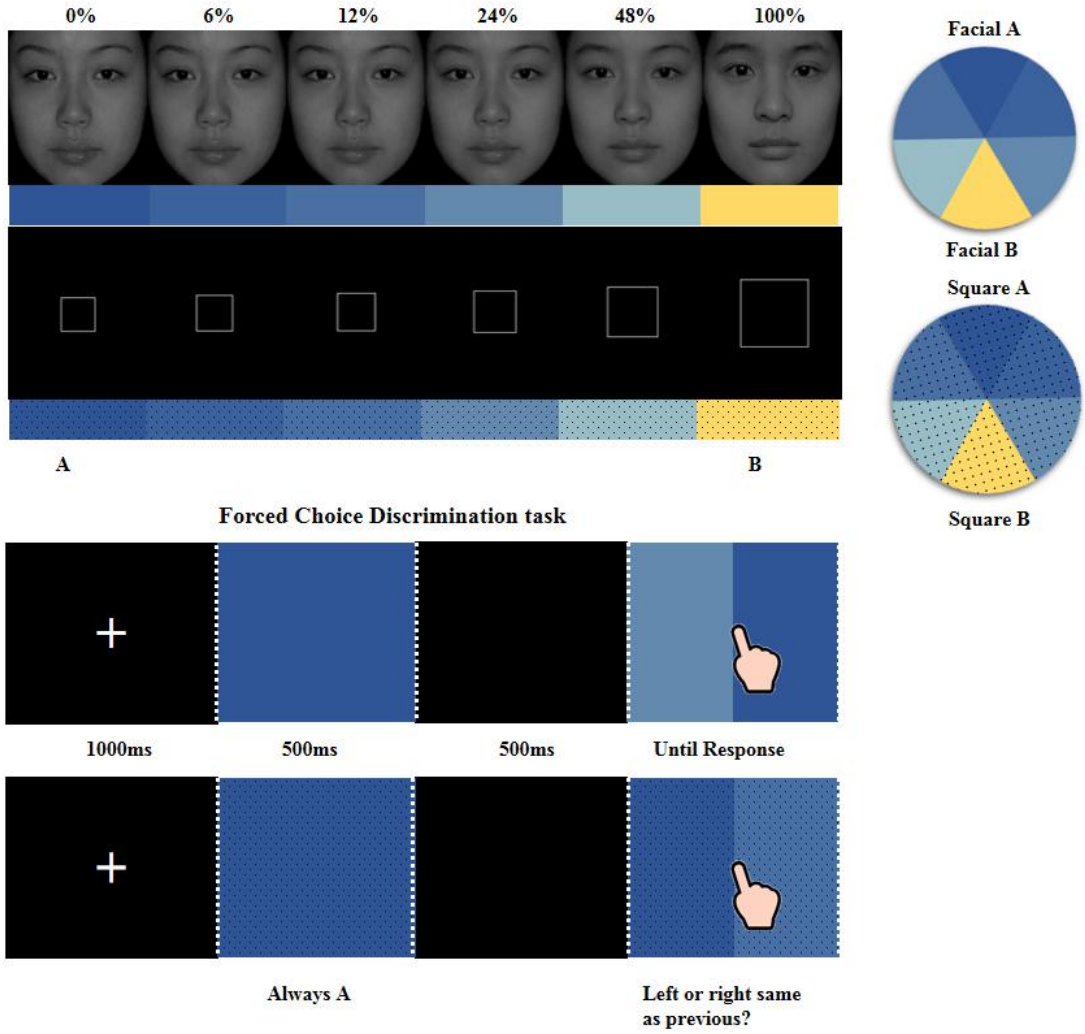


Supplementary figure 1. Experimental procedures: The participants came the lab twice. During the first visit they underwent a 15min Forced Choice Discrimination Task (FCDT). In order to decrease the effect of FCDT on fear learning and generalization, they were asked to come back after an interval of 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.