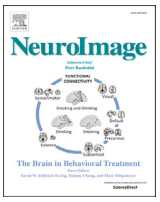
NeuroImage 218 (2020) 116735

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage



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

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ARTICLE INFO

Keywords:
Fear acquisition
Fear generalization
Social exclusion
mPFC
fNIRS
SCR

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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https://doi.org/10.1016/j.neuroimage.2020.116735
Received 31 March 2019; Received in revised form 3 March 2020; Accepted 10 March 2020
Available online 3 April 2020

(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

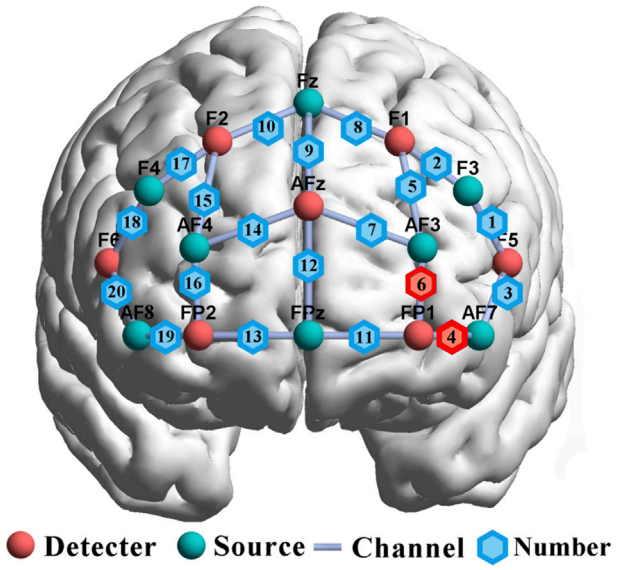

Fig. 2. Region of interest (ROI) in the PFC. Red and green balls indicate detectors and sources, respectively, while the branches represent channels. The red channels indicate the ROI (region of interest) of left mPFC.

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:$F(1, 42) = 2.439$, $p = 0.126$, $\eta^2_p = 0.055$).

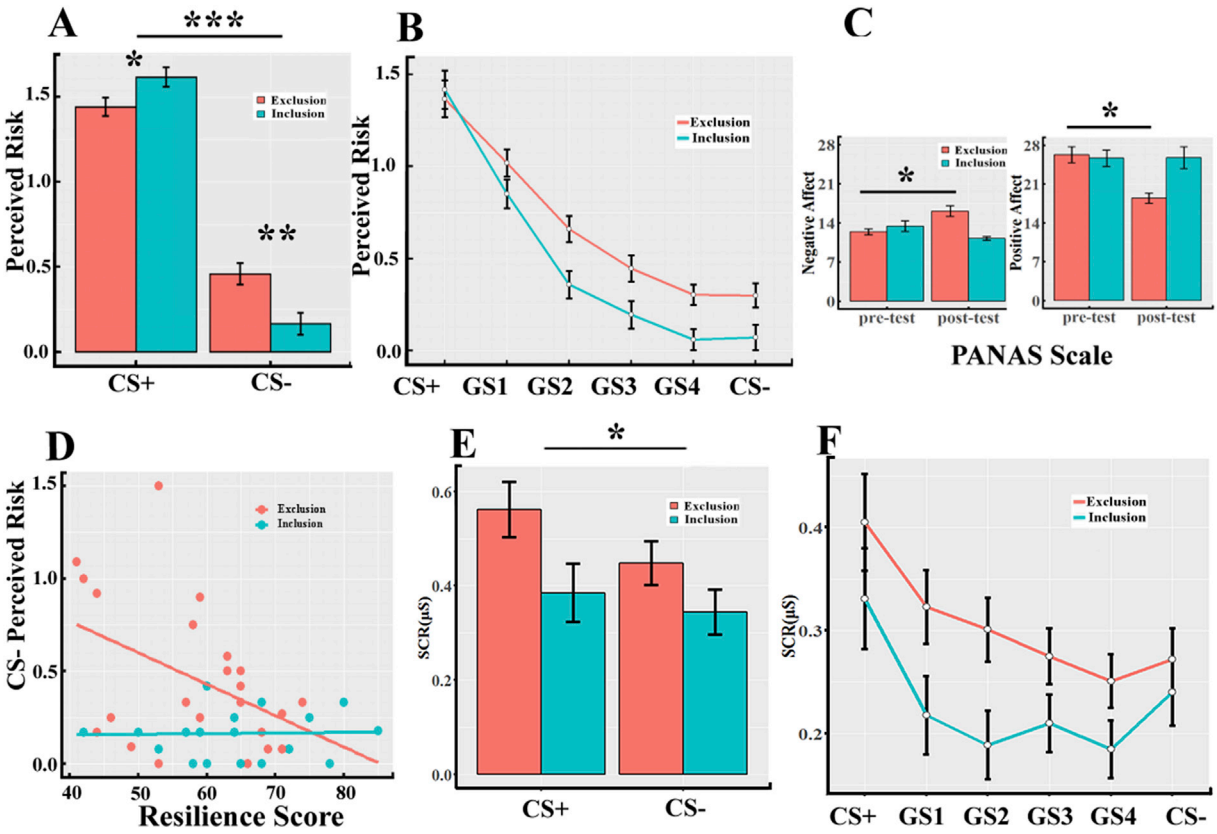

Fig. 3. A, shock-risk ranking during fear acquisition. The social exclusion group showed a larger risk to CS- and a smaller risk to CS + compared to the social inclusion group. B, shock-risk ranking during fear generalization. The social exclusion group felt a greater risk compared with social inclusion 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.

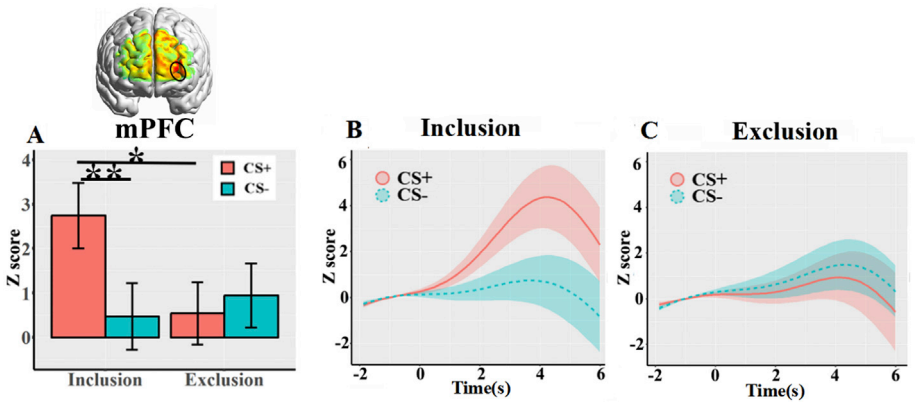

Fig. 4. fNIRS results obtained during fear acquisition. A, bar graph of oxy-Hb in the left mPFC during fear acquisition between 0.5 and 5 s. The topographic map showed the location activated by CS+ in the left mPFC in the inclusion group. B, time sequence of oxy-Hb of inclusion group during fear acquisition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

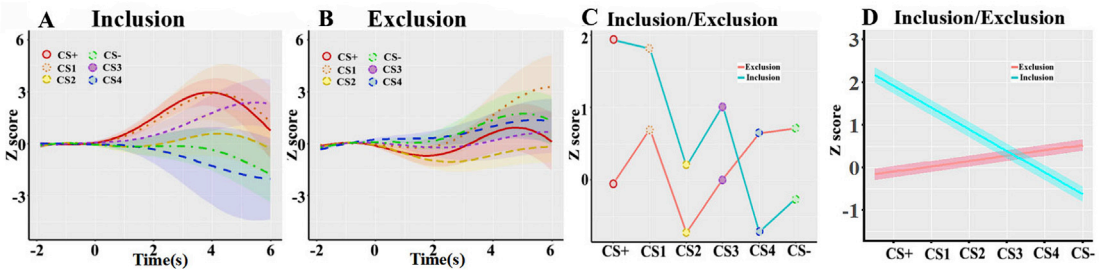

Fig. 5. fNIRS results obtained during fear generalization. A, time sequence of oxy-Hb concentration of the left mPFC in inclusion group during fear generalization; B, time sequence of oxy-Hb concentration of the left mPFC in exclusion group during fear generalization; C, a line graph of oxy-Hb in left 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

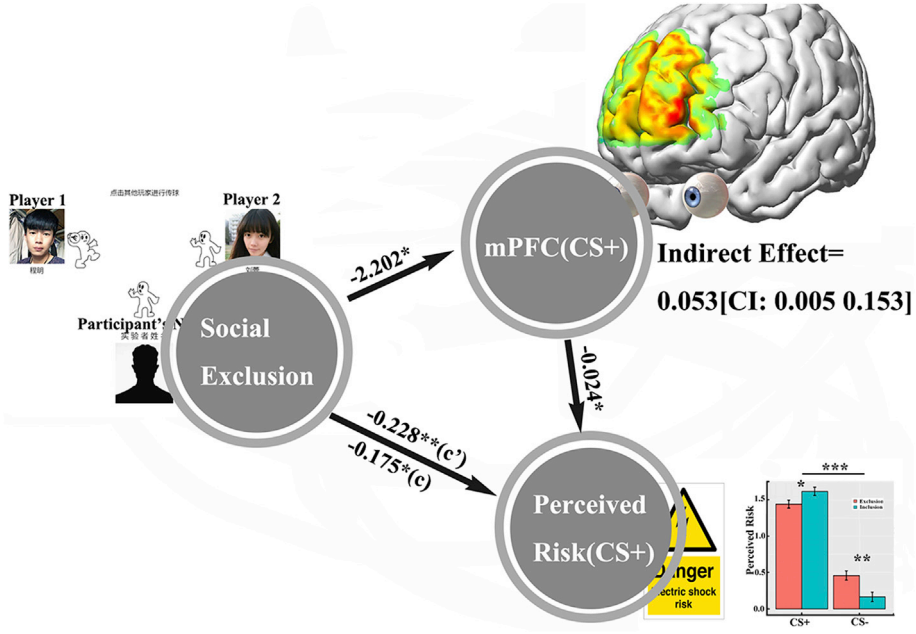

Fig. 6. Mediation analysis to explore the left mPFC influencing the relationship between social exclusion and fear acquisition. The oxy-Hb of left mPFC of CS + mediated the effect on the association between social exclusion and perceived risk of CS+ in the fear acquisition. "*", $p < 0.05$; "**", $p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

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.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NSFC31571153, NSFC31871130, NSFC31671150); the (Key) Project of DEGP (2015KCXTD009); Guangdong Key Project in "Development of new tools for diagnosis and treatment of Autism " (2018B030335001); Shenzhen Peacock Plan (KQTD2015033016104926); Major Program of Guangdong, China (2016KZDXM009); JCYJ20150729104249783; the Foundation for Distinguished Young Talents in Higher Education of Guangdong (2017KQNCX172); the Natural Science Foundation of SZU (2018053); Shenzhen-Hong Kong Brain Science Innovation Institute Project (2019SHIBS0003). The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2020.116735.

References

Ahrens, L.M., Mühlberger, A., Pauli, P., Wieser, M.J., 2014. Impaired visuocortical discrimination learning of socially conditioned stimuli in social anxiety. Soc. Cognit. Affect Neurosci. 10 (7), 929–937.
Ahrens, L.M., Pauli, P., Reif, A., Mühlberger, A., Langs, G., Aalderink, T., Wieser, M.J., 2016. Fear conditioning and stimulus generalization in patients with social anxiety disorder. J. Anxiety Disord. 44, 36–46.
Artuch-Garde, R., González-Torres, M.D.C., de la Fuente, J., Vera, M.M., Fernández-Cabezas, M., López-García, M., 2017. Relationship between resilience and self-regulation: a study of Spanish youth at risk of social exclusion. Front. Psychol. 8, 612.
Baumeister, R.F., Tice, D.M., 1990. Point-counterpoints: Anxiety and social exclusion. Journal of social and clinical Psychology 9 (2), 165–195.
Beck, A.T., Steer, R.A., Brown, G.K., 1996. Beck depression inventory-II. San Antonio 78 (2), 490–498.
Bolling, D.Z., Pitskel, N.B., Deen, B., Crowley, M.J., McPartland, J.C., Mayes, L.C., Pelphrey, K.A., 2011. Dissociable brain mechanisms for processing social exclusion and rule violation. Neuroimage 54 (3), 2462–2471 (left vlPFC).
Boucsein, W., Fowles, D.C., Grimnes, S., Ben-Shakhar, G., Roth, W.T., Filion, D.L., 2012. Publication recommendations for electrodermal measurements. Psychophysiology 49 (8), 1017–1034.
Burns, S.M., Barnes, L.N., Katzman, P.L., Ames, D.L., Falk, E.B., Lieberman, M.D., 2018. A functional near infrared spectroscopy (fNIRS) replication of the sunscreen persuasion paradigm. Soc. Cognit. Affect Neurosci. 13 (6), 628–636.
Cha, J., Greenberg, T., Carlson, J.M., DeDora, D.J., Hajcak, G., Mujica-Parodi, L.R., 2014. Circuit-wide structural and functional measures predict ventromedial prefrontal cortex fear generalization: implications for generalized anxiety disorder. J. Neurosci. 34 (11), 4043–4053.
Chester, D.S., Dewall, C.N., 2014. Prefrontal recruitment during social rejection predicts greater subsequent self-regulatory imbalance and impairment: neural and longitudinal evidence. Neuroimage 101, 485–493.
Clark, D.M., Wells, A., 1995. A cognitive model of social phobia. Social Phobia Diagn. Assess. Treat. 4 (4), 69–93.
Cohen, S., 2004. Social relationships and health. Am. Psychol. 59 (8), 676.
Connor, K.M., Davidson, J.R., 2003. Development of a new resilience scale: the Connor-Davidson resilience scale (CD-RISC). Depress. Anxiety 18 (2), 76–82.
Davydov, D.M., Stewart, R., Ritchie, K., Chaudieu, I., 2010. Resilience and mental health. Clin. Psychol. Rev. 30 (5), 479–495.
DeWall, C.N., MacDonald, G., Webster, G.D., Masten, C.L., Baumeister, R.F., Powell, C., et al., 2010. Acetaminophen reduces social pain: behavioral and neural evidence. Psychol. Sci. 21 (7), 931–937.
Dibbets, P., van den Broek, A., Evers, E.A., 2015. Fear conditioning and extinction in anxiety-and depression-prone persons. Memory 23 (3), 350–364.
Downey, G., Feldman, S.I., 1996. Implications of rejection sensitivity for intimate relationships. J. Pers. Soc. Psychol. 70 (6), 1327.
Duits, P., Cath, D.C., Lissek, S., Hox, J.J., Hamm, A.O., Engelhard, I.M., et al., 2015. Updated meta-analysis of classical fear conditioning in the anxiety disorders. Depress. Anxiety 32 (4), 239–253.
Duits, P., Richter, J., Baas, J.M., Engelhard, I.M., Limberg-Thiesen, A., Heitland, I., Cath, D.C., 2017. Enhancing effects of contingency instructions on fear acquisition and extinction in anxiety disorders. J. Abnorm. Psychol. 126 (4), 378.
Dunsmoor, J.E., Murphy, G.L., 2015. Categories, concepts, and conditioning: how humans generalize fear. Trends Cognit. Sci. 19 (2), 73–77.
Dunsmoor, J.E., Paz, R., 2015. Fear generalization and anxiety: behavioral and neural mechanisms. Biol. Psychiatr. 78 (5), 336–343.
Dymond, S., Dunsmoor, J.E., Vervliet, B., Roche, B., Hermans, D., 2015. Fear generalization in humans: systematic review and implications for anxiety disorder research. Behav. Ther. 46 (5), 561–582.
Eisenberger, N.I., 2012. The pain of social disconnection: examining the shared neural underpinnings of physical and social pain. Nat. Rev. Neurosci. 13 (6), 421–434.
Eisenberger, N.I., Gable, S.L., Lieberman, M.D., 2007. Functional magnetic resonance imaging responses relate to differences in real-world social experience. Emotion 7 (4), 745.
Eisenberger, N.I., Lieberman, M.D., Williams, K.D., 2003. Does rejection hurt? An fMRI study of social exclusion. Science 302 (5643), 290–292.
Faul, F., Erdfelder, E., Buchner, A., Lang, A.G., 2009. Statistical power analyses using G* Power 3.1: tests for correlation and regression analyses. Behav. Res. Methods 41 (4), 1149–1160.
Foa, E.B., Zinbarg, R., Rothbaum, B.O., 1992. Uncontrollability and unpredictability in post-traumatic stress disorder: an animal model. Psychol. Bull. 112 (2), 218.
Fung, K., Alden, L.E., 2017. Once hurt, twice shy: social pain contributes to social anxiety. Emotion 17 (2), 231.
Gervain, J., Mehler, J., Werker, J.F., Nelson, C.A., Csibra, G., Lloyd-Fox, S., Aslin, R.N., 2011. Near-infrared spectroscopy: a report from the McDonnell infant methodology consortium. Dev. Cognit. Neurosci. 1 (1), 22–46.
Gradin, V.B., Waiter, G., Kumar, P., Stickle, C., Milders, M., Matthews, K., et al., 2012. Abnormal neural responses to social exclusion in schizophrenia. PloS One 7 (8), e42608.
Gazendam, F.J., Kindt, M., 2012. Worrying affects associative fear learning: a startle fear conditioning study. PloS One 7 (4), e34882.
Greenberg, T., Carlson, J.M., Cha, J., Hajcak, G., Mujica-Parodi, L.R., 2013. Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization. Depress. Anxiety 30 (3), 242–250.
Guhn, A., Dresler, T., Andreatta, M., Müller, L.D., Hahn, T., Tupak, S.V., et al., 2014. Medial prefrontal cortex stimulation modulates the processing of conditioned fear. Front. Behav. Neurosci. 8, 44.
Guhn, A., Dresler, T., Hahn, T., Mühlberger, A., Ströhle, A., Deckert, J., et al., 2012. Medial prefrontal cortex activity during the extinction of conditioned fear: an investigation using functional near-infrared spectroscopy. Neuropsychobiology 65 (4), 173–182.
Gutz, L., Renneberg, B., Roepke, S., Niedeggen, M., 2015. Neural processing of social participation in borderline personality disorder and social anxiety disorder. J. Abnorm. Psychol. 124 (2), 421.
Gutz, L., Roepke, S., Renneberg, B., 2016. Cognitive and affective processing of social exclusion in borderline personality disorder and social anxiety disorder. Behav. Res. Ther. 87, 70–75.
Haaker, J., Lonsdorf, T.B., Thanellou, A., Kalisch, R., 2013. Multimodal assessment of long-term memory recall and reinstatement in a combined cue and context fear conditioning and extinction paradigm in humans. PloS One 8 (10), e76179.
Hayes, A.F., 2017. Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. Guilford Publications.
Heeren, A., Dricot, L., Billieux, J., Philippot, P., Grynberg, D., De Timary, P., Maurage, P., 2017. Correlates of social exclusion in social anxiety disorder: an fMRI study. Sci. Rep. 7 (1), 260.
Hjemdal, O., Vogel, P.A., Solem, S., Hagen, K., Stiles, T.C., 2011. The relationship between resilience and levels of anxiety, depression, and obsessive–compulsive symptoms in adolescents. Clin. Psychol. Psychother. 18 (4), 314–321.
Hornstein, E.A., Fanselow, M.S., Eisenberger, N.I., 2016. A safe haven: investigating social-support figures as prepared safety stimuli. Psychol. Sci. 27 (8), 1051–1060.
Jasper, H.H., 1958. The 10-20 electrode system of the International Federation. Electroencephalogr. Clin. Neurophysiol. 10, 370–375.
Klemenhagen, K.C., Sahay, A., Hen, R., 2012. Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. Nat. Neurosci. 15 (12), 1613–1620.
Koessler, L., Maillard, L., Benhadid, A., Vignal, J.P., Felblinger, J., Vespignani, H., Braun, M., 2009. Automated cortical projection of EEG sensors: anatomical correlation via the international 10–10 system. NeuroImage 46 (1), 64–72.
Krabbe, S., Gründemann, J., Lüthi, A., 2018. Amygdala inhibitory circuits regulate associative fear conditioning. Biol. Psychiatr. 83, 800–880.
Laux, L., Glanzmann, P., Schaffner, P., Spielberger, C.D., 1981. Das State-Trait-Angstinventar: STAI. Beltz, Weinheim.
Leary, M.R., 1990. Responses to social exclusion: Social anxiety, jealousy, loneliness, depression, and low self-esteem. J. Soc. Clin. Psychol. 9 (2), 221–229.
Legate, N., DeHaan, C.R., Weinstein, N., Ryan, R.M., 2013. Hurting you hurts me too: the psychological costs of complying with ostracism. Psychol. Sci. 24 (4), 583–588.
Lei, Y., Wang, J., Dou, H., Qiu, Y., Li, H., 2019. Influence of typicality in category-based fear generalization: diverging evidence from the P2 and N400 effect. Int. J. Psychophysiol. 135, 12–20.
Levinson, C.A., Langer, J.K., Rodebaugh, T.L., 2013. Reactivity to exclusion prospectively predicts social anxiety symptoms in young adults. Behav. Ther. 44 (3), 470–478.
Likhtik, E., Paz, R., 2015. Amygdala–prefrontal interactions in (mal) adaptive learning. Trends Neurosci. 38 (3), 158–166.

Likhtik, E., Stujenske, J.M., Topiwala, M.A., Harris, A.Z., Gordon, J.A., 2014. Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. Nat. Neurosci. 17 (1), 106.
Linnman, C., Rougemont-Bücking, A., Beucke, J.C., Zeffiro, T.A., Milad, M.R., 2011. Unconditioned responses and functional fear networks in human classical conditioning. Behav. Brain Res. 221 (1), 237–245.
Lissek, S., Biggs, A.L., Rabin, S.J., Cornwell, B.R., Alvarez, R.P., Pine, D.S., Grillon, C., 2008. Generalization of conditioned fear-potentiated startle in humans: experimental validation and clinical relevance. Behav. Res. Ther. 46 (5), 678–687.
Lissek, S., Bradford, D.E., Alvarez, R.P., Burton, P., Espensen-Sturges, T., Reynolds, R.C., Grillon, C., 2013. Neural substrates of classically conditioned fear-generalization in humans: a parametric fMRI study. Soc. Cognit. Affect Neurosci. 9 (8), 1134–1142.
Lissek, S., Kaczkurkin, A.N., Rabin, S., Geraci, M., Pine, D.S., Grillon, C., 2014. Generalized anxiety disorder is associated with overgeneralization of classically conditioned fear. Biol. Psychiatr. 75 (11), 909–915.
Lissek, S., Powers, A.S., Mcclure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., et al., 2005. Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav. Res. Ther. 43 (11), 1391.
Lissek, S., Rabin, S., Heller, R.E., Lukenbaugh, D., Geraci, M., Pine, D.S., Grillon, C., 2010. Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. American Journal of Psychiatry 167 (1), 47–55.
Lissek, S., Rabin, S., Heller, R.E., Lukenbaugh, D., Geraci, M., Pine, D.S., Grillon, C., 2009. Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. Am. J. Psychiatr. 167 (1), 47–55.
Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kössl, M., Holsboer, F., Wotjak, C.T., 2007. Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J. Neurosci. 27 (4), 832–839.
Masten, C.L., Eisenberger, N.I., Borofsky, L.A., Pfeifer, J.H., Mcnealy, K., Mazziotta, J.C., et al., 2009. Neural correlates of social exclusion during adolescence: understanding the distress of peer rejection. Soc. Cognit. Affect Neurosci. 4 (2), 143.
Matthias, S., Giuseppe, C., Bach, D.R., 2015. Optimising a model-based approach to inferring fear learning from skin conductance responses. J. Neurosci. Methods 255, 131–138.
McDevitt, R.A., Hiroi, R., Mackenzie, S.M., Robin, N.C., Cohn, A., Kim, J.J., Neumaier, J.F., 2011. Serotonin 1B autoreceptors originating in the caudal dorsal raphe nucleus reduce expression of fear and depression-like behavior. Biol. Psychiatr. 69 (8), 780–787.
Moor, B.G., Güroğlu, B., de Macks, Z.A.O., Rombouts, S.A., Van der Molen, M.W., Crone, E.A., 2012. Social exclusion and punishment of excluders: neural correlates and developmental trajectories. Neuroimage 59 (1), 708–717.
Morin, A., Michaud, J., 2007. Self-awareness and the left inferior frontal gyrus: inner speech use during self-related processing. Brain Res. Bull. 74 (6), 387–396.
Motzkin, J.C., Philippi, C.L., Wolf, R.C., Baskaya, M.K., Koenigs, M., 2015. Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans. Biol. Psychiatr. 77 (3), 276–284.
Nili, U., Goldberg, H., Weizman, A., Dudai, Y., 2010. Fear thou not: activity of frontal and temporal circuits in moments of real-life courage. Neuron 66 (6), 949–962.
Öhman, A., Mineka, S., 2001. Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. Psychol. Rev. 108 (3), 483.
Olsson, A., Carmona, S., Downey, G., Bolger, N., Ochsner, K.N., 2013. Learning biases underlying individual differences in sensitivity to social rejection. Emotion 13 (4), 616–621.
Onat, S., Büchel, C., 2015. The neuronal basis of fear generalization in humans. Nat. Neurosci. 18 (12), 1811.
Pavlov, P.I., 2010. Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. Ann. Neurosci. 17 (3), 136.
Peng, L., Zhang, J., Li, M., Li, P., Zhang, Y., Zuo, X., et al., 2012. Negative life events and mental health of Chinese medical students: the effect of resilience, personality and social support. Psychiatr. Res. 196 (1), 138–141.
Perusini, J.N., Fanselow, M.S., 2015. Neurobehavioral perspectives on the distinction between fear and anxiety. Learn. Mem. 22 (9), 417–425.
Piper, S.K., Krueger, A., Koch, S.P., Mehnert, J., Habermehl, C., Steinbrink, J., et al., 2014. A wearable multi-channel fNIRS system for brain imaging in freely moving subjects. Neuroimage 85, 64–71.
Pollak, D.D., Rogan, M.T., Egner, T., Perez, D.L., Yanagihara, T.K., Hirsch, J., 2010. A translational bridge between mouse and human models of learned safety. Ann. Med. 42 (2), 127–134.
Redondo, J., Marcos, J.L., 2003. Effects of CS-US interval on unconditioned response diminution in human heart rate classical conditioning. J. Psychophysiol. 17 (1), 30.
Sebastian, C.L., Tan, G.C., Roiser, J.P., Viding, E., Dumontheil, I., Blakemore, S.J., 2011. Developmental influences on the neural bases of responses to social rejection: implications of social neuroscience for education. Neuroimage 57 (3), 686–694.
Shergill, S.S., Brammer, M.J., Fukuda, R., Bullmore Jr., E., Edson, Amaro, Murray, R.M., et al., 2002. Modulation of activity in temporal cortex during generation of inner speech. Hum. Brain Mapp. 13 (6), 600-600.
Sotres-Bayon, F., Quirk, G.J., 2012. Prefrontal control of fear: more than just extinction. Curr. Opin. Neurobiol. 20 (2), 231–235.
Stevens, J.S., Jovanovic, T., Fani, N., Ely, T.D., Glover, E.M., Bradley, B., Ressler, K.J., 2013. Disrupted amygdala-prefrontal functional connectivity in civilian women with posttraumatic stress disorder. J. Psychiatr. Res. 47 (10), 1469–1478.
Themanson, J.R., Ball, A.B., Khatcherian, S.M., Rosen, P.J., 2014. The effects of social exclusion on the ERN and the cognitive control of action monitoring. Psychophysiology 51 (3), 215–225.
van der Kant, A., Biro, S., Levelt, C., Huijbregts, S., 2018. Negative affect is related to reduced differential neural responses to social and non-social stimuli in 5-to-8-month-old infants: a functional near-infrared spectroscopy-study. Dev. Cognit. Neurosci. 30, 23–30.
Vijayakumar, N., Cheng, T.W., Pfeifer, J.H., 2017. Neural correlates of social exclusion across ages: a coordinate-based meta-analysis of functional MRI studies. Neuroimage 153, 359–368.
Wesselmann, E.D., Ren, D., Swim, E., Williams, K.D., 2013. Rumination hinders recovery from ostracism. Int. J. Dev. Sci. 7 (1), 33–39.
Williams, K.D., Jarvis, B., 2006. Cyberball: a program for use in research on interpersonal ostracism and acceptance. Behav. Res. Methods 38 (1), 174–180.
Williams, K.D., Yeager, D.S., Cheung, C.K.T., Choi, W., 2012. Cyberball 4.0 [Computer Software]. Retrieved from. https://cyberball.wikispaces.com/.
Xu, W., Südhof, T.C., 2013. A neural circuit for memory specificity and generalization. Science 339 (6125), 1290–1295.
Xu, Y., Graber, H.L., Barbour, R.L., 2014. nirsLAB: a computing environment for fNIRS neuroimaging data analysis. In: Biomedical Optics. Optical Society of America, Miami. BM3A-1.
Yang, J., Kanazawa, S., Yamaguchi, M.K., Kuriki, I., 2016. Cortical response to categorical color perception in infants investigated by near-infrared spectroscopy. Proc. Natl. Acad. Sci. Unit. States Am. 113 (9), 2370–2375.
Zadro, L., Boland, C., Richardson, R., 2006. How long does it last? the persistence of the effects of ostracism in the socially anxious. J. Exp. Soc. Psychol. 42 (5), 692–697.
Zhang, D., Zhou, Y., Yuan, J., 2018. Speech prosodies of different emotional categories activate different brain regions in adult cortex: an fNIRS study. Sci. Rep. 8 (1), 218.
Zwolinski, J., 2012. Psychological and neuroendocrine reactivity to ostracism. Aggress. Behav. 38 (2), 108–125.