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Exaggerated sensitivity to threat and reduced medial prefrontal engagement during threat generalization in reactive aggressive adolescents

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ABSTRACT

Aggressive adolescents tend to exhibit abnormal fear acquisition and extinction, and reactive aggressive adolescents are often more anxious. However, the relationship between fear generalization and reactive aggression (RA) remains unknown. According to Reactive-Proactive Aggression Questionnaire (RPQ) scores, 61 adolescents were divided into two groups, namely, a high RA group ($N = 30$) and a low aggression (LA) group ($N = 31$). All participants underwent three consecutive phases of the Pavlovian conditioning paradigm (i.e., habituation, acquisition, and generalization), and neural activation of the medial prefrontal cortex (mPFC) was assessed by functional near-infrared spectroscopy (fNIRS). The stimuli were ten circles with varying sizes, including two conditioned stimuli (CSs) and eight generalization stimuli (GSs). A scream at 85 dB served as the auditory unconditioned stimulus (US). The US expectancy ratings of both CSs and GSs were higher in the RA group than in the LA group. The fNIRS results showed that CSs and GSs evoked lower mPFC activation in the RA group compared to the LA group during fear generalization. These findings suggest that abnormalities in fear acquisition and generalization are prototypical dysregulations in adolescents with RA. They provide neurocognitive evidence for dysregulated fear learning in the mechanisms underlying adolescents with RA, highlighting the need to develop emotional regulation interventions for these individuals.

1. Introduction

Maladaptive aggression presents a significant concern within both clinical and societal contexts (van Goozen et al., 2007), frequently coinciding with various psychiatric and neurological disorders. Adolescents exhibiting maladaptive aggression are at an elevated risk of criminal behavior in adulthood (Connor et al., 2019). Deficits in fear conditioning have been linked to an increased risk of maladaptive aggression and antisocial behavior in adult psychopaths and criminals (Cohn et al., 2013). Nonetheless, the relationship between fear conditioning mechanisms and aggressive behaviour in adolescents remains inadequately elucidated.

The fear-conditioning paradigm, which includes fear acquisition, extinction, and generalization, is a prevalent method for investigating behavioral performance in fear learning, along with its underlying neural basis and psychopathological alterations (Lissek et al., 2008; Lonsdorf et al., 2017; Zhou et al., 2019; Dou et al., 2021). This paradigm is based on Pavlovian conditioning, using two neutral stimuli as conditioned stimuli (CSs), differentially paired with an unconditioned (aversive) stimulus (US) during fear acquisition (Pavlov, 1927). The CS+ is consistently paired with the US, While the CS− remains unpaired. This probabilistic pairing (CS−US) leads to the CS+ eliciting a conditioned response (CR) alone. The CR diminishes as the CS+ is presented repeatedly without the US, termed fear extinction. Fear generalization refers to a fear response extending beyond the stimulus initially inducing fear to other neutral stimuli resembling the CS+, known as generalization stimuli (GSs) (Hovland, 1937). Individuals confronted with potential threats consider cues predicting safety or danger, selecting appropriate defensive responses based on past experiences (Webler et al., 2021). Moderate fear generalization aids survival in complex

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environments, while excessive generalization may impair daily functioning (Glenn et al., 2020).

Previous studies exploring fear conditioning in adolescents with aggression presented divergent results. Some findings indicated a reduction in fear responses, while others suggested an increase. This discrepancy may be attributed to the existence of different subtypes of aggression. Aggression can be categorized into reactive aggression (RA) and proactive aggression (PA). RA is an immediate aggressive response to provocation, while PA refers to predatory and organized behaviour aimed at obtaining rewards (Dodge and Coie, 1987; Raine et al., 2006; Blair, 2018). Despite correlations between these aggression types, such as heredity, psychophysiology, cortex, and hormones, they differ conceptually and functionally (Vitaro et al., 2002). The clinical diagnosis of conduct disorder in adolescents is divided into two subgroups: one exhibiting callous-emotional traits associated with an increased risk of persistent antisocial behavior, and the other demonstrating enhanced threat sensitivity and RA (Blair et al., 2014).

Previous longitudinal studies have shown that adolescents with low autonomic reactivity during fear acquisition, measured by skin conductance response (SCR), are more likely to exhibit aggression in the future (Gao et al., 2010a, 2010b). Gao et al. (2015) found distinct differences in SCR during fear acquisition among individuals with high levels of reactive and proactive aggression. Specifically, adolescents with proactive aggression demonstrated poor fear acquisition, whereas this impairment was not observed in those with RA. Clinical studies have demonstrated deficient fear acquisition in adolescents with aggression-related disorders, such as disruptive behavior disorder (DBD) and conduct disorder (CD) (Fairchild et al., 2008, 2010). Moreover, Cohn et al. (2013) provided initial evidence of neural changes underlying alterations in fear acquisition in adolescents with DBD, showing greater activation in fear processing related brain areas compared to healthy controls. However, findings on alterations in fear extinction among aggressive adolescents remain inconsistent (Veit et al., 2002; Birbaumer et al., 2005; Fairchild et al., 2008, 2010; Cohn et al., 2016). Moreover, behavioral and neural changes associated with fear generalization in aggressive adolescents are unclear.

The neural network involved in fear learning primarily centers on the amygdala, hippocampus, and the medial prefrontal cortex (mPFC) (Lissek et al., 2012; Hennings et al., 2022; Fullana et al., 2020; Likhtik & Paz, 2015; Webler et al., 2021). Specific changes in these brain systems have been reported during fear overgeneralization (e.g., Struyf et al., 2015; Spalding, 2018; Likhtik et al., 2014; Asok et al., 2019). The mPFC plays a critical role in regulating acquired threat responses to discriminate fear/threat from safety signals, particularly in fear inhibition (Guhn et al., 2012; Tuominen et al., 2019; Fullana et al., 2020; Zhou et al., 2019; Dou et al., 2020). Guhn et al. (2012) investigated fear extinction in healthy human subjects using functional near-infrared spectroscopy (fNIRS), revealing an inverse correlation between reduced fear association and mPFC activity during extinction. Furthermore, pharmacological challenges with medications like valproic acid have been shown to enhance synaptic plasticity effects in the mPFC, affecting trace and delay fear memories (Sui & Chen, 2012). Therefore, stronger activations in the mPFC indicate better inhibitory control and emotion regulation (Bertsch et al., 2020). This network partially overlaps with those involved in RA, reflecting an association with emotional dysregulation, including an imbalance between excessive negative emotional signals from bottom-up limbic regions and deficient prefrontal cortical control (Blair, 2013; McCloskey et al., 2016; Siep et al., 2019).

Prior studies on fear generalization predominantly involved adult participants (Lissek et al., 2014; Lissek et al., 2010; Lenaert et al., 2014), with relatively few focusing on children and adolescents (e.g., Lau et al., 2011; Glenn et al., 2012; Michalska et al., 2016; Schiele et al., 2016; El-Bar et al., 2017; Glenn et al., 2020; Reinhard et al., 2021). Adolescence represents a period of significant neurobiological change, particularly in the brain systems associated with RA (Papasideris et al., 2021). To develop effective prevention strategies for reducing reactive aggression in adolescents, it is essential to identify biomarkers of reactive aggression that can be targeted for intervention. One such biomarker is the mPFC. fNIRS has been frequently employed to investigate the regulatory function of the mPFC in fear learning (e.g., Dou et al., 2020; Guhn et al., 2012). It adept at detecting biomarkers of fear learning in adolescents, facilitates quantifying of blood oxygenation-related cortical hemodynamic changes. Moreover, fNIRS demonstrates greater reliability in measuring brain activation in naturalistic settings with reduced sensitivity to motion compared to magnetic resonance imaging (MRI)-based methods (Ayaz et al., 2012; Pinti et al., 2020; Quaresima et al., 2012; Yang et al., 2021; Li et al., 2019).

The current study investigated a sample of youth in middle childhood using a classic fear generalization paradigm based on perceptual size generalization. Building upon existing literature, we hypothesize that adolescents exhibiting elevated RA will display excessive fear during generalization, manifesting as increased US expectancy and concurrent reduced mPFC engagement. Our research aimed to enhance understanding of RA by exploring abnormalities in neurobehavioral mechanisms during fear acquisition and generalization among reactive aggressive adolescents.

2. Methods

2.1. Participants

A cohort of 443 adolescents (236 boys, age: $M = 13.31$, $SD = 0.92$) with normal or corrected-to-normal vision and devoid of neurological or psychiatric disorders, was recruited from a public school in southwestern China. Participants were screened and categorized into two groups based on their scores on the Reactive-Proactive Aggression Questionnaire (RPQ, Raine et al., 2006; RA: 8.47±4.45, PA: 1.03±1.83). Subsequently, participants were sorted based on their RA scores, with 20 % scoring below 4 and 80 % scoring above 12. Adolescents scoring below 4 were assigned to the low aggression (LA) group, while those scoring above 12 were assigned to the RA group. To mitigate the confounding effects of proactive aggression on fear generalization, proactive aggression scores were restricted to 0 in the LA group and less than 2 in the RA group.

Prior to conducting the study, a power analysis using G*Power for repeated measures ANOVAs was performed to ascertain group differences (RA and LA) (Perugini et al., 2018). Assuming a medium effect size (Cohen's $f = 0.25$), a significance level of $\alpha = 0.05$ and a power level of $1 - \beta = 0.8$, the analysis indicated a required total sample size of 24. To address potential technical challenges associated with psychophysiological indices (Lonsdorf et al., 2017), 61 participants were recruited. However, five participants were excluded from the study: two due to significant changes in aggression scores and three due to failure to exhibit fear acquisition (i.e., US expectancy CS− < CS+). Consequently, the final behavioral sample comprised 56 adolescents (30 boys; LA: 14 boys, RA: 16 boys, $p = 0.593$; for further descriptive data, refer to Table 1). Furthermore, nine additional participants were excluded from the fNIRS analysis due to technical recording errors, resulting in a final

Table 1
Demographics characteristics across samples.

	LA($n = 27$)		RA($n = 29$)		Significance[a]
Variable	**Mean**	**SD**	**Mean**	**SD**	
age	13.00	0.92	13.17	0.89	$p = 0.479$
RA $^{(1)}$	2.52	1.42	14.59	2.63	$p<0.001$
RA $^{(2)}$	5.12	2.92	12.00	3.33	$p<0.001$
SCARED	10.44	10.80	39.76	18.65	$p<0.001$

[a] Two-tailed p values reflect the significance of group differences derived from independent samples *t*-tests for all variables. RA$^{(1)}$ = reactive aggression scores for the first time; RA$^{(2)}$ = reactive aggression scores on the experiment day; SCARED = Screen for Child Anxiety Related Emotional Disorders.

fNIRS sample of 47 adolescents (25 boys; Age: $M = 13.19$, $SD = 0.90$).

Prior to commencement of the experiment, both participants and their parents provided written informed consent. The study protocol received approval from the Medicine Ethics Committee of Shenzhen University and adhered to the latest version of the Declaration of Helsinki.

2.2. Materials

The experimental paradigm employed in this study closely followed that of Lissek (2008), wherein ten rings of progressively varying sizes (Fig. 1) displayed on a computer monitor served as conditioned stimuli (CS) and generalization stimuli (GS). In this task, the largest and smallest rings were designated as either CS+ or CS−, with the former being paired and the latter unpaired with an aversive unconditioned stimulus (US). The assignment of rings to CS+ and CS− was counterbalanced across participants. Four classes of generalization stimuli (GS1, GS2, GS3, GS4) comprised eight intermediate-sized rings forming a size continuum between CS+ and CS−. The US consisted of an 85-dB female scream, which terminated simultaneously with CS+.

2.3. Study design and procedure

The experimental paradigm comprised three phases: habituation, acquisition, and generalization. The habituation phase consisted of four presentations each of CS− and CS+. During the acquisition phase, participants were presented with CS− and CS+ in 12 trials each, with nine of the CS+ trials accompanied by the scream (reinforcement rate: 75 %). In the generalization phase, participants encountered CS−, CS+ (reinforcement rate: 50 %), and the four GSs in 12 trials (refer to Table 2). To observe fear learning dynamics, we divided the generalization phase into three blocks, each containing an equal number of trials for CSs and GSs. Participants were allowed to rest between every two blocks. Subsequent to each phase, participants were prompted to rate the subjective fear ("*How fearful do you feel when looking at this picture*?";1= "*not fearful at all*", 9= "*very fearful*"), valence ("*How pleasant do you feel when looking at this picture*?";1= "*very unpleasant*", 9= "*very pleasant*") and arousal ("*How aroused do you feel when looking at this picture*?"; 1= "*very calm*" to 9= "*very arousing*") for each CS on a 9-point scale.

Stimuli were presented using E-prime 3.0 software against a grey background, with each stimulus displayed for 6 s on a computer monitor. A fixation cross (+) appeared at the screen's center for 1 s at the beginning of each trial. Subsequently, the CS or GS was presented, and participants rated the likelihood of the US occurrence on a three-point Likert scale (1= not likely at all, 3= very likely). Contingencies between the CS/GS and the US were not disclosed, and participants were instructed: *"Please rate the likelihood that you will be exposed to a scream."* Participants were required to provide ratings promptly based on their immediate feelings using a computer keyboard. The US was presented for 1 s and terminated concurrently with the CS offset. All stimuli were presented in a quasi-random order, with an inter-trial interval (ITI) ranging from 4 - 7 s (Fig. 2).

Table 2
Trial types and frequencies during the habituation, acquisition, and generalization phases of the study.

	Conditioned and Generalization Stimuli							
						CS+		
Phase	CS−	GS4	GS3	GS2	GS1	Yes US	No US	ITI
Habituation	4					0	4	4–7
Acquisition	12					9	3	4–7
Generalization	12	12	12	12	12	6	6	4–7

CS+= conditioned stimulus paired with scream; CS−= conditioned stimulus unpaired with scream; GS1, GS2, GS3, and GS4= generalization stimulus 1, 2, 3, and 4; US= unconditioned stimulus; ITI= intertrial interval. Half of the CS+ was reinforced with a scream during the generalization test to avoid extinction of the conditioned response during the generalization sequence.

2.4. Measures

2.4.1. Demographic information. basic demographic data were gathered via self-report

Reactive-Proactive Aggression Questionnaire (RPQ). The RPQ is a 23-item self-report instrument designed to evaluate aggression. It comprises 11 items assessing RA (e.g., "*When someone makes fun of me, I end up getting into a fight or angry*") and 12 items assessing PA (e.g., "*Take things from other students*"). Responses are provided on a 3-point scale (0= never, 1= sometimes, 2= often), and scores are aggregated to gauge levels of reactive and proactive aggression. The factor structure of the RPQ has been validated through extensive data analysis involving large adolescent samples (Raine et al., 2006). In our sample, internal consistency was deemed high (RA: Cronbach's α= 0.836; PA: Cronbach's α= 0.730).

The Screen for Child Anxiety Related Emotional Disorders (SCARED). The SCARED (Birmaher et al., 1997) is a 41-item self-report tool developed to evaluate anxiety levels in children and adolescents. Responses are recorded on a 3-point scale (0= never, 1= sometimes, 2= often), and scores are aggregated to quantify anxiety levels. The factor structure of the SCARED has been validated in a sizable adolescent population (Raine et al., 2006; Su et al., 2008).

Functional near-infrared spectroscopy: We employed a continuous wave system (NirScan, HuiChuang, China) to capture fNIRS signals. The system featured a 3 × 11 channel array (Hitachi Medical Co, Tokyo, Japan) comprising 16 detectors and 15 sources, yielding a total of 48 channels spaced approximately 3 cm apart. Detectors operated at two distinct wavelengths (730 and 850 nm). Signal acquisition occurred at a sampling rate of 11 Hz and was converted to changes in O_2Hb concentration. The medial prefrontal cortex (mPFC) was designated as the region of interest (ROI) based on extensive prior literature elucidating its

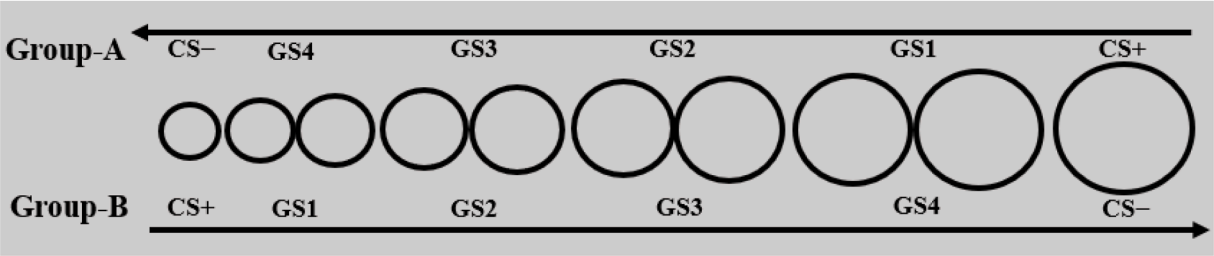

Fig. 1. Conditioned and generalization stimuli for counterbalancing Groups A and B. Half of the participants (Group A) were presented with the largest ring as the CS+ and the smallest as the CS−, while the other half (Group B) had this arrangement reversed. Additionally, each pair of intermediate rings constituted a single class of stimuli, resulting in four classes of GSs. The diameter of the smallest ring was 2.00 inches, with subsequent rings increasing by 15 %. Thus, the diameters of the rings, from smallest to largest, were 2.00, 2.30, 2.60, 2.90, 3.20, 3.50, 3.80, 4.10, 4.40, and 4.70 inches. Notations: CS+= conditioned stimulus paired with a scream; CS−= conditioned stimulus unpaired with a scream; GS1, GS2, GS3, and GS4= generalization stimulus 1, 2, 3, and 4.

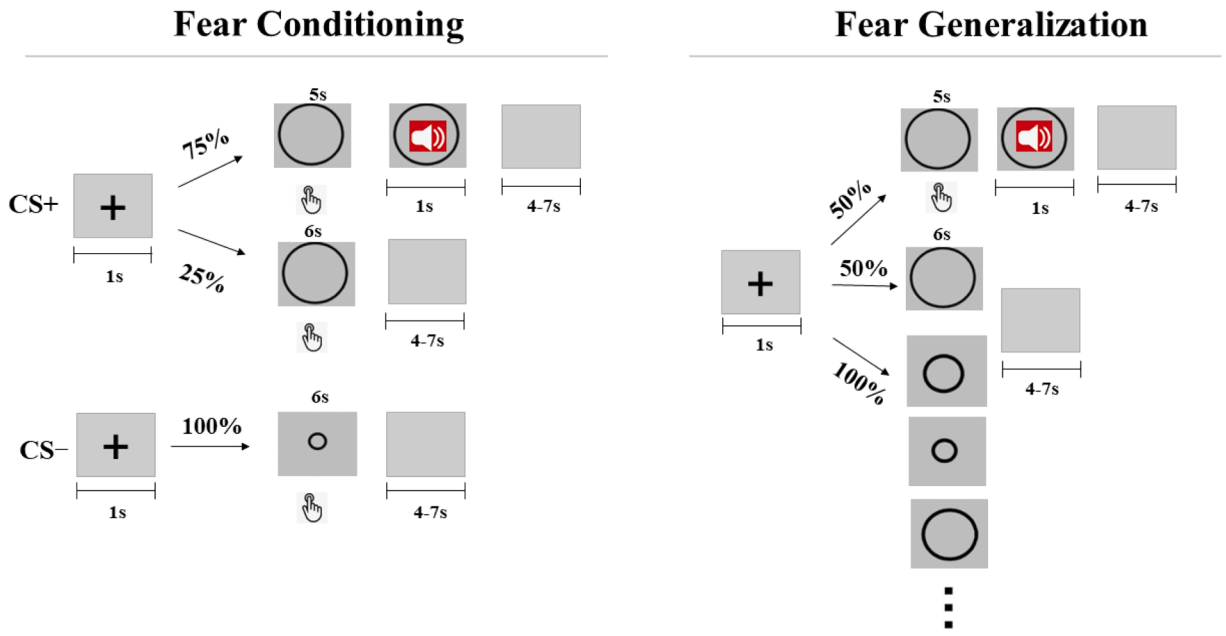

Fig. 2. Example of fear conditioning and generalization. At the onset of each trial, a fixation cross (+) was displayed at the center of the screen for 1 s. Subsequently, the conditioned stimulus (CS) or generalization stimulus (GS) was presented for 6 s, during which participants rated the likelihood of a scream occurring next. Ratings were provided on a three-alternative forced-choice scale (1= not likely at all, 3= very likely) using a computer keyboard. Participants were instructed to make their ratings promptly based on their immediate feelings. Following this, the unconditioned stimulus (US) was presented for 1 s and terminated concurrently with the offset of the CS. All stimuli were presented in a quasi-randomized order. The inter-trial interval (ITI) ranged from 4 to 7 s.

involvement in fear and aggression (Rosell & Siever, 2015; Alegria et al., 2016; Hofhansel et al., 2020; Webler et al., 2021; Zhou et al., 2019). Fig. 3 illustrates the ROI and corresponding channels.

For data preprocessing of the fNIRS signals, we used the HomER2 NIRS processing package (Huppert et al., 2009), which involved the following steps: 1) intensity data correction (HomER script: enPruneChannels; dRange=[1e+04 1e+07], SNRthresh= 2, SDrange= [0 45]); 2) conversion of optical intensity to optical density (HomER script: hmrIntensity2OD); 3) motion artifact detection (HomER script: hmrMotionArtifactByChannel; tMotion= 0.5, tMask= 1.0, STDEVthresh= 50.0, AMPthresh= 5.0) and subsequent correction (HomER script: hmrMotionCorrectSpline; p = 0.99, turnon= 1); 4) bandpass filtering (HomER script: hmrBandpassFilt; HPF= 0.01, LPF= 0.5); 5) conversion of optical density to oxygenated, deoxygenated, and total hemoglobin units using the modified Beer-Lambert Law (HomER script: hmrOD2Conc; PPF= 6); 6)calculation of the HbO blood oxygen concentration change (△HbO). To mitigate the impact of individual variations on results, we normalized the original HbO signal values in each channel and trial as Z-scores (Yang et al., 2016). The Z-scores (z) were computed by subtracting the baseline mean (μ2) from the concentration value (μ1) and dividing the result by the standard deviation (σ): z= (μ1 - μ2) / σ. Concentration time series were extracted from 2 s before stimulus onset to 5 s after stimulus onset, with the baseline defined as −2 s to 0.

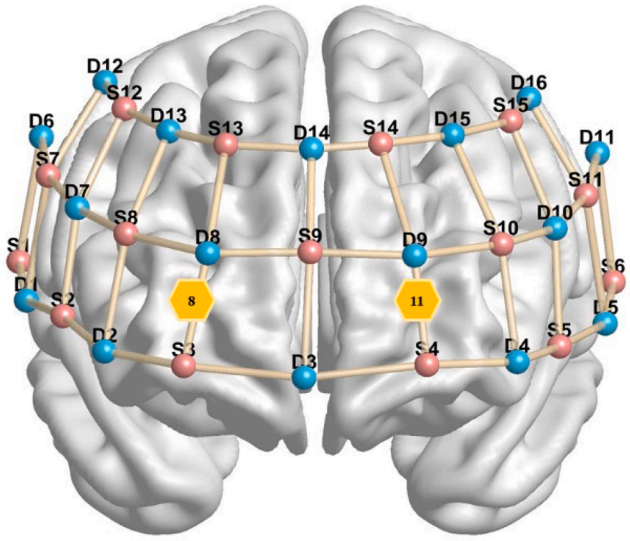

Fig. 3. Region of interest (ROI) in the Prefrontal Cortex (PFC). Red and blue spheres represent sources and detectors, respectively, while the lines indicate channels. The yellow hexagons (channel 8 and channel 11) highlight the medial prefrontal cortex (mPFC).

2.5. Data analysis

All statistical analyses were conducted using IBM SPSS 22 (IBM Corporation, Armonk, NY, USA) and Matlab software (Version 18, Natick, Mass., USA). For the analysis of US expectancy ratings during fear acquisition and generalization, we employed a 2 (Stimulus type: CS+, CS–) × 2 (Group: RA, LA) repeated measures ANOVA, and a 6 (Stimulus type: CS+, CS–, GS1, GS2, GS3, GS4) × 2 (Group: RA, LA) × 3 (Block: B1, B2, B3) repeated measures ANOVA. To control for the potential influence of grade, it was included as a covariate.

Regarding the fNIRS data collected from the mPFC during fear acquisition and generalization stages, we conducted a 2 (Stimulus type: CS+, CS–) × 2 (Group: RA, LA) repeated measures ANOVA and a 6 (Stimulus type: CS+, CS–, GS1, GS2, GS3, GS4) × 2 (Group: RA, LA) repeated measures ANOVA. Data analysis was performed using the PROCESS plug-in of SPSS statistics software (IBM, Hayes, 2017). In instances where the sphericity assumption was violated, the Greenhouse-Geisser (1959) correction was applied for repeated-measures ANOVAs. Subsequent follow-up analyses used Bonferroni correction for multiple comparisons. The alpha threshold for statistical significance was set at 0.05.

3. Results

3.1. Behavioral results

The fear acquisition task was evaluated to determine whether the stimuli elicited significantly different US expectancy in the two groups. The 2 × 2 repeated-measures ANOVA analysis of US expectancy results in the acquisition phase revealed significant main effects of Stimulus type ($F(1, 53) = 57.953$, $p < 0.001$, $\eta^2 = 0.522$) and Group ($F(1, 53) = 4.723$, $p = 0.034$, $\eta^2 = 0.082$), but the Stimulus type × Group interaction ($F(1, 53) = 0.012$, $p = 0.915$, $\eta^2 < 0.001$) did not reach significance. The ratings for the CS+ were significantly higher than those for CS– in both groups, and the ratings of the RA group were significantly higher compared to the LA group (Fig. 4A).

In addition, we examined differences in US expectancy between the two groups during fear generalization. The 6 × 2 × 3 analysis of US expectancy results revealed significant main effects of Stimulus type ($F(1.662, 88.109) = 19.314$, $p < 0.001$, $\eta^2 = 0.267$), Block ($F(2, 106) = 12.848$, $p < 0.001$, $\eta^2 = 0.195$), Group ($F(1, 53) = 5.418$, $p = 0.024$, $\eta^2 = 0.093$), and a CS Type × Block interaction ($F(6.839, 362.481) = 2.459$, $p = 0.019$, $\eta^2 = 0.044$). It was found that the perceived ratings of CS+, GS1, GS2, GS3 were significantly higher than those of CS– ($p < 0.001$), and there was no significant difference in the perceived ratings of GS4 and CS– ($p = 0.137$) (Fig. 4B). Additionally, the RA group reported significantly higher ratings than the LA group. Additionally, ratings in Block 1 (B1) were higher than those in Block 2 (B2), and ratings in B2 were higher than those in Block 3 (B3) ($p < 0.004$). Further post hoc *t*-tests demonstrated that the perceived ratings of CS+, GS1, GS2, and GS3 in B1 were significantly higher than those in B2 ($p < 0.009$), and merely ratings of GS1 in B2 were significantly higher than those in B3($p < 0.001$). The remaining omnibus effects did not reach significance ($p > 0.124$) (Fig. 4C).

3.2. fNIRS results

The subsequent analyses focused on mPFC activation in response to different stimuli. The 2 × 2 repeated-measures ANOVA of mPFC data during acquisition did not reach significance. However, the 6 × 2 analysis of mPFC data in the generalization phase revealed significant main effects of Stimulus type ($F(4.16, 187.199) = 2.749$, $p = 0.028$, $\eta^2 = 0.058$) and Group ($F(1, 45) = 5.774$, $p = 0.020$, $\eta^2 = 0.114$). The oxy-Hb activation of GS3 in the two groups was significantly higher than that of GS2 ($p = 0.001$), and that of RA group was significantly lower than the LA group ($p = 0.020$) (Fig. 5A). The Stimulus type × Group interaction ($F(4.16, 187.199) = 0.411$, $p = 0.808$, $\eta^2 = 0.009$) did not reach significance.

The main effect of Group was significant in channel 8 [$F(1, 45) = 7.684$, $p = 0.008$, $\eta^2 = 0.146$]. Channel 11 exhibited a marginal significant change [$F(1, 45) = 3.322$, $p = 0.075$, $\eta^2 = 0.069$], indicating that the active oxy-Hb value in the RA group was lower than that in the LA group (Fig. 5B). However, no significant differences were observed between the results of the other channels in the two groups ($p > 0.11$).

3.3. Moderation analyses results

To examine whether reduced mPFC activation could be attributed to higher levels of general anxiety within the RA group, we conducted an exploratory moderation analysis. This analysis aimed to explore the interplay between RA, anxiety, and fear generalization, with anxiety scores serving as the moderator. The sample size for the Bootstrap analysis was 5000. The RA × moderation-of-anxiety interaction term significantly contributed to the mPFC activation for GS2 and GS3 (β=0.013 [0.006, 0.019], $t = 3.974$, $p < 0.001$; β=0.009 [0.002, 0.015], $t = 2.504$, $p = 0.016$). The Johnson-Neyman technique revealed a significant moderation between RA and mPFC activation for GS2 when anxiety levels were below −7.62 ($p < 0.05$; 47 % of the sample), but not at higher levels ($p > 0.05$; 53 % of the sample). Similarly, a significant relationship between RA and mPFC activation for GS3 was observed when anxiety levels were below −23.30 ($p < 0.05$; 9 % of the sample), but not at higher levels ($p > 0.05$; 91 % of the sample) (Fig. 6). The mPFC activation for GS2 and GS3 in individuals with low anxiety decreased as RA levels increased.

4. Discussion

The present study provided preliminary evidence of dysregulation in fear generalization and related neurofunctional alterations in the mPFC among adolescents with high RA. Utilizing Pavlovian fear conditioning coupled with fNIRS, we assessed mPFC activity and investigated dysfunctions in fear generalization among adolescents with high RA. During fear acquisition and generalization, the RA group rated US expectancy significantly higher than the LA group. Moreover, fNIRS data revealed reduced mPFC activation in the RA group during fear generalization. Our findings suggest that adolescents with RA exhibit heightened threat sensitivity during generalization.

Behavioral data demonstrated successful acquisition of conditioned fear in both groups, as evidenced by higher US expectancy ratings for threat stimuli compared to safety stimuli. Furthermore, the RA group exhibited higher US expectancy ratings for the CS during fear acquisition compared to the LA group. Notably, ratings of US expectancy may serve to pinpoint subjective CS discrimination, highlighting potential differences in threat expectancy and sensitivity between adolescents with RA and those with LA (Lonsdorf et al., 2017). Moreover, compared to the LA group, the RA group displayed lower valence and higher fear ratings (Figure S2). These findings indicate that while adolescents with RA were able to differentiate between the CS+ and CS− during acquisition similar to LA adolescents, their fear response surpassed that of LA adolescents. Previous research has consistently associated RA with

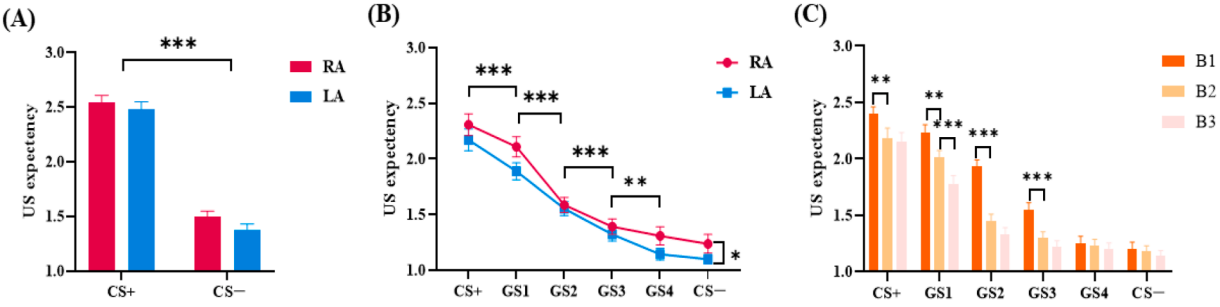

Fig. 4. Behavioral results for the self-reported likelihood to scream. (A) Acquisition across groups and stimulus type. (B) Generalization across groups and stimulus type. (C) Generalization across blocks. Generalization trials were divided into three blocks according to trial sequence (i.e., B1, B2, and B3) and stimulus type, in which 1= not likely at all and 3= very likely. RA, reactive aggression; LA, low aggression; CS+, conditioned stimulus paired with a scream; CS−, conditioned stimulus unpaired with a scream. Four classes of GSs (GS1, GS2, GS3, and GS4) formed a similarity continuum between CS+ and CS−.

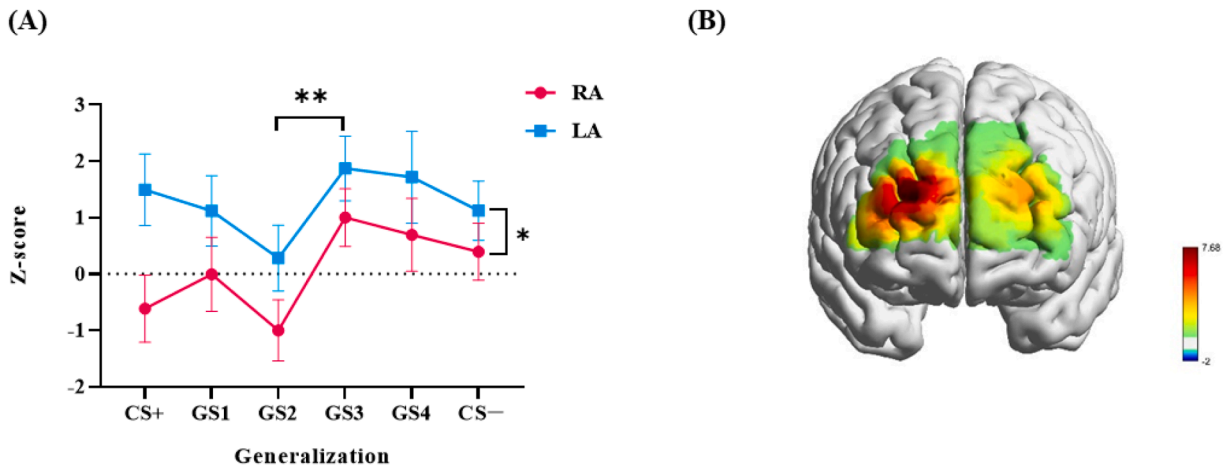

Fig. 5. Generalization fNIRS results. (A) mPFC activity across groups and stimulus type. (B) mPFC activity was significant in channel 8 and marginal significant in channel 11.

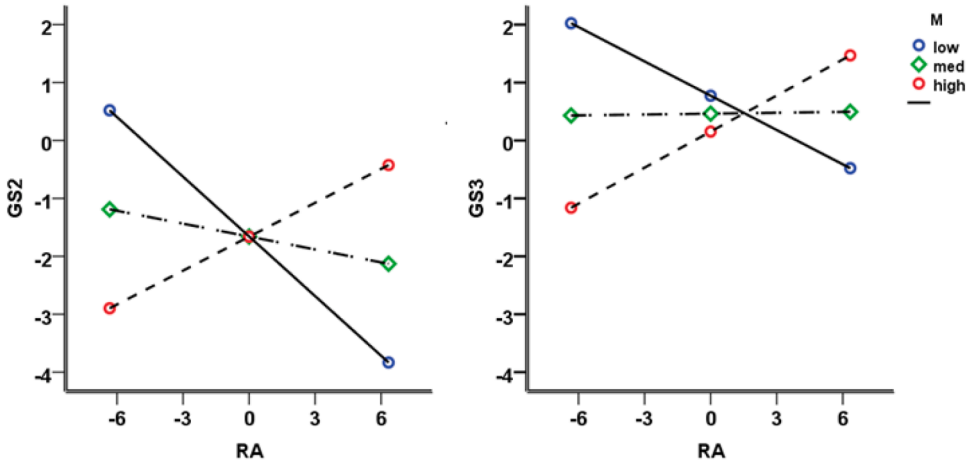

Fig. 6. Conditional effect of RA on mPFC activity for GS2 and GS3 as a function of moderation-of-anxiety. The blue circle line represents where the relationship between RA and mPFC activity is significant for individuals with low anxiety, as determined using the Johnson-Neyman technique.

heightened threat sensitivity, suggesting that individuals with RA may inaccurately classify stimuli as threats due to their excessive sensitivity to threats (Martinelli et al., 2018; Blair, 2018; Bertsch, Florange, & Herpertz, 2020; Glenn et al., 2020). However, there are contradictory findings regarding fear acquisition in aggressive adolescents. For instance, Gao and colleagues (2015) found that adolescents with elevated RA did not exhibit impairments compared to their proactive counterparts during fear acquisition. This discrepancy may stem from limited comparable studies and differences in methodologies, including variations in subject selection criteria and measurement indicators. While prior studies commonly categorized aggression as proactive or reactive, few researchers have explored the contrast between RA and LA in fear learning.

The US expectancy ratings for the CS+ and GS exhibited a gradual decrease across participants, reflecting a typical generalization gradient and confirming successful implementation of the fear generalization paradigm. Glenn et al. (2012) conducted a study on the development of fear learning and generalization in children aged 8–13 years old, and they reported that 11- to 13-year olds display the usual generalization pattern. Consistent with our hypothesis, the US expectancy ratings for all stimuli (CS+, CS−, GSs) were higher among the RA group compared to the LA group. This suggests that adolescents with RA are not only responsive to ambiguous threats but also sensitive to perceptual cues signaling potential threats. This finding supports the notion of heightened threat sensitivity among adolescents with RA, who may interpret a safe cue as threatening unlike their counterparts (Verona and Bresin, 2015; Bertsch, Florange, & Herpertz, 2020). Furthermore, the reinforcement rate of the CS+ decreased during the generalization phase compared to the acquisition phase, leading to a decline in US expectancy ratings over time. This indicates the occurrence of fear extinction in both groups (Wang et al., 2021).

The fNIRS results pertaining to fear generalization revealed lower activation of the mPFC towards GS2 compared to GS3 in both groups. This finding suggests the involvement of the mPFC in activating safety cues and inhibiting threat cues. However, this pattern differs from the typical generalization gradient observed in the behavioral results. This difference may be attributed to GS2 and GS3 being the most ambiguous stimuli compared to GS1 (closest to CS+) and GS4 (closest to CS−). Moreover, similar generalization patterns have been reported in some studies examining SCR results in adolescents (Klein et al., 2021; Schiele et al., 2016). This divergence from typical generalization gradients may be explained by the fact that adolescence is characterized by rapid development of the prefrontal cortex, which could contribute to these dissimilar results. These findings align with previous research indicating the crucial role of the mPFC in fear inhibition (Guhn et al., 2012; Xin et al., 2014; Kenwood et al., 2022; Zhou et al., 2019) and discrimination between threat and safety cues (Likhtik et al., 2014; Likhtik & Paz, 2015; Dou et al., 2020).

Furthermore, mPFC activation in the RA group was consistently lower than that of the LA group across all stimuli (CS+, CS−, GSs). Our fNIRS findings were in line with the behavioral results, indicating that adolescents with RA may encounter difficulties in inhibiting their responses to cues perceived as threatening. Previous research has indicated that fear generalization in both animals and humans is associated with decreased activity in the mPFC (Likhtik et al., 2014). Furthermore, neurobiological models propose that RA stems from a breakdown in emotion regulation, characterized by an imbalance between prefrontal cortical control and excessive bottom-up signals of negative affect from limbic regions (Siep et al., 2019; da Cunha-Bang et al., 2017, 2018; Diano et al., 2017; Heesink et al., 2018). Therefore, our study proposes a correlation between impaired fear regulation and adolescents with RA. Further research is necessary to elucidate the causal dynamics between these variables, potentially informing targeted interventions.

Our fNIRS data indicated no significant neural differences in the mPFC between groups during fear acquisition for both CS+ and CS–. This finding is consistent with a previous study involving adolescents with DBD. Cohn and colleagues (2013) suggested that adolescents with DBD display increased activation in brain regions associated with fear conditioning, such as the insula and anterior cingulate cortex, rather than the mPFC. However, a notable disparity was observed between the fNIRS data and the behavioral outcomes during the acquisition phase. This discrepancy may be attributed to the relatively less complex nature of the experimental task, which primarily demonstrated cognitive differences between the two groups without revealing differences in mPFC activation. This observation aligns with the findings of Birbaumer et al. (2005), who reported a distinction between affective and cognitive processing among psychopathic offenders. While psychopathic offenders were able to acquire the CS-US contingency, they did not exhibit heightened activation in the limbic-frontal circuitry compared to the control group.

Furthermore, adolescents displaying RA often exhibit higher levels of anxiety (Fite et al., 2014; Smeets et al., 2017), and anxiety disorders are characterized by an overgeneralization of conditioned fear (Fraunfelter et al., 2022; Cooper et al., 2022; Lonsdorf et al., 2017; Dymond et al., 2015). To explore the potential moderation of the relationship between RA and both US expectancy and mPFC activity by anxiety, separate regression models were constructed, including an interaction term. Our analysis revealed that mPFC activation for GS2 and GS3 decreased as RA levels increased among individuals with low anxiety (Figure S3). Specifically, a negative predictive effect of RA was observed on mPFC activation for GS2 and GS3 in low-anxiety adolescents, indicating greater difficulty in inhibiting fear responses. Importantly, these alterations appear to be specific to RA and not merely a reflection of anxiety levels.

As mentioned above, our findings provide further evidence that adolescents with RA show oversensitivity to threat. From a clinical perspective, these findings indicate that oversensitivity of potential threat and deficient prefrontal engagement serve as the neurocognitive basis and potential treatment target for RA during adolescence. Initial studies have explored behavioural strategies to modulate generalization (Sevenster et al., 2017), and an increasing number of studies have successfully employed neurofeedback training to enhance prefrontal emotion regulation (Zhao et al., 2019; Zweerings et al., 2020; Linhartová et al., 2019). This includes fNIRS-based training, which has been shown to allow subjects to gain control over prefrontal activity with effects being maintained for several days and under cognitive challenge (Li et al., 2019; Yu et al., 2021, 2023; Yang et al., 2023). And our future studies would further explore the impact of increased mPFC activation on reducing reactive aggressive behaviour.

The present study had several limitations. First, the selection and grouping of participants relied on self-report measures. While similar trait-based self-reporting grouping approaches have been used in previous studies (e.g., Kou et al., 2022; Blair et al., 2018), future research could benefit from incorporating behavioral paradigms, such as provocation tasks, to define individuals with high RA at the behavioral level. Furthermore, the limited information provided about the sample in this study restricts the generalizability of the findings to other adolescent populations. Second, the measurements employed, including US expectancy ratings and fNIRS, have inherent limitations. While fNIRS is considered safe for use with youth, it only captures superficial brain activation. Future studies could enhance the understanding of fear responses by incorporating multidimensional measurements, such as SCR and functional magnetic resonance imaging (fMRI). fMRI offers higher spatial resolution and can detect the engagement of limbic regions associated with fear, such as the amygdala and hippocampus, or distributed whole-brain signatures of subjective fear. (Rosell & Siever, 2015; Zhou et al., 2021; Zhang et al., 2023). Third, the present study used visual stimuli exclusively, overlooking the role of auditory stimuli, which are crucial in perceiving threats. Previous research has demonstrated perceptual generalization across auditory, tactile, and olfactory stimuli (Lonsdorf et al., 2017). Future investigations should incorporate various perceptual stimuli to ascertain whether abnormalities in fear learning extend across different modalities in adolescents with RA. Finally, perceptual variability may influence the ability to differentiate between stimuli (Struyf et al., 2015; Zaman et al., 2021). To enhance the accuracy of the obtained generalization gradient and the ability to infer the underlying mechanisms, future studies should consider incorporating perceptual tasks prior to the generalization phase. This approach would provide valuable insights into how perceptual variability influences fear learning.

5. Conclusion

The present study employed a classical fear conditioning paradigm combined with fNIRS to investigate fear acquisition and generalization in adolescents. The findings revealed that adolescents with reactive aggression exhibited higher US expectancy ratings for both CS and GS, along with lower mPFC engagement compared to adolescents with low aggression. Moreover, mPFC activation induced by GS2 was notably lower than GS3. These findings suggest that adolescents with high levels of reactive aggression may encounter challenges in regulating fear responses towards ambiguous safety cues. Understanding the potential neurocognitive mechanisms underlying high RA in adolescents may inform novel interventions that can target generalization or the underlying brain alterations.

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CRediT authorship contribution statement

Yizhen Wang: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benjamin Becker:** Writing – review & editing. **Jinxia Wang:** Writing – review & editing, Supervision. **Yuanyuan Wang:** Writing – review & editing. **Liangyou Zhang:** Writing – review & editing. **Ying Mei:** Conceptualization. **Hong Li:** Writing – review & editing, Supervision, Resources. **Yi Lei:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All authors have approved this manuscript.

Data availability

Data will be made available on request.

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Supplementary materials

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