

Fear responses to safety cues in anxious adolescents: preliminary evidence for
atypical age-associated trajectories of functional neural circuits

Anneke D.M. Haddad^{a,*}

Amy Bilderbeck^b

Anthony C. James^b

Jennifer Y.F. Lau^{a,c}

^aDepartment of Experimental Psychology, University of Oxford, Oxford, OX1 3UD,
United Kingdom; ^b Department of Psychiatry, University of Oxford, United Kingdom; ^c
Psychology Department, Institute of Psychiatry, Kings College London, PO77, De Crespigny
Park, London SE5 8AF, United Kingdom.

*Now also at the School of Psychology, University of Nottingham.

ABSTRACT

Adolescent anxiety is common and impairing and often persists into adulthood. There is growing evidence that adult anxiety is characterized by abnormal fear responses to threat and safety cues, along with perturbations in fear-related neural circuits. Although some of this work has been extended to adolescents, with promising results, it is not yet clear whether changes in these circuits across developmental age varies between anxious and non-anxious adolescents. Here we used fMRI to examine how age modulates neural responses as adolescents are exposed to threat and safety cues. Participants were 15 anxious and 11 non-anxious adolescents (age 12-17) who completed a fear conditioning paradigm. The paradigm incorporated a threat cue comprising a neutral face which was paired with a fearful, screaming face, a safety cue comprising a different neutral face, and a control stimulus. Across the whole sample, neural activation to the threat cue (relative to the control cue) correlated positively with age in a number of regions, including the dorsal anterior cingulate and bilateral dorsolateral prefrontal cortex (PFC). However, neural activation to the safety cue (relative to the control cue) was modulated differently by age in the two groups: a more positive association between activation and age was observed in the control group compared to the anxious group in various regions including medial and dorsolateral PFC, anterior insula, and amygdala. These findings suggest that maturation of the neural substrates of fear responses to safety cues may be perturbed in anxious adolescents, potentially contributing to the emergence and maintenance of anxiety disorders in adulthood.

Keywords: adolescent, anxiety, magnetic resonance imaging, anxiety disorders, prefrontal cortex, conditioning

INTRODUCTION

Adult anxiety is characterised not only by behavioural, cognitive, and neural abnormalities in fear responses to threat cues but also inappropriate fear of safety cues. Anxiety disorders often have their onset during adolescence (Pine et al. 1998), and improved understanding of how they emerge could inform early interventions to attenuate their progression. Substantial differences between adults and adolescents in terms of brain structure and function, cognition, and social environment mean that studies of adolescents are crucial. Here we examined how neural responses to threat and safety cues differ between anxious and non-anxious adolescents and in particular how these differences may emerge across development.

Studies using conditioning paradigms, where an initially neutral stimulus becomes a reinforced conditioned stimulus (CS+) through repeated pairings with an aversive unconditioned stimulus (UCS), have established that high anxious adults show exaggerated self-reported and physiological fear responses to threat cues (Lissek et al. 2005). Yet anxious individuals do not only fear cues that predict threat; they also show generalized fear responses to safety cues. *Safety* learning occurs when neutral stimuli which are never followed by the UCS (i.e., non-reinforced conditioned stimuli, CS-) come to signal the *absence* of the threat. Fear responses to safety cues may sometimes be adaptive but can also be costly, because individuals expend energy on unnecessary fear and/or avoidance behaviours and cannot benefit from opportunities or resources associated with safety cues.

Healthy adults show a relatively steep drop-off in self-reported and physiological fear as graduated safety cues become less perceptually similar to the threat cue, (Lissek et al. 2008; Lissek, Bradford, et al. 2013; Dunsmoor, White, et al. 2011; Dunsmoor & Schmajuk 2009). In contrast, anxious individuals fear a wider range of safety cues - even those less

ADOLESCENTS

perceptually similar to threat cues (Dunsmoor, White, et al. 2011; Haddad et al. 2012; Lissek 2012; Lissek et al. 2009; Lissek et al. 2010; Lissek, Kaczkurkin, et al. 2013).

These difficulties in inhibiting fear responses to safety cues may be driven by anxiety-associated abnormalities in prefrontal-subcortical circuitry (M. J. Kim et al. 2011).

Ventromedial prefrontal cortex (vmPFC) activation in healthy adults diminishes as safety cues become more similar to a threat cue. The vmPFC response is less discriminating in anxious patients (Greenberg et al. 2013b), or generally lower (Britton et al. 2013), suggesting deficient vmPFC recruitment in response to safety cues. In contrast, in regions where healthy volunteers show ‘positive’ generalization gradients (i.e., greater activity as safety cues increase in resemblance to threat, which occurs in the insula, supplementary motor area (SMA), and dorsomedial prefrontal cortex (dmPFC) (Lissek, Bradford, et al. 2013), as well as anterior cingulate (ACC), and caudate (Greenberg et al. 2013a)), no anxiety-linked differences have been found (Greenberg et al. 2013b). Together these data suggest that at the neural level, generalization abnormalities in anxiety are found in regions that are involved in inhibitory but not excitatory responses.

Although this emerging evidence sheds light on the nature of fear learning difficulties in anxious adults, extending conclusions from adults to adolescents is not straightforward and the few studies of fear generalization in youth have mainly focussed on younger children. What has been shown in anxious adolescents is that, like anxious adults, they may have elevated self-reported fear of both threat and safety cues (Lau et al. 2008) and at the neural level, they may have reduced subgenual ACC activity across a range of safety stimuli (Britton et al. 2013). The vmPFC may show a more subtle pattern: anxious adolescents in this study had higher vmPFC activation for the extreme ends of the gradient (CS+ and CS-) and lower activation for intermediate stimuli, which may reflect heightened sensitivity to both threat and safety.

ADOLESCENTS

Studies of the *age trajectory* of these responses in anxious and non-anxious adolescents are, however, lacking. Yet adolescence is a period of protracted neurocognitive maturation of key brain circuits involved in fear regulation, and so anxiety-associated differences are likely to emerge gradually as a perturbation of these age-typical changes. Age-related changes have been observed in emotional processing in general through normal adolescence (Cohen Kadosh et al. 2012; Deeley et al. 2008; Glenn et al. 2012; Moore et al. 2012; Somerville et al. 2011; Van Den Bulk et al. 2013; Vink et al. 2014; Yurgelun-Todd 2007). With regard to fear learning specifically, studies of rodents suggest greater sensitivity in the acquisition of fear associations during adolescence compared to other developmental stages (Den & Richardson 2013). An inability to attenuate these fear responses through a process of new (extinction) learning has been reported as well (J. H. Kim et al. 2011)(McCallum et al. 2010), a finding that extends to humans (Pattwell et al. 2012). In relation to safety learning,, one study found stronger fear of safety cues in older than younger adolescents (Glenn et al., 2012). Some studies have investigated the neural substrates of these age-associated changes, and have found correlations between adolescents' age and activation in key areas including hippocampus, amygdala, ventrolateral PFC, dorsomedial PFC, thalamus, and caudate while viewing emotional pictures or faces (Vink et al. 2014; Deeley et al. 2008). This indicates that patterns of emotional processing in general, and perhaps fear responding in particular, change through childhood and early adolescence, lending credibility to the idea that the emergence of anxiety could be due to perturbed age-associated changes in fear responses at the behavioural and/or neural level.

This study

In this study, we sought to examine this idea by investigating age trajectories in neural responses during fear responding in anxious and non-anxious adolescents. We employed a threat/safety learning paradigm based on the “screaming lady” task (Lau et al. 2008). During

ADOLESCENTS

fMRI, participants viewed a threat cue (CS+, neutral face) that was paired with an unconditioned stimulus (UCS, fearful face and scream), a safety cue (CS-, different neutral face), and a control cue (oval). Conditioning studies often compare CS+ and CS- responses directly. However, we expected that anxious adolescents would show elevated fear of the CS- (Lau et al., 2008) as well as perturbed neural responses to both threat and safety (Britton et al. 2013). Directly comparing CS+ and CS- responses would not allow us to consider elevated fear of threat and elevated fear of safety separately. We therefore considered responses to each CS separately in order to probe group and age-related differences in fear of threat (i.e., CS+ relative to control) and fear of safety (i.e., CS- relative to control). One potential difficulty of this approach is that both comparisons could merely index face processing, because both involve comparing a neutral face with an oval. We checked whether this was plausible by comparing the pattern of findings for the two contrasts; regions active due solely to face processing should elicit the similar responses for both contrasts. Based on previous studies, we expected anxiety-associated differences and age-related changes across the whole group during fear of threat and safety in hippocampus, amygdala, and PFC. We also explored a new hypothesis: that anxious and non-anxious groups show age-related divergence in these patterns of neural activation.

METHOD

Participants and Procedure

The final sample consisted of 26 participants aged 11-17 recruited through the community. Fifteen were anxious (2 males; mean age =15.2±1.5 years, range 154 to 212 months) and 11 were healthy (5 males; mean age=15.6±1.3 years, range 168 to 212 months). The group-by-gender interaction was not significant, Fisher's exact test $p=.095$.

Data from nine other participants were excluded: three withdrew prior to or during scanning; three had excessive movement during scanning (>3mm in any direction) or gross structural abnormalities; one reported seeing only one face; and two showed no behavioural evidence of conditioning (no increase in CS+ ratings from pre-acquisition to acquisition and no elevated ratings of CS+ above CS- during acquisition).

Based on Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS), 11 of the anxious participants met criteria for one or more anxiety disorders and 4 had subclinical symptoms. Seven had concurrent major depression. Non-anxious participants had no current or past psychological disorders. Exclusion criteria included IQ < 70; current psychotropic medication; and conditions that would increase the risks of MRI. The local ethics committee approved the study and we obtained written informed consent/assent from parents and participants respectively. Participants completed the KSADS and measures of IQ and trait anxiety at an initial visit and completed scanning procedures during a second visit at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). Participants and parents were reimbursed for their participation.

ADOLESCENTS

Materials

IQ

IQ was measured with the two subscale version of the Weschler Abbreviated Scale of Intelligence (WASI). WASI score was missing for one anxious participant. Scores did not differ significantly between the groups (anxious: mean=110.71±12.17; healthy: mean=112.27±8.42), $t(23)=.362$, $p=.721$.

Trait anxiety

Trait anxiety score was assessed using the Spielberger Trait Anxiety Inventory for Children (STAI-C). The anxious group (mean=47.87±5.71) was, unsurprisingly, significantly more anxious than the non-anxious group (mean=31.36±7.07), $t(24)=6.59$, $p < .001$.

Conditioning paradigm

The classical fear conditioning paradigm comprised pre-acquisition, conditioning, and extinction phases (Lau et al. 2008). Results from the extinction phase are not reported here. The threat cue (CS+) was a photograph of a neutral female face. The UCS was a photograph of the same female showing fear and a 95 Db scream. The safety cue (CS-) was a different neutral female photograph. CS+ and CS- identities were counterbalanced. The control cue was a grey oval of similar proportions to the faces. Photographs were from the NimStim set (Tottenham et al. 2009).

Stimuli were presented in a different pseudorandom order for each participant. Pre-acquisition comprised five trials of each stimulus type, with no UCSs. Acquisition comprised 30 CS+ trials (50% reinforced), 15 CS- trials, and 15 control cue trials. The 50% reinforcement schedule was chosen to minimize habituation to the UCS and to allow analysis of unreinforced trials only (to avoid confounding by neural response to the UCS on reinforced trials). Within acquisition, the first trial was always a reinforced CS+ trial and the

ADOLESCENTS

60 trials were presented in three blocks each comprising 5 trials of each type. Each trial comprised presentation of the given CS alone for 3s (“view” period), after which the words “How nervous are you?” and a red bar appeared below the CS for 3s (“rate” period). During the “rate” period, participants changed the bar length (implicit 0-10 scale) using a button box; their response was recorded after 3s. On reinforced trials only, the UCS was presented for 1s immediately after CS+ offset; thus non-reinforced trials were 6s long and reinforced trials were 7s long. The inter-trial interval (ITI) varied between 2 and 4s. The task lasted approximately 17 minutes.

MRI acquisition parameters

Whole-brain blood oxygen level dependent (BOLD) contrast functional images were acquired with an echo-planar T2*-weighted image sequence in a Siemens TIM Trio 3T scanner. Each volume consisted of 45 interleaved 3mm thick slices (in-plane resolution=3mm x 3mm; flip angle=87°; TE=30ms; TR=3s; echo spacing=0.49ms; bandwidth=2368 Hz). Field maps were acquired for registration using dual echo 2D-gradient echo sequences with echoes at 5.19ms and 7.65ms, and TR=488ms (64x64x64 voxel grid; voxel resolution of 3mm isotropic; flip angle=60°). High-resolution (1mm x 1mm x 1mm) T1 weighted structural images were also acquired (flip angle=8°; TE=4.7ms; TR=2.04s; bandwidth=130 Hz).

Data analysis

We examined nervousness ratings using a mixed design ANOVA in SPSS 22 with phase (pre-acquisition, acquisition), stimulus (CS+, CS-, control) and group (anxious, control) as within- and between-subjects factors. The Greenhouse Geisser correction was used where the assumption of sphericity was violated.

FMRI data processing was carried out using FSL, FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl (Jenkinson et al. 2012; Smith et al. 2004; Woolrich et al. 2009). The

ADOLESCENTS

following pre-statistics processing steps were applied: motion correction using MCFLIRT (Jenkinson et al. 2002); slice-timing correction using Fourier-space time-series phase-shifting; removal of non-brain tissue using BET (Smith 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor; and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with $\sigma=50.0s$).

Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich et al. 2001). Registration to high resolution structural and/or standard space images was carried out using FLIRT (Jenkinson et al. 2002; Jenkinson & Smith 2001). Registration from high resolution structural to standard space was then further refined using FNIRT nonlinear registration (Andersson, Jenkinson & S. M. Smith 2007; Andersson, Jenkinson & S. Smith 2007). Volumes contaminated by large movements were excluded using the fsl script *fsl_motion_outliers*, which produces a confound matrix that was included in the GLM to remove these timepoints.

Whole-brain analysis using a General Linear Model was conducted to identify which brain regions were associated with each CS contrast, and whether BOLD activity in these regions varied by anxiety group. Trials were separated into “view” events (first 3s, when participants viewed the stimulus), and “rate” events (subsequent 3s, when participants rated their nervousness). We examined responses to “rate” events because we expected group differences to be stronger as the (possible) UCS became imminent. First-level analyses included the following explanatory variables (EVs): pre-acquisition “rate” events; acquisition “rate” events (one EV for each CS type, with reinforced and unreinforced CS+ trials modelled separately); extinction “rate” events; all UCSs; and all “view” events. The main contrasts of interest were [CS+ > control cue] (including unreinforced CS+ trials only), and

ADOLESCENTS

[CS- > control cue]. As explained earlier, the separate comparisons of CS+ and CS- to the control cue allowed us to evaluate group- and age-related differences in response to threat and safety separately, which is not possible if CS+ to CS- responses are compared directly. However, the results from the [CS+ > CS-] contrast are included in the Supplementary Information.

Higher-level (group) analyses, including age (in months) as a covariate were run using a cluster-forming threshold of $Z > 2.3$ and a corrected cluster threshold of $p < 0.05$ across the whole brain and for each region of interest (ROI), defined using the Harvard-Oxford cortical and subcortical probability atlases (25% threshold): left/right amygdala, left/right hippocampus, and bilateral frontal medial cortex (i.e., vmPFC). Mean percentage signal change values for significant clusters were extracted using *featquery* and imported into SPSS 22 for further analysis.

RESULTS

Self-reported nervousness

For the whole experiment, the 2 (phase) x 3 (stimulus) x 2 (group) mixed design ANOVA on self-reported trial-by-trial nervousness ratings yielded main effects of each of the factors; the three two-way interactions, but not the three-way interaction, were also significant (see Supplementary Information for details). In order to probe the origins of these effects, we performed a separate ANOVA for each phase.

For pre-acquisition, the 3 (stimulus) x 2 (group) mixed design ANOVA yielded a main effect of stimulus, $F(1.426, 34.221)=25.516, p < .001$, partial $\eta^2=.515$ and a stimulus-by-group interaction, $F(1.426, 34.221)=4.438, p=.030$, partial $\eta^2=.156$. These effects arose because the anxious group's ratings of the CS+ were higher than the control group's, $t(22.349)=2.085, p=.049$ (corrected for unequal variance), whereas the groups' ratings for the CS- and control cues did not differ (see Figure 1). This effect was not specific to one of the faces, because including task version (i.e., which identity was the CS+) did not change the pattern of results. Importantly, however, within each group, the CS+ and CS- ratings did not differ significantly.

For acquisition, the 3 (stimulus) x 2 (group) mixed design ANOVA yielded main effects of group, $F(1, 23)=7.603, p=.011$, partial $\eta^2=.241$ and stimulus, $F(2,48)=2.251, p < .001$, partial $\eta^2=.497$. The anxious group reported significantly more nervousness than did the control group, and the CS+ received significantly higher nervousness ratings than the CS-, paired $t(25)=3.188, p=.004$, which in turn received significantly higher nervousness ratings than the control cue, paired $t(25)=4.375, p < .001$, see Figure 2.

Age did not significantly moderate these findings, nor did age correlate with nervousness ratings for any of the stimuli for either phase.

[Insert Figures 1 & 2 about here.]

ADOLESCENTS

fMRI results

The fMRI results are organised in three sections. First, we examine regions where, for each contrast of interest (i.e., [CS+ > control cue] and [CS- > control cue]), there was a main effect of anxiety; second, we consider areas showing a main effect of age; and third, we examine where the relationship between age and activation differs between the two groups (i.e., age by group interaction).

Anxiety group differences

For the [CS+ > control cue] contrast in the whole brain analysis, the non-anxious group had more robust activation than the anxious group in one region in the medial PFC/paracingulate (913 voxels, $p = .000262$, peak voxel (MNI co-ordinates) at [-18, 48, 42]; see Figure 3). There were no clusters showing significant group differences for the [CS- > control cue] contrast in the whole brain analysis.

ROI analyses revealed more robust activation in the control group than in the anxious group for the [CS+ > control cue] contrast in clusters within left and right amygdala and right (but not left) hippocampus, as well as within the vmPFC. There were no significant clusters for the [CS- > control cue] contrast in any of the ROI analyses.

Age modulations across the whole group

We next examined regions where age correlated with activation across the whole sample. For the [CS+ > control cue] contrast, the whole brain analysis revealed significant correlations with age in several regions including dorsal ACC, an extensive area of the right insula/operculum extending into the right putamen/caudate, and bilateral dorsolateral PFC

ADOLESCENTS

(dlPFC), extending on the left into the insula (see Figure 4, Table 1 and Supplementary Table S1 for details). There were no significant associations with age for any of the ROI analyses. There were no regions showing significant age correlations across the whole group for the [CS- > control cue] contrast.

[Insert Figures 3 and 4 and Table 1 about here]

Group differences in age modulation

We finally examined regions where there were group differences in age correlation. For the [CS+ > control cue] contrast, there were no such regions. However, the correlation between activation for the [CS- > control cue] contrast and age was higher for the non-anxious than the anxious group in several notable regions, including bilateral anterior insula (extending into lateral OFC on the right and into striatum on the left) and right dlPFC (see Figure 5 and Table 2). ROI analyses yielded a similar pattern in the left amygdala (but not right amygdala, either hippocampus, or vmPFC).

To explore these effects, we extracted percent signal change for the CS- and control cues separately for each participant in each cluster where we found a group difference in age modulation (both for whole brain and ROI analyses). Amongst the non-anxious there tended to be positive associations between age and percent signal change to the CS- in each of these regions, whereas these associations tended to be negative in the anxious group, albeit many individual correlations were non-significant (see Supplementary Table S2 and Supplementary Figure S1). Correlations for the control cue were not significantly different from zero and did not differ between the groups, suggesting that the differences in age trajectories were primarily driven by differential association with age for CS- response, rather than control cue responses. However, it is worth noting that a whole brain analysis of group differences in age

ADOLESCENTS

correlation for the CS- alone (i.e., compared to implicit baseline) did not yield any significant clusters.

[Insert Figure 5 and Table 2 about here]

DISCUSSION

In this study, we examined how age trajectories in the neural correlates of fear generalization differ between anxious and non-anxious adolescents. This work extends previous studies investigating group differences between anxious and non-anxious adolescents, and age differences across all adolescents. We found several key regions where, relative to a control cue, neural activation to the safety cue (CS-), but not the threat cue (CS+), showed positive correlations with age in non-anxious adolescents; in contrast, the same correlations tended to be negative in the anxious participants. Importantly, results from the [CS+ > control] contrast and the [CS- > control] contrast showed *different* patterns, even though both involve a neutral face > oval comparison. This indicates that that results do not merely reflect differences in face processing between healthy and anxious adolescents across age.

A number of regions, including regions of the PFC, bilateral amygdalae, left hippocampus, and striatum showed more robust activation to the threat cue (relative to control) in the non-anxious compared with the anxious group. This was surprising given previous studies suggesting elevated activation in such areas in anxious relative to non-anxious adults and adolescents in response to threat relative to safety (Britton et al. 2013; Greenberg et al. 2013b; Lau et al. 2011). The fact that we found higher activation in these regions in the non-anxious group suggests that anxious adolescents may not show the usual differential activation of fear-related neural structures for stimuli of differential threat value, though notably, both groups did show the expected changes in subjective nervousness towards the stimuli.

Age-related increases in activation across the whole sample in response to the threat cue (relative to control) were evident in a number of regions – in particular, the insula and dlPFC bilaterally – suggesting that maturation of threat-related processing is supported by

ADOLESCENTS

increased activation in these regions. Previous studies have similarly found age-related increases in dlPFC activity while viewing fearful faces (Yurgelun-Todd & Killgore 2006), although it has also been reported that this pattern occurs only in females and only on the left (Killgore et al. 2001).

Importantly, however, *safety* cue responses showed group differences in the correlation with age in several key regions, including the dlPFC, bilateral insula, and left striatum. Thus unlike in the anxious group, the tendency towards an increasing response to the CS- relative to the control cue in these regions with age amongst the non-anxious group may reflect normal developmental changes in processing of a safety cue. The pattern of absent or negative associations with age in response to the safety cue in the anxious group suggests that these changes may be perturbed in those with anxiety. It has been suggested that dlPFC activation during conditioning reflects modulation of fear by cognitive emotional regulation (Delgado et al. 2008), categorization of stimuli as threatening or safe (Lau et al. 2011), or uncertainty about receiving the UCS (Dunsmoor et al., 2007, Dunsmoor and Schmajuk, 2009). Thus the observation of group differences in age correlation in this region could be due to improved regulation of emotional response to safety cues, better categorization of stimuli as threatening or safe, and/or increased contingency awareness with increased age in the control group. Although our data cannot distinguish between these possibilities, the results suggest that the age-related dlPFC changes which we observed in healthy adolescents are different in anxious adolescents. Whether changes in dlPFC activation to safety cues in anxious adolescents occurs earlier, later, or not at all remains unclear, but it is noteworthy that abnormal dlPFC activation to safety cues has not been found in anxious adults. Studies covering a wider range of ages might allow a clearer understanding of when the age trajectory of dlPFC activation to safety cues in anxious adolescents diverges from normal. Such studies might also examine whether, when, and in whom dlPFC activation

ADOLESCENTS

to safety cues subsequently “normalizes” (which might explain the lack of evidence for abnormal dlPFC responses in anxious adults).

We found similar patterns in the bilateral insula and left striatum, such that the non-anxious group tended to show age-related increases in activation in these regions in response to the CS- (relative to the control) whereas these relationships were non-significant or negative in the anxious group. Adult studies implicate the insula and striatum in learning and generalization (Dunsmoor, Prince, et al. 2011; Greenberg et al. 2013a; Greenberg et al. 2013b). Thus with increasing age, the non-anxious adolescents, unlike the anxious adolescents, may be showing activation patterns in these areas that are increasingly like those of adults. However, Greenberg and colleagues (2013b) found no evidence for differential activation between adults with and without generalized anxiety disorder in these areas during fear generalization. This suggests that by adulthood, anxiety-linked differences in these regions may have attenuated. Of course, not all anxious adolescents continue to have such difficulties into adulthood, and differential patterns of activation with age may relate to varying longitudinal trajectories.

One implication of diverging age trajectories in safety cue responses is that older anxious adolescents’ safety cue responses differ more substantially from their non-anxious counterparts than those of younger adolescents. Thus interventions to improve discrimination between threat and safety cues (Dunsmoor & LaBar 2013; Vervliet et al. 2011) might be more effective in alleviating anxiety amongst older adolescents. Future research should also investigate gender differences in adolescent fear responses to safety cues, especially given evidence suggesting adolescent gender differences in PFC activation while viewing fearful faces (Yurgelun-Todd & Killgore 2006).

A number of limitations apply to this study. First, the number of participants was small, and as a result, although group differences in the size of the correlations were

ADOLESCENTS

significant, within each group, significant correlations did not always emerge. Moreover, diagnoses in the anxiety group were heterogeneous. It is therefore important to replicate these results in future studies. Second, there was an unexpected difference between anxious and non-anxious participants' ratings of the CS+ at pre-acquisition, regardless of which face was the CS+. However, within each group, pre-acquisition ratings for the CS+ and CS- were not significantly different, and ratings of the CS+ during acquisition were above those for the CS- in both groups. Third, we did not collect autonomic measures of behaviour. As there was a discrepancy between the subjective nervousness ratings and the fMRI data (i.e., higher reported fear in anxious vs non-anxious participants, yet reduced amygdala activation in anxious vs non-anxious participants), having an additional psychophysiological measure of fear may have shed light on the nature of this discrepancy. Finally, in common with many other neuroimaging studies, it must be acknowledged that it is difficult to know how to interpret differences in activation between the anxious and non-anxious groups. For example, the fact that anxious adolescents showed reduced activation compared to the non-anxious group for the CS+ > control cue contrast in some regions may suggest that these areas are not working as efficiently, though this is perhaps unlikely in the case of the amygdala. More generally, studies of adolescents have found patterns of both increased and decreased activation and precisely what these patterns reflect remains unclear.

In summary, our findings point to changes in brain activity during fear responding across adolescence, and also suggest that several regions, including anterior insula and right dlPFC, show differential age trajectories in fear generalization response in anxious and non-anxious adolescents. Further investigation of the relationship between adolescent trajectories and (risk of) adult anxiety is needed; however, our findings support the suggestion that clinical interventions to promote better discrimination between threat and safety cues

ADOLESCENTS

(Vervliet et al. 2011; Dunsmoor & LaBar 2013) could prove increasingly useful through adolescence.

ACKNOWLEDGEMENTS

This study was funded by a John Fell Fund grant to Jennifer Lau. The authors gratefully acknowledge the contributions of Belinda Platt, Steve Knight, Nicola Filippini, Jan Scholz, and Alexandra May. We also wish to thank the participants and their families, as well as local CAMHS and PCAMHS teams and schools.

DISCLOSURE STATEMENT

The authors have no actual or potential conflicts of interest to disclose.

REFERENCES

- Andersson, J.L.R., Jenkinson, M. & Smith, S., 2007. Non-linear registration aka Spatial normalisation FMRIB Technical Report TR07JA2.
- Andersson, J.L.R., Jenkinson, M. & Smith, S.M., 2007. *Non-linear optimisation. FMRIB Technical Report TR07JA1*,
- Britton, J.C. et al., 2013. Response to learned threat: An FMRI study in adolescent and adult anxiety. *The American Journal of Psychiatry*, 170(10), pp.1195–204.
- Van Den Bulk, B.G. et al., 2013. How stable is activation in the amygdala and prefrontal cortex in adolescence? A study of emotional face processing across three measurements. *Developmental Cognitive Neuroscience*, 4, pp.65–76.
- Cohen Kadosh, K. et al., 2012. Effects of Age, Task Performance, and Structural Brain Development on Face Processing. *Cerebral cortex New York NY 1991*, 23, pp.1630–1642.
- Deeley, Q. et al., 2008. Changes in male brain responses to emotional faces from adolescence to middle age. *NeuroImage*, 40(1), pp.389–97.
- Delgado, M.R. et al., 2008. Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*, 59(5), pp.829–838.
- Den, M.L. & Richardson, R., 2013. Enhanced sensitivity to learning fearful associations during adolescence. *Neurobiol Learn Mem*, 104, pp.92–102.
- Dunsmoor, J.E., Prince, S.E., et al., 2011. Neurobehavioral mechanisms of human fear generalization. *Neuroimage*, 55(4), pp.1878–1888.
- Dunsmoor, J.E. & LaBar, K.S., 2013. Effects of discrimination training on fear generalization gradients and perceptual classification in humans. *Behavioral neuroscience*, 127(3), pp.350–6.

ADOLESCENTS

- Dunsmoor, J.E. & Schmajuk, N., 2009. Interpreting patterns of brain activation in human fear conditioning with an attentional-associative learning model. *Behavioral neuroscience*, 123(4), pp.851–5.
- Dunsmoor, J.E., White, A.J. & LaBar, K.S., 2011. Conceptual similarity promotes generalization of higher order fear learning. *Learning & Memory*, 18(3), pp.156–160.
- Glenn, C.R. et al., 2012. The development of fear learning and generalization in 8-13 year-olds. *Developmental psychobiology*, 54, pp.675–84.
- Greenberg, T. et al., 2013a. Neural reactivity tracks fear generalization gradients. *Biological psychology*, 92(1), pp.2–8.
- Greenberg, T. et al., 2013b. Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization. *Depression and Anxiety*, 30(3), pp.242–250.
- Haddad, A.D.M. et al., 2012. Trait anxiety and fear responses to safety cues: Stimulus generalization or sensitization? *Journal of Psychopathology and Behavioral Assessment*.
- Jenkinson, M. et al., 2012. FSL. *NeuroImage*, 62(2), pp.782–90.
- Jenkinson, M. et al., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*, 17(2), pp.825–841.
- Jenkinson, M. & Smith, S.M., 2001. A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, 5(2), pp.143–156.
- Killgore, W.D., Oki, M. & Yurgelun-Todd, D.A., 2001. Sex-specific developmental changes in amygdala responses to affective faces. *Neuroreport*, 12, pp.427–433.
- Kim, J.H., Li, S. & Richardson, R., 2011. Immunohistochemical analyses of long-term extinction of conditioned fear in adolescent rats. *Cerebral Cortex*, 21(3), pp.530–538.
- Kim, M.J. et al., 2011. The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behavioural Brain Research*, 223(2), pp.403–410.
- Lau, J.Y.F. et al., 2011. Distinct neural signatures of threat learning in adolescents and adults. *Proceedings of the National Academy of Sciences*, 108(11), pp.4500–4505.
- Lau, J.Y.F. et al., 2008. Fear conditioning in adolescents with anxiety disorders: results from a novel experimental paradigm. *Journal of the American Academy of Child and Adolescent Psychiatry*, 47(1), pp.94–102.
- Lissek, S. et al., 2005. Classical fear conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research and Therapy*, 43(11), pp.1391–424.
- Lissek, S. et al., 2008. Elevated fear conditioning to socially relevant unconditioned stimuli in social anxiety disorder. *The American journal of psychiatry*, 165(1), pp.124–32.
- Lissek, S., Kaczkurkin, A.N., et al., 2013. Generalized Anxiety Disorder is associated with overgeneralization of classically conditioned fear. *Biological Psychiatry*, pp.1–7.
- Lissek, S. et al., 2009. Impaired discriminative fear-conditioning resulting from elevated fear responding to learned safety cues among individuals with panic disorder. *Behaviour research and therapy*, 47(2), pp.111–8.

ADOLESCENTS

- Lissek, S., Bradford, D.E., et al., 2013. Neural substrates of classically conditioned fear-generalization in humans: a parametric fMRI study. *Social cognitive and affective neuroscience*.
- Lissek, S. et al., 2010. Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *American Journal of Psychiatry*, 167(1), pp.47–55.
- Lissek, S., 2012. Toward an account of clinical anxiety predicated on basic, neurally mapped mechanisms of pavlovian fear-learning: the case for conditioned overgeneralization. *Depression and Anxiety*, 29(4), pp.257–263.
- McCallum, J., Kim, J.H. & Richardson, R., 2010. Impaired extinction retention in adolescent rats: effects of D-cycloserine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 35(10), pp.2134–2142.
- Moore, W.E. et al., 2012. Facing puberty: associations between pubertal development and neural responses to affective facial displays. *Social cognitive and affective neuroscience*, 7, pp.35–43.
- Pattwell, S.S. et al., 2012. Altered fear learning across development in both mouse and human. *Proc Natl Acad Sci U S A*, 109(40), pp.16318–16323.
- Pine, D.S. et al., 1998. The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry*, 55(1), pp.56–64.
- Smith, S.M. et al., 2004. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, 23 Suppl 1, pp.S208–19.
- Smith, S.M., 2002. Fast robust automated brain extraction. *Human Brain Mapping*, 17(3), pp.143–155.
- Somerville, L.H., Fani, N. & Clure-Tone, E.B., 2011. Behavioral and neural representation of emotional facial expressions across the lifespan. *Developmental Neuropsychology*, 36, pp.408–428.
- Tottenham, N. et al., 2009. The NimStim set of facial expressions: judgments from untrained research participants. *Psychiatry Research*, 168(3), pp.242–249.
- Vervliet, B. et al., 2011. Generalization gradients in human predictive learning: Effects of discrimination training and within-subjects testing. *Learning and Motivation*, 42(3), pp.210–220.
- Vink, M. et al., 2014. Functional differences in emotion processing during adolescence and early adulthood. *NeuroImage*, pp.1–7.
- Woolrich, M.W. et al., 2009. Bayesian analysis of neuroimaging data in FSL. *NeuroImage*, 45(1 Suppl), pp.S173–86.
- Woolrich, M.W. et al., 2001. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage*, 14(6), pp.1370–1386.
- Yurgelun-Todd, D.A., 2007. Emotional and cognitive changes during adolescence. *Current Opinion in Neurobiology*, 17(2), pp.251–257.

Yurgelun-Todd, D.A. & Killgore, W.D.S., 2006. Fear-related activity in the prefrontal cortex increases with age during adolescence: a preliminary fMRI study. *Neuroscience letters*, 406, pp.194–199.