Distinct neural signatures of threat learning in adolescents and adults

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Most teenage fears subside with age, a change that may reflect brain maturation in the service of refined fear learning. Whereas adults clearly demarcate safe situations from real dangers, attenuating fear to the former but not the latter, adolescents' immaturity in prefrontal cortex function may limit their ability to form clear-cut threat categories, allowing pervasive fears to manifest. Here we developed a discrimination learning paradigm that assesses the ability to categorize threat from safety cues to test these hypotheses on age differences in neurodevelopment. In experiment 1, we first demonstrated the capacity of this paradigm to generate threat/safety discrimination learning in both adolescents and adults. Next, in experiment 2, we used this paradigm to compare the behavioral and neural correlates of threat/safety discrimination learning in adolescents and adults using functional MRI. This second experiment yielded three sets of findings. First, when labeling threats online, adolescents reported less discrimination between threat and safety cues than adults. Second, adolescents were more likely than adults to engage early-maturing subcortical structures during threat/safety discrimination learning. Third, adults' but not adolescents' engagement of late-maturing prefrontal cortex regions correlated positively with fear ratings during threat/safety discrimination learning. These data are consistent with the role of dorsolateral regions during category learning, particularly when differences between stimuli are subtle [Miller EK, Cohen JD (2001) *Annu Rev Neurosci* 24:167–202]. These findings suggest that maturational differences in subcortical and prefrontal regions between adolescent and adult brains may relate to age-related differences in threat/safety discrimination.

amygdala | brain development | discriminative fear conditioning | threat responding | anxiety

Growing up entails dramatic changes in reported fears to naturally occurring threats (1). Childhood fears focus on specific situations, whereas adolescent fears relate more to abstract concepts (1, 2). Typically, both subside in adulthood, possibly as the adolescent learns to differentiate threat from safety with increasing skill (3). This emerging capacity may reflect prefrontal cortex (PFC) maturation, which facilitates performance on category-learning tasks (4). Thus, in adults, the PFC may support the formation of precise threat/safety distinctions, but in adolescents, PFC immaturity may produce less clearly reported threat/safety boundaries. Instead, in adolescents, such threat/safety distinctions may rely on simpler, cruder forms of discriminatory learning that rely on subcortical activity, such as in the amygdala and hippocampus (5–8).

Although data suggest that threat-sensitive brain systems operate differently in adolescents and adults (9, 10), these data only indirectly link brain development to age differences in threat/safety discrimination learning. Here we used a unique paradigm to chart age differences in the neural signature of threat/safety category learning, as expressed through fear learning. During fear learning, one neutral conditioned stimulus (CS+) becomes a threat through pairing with an aversive unconditioned stimulus (UCS), whereas a second stimulus (CS−) acquires a safety value by predicting the absence of the UCS.

Converging data implicate the amygdala and hippocampus in fear learning (5–8). Subregions of the PFC also participate during fear learning (11, 12). Whereas consistent data link greater ventromedial PFC (VMPFC) activity to reduction in fear during extinction learning (13–15), more variable findings characterize lateral regions of the PFC. Dorsolateral PFC (DLPFC) has tentatively been implicated in fear learning through its role in category learning (4) and fear regulation (16–20), although inconsistent findings exist on the direction of the DLPFC–fear relationship. Based on prior work on the DLPFC, typically studied in nonemotional contexts (21), we propose that one important function of the DLPFC during fear learning is to demarcate the boundaries separating stimuli into categories of fear-relevant (i.e., threat cues) and fear-irrelevant (i.e., safe cues) types. Whereas this function also is expected to draw on subcortical structures, such as the amygdala and hippocampus, when a person needs to verbally label their response to a stimulus, refined levels of threat/safety category learning are expected to rely on the DLPFC.

Because subcortical structures, such as the amygdala and hippocampus, mature early (9) and PFC regions, particularly DLPFC, mature late, differences are expected in the degree to which these regions participate in fear learning in adolescents versus adults. In adolescents, fear learning is expected to rely predominantly on simpler forms of discrimination learning supported by subcortical activity (22). However, adult fear learning is expected to involve other additional brain regions. Specifically, the more complex category-learning skill of the adult, such as when verbally labeling well-demarcated threat/safety categories, is expected to reflect mature functioning supported by the DLPFC (4).

These findings set the stage for the current report comparing the neural profile engaged by fear learning in adolescents and adults. Ethical factors complicate this work. Electrical shocks, the most widely used UCS in adults, may not be appropriate for adolescents, yet less noxious UCSs typically provoke minimal fear in adolescents (23). To overcome these barriers, we developed a unique task that capitalizes on the intrinsic aversiveness of witnessing fear in others (24). Thus, the task uses

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a fearful facial expression paired with a scream as the UCS (23). This UCS is paired with a photograph of one actor, the CS+ (threat stimulus), but is never paired with a photograph of another actor, the CS− (safety stimulus) (Fig. 1). Given our interest in assessing developmental differences in threat/safety discrimination during fear learning and the neural bases for these differences, we first had to test the ability of this fear-learning paradigm to produce learned threat/safety categories in both adolescents and adults. We did this in experiment 1. As our dependent variable, we used an autonomic marker, galvanic skin response (GSR) to a passively viewed CS, a method used in prior research with adults (17, 25).

In experiment 2, with a different sample of adolescents and adults, we mapped age differences in trial-by-trial verbal reports of learned fear and their neural correlates. Verbally reported fear to each CS indexes the precision with which threat/safety distinctions are drawn in adolescents versus adults, reflecting category learning. Moreover, the literature suggests that developmental differences in human fear are found most consistently in verbal fear reports, although thus far studied only to naturally occurring, rather than experimental, threats (1). Based on these data (1), we predicted that adolescents would have less distinct verbal categories for stimuli labeled as fear and safety than adults, extending our broader hypothesis that maturity brings better categorization skills. We also predicted that threat/safety category learning in adolescents would rely more on subcortical than cortical structures, reflected in greater responses in the amygdala and hippocampus to the CS+ versus CS− in adolescents than adults when performing fear categorization.

Based on findings of an increasing "frontalization" with age (10), we predicted that this same categorization process would engage the DLPFC more in adults than adolescents. According to theories of PFC function, category learning is facilitated by dorsolateral regions, which serve to resolve competition created by representations of similar-appearing stimuli (4, 22). In the present study, such competition was expected to arise between the similar-appearing CS+ and CS−. Unlike subcortical structures, which were expected to respond more to the CS+ than CS−, greater DLPFC response was expected to the CS− than CS+. This expectation was also based on prior findings of greater human DLPFC responses to safety than threat cues (17).

We also expected DLPFC engagement during category learning to vary positively with levels of reported fear in CS− trials. Such a positive correlation was predicted, because increasingly high fear to the CS− reflects instances where the CS− is experienced as increasingly similar to the CS+. Under these circumstances, greater DLPFC response is expected, as it is required to resolve this ambiguity (22). Given DLPFC immaturity, we expected no correlation between adolescent DLPFC responding and fear ratings to the CS−. Thus, differences in the DLPFC–fear relationship may parallel differences in rating data, reflecting the more refined ability to detect boundaries between threat and safety among adults than adolescents.

Results

Experiment 1. Demographic information for adolescents and adults is shown in Table S1. Adult and adolescent GSR data were analyzed during fear learning using analysis of variance (ANOVA), with one between-subjects factor (age group: adolescents, adults) and one within-subjects factor (CS-type: CS+, CS−). We found a main effect of CS-type [$F(1,40) = 21.49$, $P < 0.001$], where all participants manifested greater GSRs to the CS+ than CS− (CS+: mean (M) = 0.29, SD = 0.24; CS−: M = 0.17, SD = 0.17). A main effect of age group [$F(1,40) = 16.15$, $P < 0.001$] showed that adolescents manifested greater responses than adults overall (adolescents: M = 0.33, SD = 0.20; adults: M = 0.13, SD = 0.11). However, there was no age-group-by-CS-type interaction ($P > 0.54$). Data acquired during preconditioning revealed no age-group ($P > 0.86$) or CS-type ($P > 0.72$) differences.

As with GSR analyses, the postconditioning fear-rating data also yielded a CS-type main effect [$F(1,40) = 29.89$, $P < 0.001$]: All adolescents and adults reported more fear to the CS+ after con-

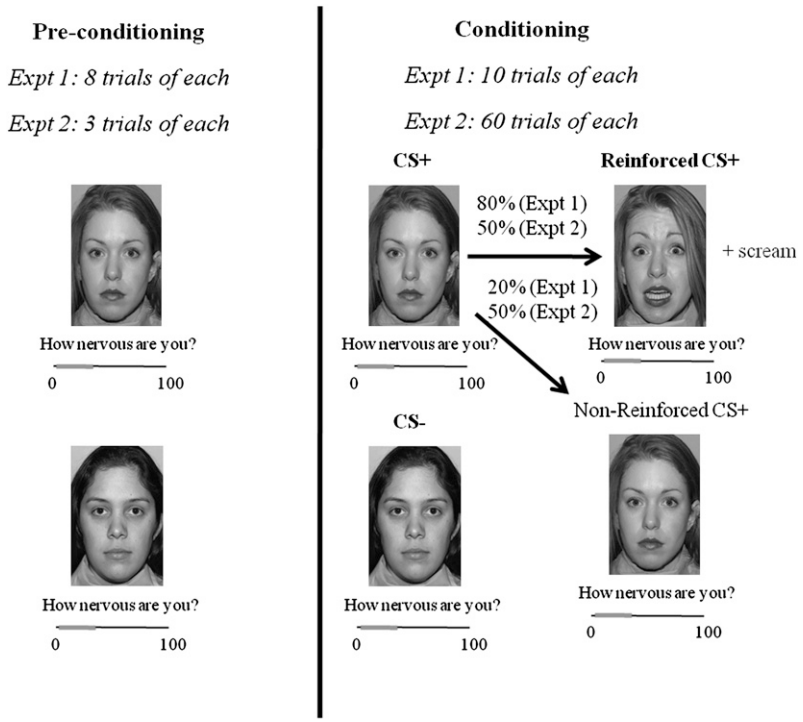

Fig. 1. A discrimination learning paradigm for studying threat classification.

ditioning. There was no main effect of age group ($P > 0.26$) or age-group-by-CS-type interaction ($P > 0.43$). Correlations between GSR and reported fear were not significant: Pearson's r (r) = 0.23 for the CS+ ($P > 0.14$) and $r = 0.12$ for the CS− ($P > 0.46$).

Experiment 2. Adolescent and adult fear ratings collected on a trial-by-trial basis (Fig. 2) were analyzed with an ANOVA that included one between-subjects factor (age group: adolescents, adults) and two within-subjects factors (CS-type: CS+, CS−; time: trial 1, trial 2. . .trial 30). This analysis revealed a significant CS-type main effect [$F(1,33) = 182.99$, $P < 0.001$] and a significant age-group-by-CS-type interaction [$F(1,1917) = 37.59$, $P < 0.001$]. Decomposing the interaction showed that whereas adults and adolescents both reported greater fear to the CS+ than CS−, adolescents showed a markedly smaller difference in their rated fear to the CS+ versus CS− [Means = 36.93 versus 29.52, $F(1,33) = 24.48$, $P < 0.001$] than did adults [Means = 34.92 versus 15.24, $F(1,1917) = 218.99$, $P < 0.001$] (Fig. 2). Thus, the interaction established that the rating difference between CS stimuli varied by age. This interaction effect was not present at preconditioning ($P > 0.35$). Nor were effects of age group at pre-conditioning ($P > 0.54$) or CS type ($P > 0.21$).

We next generated subject-level contrasts between BOLD (blood oxygen level-dependent) responses to nonreinforced CS+ and CS−. Significant group differences in this contrast emerged in the right amygdala and bilateral hippocampus (Fig. 3), again representing significant age-group-by-CS-type interactions. To further understand these interactions, for each participant we computed an average of all voxels within functionally defined regions of interest (ROIs) to each event type (nonreinforced CS+, CS−). t tests showed significant differences between CS+ and CS− in all ROIs, but only in the right hippocampus in adults (Fig. 3). Thus, these interactions demonstrated that differences in subcortical responses between CS-types varied by age and that, as predicted, subcortical discrimination of threat versus safety cues was generally greater in adolescents than adults. Importantly, no group differences in brain responses to CSs manifested before conditioning (*SI Materials and Methods*).

As noted above, in adults, amygdala activation was not present in the ROI identified by the between-group analysis. However, in

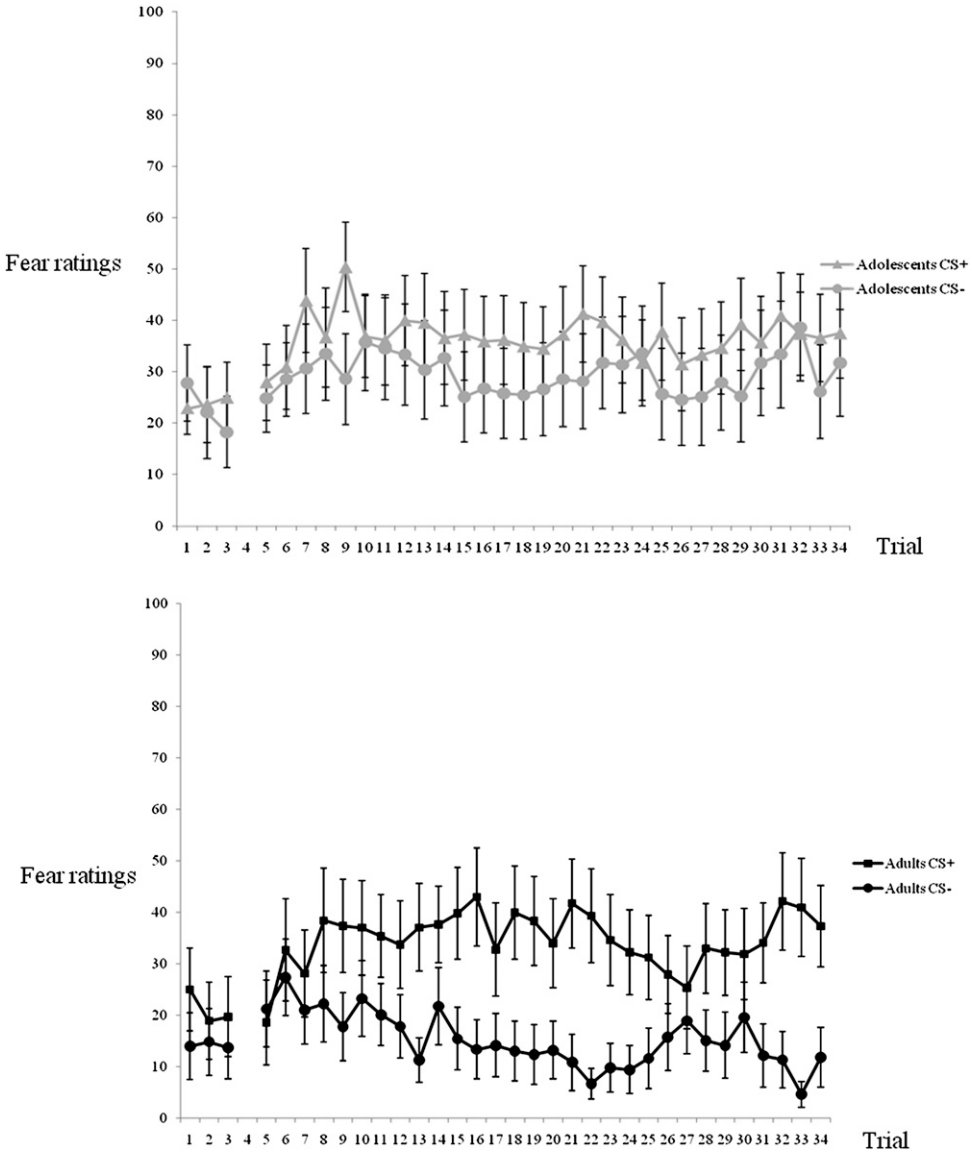

Fig. 2. Adolescent and adult fear ratings to CS+, CS− after each CS presentation during preconditioning and conditioning trials of experiment 2.

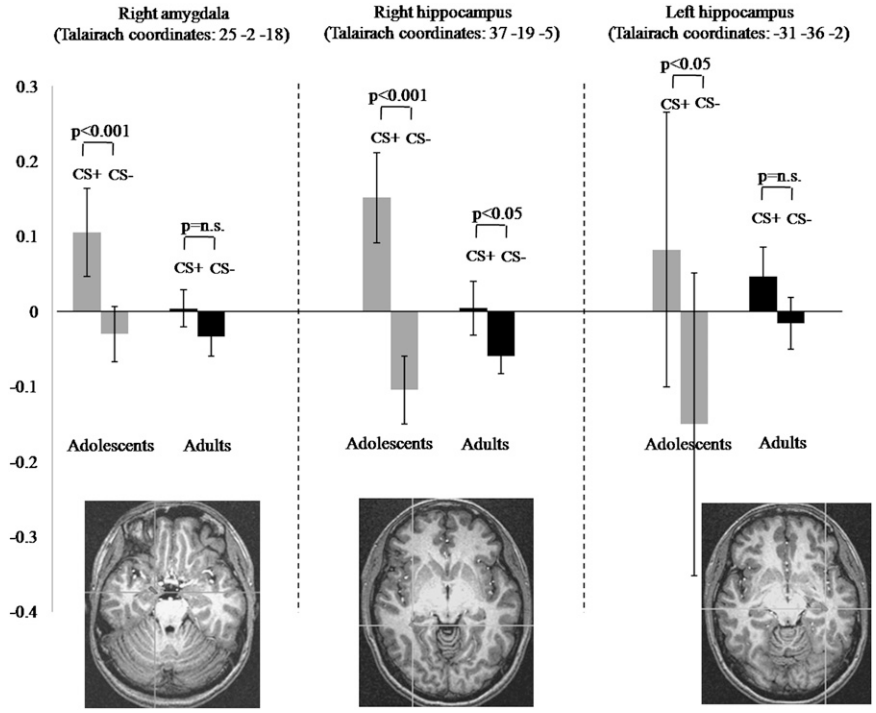

Fig. 3. Adolescent and adult responses in the right amygdala and right and left hippocampus to nonreinforced CS+ and CS− (experiment 2). n.s., not significant.

another amygdala location, medial and superior to the location where age differences were found, main effects of CS type [$F(1,33) = 19.46$, $P < 0.05$] occurred in the absence of an age-group-by-CS-type interaction (Fig. S1). Thus, in this amygdala location, similar levels of differential conditioning to the nonreinforced CS+ versus CS− occurred in adults and adolescents.

We next explored DLPFC responses to the CS− relative to the nonreinforced CS+. We had two a priori interests: first, in confirming that greater DLPFC responses occur to the CS− relative to the nonreinforced CS+, and second, in establishing age-group differences in rating–activation associations. In our whole-brain analysis of the BOLD contrast between nonreinforced CS+ and CS−, a single cluster in the DLPFC showed greater activity to the CS− compared with the nonreinforced CS+ [Talairach coordinates: 16, 53, 26, $t(32) = -3.32$, $P < 0.01$]. However, this cluster did not vary across age, even at low statistical thresholds.

Instead, age-group differences in this cluster emerged in our second whole-brain analysis of rating–activation associations. We used regression analysis to model correlations between activation and fear ratings to each CS. Subject-level coefficients reflecting this correlation were submitted to between-group comparisons, generating tests of an age-group-by-CS-type interaction on brain–behavior associations. A cluster in the DLPFC (Talairach coordinates: 24, 50, 24) again emerged, reflecting age-specific patterns in the correlation between activation and fear ratings to the CS−. Of note, this cluster was encompassed by the region that was differentially activated by the CS− relative to the nonreinforced CS+ across all participants in our first whole-brain analysis. Extracted coefficients showed that in adults, positive covariance emerged ($\beta = 0.008$), but in adolescents, this relationship was negative ($\beta = -0.008$). These associations differed between groups [$t(32) = 3.88$, $P < 0.05$], and in each group differed from zero ($t > 2.49$). Thus, greater activity in this region correlated with higher fear ratings to the CS− in adults, but lower activity predicted more fear to the CS− in adolescents.

No between-group differences characterized covariation between this region and fear to the CS+. Nor did group differences characterize covariation between other brain regions and rated fear to the CSs.

Discussion

Distinguishing threat from safety is adaptive, yet prior data on reported fears to naturally occurring threats suggest that this ability changes in the transition from adolescence to adulthood (1). Here we tested the hypothesis that, in adolescents and adults, age differences in subcortical and prefrontal function relate to age differences in the capacity to discriminate learned threats from learned safety cues. To test this hypothesis, we first developed a fear-learning task that generates threat/safety category learning in both adolescents and adults. Experiment 1 established the capacity of this task, using face-scream CS/UCS pairings, to produce robust CS differentiation using autonomic and subjective measures in both age groups, albeit occurring in the context of overall higher levels of autonomic responding in adolescents.

Using this task in a second study, we then showed that whereas adolescents could verbally discriminate between safety and threat cues, adults formed more differentiated threat/safety boundaries than did adolescents, consistent with data on fear to naturally occurring threats (1). These rating differences were paralleled by findings in our imaging data: Age differences emerged in the degree to which subcortical and prefrontal structures supported fear learning. In adolescents, relative to adults, larger differential amygdala and hippocampal responses emerged to CS+ versus CS− trials. Of note, both adolescents and adults showed differential CS-related responding in a right amygdala region, medial and superior to the location where age differences were found. Such findings are consistent with prior neuroimaging reports showing that adults and adolescents tend to exhibit activation in similar brain regions, but with activation in adults manifesting in a more spatially restricted manner (26), possibly reflecting age-

related differences in the intrinsic functional architecture of the brain (27).

Unlike in adolescents, adults' formation of threat/safety categories was positively linked to DLPFC activity. Incorporation of fear-rating data into an event-based analysis focused on trial-by-trial associations; this allowed experiment 2 to map age differences in the relationship between DLPFC activation and an individual's ability to differentiate threat from safety cues, the very same capacity where, overall, adults showed better discriminative ability than adolescents. Similar to prior data, DLPFC responded more to the safety than threat cues (17). However, intriguingly, adults' DLPFC activation correlated positively with fear rating to the CS−. Thus, in adults, stronger DLPFC engagement may have emerged to CS− events that were progressively more "ambiguous," due to their similarity to the CS+ in terms of fear-evoking capacity (22). This concurs with theories suggesting that mature DLPFC function disambiguates competition among similar-appearing stimuli, enabling the formation of precise categories. This positive correlation could directly underpin adults' increased verbal demarcation of threat/safety categories.

In contrast, DLPFC findings in adolescents exhibited a negative association with fear ratings. Immaturity of this region would generally be expected to manifest as an absent rather than a negative DLPFC–fear association. One possible explanation for this negative relationship is that an immature DLPFC, although generally capable of drawing categorical distinctions, fails to adapt appropriately under conditions of increased ambiguity between categories. In this context, categorization procedures draw on functions in subcortical structures. Given the preliminary nature of these data, cautious interpretations are warranted.

Our data extend a rich developmental literature (1). By suggesting that immature PFC shapes the less nuanced threat/safety discrimination in adolescents, our data may explain tentatively why, relative to adults, adolescents report more pervasive fears and worries and are more vulnerable to stress-related problems (2, 28–35). Despite these implications, our study has various limitations and raises some broader questions.

First, prior studies found atypical amygdala responses to neutral faces in children (35, 36), raising questions about whether our use of neutral faces here could confound reported group differences. However, these studies relied on different designs and recruited younger children than examined here. More similar studies have found no age differences in responses to neutral faces (33, 34). The current study also found no age differences in amygdala response to neutral CS events during preconditioning, findings echoed by the absence of preconditioning GSR differences.

A second set of questions arises given the absence of direct anatomical connections between the amygdala and DLPFC (37). However, considerable prior work finds similar functional parallels between DLPFC and amygdala activity in emotionally evocative imaging paradigms (38), including fear-conditioning tasks (17). These data suggest that these relationships may emerge through complex anatomical intermediaries (37).

The usual functional MRI caveats apply: small samples, lack of test–retest reliability, and imprecise estimation of correlations, all contributing to controversy (39, 40). To minimize spurious correlations, we only examined PFC–rating relationships in regions that responded differentially to the CSs. Nevertheless, our data might be best viewed as lower-bound estimates of between-group differences and in need of replication.

Finally, we examined age trends using cross-sectional data in two broadly defined but clearly demarcated "adolescent" and "adult" age groups, with mean ages of 13 and 28 y, respectively. Such a "categorical" approach maximizes the potential for observing age differences. However, limitations of this approach include the fact that it combines adolescents at different stages of maturity into a single age group and that it ignores the considerable individual variability that likely characterizes maturation in fear learning. It also assumes that there is a precise age in which an adolescent becomes a fully mature adult. Instead, data on the maturation of various other complex, emotion-related psychological constructs (41, 42) suggest a smooth, gradually emerging age curve with a plateau in the late 20s. To better identify the precise timing of these adolescent-to-adult transitions in fear learning and individual differences within this, future research should include longitudinal studies of large groups of similarly aged individuals at this developmental transition and follow them well into adulthood.

In summary, we present human data to link age differences in brain function to age differences in threat/safety category learning. Although our data do not inform the origins of the developmental divergence in DLPFC function, it is likely that these maturational changes reflect both genetically driven programs of biological change (43) and experience-dependent changes that occur from interactions with the wider social environment (44). Indeed, gene-by-environment interactions have been reported in more general behavioral changes across development (45), and more recently these have been extended to our understanding of adolescent brain development. Within this context, our data suggest that the relative maturation of subcortical and prefrontal systems in adolescents versus adults contributes to differences in the neural architecture of category learning. In turn, these age-related differences in category learning may underlie previously observed age differences in reported fears to naturally occurring threats (1).

Materials and Methods

This work was approved by the National Institute of Mental Health Institutional Review Board. Participants and legal guardians provided assent/consent.

Experiment 1. Twenty-one adolescents and 21 adults recruited through local schools and newspapers were studied successfully (Table S1). An additional five subjects between the ages of 8 and 11 were studied unsuccessfully when they asked to terminate the conditioning procedures, which they experienced as excessively fear-provoking. All participants were free of psychopathology based on clinical interview (46). There were no differences between age-groups in sex, IQ, or SES ($P > 0.10$).

Participants completed the discriminative fear-conditioning task during GSR data acquisition. The task consists of preconditioning, conditioning, and extinction phases. Only preconditioning and conditioning data pertain to the formation of threat categories and will be discussed here. During preconditioning trials ($n = 8$), participants view two female actresses displaying neutral expressions. During conditioning trials ($n = 20$), the neutral expression of one actress is randomly selected as the conditioned stimulus (CS+), terminating in an expression of intense fear accompanied by a 90-dB female scream (UCS), on 80% of trials. In contrast, the photograph of the second actress is never followed by the UCS (CS−). Stimuli are shown for 8 s. The UCS comprises a fearful face presented for 3 s and coterminates with a 1-s scream. Intertrial intervals range from 8 to 10 s. Conditioning trials were administered over one block of 20 trials with Presentation (Neurobehavioral Systems) and Psylab (Contact Precision Instruments). CSs were viewed passively. GSRs were recorded from the index and middle finger of the left hand using published recommendations (47). Self-reported fear ratings to each CS were collected after conditioning.

Experiment 2. Fifteen healthy adolescents (mean age, 13.33 y; age range, 10–17 y; 67% male) and 20 adults (mean age, 28.90 y; age range, 18–50 y; 65% male) were recruited through local schools and newspapers (Table S1). No differences in sex, IQ, and SES emerged between adolescents and adults. All were confirmed to be free of psychopathology based on clinical interview.

During MRI acquisition, participants completed the original discriminative fear-conditioning task (23) but with three modifications (Fig. 1). First, the number of conditioning trials was increased from 20 to 60 to enhance power. Second, alterations were made to task parameters. The number of CS+ trials that terminated in the UCS was decreased to 50%, resulting in more nonreinforced CS+ trials where neural activations are not confounded by UCS presentation. Trials were presented for 6 s, consisting of face presentation (3 s) and rating (3 s). Reinforced CS+ trials were 7.1 s, including

fearful faces that terminated with the scream (1.1 s). Intertrial intervals ranged from 2 to 4 s. Finally, we were interested in comparing the degree to which online, real-time changes in behavior are modulated by changes in brain activity. Thus, fear ratings in experiment 2 were collected online, concomitant with each CS presentation, rather than postconditioning, as in experiment 1. A response box recorded behavior, requiring both hands to move a slider and one of these to make a button response, making monitoring of GSR data difficult. Ratings were made on a 0 (not nervous) to 100 (extremely nervous) scale. The acquisition of fear ratings during imaging also allowed close monitoring of subjects, addressing ethical concerns about inducing fear in unmonitored juveniles, studied in the confines of an MRI scanner. Behavioral ratings were analyzed with ANOVAs with Greenhouse–Geisser adjustment. Fear ratings ascertained during preconditioning trials provided data on differences between adolescents and adults in response to neutral faces in the absence of UCS.

Whole-brain blood oxygen level-dependent MRI data were acquired in a General Electric Signa 3T scanner. Standard preprocessing of echo-planar imaging data was conducted using Analysis of Functional and Neural Images (AFNI) software (*SI Materials and Methods*). Statistical models set to the onset of each event type, convolved with the hemodynamic response function of each participant, produced subject-specific β-coefficients for each event type: CS+, CS− (*SI Materials and Methods*). Comparing coefficients for event types generated subject-level "nonreinforced CS+ versus CS−" contrast values. These were submitted to two-tailed *t* tests to assess age-group differences. Significant activations exceeded initial thresholds of $P < 0.05$ whole-brain uncorrected and cluster sizes of 195 and 130 voxels within the amygdala and hippocampus, respectively, and 216 voxels within the PFC, corresponding to ROI-corrected $P < 0.05$.

In another model, other basis functions containing stimulus onset time weighted by "relative" fear ratings were incorporated in regression models of functional data. Relative fear ratings were deviations of fear ratings per trial from mean ratings of each CS. β-Coefficients for each event type were produced, reflecting covariation between neural activations and reported fear. Two subject-level contrasts were generated by comparing β-coefficients of nonreinforced CS+ events and CS− events with baseline values. Subject-level contrasts were submitted to group-level *t* tests. We used standard whole-brain $P < 0.05$ two-tailed *t* test uncorrected for multiple comparisons throughout the brain to explore regions where significant group differences emerged to either CS. Again significant activations exceeded a cluster size of 216 voxels within the PFC, corresponding to ROI-corrected $P < 0.05$.

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