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Effective Connectivity Reveals Hippocampal Disruptions During Conditioning and Extinction are Associated with Adolescent Anxiety

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Abstract

Anxiety disorders peak during adolescence alongside rapid neural development, yet the neural mechanisms underlying adolescent threat learning remain unclear. Using fMRI and dynamic causal modeling, we mapped effective connectivity during threat conditioning and extinction in 87 adolescents (ages 11-17, *M*=15.43 years; 71.68% female), focusing on the amygdala, hippocampus, and posterior ventromedial prefrontal cortex (p-vmPFC) circuit. The adolescent brain showed distinct circuit strategies for threat versus safety learning. Threat learning (CS+) initially engaged only amygdala and p-vmPFC excitatory coupling without hippocampal involvement, while early safety learning (CS-) recruited hippocampal inhibition of amygdala and p-vmPFC. By late conditioning, bidirectional inhibitory connectivity emerged between amygdala and p-vmPFC for both stimulus types, suggesting homeostatic regulation once associations consolidated. During extinction, the network transitioned from complex mutual amygdala-hippocampus inhibition during early CS+ trials to streamlined hippocampal-prefrontal pathways by late extinction, reflecting safety consolidation. Adolescents with higher trait anxiety demonstrated stronger hippocampal to p-vmPFC connectivity during early conditioning and altered hippocampal modulation during extinction, revealing temporally dynamic disruptions in directional signaling. This study provides the first effective connectivity map of adolescent threat learning. The identification of temporally dynamic vulnerabilities has direct implications for adolescent anxiety treatment.

Keywords: adolescence, anxiety, dynamic causal modeling, fMRI, hippocampus

Anxiety disorders peak during adolescence, with over half of cases emerging before age 18 years.[1,2] Anxiety symptoms that emerge during adolescence lead to mental health symptoms across the lifespan,[3,4] yet critically the neural mechanisms underlying adolescent anxiety vulnerability remain poorly understood. Contemporary models converge on the view that anxiety reflects alterations in how threat- and safety-related information is encoded across interconnected corticolimbic circuits, rather than dysfunction in any single region in isolation.[5] However, most neuroimaging work in adolescents has focused on regional activation or correlations in activity, providing limited insight into how these circuits influence one another over time. There is a critical need for approaches that characterize the directional, causal interactions between key nodes of threat circuitry during learning, and to test how variation in these directed influences relates to adolescent anxiety.

Pavlovian threat conditioning and extinction (also referred to as fear conditioning[6]) paradigms provide experimental models for understanding anxiety pathophysiology.[7–9] In a typical threat conditioning and extinction paradigm, a neutral conditioned stimulus (CS; e.g., an image) is paired with an aversive unconditioned stimulus (US; e.g., an aversive noise). After one or more conditioning trials, presentation of the CS alone comes to elicit a conditioned response considered to reflect fear. These conditioned responses can be extinguished by repeated presentations of the CS in the absence of the US. Within this framework, individual differences in how threat associations are acquired and updated can be linked to the functioning of specific neural circuits, providing a tractable bridge between mechanistic models of threat learning and the neural basis of adolescent anxiety.

Human functional magnetic resonance imaging (fMRI) studies implicate a core network comprising the amygdala, ventromedial prefrontal cortex (vmPFC), and hippocampus in threat processing in adolescents and adults.[10–19] The amygdala encodes stimulus-threat associations, drives conditioned responses, and facilitates extinction through

synaptic plasticity.[20,21] The vmPFC regulates threat responses through connections to the amygdala,[10] with its posterior subregion (p-vmPFC) specifically implicated in both threat acquisition and extinction.[11,22–24] The hippocampus integrates contextual information and, in trace conditioning paradigms where the CS and US are temporally separated, is essential for associative learning.[25] Increased amygdala threat reactivity and reduced vmPFC regulatory recruitment represent two independent dimensions of neurocognitive function that may confer vulnerability to anxiety.[26,27] In parallel, hippocampal involvement may index a third, context-dependent mechanism of vulnerability, as alterations in hippocampal structure and function during adolescence have been linked to impaired use of contextual cues to constrain threat responses[28] and to heightened anxiety symptoms.[29]

These regions undergo substantial structural and functional reorganization during adolescence,[30–33] with delayed maturation of amygdala-prefrontal connectivity proposed as a neural substrate for adolescent anxiety risk.[14,34–36] Adolescents show impaired extinction and reduced PFC activity compared to adults, partially explaining the peak in prevalence of anxiety disorders during this developmental period.[32,37–41] Critically, anxious adolescents display more negative amygdala-PFC coupling during threat processing, whereas anxious adults show more positive coupling.[36] In adolescence, the hippocampus continues to undergo structural maturation that supports the refinement of contextual memory and threat learning.[42–44] At the same time, hippocampal connectivity with the amygdala and PFC shifts from a pattern of less efficient, sometimes excessive or uncoordinated coupling toward more integrated, context-sensitive communication across development.[45,46] Adolescent studies show reduced hippocampal-PFC connectivity, increased amygdala-hippocampus coupling, and decreased hippocampal volume are all linked to deficits during threat conditioning and extinction (as well as anxiety).[29,37,47] Despite the importance of identifying *where* threat processing occurs, existing neuroimaging research in adolescence has not yet characterized

how information flows between these regions. Conventional functional connectivity (i.e., associative) analyses cannot characterize how one region drives another or responds to a common input.[48] This is a fundamental limitation for testing mechanistic hypotheses of how regulatory control emerges or fails within threat circuitry.[49–51]

Effective connectivity methods address this gap by estimating directional causal influences between brain regions.[52–54] Dynamic causal modeling (DCM) provides a hypothesis-driven framework for testing specific models of directional interactions within neural networks.[55] Previous DCM studies in adults demonstrate that threat conditioning modulates connectivity from the amygdala to the vmPFC, while extinction engages top-down prefrontal control over amygdala responses.[56–58] Despite these advances, no studies to date have applied DCM to characterize threat circuit dynamics during adolescence, leaving unresolved whether adolescent anxiety vulnerability reflects aberrant bottom-up threat signaling from the amygdala, impaired top-down regulation by the vmPFC, or disrupted contextual gating by the hippocampus.

The current study provides the first *effective connectivity* map of adolescent threat and safety learning using DCM. We focused on the p-vmPFC-amygdala-hippocampus circuit during trace conditioning and extinction, a paradigm that engages hippocampal temporal integration alongside amygdala threat processing and p-vmPFC regulation.[25,59–61] We tested four hypotheses grounded in adult connectivity literature and developmental models of anxiety: (1) *Extinction shifts network architecture*. We hypothesized that extinction would reconfigure p-vmPFC and amygdala connectivity compared to conditioning, engaging inhibitory regulatory pathways as the network learns new safety associations; (2) *Hippocampal contextual gating.* We predicted the hippocampus would serve as a contextual gate by bidirectionally modulating amygdala activity and exciting the p-vmPFC, with the direction and strength of hippocampal-amygdala connectivity varying based on whether

contextual information signals threat versus safety; (3) *Early phase sensitivity.* We hypothesized that the network would show dynamic reconfiguration across learning stages. We expected early phases to be characterized by complex connectivity patterns reflecting learning (during conditioning) and circuit conflict between residual associations and emerging learning (during extinction). We expected late phases to transition to simplified, stabilized connectivity as contingencies are learned; (4) *Anxiety-related dysregulation.* We predicted high-anxiety adolescents would show: (a) increased amygdala to p-vmPFC excitation during conditioning, reflecting heightened bottom-up threat signaling; and (b) altered hippocampal modulation of p-vmPFC during conditioning and extinction, reflecting disrupted contextual processing.

Results

After exclusion for image quality and excessive in-scanner head motion (see Methods), our final usable sample included 87 adolescent participants (*M*age=15.66, *SD*=0.94, Range=11.82-17.30 years; 71.26% female). All participants completed a threat conditioning task while undergoing fMRI. During the task, participants underwent conditioning and extinction phases using female faces from the NimStim set.[62] During conditioning, one of two neutral faces was conditioned as a threat stimulus (CS+). The CS+ was reinforced 100% with a female scream as the unconditioned stimulus (US). The safe stimulus (CS-) was never paired with the US. Extinction immediately followed conditioning with presentation of the CS+ and CS- both in the absence of any US.

Anxiety was assessed using the State-Trait Anxiety Inventory for children, trait subscale (STAI-T). High and low anxiety groups were identified with individuals scoring less than 40 grouped as low anxiety (*N*=46, *M*age=15.48, *SD*=0.98, Range=11.82-17.30 years; 63.04% female; average score 31.83, Range 20-39) and those scoring 40 or higher grouped as high anxiety (*N*=41, *M*age=15.86, *SD*=0.85, Range=13.83-16.98 years; 80.49% female;

average score 47.68, Range 40-62). This threshold is typical for establishing baseline groups of high and low trait anxiety.[63] Age did not differ between the two groups, $t(85)=1.88$, 95%CI[-0.77, 0.02], $p=.06$, nor did the proportion of females, $X^2(1, N=87)=3.22$, $p=.073$. STAI-T significantly differed between the groups, $t(85)=16.52$, 95%CI[-17.77, -13.95], $p<.001$.

Dynamic Causal Modeling specifications

DCM models the rate of change in activity of a given brain region as a function of the activity in another brain region. To test different hypotheses these models were allowed to vary in three components: the endogenous connections between nodes (intrinsic parameters); the stimulus input that drives specific areas directly (extrinsic parameters); and the modulation of the strengths of functional coupling between regions induced by an experimental condition. Connectivity effect sizes are measured in hertz (Hz). Positive values indicate excitatory connections between regions while negative values indicate inhibitory connections. For the second-level parametric empirical Bayes (PEB) model, a posterior probability threshold of greater than 0.95 was used to identify parameters that had strong supporting evidence. We modeled early and late trials separately due to evidence of differential neural engagement in different phases of conditioning and extinction.

Given our aim to examine effective connectivity within regions of an established threat processing network, we constructed a DCM network comprised of three regions-of-interest (ROIs): amygdala, hippocampus, and p-vmPFC (for more details on these ROIs, see Methods) (**Fig. 1a**). We compared variations of our full connectivity model that differed in which regions of the threat circuit received task-related driving inputs. We compared seven models in total, which spanned all possible configurations of driving inputs. Bayesian Model Selection showed that Model 3, which featured driving input only to the amygdala, outperformed all other models with posterior probability (Pp) = 0.50 (**Fig. 1b-c**).

We applied Bayesian Model Averaging to our full model for the endogenous (non-task related) connections between regions, identifying bidirectional connections among all regions (**Fig. 1d, Table 1**). All connections were excitatory except for the hippocampus to amygdala inhibitory connection.

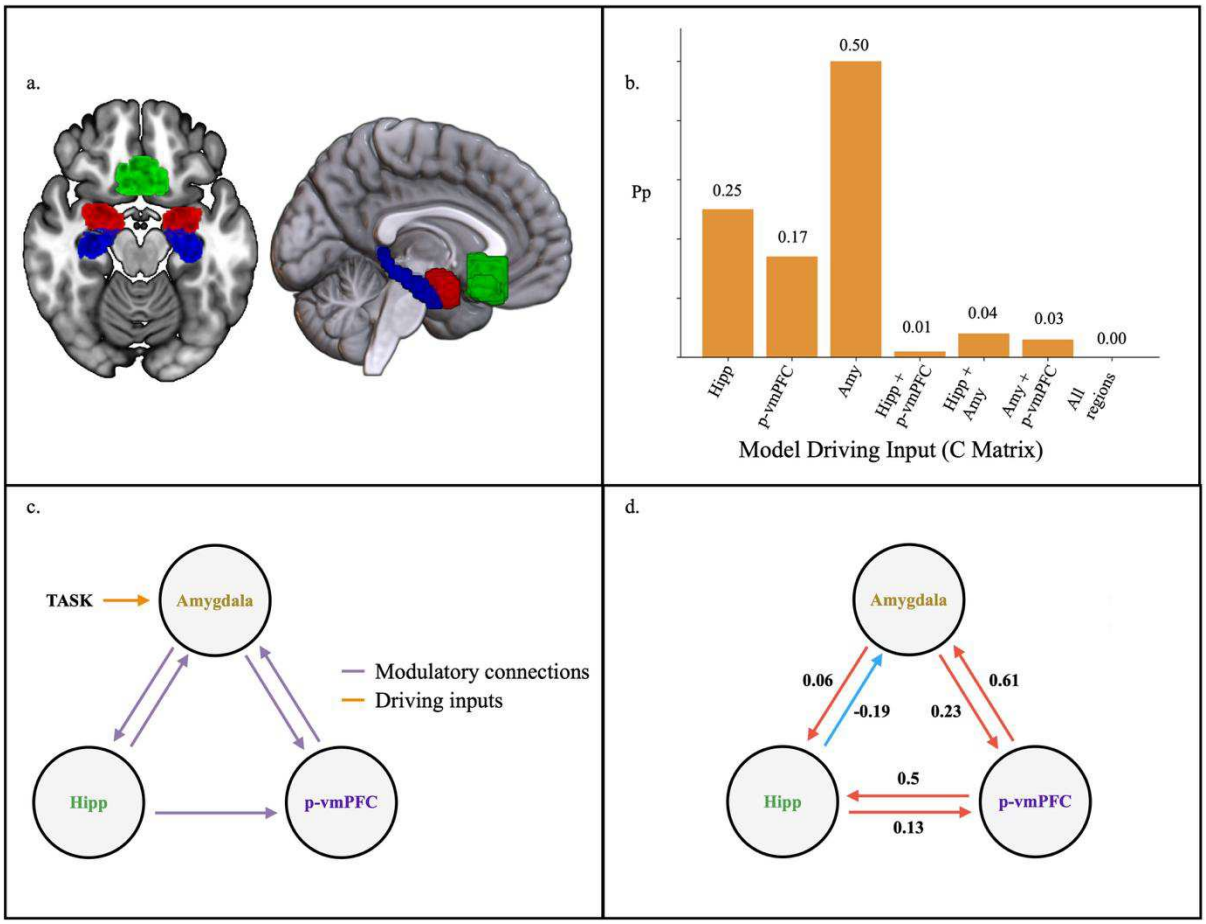


Fig. 1. DCM model specification. (a) Volumes of interest (VOIs), including all voxels for each subject with intensity reflecting the number of times each voxel appears in different subject VOIs (brighter colors indicate more participants are represented). Red VOIs = amygdala, Blue VOIs = hippocampus, Green VOIs = p-vmPFC. **(b)** Driving input Bayesian Model Selection. Model 3, which featured driving input to the amygdala only, was superior fit for the data. **(c)** Model specification for modulatory connections (B matrix) and driving input (C matrix) for the final model. **(d)** Intrinsic connectivity. Red arrows indicate connections with excitatory connectivity (increased Ep); blue arrows indicate connections

with inhibitory connectivity (decreased Ep). All displayed connections meet posterior probability threshold (Pp>0.95). Ep = Expected value (posterior mean). Pp = Posterior probability. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex.

Table 1. Bayesian Model Average intrinsic connections (A matrix).

Source	Target	Ep	Cp	Pp
Hipp	p-vmPFC	0.13161	0.00045	1.00000
Hipp	Amygdala	-0.18679	0.00058	1.00000
p-vmPFC	Hipp	0.49851	0.00052	1.00000
p-vmPFC	Amygdala	0.60944	0.00073	1.00000
Amygdala	Hipp	0.06394	0.00055	0.96480
Amygdala	p-vmPFC	0.23115	0.00041	1.00000

Note. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex. Ep = Expected value (posterior mean), Cp = Posterior covariance, Pp = Posterior probability (range 0-1), values >0.95 indicate strong supporting evidence.

Conditioning

During conditioning, distinct connectivity patterns emerged between CS+ and CS- trials. For early conditioning trials, threat stimuli (CS+) resulted in bidirectional excitatory connections between amygdala and p-vmPFC, which were sustained for safe stimuli (CS-) (**Fig. 2a-b, Table 2**). CS- stimuli also resulted in inhibitory connections from the hippocampus to both the amygdala and p-vmPFC. This pattern suggests that initial safety learning is characterized by hippocampal involvement that is not observed during threat learning. In contrast, late conditioning trials resulted in inhibitory connections between p-

vmPFC and amygdala for CS+ and CS- trials. Hippocampal inhibition of the amygdala and p-vmPFC also reversed to excitatory. Additionally, the amygdala excited the hippocampus during late conditioning CS- trials (**Fig. 2c-d, Table 2**). By late conditioning, the network showed full tripartite engagement.

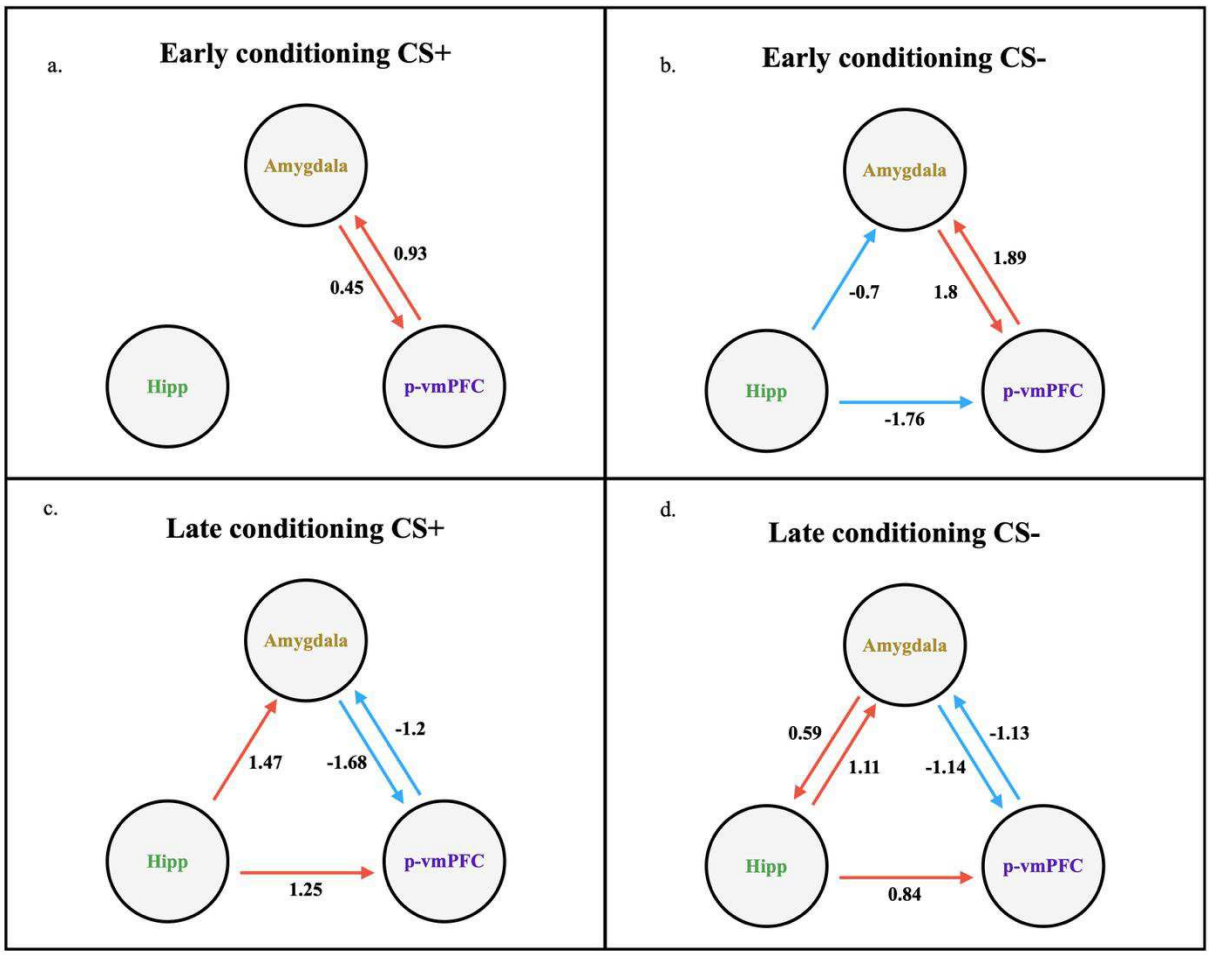


Fig. 2. Bayesian Model Averaging modulatory connection strengths during conditioning phases. (a) Early conditioning CS+ **(b)** Early conditioning CS- **(c)** Late conditioning CS+ **(d)** Late conditioning CS-. Red arrows indicate connections with excitatory modulation (increased Ep); blue arrows indicate connections with inhibitory modulation (decreased Ep). All displayed connections meet posterior probability threshold (Pp>0.95). Ep = Expected value (posterior mean). Pp = Posterior probability. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex.

Extinction

Extinction showed dynamic network reconfiguration across learning stages. During early CS+ extinction trials, the network displayed mutual inhibition between the hippocampus and amygdala alongside strong excitatory hippocampal drive to the p-vmPFC, suggesting active conflict between residual threat associations and emerging contextual safety signals (**Fig. 3a, Table 2**). Early CS- extinction trials resulted in a different pattern, where the amygdala and hippocampus showed bidirectional excitatory connections and the p-vmPFC inhibited the amygdala (**Fig. 3b, Table 2**). The excitation of the p-vmPFC by the hippocampus and the inhibition of the p-vmPFC by the amygdala remained consistent. By late extinction, the network simplified considerably for both CS+ and CS-. Fewer connections at late extinction meant the network returned closer to a baseline state indicating more stability. Late CS+ trials showed excitation of the p-vmPFC by both the amygdala and hippocampus, as well as inhibition of the hippocampus by the amygdala (**Fig. 3c, Table 2**). Late CS- showed only bidirectional hippocampal-amygdala inhibition (**Fig. 3d, Table 2**).

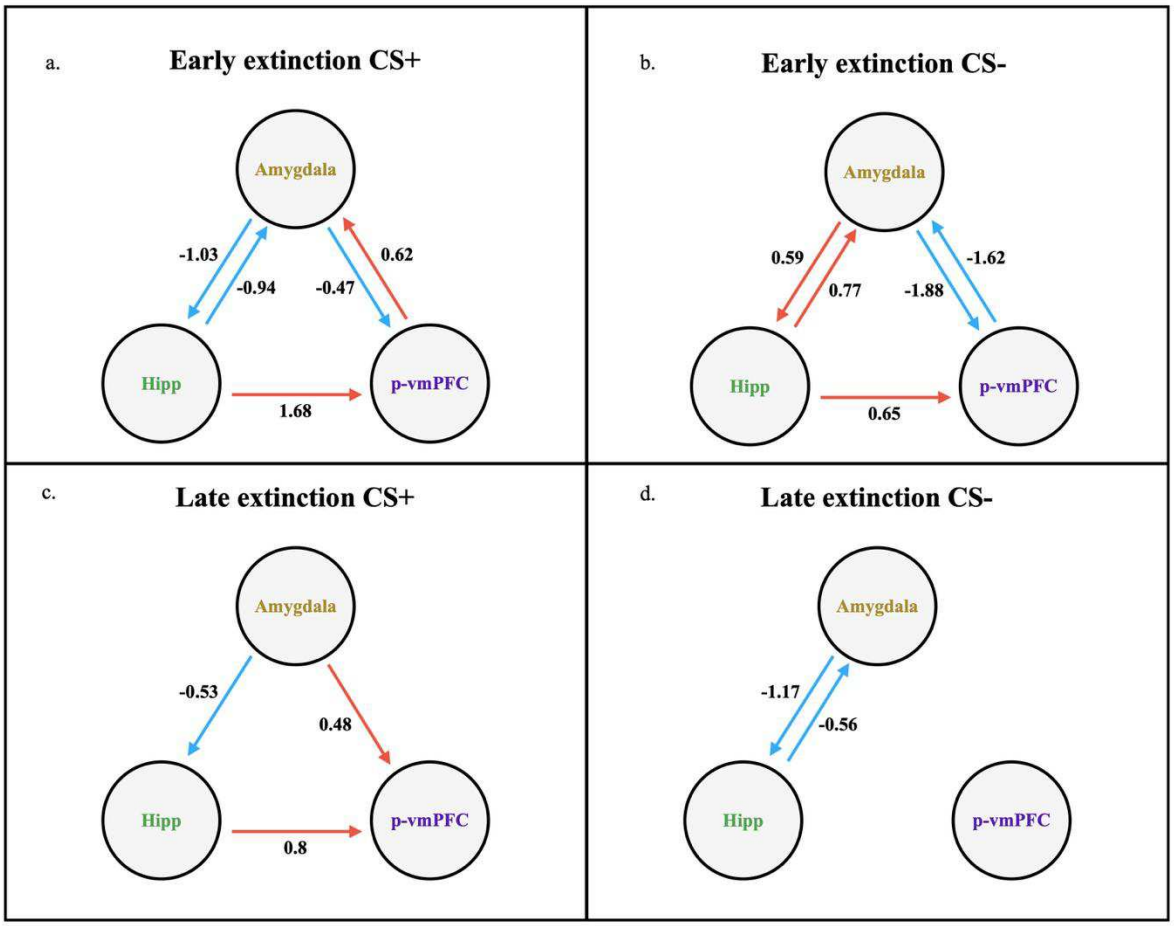


Fig. 3. Bayesian Model Averaging modulatory connection strengths during extinction phases. (a) Early extinction CS+ **(b)** Early extinction CS- **(c)** Late extinction CS+ **(d)** Late extinction CS-. Red arrows indicate connections with excitatory modulation (increased Ep); blue arrows indicate connections with inhibitory modulation (decreased Ep). All displayed connections meet posterior probability threshold (Pp>0.95). Ep = Expected value (posterior mean). Pp = Posterior probability. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex.

Table 2. BMA modulatory connections (B matrix) for all task conditions.

Task Condition	Source	Target	Ep	Cp	Pp
Early Conditioning CS+	p-vmPFC	Amygdala	0.93224	0.01652	1.00000
	Amygdala	p-vmPFC	0.45447	0.00943	1.00000

Early Conditioning CS-	Hipp	p-vmPFC	-1.76086	0.02403	1.00000
	Hipp	Amygdala	-0.69988	0.02455	0.99965
	p-vmPFC	Amygdala	1.89304	0.01679	1.00000
	Amygdala	p-vmPFC	1.80075	0.01095	1.00000
Late Conditioning CS+	Hipp	p-vmPFC	1.24674	0.02167	1.00000
	Hipp	Amygdala	1.47202	0.03080	1.00000
	p-vmPFC	Amygdala	-1.20272	0.03216	1.00000
	Amygdala	p-vmPFC	-1.67733	0.02402	1.00000
Late Conditioning CS-	Hipp	p-vmPFC	0.83668	0.01763	1.00000
	Hipp	Amygdala	1.11249	0.03483	1.00000
	p-vmPFC	Amygdala	-1.12872	0.03290	1.00000
	Amygdala	Hipp	0.58568	0.01510	1.00000
	Amygdala	p-vmPFC	-1.13947	0.02019	1.00000
Early Extinction CS+	Hipp	p-vmPFC	1.67560	0.01802	1.00000
	Hipp	Amygdala	-0.94168	0.04059	1.00000
	p-vmPFC	Amygdala	0.61981	0.02498	0.99749
	Amygdala	Hipp	-1.03289	0.01088	1.00000
	Amygdala	p-vmPFC	-0.47126	0.01777	0.99341
Early Extinction CS-	Hipp	p-vmPFC	0.65215	0.01458	1.00000
	Hipp	Amygdala	0.76791	0.02018	1.00000
	p-vmPFC	Amygdala	-1.61672	0.02166	1.00000
	Amygdala	Hipp	0.58676	0.01363	1.00000
	Amygdala	p-vmPFC	-1.88019	0.01492	1.00000
Late Extinction CS+	Hipp	p-vmPFC	0.79836	0.01671	1.00000
	Amygdala	Hipp	-0.53500	0.02196	0.99368
	Amygdala	p-vmPFC	0.47789	0.02378	0.98197
Late Extinction CS-	Hipp	Amygdala	-0.56018	0.02887	0.98728
	Amygdala	Hipp	-1.17094	0.02001	1.00000

Note. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex. Ep = Expected value (posterior mean), Cp = Posterior covariance, Pp = Posterior probability (range 0-1), values >.95 indicate strong supporting evidence.

Trait anxiety

We tested whether modulatory connection strengths predicted trait anxiety by adding participants' group classification as a covariate in PEB analysis. Participants were classified as high or low trait anxiety based on their STAI-T score. Four connections predicted anxiety classification with Pp>0.95 (**Fig. 4a-b, Table 3**). During early conditioning to CS+, high-anxiety adolescents showed two distinct patterns of circuit modulation, increased excitatory modulation of the p-vmPFC by the amygdala and increased inhibitory modulation of the p-vmPFC by the hippocampus. During late extinction to CS-, high-anxiety adolescents exhibited increased excitation of the p-vmPFC by both the amygdala and hippocampus. Notably, while the hippocampus to p-vmPFC connection showed inhibitory modulation in high-anxiety individuals during conditioning, this same connection reversed to excitatory modulation during extinction. Leave-one-out cross validation (**Fig. 4c**) further confirmed the predictive validity that an increase in the strength of the modulatory connection from the hippocampus to the p-vmPFC elicited in response to early threat conditioning predicted higher anxiety symptoms ($p = 0.007$).

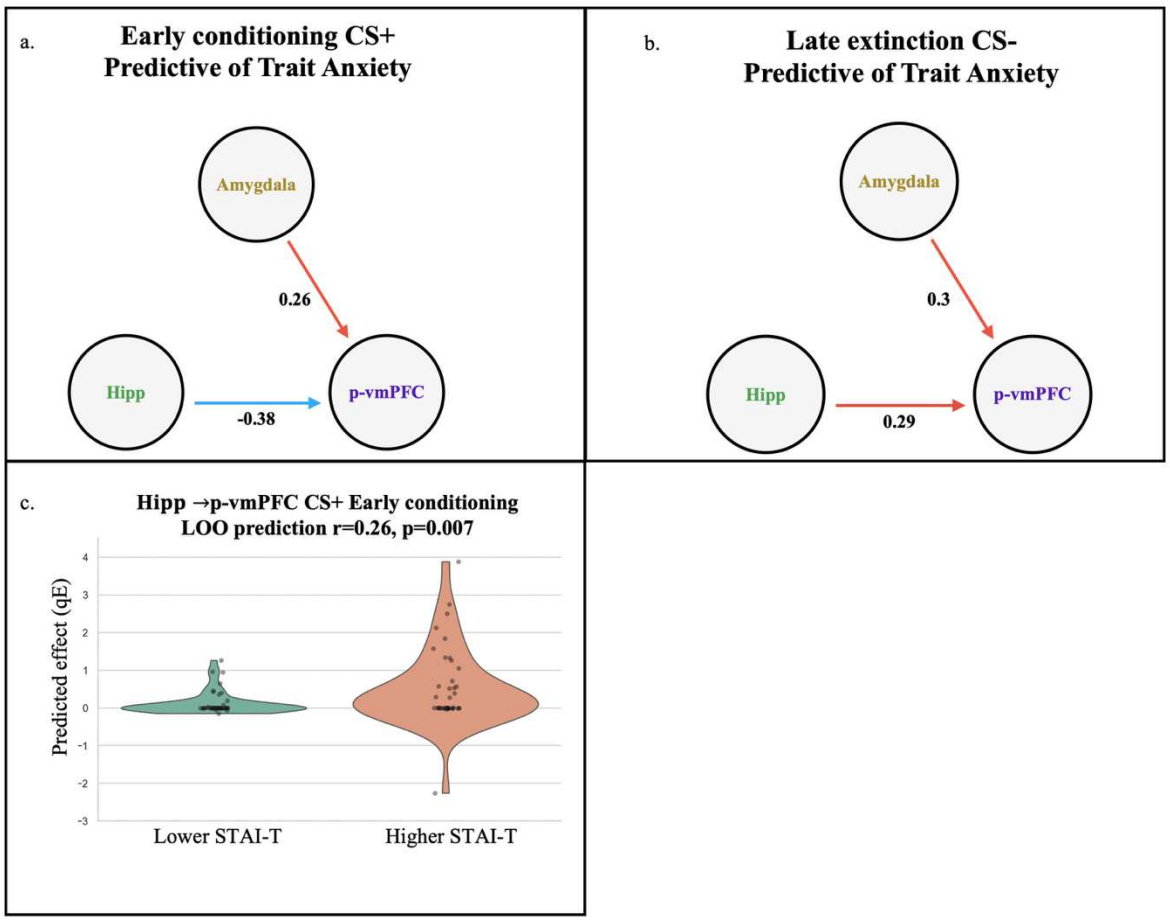


Fig. 4. Effective connectivity differences associated with trait anxiety. Task conditions modulated threat connectivity, with modulations associated with trait anxiety during **(a)** early conditioning to the threat stimulus (CS+) and **(b)** late extinction to the safety stimulus (CS-). Red arrows indicate connections where higher trait anxiety predicted increased excitatory modulation (increased Ep); blue arrows indicate connections where higher trait anxiety predicted increased inhibitory modulation (decreased Ep). All displayed connections meet posterior probability threshold (Pp>0.95). **(c)** Significant out-of-sample correlation of actual anxiety scores against predicted anxiety scores for each left-out subject. Each dot represents the estimate of a participant's anxiety symptom score. Ep = Expected value (posterior mean). Pp = Posterior probability. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex. STAI-T=State-Trait Anxiety Inventory–Trait scale. qE = Posterior density. LOO = leave-one-out cross validation.

Table 3. BMA modulatory connections predictive of higher trait anxiety classification.

Task Condition	Source	Target	Ep	Cp	Pp	*r*
Early Conditioning						
CS+	Hipp	p-vmPFC	-0.38152	0.00908	1.00000	0.26*
	Amygdala	p-vmPFC	0.26255	0.00891	0.96096	-0.09
Late Extinction						
CS-	Hipp	p-vmPFC	0.29291	0.00898	0.97531	0.14
	Amygdala	p-vmPFC	0.30164	0.01065	0.96972	0.09

Note. Hipp = hippocampus. p-vmPFC = posterior ventromedial prefrontal cortex. Ep = Expected value (posterior mean), Cp = Posterior covariance, Pp = Posterior probability (range 0-1), values >0.95 indicate strong evidence., r=correlation. *indicates that condition survived leave-one-out cross validation with $p<0.05$.

Discussion

This study revealed dynamic network reconfigurations that distinguish adolescent threat from safety learning and shift across acquisition and extinction phases. Using DCM, we found that the adolescent brain employs distinct strategies for learning threat versus safety. Threat learning (CS+) initially engaged only amygdala-prefrontal excitatory coupling without hippocampal involvement, while safety learning (CS-) rapidly recruited hippocampal inhibition of threat-processing regions, demonstrating neural contextual discrimination in early trials. By late conditioning, bidirectional inhibitory connectivity emerged between the amygdala and p-vmPFC for both stimulus types, suggesting homeostatic regulation once associations consolidated. During extinction, CS+ trials showed initial circuit conflict between hippocampal safety signals and prefrontal threat maintenance that resolved through network simplification. In contrast, CS- trials displayed cooperative processing that

streamlined to minimal hippocampal-amygdala regulation, demonstrating distinct mechanisms for maintaining safety versus updating threat information. Critically, adolescents with higher trait anxiety displayed stronger inhibitory hippocampal to prefrontal connectivity during early threat conditioning, with a pattern reversal to excitatory modulation during late safety extinction, suggesting anxiety vulnerability reflects altered hippocampal contextual processing. These findings identify specific directional circuit mechanisms underlying threat learning in adolescence and reveal how individual differences in anxiety map onto network communication patterns.

A particularly notable finding was the emergence of bidirectional inhibitory connectivity between the amygdala and p-vmPFC during late conditioning trials for both CS+ and CS- stimuli. For CS+ trials. This bidirectional inhibition may reflect a homeostatic regulatory mechanism that modulates defensive responses after learning is established, preventing excessive or dysregulated threat reactivity. The mutual inhibition could represent an adaptive 'checks and balances' system wherein the p-vmPFC attempts to regulate amygdala output, while the amygdala simultaneously constrains excessive prefrontal suppression of threat responses. The bidirectional inhibitory pattern for CS- trials during late conditioning likely serves a different functional role. Unlike CS+ trials, which require balanced expression and regulation, CS- trials benefit from comprehensive suppression of threat-processing circuits. The mutual inhibition between amygdala and p-vmPFC for CS- may reflect the network actively maintaining a low-threat state, with both regions dampening each other's activity to prevent inappropriate responses to safety signals. These patterns may be particularly significant during ongoing maturation of prefrontal-limbic circuits during adolescence. Rather than relying solely on mature top-down prefrontal inhibition of the amygdala, which may be less reliable in adolescents, the reciprocal inhibitory architecture we

observed may serve as a transitional regulatory mechanism that balances threat sensitivity with emerging control capacities during this vulnerable developmental window.

The hippocampus demonstrated stimulus-specific and stage-dependent connectivity patterns, which highlight its role as a contextual gating mechanism. During early conditioning, the hippocampus was selectively engaged for CS- trials, where it inhibited both the amygdala and p-vmPFC. This immediate hippocampal involvement in safety learning suggests that the hippocampus rapidly processes contextual information to distinguish stimuli and modulate defensive response expression.[10,64] The inhibitory hippocampal to amygdala connectivity during early CS- trials may reflect the hippocampus signaling contextual safety, actively dampening amygdala reactivity to prevent inappropriate defensive responses. In contrast, the absence of hippocampal connectivity during early CS+ learning suggests that initial threat encoding relies primarily on amygdala-prefrontal circuits, with contextual integration occurring later. By late conditioning, CS+ trials recruited strong excitatory hippocampal drive to both the amygdala and p-vmPFC, while CS- trials showed bidirectional excitatory hippocampal-amygdala coupling. This temporal engagement pattern aligns with the hippocampus's established role in trace conditioning, where it supports the temporal bridging required to associate stimuli separated by a delay.[10] The delayed hippocampal recruitment for threat learning may reflect the time needed to integrate temporal and contextual features of the CS+ and US association, whereas safety signals benefit from immediate contextual discrimination.

Our findings indicate a more complex role for p-vmPFC than simple inhibition of the amygdala. The bidirectional excitatory connections between amygdala and p-vmPFC during early conditioning suggest the p-vmPFC actively participates in threat encoding, not just threat suppression. This aligns with recent lesion evidence showing the vmPFC is necessary for threat acquisition,[11] and functional work implicating the p-vmPFC in threat prediction.[22–

[24] The adolescent p-vmPFC may therefore serve dual roles in both constructing threat representations and later inhibiting them. During early CS+ extinction, the hippocampus inhibited the amygdala, signaling the new safe context, while p-vmPFC simultaneously excited the amygdala, appearing to maintain the previously learned threat association. This opposing pattern may reflect incomplete context updating, where the p-vmPFC's role in constructing threat representations during conditioning interferes with its ability to immediately support extinction. By late CS+ extinction, this conflict resolved through network simplification with neither hippocampus nor p-vmPFC maintaining connections to the amygdala. In contrast to the circuit conflict observed during CS+ extinction, CS- extinction reflected processing of an already-established safety signal through distinct connectivity patterns. Early CS- extinction displayed bidirectional excitatory coupling between amygdala and hippocampus alongside mutual amygdala-p-vmPFC inhibition, reflecting cooperative processing rather than the threat-safety conflict observed for CS+. By late extinction, network connectivity to both CS+ and CS- simplified, but through different mechanisms. CS+ resolution involved eliminating hippocampal and p-vmPFC connections to the amygdala, while CS- required only bidirectional hippocampal-amygdala inhibition to maintain consolidated safety responding, with the p-vmPFC disengaged.

The anxiety-related connectivity patterns we identified reveal specific mechanistic vulnerabilities. High-anxiety adolescents showed increased amygdala to p-vmPFC excitation during conditioning, consistent with models of anxiety as excessive bottom-up threat signaling.[32,36,37,41] The pattern of increased excitation from the amygdala and hippocampus to the p-vmPFC for CS- in high anxiety adolescents is similar to the pattern of connectivity observed in the full sample for late CS+ extinction, indicating that high anxiety adolescents may have impaired threat-safety discrimination during extinction. High anxiety adolescents also showed increased hippocampus to p-vmPFC inhibition during early CS+ conditioning

and hippocampus to p-vmPFC excitation during late CS- extinction. Hippocampal inhibition of the vmPFC during conditioning likely disrupts the vmPFC's ability to modulate amygdala reactivity whereas excitation of the vmPFC during extinction suggests the hippocampus is continuing to provide contextual information to the vmPFC despite this being a long-established safety association. These findings reveal a pattern of hippocampal connectivity instability that has not been previously reported and may reflect anxious adolescents' failure to establish stable contextual gating. The specificity of these connectivity disruptions, with phase-dependent modulation during conditioning versus extinction, reveals that anxiety vulnerability involves temporally dynamic circuit differences rather than simple hypo- or hyper-connectivity.

Clinical implications

Our findings have direct implications for understanding why adolescence represents a peak period for anxiety disorder onset and why exposure-based treatments may show age-specific challenges.[32,37,41] For example, the hippocampal connectivity instability we identified may explain why anxious adolescents show poor extinction retention.[37] If the hippocampus cannot maintain stable contextual representations across learning phases, extinction memories may be especially vulnerable to interference or decay. Current exposure protocols are predicated on the assumption that extinction success depends primarily on top-down vmPFC to amygdala inhibition. Our results reveal that hippocampal contextual gating may play an equally critical, and potentially more disruptive, role in adolescent anxiety. Interventions specifically targeting hippocampal function, such as retrieval practice to strengthen hippocampal-dependent memory consolidation or contextual variation protocols that enhance hippocampal discrimination between threat and safety contexts, may augment therapeutic outcomes in anxious youth. For example, protocols could incorporate deliberate context-switching during exposure sessions (e.g., alternating room locations, varying sensory

features) to strengthen hippocampal discrimination training, paired with brief post-session recall exercises to promote consolidation of safety memories. Such modifications would directly target the hippocampal instability patterns we observed in high-anxiety adolescents rather than relying solely on repeated exposure to reduce amygdala reactivity. Our findings also suggest that extinction requires progression through distinct network states, from initial conflict resolution to consolidated safety circuits. Therapeutic protocols that support this natural trajectory, allowing time for networks to transition from complex regulatory engagement to streamlined safety signaling, may be more effective than approaches optimizing initial exposures. Importantly, our sample was predominantly subclinical, suggesting these anxiety-related differences represent vulnerability markers that likely precede disorder onset rather than reflecting consequences of enduring psychopathology.

Limitations and future directions

Several limitations warrant consideration. We examined short-term extinction within a single session rather than assessing long-term extinction memory retrieval. Whether the connectivity patterns we identified predict extinction recall tested days later remains to be determined. Trace conditioning, while ecologically relevant and necessary for engaging hippocampal processes, introduces additional working memory demands that may interact with anxiety effects. Replication controlling for working memory abilities would increase generalizability across paradigms. Our cross-sectional design cannot determine whether the anxiety-related connectivity patterns we observed represent stable vulnerability traits, developmental lags that might resolve, or early manifestations of disorder onset. Longitudinal work tracking adolescents through potential disorder development would clarify whether these connectivity signatures predict future diagnosis. Similarly, whether the hippocampal instability we identified normalizes with successful treatment or represents a marker resistant to intervention remains unknown.

Conclusions

This study provides the first effective connectivity architecture of adolescent threat learning, revealing dynamic network reconfigurations that distinguish threat from safety learning and evolve across conditioning and extinction phases. The adolescent hippocampus demonstrated differential engagement for safety versus threat learning, with immediate inhibitory modulation during safety conditioning but delayed recruitment during threat learning. Network simplification from early to late extinction phases suggests that successful safety learning in adolescents is marked by efficient circuit resolution rather than sustained multi-pathway regulatory control. Critically, anxiety vulnerability involves disrupted directional signaling, with hippocampus to p-vmPFC connectivity shifting from excessive inhibition during conditioning to excitation during extinction. These findings identify hippocampal contextual gating, not solely prefrontal inhibitory control, as a key mechanism underlying anxiety risk. The identification of these temporally dynamic vulnerabilities has direct implications for developing targeted interventions that support the progression from conflicting circuit communication to circuit consolidation in adolescent anxiety treatment.

Methods

Participant information

One-hundred and thirteen participants completed the study (Mage=15.43, SD=1.18, Range=11.74-17.30 years; 71.68% female). Twenty-six participants were removed, 12 due to incomplete MRI data and 14 due to data quality issues related to motion artifacts (mean framewise displacement > 0.3 mm). This resulted in a final sample of 87 participants (Mage=15.66, SD=0.94, Range=11.82-17.30 years; 71.26% female). Consistent with epidemiological research indicating that anxiety disorders are approximately 1.7 times more

prevalent in females than males,[2,65] our sample was recruited with a bias toward more female participants (~2:1).[66]

Ethics

Ethics approval was granted through the Royal Children's Hospital Human Ethics Research Office (#1955580).

Inclusion criteria

Inclusion criteria were as follows: adequate comprehension of written and spoken English; living in Greater Melbourne or able to travel to Royal Children's Hospital; aged between 11-16 years old at the time of recruitment; consent provided by adolescent and parent/caregiver.

Exclusion criteria

Exclusion criteria were as follows: reported diagnosis of a developmental disorder; reported medical or neurological condition for which the adolescent participant was taking psychoactive medication; current or previous pregnancy; history of loss of consciousness for 5 minutes or more as the result of a head injury; magnetic resonance imaging contraindications (e.g., metal); hormone based contraceptive use; reported diagnosis of an endocrine disorder, premature adrenarche or precocious puberty, or reported use of hormonal medication; body mass index $<5^{th}$ or $>95^{th}$ percentiles; adolescents with siblings who previously participated in the study.

Experimental procedures

Participant data were collected as a combination of at-home tasks and in-person MRI scan visit to the Royal Children's Hospital, Melbourne, Australia. Participant questionnaire response data were collected using REDCap and Qualtrics software prior to the in-person appointment. At the in-person visit, adolescents were prepared for the MRI scan using a mock scanner. During the mock scan, adolescents lay supine in the practice MRI scanner and were

familiarized with the procedure, including receiving instructions for the fMRI tasks. Adolescents then participated in an MRI brain scan where they complete a structural scan, a resting state functional sequence, and fMRI tasks. At the end of the in-person visit, researchers conducted a debriefing interview with participants where adolescents were provided with a summary of their activities and mental health resources. Participants were reimbursed for their time and provided with a personalized 3D model of their brain.

Anxiety questionnaires

Anxiety was assessed using the State-Trait Anxiety Inventory–Trait scale (STAI-T). The STAI is a validated self-report inventory consisting of 20 items rated on a 4-point Likert scale.[67] The range for responses spans 20-80, with higher scores indicating greater anxiety.

fMRI paradigm

Adolescents were administered a threat conditioning task (**Fig. 6**) in the MRI scanner immediately after the structural scan. Participants underwent pre-conditioning / familiarization, conditioning and extinction learning phases. All faces presented in the task were female faces from the NimStim set.[62] During pre-conditioning, participants were presented with two female adult neutral faces (counterbalanced across participants) and played a scream sound through headphones. During conditioning, one of the two neutral faces (conditioned threat stimulus; CS+) was presented for 3 seconds followed by 2-4 second jittered trace period then a face with a fearful expression (1 s) and an aversive female scream (unconditioned stimulus; US, ~95-100 dB). The jittered interval was based on prior work [37,39,68] for optimal fMRI analyses and to reduce US habituation.[69] The CS+ was reinforced 100% with the US during conditioning based on prior trace conditioning studies.[37,70] The other face (conditioned safety stimulus; CS-) was presented for 3 seconds and was never paired with the US. CS+US trials and CS- trials were interleaved and presented 15 times each. The inter-trial interval was a white fixation cross on a black background, jittered for 8-

12 seconds. Extinction followed immediately, with presentation of 15 CS+ (3 seconds) trials in the absence of the US, interleaved with 15 CS- (3 seconds) trials.

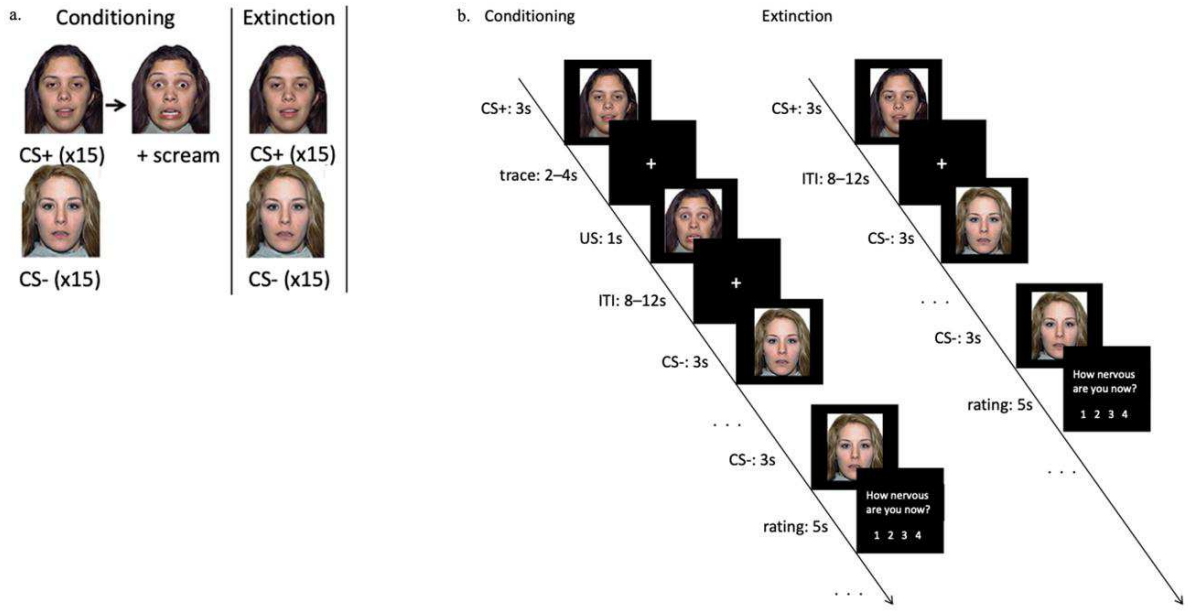


Figure 6. Threat conditioning and extinction task. (a) Overall schematic of the task phases. x15 refers to the number of stimuli presented during each task phase. **(b**) Sequence and timing of stimuli presented in different phases. Note: CS+ = conditioned stimulus paired with the US, CS- = conditioned stimulus never paired with the US, ITI = inter-trial-interval, s = seconds, US = unconditioned stimulus.

Imaging acquisition

Neuroimaging data was acquired on the 3T Siemens TIM Trio scanner (Siemens, Erlangen, Germany) at the Royal Children's Hospital. Participants lay supine with their head supported in a 32-channel head coil.

Functional data were collected with T2*-weighted echo planar imaging images with the following parameters: TR = 3000ms, TE = 40ms, flip angle = 85◦, field of view = 216 x 216 mm; producing 40 slices and 296 volumes. The total sequence was 14:48 for the threat

conditioning task. Auditory stimuli, including task instructions, were delivered through MRI-compatible headphones.

Adolescent participants also underwent a T1-weighted structural scan in the MRI scanner while they watched part of a film. A software upgrade occurred after the first 23 participants. This resulted in slightly different sequence parameters for the T1-weighted scan before versus after the upgrade (details below). Prior to the upgrade: T1-weighted 3D MPRAGE images were acquired with motion correction with the following parameters: repetition time = 2350ms; echo time1 = 2.18ms; flip angle = 8°; field of view = 187 x 256 x 256 mm;) producing 208 contiguous 0.9 mm thick slices (voxel dimensions = 0.9mm^3). Sequence duration 6:47 min. Since the upgrade: T1-weighted 3D MPRAGE images are acquired with the following parameters: repetition time = 2500ms; echo time1 = 1.72ms; flip angle = 8°; field of view = 187 x 240 x 256 mm; producing 208 contiguous 0.9 mm thick slices (voxel dimensions = 0.9mm^3). Sequence duration = 7:32 min.

Image preprocessing

Image pre-processing and analysis were conducted using Statistical Parametric Mapping (SPM) (development version SPM25, 00.00; The Wellcome Centre for Human Neuroimaging, London, UK; https://www.fil.ion.ucl.ac.uk/spm/) software implemented within a MATLAB environment (The Mathworks, Inc. MATLAB, Version 9.14.0, R2023a Update 1). Twenty-four standard and extended motion regressors were created for each participant using fMRIPrep (v23.2.1).[71] Participants were excluded if mean framewise displacement exceeded 0.3 mm. Each participant's T1-weighted image was skull-stripped and segmented; BOLD images were co-registered to the T1w image using boundary-based registration, and structural and BOLD images were normalized to MNI152NLin2009cAsym (2 mm) using ANTs, all as implemented in fMRIPrep. These functional images were smoothed with a 6-mm full-width-at-half-maximum (FWHM) Gaussian kernel. Smoothed

images were only used to inspect group-level activation. DCM analyses were conducted on unsmoothed images.

Pre-processed timeseries were entered into a first-level SPM GLM where image onsets (CS+ / CS- for early and late conditions, US, ITI, events of non-interest) were convolved with canonical hemodynamic response function and included in the model as the regressors of interest. Timeseries were high-pass filtered with a cut-off period of 128s to account for low-frequency noise. The primary contrast of interest at the first level was the direct comparison of threat (CS+) and safety (CS-) cues during conditioning and extinction. Following prior research, four task phases were examined: early conditioning (first 5 CS+ trials), late conditioning (last 5 CS+ trials), early extinction (first 5 CS- trials), and late extinction (last 5 CS- trials).[37,72,73] Contrast images were estimated for each participant and entered to a second-level random-effects GLM using one sample t-test, which was corrected for multiple comparisons using a whole-brain, voxel-wise false discovery rate (FDR) threshold ($p < .05$, $K_E \geq 10$).

Dynamic Causal Modeling analysis

DCM is a technique that estimates interactions between neural populations and estimates influence one neural population exerts on another (i.e., effective connectivity).[52] Unlike functional connectivity, which indicates statistical dependencies between temporally correlated activity, effective connectivity estimates the directionality of observed dependencies.[54] DCM uses generative models to predict how neural activity is transformed into observed hemodynamic response timeseries.[55] The strength of the connectivity parameters from these models are estimated through model inversion, a process of estimating the fit of the model generated timeseries with observed timeseries while penalizing the models for complexity.[74] A Bayesian model comparison framework is then used to evaluate the relative evidence to find the model architecture that offers the best trade-off between the

model fit and complexity. DCM enables inferences to be made about intrinsic connections (invariant to experimental changes) and modulation of these connections in response to experimental manipulation (presentation of CS+ or CS-). Model parameters are reported in Hz, which represent the rate of change in activity in one region due to the influence of another. Positive and negative connectivity parameters indicate excitatory and inhibitory influences of one region over another, respectively.

Regions of interest (ROIs)

Our model space consisted of three ROIs: the amygdala, hippocampus, and p-vmPFC. ROIs were restricted to larger regions defined using the Harvard-oxford subcortical atlas (amygdala and hippocampus) and the Harvard-oxford cortical atlas (p-vmPFC; subcallosal cortex).[24] Regions of interest (ROIs) were defined per participant by intersecting the thresholded SPM T-map for the Task condition (at a threshold of $p<.05$, uncorrected) with a priori anatomical masks. The principle eigenvariate from each ROI was extracted from the resulting suprathreshold voxels. All timeseries were adjusted using an F-contrast to mean-correct. For timeseries extraction and to specify conditions for our model space, a separate GLM (other than the one specified above) was estimated at the first level for eight conditions; CS+ during early conditioning, CS- during early conditioning, CS+ during early extinction, CS- during early extinction, CS+ during late conditioning, CS- during late conditioning, CS+ during late extinction, CS- during late extinction. Representative timeseries were extracted from each ROI at the participant level.[74]

Model specification

Our candidate model space was specified using DCM as implemented in SPM25. Intrinsic between-region connections within the model were defined bidirectionally between all regions (See **Fig. 1c**). The task driving input was modelled into all regions and each

combination of regions. The input matrix was mean-centered, therefore the intrinsic connectivity represented the average connectivity irrespective of experimental condition.

Model estimation and inference

The full model was fitted to each participant's time series (*spm_dcm_peb_fit*) to obtain posterior probability densities. The Parametric Empirical Bayes (PEB) framework was used to estimate the group-level connectivity parameters. PEB is a hierarchical modelling approach, which consists of participant specific DCMs at the first level and a GLM of connectivity parameters at the second level, which links participant specific connection strengths to the group. The posterior probability densities of the model parameters in each participant's DCM were then re-estimated by *spm_dcm_peb_fit* using the group average parameter estimates as their prior. Re-estimated participant level parameter estimates were then taken to the second level and modelled, dividing participant-level variance into shared group-level effects and additive random effects (*spm_dcm_peb*). Following PEB model estimation, Bayesian model reduction (BMR) was used to remove parameters that did not contribute to the model evidence (*spm_dcm_peb_bmc*). A Bayesian model average (BMA) was then calculated from these reduced models to obtain parameter estimates. Posterior probabilities of (PP)>0.95 were used to identify parameters with strong supporting evidence.

Data availability

Science Data Bank, https://doi.org/10.57760/sciencedb.31785

Code availability

https://github.com/ANDLabUniMelb/PANDA_fear_conditioning

CRediT Authorship Contribution Statement

Sarah M. Tashjian: Conceptualization, Methodology, Validation, Resources, Writing- Original Draft, Writing- Reviewing and Editing, Visualization, Supervision.

Joseph Cussen: Methodology, Software, Validation, Formal analysis, Resources, Writing- Reviewing and Editing, Visualization.

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Orli S. Schwarz: Investigation, Writing- Reviewing and Editing.

Julian G. Simmons: Investigation, Writing- Reviewing and Editing.

Isabel S. Zwaan: Investigation, Writing- Reviewing and Editing.

Sarah Whittle: Conceptualization, Investigation, Data curation, Writing- Reviewing and Editing, Supervision, Project administration, Funding acquisition.

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