

Negative overgeneralization is associated with anxiety and mechanisms of pattern completion in peripubertal youth

Dana L. McMakin,^{1,2,3} Adam Kimbler,¹ Nicholas J. Tustison,⁴ Jeremy W. Pettit,^{2,3} and Aaron T. Mattfeld^{1,3}

¹Cognitive Neuroscience Program, Department of Psychology, Florida International University, Miami, FL 33199, USA

²Clinical Science Program, Department of Psychology, Florida International University, Miami, FL 33199, USA

³Center for Children and Families, Florida International University, Miami, FL 33199, USA

⁴Department of Radiology and Medical Imaging, University of Virginia, Charlottesville, VA 22903, USA

Correspondence should be addressed to Aaron T. Mattfeld, Department of Psychology, Florida International University, 11200 SW 8th Street, AHC4-462, Miami, FL 33199, USA. E-mail: amattfel@fiu.edu.

Abstract

This study examines neural mechanisms of negative overgeneralization, the increased likelihood of generalizing negative information, in peri-puberty. Theories suggest that weak pattern separation [overlapping representations are made distinct, indexed by dentate gyrus/ cornu ammonis (CA)3 hippocampal subfield activation] underlies negative overgeneralization. We alternatively propose that neuro-maturational changes that favor pattern completion (cues reinstate stored representations, indexed by CA1 activation) are modulated by circuitry involved in emotional responding [amygdala, medial prefrontal cortices (mPFC)] to drive negative overgeneralization. Youth ($n = 34$, 9–14 years) recruited from community and clinic settings participated in an emotional mnemonic similarity task while undergoing magnetic resonance imaging. At study, participants indicated the valence of images; at test, participants made recognition memory judgments. Critical lure stimuli, which were similar to images at study, were presented at test, and errors ('false alarms') to negative relative to neutral stimuli reflected negative overgeneralization. Negative overgeneralization was related to greater and more similar patterns of activation in CA1 and both dorsal mPFC (dmPFC) and ventral mPFC (vmPFC) for negative relative to neutral stimuli. At study, amygdala exhibited greater functional coupling with CA1 and dmPFC during negative items that were later generalized. Negative overgeneralization is rooted in amygdala and mPFC modulation at encoding and pattern completion at retrieval.

Key words: anxiety; pattern completion; generalization; hippocampus; mPFC; fMRI

Introduction

Negative overgeneralization is a core feature of anxiety when responses to one aversive situation (e.g. severe weather) spread to non-aversive situations that share stimulus features (e.g. breezy day). A similar term, threat generalization, has been studied extensively using classical conditioning paradigms wherein behavior is characterized by shallow response decay gradients—conditioned responses are evoked by stimuli that are increasingly dissimilar from the conditioned stimulus (Greenberg et al., 2013a; Dymond et al., 2015; Laufer et al., 2016).

Both negative overgeneralization and threat generalization can theoretically involve either weak pattern separation (a neuro-computational process by which overlapping or similar representations are made distinct) leading to poor behavioral discrimination between threat and safety cues/contexts or strong pattern completion (a neuro-computational process by which a partial match of cues can reinstate previously stored

representations) leading to overactive behavioral generalization from threat to safety cues and contexts. There has been a strong focus on pattern separation as a key driver of poor discrimination, with some empirical support in adult populations (for review, see Leal and Yassa, 2018). However, neuro-maturational changes during the transition from childhood to adolescence (peri-puberty) potentiate neural mechanisms (e.g. hippocampal subregions and amygdala) that normatively favor pattern completion (e.g. Lavenex and Banta Lavenex, 2013; Lee et al., 2014; Keresztes et al., 2017) and therefore could drive negative overgeneralization among vulnerable youth. Ultimately, these mechanisms may prove to reflect etiological roots of anxiety that immediately precede the known escalation of symptoms across adolescence (Beesdo et al., 2009).

We draw specifically on three lines of converging evidence to support and test our theory that pattern completion and related hippocampal and modulatory neural mechanisms drive negative

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overgeneralization in peri-puberty. First, normative developmental changes enhance pattern completion and generalization, relative to separation and discrimination, in peri-puberty. Young children have a strong tendency to generalize information to allow for rapid learning and broad threat and reward detection (Hudson *et al.*, 1992; Bauer *et al.*, 2000; Ramsaran *et al.*, 2019). As children transition through adolescence, generalization is increasingly complemented by precision in the ability to discriminate from among similar events (Price and Goodman, 1990; Farrar and Goodman, 1992; Ramsaran *et al.*, 2019). These behavioral changes coincide with neuro-maturational changes in hippocampal subfields that contribute to pattern completion and separation. The cornu ammonis (CA)3 subfield heavily supports pattern completion, while the dentate gyrus (DG) supports pattern separation (Marr, 1971; Yassa and Stark, 2011). In human functional Magnetic resonance imaging (fMRI) studies, activations in CA3/DG subregions are combined to reflect pattern separation, while activations in CA1, which receives input from CA3, reflect pattern completion (Bakker *et al.*, 2008). The CA1 develops earlier, while the DG/CA3 exhibits a protracted developmental trajectory (Lavenex and Banta Lavenex, 2013) and recent studies suggest that changes in DG/CA3 volume are associated with the developmental shift from generalization to discrimination from late childhood through early adulthood (Lavenex and Banta Lavenex, 2013; Lee *et al.*, 2014; Keresztes *et al.*, 2017). However, whether or not a relative strength in pattern completion at the neuromaturational inflection point of peri-puberty contributes to overgeneralization in youth with, or at risk for, anxiety is unknown.

Second, neural regions that modulate hippocampal function [e.g. amygdala, medial prefrontal cortices (mPFC)] also evidence protracted development in ways that may further tip systems toward pattern completion of emotional stimuli. Amygdala-based emotional arousal has been shown to enhance the creation of long-lasting memories (McGaugh, 2013) and increases in generalization are reliably identified following negative (Schechtman *et al.*, 2010), aversive (Resnik *et al.*, 2011) or threatening (Starita *et al.*, 2019) events. Moreover, lesions of the amygdala result in selective deficits of gist but not detailed memories (Adolphs *et al.*, 2005). Protracted maturation of the amygdala and related modulatory circuitry leads to a greater reactivity of amygdala in peri-puberty, relative to mid- to late- adolescence and adulthood (Silvers *et al.*, 2017), which could amplify tendencies toward generalization. The mPFC also undergoes protracted development and exercises control over the specificity of hippocampal memories (Xu and Südhof, 2013) and can both potentiate [dorsal mPFC (dmPFC)] and inhibit [ventral mPFC (vmPFC)] fear memory (Giustino and Maren, 2015; Spalding, 2018)—both have been implicated in negative generalization during fear conditioning (e.g. Antoniadis and McDonald, 2006; Cullen *et al.*, 2015; Dymond *et al.*, 2015; Greenberg *et al.*, 2013a, 2013b; Laufer *et al.*, 2016; Lissek *et al.*, 2014; Onat and Büchel, 2015; Zelikowsky *et al.*, 2014). Taken together, these maturational changes could lead to a mnemonic system that is readily influenced by emotional arousal and shows a tendency toward generalization.

Finally, youth with anxiety evidence differences in generalization and related neural mechanisms in ways that could lead to a maturational inflection point and contribute to worsening symptoms. For example, a recent study demonstrated a wider generalization gradient for anxious youth in response to a conditioning task where a loss or gain was associated with well-separated tones and a tone probe was subsequently used to assess generalization curves (Laufer *et al.*, 2016). Moreover, this study demonstrated

normative elevation in generalization among the child group, relative to adolescents, but in youth with anxiety the pattern was reversed. This reversal may suggest a neuro-maturational inflection point for negative overgeneralization that uniquely affects vulnerable youth. Although there are no available neuroimaging studies of negative overgeneralization in youth, it is known that youth with anxiety show greater activation of the amygdala and reduced connectivity with vmPFC in response to threatening or ambiguously threatening stimuli (Forbes *et al.*, 2011) that could amplify maturational tendencies toward pattern completion and negative overgeneralization during this sensitive period.

This study aims to examine neural mechanisms of negative overgeneralization in peripubertal youth (ages 9–14 years)—a key developmental window when mechanisms related to memory specificity and escalation of internalizing symptoms take root. We sample youth, across a continuum of anxiety symptoms to maximize variability in negative overgeneralization. We use a declarative memory paradigm with high-resolution fMRI that is well established for distinguishing behavioral generalization and discrimination and for visualizing hippocampal subfields associated with pattern completion and separation. We hypothesize that negative overgeneralization will be associated with neural mechanisms of pattern completion (greater CA1 activation) and modulatory effects of amygdala and mPFC and that anxiety severity will be associated with greater negative overgeneralization in terms of behavior and activation in proposed neural mechanisms.

Methods

Participants

Volunteers, ages 9–14 years, were recruited from anxiety clinic referrals and the community and paid for their participation. The local institutional review board approved study procedures and participants completed informed consent and assent. Participants were screened for major medical and psychiatric exclusionary comorbidities (e.g. current depressive episode, bipolar disorder, post-traumatic stress disorder, attention-deficit/hyperactivity disorder, conduct disorder, oppositional defiant disorder, psychotic disorders and obsessive compulsive disorder). Forty-eight participants met eligibility requirements. All participants were right-handed and had normal or corrected to normal vision. Participants were dropped from final analyses due to errors in data collection at either study or test ($n = 4$), excessive motion ($n = 2$; >20% of trials were flagged for removal), failed to show up for their scheduled study or test session ($n = 2$) or poor task performance ($n = 6$; hit rate for targets was 1.5 s.d. below the average performance), leaving $n = 34$ (11.4 ± 2.0 years, 16 female) for the final sample. Anxiety severity was assessed using the Pediatric Anxiety Rating Scale (PARS-6; The Pediatric Anxiety Rating Scale (PARS), 2002). Pubertal development was assessed using the Pubertal Developmental Scale, a reliable and valid self-report measure (Petersen *et al.*, 1988).

Emotional memory task procedures

The emotional mnemonic similarity task consisted of two phases: study and test (Figure 1A). During study, participants viewed (2s) emotional and non-emotional scenes. The same set of images was presented to all participants in a randomized order. Images were obtained from the authors of a study investigating the effect of emotion on mnemonic interference in aging (Leal *et al.*, 2014). In the original study, valence, arousal and similarity were independently assessed. Emotional images were more arousing than neutral, and negative was more arousing than positive

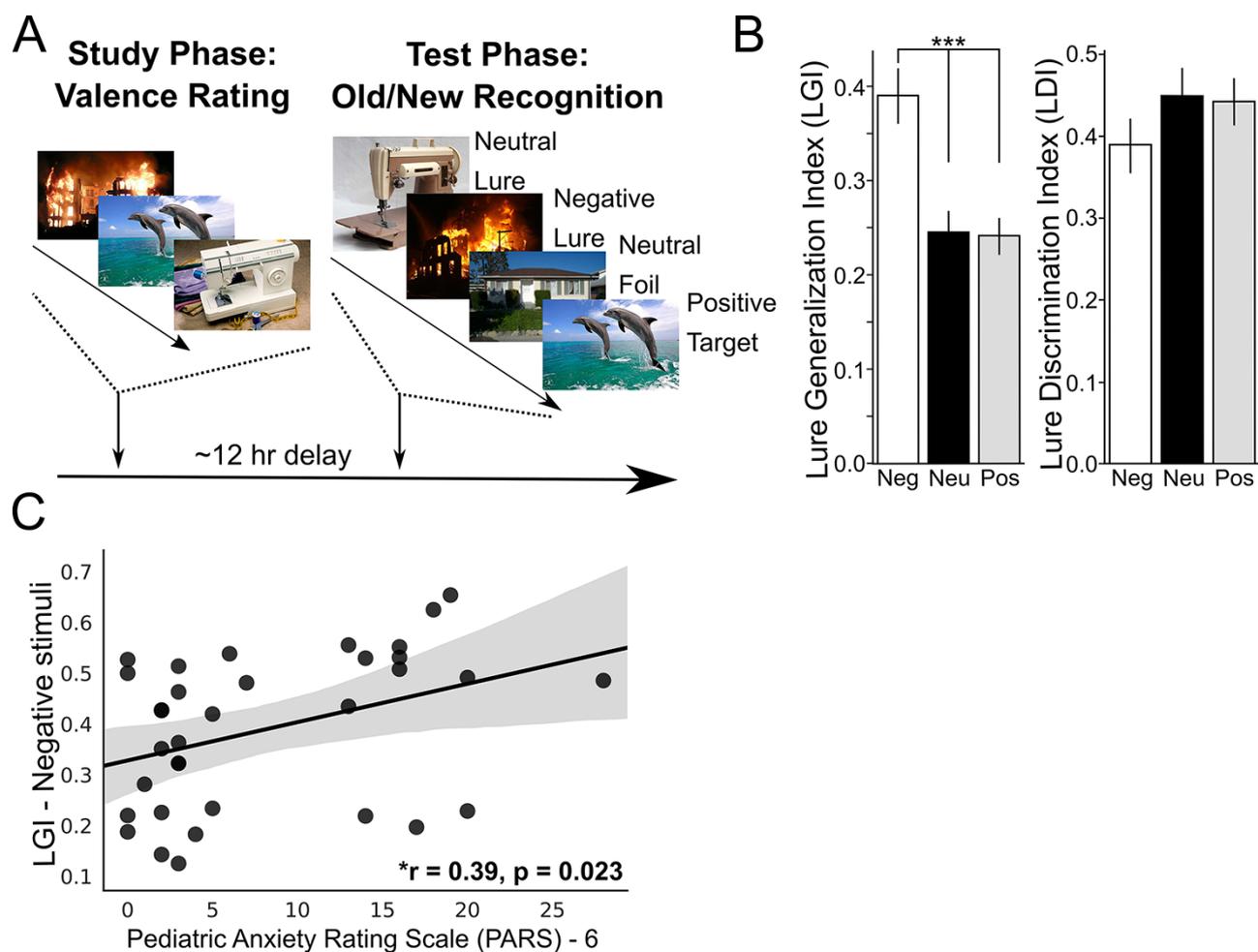


Fig. 1. Greater FAs to negative lures in peripubertal youth. Timeline of experimental procedures and lure behavioral performance. (A) The scanning portion of the experiment consisted of two phases: 1) A study phase during which participants rated the valence of each image. Images were presented for 2 s and were separated by a jittered inter-stimulus-interval (ISI: white fixation cross; 2–6 s). 2) During the test phase participants performed an old/new recognition memory task. Target (images from the Study phase), foil (new pictures) and lure (new images that were similar but not exactly the same as images from the Study phase) stimuli were presented for 2 s and separated by a jittered ISI (2–6 s). (B) The LGI: $p(\text{'Old'|Lure}) - p(\text{'Old'|Foil})$ —was significantly greater for negative compared to neutral [$t(33) = 5.07, P = 1.4 \times 10^{-5}$] and positive [$t(33) = 6.44, P = 2.6 \times 10^{-7}$] stimuli. A similar difference was not observed (all $P > 0.10$) for the LDI: $p(\text{'New'|Lure}) - p(\text{'New'|Target})$. Data are represented as a mean \pm SEM. (C) Negative generalization (Negative LGI) was positively associated with anxiety severity (PARS-6) ($r = 0.39, P = 0.023$; $\rho = 0.29, P = 0.09$). Neg = Negative; Neu = Neutral; Pos = Positive; *** $P < 0.0001$.

(Leal et al., 2014). Participants were instructed to indicate the valence (negative, neutral or positive) of each image with one of three button responses. Each scene was followed by a white fixation cross in the middle of a black screen of variable duration (2–6 s) before the onset of the next trial. At study, participants saw 145 stimuli (48 negative, 47 neutral and 50 positive) divided between two scanning runs lasting ~ 8 min. Participants returned to the scanner after a 12 h delay for test, at which point they were given a surprise memory test. Participants were presented with scenes that were viewed at study (targets), scenes that were similar but not identical to the images presented at study (lures), and completely new scenes (foils). Each scene was presented in the middle of the screen for 2 s, followed by a variable inter-stimulus interval (2–6 s) during which a white fixation cross was presented in the middle of a black screen. Participants were instructed to indicate whether items were 'old' or 'new' by pressing one of two buttons. The instructions indicated that for an item to be endorsed as 'old', it had to be the exact same image that was presented at study. A total of 284 stimuli were presented at test: 16 each negative, neutral and positive targets; 32 negative, 33 neutral

and 33 positive lures; and 42 negative, 49 neutral and 48 positive foils.

Neuroimaging data collection and preprocessing

Neuroimaging

Neuroimaging data were collected on a 3 T Siemens MAGNETOM Prisma scanner with a 32-channel head coil at the Center for Imaging Science at Florida International University. A T2*-weighted Echo Planar Imaging (EPI) sequence (Repetition Time (TR) = 993 ms, Echo Time (TE) = 30 ms, flip angle = 52°, Field-of-View (FOV) = 216 mm, 56 axial slices, slice acceleration = 4, voxel size = 2.4 mm isotropic) in addition to a T1-weighted magnetization-prepared rapid gradient echo sequence (MPRAGE: Repetition Time (TR) = 2500 ms, Echo Time (TE) = 2.9 ms, flip angle = 8°, Field-of-View (FOV) = 256 mm, 176 sagittal slices, voxel size = 1 mm isotropic) were collected. During each run of the emotional memory task acquisition began after the first four volumes were collected to allow for T1-equilibration.

Standard preprocessing procedures were implemented including motion correction, co-registration, outlier identification,

spatial smoothing and normalization using the following software packages scripted in a Neuroimaging in Python (Nipype version 0.12.1; Gorgolewski et al., 2011) pipeline: Analysis of Functional Neuroimages (AFNI version 16.3.18 Cox, 1996), FMRIB Software Library (FSL version 5.0.10; Smith et al., 2004), FreeSurfer (version 6.0.0; Fischl, 2012) and Advanced Normalization Tools (ANTs version 2.1.0, Avants et al., 2008). See Supplementary Materials for a detailed description of the preprocessing steps.

Behavioral data analysis

To assess generalization, the primary behavioral outcome measure for the emotional memory task was the lure generalization index (LGI). The LGI score provided a bias-corrected measure of how likely participants were to false alarm (FA) to similar lure items. LGI was defined as $p(\text{'Old'|Lure}) - p(\text{'Old'|Foil})$, correcting for a general tendency to call new stimuli 'Old'. Similar corrections have been used in prior work (Lacy et al., 2010; Yassa et al., 2010; Yassa and Stark, 2011). Similarly, to assess valence related changes to discrimination, we calculated the lure discrimination index [LDI: $p(\text{'New'|Lure}) - p(\text{'New'|Target})$]. The LDI score corrected for the propensity of participants to endorse items as new. LGI and LDI scores were calculated for each valence separately. Pairwise comparisons were performed across the different stimulus valences for both the LGI and LDI scores. The relation between anxiety severity (PARS-6) and negative generalization indexed by negative LGI was explored using both Pearson's and Spearman's rank correlation. A Bonferroni correction was used to account for Type I error inflation following multiple comparisons.

MRI data analysis

Anatomical regions of interest

Our study was motivated to understand the contributions of *a priori* anatomical ROIs to negative generalization: CA1 subfield of the hippocampus, CA3/DG combined subfield of the hippocampus, amygdala and dmPFC/vmPFC. Cortical and amygdala ROIs were obtained from FreeSurfer's *aparc + aseg.mgz* file. Hippocampal subfield ROIs were generated following a consensus labeling approach. For details on anatomical ROI generation and hippocampal subfield segmentation see the Supplementary Materials. All masks were back-projected to functional space using the inverse of the co-registration transformation for data analyses.

Task neuroimaging data analysis

Functional neuroimaging data were analyzed using a general linear model approach in FSL. Separate models were created for the study and test data to evaluate the neurobiological correlates of negative generalization. Both the study and test models included the following regressors of no interest: motion (*x*, *y*, *z* translations; pitch, roll, yaw rotation), the first and second derivatives of the motion parameters, normalized motion, first through third-order Lagrange polynomials to account for low-frequency changes in the signal, as well as a regressor for each outlier timepoint that exceeded outlier thresholds. The test model included 18 regressors of interest: lure FAs, lure correct rejections (CRs), target hits, target misses, foil CRs and foil FAs. All regressors were separately modeled for the three different valences (negative, neutral and positive). The study model included a similar combination of regressors; however, these were scenes that would subsequently be target hits and misses or be replaced by similar

lures and subsequently correctly rejected or false alarmed. To investigate signals related to negative generalization, we focused our analyses on negative compared to neutral lure trials that were (subsequently) false alarmed.

Task-based functional connectivity analysis

We used a beta-series correlation analysis (Rissman et al., 2004) to assess the functional interactions between the amygdala and the CA1, dmPFC and vmPFC during encoding for negative and neutral stimuli that were subsequently replaced by lures and either false alarmed or correctly rejected. We followed a least-square single approach (Mumford et al., 2012). Correlations between regions of interest were calculated. The arctangent of the resulting Pearson's correlation coefficients was calculated and pair-wise comparisons were made. See Supplementary Materials for a detailed description of our approach.

Representational similarity analysis

Neurobiological correlates of generalization may arise as similar/dissimilar patterns of activation. To evaluate this possibility, we performed a representational similarity analysis (RSA) using the bilateral CA1 subfield of the hippocampus and dmPFC/vmPFC as our anatomical regions of interest. The original test phase model was re-run; however, this time using un-spatially smoothed functional data—as fine differences in activation patterns were the goal of the RSA analysis. Correlations were calculated between patterns of activation across all voxels in our *a priori* anatomical regions of interest for target hits and lure FAs for each valence as our neural measures of representational similarity. Large values indicated similar patterns of activation and small values related to dissimilar patterns of activation between target hits and lure FAs. To evaluate the relation between behavioral generalization and neural measures of pattern similarity, correlations were performed between negative LGI and our neural measures of representational similarity across separate regions of interest. Bonferroni corrections were used to correct for inflated Type I error due to multiple comparisons.

Results

Greater FAs to negative lures in peripubertal youth

To examine negative overgeneralization behaviorally, we compared LGI between negative, neutral and positive stimuli. Generalization was significantly greater for negative compared to both neutral [$t(33) = 5.07$, $P = 1.4 \times 10^{-5}$] and positive [$t(33) = 6.44$, $P = 2.6 \times 10^{-7}$] stimuli, which were not significantly different from one another [$t(33) = -0.16$, $P = 0.87$] (Figure 1B). The same comparisons were performed using LDI. No significant differences in discrimination were evident when comparing the different stimulus valences (LDI_{Neg v. Neu}: $t(33) = -1.56$, $P = 0.12$; LDI_{Neg v. Pos}: $t(33) = -1.66$, $P = 0.106$; LDI_{Pos v. Neu}: $t(33) = -0.19$, $P = 0.85$). To evaluate the relationship between negative overgeneralization and anxiety, we correlated negative LGI with PARS-6. Participants with greater anxiety severity were more likely to generalize negative information ($r = 0.39$, $P = 0.023$) (Figure 1C). Noting the non-normal distribution of our anxiety severity measure (PARS-6), we re-ran this analysis using a Spearman's rank correlation and identified a similar yet only trending association between greater anxiety and negative generalization ($\rho = 0.29$, $P = 0.09$). Percentage of hits, misses, correct rejections and FAs by valence and stimulus type can be found in Table 1.

Table 1. Percentage of hits, misses, correct rejections and FAs by valence and stimulus

| Trial type | Mean(%) | s.d.(%) | Range(%) |
|---------------|---------|---------|----------|
| Negative | | | |
| Target hits | 81.9 | 21.1 | 6.3–100 |
| Target misses | 18.1 | 21.1 | 0–93.7 |
| Lure CR | 52.5 | 19.2 | 0–93.3 |
| Lure FA | 47.5 | 19.2 | 6.6–100 |
| Foil CR | 87.1 | 21.0 | 0–100 |
| Foil FA | 12.9 | 21.0 | 0–100 |
| Neutral | | | |
| Target hits | 74.4 | 24.4 | 7.1–100 |
| Target misses | 25.6 | 24.4 | 0–92.8 |
| Lure CR | 67.3 | 18.8 | 20–100 |
| Lure FA | 32.7 | 18.8 | 0–80 |
| Foil CR | 87.4 | 16.5 | 16–100 |
| Foil FA | 12.6 | 17.8 | 0–83.6 |
| Positive | | | |
| Target hits | 74.6 | 21.0 | 0–100 |
| Target misses | 25.4 | 21.0 | 0–100 |
| Lure CR | 64.1 | 14.0 | 30–100 |
| Lure FA | 35.9 | 14.0 | 0–70 |
| Foil CR | 86.7 | 16.5 | 16–100 |
| Foil FA | 13.3 | 16.5 | 0–83.3 |

MRI results

Negative generalization is associated with greater activation in the mPFC and CA1

To investigate neurobiological mechanisms supporting negative overgeneralization, we examined differences in activations for negative relative to neutral lure stimuli that were incorrectly identified as old in anatomically defined ROIs. We found greater activation in the CA1 for negative compared to neutral lures [$t(33) = 3.51$, $P = 0.001$] (Figure 2A). Activations between negative and neutral lures in the DG/CA3 did not significantly differ following corrections for multiple comparisons but exhibited a trend toward greater activation for negative relative to neutral items [$t(33) = 2.29$, $P = 0.03$], in the opposite direction predicted based on a failure to pattern separate (Figure 2B). Both the dmPFC

[$t(33) = 2.74$, $P = 0.009$] and vmPFC [$t(33) = 3.24$, $P = 0.002$] exhibited greater activation for negative when compared to neutral lures (Figure 2C, D).

To further evaluate whether negative overgeneralization was a result of impaired discrimination resulting from poor pattern separation, we compared activations in the same regions for negative and neutral lure stimuli that were identified as ‘New’ (e.g. CR). Amongst ROIs no regions exhibited a significant difference between negative and neutral lures following corrections for multiple comparisons [CA1: $t(33) = 2.32$, $P = 0.03$; DG/CA3: $t(33) = 2.50$, $P = 0.02$; vmPFC: $t(33) = 0.29$, $P = 0.76$; dmPFC: $t(33) = 0.39$, $P = 0.69$] (Supplemental Figure S1). In fact, similar to FAs in the DG/CA3, there was a trend toward greater activation in the CA1 and DG/CA3 for negative relative to neutral lures—opposite the predicted difference based on an assumption of impaired pattern separation.

A subcortical and cortical network contribute to negative overgeneralization

To evaluate the contribution of other brain areas to negative overgeneralization outside our anatomical ROIs, we performed an exploratory whole-brain analysis. We compared negative to neutral lure FAs. Similar to our anatomical ROI approach, clusters in the hippocampus survived corrections for multiple comparisons. Specifically, 29 voxels in the right CA1, 376 voxels in the left CA1, 238 voxels in the left subiculum and 184 voxels in the left DG/CA3. We also observed clusters in the bilateral amygdala and midline thalamus. Clusters in the bilateral mPFC, posterior cingulate cortex extending into the precuneus, inferior parietal lobule and middle temporal gyrus were observed (Figure 3).

Enhanced functional coupling with amygdala during encoding

We next assessed the functional correlations between amygdala and target regions at Study. We observed enhanced functional coupling between the amygdala and both the CA1 [amygdala ↔ CA1: $t(33) = 2.9$, $P = 0.007$] and the dmPFC [amygdala ↔ dmPFC: $t(33) = 2.6$, $P = 0.013$] for negative stimuli compared to neutral stimuli that were subsequently FA but only a trend for

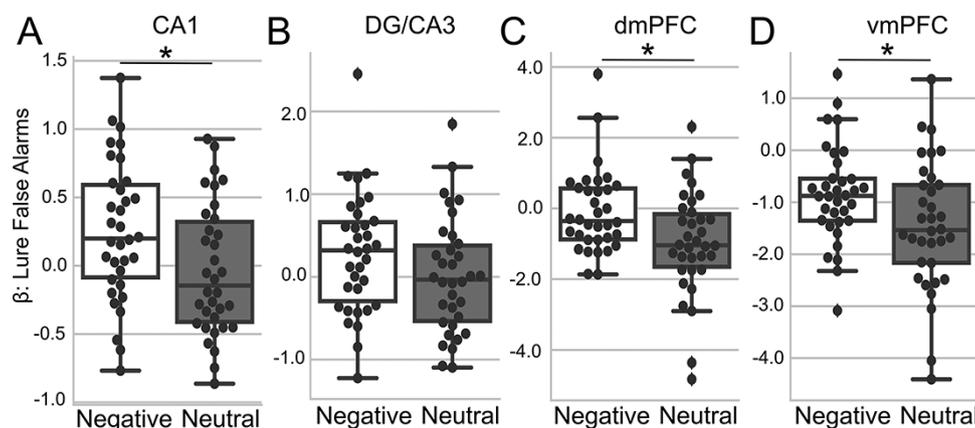


Fig. 2. Negative generalization is associated with greater activation in the CA1 and mPFC. Anatomical region-of-interest analysis showing hippocampal subfield (CA1 and DG/CA3) and both dmPFC and vmPFC activations. (A) The CA1 hippocampal subfield ($P = 0.001$) exhibited greater activations for negative lure FAs relative to neutral lure FAs. (B) The DG/CA3 subfields ($P = 0.03$) exhibited a trend toward greater activations for negative lure FAs relative to neutral lure FAs. (C, D) Both the dmPFC ($P = 0.009$) and the vmPFC ($P = 0.002$) showed greater activations for negative compared to neutral lure FAs. Boxplots reflect median and interquartile range. Error bars reflect maximum and minimum range. *Significant following Bonferroni correction for multiple comparisons.

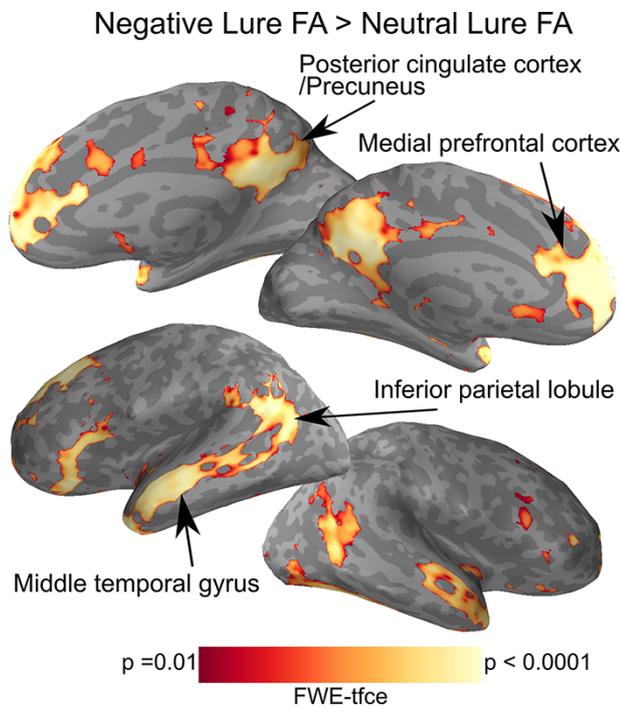


Fig. 3. A subcortical and cortical network contribute to negative overgeneralization. Whole brain exploratory analysis showing regions that exhibited greater activations for negative relative to neutral lure FA in the posterior cingulate cortex extending into the precuneus, medial prefrontal cortex, inferior parietal lobule and middle temporal gyrus. FWE-tfce corrected $P < 0.01$.

the amygdala and the vmPFC [amygdala \leftrightarrow vmPFC: $t(33) = 1.6$, $P = 0.13$] (Figure 4). No significant difference in functional connectivity between the same regions was observed when comparing negative and neutral stimuli that were subsequently CR [amygdala \leftrightarrow CA1: $t(33) = 1.03$, $P = 0.32$; amygdala \leftrightarrow dmPFC: $t(33) = -0.46$, $P = 0.64$; amygdala \leftrightarrow vmPFC: $t(33) = -0.76$, $P = 0.45$] (Supplemental Figure S2).

Greater representational similarity in the mPFC and left CA1 associated with enhanced negative overgeneralization

To evaluate the relationship between patterns of brain activation and our behavioral indices of generalization (i.e. LGI), we utilized RSA to examine the correlation between target hit and lure FA similarity and LGI scores. Negative LGI was elevated as both vmPFC ($r = 0.53$, $P = 0.001$) and dmPFC ($r = 0.44$, $P = 0.009$) similarities between negative target hits and lure FA were greater (i.e. patterns of activation were more similar) (Figure 5A, B). A similar pattern was observed in the left CA1 ($r = 0.46$, $P = 0.006$) (Figure 5C)—negative LGI was greater as similarity in the left CA1 was elevated—but not in the right CA1 ($r = 0.08$, $P = 0.65$) (Figure 5D).

Both lure FAs and target hits were endorsed with an ‘old’ response; thus, the similarity in activations may be related to the same responses across the two conditions. To evaluate this confound, we performed the same analyses using positive and neutral stimuli. No significant relationship between pattern similarity and behavior, despite the same response type, was observed for positive stimuli (left CA1: $r = -0.015$, $P = 0.93$; right CA1: $r = -0.16$, $P = 0.35$; vmPFC: $r = 0.09$, $P = 0.59$; dmPFC: $r = 0.19$, $P = 0.27$) (Supplemental Figure S3), and only a nonsignificant trend for neutral stimuli was observed (left CA1: $r = 0.40$,

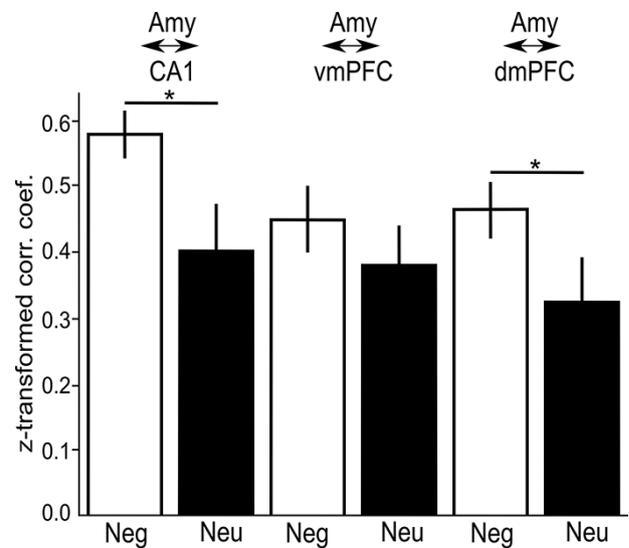


Fig. 4. Enhanced functional coupling with amygdala during study. Task-based functional connectivity during the study phase between the amygdala (Amy) and three target regions: CA1, vmPFC and dmPFC for items that were subsequently replaced by lures and false alarmed. Greater functional connectivity was observed between the Amy \leftrightarrow CA1 ($P = 0.007$) and the Amy \leftrightarrow dmPFC ($P = 0.013$) for negative (Neg) relative to neutral (Neu) stimuli that were replaced by similar lure stimuli and subsequently false alarmed. Data are represented as a mean \pm SEM. *Significant following Bonferroni correction for multiple comparisons.

$P = 0.018$; right CA1: $r = 0.30$, $P = 0.08$; vmPFC: $r = 0.29$, $P = 0.09$; dmPFC: $r = 0.13$, $P = 0.46$; Supplemental Figure S4).

Relationship between anxiety severity and brain measures

Lastly, we explored the relationship between the identified neurobiological mechanisms of negative overgeneralization and anxiety symptom severity. No significant relationship between anxious symptoms (e.g. PARS-6) and univariate activations amongst our ROIs was evident (CA1: $r = 0.04$, $P = 0.84$; vmPFC: $r = 0.18$, $P = 0.32$; dmPFC: $r = -0.03$, $P = 0.87$). Functional connectivity during the Study phase between the amygdala \leftrightarrow CA1 and the amygdala \leftrightarrow dmPFC for negative items that were subsequently false alarmed exhibited a trend, whereby greater connectivity was associated with more severe symptoms of anxiety ($r_{AMY \leftrightarrow CA1} = 0.29$, $P = 0.09$; $r_{AMY \leftrightarrow dmPFC} = 0.35$, $P = 0.04$) (Supplemental Figure S5A). A similar trend in our multivariate measure was noted, wherein representational similarity in the left CA1 between negative target hits and negative lure FAs was elevated for participants with greater levels of anxiety ($r = 0.32$, $P = 0.06$) (Supplemental Figure S5B). This relationship was not evident in the vmPFC ($r = 0.02$, $P = 0.85$) or the dmPFC ($r = 0.44$, $P = 0.80$).

Discussion

Behavioral and neurobiological data from an emotional mnemonic similarity task converged to support the notion that negative overgeneralization during peri-puberty results from enhanced pattern completion. Specifically, results indicated greater behavioral generalization (e.g. LGI) and neural activation in CA1 and mPFC when generalizing negative relative to neutral lures. Neurobiological mechanisms of negative overgeneralization are modulated by interactions between the amygdala with target regions during encoding, as evidenced by greater

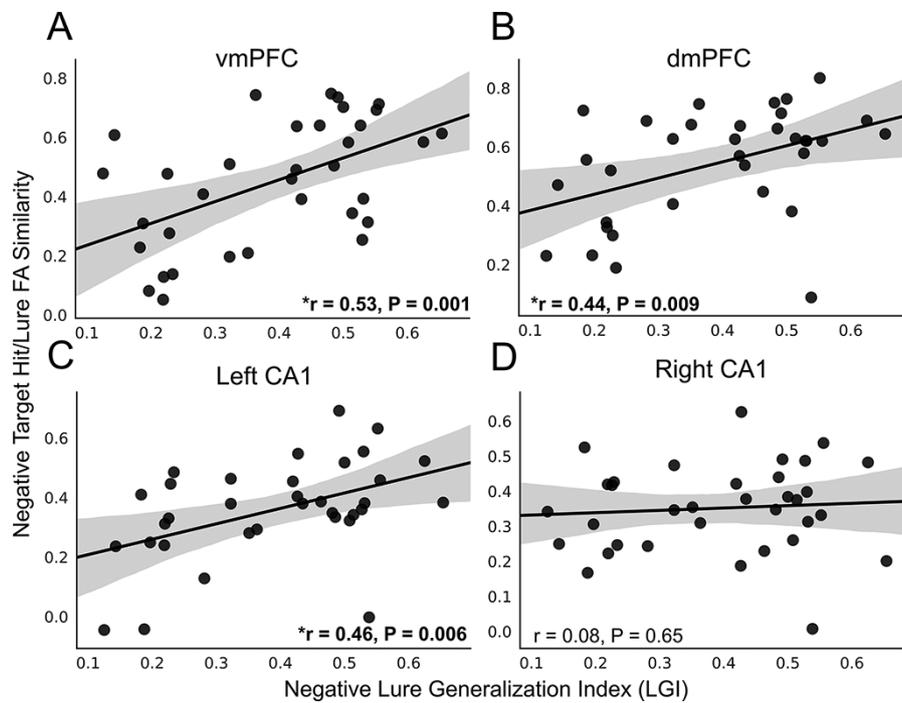


Fig. 5. Greater representational similarity in the mPFC and left CA1 associated with enhanced negative overgeneralization. Correlations between negative target hit and lure FA similarity in the vmPFC, dmPFC and bilateral CA1 and LGI behavioral performance. (A, B) Both the vmPFC ($P = 0.001$) and the dmPFC ($P = 0.008$) exhibited a significant correlation between negative target hit/lure FA similarity and negative LGI performance. As the similarity between target hit and lure FA activations was elevated (i.e. became more similar) negative LGI performance was greater. (C, D) A similar correlation was identified in the left CA1 ($P = 0.006$) but not the right CA1 ($P = 0.65$). *Significant following Bonferroni correction for multiple comparisons.

amygdala ↔ CA1 and dmPFC connectivity during the study phase for negative (relative to neutral) stimuli that were subsequently generalized. Representational similarity analyses also showed that more similar neural activation patterns in left CA1 and mPFC were associated with greater behavioral generalization for negative stimuli. Greater negative overgeneralization was associated with elevated anxiety; however, only a trend with neurobiological mechanisms was identified. Together, these results outline candidate neurobiological mechanisms underlying negative overgeneralization. Contributions from these neural circuits and increasing symptoms of anxiety during peri-puberty highlight the potential susceptibility and plasticity of these mechanisms during this developmental window, providing a substrate from which anxiety and related disorders could emerge or escalate.

Activations in *a priori* ROIs were associated with the computational process of pattern completion. Prior studies have identified the hippocampus as an important contributor to negative overgeneralization (Antoniadis and McDonald, 2006; Lissek et al., 2014; Zelikowsky et al., 2014; Baldi and Bucherelli, 2015; Cullen et al., 2015; Onat and Büchel, 2015); however, the contributions of hippocampal subfields have remained underspecified. Using high-resolution imaging, we observed greater activation in the CA1 for negative versus neutral lure FAs—consistent with generalization through pattern completion. The ‘generalization’ interpretation of CA1 activation in our study is in line with computational models that have proposed CA1 activity may generalize following partial or incomplete pattern completion in the CA3 (Rolls, 2007, 2013). In contrast, the DG/CA3, a subfield of the hippocampus that contributes to successful discrimination through pattern separation (Marr, 1971; Yassa and Stark, 2011), exhibited a trend toward greater activation for negative compared to

neutral lure FAs and correct rejections—a directionality that is inconsistent with predictions based on impairments in pattern separation.

Theoretical models have emphasized poor pattern separation and related deficits in behavioral discrimination in models of aging as well as both typical and clinical adult populations (Leal and Yassa, 2018). Our results challenge the transcendence of pattern separation, supporting the notion that neural mechanisms underlying the tendency to generalize or the failure to discriminate vary across developmental periods. The divergent mechanisms across development may be further complicated by pathology. For example, negative generalization is exacerbated in adolescents compared to children but only in those with a diagnosis of anxiety (El-Bar et al., 2017).

We also found support for involvement of mPFC in negative overgeneralization. However, both the dmPFC and the vmPFC exhibited greater activation for negative compared to neutral lure FAs suggesting that the functional distinction between these regions identified during aversive conditioning paradigms (Lissek et al., 2014; Baldi and Bucherelli, 2015; Dymond et al., 2015; Giustino and Maren, 2015; Onat and Büchel, 2015; Jasnow et al., 2017; Spalding, 2018) may not hold for more complex tasks and/or this developmental period. Similar conclusions have been drawn in rodent models using context fear conditioning paradigms (Antoniadis and McDonald, 2006; Baldi and Bucherelli, 2015; Cullen et al., 2015; Giustino and Maren, 2015; Jasnow et al., 2017). Negative overgeneralization was not limited to the CA1 and mPFC, rather a broad network including regions like the amygdala, posterior cingulate cortex/precuneus and midline thalamus also exhibited greater activations for negative relative to neutral lure stimuli that were false alarmed lending support to the hypothesis that a broad network governs negative overgeneralization.

Taken together, these findings stand in contrast to existing literature pointing to failures in pattern separation as a neuro-computational driver of negative overgeneralization. However, prior studies have been conducted primarily with rodent models, healthy adults, aged adults or adults with subclinical depressive symptoms (see [Leal and Yassa, 2018](#) for a review). Our sampling strategy focusing on peri-pubertal youth and a full range of anxiety symptoms to maximize variability in the construct of interest (i.e. negative overgeneralization), likely account for these differences.

During the study phase of our experiment, we observed greater functional correlations between the amygdala and both the CA1 and dmPFC for negative relative to neutral items that were subsequently replaced by a lure and false alarmed. These regions are important for generalization and fear potentiation ([Schafe et al., 2005](#); [Spalding, 2018](#)). Greater functional coupling for similar stimuli that were correctly rejected was not observed, discounting the possibility that the amygdala is more correlated with negative stimuli regardless of behavioral performance. These results support our theory that the amygdala at encoding biases neurobiological mechanisms toward generalization, consistent with prior studies of emotions preferentially enhancing gist rather than detailed memories ([Mather and Sutherland, 2011](#)).

As a complementary analytic approach, we used representational similarity analyses to investigate the contributions of the CA1 and mPFC. We found support for our hypothesis that negative overgeneralization is strongest when the representations of novel lure stimuli resemble the representations of previously encountered items. Consistent with this prediction, negative LGI was greater among individuals with highly similar patterns of activation between negative target hits and lure false alarms. These results are important given that both the hippocampus and mPFC play a role in memory integration and concept formation ([van Kesteren et al., 2012](#); [Schlichting and Preston, 2015](#); [Bowman and Zeithamova, 2018](#); [Mack et al., 2018](#)). Thus, the relation between the convergent patterns of brain activation and negative overgeneralization behaviorally may reflect mechanisms of pattern completion and memory integration, respectively.

Finally, our exploratory analyses of associations between neurobiological mechanisms of negative overgeneralization and anxiety severity evidenced either weak or absent effects. We believe there could be at least two explanations. First, anxiety disorders are highly heterogeneous, and although negative overgeneralization is one common symptom dimension, it is not directly assessed in the anxiety severity interview (PARS-6). Therefore, the summary score of anxiety severity may be diffuse, contributing to weak associations with neurobiological mechanisms of negative overgeneralization. Additionally, the sample size is likely underpowered to support multiple interactions across response systems. Future work could use measures specifically designed to assess the symptom dimension of negative overgeneralization in a larger sample in order to clarify symptom-mechanism associations.

Three limitations of the current study warrant attention. First, the design of the experiment precludes our ability to evaluate whether effects on negative overgeneralization were driven by recognition of previously encoded stimuli versus familiarity with those stimuli. The hippocampus is important for pattern separation and completion according to complementary learning systems models ([Norman, 2010](#)). How subjects use hippocampal memory signals to make recognition memory decisions is a step removed from the focus of the current analyses but an important topic of future research. Second, stimulus sets were

neither matched on arousal nor form and figure. Primary analyses examined negative relative to neutral images and are consistent with work that suggests emotional arousal plays an important role both behaviorally and neurobiologically ([Lin et al., 2020](#)). The null findings comparing positive to neutral LGI behaviorally may be a result of the lower arousal of the positive images, and therefore, specificity of results to negatively valenced stimuli requires further study. Lastly, the hippocampal segmentation atlas was originally created using an adult sample. Future work should aim to use developmentally matched atlases ([Schlichting et al., 2019](#)).

Despite these limitations, these data support a neurobiological mechanism underlying negative overgeneralization. Interactions between the amygdala and target regions during initial encoding contribute to the formation of gist like representations. At retrieval, enhanced univariate as well as convergence of multivariate patterns in activation in both the mPFC and CA1 reflect enhanced pattern completion. In this way interactions between the amygdala and mPFC and CA1 at encoding determine their contribution to negative overgeneralization at retrieval. These findings emphasize the importance of considering developmental stages when defining mechanisms of negative overgeneralization and can further detail working models of anxiety pathogenesis during the transition from childhood to adolescence. Findings such as these also carry implications for novel treatment development. For example, strategies that aim to improve the balance of pattern completion relative to separation processes in relation to emotional experiences in vulnerable youth could help to mitigate overgeneralization at this key maturational inflection point. Such strategies may include those that aim to reduce emotional reactivity and associated amygdala response to aversive experiences (i.e. in the context of exposure therapy) as a means to mitigate modulatory influences on pattern completion and generalization and/or strategies such as memory specificity training (thus far evaluated mostly in relation to adult depression; e.g. [Ahmadi Forooshani et al., 2020](#)) to reduce strong completion tendencies and related overgeneralization of past negative memories. The neural mechanisms of negative overgeneralization in peripubertal youth that were identified in this study provide defined mechanistic targets for developing and refining such treatment strategies from an experimental therapeutics framework.

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Conflict of interest

The author declared that they had no conflict of interest with respect to their authorship or the publication of this article.

Supplementary data

Supplementary data are available at SCAN online.

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