STRENGTH OF FAMILY BOND PREDICTS DISRUPTED NEURAL HABITUATION TO VISUAL FAMILIAL THREAT IN ADOLESCENCE

BY

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THESIS

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ABSTRACT

BACKGROUND: Familial stressors, such as low family connectedness, confer risk for affective pathologies, yet little is known regarding how they uniquely impinge on neural systems involved in adaptive threat processing.

METHODS: MVPA analysis of fMRI data was used in a novel way to measure disrupted neural habituation in a threat network comprising nodes of the limbic system across several classes of stimuli including unpredictable familial and nonfamilial threat and joy. In the present longitudinal design, measures of family connectedness were collected for adolescents (n=22, age=14.38) at the study's outset, and a year later, a neuroimaging protocol was carried out.

RESULTS: For those low in family connectedness, elevated sensitivity scores derived from MVPA reflected that the encoding of unpredictable familial threat was more stable across time, indicating reduced habituation to familial threat. Reduced habituation was specific to familial threat, as null relationships were found between family connectedness and MVPA sensitivity to familial joy, nonfamilial threat, and nonfamilial joy.

CONCLUSIONS: Results suggest that familial stressors may confer specific biological disruptions to risk-related stimuli, in this case, threatening maternal images. The present novel experimental paradigm and use of MVPA provide the foundation for further exploring how MVPA can be used to test for selective habituation to specific stimuli germane to other environmental risk factors for internalizing pathologies.

ACKNOWLEDGEMENTS

This project is the culmination of the exceptional scientific training I have received at the University of Illinois. Although I am departing the University before I expected I would, I will always consider the University of Illinois, and my mentors, including Dr. Wendy Heller, Dr. Gregory A. Miller, Dr. Bradley P. Sutton, and Dr. Eva H. Telzer, as centerpieces in my foundation in science.

I'm beyond appreciative, and feel deeply fortunate, to have had Dr. Wendy Heller as my first mentor in science. Wendy has gone above and beyond to support my research niche in developmental neuroscience, providing me with an opportunity to investigate how mindfulness shapes the developing brain, and encouraging me to collaborate with several researchers across the University who have similar interests. More importantly, I am so thankful for her willingness to devote large amounts of time helping me with my first foray into scientific writing, which would become my first publication and one of the richest intellectual experiences I've ever been part of. Wendy allowed me to take the lead on writing a review paper, and modeled for me how meticulous reading and writing in science should be done. I developed fundamental skills through this process that I believe will be a boon to attaining my career aspirations in academic science. And most importantly, during a time of great uncertainty in my first year in graduate school when I found out my dad was diagnosed with a serious illness, Wendy was immediately supportive. Both in her heartfelt correspondences by email when I had to abruptly leave campus, and through her genuine and candid empathy and sympathy when we met in person, Wendy made me feel at home in her office and in our program after being her student for only 4 months. It is this most cherished time that made me feel most proud to be her student, and exemplified the kind of mentor I hope to be one day.

Additionally, this work could not have been done without Dr. Bradley P. Sutton's mentorship. Before I arrived on campus for my first year of graduate school, I was fascinated by the human neuroscience literature I was reading, and reached out to Brad to inquire about the kind of neuroimaging I wanted to learn, called diffusion weighted imaging. Brad answered all the questions I had and gave me the opportunity to meet weekly with him once I arrived on campus that fall. Over the past three years, Brad has devoted time to help me learn different analysis methods, allowed me to conduct pilot neuroimaging with his lab, connected me to other researchers at the Beckman Institute, and has even helped with projects of mine that were unrelated to our collaboration. Indeed, Brad has gone above and beyond to be supportive of my scientific training, and has helped me grasp neuroimaging analysis methods to an extent that would not be possible without his guidance. Working with an engineer like Brad has instilled in me the touchstone of knowing the *meaning* of the operations underlying these complex methods I wield as a developmental neuroscientist. Without a doubt, this has provided me with the skills to discern the appropriate usage of these tools, and the adroitness to adapt to a world of rapidly changing technology.

Similarly, the roots of my interest in psychology and neuroscience and the role they play in understanding the development of psychopathology were formed through the many correspondences I have had with Dr. Gregory A. Miller. My professional relationship with Greg began after reading his 2010 review on the relationship between psychology and biology. Greg's multifaceted argument left me with many questions, and ignited my interest in philosophy of science after recognizing the central role it plays in all of science. The rich debates we've had on

issues in clinical neuroscience has provided me with a depth of learning that is a pillar of my scientific training and a gift for my intellectual curiosity. The willingness, passion, joy, and rigor Greg brings to exploring science with others is infectious, and I hope to exude a similar kind of passion and curiosity with my future mentors, colleagues, and students.

And last but not least, I am deeply grateful for Dr. Eva H. Telzer for all of her help on this project and on helping me refine my developing program of research. When I first wanted to gain more experience in developmental neuroscience about a year and a half ago, I emailed Eva, and we began meeting regularly. She invited me to come to her lab meetings, and eventually got me involved in some of the ongoing projects her lab was running. Her expertise in neuroimaging and developmental science was evident when we started meeting, and her passion for the kind of area I wanted to learn most about – how social experiences for youth influence neurodevelopment – made me feel so grateful for our nascent collaboration. This project for my master's thesis has felt like the first step down the path towards my unique research identity, and I would not have been able to pursue this kind of project with this rich dataset without Eva's guidance and willingness to work with me. I am so excited for what's to come in the next few years as I start the next chapter of my training and career in developmental neuroscience with Eva.

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BACKGROUND

Although the majority of youth benefit from rapid biological, psychological and social changes during adolescence, this "tipping point" in life is when many are initially diagnosed with clinical depression and anxiety (1). Investigations are beginning to determine the circumstances under which adolescents develop such pathologies (2), exploring how genetic and social risk factors affect the biology of endophenotypes (3) such as maladaptive threat processing (4,5). Forms of maladaptive threat processing, such as reduced habituation to threat and a lower threshold to appraise environmental stimuli as threatening, are involved in the pathogenesis of anxiety and depression (4). To date, many biological correlates of maladaptive threat processing, including hypersensitivity and reduced habituation to threat in the amygdala, are associated with a variety of etiological antecedents (6-9). It thus remains imperative for the field of clinical neuroscience to better understand the pathophysiology of various risk factors for depression and anxiety. The present study examined how family connectedness, or the extent to which an individual feels close to their family, may be uniquely associated with altered habituation to familial threats in neural mechanisms of threat processing.

Adolescents' perceptions of family connectedness are influenced by the quality of early bonds formed with their mother (10). Adolescents' self-reported family connectedness (5,10), perceptions of parental rejection (11), and attachment style (12) are related to concurrent and longitudinal increases in depression and anxiety symptoms. In rodent, non-human primate, and human studies, the formation of strong bonds between mother and child greatly affects the development of limbic circuitry (13,14) involved in emotion regulation and adaptive threat processing (15). Moreover, in the presence of negatively-valenced stimuli, adolescents who

endorse low family connectedness and early child maltreatment show hypersensitivity (16) and reduced habituation in the amygdala (15). Despite these advances in understanding how parental dynamics are associated with neurodevelopment, more work is needed to determine if those with familial stressors are hypersensitive to threatening familial stimuli above and beyond other types of threatening social stimuli (17). Such findings would contribute to understanding the pathophysiology of anxiety and depression in individuals with a history of familial stressors, in which disrupted habituation in the amygdala and concomitant protracted anxiety may be related to mechanisms giving rise to memory of and learned associations about one's social environment.

Leveraging multivoxel pattern analysis (MVPA) of fMRI data, we tested a theory of disrupted neural habituation to familial threats in adolescents with perceived weak family connectedness. We hypothesized a linear relationship between family connectedness and habituation to familial threat, such that as family connectedness decreases, so too does habituation to familial threat. MVPA is an ideal method because we conceptualize familial threat as a multidimensional construct (Figure 1). Familial threat comprises lower-level dimensions regarding valence, familiarity and previous associative learning about the familial or non-familial stimulus. Although the latter two dimensions may seem quite similar, adolescents equally familiar with a stimulus of their mother may have very different associations with said stimulus based on prior learning/memory that influences how familial threat is encoded.

In particular, we tested how habituation (i.e. the attenuation of threat signaling in response to repeated exposure of a given stimulus) to uncertain familial threat differed across levels of perceived family connection. We used a block design, training the MVPA classifier on the persistent signal across blocks either of unpredictable angry maternal or stranger faces

embedded within more frequently presented neutral faces. Because the angry faces occurred less frequently, their presentation was unpredictable and therefore constituted uncertain familial and nonfamilial threat. Thus, we were not interested in the transient signal elicited by individual stimuli, but rather, the signal across a block in which it is uncertain whether an angry or neutral maternal face will appear.

The MVPA classifier was trained on the first half of the block of unpredictable, familial threat and tested on the second half of the block. Habituation was measured in a novel way using sensitivity metrics gleaned from MVPA. Sensitivity measures the percentage of correct classifications for a given stimulus type. For those habituating normatively, sensitivity would be low because the classifier expects the threat-encoding signal in the second half of the block to be similar to the signal in the first half of the block, which is not the case if threat-processing regions habituate to a given threat. Conversely, for those with reduced habituation, sensitivity should be high, because there is a greater similarity between the signals from the first and second halves of the block. We therefore expected sensitivity to vary inversely with family connectedness for unpredictable, familial threat. As family connectedness decreases, sensitivity to familial threat should increase, which is conceived here as a marker of reduced habituation.

METHODS AND MATERIALS

Participants

Thirty adolescents participated in a two-wave longitudinal design. Two participants were excluded due to excessive movement (>3 mm), two participants did not complete the scan, three did not complete this particular task and one participant's behavioral responses were not recorded due to technical issues. Our final sample included 22 adolescents (13 males). When participants were in the 8th grade (mean age=14.38 years), they completed self-report measures of family connection. One year later, in the 9th grade (mean age=15.19 years), participants underwent a brain scan during which they completed a task depicting familial and stranger threat cues. Adolescent participants were European-American (n=16), African-American (n=3), Asian-American (n=1) or Hispanic (n=1). All participants provided written assent and consent in accordance with the Institutional Review Board.

Perceived family connection

In 8th grade, participants completed a family connectedness measure (18) in which they answered 10 questions using a 5-point scale (1=strongly disagree to 5=strongly agree) to indicate how strongly connected they felt to their family (e.g., "I feel a sense that I personally belong in my family," and "I do not feel like an important part of my family"). The reliability across items of the scale was high (α=87).

Maternal vs. Stranger Go No-Go Task

One year after completing the self-report measure of familial connection, participants completed a brain scan. The fMRI task was a Go No-Go task, in which participants were

instructed to press a button as quickly as possible during "go" trials and inhibit this response by not hitting any button during "no-go" trials (Figure 2). The task had five blocks each consisting of 80 trials, in which 75% of trials were "go" trials of neutral faces, intentionally outnumbering no-go trials in order to generate a prepotent response that is difficult to inhibit. The remaining "no-go" trials comprised emotional faces. Blocks consisted of (1) happy vs. neutral stranger faces, (2) angry vs. neutral stranger faces, (3) happy vs. neutral maternal faces, (4) angry vs. neutral maternal faces, and (5) blue vs. orange houses. Maternal images were taken on-site against a white background. Female strangers' faces were taken from the NimStim (19) stimuli.

Each stimulus was presented for 500 ms, followed by a fixation that was randomly jittered to vary the inter-stimulus interval increasing the experimental design efficiency. Each block included the same amount of TRs, followed by 7 TRs (14 seconds) of rest between blocks.

fMRI data collection

Imaging data were collected using a 3 Tesla Siemens Trio MRI scanner. The Faces Go No-Go task included T2*-weighted echoplanar images (EPI) (slice thickness 3 mm; 38 slices; repetition time (TR) 2 s; echo time (TE) 25 ms; matrix 92 x 92; FOV 230 mm; voxel size 2.5 x 2.5 x 3 mm^3). Structural scans consisted of a T2*weighted, matched-bandwidth (MBW), high-resolution, anatomical scan (TR 4 s; TE 64 ms; FOV 230; matrix 192 x 192; slice thickness 3 mm; 38 slices) and a T1* magnetization-prepared rapid-acquisition gradient echo (MPRAGE; TR 1.9 s; TE 2.3 ms; FOV 230; matrix 256 x 256; sagittal plane; slice thickness 1 mm; 192 slices). The orientation for the MBW and EPI scans was oblique axial to maximize brain coverage.

fMRI preprocessing for MVPA

Minimal preprocessing was done on NIFTI images in preparation for the MVPA analysis. FSL's MCFLIRT (20) with default settings was used for motion correction, followed by using FEAT to remove scanner drift using a high pass filter that eliminated frequencies below .01 Hz contributing to the BOLD signal (Gaussian-weighted least-squares straight line fitting, with sigma=50.0s). Smoothing was not used to avoid reducing the amount of information (by blurring distinct voxel-level signals) in the hypothesized multidimensional coding instantiated in small regions such as the amygdala (21).

MVPA block design

Using AFNI's 3dSVM algorithm (22), a linear classifier determined a decision boundary between the encoding of blocks of unpredictable familial threat from unpredictable non-familial (strangers) threat. 3dSVM implements a type of supervised learning, a support vector machine, to determine a hyper plane in high-dimensional space (each dimension representing a voxel within the region of interest (ROI)) that differentiates the encoding of experimental conditions. Each instance of the class (synonymous with block) that trained the classifier was a TR representing each time point of data collection in the 4D BOLD NIFTI file. Because these blocks contained unpredictable presentations of angry maternal or stranger faces, we operationalized the consistent signal across these blocks as unpredictable familial or non-familial threat. There is precedent for such modeling of protracted anxiety signals in univariate mixed block/event-related fMRI designs using unpredictable, threatening visual stimuli (23).

To measure habituation, the classifier was trained on the first half of the block and tested on the second half of the block. The experimental design was optimized to measure habituation effects given that stimuli were the same within each class. That is, a hypothetical MVPA design that tested if regions differentiate threatening from non-threatening stimuli could and usually do

use several *different* valenced images. The present study, in contrast, used repeated exposure within each block to the *same* maternal or stranger faces.

Stranger stimuli were used as a comparison to maternal stimuli (the MVPA models estimated a function that separates the stranger from maternal threat) because their cognitive representations share characteristics (both are human faces and share similar valence). Habituation, which is operationalized as a change in the multivoxel pattern encoding such stimuli, would lead to misclassifications. In contrast, if we chose to train an MVPA classifier to distinguish angry maternal faces from dissimilar stimuli such as household items, the distance in multivoxel space between the encoding of such representations would be so great that the classifier would likely do well regardless of habituation effects.

To ensure that the correlation between sensitivity measures and family connectedness was not being driven by the ratio of angry to neutral stimuli within training vs. testing phases of unpredictable maternal threat (to rule out that low sensitivity was related to how far the ratio of angry maternal stimuli in training and testing phases was from 1:1), we inspected the data and found no such correlation.

3dSVM was run on previously described preprocessed BOLD images. For each subject, 3dSVM was run on five different models a priori defined. Four ROIs (right and left amygdala, rITG and rMTG) were tested separately. Individually, each region was selected because of its association with various types of threat processing (measured by univariate fMRI), including negatively-valenced facial stimuli (25). ROIs were defined structurally using the Harvard Oxford atlas, and were transformed into subjects' BOLD space using FSL's linear, affine registration tool, FLIRT. The 1mm atlas space ROIs were transformed first to each subject's high-resolution T1-weighted structural image, and subsequently were transformed into native subject's BOLD

space. We tested 3dSVM on a concatenation of all ROIs (which we henceforth refer to as the "threat network"). Each of these regions (amygdala and regions in anterior cortical temporal lobe) is part of a putative functional network, the limbic system, which has been shown to have resting-state and task-based functional and structural connectivity (26,27). Lastly, we also tested 3dSVM at the whole-brain level to rule out the explanation that sensitivity increases as a function of more voxels used to train the classifier. To determine if the relationship between multivariate activity patterns in threat-processing neural regions and family connectedness was specific (as was hypothesized) to threatening stimuli (angry faces), we also ran the same five MVPA models on blocks containing happy expressions for both maternal and stranger faces.

3dSVM computes a single, overall accuracy metric, which denotes in this study the percentage of correct classifications for both familial and non-familial faces. Since this number does not contain information about accuracy within each stimulus class, sensitivity analyses were conducted using an in-house created Python algorithm. Sensitivity is a metric from signal-detection theory that provides the fraction of correct classifications (true positives) of the total amount of opportunities to classify a specified type of true signal (true positives + false negatives). We conceptualized sensitivity to maternal threat as a measure of habituation (Figure 2). Lower sensitivity would indicate a greater attenuation of threat (i.e. greater habituation) in threat-sensitive neural regions between the training (first half of block) and testing (second half of block) phases.

Univariate, event-related design: Is familial threat multidimensionally coded?

Despite known issues regarding the comparison of MVPA and univariate analyses (28), we carried out an event-related, univariate design to see whether such an analysis could detect a similar relationship between family connectedness and sensitivity to familial threat. Significant

results would support that a single dimension (e.g., familiarity) drives hypersensitivity to maternal compared to stranger angry faces for those low in family connectedness.

Univariate fMRI preprocessing and analysis

The same preprocessing that was used with MVPA was used with univariate, voxel-wise analysis, with the addition of smoothing (full width at half maximum=6mm) and registration to the 2mm Montreal Neurological Institute (MNI) standard space brain.

FSL's FEAT was used to carry out the hierarchical general linear model (GLM). At the first level of the GLM, the canonical hemodynamic response function was convolved with each event (angry/happy/neutral maternal faces, angry/happy/neutral stranger faces) to derive beta estimates. Additionally 6 motion regressors were used as covariates to account for motion effects that MCFLIRT did not detect (29).

At the second level, we carried out ROI and whole brain tests. Using the same ROIs in the MVPA analysis, Pearson correlations were computed between the mean signal within all ROIs and family connectedness scores. Mean signals were extracted from uncorrected z-score maps of each individual's contrast image (maternal angry face minus stranger angry face). The large number of voxel-wise tests was reduced in this model to only five tests, thus quasi-eliminating the problem of correcting for multiple comparisons. At the whole brain level, FSL's FLAME1 was used to test for voxels that significantly correlated with family connectedness. We used default settings for a cluster-wise z-score threshold ($z=2.3$, $p<.05$).

RESULTS

Habituation to unpredictable familial threat and Family Connectedness

As mentioned above, we conceptualize sensitivity as a measure of habituation. As sensitivity increases, the signal in the second half of a block is more similar to the signal in the first half of the block. Because habituation should make it more likely that multidimensional signal in the second half of the block should differ from that of the first, habituation is inversely related to sensitivity. To measure sensitivity to maternal (familial) threat, we conducted Pearson correlation tests between the MVPA-derived sensitivity metrics and all a priori defined ROIs and networks. Sensitivity of the threat network (Figure 4) was inversely related to family connectedness with the strongest effect size of all tests (r = -0.59, p=.004), followed by the left amygdala (r = -.47, p=.027). Although subsequent correlations of ROIs and family connectedness did not pass 2-tailed significance thresholds, their effect sizes are in the medium-high range and are indicative of the same theory of how family connectedness relates to sensitivity in threat-processing regions (rITG = -.38; rMTG = -.37). Additionally, family connectedness was correlated inversely with sensitivity at the whole brain-level, albeit with a lower effect size than the threat network (r= -.48, p=.024).

MVPA sensitivity: unpredictable, familial joy

We tested the same 5 ROIs on blocks containing unpredictable familial or nonfamilial joy (i.e., happy faces) in order to rule out that those with low family connectedness had higher sensitivity to familiar faces *regardless* of valence. Correlations were insignificant and in the opposite direction as the relationships supported by sensitivity to unpredictable threat (for threat network and maternal joy: r =.354, p =.107). The reversal of the sign of the correlation is

supported by extant evidence, which shows that those with high attachment security show enhanced amygdala responsivity to maternal happy stimuli compared to stranger happy stimuli in childhood and adolescence (Tottenham et al., 2012).

MVPA sensitivity: Nonfamilial threat

We also ran sensitivity analyses of the same five models for unpredictable nonfamilial threat (stranger angry faces) and unpredictable nonfamilial joy (stranger happy faces). No significant correlations were found. In the threat network, sensitivity to nonfamilial joy and family connectedness were unrelated (r = 0.05, p=.82) as were sensitivity to nonfamilial threat and family connectedness (r = -0.08, p=.74).

Is sensitivity to familial threat greater than chance?

Since a two-class classifier has a 50% chance of correctly classifying a given instance of an independent, testing dataset, we tested if individuals low in family connectedness that had higher sensitivity to maternal threat surpassed sensitivity levels as predicted by chance. Thus, we conducted a one-sample t-test (test value = 0.5 representing chance level) for both high and low family connectedness groups (mean split) in the threat network. As predicted, those low in family connectedness were classifying greater than chance (t(10)= 2.912, SE = .027). This result suggests that for those with low family connectedness, the encoding of unpredictable familial threat was similar between the beginning and end of the block, further substantiating our theory that those who perceive themselves to be weakly connected to their family show reduced habituation to unpredictable, familial threat. Surprisingly, those with high family connectedness had a mean sensitivity significantly *less* than chance (t(10) = -2.512, SE= .036). This may be explained by greater habituation. (Figure 5).

MVPA differential sensitivity to familial threat and nonfamilial threat

Sensitivity is a measure of within-category classification. It is not necessarily the case for a dataset trained on two categories of stimuli that sensitivity for one category correlates with sensitivity for the other category (Figure 6). Thus, habituation to familial threat need not correlate with sensitivity to nonfamilial threat (30). However, we tested if adolescents low in family connectedness had less habituation (higher sensitivity) to familial *relative to* nonfamilial threat. To do so, we subtracted nonfamilial sensitivity scores from familial sensitivity scores, which represents the within-subject difference in sensitivity across familial and nonfamilial threat (the higher the score per subject, the greater the sensitivity to maternal threat relative to nonfamilial threat), and computed a correlation with family connectedness. Although marginally significant, there was still a substantial effect size between family connectedness and differential sensitivity in the threat network (r = -.414, p=.055), with a significant effect in the left amygdala (r =-.441, p=.04). Taken together, as family connectedness decreases, individuals show greater sensitivity (reduced habituation) in limbic circuitry to familial threat relative to sensitivity to nonfamilial threat.

Univariate, event-related fMRI

Finally, to test whether our effects are specific to multivariate analyses, we examined univariate tests with the same ROIs. When examining the contrast of angry maternal versus angry stranger faces, no ROIs significantly correlated with family connectedness in the (1) threat network (r=.13, p=.57), (2) rAmyg (r=.26, p=.25), (3) lAmyg (r=.21, p=.35), (4) rITG (r=.05, p=.82), or (5) mITG (r=-.10, p=.66). At the whole brain level, no significant clusters were associated with family connectedness.

DISCUSSION

Although familial stressors, such as low family connectedness, are putative risk factors for depression and anxiety (10), little is known regarding how family stress affects neurodevelopment. If family stressors are shown to result in unique mechanisms of dysfunction, precise interventions can be created to target these specific deficits. The present study sought to contribute to this enterprise by testing the hypothesis that weak family connectedness would be associated with reduced habituation to familial threats.

Results supported our hypotheses and established a new way to use MVPA machine learning tools such as support vector machines to test for habituation. In a threat network comprising key nodes in the limbic system, reduced habituation (elevated sensitivity) to unpredictable, familial threat was significantly related to weak family connectedness. The present study rests on a conceptualization of threat processing as a system that encodes multiple dimensions comprising threatening stimuli. Thus, during the fMRI task in which adolescents were exposed to repeated presentations of maternal, angry faces, it is theorized that they rapidly updated the encoding of the valence of the face, the current familiarity of the stimulus, the prior familiarity of the stimulus based on memory, relevant contents of autobiographical memory (27), and likely many other dimensions.

We also conducted several tests to rule out alternative explanations for our results. Null results of models that used positive familial and positive/negative nonfamilial stimuli ruled out the possibility that our main finding was driven by the familiarity of maternal stimuli and supported the conclusion that those low in family connectedness are uniquely sensitive and habituate less to familial threats. We also tested event-related univariate models in the same

ROIs and at the whole-brain level to disconfirm that hypersensitivity to unpredictable familial threat in those low in family connectedness was being driven by a single dimension such as greater negative valence.

More work is needed to better understand how reduced habituation to familial threat is related to the emergence of clinical pathologies. Future research should seek to understand how other neural systems contribute to specific types of reduced habituation using techniques such as dynamic causal modeling (31). Extant theories suggest that memory structures formed by early adverse experience (e.g., parental neglect and abuse) may influence psychopathological behavior (32), which may be implemented in neural interactions between threat processing and memory systems. Finally, it should be noted that our interpretation of the results is limited because we cannot be sure that MVPA sensitivity is explained better by how distal the encoding of each stimulus class was in both the testing and the training phases. Thus, it is possible that high sensitivity does not necessarily indicate poor habituation, and those with low family connectedness are merely hypersensitive to unpredictable, maternal threat. Future studies should analyze such data using representational similarity analysis (33,34), which can quantify how dissimilar neural patterns are across individuals and conditions.

In conclusion, our results suggest that familial stressors may impinge on threat processing neural mechanisms in a specific way such that those with weak family bonds show protracted threat processing in the presence of maternal threat. Disrupted habituation to familial threat for those with familial stressors may be a biomarker that precedes clinical emergence of internalizing disorders. Thus, present findings may contribute to the eventual goal of using in vivo neuroimaging as a screening tool for familial risk for depression and anxiety. Similarly, the methodological innovation presented here in which MVPA-derived within-class sensitivity

scores are used as a measure of habituation to specific stimuli may be a boon to understanding how anxiety manifests differently across individuals. Indeed, the use of MVPA as deployed here may be an innovative way to determine which classes of stimuli exposure therapy should target for individuals showing selective disrupted habituation to certain stimuli.

FIGURES

Figure 1. (A) Represents how we conceptualize familial vs. nonfamilial threat as multidimensional concepts, comprising two dimensions (non-exhaustive) of familiarity and valence. Holding valence constant, familial threat is always more familiar. Similarly, when holding familiarity constant, familial threat tends to elicit greater negative valence. However, the difference in either dimension may be minimal, which may be why it is difficult to distinguish these closely related cognitive representations using traditional univariate fMRI analyses. We believe this conceptual structure is implemented in neural systems associated with threat encoding as depicted in (B). Assume the 2 voxels in the axes comprise the entire amygdala. If one used traditional voxel-wise univariate analysis of fMRI data to test the hypothesis that voxels in the amygdala distinguish familial from nonfamilial threat, the mean voxel-wise activations for a given population for familial (red circles) and non-familial (yellow circles) stimuli may be insignificantly different from each other. Conversely, an MVPA algorithm that takes into account the distributed pattern of activity across voxels could estimate the red-dotted line that best distinguishes these types of stimuli.

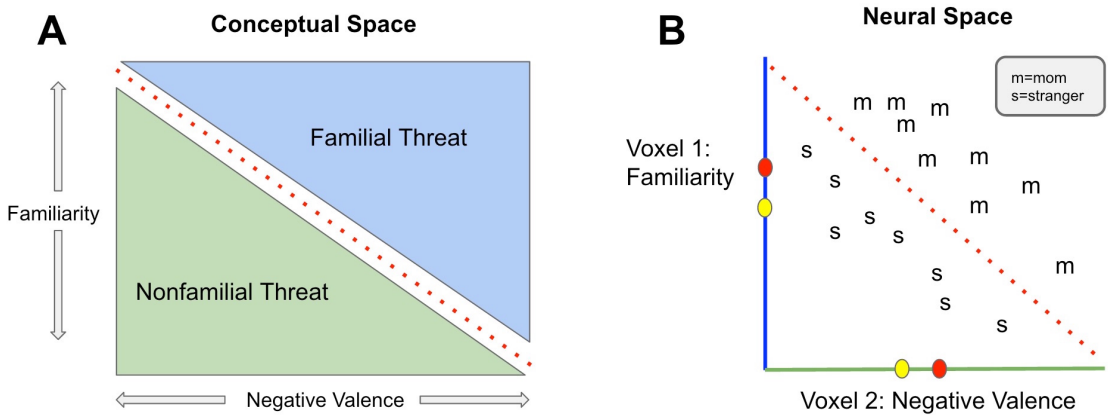


Figure 2. Mother vs. Stranger Go No-Go fMRI task.

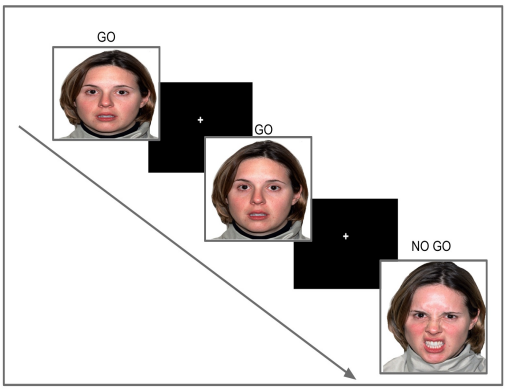


Figure 3. The images on the top row consist of three square voxels and represent the case in which both MVPA and univariate fMRI are capable of detecting habituation effects. At the start of stimulus presentation (time 1) signal across voxels is relatively high. With univariate cluster-wise analysis, in order for the contrast image of Time1 - Time 2 to yield significant clusters, it is necessary that all voxels at time 2 decline precipitously to pass the a priori set z-score value. However, the images on the bottom row represent a habituation effect that is *only* detectable by MVPA. If such voxels are encoding threat, the present study theorizes that levels of threat that change via habituation vary as a function of a distributed pattern across voxels, and are undetectable to analyses that require steep voxel-wise changes or unidirectional changes across contiguous voxels to pass cluster-wise thresholds.

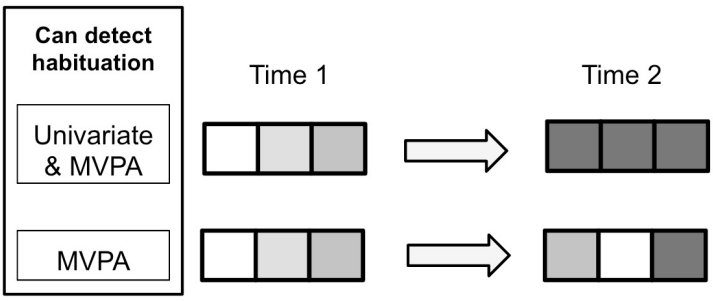


Figure 4. Family connectedness and Sensitivity to unpredictable, familial threat in the threat network.

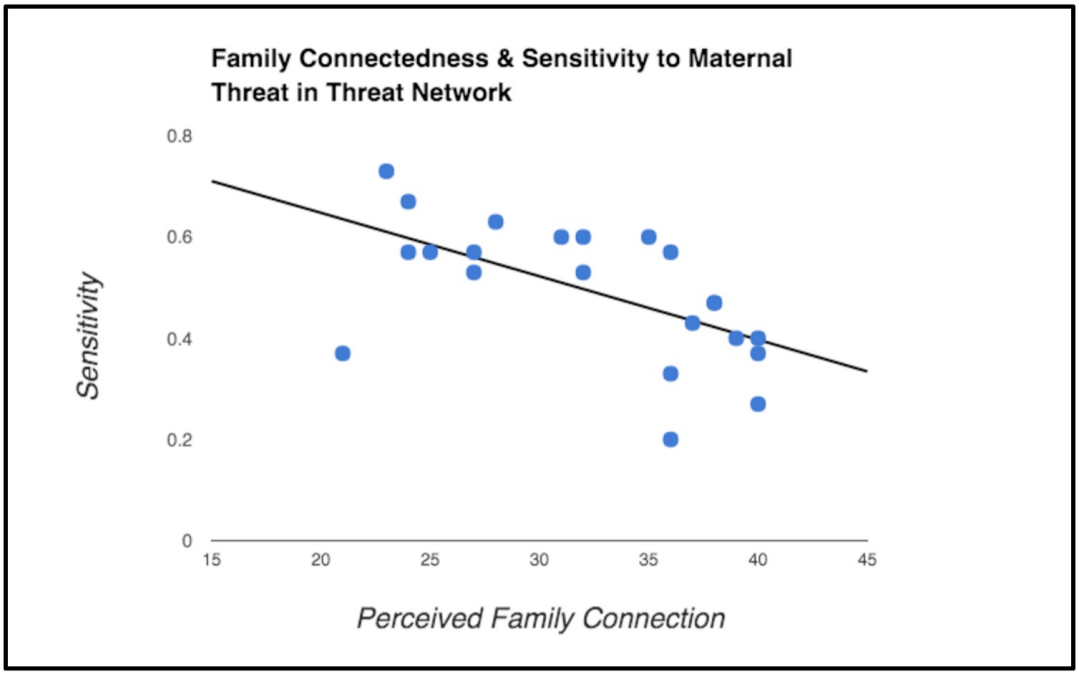


Figure 5. Sensitivity to familial threat greater than chance levels.

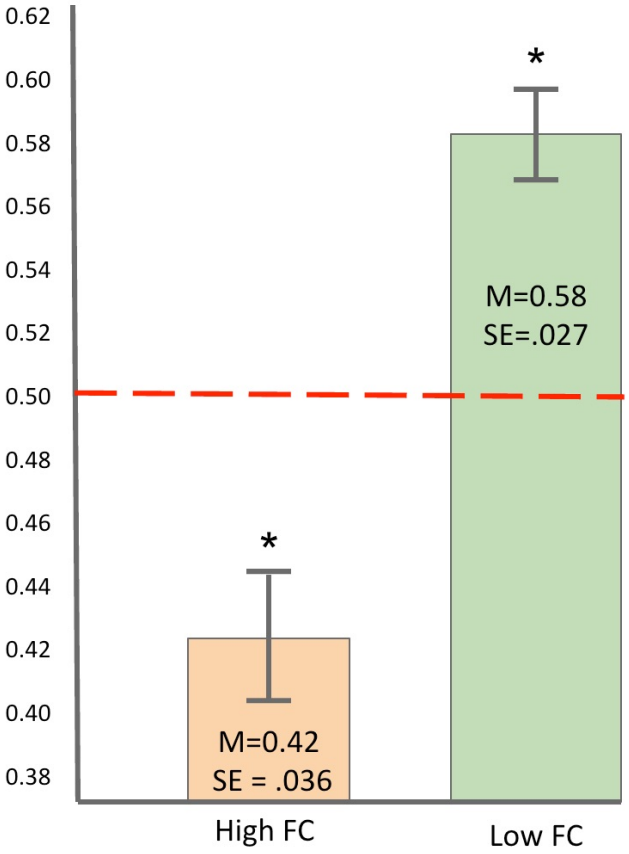
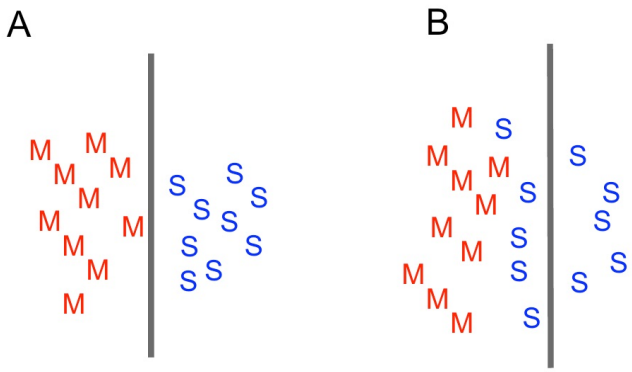


Figure 6. (A) represents the case in which both classes of stimuli (Maternal - M and Stranger - S) have 100 percent sensitivity. (B) displays how one class's sensitivity is not necessarily correlated with the other class's sensitivity. Indeed, for maternal stimuli, the sensitivity is 100 percent and for the stranger stimuli, the sensitivity is 50%.



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