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Neural reactivity tracks fear generalization gradients

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ABSTRACT

Recent studies on fear generalization have demonstrated that fear-potentiated startle and skin conductance responses to a conditioned stimulus (CS) generalize to similar stimuli, with the strength of the fear response linked to perceptual similarity to the CS. The aim of the present study was to extend this work by examining neural correlates of fear generalization. An initial experiment ($N = 8$) revealed that insula reactivity tracks the conditioned fear gradient. We then replicated this effect in a larger independent sample ($N = 25$). Activation in the insula, anterior cingulate, right supplementary motor cortex and caudate increased reactivity as generalization stimuli (GS) were more similar to the CS, consistent with participants' overall ratings of perceived shock likelihood and pupillary response to each stimulus.

1. Introduction

Paradigms that assess fear learning have provided valuable translational tools for understanding the etiology, maintenance and treatment of anxiety disorders (Milad et al., 2006; Mineka and Oehlberg, 2008). The acquisition and extinction of conditioned fear responses involve a common neurocircuitry across species that includes the amygdala, insula, anterior cingulate cortex, hippocampus, sensory areas, and ventromedial prefrontal cortex (Büchel and Dolan, 2000; LeDoux, 2000; Phelps et al., 2004). In addition to acquisition and extinction, there is increasing interest in fear generalization, which describes the transfer of a conditioned fear response to stimuli that are perceptually similar to the conditioned stimulus (CS). Insofar as the transfer of fear responses from threat-related stimuli to potentially innocuous cues is a common feature in anxiety disorders (Lissek et al., 2008), fear generalization may be a key learning process in the development and maintenance of pathological anxiety.

Recent studies have validated laboratory-based procedures for testing fear generalization, which involves the assessment of fear responses to a CS and to generalization stimuli (GS) that vary in perceptual similarity to the CS (Hajcak et al., 2009; Lissek et al., 2008). In these paradigms, fear responses were quantified with the fear-potentiated startle reflex, which followed a generalization gradient: the strongest startle reflex was elicited during the CS, with a steep decline corresponding to the relative decrease in similarity of the GS to the CS[1] (Hajcak et al., 2009; Lissek et al., 2008). Lissek and colleagues assessed fear generalization in a paradigm in which participants had to learn which stimulus was the CS and which were the GS. On the other hand, Hajcak and colleagues found comparable results even when participants were explicitly instructed regarding the identity of the CS and the reinforcement contingencies to the CS and GS. Despite being told explicitly which stimulus was the CS, and never being shocked following a GS, participants in the Hajcak et al. study had larger startle responses and reported greater shock likelihood as GS were more perceptually similar to the CS.

Fear generalization paradigms could be useful for assessing pathological fear and risk for anxious psychopathology. For instance, patients with panic disorder exhibit a flatter fear gradient with more gradual decreases in fear response to the GS (Lissek et al., 2010). Hajcak et al. (2009) reported fear generalization deficits in a generalization paradigm as a function of variation in the brain-derived neurotrophic factor (*BDNF*) genotype, which has been related to both learning and anxiety-related behaviors.

In the current study, we sought to extend this work by examining neural activity using fMRI in a fear generalization paradigm that we previously employed (Hajcak et al., 2009). The aim was to elucidate the brain regions associated with generalization, which have

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[1] Comparable results have also been obtained using skin conductance (Dunsmoor et al., 2009; Vervliet et al., 2010), which is a more general measure of arousal that is not specific to defensive motivation.

received little attention in the literature, and to examine whether reactivity in these regions exhibit a similar generalization gradient to that reported with peripheral measures of fear. These neural gradients may be useful in identifying deficits in the generalization process and may be relevant to future work on pathological anxiety (e.g., Lissek et al., 2010). In the current study, the CS was a middle-sized rectangle and the GS were six additional rectangles varying in width from the CS by ±20%, ±40% or ±60%.

In an initial experiment ($N = 8$), we examined regions of interest (ROIs) based on neuroimaging studies of fear learning that have implicated key areas in the expression and inhibition of autonomic and behavioral fear responses (Dunsmoor et al., 2011; Sehlmeyer et al., 2009). These ROIs included the *amygdala*, *insula*, *thalamus*, *caudate*, *anterior cingulate cortex (ACC) and ventromedial prefrontal cortex (vmPFC)*. We hypothesized that reactivity in one or more of these regions would demonstrate a similar gradient response to the pattern reported in previous laboratory-based studies. In a second experiment[2] ($N = 25$), we conducted a whole-brain analysis and obtained additional self-report ratings and physiological measures.

2. Experiment 1

2.1. Methods

2.1.1. Participants

Eight individuals (6 females and 2 males) participated in the study (Mean age = 23.2; SD = 4.7). All reported being right handed. Potential participants were screened for prescription and recreational drug usage, as well as neurological and psychological histories. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

2.1.2. Procedure

Prior to the scan, an electric shock, delivered to the left wrist (Constant Voltage Stimulator STM 200; Biopack Systems Inc.), was individually set for each participant to a level that was "uncomfortable but not painful". Instructions for the task were then provided. Participants were told that the middle-sized rectangle (CS) indicated a 50% probability that they would receive a subsequent electric shock, but that shocks would never follow rectangles of greater or lesser size. A conditioning phase was administered next, which included five presentations of the CS with electric shock (i.e., CS_{paired}) and one presentation of each of the other six rectangles. The task immediately followed. Thus, the current study examined generalization within the context of a paradigm that combined instructed and associative fear learning.

2.1.3. Task

The task consisted of three blocks presented consecutively. Each block included 40 trials (5 trials × 8 conditions) for a total of 120 trials. The stimuli were seven red rectangles with identical height (56 pixels) and varying width (112–448 pixels) presented against a black background. The middle-sized rectangle (280 pixels) was the conditioned stimulus (CS). Half of the time the CS co-terminated with a 500 ms electric shock (CS_{paired}), while half of the time it did not ($CS_{unpaired}$). The six remaining rectangles differed by ±20%, ±40% or ±60% in width from the CS and served as the generalization stimuli (GS). Stimuli were presented pseudorandomly for 2 s with a jittered interstimulus interval ranging from 4 to 10 s, during which a white fixation cross was shown on a black background. The task was programmed with E-prime 1.2 (Psychology Software Tools, Inc. Pittsburg, PA) and presented with an MRI compatible 60 Hz projector with 1024 × 768 resolution. The duration of the task was 15 min and 12 s.

2.1.4. Image acquisition

Participants were scanned with a 3 tesla Siemens Trio scanner at the Stony Brook Social, Cognitive and Affective Neuroscience (SCAN) center. A total of 456 T2*-weighted echoplanar images were acquired with an oblique coronal angle and TR = 2000 ms, TE = 22 ms, flip angle = 83°, matrix = 96 × 96, FOV = 224 mm × 224 mm, slices = 36 and slice thickness = 3.5 mm. In addition, we obtained T1-weighted structural scans with TR = 1900 ms, TE = 2.53, flip angle = 9°, FOV = 176 mm × 250 mm × 250 mm, and matrix = 176 mm × 256 mm × 256 mm.

2.1.5. Image analysis

Preprocessing procedures were performed in SPM8 and included slice time correction, motion correction, normalization and smoothing with a 6-mm full width at half maximum Gaussian kernel. Preprocessed images were entered into a general linear model in which each rectangle was modeled as an event with no duration; CS_{paired} and $CS_{unpaired}$ were modeled separately. The six motion parameters estimated during realignment were included as regressors of no interest and serial autocorrelations were modeled using an AR (1) process. First-level single subject statistical parameter maps were created for the 'CS_{paired} − Baseline' (i.e., fixation), '$CS_{unpaired}$ − Baseline' and each of the 'GS − Baseline' contrasts. These contrasts, except for 'CS_{paired} − Baseline', were used in a second-level random effects repeated measures analysis.

2.1.6. Gradients of neural reactivity

Individual bilateral masks were created for the amygdala, insula, thalamus, caudate nucleus, anterior cingulate cortex (ACC) and ventromedial prefrontal cortex (vmPFC) using the Masks for Regions of Interest Analysis software (Walter et al., 2003). A region of interest (ROI) analysis for the F-contrast (main group effect) was performed using an initial threshold of $\alpha = .01_{(uncorrected)}$ and extent threshold = 20 contiguous voxels, and a small volume familywise error rate corrected $\alpha = .05$, for each mask.

Neural gradients were generated for the right and left insula (which were the only regions that showed significant activation with these thresholds) by extracting the first eigenvariate (i.e., the principal component) from a 6 mm sphere centered on the local maxima within each region, for each of the '$CS_{unpaired}$ − Baseline' and 'GS − Baseline' contrasts, across all participants. Mean values for $CS_{unpaired}$, as well as GS ± 20%, GS ± 40% and GS ± 60%, were plotted as a four-point gradient.

2.2. Results

2.2.1. Gradients of neural reactivity

Generalization gradients for the right and left insula are shown in Fig. 1b and c, respectively. Reactivity in the right ($F(3,21) = 18$, $p < .001$) and left ($F(3,21) = 13.3$, $p < .001$) insula varied as a function of stimulus type. For the right insula, pairwise comparisons revealed higher reactivity for the $CS_{unpaired}$ versus GS ± 40% ($p = .004$) and GS ± 60% ($p = .01$), and for the GS ± 20% versus GS ± 40% ($p = .02$). A comparison of the GS ± 20% to GS ± 60% was marginally significant ($p = .053$). For the left insula, reactivity was higher for the $CS_{unpaired}$ versus GS ± 40% ($p = .007$) and GS ± 60% ($p = .03$), and for the GS ± 20% versus both GS ± 40% ($p = .03$) and GS ± 60% ($p = .04$).

3. Experiment 2

3.1. Methods

3.1.1. Participants

Twenty-five women participated in the study (Mean age = 21.6; SD = 5.1). All reported being right-handed except for one participant, who reported being ambidextrous. Participants were screened for psychiatric illness with the Structured Clinical Interview for DSM-IV Axis I Disorders – Patient Edition, Version 2 (SCID-I/P; First et al., 2002). All other screening procedures were identical to Experiment 1. The study was approved by the Stony Brook University Institutional Review Board; all participants provided informed consent.

3.1.2. Experimental paradigm

The experimental paradigm was identical to Experiment 1 except for the addition of post-task ratings of shock likelihood for each rectangle, obtained on a Likert scale of 1 ("certainly not shocked") to 5 ("certainly shocked"), acquisition of pupillary response with the Eyelink-1000 (SR Research Ltd., Ontario) as a measure of activation of the sympathetic nervous system, as well as a 12 s increase in task length to accommodate a change in TR and TE due to scanner requirements.

3.1.3. Image acquisition and analysis

A total of 440 T2*-weighted echoplanar images were acquired with an oblique coronal angle and TR = 2100 ms, TE = 23 ms, flip angle = 83°, matrix = 96 × 96, FOV = 224 mm × 224 mm, slices = 37 and slice thickness = 3.5 mm. Parameters for acquisition of structural images, as well as preprocessing procedures and statistical analysis were identical to Experiment 1.

3.1.4. Gradients of neural reactivity

Gradients of neural reactivity were generated for all brain regions for which we found significant clusters for the main effect group F-contrast using a whole brain threshold of $\alpha = .001_{(uncorrected)}$ and extent threshold of 20 contiguous voxels.

3.1.5. Preprocessing of pupil data

Pupil data was processed using custom MATLAB codes (MathWorks). First, we excluded periods of eye blinks detected by an on-line parsing system (Eyelink; SR Research Ltd., Ontario). We used a window of 100 ms prior to onsets of eye blinks and 300 ms following their offset in order to minimize after-blink constriction effects. Missing values were linearly interpolated. We adopted pre-processing procedures from Hupé et al. (2009). Specifically, a baseline for each trial was calculated by averaging data points from 500 ms immediately preceding the onset of the stimulus and then subtracting this mean from each trial. The baseline corrected values were

[2] Experiment 2 was run separately due to changes in imaging parameters required by our imaging center (see Section 3.1.2).