A Neuroimaging Method for the Study of Threat in Adolescents

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ABSTRACT: Little is understood about the brain basis of anxiety, particularly among youth. However, threat paradigms with animals are delineating the relationship between anxietylike behaviors and brain function. We adapted a threat paradigm for adolescents using functional magnetic resonance imaging. The aim was to examine amygdala activation to fear. The threat was an aversive air blast directed to the larynx. Participants were explicitly informed that they might receive the air blast when viewing one stimulus (threat condition) and would not receive the blast when viewing the other stimulus (safe condition). Participants provided fear ratings immediately after each trial. Based on the relatively mild nature of the air blast, we expected participants to report varying degrees of fear. Those who reported increased fear showed right amygdala activation during the threat condition and left amygdala activation in the safe condition. These procedures offer a promising tool for studying youth with anxiety disorders.

Keywords: functional magnetic resonance imaging; fMRI; amygdala; fear; anxiety

Despite their high prevalence and associated risk for later debilitating psychopathology, very little is understood about the neurobiology of adolescent anxiety disorders. However, using threat paradigms, animal research provides an effective pathophysiological model for fear and anxietylike behaviors (Hitchcock & Davis, 1986; Lee & Davis, 1997; Morgan & LeDoux, 1995; Timms, 1977). In a typical threat paradigm, a rat is placed in a context in which a shock is anticipated. Shock anticipation leads to a number of physiological and behavioral signs of fear such as freezing (Morgan & LeDoux, 1995; Morgan, Romanski, & LeDoux, 1993) and increased startle (Hitchcock & Davis, 1986). Work in animals with such paradigms most consistently implicates the amygdala in this fear response (Campeau et al., 1991; Hitchcock & Davis, 1986). Because such fear behaviors may relate to anxiety symptoms in humans (Fowles, 1980; Grillon, Dierker, & Merikangas, 1998; Kagan, Reznick, & Snidman, 1988), methods for inducing and monitoring fear in a controlled laboratory environment may reveal psychological and neurophysiological processes associated with anxiety disorders.

In an effort to measure comparable behaviors across species, threat paradigms have been adapted for use with humans (Grillon, Ameli, Merikangas, Woods, & Davis,
1993; Grillon, Ameli, Woods, Merikangas, & Davis, 1991). Threat paradigms are excellent candidates for cross-species research because the threat response increases in humans as they anticipate an aversive shock, much like it does in rodents (Hamm & Vaitl, 1996; Lipp, Sheridan, & Siddle, 1994). Such a translational approach permits the relatively well-specified models of anxietylike behaviors in animals to inform perspectives on comparable behaviors in humans with anxiety disorders. Indeed, startle threat paradigms differentiate between participants with and without anxiety disorders (Grillon, Falls, Ameli, & Davis, 1994; Grillon & Morgan, 1999; Grillon, Morgan, Davis, & Southwick, 1998).

Given that most anxiety disorders in adults have strong antecedents in childhood (Pine, Cohen, Gurley, Brook, & Ma, 1998), it is vital to extend such research to pediatric populations. This will facilitate examination across development of the relationship between anxiety and associated neurophysiology. Furthermore, a developmental approach might reveal factors that predict differential risk for chronic impairment or health in later life. However, highly anxiogenic stimuli such as shock may not be ethically permissible for use with children. Moreover, such stimuli may not be optimal for differentiating anxious and healthy individuals because they may elicit strong fear in both patients and controls. If anxiety disorders involve experiencing fear in situations that are only mildly challenging to healthy individuals, utilizing a milder stressor may offer advantages in evaluating the symptoms of interest.

In a study with adults, patients with panic disorder and healthy controls showed similar anxiety levels during shock anticipation (Grillon, Ameli, Goddard, Woods, & Davis, 1994). However, in the same study, the milder contextual fear from participating in a stressful experiment differentiated the two groups (Grillon, Ameli, et al., 1994). Similar results have been reported with patients with posttraumatic stress disorder (Grillon, Ameli, et al., 1994). These findings indicate that shock anticipation may not reveal large differences between anxious and nonanxious individuals. Finally, in a longitudinal study of healthy children and adolescents over a 3-year period, younger subjects were more fearful on a range of issues relative to older subjects, and the fear levels diminished with age (Gullone, King, & Ollendick, 2001). Therefore, a milder stressor may be more appropriate for examining healthy adolescents and those with anxiety disorders.

As a less anxiogenic unconditioned stimulus for children, investigators have used a strong blast of air to the larynx (Grillon & Ameli, 1998; Grillon et al., 1999). Grillon et al. (1999) used this procedure with adolescents in a multisite study and found consistent startle potentiation in the threat-of-air-blast condition. Furthermore, adolescents at risk for anxiety disorders were shown to display larger startle responses than healthy adolescents with this procedure (Grillon, Dierker, & Merikangas, 1997, 1998).

Relative to startle, neuroimaging methods provide a means for more directly evaluating neural structures involved in fear processing in humans. Existing neuroimaging research on healthy humans often employs facial expressions to elicit fear. Many of these studies focus on the relation between amygdala activation and fear-related stimuli. In particular, the presentation of fearful faces to healthy individuals is associated with increased amygdala engagement (Breiter et al., 1996; Hariri, Bookheimer, & Mazziotta, 2000; Morris et al., 1998; Morris et al., 1996). In children and adolescents, presentation of fearful faces has been shown to produce increased activation in the amygdala (Baird et al., 1999; Killgore, Oki, & Yurgelun-Todd, 2001; Monk et al., 2003; Thomas, Drevets, Whalen, et al., 2001).

In individuals with anxiety disorders, previous imaging studies implicate alterations in amygdala activation when fear-triggering stimuli that are relevant to the disorder are presented (Birbaumer et al., 1998; Fredrikson, Fischer, & Wik, 1997; Fredrikson, Wik, Annas, Ericson, & Stone-Elander, 1995; Furmark et al., 2002; Rauch et al., 1995; Tillfors et al., 2001). Consistent with these findings, the one imaging study to examine anxious children revealed greater amygdala activation in those with generalized anxiety or panic disorders, relative to healthy children (Thomas, Drevets, Dahl, et al., 2001). However, while these findings demonstrate that fear-provoking paradigms engage analogous brain structures in humans and animals, the procedures utilized in the neuroimaging studies are not comparable to those used in the animal work. This complicates efforts to generate cross-species comparisons.

Two recent neuroimaging studies with humans developed paradigms that more closely approximate fear provocation procedures in animals. Phelps et al. (2001) informed subjects that they might receive a shock when they viewed one stimulus (threat condition), but would definitely not be given a shock when they viewed a different stimulus (safe condition). The authors reported activation in the left amygdala to the threat relative to the safe condition. Similarly, in an effort to develop a fear-conditioning paradigm that utilizes a milder stressor than shock, one of us (Pine et al., 2001) repeatedly paired an air blast to the larynx with a visual stimulus in adult healthy participants. A region-of-interest analysis showed increased amygdala activation among adults to the conditioned stimulus relative to the stimulus that was not paired with the air blast. Such findings demonstrate that fear paradigms adapted for neuroimaging with humans have the capacity to elicit activation of the amygdala, a struc-
tured that the animal work suggests is involved in fear processing.

Because the air blast poses a milder threat than shock, this aversive stimulus may be a particularly useful tool for differentiating between healthy adolescents and those with anxiety disorders. To establish the methodological reliability of this approach, we studied the threat of airblast procedure with a sample of healthy adolescents using functional magnetic resonance imaging (fMRI). Based on the relatively mild nature of the air blast, we anticipated variations across participants in subjective fear to the threat of the air blast. We investigated whether participants who reported experiencing fear would show amygdala activation.

**METHODS**

**Subjects**

Fifteen adolescents were scanned with a 3 Tesla scanner. Of the 15 adolescents who completed the MRI scanning session, 1 subject produced unacceptable movement (i.e., movement that was greater than 3 mm along the x, y, or z planes). Adolescents (7 females, 7 males) were between the ages of 10 and 17 years ($M = 14.9, SD = 2.3$). The age range among females was 10 to 17 ($M = 14.57, SD = 2.76$); among males it was 12 to 17 ($M = 15.14, SD = 1.77$). All participants had age-adjusted IQs greater than 70 ($M = 112.1, SD = 11.8$) as determined by the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler 1999). In addition, all participants were determined to be healthy based on a physical and a standardized, structured psychiatric interview (Kaufman et al., 1997).

**Procedures**

Participants were acclimated to the MRI environment with an MRI simulator. Before the scanning began, participants were informed that they would view blue and green squares presented sequentially. Half of the participants were told that they might receive a blast of air to the larynx while viewing the blue square (threat condition) and that they would never receive such a blast when they viewed the green screen (safe condition). The other half was told that the blue square represented the safe condition and the green square was for the threat condition. Before participants were positioned in the MRI, they were given a sample blast of air to the larynx. After they were placed in the MRI and immediately before the task began, the experimenter reviewed the procedures with the participants and reminded them which colored square was associated with the threat and safe conditions.

The procedures for the air blast followed the work of one of us (Grillon et al., 1999). The apparatus used for producing the blast of air consisted of a compressed air cylinder, a regulator, and a solenoid that received a signal from the task computer. These items were placed in the control room adjacent to the MRI. A tube was connected to the regulator, passed through the wave guide, and placed approximately 1 cm from the larynx of each participant. The duration of the blast was 10 ms, and the intensity of the air blast was designed to be 60 psi, comparable to Grillon et al. (1999).

The fMRI task consisted of 17 trials. Randomly intermixed among these 17 trials were nine threat trials and eight safe trials. In the third threat trial, a blast of air was delivered to the larynx at the offset of the colored square. This trial was not included in the analysis. The blast of air was delivered only once due to concerns that the aversive stimulus might induce movement. This left eight threat trials and eight safe trials for inclusion in the analysis.

As in other studies of threat or fear conditioning, duration of trials varied from 25 to 39 s. The colored square was presented for 10 s, followed by a 4-s fixation. After fixation, the visual display system presented the following question: “How afraid were you?” on a 1 to 5 scale. During training, participants were instructed to indicate how afraid they had felt while they had been viewing the square moments earlier. The fear-rating screen was presented for 4 s. Following this, there was an intertrial interval with a fixation that was presented for 7 to 21 s. Such variability of the intertrial interval serves to decrease expectancy of the onset of the subsequent trial. Stimuli were displayed on the Avotec Silent Vision Glasses (Stuart, FL), and the five-key button box was developed by MRI Devices (Waukesha, WI).

**Imaging and Data Analysis**

Imaging acquisition was performed on a General Electric Signa 3 Tesla scanner. We acquired a sagittal localizer scan to orient subsequent scans. For functional imaging scans, we used a series of 23 contiguous, 5-mm axial slices covering the entire brain, parallel to the AC–PC. The scans employed an echo-planar single-shot gradient echo T2* weighting (TR = 2000 ms, TE = 40 ms, FOV = 240 mm; 64 x 64 matrix, 3.75-mm voxels). In addition, high-resolution T1-weighted volumetric scans used a magnetization prepared gradient echo sequence (MP-RAGE) [180 1.0-mm sagittal slices; FOV = 256 mm, NEX = 1, TR = 11.4 ms, TE = 4.4 ms; matrix = 256 x 256; TI = 300 ms, bandwidth 130 Hz/pixel =33 kHz for 256 pixels in-plane resolution = 1 mm3].

Functional imaging data were analyzed using SPM (SPM99b, Wellcome Department of Neurology, London, England) (Frackowiak, Friston, Frith, Dolan, & Mazziotta, 1997) with an event-related model. In addition, other routines were implemented with Matlab 5.3 from Mathworks (Natick, MA). For anatomical definitions of the amygdala, we followed established procedures (Szeszko et al., 1999). Functional data were corrected for slice timing and motion, coregistered to the anatomical data, aligned to the first volume for each participant, spatially normalized to a Montreal Neurological Institute (MNI) T1-weighted template image supplied with SPM99, and smoothed with an 8-mm full width at half-maximum Gaussian kernel. Within-subject time series modeling accounted for threat as well as safe conditions, fear rating period, and intertrial interval (rest).

In each condition, the fMRI response to each event type was modeled as a rectangular pulse convolved with the hemodynamic response function specified in SPM99 (default para-
RESULTS

Behavioral Results

The mean rating for fear was 1.92 (SD = .79) to the threat condition and 1.05 (SD = .14) to the safe condition, t(13) = 4.45, p < .002. Since the purpose of this study was to better understand the relationship between fear and amygdala engagement, we focused the analyses on those who reported increased fear to the threat stimulus (fearful group). A participant was selected for the fearful group if the individual reported increased fear to the threat stimulus in more than half of the threat trials. This yielded 8 participants for the fearful group and 6 participants for the no fear or minimally fearful group. The fearful group was comprised of 5 females and 3 males with an age range of 10 to 17 years (M age = 14.25, SD = 2.60); the not fearful/minimally fearful group had 2 females and 4 males with an age range of 14 to 17 years (M age = 15.67, SD = 1.51). The mean rating of fear for the fearful group was 2.52 (SD = .51) to the threat condition and 1.08 (SD = .18) for the safe condition. Of the other 6 participants who reported less fear, only 2 reported any increase in fear to the threat stimulus, and this increase was minimal. The mean rating of fear for those who reported no fear or minimal fear was 1.18 (SD = .28) to the threat condition and 1.02 (SD = .05) for the safe condition.

fMRI Results

Within the fearful group, the contrast of the threat condition relative to rest revealed activation in the right amygdala (Table 1, Figure 1). Furthermore, the contrast of the safe condition compared to rest showed left amygdala activation (Table 1, Figure 2). No significant differences in amygdala activation were found in the group with no or minimal fear even when the p value was relaxed to 0.2. In addition, no significant differences emerged in direct comparisons of the fearful versus no or minimally fearful groups. However, this comparison possesses limited statistical power due to small sample sizes. Therefore, an exploratory analysis was carried out to further evaluate the amygdala engagement between the fearful and no or minimally fearful groups. The unsmoothed voxel activation for each participant from the contrast of the threat and rest conditions was evaluated by group with a t test. The voxel (30 6 –22) that was evaluated was the same one that was found to be significant in the analysis of the fearful participants alone. However, the t test was not protected for the multiple comparisons within the amygdala. These exploratory procedures revealed that the fearful group showed greater right amygdala activation relative to the no or minimally fearful group, t(12) = 2.20, p < 0.05. Figure 3 presents a scatter plot of amygdala activation separated by the fearful and no or minimal fear groups.

While the threat and safe conditions were conceptualized as both containing emotional content, we performed a direct comparison of the threat relative to the safe conditions among the fearful participants to more closely approximate the work of Phelps et al. (2001). In this exploratory analysis, a 3-mm sphere correction was placed around the location of peak activation in the right amygdala from the threat minus rest contrast (voxel coordinate = 30 6 –22), and a significant effect was found, t(12) = 2.03, p < 0.05. Furthermore, this same contrast did not approach significance in the left amygdala. Finally, no gender or age effects were found for amygdala activation in the threat condition.

Table 1. fMRI Activation Among Adolescents Who Reported Increased Fear to the Threat Condition. Coordinates are Reported in Montreal Neurological Institute Space (Collins et al., 1998)

<table>
<thead>
<tr>
<th>Contrast</th>
<th>ROI</th>
<th>x y z</th>
<th>Cluster Size</th>
<th>p Corrected</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threat minus Rest</td>
<td>Right amygdala</td>
<td>30 6 –22</td>
<td>71</td>
<td>.045</td>
<td>2.34</td>
</tr>
<tr>
<td>Safe minus Rest</td>
<td>Left amygdala</td>
<td>–14 2 –16</td>
<td>220</td>
<td>.015</td>
<td>3.48</td>
</tr>
</tbody>
</table>

ROI = region of interest.
DISCUSSION

The purpose of this study was to determine whether subjective fear related to engagement of the amygdala. Participants who reported fear in response to threat revealed activation in the right amygdala during the threat condition. In addition, these participants engaged the left amygdala during the safe condition. In contrast, adolescents who reported no or minimal fear during the threat presentation did not show amygdala engagement to the threat or safe conditions.

The overarching objective of this work is to develop a neuroimaging task that is appropriate for studying juveniles with anxiety disorders. These procedures successfully identified a subgroup of participants who reported experiencing elevated fear; these participants had differential amygdala engagement to the threat and safe stimuli. Nevertheless, the increase in fear, even among those who reported it, was mild. This may carry advantages in terms of the tolerability of this procedure for pediatric clinical populations. People with anxiety disorders have been shown to exhibit greater subjective fear.
levels to threats (Klein & Pine, 2002; Pine et al., 2000), raising concerns about tolerability of more extreme threats. Thus, even though it is anticipated that anxious adolescents will find the threat of the air blast more frightening than the fearful participants in the present study, this approach is expected to still be tolerable.

The results of this study also are relevant to findings on laterality effects of affective processing. Evidence from electrophysiology, neuroimaging, and neuropsychology indicates that positively valenced stimuli are associated with processing in the left hemisphere and negatively valenced stimuli are implicated in processing in the right hemisphere (Canli, Desmond, Zhao, Glover, & Gabrieli, 1998; Davidson, Ekman, Saroiu, Senulis, & Friesen, 1990; Sackeim et al., 1982). Enhanced activation of the left amygdala is related to winning in a game, and right amygdala engagement is linked to losing (Zalla et al., 2000). In addition, the degree of left amygdala activation to happy faces is associated with the personality trait of extroversion (Canli, Sivers, Whitfield, Gotlib, & Gabrieli, 2002). Finally, in a recent developmental study, adolescents showed activation in the right amygdala in response to negatively valenced stimuli, fearful faces (Monk et al., 2003).

Investigations of clinical disorders also suggest that right amygdala activation may relate to negative affect. Posttraumatic stress disorder and social phobia are associated with enhanced right amygdala activation relative to that seen in healthy controls in emotionally engaging tasks (Rauch et al., 1996; Tillfors et al., 2001). Among a group of depressed adults, resting metabolic rate in the right amygdala is positively correlated with negative affect (Abercrombie et al., 1998). Finally, in a study of children with generalized anxiety or panic disorder, those with anxiety showed greater right amygdala activation compared to healthy children (Thomas, Drevets, Dahl, et al., 2001).

However, these laterality findings in the amygdala from healthy participants and patients with mood and anxiety disorders are not entirely consistent (Birbaumer et al., 1998; Blair, Morris, Frith, Perrett, & Dolan, 1999; Hariri et al., 2000; Sheline et al., 2001; Thomas, Drevets, Whalen, et al., 2001). Indeed, as described earlier, threat of shock was associated with increased left amygdala activation among healthy adults (Phelps et al., 2001). This suggests that differences in the procedures may account for the disparity in the results between the present work and that of Phelps et al. For example, differences in participants’ ages across the two studies may account for differences in laterality. Indeed, since pronounced differences in the neurophysiological processing of emotional information have been documented between adolescents and adults (Monk et al., 2003; Thomas, Drevets, Whalen, et al., 2001), one might expect threat tasks to recruit different neural structures in different age groups. In addition, it is possible that threat of a puff of air and threat of shock may be processed differently at a neuroanatomical level. While these stimuli are conceptualized as differing only in degree of aversiveness, the threat of these stimuli may differ qualitatively. The findings in the present study also are inconsistent with some neuroimaging studies using emotional facial expressions with adults. Across a range of procedures, such studies often document bilateral amygdala activation in response to negative emotional expressions (Baird et al., 1999; Hariri et al., 2000; Pessoa, McKenna, Gutierrez, & Ungerleider, 2002; Phillips et al., 2001). However, other studies reported left amygdala activation to negative facial expressions (Blair et al., 1999; Iidaka et al., 2001; Thomas, Drevets, Whalen, et al., 2001). Thus, while the amygdala appears to be engaged during the presentation of affective information, specialization of the left and right amygdalae is not certain.

In summary, these procedures offer a tool to examine neural circuits engaged in the processing of fear among adolescents. Those who reported experiencing fear showed increased right amygdala activation to the threat stimulus and increased left amygdala activation to the safe stimulus. Since these procedures induced only moderate fear in healthy adolescents, it is likely that anxious adolescents will tolerate them. In addition to using these procedures to examine adolescents with anxiety disorders, an important direction for future research will be to extend this work to populations at high risk for anxiety disorders, but who are not currently presenting symptoms. Such work may help uncover how changes in neural function at adolescence relate to both healthy development and risk for psychopathology.

NOTES

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