Methods for Developmental Studies of Fear Conditioning Circuitry

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Psychophysiologic studies use air puff as an aversive stimulus to document abnormal fear conditioning in children of parents with anxiety disorders. This study used functional magnetic resonance imaging (fMRI) to examine changes in amygdala activity during air-puff conditioning among adults. Blood oxygen level–dependent (BOLD) signal was monitored in seven adults during 16 alternating presentations of two different colored lights (CS+ vs. CS−), one of which was consistently paired with an aversive air puff. A region-of-interest analysis demonstrated differential change in BOLD signal in the right but not left amygdala across CS+ versus CS− viewing. The amygdala is engaged by pairing of a light with an air puff. Given that prior studies relate air-puff conditioning to risk for anxiety in children, these methods may provide an avenue for directly studying the developmental neurobiology of fear conditioning. Biol Psychiatry 2001;50: 225–228 *© 2001 Society of Biological Psychiatry*

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Introduction

The amygdala mediates fear conditioning in a range of mammalian species (Davis 1998; Kapp et al 1984; LeDoux 1996). Amygdala involvement in human fear conditioning has been demonstrated in functional magnetic resonance imaging (fMRI) studies and in studies of brain injury (Adolphs et al 1995; Bechara et al 1995; Buchel and Dolan 2000). Clinical research on fear conditioning provides intriguing insights on the nature of risk for anxiety disorders (Gorman et al 2000; Merikangas et al 1999). Because most adult anxiety disorders begin during childhood (Pine et al 1998), there is a great need for research linking adult anxiety disorders to risk factors in children, including perturbations in brain systems regulating fear conditioning. Nevertheless, complications arise in such research, based partly on ethical limitations in the use of unconditioned stimuli (UCS) employed in adult fear conditioning experiments. Grillon et al (1998, 1999) recently used an air-puff UCS to demonstrate enhanced fear conditioning in children of parents with an anxiety disorder. These data suggest that aspects of amygdala function may index childhood risk for anxiety.

Recent studies in rodents have begun to implicate genetic factors in amygdala-based functions, including fear conditioning (Rogan et al 1997; Tang et al 1999). Taken together, available fear conditioning data in rodents and humans raise questions on the relationship between genetic and neurophysiologic risk factors for anxiety in humans. As such, these data might encourage efforts to study the nature of risk for anxiety by conducting comparable fear conditioning experiments across species and across developmental periods (Merikangas et al 1999). Efforts to conduct such integrative studies will benefit from research examining the degree to which the human amygdala is engaged by conditioning paradigms acceptable for use in children. Our study examines changes in amygdala activity among adults during light conditioned stimulus (CS) and air-puff UCS methods from Grillon et al (1998, 1999).

Methods and Materials

Subjects

Seven right-handed volunteers (four men, three women) were recruited from medical center advertisements and from pools of volunteers participating in prior studies. All subjects provided consent and were screened for medical, neurologic, or mental conditions. Subjects were 33.6 ± 4.3 years old (range 23–46).

Conditioning Procedures

Blood oxygen level dependent (BOLD) signal was compared across 16 conditioning trials, including eight alternating trials

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Table 1. Mean BOLD Signal Intensity of Right and Left Amygdala during Conditioning

	Trial number							
	1	2	3	4	5	6	7	8
Right amygdala [$F(7, 1200) = 2.48$, $p < .02$.]								
CS+	711.86	714.38	711.34	709.00	706.93	704.10	701.73	703.50
CS−	712.04	712.92	711.90	709.63	708.65	706.01	703.63	703.00
Left amygdala [$F(7, 1200) = 1.50$. $p < .16$.]								
CS+	607.89	607.03	609.95	609.53	608.72	606.89	606.42	606.29
CS−	606.21	609.07	611.30	610.05	609.22	607.50	607.51	605.84

BOLD, blood oxygen level–dependent.

with either conditioned or with unconditioned stimulus presentation (CS+ vs. CS−). Conditioned and unconditioned stimuli were one of two different colored lights. One colored light was randomly selected to be the conditioned stimulus, and the other was selected to be the unconditioned stimulus. Stimuli were presented within the context of a virtual reality city used in previous imaging studies on spatial navigation (Maguire et al 1998). Subjects navigated across 16 trials between two city locations, where either a CS+ or CS− appeared. The virtual-reality-based format was developed for two reasons. First, because subjects were required to navigate after each trial, the procedures engaged subjects and allowed the research team to continuously monitor their engagement. In general, such features facilitate fMRI research with juveniles. Second, if conditioning could be demonstrated in this environment, this might lead to future experiments that use virtual reality to systematically manipulate characteristics of both the CS and the experimental context.

Subjects navigated using a computer mouse pad between locations after each conditioning trial, during which time fMRI data were not acquired. Upon arrival at one or the other virtual reality location, subjects stopped navigating and fMRI data collection commenced. Each conditioning trial lasted 30 sec, with 10-sec CS presentation and 20 sec when subjects remained motionless in the virtual reality location with no CS. The precise timing of the 10-sec CS window within the 30-sec trial varied randomly across the 16 trials. The eight presentations of the CS+, but not the CS−, coterminated with an air-puff UCS. Pressure at the throat was matched to that in Grillon et al (1999), and the UCS was delivered for 10 msec. The choice of CS color and order was counter-balanced across subjects. Because the last scan in each CS+ trial was acquired immediately before the UCS, scans acquired during CS+ presentation reflect brain activity during anticipation of the UCS as opposed to delivery of the UCS.

Functional Magnetic Resonance Imaging Procedures

All subjects were scanned in a 1.5 T Siemens MRI scanner. A set of 30 functional scans was acquired during each of the 16 conditioning trials. These scans consisted of 10 contiguous 5-mm interleaved coronal slices, positioned perpendicular to the AC–PC line and centered on the amygdala. The scans used a 64 × 64 matrix with echoplanar single shot gradient echo T2* weighting (TR = 1000 m/sec; TE = 40 m/sec; 90° flip; FOV = 200 mm; 3.125 × 3.125 × 5 mm voxels). This approach, as opposed to a whole-brain approach, was chosen to maximize the number of coronal, amygdala-centered scans. A high-resolution T1-weighted whole-brain volumetric scan also was acquired using a magnetization prepared gradient echo sequence. This used 180 1.0 mm sagittal slices (FOV = 200 mm, NEX = 1, TR = 11.4 m/sec, TE = 4.4 m/sec; matrix = 128 × 128; TI = 300 m/sec, bandwidth 130 HZ/pixel).

Data Analysis

Data sets of BOLD images were aligned to the mean volume for each subject, and intensity of BOLD signal was compared using a region-of-interest approach across 80 scans acquired during the presentations of CS+ and CS−. The amygdala was delineated on each subject's high-resolution scan using criteria from Szeszko et al (1999) and Bogerts et al (1993). High-resolution scans were coregistered with each subject's series of BOLD scans. Mean values of BOLD activity in each amygdala were determined, and changes in mean amygdala BOLD signal were analyzed using a mixed effects linear model, with CS presentation (CS+ vs. CS−), order (starting with CS+ vs. starting with CS−), as well as time (10 data points per CS trial) treated as fixed effects and person treated as a random effect (Gibbons et al 1993). Conditioning was evaluated by tests of the CS-by-trial and CS-by-trial-by-time interactions. Data for the right and left amygdala were analyzed individually, based on data from prior studies, including LaBar et al (1998), which used methods similar to those in our study. These prior studies suggest that conditioning-related amygdala activations may be biased toward one or the other hemisphere (Buchel and Dolan 2000; Furmark et al 1997; Morris et al 1998).

Results

No evidence of CS type-by-trial-by-time interactions or order effects emerged. Table 1 presents mean BOLD signal in the right and left amygdala during each of the 16 conditioning trials across the seven subjects. As shown in the table, there was a significant CS type-by-trial interaction in the right [$F(7, 1200) = 2.48$; $p < .02$] but not left amygdala [$F(7, 1200) = 1.50$; $p = .16$]. Post hoc linear models examined the nature of this interaction. These

models showed that the interaction reflected a greater cross-trial decrease in BOLD signal for CS+ than CS− trials between trials 2 and 5 ($t_{1200} = 2.9$; $p < .005$), trials 2 and 6 ($t_{1200} = 3.1$; $p < .005$), as well as trials 2 and 7 ($t_{1200} = 3.1$; $p < .005$).

Discussion

Our study assessed changes in BOLD signal activity during a series of CS+–CS− presentations. Across-time changes in right amygdala signal intensity differed in CS+ versus CS− trials, due to a somewhat greater increase between the first and second CS+ trial followed by greater decreases between the second and later trials. These differential across-time changes within a region of interest, documented in a linear model, are consistent with fMRI conditioning data from other studies using voxelwise statistical approaches. Namely, prior fMRI studies found early increases in amygdala BOLD signal during initial CS+ presentations, followed by decreases, reflecting amygdala deactivation, during later CS+ presentations (Buchel et al 1998, 1999). This pattern of transient amygdala responses in fMRI experiments is thought to reflect rapid habituation to predictable stimuli within amygdala-based circuits (Buchel and Dolan 2000; LeDoux 1996).

Specific engagement of the right amygdala in our report is consistent with LaBar et al (1998), who found rightward-biased amygdala activation during light-CS–shock-UCS conditioning, and with Furmark et al (1997), who found correlations between electrodermal fluctuations and right amygdala activity during conditioning. Each finding is consistent with other evidence of lateralization in neural contributions to emotional processes. For example, Davidson et al (1999) noted consistent rightward bias in brain systems mediating withdrawal from potentially dangerous stimuli. Alternatively, Morris et al (1998) suggested that rightward bias characterizes brain systems mediating conditioning-related processes where verbal functions are not critical.

Our study is unique in at least two respects. First, we used air-puff and light conditioning techniques explicitly developed for psychophysiologic research with children. Using these methods, Grillon et al (1998) demonstrated enhanced fear-potentiated startle in children at high versus low familial risk for anxiety disorders. This psychophysiological difference was hypothesized to result from differences in amygdala function, and the current study provides direct evidence of amygdala involvement in air-puff/light CS conditioning. Second, the current study used a virtual reality format to present conditioned stimuli, since virtual reality may engage children and provide considerable flexibility in future studies of conditioning. For example, the documentation of differential amygdala involvement might encourage future studies that manipulate various aspects of both cue and contextual conditioned stimuli. This may be particularly important given the possibility that clinical factors may show differential associations with conditioned cues as opposed to contexts (Davis 1998; Grillon et al 1999).

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