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Functional MRI of Human Amygdala Activity During Pavlovian Fear Conditioning: Stimulus Processing Versus Response Expression

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Although laboratory animal studies have shown that the amygdala plays multiple roles in conditional fear, less is known about the human amygdala. Human subjects were trained in a Pavlovian fear conditioning paradigm during functional magnetic resonance imaging (fMRI). Brain activity maps correlated with reference waveforms representing the temporal pattern of visual conditional stimuli (CSs) and subject-derived autonomic responses were compared. Subjects receiving paired CS–shock presentations showed greater amygdala activity than subjects receiving unpaired CS–shock presentations when their brain activity was correlated with a waveform generated from their behavioral responses. Stimulus-based waveforms revealed learning differences in the visual cortex, but not in the amygdala. These data support the view that the amygdala is important for the expression of learned behavioral responses during Pavlovian fear conditioning.

The amygdala is a crucial structure in Pavlovian fear conditioning and other forms of emotional expression (Davis, 1997; LeDoux, 1995, 2000). This structure appears to be a critical component in a distributed network essential for associating emotional responses and environmental stimuli. Although the majority of the data on the amygdala's role in fear conditioning come from laboratory animal studies, observations of patients with damage to this region suggest that it plays a similar, if not identical, role in humans expressing autonomic correlates of fear (Aggleton, 1992; Bechara et al., 1995; LaBar, LeDoux, Spencer, & Phelps, 1995).

Functional neuroimaging techniques have been used to describe the amygdala's contributions to emotion and memory in healthy humans. Initial positron emission tomography (PET) studies using Pavlovian fear conditioning demonstrated differential activation in the orbitofrontal cortex, anterior and posterior cingulate cortices, and visual cortices, but not the amygdala (Fredrikson, Wik, Fischer, & Andersson, 1995; Hugdahl et al., 1995). However, right amygdala activity as inferred with PET was better correlated with performance during recall of emotionally arousing material compared to non-emotionally arousing stimuli (Cahill et al., 1996). These data suggest the amygdala is involved in processing emotional information but do not directly address its role in human fear conditioning. The limited temporal and spatial resolution of PET may be one explanation for why differences in amygdala activity during fear conditioning were difficult to observe with this technique.

Detailed analysis of the amygdala with functional imaging has been challenging because of its size and location. Functional magnetic resonance imaging (fMRI) has recently received increased use in neuroimaging studies because of its excellent spatial and temporal resolution and the ability to produce images without using radiolabeled substances (Cohen & Bookheimer, 1994). Several laboratories have successfully detected changes in blood flow within the amygdala using fMRI (Büchel, Morris, Dolan, & Friston, 1998; Canli, Zhao, Desmond, Glover, & Gabrieli, 1999; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; Whalen et al., 1998). Although learning-related differential amygdala activity has been demonstrated during Pavlovian fear conditioning (e.g., Büchel et al., 1998; LaBar et al., 1998), other fMRI studies of fear conditioning have not detected changes within the amygdala (e.g., Knight, Smith, Stein, & Helmstetter, 1999; Smith, Knight, Cheng, Stein, & Helmstetter, 1998).

This discrepancy may be due in part to different experimental designs and data analysis techniques. A range of approaches to fMRI data analysis is currently used, including various forms of multiple regression, subtraction techniques, and cross-correlation methods (Bandettini, Jesmanowicz, Wong, & Hyde, 1993; Cahill et al., 1996; Courtney, Petit, Maisog, Ungerleider, & Haxby, 1998; LaBar et al., 1998). LaBar et al. (1998) used a differential fear

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conditioning paradigm and were able to demonstrate activity in the amygdala with a double subtraction method in which baselines (pre-conditional stimulus [CS] resting periods) were first subtracted from CS+ and CS− periods followed by a second subtraction of stimulus-evoked responses from each other (CS+ minus CS−). In a between-subjects design, Knight et al. (1999) used a cross-correlation approach in which fMRI signal amplitude and an ideal reference vector representing the temporal pattern of visual stimuli within training blocks were compared. Although the latter study showed that a number of brain structures showed clear changes in patterns of activity during acquisition, differential responses in the amygdala were not detected.

Laboratory animal studies have established that the amygdala is important not only for learning the relationship between the CS and unconditional stimulus (UCS), but also for response expression during fear conditioning as well as during nonassociative aversive experiences (Bellgowan & Helmstetter, 1996; Helmstetter, 1992; Helmstetter & Bellgowan, 1994; LeDoux, 1995; Maren, 1999). The amygdala's roles in forming associations between CS and UCS and in generating emotional responses appear to be mediated through different amygdaloid nuclei. The lateral and basolateral nuclei are thought to be critical for the acquisition of fear responses, whereas the central nucleus and its diverse projections to the hypothalamus, central gray, and other brainstem regions are important for the expression of autonomic and behavioral reactions used as indices of learning in this paradigm (Campeau & Davis, 1995; Davis, 1997; LeDoux, Iwata, Cicchetti, & Reis, 1988). Although current imaging techniques are often unable to anatomically dissociate different nuclei in the amygdala, investigation of the amygdala's multiple roles in fear conditioning is possible.

Metabolic signals changing within the amygdala during exposure to the procedures used in fear conditioning may be related to one or more of a variety of processes including alterations in attention (Holland & Gallagher, 1999; Knight, Smith, Cheng, Stein, & Helmstetter, 2001), recognition of environmental signals related to emotion (Whalen et al., 1998), formation of associative links between the CS and UCS (Bailey, Kim, Sun, Thompson & Helmstetter, 1999; Maren, 1999), modulatory influences on the encoding of this new information (Cahill et al., 1996), or the autonomic or behavioral expression of the conditional response (CR). The present study used fMRI to more closely examine the relationship of dynamic changes in activity within the amygdala to its role in the expression of conditional fear by correlating multiple reference functions with amygdala activity. Statistical brain maps generated by cross-correlation were compared by using three reference vectors: an ideal stimulus waveform representing the CS (light), a hemodynamically convolved waveform based on an empirical model of the brain blood flow response (Cohen, 1997) to the CS (light), and a waveform that represented subjects' individual galvanic skin response (GSR) reflecting CRs expressed during training.

Method

Subjects

Participants in this study consisted of 20 healthy, right-handed subjects (8 men, 12 women) ranging from 18 to 39 years of age ($M = 24.85 \pm 5.51$ years). Handedness was defined by the Edinberg Inventory (Oldfield, 1971). Volunteers were paid $6/hr and offered extra credit in their psychology courses. Informed consent was obtained from all subjects. All procedures were approved by the Institutional Review Boards for human subject research at both the Medical College of Wisconsin and the University of Wisconsin—Milwaukee.

Apparatus

Scanner

Whole brain imaging was performed on a GE 1.5 Tesla Signa scanner with a multislice gradient-echo, echo-planar pulse sequence. Contiguous 8-mm sagittal slices were collected (TR = 3 s, TE = 40 ms, FOV = 24 cm, flip angle = 90°) in a series of 86 sequential images (258 s) during each block of stimulus presentations. High-resolution spoiled gradient recalled acquisition at steady state (SPGR) anatomical images were also obtained, over which echoplanar (functional) images were superimposed.

GSR

A J&J thermal monitoring system (Model T-68) with GSR Preamp (Model IG-3) were used to monitor the subject's skin conductance throughout the study (Bio-Medical Instruments, Warren, MI). A pair of surface cup electrodes (silver/silver chloride, 1 cm diameter; Beckman Instruments, Fullerton, CA) filled with electrolyte gel (Teca Corp., Pleasantville, NY) were attached 2 cm apart to the sole of the subject's left foot. Skin conductance data were digitized and stored continuously at 250 Hz with Asyst software (Version 3.10, Rochester, NY) and stored on an IBM compatible computer.

Electrical Stimulus

The UCS was electrical stimulation presented by means of a custom-made AC (60 Hz) source through two aluminum surface electrodes (2 cm diameter) positioned over the right tibial nerve over the right medial malleolus. Subjects were instructed to rate the level of electrical stimulation on a scale from 0 to 5 (0 = *no sensation*, 5 = *painful, but tolerable*) prior to training. The stimulus intensity that elicited a subjective report of 5 was presented intermittently throughout the study. The maximum possible current used for the UCS was 7.5 mA.

Visual Stimulus

The CS was a flashing (0.5 Hz) red 25-W light bulb housed in a black plastic fixture and mounted on a wooden frame 5.5 m from the scanner. The subject viewed the visual stimulus from a supine position through prismatic glasses attached to the radio frequency coil. Lights in the scanning room were turned off to reduce extraneous visual stimulation. A small LED situated 16 cm below the CS served as a constant fixation point for the subject. Timing and presentation of the visual and electrical stimuli were controlled by an IBM compatible computer and interface using custom software.

Procedure

Scanning Procedure

Subjects underwent an anatomical scan lasting approximately 20 min in the absence of visual and electrical stimulation. After this scan, subjects were randomly assigned to one of two groups: paired ($n = 10$) or control ($n = 10$). All subjects were exposed to four blocks (five trials per block) of CS−UCS presentations while functional images were obtained. The paired group received CS (15-s duration) and UCS presentations (0.5-s duration) that coterminated. The control group received explicitly unpaired

UCS presentations 15 s after termination of the CS. The intertrial interval (ITI) for both groups was 30 s.

Data Analysis

Behavioral data. We focused on the second interval response (SIR) after CS presentation as an index of CR amplitude. The SIR is generally considered to be an emotional anticipatory response to the predicted UCS and should accurately reflect subjects learning the relationship between the CS and the UCS (Prokasy & Raskin, 1973; Wolter & Lachnit, 1993). Behavioral analysis used the SIR evoked by CS presentations on Block 1. The SIR was defined as the peak skin conductance response (average of 1-s sample) during the final 5 s of the CS presentation and was compared with a baseline value defined as the average skin conductance response of the subject during the 5 s prior to CS onset. These results are reported as a percentage change from baseline.

Imaging data. In-plane motion correction and edge-detection algorithms were applied to the functional dataset to correct for minor movement artifacts. Functional images were registered onto high-resolution anatomical images (SPGR) by using Analysis of Functional NeuroImages (AFNI) software (Cox, 1996). Functional images were generated by a cross-correlation of the subjects' fMRI signal with one of three reference vectors. For each voxel, a correlation coefficient was calculated, indicating the strength of relationship between the subjects' BOLD signal and the target reference function.

An ideal stimulus reference waveform (see Figure 1A) representing the temporal pattern of visual stimulation served as one reference function. On-periods coincided with CS presentations, and off-periods coincided with baseline (no visual stimulation). To account for the predicted delay between stimulus onset and fMRI response, the waveform was shifted one image (3 s) forward in time. This phase shift was based on prior work from our laboratory (e.g., Knight et al., 1999) and the temporal features of cerebral blood flow (Kwong et al., 1992).

A second reference function used in the analysis was a convolved stimulus waveform (see Figure 1B). We used a hemodynamic model based on Cohen (1997), modified to include poststimulus undershoots, that is part of the AFNI distribution (Cox, 1996). The convolution was performed to account for two features of cerebral blood flow: time delay and dispersion. *Time delay* is the latency between neural activation and oxygen delivery through increased cerebral blood flow, and *dispersion* represents the temporal flattening of the hemodynamic response (Rajapakse, Kruggel, Maisog, & Cramon, 1998). A manual phase shift was not applied because this model takes the hemodynamic delay into account.

The final reference function used was based on each individual subject's GSR (see Figure 1C). The raw values recorded during Block 1 of training were utilized in a cross-correlation analysis identical to that used with the stimulus-based waveforms. The latency of the subjects' GSR to the CS and UCS is comparable to that of the hemodynamic delay (Kwong et al., 1992; Lim et al., 1999; Prokasy & Raskin, 1973), and therefore the reference function was not temporally phase shifted.

For all three reference functions, four images (12 s) after each UCS presentation were omitted in the cross-correlation analysis to account for potential UCS artifacts. The 12-s period was selected on the basis of the average amount of time (10 s) needed for the subjects' GSR to return to baseline after the UCS was presented.

Functional and anatomical images for each participant were transformed into stereotaxic coordinate space relative to the line between the anterior and posterior commissures (Talairach & Tournoux, 1988). To best compensate for anatomical variability between subjects, a 3-mm Gaussian blur was performed on each subject's functional data set after transformation to common coordinate space. Finally, *t* test comparisons were performed on correlation coefficients between the paired and control groups during Block 1.

Results from the *t* test comparisons were used in a voxel-based analysis. Functional maps with an associated *p* value of .01 were used as a mask to sample correlation coefficients from the paired and control groups.

Although whole-brain imaging was performed, our analysis focused primarily on the amygdala. In addition, Block 1 was selected for analysis on the basis of prior studies observing amygdala activity early, but not late,

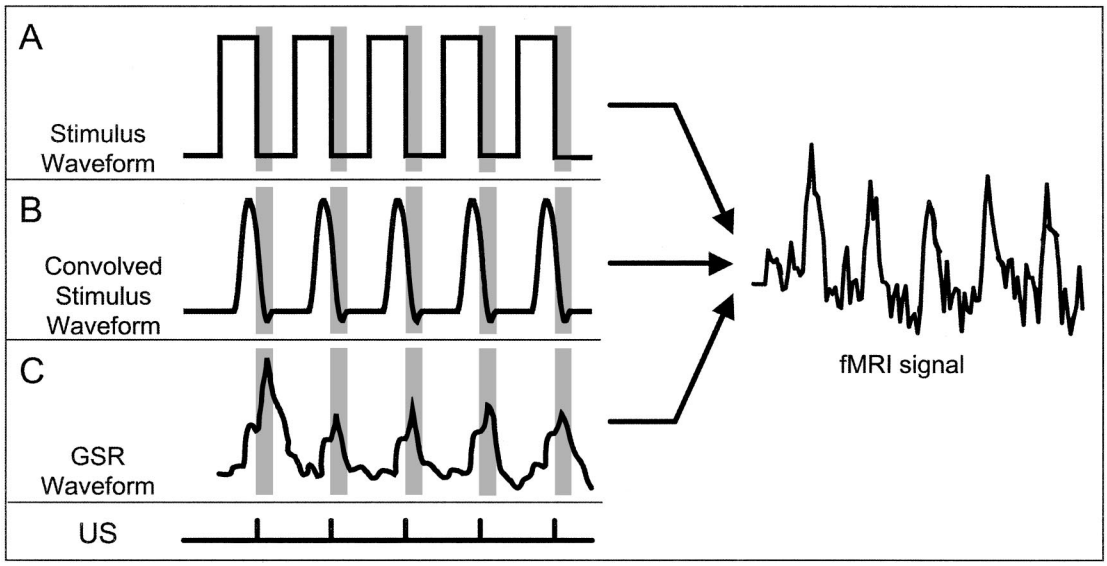

Figure 1. Three reference waveforms used in identical cross-correlation analyses. A: A conditional stimulus (CS)-derived waveform representing the temporal pattern of visual stimulation during training. B: A CS-derived waveform that has been convolved with a model of the predicted hemodynamic response. C: Sample individual galvanic skin response (GSR) waveform. Shaded areas indicate sections of the functional time series excluded from the cross-correlation analysis to account for unconditional stimulus (US) artifacts. fMRI = functional magnetic resonance imaging.

in acquisition (Büchel et al., 1998; LaBar et al., 1998). The visual cortex was selected a priori for comparison on the basis of previous results from our laboratory that indicated a strong learning-related evoked response corresponding to the pattern of visual CS presentation (Knight et al., 1999).

Results

Analysis of the GSR data indicated that subjects demonstrated learning-related changes during training. Although group differences were not significant on Trials 1 and 2, $t(18) = 0.04$, $p > .05$, paired subjects demonstrated significantly larger CRs than controls on Trials 4 and 5, $t(18) = 3.36$, $p < .01$. Figure 2 shows the mean CR amplitude for each group over the five acquisition trials.

As can be seen in the figure, subjects in the paired group maintained or slightly increased their autonomic response amplitude over trials, whereas control group subjects showed initially large evoked responses followed by a gradual decrease in amplitude over trials. Baseline skin conductance recorded during the 5 s prior to CS onset did not differ between the groups on any of the trials, *t*s(18) = 0.71–1.02, *p*s > .05.

Figure 3 shows the pattern of activation in two brain regions of interest that show differences in stimulus- versus response-based activity. Warm colors (positive *t* statistics) denote voxels in the paired group that have significantly higher correlation values than voxels in the control group. The cool colors (negative *t* statistics) signify voxels in the control group that possess higher correlation values than voxels in the paired group.

Similar general patterns of activation were noted when comparing statistical maps of the ideal stimulus reference vector and the convolved stimulus reference function throughout the brain. For example, paired subjects demonstrated a significantly stronger correlation between their fMRI signal and the ideal stimulus reference vector, $t(18) = 3.03$, $p < .01$, and the convolved stimulus reference function, $t(18) = 2.95$, $p < .01$, in the right middle occipital gyrus (Talairach coordinates: 28, −95, 4) relative to controls. This pattern of activation in the middle occipital gyrus was not observed with the GSR reference waveform (see top of Figure 3).

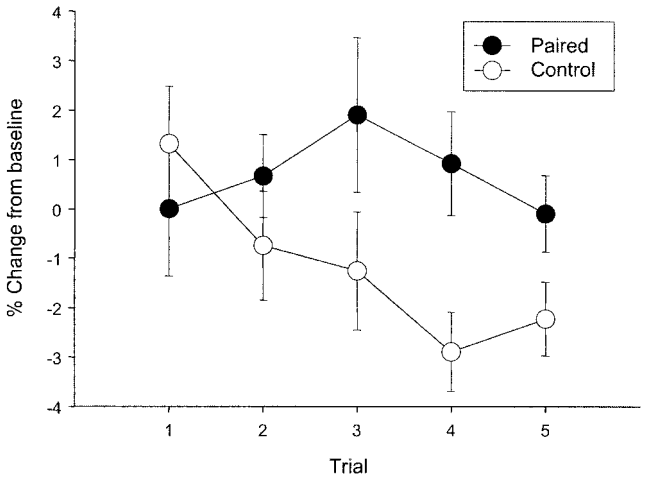

Figure 2. Conditional stimulus-evoked galvanic skin response of paired and control groups during training. Differences on Trials 4 and 5 reflect significant training-related changes.

A different pattern of activation was detected with the GSR reference waveform. The paired group showed significantly stronger right amygdala activity (Talairach coordinates: 24, −9, −16) than the control group when GSR was used ($t = 2.88$, $p < .01$). Differences in amygdala activity were not seen when the ideal stimulus or convolved stimulus waveforms were used (see bottom of Figure 3).

Active areas were used as functional regions of interests (ROI) to calculate the average correlation coefficients for the paired and control groups. Figure 4 shows the averaged correlation values for the paired and control groups for each ROI. Voxels within the functional mask for the middle occipital gyrus revealed differentiation between the paired and control group only when the reference function used was either the ideal visual stimulus (paired: $M = 0.20 \pm 0.02$, control: $M = 0.06 \pm 0.01$) or the convolved visual stimulus (paired: $M = 0.18 \pm 0.02$, control: $M = 0.05 \pm 0.02$), and not the GSR waveform (paired: $M = -0.02 \pm 0.04$, control: $M = 0.01 \pm 0.02$). For each of the two stimulus-derived waveforms, paired subjects showed larger positive correlations in visual cortex (see top series on Figure 4). When selecting tissue within the amygdala, differential responding was observed between paired and control groups only when correlation values based on GSR reference functions were used (paired: $M = 0.11 \pm 0.04$; control: $M = -0.06 \pm 0.04$) and not values based on ideal stimulus (paired: $M = 0.02 \pm 0.03$, control: $M = 0.01 \pm 0.03$) or convolved stimulus (paired: $M = 0.01 \pm 0.02$, control: $M = 0.02 \pm 0.03$) reference functions (see bottom series on Figure 4).

Discussion

The neural circuits subserving Pavlovian fear conditioning include brain regions that process environmental danger signals and those that control the expression of autonomic and behavioral fear responses. Using fMRI, we were able to demonstrate clear patterns of activation that may reflect brain activity related to learning-induced changes in stimulus processing versus expression of behavioral responses.

Subjects receiving paired CS−UCS training showed larger amplitude autonomic responses during the five training trials than did unpaired controls (Figure 2). Although differential responding between the groups was clearly observed, the significant separation was due to a slight increase in the paired group and a trial-related decrease in the control group. There are at least two processes that may have contributed to this response pattern. Our subjects were not given CS-alone presentations during a habituation phase prior to conditioning as is often done in this type of study. We chose this approach because of the potential problems in interpreting brain activation due to detection of an altered contingency (as when the now familiar nonreinforced CS begins to signal shock at the onset of the acquisition phase), as opposed to activity uniquely driven by the acquisition process itself. Novel stimuli can elicit "orienting" responses which gradually diminish when evoked in the absence of the UCS. One important index of an organism's encoding of the predictive relationship between CS and UCS is the preservation or maintenance of these CS-evoked responses as a function of learning (Holland, 1984; Stern & Walrath, 1977). As was the case in the present behavioral data, this account predicts that responses in the unpaired group should gradually decrease, whereas in the paired condition, CR magnitude increases

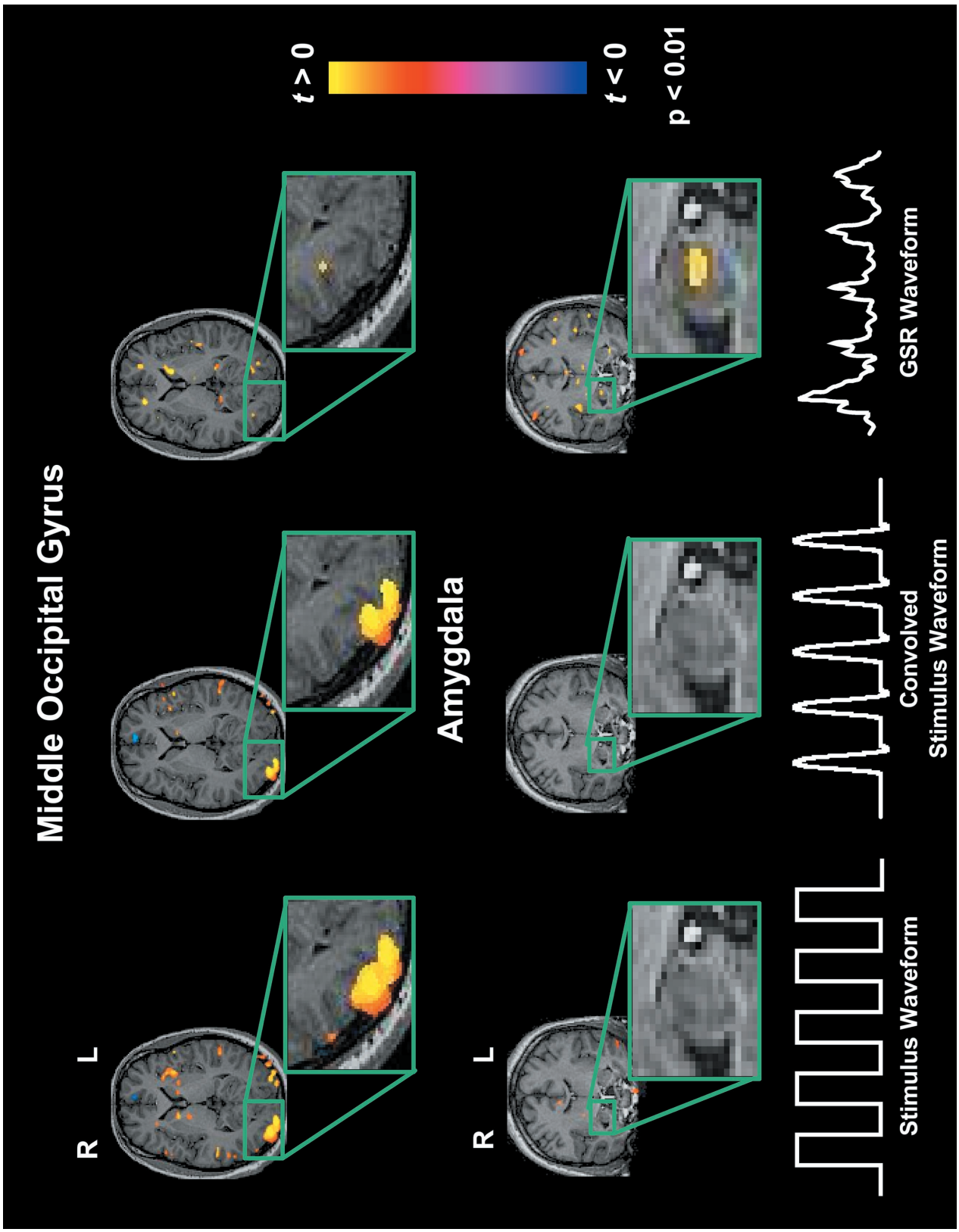

Figure 3. Unilateral patterns of activation detected with each of the three waveforms (ideal stimulus, convolved stimulus, and individual galvanic skin response [GSR]) in a cross-correlation analysis. The right middle occipital gyrus (28, −95, 4; top) of subjects receiving paired presentations responded best to the stimulus canonicals, whereas the right amygdala (24, −9, −16; bottom) of subjects receiving paired presentations responded best to the GSR waveform.

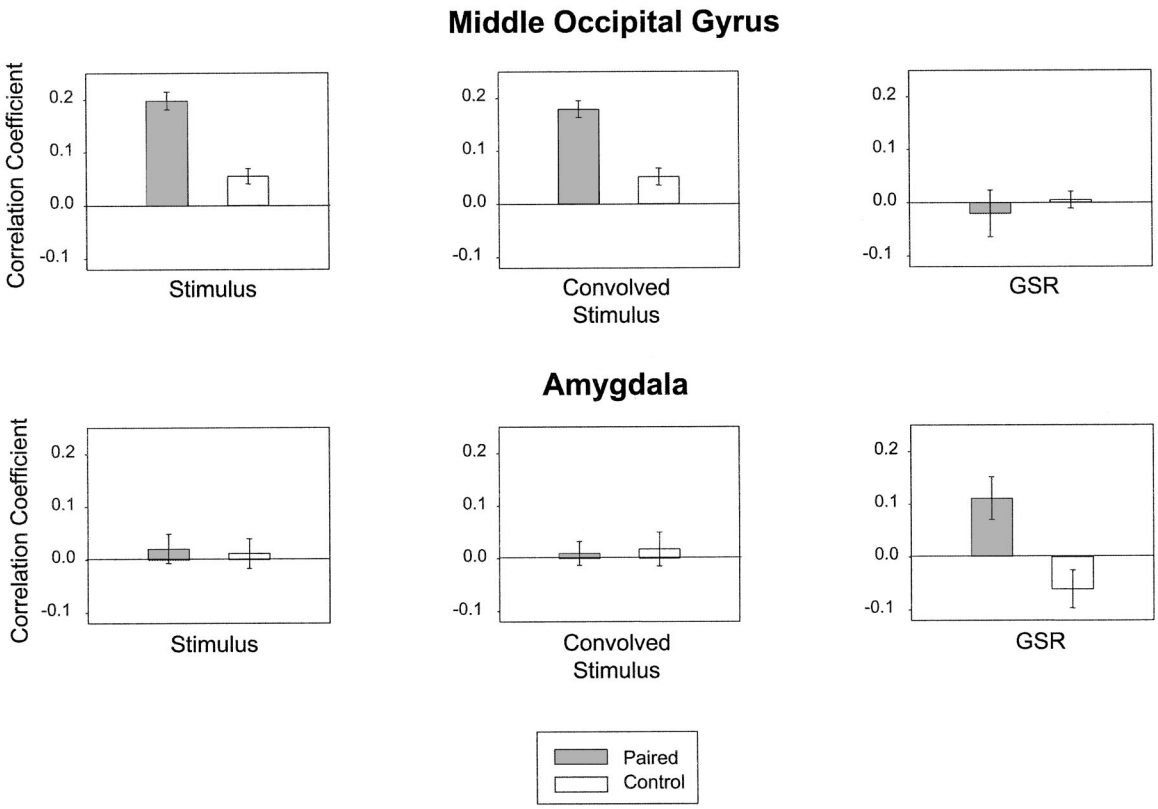

Figure 4. Averaged correlation coefficients sampled in a voxel-based analysis using functional *t* maps. Differences in the visual cortex were a result of the paired group's BOLD responses being more positively correlated with visual conditional stimulus presentations than the control group's (top). Amygdala differences were a result of both positive correlation in the paired group and negative correlation in the control group (bottom). GSR = galvanic skin response.

or is maintained at the initial level (see Figure 2). It is also possible that some of the difference between the paired and control subjects' GSR was related to the subjects in the control condition learning that the CS was a valid predictor of the nonoccurrence of shock. The possibility that the CS served as a "safety signal" in the unpaired condition is supported by the observation that, by the end of training, these subjects showed large decreases in GSR relative to pre-CS baseline, even though baseline values did not differ significantly between the groups.

In the visual cortex, differences in evoked activity produced by paired versus unpaired training were readily detected through comparisons of time-dependent BOLD variation with waveforms coding for presentation of the visual CS.

Significant differential activity between paired and control subjects was a function of the paired subjects demonstrating a larger increase in the magnitude of their correlation values relative to the control subjects (Figure 4). Whereas simple exposure to visual stimuli results in large-amplitude cortical BOLD responses (e.g., DeYoe et al., 1996), the significantly greater correlation observed in subjects given paired training may represent a selective modification of cortical processing of stimuli that signal a biologically significant UCS (Knight et al., 1999), comparable to that seen in other sensory systems during aversive Pavlovian conditioning (e.g., Bakin & Weinberger, 1990; Fredrikson et al., 1995; Weinberger, 1998). This result suggests preferential, or at least significantly altered, processing of environmental signals based on their acquired predictive value.

Conditioning-related activity in the amygdala was better predicted by a response-derived function in the same subjects that simultaneously showed plasticity in the visual cortex, as described above. Significant differences in the amygdala were driven by an increase in correlation values in the paired subjects as well as by a decrease in correlation values in the control subjects. This finding may indicate that alterations in metabolic activity in the amygdala are more closely related to efferent control of the autonomic nervous system than to perceptual or evaluative processing of visual signals for shock. Our finding bidirectional changes in correlation magnitude related to stimuli that reliably signal the occurrence or nonoccurrence of shock is particularly interesting given recent electrophysiological data showing that unit responses recorded in the lateral amygdala show a somewhat similar pattern during fear conditioning (Collins & Pare, 2000).

Relative insensitivity to conditioning-related differences in the amygdala when using the CS-derived reference function was expected on the basis of previous experiments from our laboratory (Cheng, Knight, Smith, Stein, & Helmstetter, 1998; Knight et al.,

1999). However, it is clear that learning-related changes in the amygdala can be detected by using analyses that are linked primarily to presentation of the CS, as has been shown repeatedly (Büchel et al., 1998; Knight et al., 2001; LaBar et al., 1998). At least two issues should be considered when evaluating the differential sensitivity of these approaches. First, it is clear that after successful acquisition, the temporal pattern of CS presentation and CR production should be closely related. If the timing and amplitude of the CR is completely predicted by the CS, then the ability to detect differences in the amygdala should not differ, even if the exclusive role of this structure was to generate GSR responses to emotionally relevant stimuli. Second, a large body of data support the idea that the amygdala cannot be considered functionally homogeneous (Aggleton, 1992). Processes directly related to either CS processing or CR expression can occur simultaneously and may be differentially detected, depending on the approach to analyzing imaging data.

One would certainly expect the physiological processes underlying BOLD signals to display a greater degree of covariation with a subject-derived response such as GSR compared with the simple square wave describing CS presentations. For this reason, we convolved the stimulus canonical to better reflect the time delay and dispersion characteristics of the predicted hemodynamic response (Cohen, 1997; Rajapakse et al., 1998). Consequently, similar patterns of activation were demonstrated for the square and convolved CS waveforms, and although the convolved waveform shares some physiologically relevant components of the GSR reference function, it (similar to the square waveform) also was unable to account for a significant amount of learning-related variance within the amygdala.

Furthermore, the amygdala activation observed in this study cannot be attributed to simple covariation in physiological signals generated by the same subject since our control group's correlation values were calculated identically by using their GSR waveforms. Activity in the control group should have correlated equally as well as in the paired group if the differences were not learning-related. Critchley, Elliot, Mathias, and Dolan (2000) recently investigated the functional neuroanatomy related to the generation of spontaneous GSR fluctuations and did not find amygdala activity covarying with this autonomic response. Furthermore, patients can exhibit intact skin conductance responding after bilateral amygdala damage (Tranel & Damasio, 1989; Tranel & Damasio, 1993). This evidence suggests that the amygdala is not a necessary structure for the generation and maintenance of GSR and that the differences reported here do not reflect nonspecific physiological covariance or arousal, but rather that activity in the amygdala contributes to the production of conditioned emotional autonomic responses. This conclusion is supported by other studies that found autonomic CR amplitude to be directly related to amygdala metabolic activity (e.g., LaBar et al., 1998). Differences between waveform comparisons due to general covariation between GSR and BOLD should also have been reflected throughout the entire brain, and data from the middle occipital gyrus clearly do not support this alternative.

Although it is clear that the amygdala subserves multiple functions during fear conditioning, including active roles in both learning and performance of aversive CRs (Helmstetter, 1992; Helmstetter & Bellgowan, 1994), as well as processing "emotional" stimuli (Phelps & Anderson, 1997), the present data support the idea that patterns of activity in amygdala neurons as detected by fMRI during acquisition may be more closely related to the generation of aversive CRs. It is tempting to interpret these findings as support for the amygdala being crucial only for the expression of CRs and not for other roles during acquisition. However, it is possible that transient responses of amygdala neurons during processing, which are necessary for acquisition, preclude detection using fMRI. For example, Quirk et al. (1997) found that amygdala cells primarily respond only in the first few milliseconds of the first few CS presentations during acquisition and then decrease to baseline response levels. This lack of significant sustained activity would make fMRI differences difficult to observe.

Because previous neuroimaging studies on the acquisition of fear conditioning have yielded conflicting results, the present approach was developed to better understand the multiple potential roles of the amygdala during Pavlovian fear conditioning. The present study represents a first step in the comparison of response- versus stimulus-derived analyses of imaging data during fear conditioning. More recent work in our lab using event-related fMRI has shown that activity within the amygdala is not generally related to autonomic nervous system activity or arousal, but is specific to autonomic responses that uniquely occur in the presence of a CS+ (Cheng, Knight, Smith, Stein, & Helmstetter, 2002). Future work should include an extension of this approach to some of the other autonomic, cognitive, and behavioral responses that are reliably expressed during this form of learning, as well as to additional brain structures related to fear conditioning.

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