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Neural substrates of explicit and implicit fear memory

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Abstract

Distinct aspects of our fearful experiences appear to be mediated by separate explicit and implicit memory processes. To identify brain regions that support these separate memory processes, we measured contingency awareness, conditional fear expression, and functional magnetic resonance imaging signal during a Pavlovian fear conditioning procedure in which tones that predicted an aversive event were presented at supra and sub-threshold volumes. Contingency awareness developed in conjunction with learning-related hippocampal and parahippocampal activity on perceived conditioning trials only. In contrast, conditional fear and differential amygdala activity developed on both perceived and unperceived trials, regardless of whether contingency awareness was expressed. These findings demonstrate the distinct roles of these brain regions in explicit and implicit fear memory processes.

Separate memory systems exist within the human brain that acquire and store distinct features of our fearful experiences (Bechara et al., 1995; LeDoux, 2000; Milner et al., 1998; Moris et al., 1998; Tabbert et al., 2006). One system is responsible for consciously recalled facts and details of events (explicit memory), while another system mediates the production of learned fear responses that occur without conscious thought (implicit memory; Bechara et al., 1995; Milner et al., 1998). These separate memory systems appear to be mediated by distinguishable, but partially overlapping neural circuits (Bechara et al., 1995; LeDoux, 2000; Milner et al., 1998). However, the extent to which these memory systems are independent and the conditions in which they interact are unresolved issues in the study of emotional learning and memory.

The laboratory study of fear learning and memory has traditionally used a Pavlovian conditioning paradigm in which the presentation of a neutral stimulus (conditioned stimulus, CS) predicts an aversive event (unconditioned stimulus, UCS). An awareness of CS-UCS contingencies (explicit memory) typically develops during standard conditioning tasks (Knight et al., 2003; Knight et al., 2004; Manns et al., 2000). However, it is the autonomic conditioned responses (CR) elicited by a CS that are usually taken as evidence that an association has been formed. CR production in the absence of contingency awareness is thought to reflect an implicit memory process. However, whether these CRs can be produced in the absence of contingency awareness is an issue of continuing debate (Lovibond & Shanks, 2002; Manns et al., 2002; Shanks & Lovibond, 2002; Wiens & Öhman, 2002).

Conditioning in the absence of contingency awareness has been reported by a number of studies investigating eye blink, SCR, and fear-potentiated startle responses (Bechara et al., 1995; Clark & Squire, 1998; Esteves et al., 1994; Hamm & Weike, 2005; Knight et al., 2003 & 2006; Öhman & Soares, 1994; Weike et al., 2005 & 2007). However, the findings of many of these

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studies have been challenged because of methodological concerns with the manner in which awareness was assessed (Lovibond & Shanks, 2002). A concern with many of these studies is that contingency awareness was assessed post-experimentally. Post-experimental assessments do not evaluate contingency awareness on a trial-by-trial basis and can be susceptible to forgetting, interference, and reconstruction of events (Lovibond & Shanks, 2002; LaBar & Disterhoft, 1998). Therefore, these assessments may be insensitive to subtle evidence of CS discrimination. Assessing awareness simultaneously with conditioning is an alternative recommended by those who are critical of post-experimental assessment procedures (Lovibond & Shanks, 2002). However, subsequent work has assessed contingency awareness during the conditioning session and demonstrated CR expression with and without an awareness of CS-UCS contingencies (Knight et al., 2003 & 2006). These findings provide additional support for prior work that used post-experimental methodologies to demonstrate conditioning in the absence of awareness.

The manner in which awareness is manipulated may also influence whether it is necessary for CR production. Subjects quickly learn the stimulus contingencies in conditioning studies (Dunsmoor et al., 2007; Knight et al., 2003; Knight et al., 2004; Manns et al., 2000). Therefore, research interested in implicit memory processes has often employed distraction tasks to interfere with contingency awareness. However, the role of awareness in this type of fear learning appears to vary with the extraneous demands of the conditioning procedure (Carter et al., 2003; Biferno & Dawson, 1977; Dawson & Biferno, 1973; LaBar & Disterhoft, 1998). Therefore, contingency awareness may become necessary for CR production as a result of increasing the difficulty of the conditioning task. An alternative to increasing task difficulty is to modulate contingency awareness by presenting stimuli below the perceptual detection threshold. CRs to sub-threshold CSs can be monitored during the conditioning session without distracting participants or increasing the complexity of the conditioning procedure (Esteves et al., 1994; Knight et al., 2003 & 2006). Prior work has demonstrated behavioral, autonomic, and fMRI signal responses to subthreshold auditory, visual, somatosensory, and visceral stimuli (Blankenburg et al., 2003; Borgeat et al., 1985; Bornhövd et al., 2002; Colder & Tanenbaum, 1999; Kern & Shaker, 2002; Kotzé & Moller, 1990; Knight et al., 2003; Merikle et al., 2001; Öhman & Soares, 1994; Shevrin, 2001). Further, several of these studies have observed learning-related changes in the response to these sub-threshold stimuli, demonstrating conditioning without contingency awareness (Esteves et al., 1994; Knight et al., 2003 & 2006; Öhman & Soares, 1994; Morris et al., 1998 & 1999).

Partially independent neural circuits appear to support explicit and implicit memory processes during Pavlovian conditioning. For example, hippocampal lesions interfere with the ability of patients to report CS-UCS relationships, but do not prevent CR expression (Bechara et al., 1995; Clark & Squire, 1998). In contrast, amygdala lesions disrupt conditional fear responses, but leave contingency awareness relatively intact (Bechara et al., 1995; LaBar et al., 1995). Prior neuroimaging studies of fear conditioning have observed learning-related changes in amygdala, hippocampus, cingulate, thalamus, insula, and sensory cortex activity (Büchel et al., 1998 & 1999; Cheng et al., 2003 & 2006; Knight et al., 1999, 2004, 2005; Tabbert et al., 2005). However, these studies were not designed to determine whether the observed activity supported explicit or implicit memory functions. Studies that have explored brain activity during conditioning with and without awareness indicate the amygdala supports fear learning whether or not contingency awareness is reported (Morris et al., 1998; Tabbert et al., 2006). However, these studies did not distinguish brain regions that mediate explicit as opposed to implicit memory processes.

The present study was designed to further delineate the neural circuitry that supports explicit and implicit fear memory processes. To achieve this aim, two separate behavioral measures were recorded from participants that were exposed to a Pavlovian conditioning procedure

during functional magnetic resonance imaging (fMRI). These behavioral measures included UCS expectancy and skin conductance response (SCR). UCS expectancy was used as an index of CS-UCS contingency awareness, a form of explicit memory. SCR was used as an autonomic index of CR expression. CR expression in the absence of contingency awareness reflects a form of implicit memory (Bechara et al., 1995; Esteves et al., 1994; Knight et al., 2003 & 2006; Öhman & Soares, 1994). During the conditioning session, one tone (CS+) predicted a loud unpleasant static white noise (UCS) and a second tone (CS-) was presented alone. The ability of participants to demonstrate their awareness of the CS-UCS contingencies was varied on a trial-by-trial basis by presenting the auditory CSs at volumes just above (perceived trials) or below (unperceived trials) their perceptual detection threshold. This methodology allowed us to investigate the relationship between contingency awareness, CR expression, and human brain activity during Pavlovian fear conditioning.

Materials and Methods

Participants

Fifteen healthy right-handed volunteers (7 female and 8 male; mean age, 28.87 ± 1.69 years; range, 22 to 39 years) participated in this study. All subjects provided written informed consent in compliance with the National Institute of Mental Health Institutional Review Board.

Conditioned & Unconditioned Stimuli

Two pure tones (700 and 1300 Hz) were presented as conditioned stimuli (10 s duration, 20 s inter-trial interval) during the training session. The CS+ (60 trials) coterminated with a 500 ms loud (100 dB) white-noise UCS and the CS- (60 trials) was presented alone. Conditioned stimuli were counter-balanced and presented in a pseudo-random order such that no more than 2 trials of the same CS were consecutively presented. The volume of the CS+ and CS- were modulated on a trial-by-trial basis for each subject using an adaptive threshold estimation procedure as described below.

UCS Expectancy

A MRI compatible joystick was used to monitor subjects' perception of conditioned stimuli and expectancy of receiving the UCS. Perception of conditioned stimuli was monitored by instructing subjects to push a button on the joystick immediately upon hearing either tone. In addition, the joystick controlled a rating bar presented throughout training at the bottom of the visual display. Subjects were instructed to rate their UCS expectancy on a continuous scale from 0 to 100 (0 = certain that the UCS will not be presented, 50 = uncertain whether the UCS will be presented, 100 = certain that the UCS will be presented), and were instructed to continuously update (sampled at 10 Hz) their rating to reflect their current UCS expectancy.

Skin Conductance Response

A Contact Precision Instruments (Cambridge, MA) skin conductance monitoring system was used to monitor skin conductance response (SCR) throughout the assessment. SCR was sampled (40 Hz) with a pair of surface gel cup electrodes [silver/silver chloride, 6mm diameter, BIOPAC (Goleta, CA) model TSD203] attached to the distal phalanx of the middle and ring fingers of the nondominant hand.

Procedure

Subjects were informed that two tones would be repeatedly presented and told that the volume of each tone would vary above and below their perceptual threshold. Subjects were instructed to push the button immediately upon hearing a tone, and to update their UCS expectancy accordingly. Unknown to the subjects, the volume of each CS was controlled by their button

press responses, such that the volume of the CS was decreased by 5 dB following perceived trials (i.e. when a button press was made). CS volume was increased by 5 dB following unperceived trials (i.e. when a button press was not made). The volume of the CS+ and CS- were modulated independently.

Behavioral Data Analysis

UCS expectancy was calculated as the average (1 s sample) response beginning 0.5 s prior to UCS presentation on CS+ trials and the equivalent period of time on CS- trials. Skin conductance responses (SCR) were also monitored during the conditioning session. SCR amplitude was calculated by subtracting the average skin conductance measurement during the baseline period (5 s immediately preceding CS presentation) from the second interval response (peak response during the 5 s preceding UCS presentation on CS+ trials and the equivalent period of time on CS- trials). SCR data were square root transformed to reduce skew prior to statistical analysis. Repeated measures ANOVA and post-hoc t-test comparisons of UCS expectancy and SCR data for CS+ versus CS- presentations were completed for perceived and unperceived trials.

Functional Image Acquisition and Analysis

Structural and functional imaging was completed on a 1.5 Tesla General Electric Signa scanner using a brain-specific RF head coil (Medical Advances, Milwaukee, WI). Functional imaging of the entire brain was conducted using a gradient-echo echoplanar pulse sequence (TR = 2000 ms, TE = 40 ms, FOV = 24 cm, matrix = 64×64 , slice thickness = 6 mm) during each of four 920 s blocks of stimulus presentations. High-resolution anatomical images (SPGR) were obtained to serve as an anatomical reference. Image processing was performed with the AFNI software package (Cox, 1996). Echo-planar time series data were motion corrected, concatenated, and reregistered to the fifth volume of the first functional imaging scan. The imaging analysis modeled the hemodynamic response as the sum of the basis functions that represent the onset of each type of stimulus (i.e. perceived CS+, perceived CS-, unperceived CS+, & unperceived CS-) using the 3dDeconvolve program within AFNI. Modeling the response in this manner does not assume a fixed or predetermined hemodynamic response shape, but instead allows the shape of the response to vary (Saad et al., 2006). Additional regressors for head motion and motor processes were also included in the analysis. The percent area under images one through three of the hemodynamic response curve (AUC), which occur prior to UCS presentation (see Supplemental Figure 1), was compared to a resting baseline and used as an index of the response magnitude associated with each type of CS presentation. Functional maps reflecting the AUC associated with each type of CS presentation were converted to a standard stereotaxic coordinate system (Talairach & Tournoux, 1998) and spatially blurred using a 4 mm full-width-at-half-maximum isotropic Gaussian filter. Initial analyses were limited to regions of the medial temporal lobe that included the amygdala, hippocampus, and parahippocampal cortex as defined by the Talairach & Tournoux Atlas (1998) within the AFNI software package (Cox, 1996). Repeated measures ANOVA and post-hoc t-test comparisons of AUC data were then completed for clusters of significant ($p < 0.005$ uncorrected; $F > 11.05$) activation that were larger than 200 mm^3 . An additional analysis of whole-brain data was completed for areas of activation (volume $> 200 \text{ mm}^3$) with a more stringent significance threshold ($p < 10^{-5}$ uncorrected; $F > 28.49$). Monte Carlo simulations indicated that both medial temporal lobe and whole-brain analyses were significant at a $p < 0.05$ (corrected) level when restricted to clusters of activation larger than 200 mm^3 .

Results

Conditioned Stimuli

An independent perceptual threshold was determined for each subject using an adaptive threshold estimation procedure. By design, the volume of perceived CSs was higher than the volume of unperceived CSs ($t[14] = 8.49, p < 0.05$). However, the volume of perceived CS+ ($+2.93 \pm 0.98$ dB; all values reflect mean volume relative to threshold $\pm$ SEM) and CS- ($+2.30 \pm 1.24$ dB) presentations did not differ ($t[14] = 0.29$), nor did the volume of unperceived CS+ (-3.88 ± 1.42 dB) and CS- (-3.07 ± 1.22 dB) trials ($t[14] = -0.34$). The number of perceived CSs was higher than the number of unperceived CS presentations. However, an equal number of perceived CS+ (33.47 ± 1.17 ; all values reflect mean number of trials $\pm$ SEM) and CS- trials (33.67 ± 1.10 ; $t[14] = -0.17$) as well as unperceived CS+ (26.53 ± 1.17) and CS- (26.33 ± 1.10 ; $t[14] = 0.17$) trials were presented.

SCR & UCS Expectancy

Our behavioral results show that learning-related changes in UCS expectancy and SCR developed during the conditioning session. Repeated measures ANOVA of UCS expectancy data revealed significant main effects for CS type ($F[1, 14] = 41.11, p < 0.05$) and CS perception ($F[1, 14] = 11.21, p < 0.05$), as well as a significant CS type $\times$ CS perception interaction ($F[1, 14] = 30.14, p < 0.05$). Participants demonstrated their awareness of the CS-UCS contingencies on perceived trials, with larger UCS expectancy ratings to the CS+ than CS- ($t[14] = 5.98, p < 0.05$). These participants were unable to demonstrate an awareness of the stimulus relationships on unperceived trials as evidenced by UCS expectancies that did not differ between CS+ and CS- presentations ($t[14] = 2.05$) (Figure 1a). In contrast, learning-related changes in SCR were produced on both perceived and unperceived conditioning trials. Repeated measures ANOVA of SCR data revealed significant main effects for CS type ($F[1, 14] = 22.16, p < 0.05$) and CS perception ($F[1, 14] = 15.20, p < 0.05$), without a CS type $\times$ CS perception interaction ($F[1, 14] = 1.13$). SCRs were larger on perceived than unperceived CS+ trials ($t[14] = 3.78, p < 0.05$). Similarly, larger SCRs were observed during perceived compared to unperceived CS- presentations ($t[14] = 3.36, p < 0.05$). However, CS perception did not appear to be necessary for CR production given that larger SCRs were produced to the CS+ than CS- during perceived ($t[14] = 3.77, p < 0.05$) and unperceived conditioning trials ($t[14] = 2.46, p < 0.05$). These findings replicate prior behavioral work with this methodology (Knight et al., 2003; Knight et al., 2006) and demonstrate CR expression with and without contingency awareness (Figure 1b).

Medial Temporal Lobe fMRI Analysis

The initial fMRI analysis was limited to the medial temporal lobe (Table 1). These functional imaging results revealed learning-related changes in brain activation within the hippocampus, parahippocampal gyrus, and amygdala. Significant main effects for CS type and CS perception, as well as a significant CS type $\times$ CS perception interaction were revealed within the hippocampus bilaterally. Hippocampal activity was larger to the CS+ than CS- on perceived conditioning trials. In contrast, hippocampal responses did not differ on unperceived trials (Figure 2). A significant CS perception effect was also observed within the left and right parahippocampal gyrus demonstrating larger responses to perceived than unperceived CS presentations. However, only the left parahippocampal gyrus showed a significant CS type $\times$ CS perception interaction. The pattern of left parahippocampal activity was similar to the pattern observed within the hippocampus. Perceived CS+ trials produced a larger fMRI signal response than the CS-, whereas the response to the unperceived CS+ and CS- did not differ.

Learning-related changes in response magnitude were also observed within the right amygdala (Figure 3). Significant main effects for CS type and CS perception were observed within this

region. However, a CS type $\times$ CS perception interaction was not demonstrated. Post-hoc t-test comparisons confirmed that amygdala activity was larger during perceived than unperceived trials. On perceived trials, amygdala responses were larger to the CS+ than CS-. Similar results were demonstrated on unperceived trials with larger responses to CS+ than CS- presentations. These findings indicate that perceived stimuli elicit larger responses than unperceived stimuli within the amygdala. However, learning-related differences in the amygdala's response to the CS+ and CS- were produced on both perceived and unperceived trials. Further, the magnitude of the differential amygdala responses produced by the CS+ versus CS- did not differ between perceived and unperceived conditioning trials given that a CS type $\times$ CS perception interaction was not demonstrated. These findings indicate that learning-related amygdala activity was expressed during both perceived and unperceived CS presentations.

Whole-Brain fMRI Analysis

Our whole-brain functional imaging results showed significant CS evoked activations within a number of brain regions during the conditioning session (Table 2). A significant main effect for CS type was observed within the insula, fusiform gyrus, cuneus, caudate, cerebellum, and precentral gyrus. Response magnitude was larger to CS+ than CS- presentations within each of these regions. A significant main effect for CS perception was revealed within the superior temporal gyrus, with larger responses to perceived than unperceived CSs. Activity within the cingulate gyrus showed a significant CS type $\times$ CS perception interaction. Similar to hippocampal findings from the medial temporal lobe analysis, functional MRI signal responses within the cingulate gyrus were larger during the perceived CS+ than CS-, whereas responses to the unperceived CS+ and CS- did not differ.

Discussion

The present results demonstrate the independence of explicit and implicit memory systems. An awareness of stimulus relationships often develops during conditioning as demonstrated by the differential UCS expectancy ratings on perceived trials in the current study. Learning-related changes in SCR were also observed on these perceived conditioning trials. However, this study also demonstrated that contingency awareness can be limited by presenting CSs below the perceptual detection threshold. Even though an awareness of CS-UCS relationships was not demonstrated on these unperceived trials, conditional SCRs were expressed. CR expression in the absence of awareness reflects an implicit memory process. These findings indicate that while autonomic CRs and contingency awareness are often expressed concurrently, CRs can be produced without an awareness of stimulus relationships.

One novel aspect of our findings is that learning-related activity within the hippocampus and parahippocampal gyrus only developed on perceived conditioning trials. The fMRI signal measured within these brain regions was larger on perceived CS+ than CS- trials. However, responses to the unperceived CS+ and CS- did not differ. This pattern was also observed in participants' UCS expectancy responses, and suggests that the development of contingency awareness during Pavlovian conditioning is mediated by these medial temporal lobe regions. Further, these findings are consistent with prior conditioning studies that have shown impaired contingency awareness in patients with hippocampal lesions, providing additional support for the view that awareness of stimulus contingencies relies upon the hippocampus and medial temporal cortex (Bechara et al., 1995; Clark & Squire, 1998; Milner et al., 1998).

Although the contingency awareness associated with hippocampal activity typically develops during standard conditioning tasks, these processes appear to be unnecessary for CR expression. In this study, CRs were produced without contingency awareness and in the absence of differential hippocampal activity on unperceived trials. These findings indicate that hippocampal activity was not necessary for CR expression during the conditioning session.

Instead, CR production paralleled the differential amygdala responses observed during the study. SCR and amygdala activity were larger to the CS+ than CS- on both perceived and unperceived conditioning trials. These findings suggest the amygdala plays an important role in the production of conditional SCRs and indicate that it can mediate CR expression independent of the explicit memory system of the medial temporal lobe (Bechara et al., 1995; Milner et al., 1998; Morris et al., 1998).

We also observed learning-related fMRI signal changes within brain regions that include the insula, caudate, and fusiform gyrus (Table 2). Prior fMRI studies of Pavlovian conditioning have observed activity within many of these areas (Büchel et al., 1998 & 1999; Dunsmoor et al., 2007). However, these studies did not investigate whether the observed activity was linked to explicit or implicit memory processes. The fMRI studies that have explored the relationship between awareness and conditioning have not reported activation within these brain regions (Morris et al., 1998; Tabbert et al., 2006). In the present study however, we observed learning-related responses within these brain areas on both perceived and unperceived conditioning trials. This pattern of activation was similar to the pattern observed within the amygdala. The fMRI signal responses produced without contingency awareness in these regions could be mediated via interconnections with the amygdala. The amygdala's role in conditional fear has been well characterized, and prior studies indicate the amygdala is a critical component of the neural circuit that mediates fear acquisition, consolidation, and expression (Büchel et al., 1998 & 1999; Campeau & Davis, 1995; Cheng et al., 2003 & 2006; Helmstetter, 1992; Helmstetter & Bellgowan, 1994; Knight et al., 2005; LaBar et al., 1998; LeDoux et al., 2000; Maren, 1999). This line of study indicates that there are separate cortical and subcortical pathways to the amygdala that can independently support auditory fear conditioning (LeDoux, 2000). Subcortical projections from the auditory thalamus to the lateral amygdala may provide a pathway for CS input to reach the amygdala and elicit fear responses without passing through cortical regions that are necessary for CS perception and contingency awareness. However, it may also be possible for learning to develop through a cortical route via low level activity that does not meet the threshold for perception and contingency awareness. Sub-threshold auditory stimuli may elicit cortical activation that falls below the level required for stimulus perception (Colder & Tanenbaum, 1999), and this sub-threshold activity may in turn be projected to the amygdala where conditional fear responses are produced. However, we did not observe auditory cortex activity on unperceived trials in this study. Instead, our findings indicate that unperceived stimuli are less likely to activate the brain networks that support higher-order cognitive processes such as contingency awareness. In contrast, perceived stimuli appear to gain greater access to regions like the hippocampus and parahippocampal gyrus that mediate contingency awareness, and these medial temporal lobe regions may interact with areas within the prefrontal cortex that support more complex forms of learning such as trace conditioning (Knight et al., 2004; Kronforst-Collins & Disterhoft, 1998; Weible et al., 2000).

The present study demonstrated Pavlovian conditioning in the absence of contingency awareness. However, several prior studies have reported similar findings. Many of these initial studies explored the influence of contingency awareness on conditioning using biologically prepared visual stimuli such as snakes, spiders, and human faces as CSs (Esteves et al., 1994; Morris et al., 1998; Soares & Öhman, 1993). These stimuli were presented very briefly (~ 30 ms) then masked to limit CS perception. Findings from this prior work suggested that basic perceptual features of these images can be processed in a privileged manner, outside of awareness, by neural circuitry (i.e. the amygdala) that is selectively engaged by fear-relevant stimuli that have been threatening to humans across evolution (Öhman & Mineka, 2001). This circuitry presumably responds automatically to prepared stimuli at a subcortical level without the need of higher-order cognitive processes (Öhman & Mineka, 2001). However, the present study demonstrated similar behavioral effects using nonprepared CSs. Other human studies have also demonstrated conditioning without awareness using nonprepared visual and auditory

stimuli (Bechara et al., 1995; Jovanovic et al., 2006; Knight et al., 2003 & 2006; Wong et al., 2004). Further, the current study demonstrated learning-related amygdala activity during unperceived conditioning trials in which contingency awareness was not expressed. The amygdala is a key component of the neural circuit that prior work suggests is selectively engaged by biologically prepared stimuli (Öhman & Mineka, 2001). The findings of the present study indicate that although there may be an advantage to conditioning with prepared stimuli, nonprepared stimuli can also elicit amygdala activity and produce autonomic CRs without an awareness of stimulus contingencies.

Although conditional SCR expression has been reported in the absence of awareness in several prior studies (Bechara et al., 1995; Esteves et al., 1994; Knight et al., 2003 & 2006; Öhman & Soares, 1993 & 1994), others have argued that contingency awareness is necessary for SCR conditioning (Hamm & Weike, 2005; Weike et al., 2005 & 2007). Instead, they suggest that fear-potentiated startle responses can be produced without an awareness of stimulus contingencies (Hamm & Weike, 2005; Weike et al., 2005 & 2007). However, these studies only investigated first interval skin conductance responses (FIRs), which occurred within 4 seconds of CS onset. Due to methodological limitations, second interval responses (SIR) produced more than 4 seconds after CS onset could not be assessed. The FIR is typically interpreted as an orienting response to CS presentation, whereas the SIR is generally considered a response that is elicited by anticipation of the UCS (Prokasy & Kumpfer, 1973). Both FIRs and SIRs can serve as indices of associative learning. Further, learning-related changes in both the FIR and SIR have been observed without an awareness of CS-UCS contingencies (Esteves et al., 1994; Knight et al., 2003 & 2006; Soares & Öhman, 1993). However, prior work indicates these two types of responses are relatively independent (Prokasy & Kumpfer, 1973). In the present study, conditional SIRs were expressed without contingency awareness. These findings are consistent with prior work that has demonstrated differential SCRs without contingency awareness during the SIR, but not the FIR (Knight et al., 2003). These findings suggest that under some circumstances the SIR may be more sensitive to conditioning in the absence of contingency awareness.

The present study investigated brain activity associated with contingency awareness and CR expression to distinguish regions that mediate explicit and implicit fear memory processes. Our findings further demonstrate the independence of these memory systems, and indicate that the hippocampus supports contingency awareness, whereas the amygdala mediates conditional fear expression with and without awareness. Our findings support the view that these distinct explicit and implicit features of our fearful experiences generally develop in parallel, but are supported by neural circuits that are partially independent. The neural circuitry that supports these distinct memory processes interact in many circumstances (Richardson et al., 2004), and the interaction of these circuits appears to be necessary for successful learning under complex conditions (LeDoux, 2000; Büchel et al., 1999). However, our findings indicate that these interactions are not necessary for simple forms of emotional learning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations footnote

CS, conditioned stimulus; UCS, unconditioned stimulus; CS+, CS paired with the UCS; CS−, CS presented alone; CR, conditioned response; SCR, skin conductance response.

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Figure 1.

UCS expectancy & SCR data. a) Learning-related changes in UCS expectancy were demonstrated on perceived trials only. Larger responses were produced during the CS+ relative to the CS- on perceived, but not unperceived trials. b) In contrast, learning-related SCRs were demonstrated on both perceived and unperceived trials. The CS+ produced larger SCRs than the CS- on both perceived and unperceived conditioning trials. Asterisk indicates significant difference. Error bars represent standard error of the mean.

Figure 2. Functional MRI data. Learning-related changes in the area under the hemodynamic response curve (AUC) were observed within the hippocampus on perceived trials only. Larger responses were observed during perceived CS+ relative to perceived CS- presentations, while responses to the CS+ and CS- did not differ on unperceived trials. Asterisk indicates significant difference. Error bars represent standard error of the mean.

Figure 3.

Functional MRI data. Learning-related changes in the area under the hemodynamic response curve (AUC) were observed within the amygdala during both perceived and unperceived trials. The AUC was larger during the CS+ than CS- on both perceived and unperceived trials. Asterisk indicates significant difference. Error bars represent standard error of the mean.

Table 1

Medial temporal lobe activity during Pavlovian conditioning

Region	Hemisphere	Volume (mm ³)	RL	Talairach Coordinates AP	IS	F value
a. Regions showing a main effect for CS type						
Amygdala	Right	207	25	-5	-17	14.11
Hippocampus	Right	450	30	-29	-9	15.06
Hippocampus	Left	2420	-12	-34	0	17.63
b. Regions showing a main effect for Perception						
Amygdala	Right	609	26	-4	-17	15.39
Hippocampus	Right	432	25	-22	-13	14.88
Hippocampus	Left	252	-18	-26	-10	14.10
Parahippocampal Gyrus	Right	470	19	-39	-4	14.34
Parahippocampal Gyrus	Left	515	-21	2	-16	15.12
c. Regions showing a CS × Perception Interaction						
Hippocampus	Right	340	26	-21	-15	15.98
Hippocampus	Left	641	-29	-13	-17	14.96
Parahippocampal Gyrus	Left	560	-31	-35	-11	15.72

Locations, volumes, and Talairach coordinates (Talairach and Tournoux, 1988) for areas of activation. LF, left/right; AP anterior/posterior; IS, inferior/superior.

Table 2

Whole-brain activity during Pavlovian conditioning

Region	Hemisphere	Volume (mm ³)	RL	AP	IS	F value
a. Regions showing a main effect for CS type						
Fusiform	Right	242	22	-6	13	30.31
Insula	Right	206	41	-20	22	31.09
Cuneus	Left	247	-6	-72	31	28.66
Caudate	Left	318	-8	1	8	34.61
Cerebellum	Right	378	15	-61	-24	31.17
Precentral Gyrus	Left	324	-18	-21	60	30.71
b. Regions showing a main effect for Perception						
Superior Temporal Gyrus	Left	638	-50	-8	-8	30.95
c. Regions showing a CS × Perception Interaction						
Cingulate Gyrus	Left	218	-9	-26	31	35.63

Locations, volumes, and Talairach coordinates (Talairach and Tournoux, 1988) for areas of activation. LF, left/right; AP anterior/posterior; IS, inferior/superior.