Published in final edited form as:

Neuroscience. 2011 August 25; 189: 359–369. doi:10.1016/j.neuroscience.2011.05.067.

Fear conditioning is associated with dynamic directed functional interactions between and within the human amygdala, hippocampus and frontal lobe

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Abstract

The current model of fear conditioning suggests that it is mediated through modules involving the amygdala (AMY), hippocampus (HIP), and frontal lobe (FL). We now test the hypothesis that habituation and acquisition stages of a fear conditioning protocol are characterized by different event-related causal interactions (ERC) within and between these modules. The protocol employed the painful cutaneous laser as the unconditioned stimulus and ERC was estimated by analysis of local field potentials recorded through electrodes implanted for investigation of epilepsy.

During the pre-stimulus interval of the habituation stage FL>AMY ERC interactions were common. For comparison, in the post-stimulus interval of the habituation stage only a subdivision of the FL (dorsal lateral prefrontal cortex, dlPFC) still exerted the FL>AMY ERC interaction (dlPFC>AMY). For a further comparison, during the poststimulus interval of the acquisition stage the dlPFC>AMY interaction persisted and an AMY>FL interaction appeared.

In addition to these ERC interactions between modules, the results also show ERC interactions within modules. During the post-stimulus interval HIP>HIP ERC interactions were more common during acquisition, and deep hippocampal contacts exerted causal interactions upon superficial contacts, possibly explained by connectivity between the perihippocampal gyrus and the hippocampus. During the prestimulus interval of the habituation stage AMY>AMY ERC interactions were commonly found, while interactions between the deep and superficial amygdala (indirect pathway) were independent of intervals and stages. These results suggest that the network subserving fear includes distributed or widespread modules, some of which are themselves 'local networks'. ERC interactions between and within modules can be either static or change dynamically across intervals or stages of fear conditioning.

Keywords

Pain; Fear; Network; Human; Laser; Local Field Potentials

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None of the authors has conflicts of interest related to this work.

INTRODUCTION

Fear conditioning occurs when a neutral conditioned stimulus (CS+), such as a light, is paired with an unconditioned aversive stimulus (US), such as a foot shock. Following acquisition, the conditioned stimulus alone acquires the ability to evoke a conditioned response (CR), such as robust autonomic arousal. Modules in the brain, including parts of the amygdala (AMY), the hippocampus (HIP), and the frontal cortex (FL), are known to be involved in fear conditioning although the functional interactions between and within these modules are unclear (Davis, 1992; LeDoux, 1992; Squire, 1992).

Conditioned fear may result from the convergence of signals related to the CS and US in the lateral and basal nuclei of the amygdala. These nuclei project upon the central nucleus of the amygdala which is an output structure important for the expression of fear (Davis, 1992; Sotres-Bayon et al., 2006; Rauch et al., 2006; Pare et al., 2004; Liu et al., 2011b). This is the indirect pathway through the human amygdala, while the direct pathway transmits signals evoked by the US directly to the central nucleus (Liu et al., 2011b).

Activity in the amygdala may be regulated by inputs from structures in the frontal lobe including the dorsolateral prefrontal (dlPFC) cortex during fear conditioning protocols (Ochsner and Gross, 2005; Gottfried and Dolan, 2004; Phelps et al., 2004). Pathways within the hippocampus and related structures may be activated during acquisition, particularly during trace conditioning, in which there is a stimulus-free period between offset of the CS and onset of the US (Buchel et al., 1999).

The causal interactions between these modules may constitute a 'network' which is activated during fear conditioning protocols. A network consists of neural elements, their connections, and connectional weights, which are often equated with neurons or structures in the brain, axons, and synapses, respectively. The companion papers have provided evidence that the laser stimulus evokes pre-stimulus and post-stimulus interactions within and between the amygdala and hippocampus, as well as activation of both modules (Liu et al., 2010; Liu et al., 2011b). These results suggest that causal interactions related to a painful laser US will depend upon timing of the stimulus, and perhaps upon the stage of fear conditioning. We now test the hypothesis that stages of fear conditioning (habituation and acquisition) differentially affect causal interactions between and within the amygdala, hippocampus, and the frontal lobe. We propose to test this hypothesis by examining causal interactions involving the indirect pathway, the dlPFC, and the hippocampus plus related structures during the stages of fear conditioning.

We measured event related causality (ERC) over short time intervals and different stages of fear conditioning protocols related to the US, the laser stimulus (Korzeniewska et al., 2003; Korzeniewska et al., 2008; Liu et al., 2011b). The ERC was calculated from local field potentials (LFP) recorded from depth electrodes implanted for the investigation of epilepsy in amygdala, hippocampal and frontal lobe modules in fear conditioning protocols.

METHODS

The protocol for these studies was reviewed and approved annually by the Institutional Review Board at the School of Medicine, Johns Hopkins University. These studies were carried out after the implantation of depth electrodes in the amygdala and HIP for the investigation of medically intractable epilepsy in four subjects. We have previously published patient characteristics, laser-evoked potentials and causal interactions in response to painful stimuli in these subjects, who are identified here by the same number as in those reports (Liu et al., 2010; Liu et al., 2011b). All subjects gave informed consent for participation in these studies.

Clinical Procedures and Electrode Location

Depth electrodes were implanted in the frontal lobe, amygdala and hippocampus by a stereotactic procedure using the Leksell frame (Liu et al., 2010). Targeting was carried out using a coronal MRI scan to determine the location of implantation of the electrodes at the stereotactic target. Intraoperative radiographs confirmed that the electrode was at that target for the amygdala and hippocampus in the companion paper (Liu et al., 2010). Along electrodes through the amygdala, contacts 1 and 2 were in the ventral amygdala, while 4 and 5 were in the dorsal amygdala. Along hippocampal electrodes, contacts 1–3 were in the perihippocampal gyrus while contacts 4 and 5 were in the body of the hippocampus (Figure 1 in (Liu et al., 2010)).

Figure 1 demonstrates the position of the frontal lobe electrode which was localized by the same technique as the amygdala and hippocampal electrodes in the companion papers (Liu et al., 2010; Liu et al., 2011b). The electrode in the frontal lobe was placed 10 cm anterior, 10 mm lateral to the tip of the lateral ventricle, and with the tip of the electrode at the roof of the orbit, as confirmed by radiographs in the operating room. These landmarks resulted in implantation of contact 6 of the electrode (labeled in the target scan) where the deepest point on the superior frontal sulcus is indicated by an arrow in 1A and 1B. The Target –9 mm scan is close to base of the frontal pole as indicated by the fact that superior frontal sulcus extends from the convexity almost to the roof of the orbit. By these landmarks, atlas maps indicate that the electrode location shown in the Target scan is within dlPFC defined by Brodmann areas 9, 10, 12, 46 (Mai et al., 2007; Duvernoy et al., 1991; Pandya and Yeterian, 1985).

Seizure monitoring was carried out over the one week period between the implantation and removal of the grid of contacts, starting on the day after implantation. All seizure medications were discontinued for 36 hours after the implantation of the electrodes, so that all subjects had substantial blood levels of these drugs at all points relevant to this study (Levy et al., 2002).

Laser and Conditioning Protocols

During the laser study, the patient wore protective glasses and reclined in bed with his/her eyes open, quietly awake. Noxious cutaneous heat stimulation, that the patient expected could be painful, was delivered by a Thulium YAG laser (wavelength 2 μ m, duration 1 ms; StarMedTec, Starnberg, Germany). Stimuli were applied to the dorsum of the left or right forearm as described below for the fear conditioning protocol which included an 8 to 10 sec interval between stimuli in order to avoid sensitization or fatigue of primary nociceptive afferents (Meyer et al., 1994). The laser beam was moved at random to a slightly different position for each stimulus.

The average energy level for laser stimulation, the patients' pain intensity and unpleasantness ratings were measured at the end of each block of stimuli. The ratings were made with an intensity scale with 0 as no pain, and 10 as the most intense pain imaginable. A total of 25 laser pulses were applied during a single run and the inter-run interval time was 2 min. The signal was first amplified (12A5, Astro-Med Grass, Inc., West Warwick, RI), then band-pass filtered at 0.1–300 Hz and finally, digitized at 1000 Hz.

The trace fear conditioning protocol was administered during the patient's stay in the epilepsy monitoring unit over intervals remote from seizures. Subjects were instructed to look at an LED display, consisting of two different LED colors (red and green), which were assigned randomly to CS+ or CS– across patients. The US was the laser stimulus described above. The duration for the CS was 500 ms and the US was 1 ms. On paired trials, the CS+ was followed by a 200 ms stimulus-free period before US presentation. The time between

CS trials varied between 8 to 10 sec randomly. During the habituation stage, 40 CS+, 40 CS- and 20 US were delivered unpaired at random for each session. During the acquisition stage, there were 40 CS+ and 40 CS- of which half (20 CS+) were paired with the US. Two habituation and two acquisition sessions were administered to each subject. .

Event-Related Causality (ERC)

To evaluate the directional interactions between brain areas, we used an approach called Event-Related Causality (ERC) which was based on the concept of Granger causality (Granger, 1969). For two observed time series X and Y , it is said that X is Granger causal of Y if the past knowledge of X significantly reduces the prediction error for Y . The significant ERC reported in this study as indicated by Figures 2 and 4 can be described in terms of the contact (or brain structure) X which exerts a causal influence, or plays a driver role upon, the contact (or brain structure) Y . We refer to this as a causal pair of electrodes, or a modular causal pair between structures or modules in the brain, both described by $X > Y$. We have now used this ERC technique to address the directional causal influences between structures in the frontal and medial temporal lobe.

The ERC analysis for the multichannel LFP data was achieved using a multivariate autoregressive (MVAR) modeling technique, named *short-time direct directed transfer function* (SdDTF) (Korzeniewska et al., 2003; Korzeniewska et al., 2008; Liu et al., 2011b). The SdDTF measures the directions, intensities, and spectral contents of direct causal interactions among acquired signals and is also adapted for the signals with short durations.

In this study, the ERC analysis was applied to signals recorded from electrode contacts located over different cortical structures to evaluate the strength of causal interactions between these structures. The ERC was calculated in the 6–14 Hz frequency band which was based on the frequencies of peaks in ensemble averages of the LFP autopower, coherence and ERC (Liu et al., 2010; Liu et al., 2011b). These frequencies were consistent with those of other studies of intracranial recordings (Ohara et al., 2001; Ohara et al., 2006; Tallon-Baudry et al., 2001) and were higher than those of recordings from the scalp (Andres et al., 1999; von Stein et al., 1999).

In this study, a sliding window approach was used in the ERC analysis, the length of the sliding window was set to 0.1 sec and advanced 0.04 sec for the consecutive windows. The order for the MVAR model was determined by the Akaike information criteria (AIC) (Akaike, 1974) as an estimate for the number of coefficients which was chosen to optimize the MVAR analysis.

In addition, all signals relevant to the system under study must be observed before final conclusions can be drawn from this analysis. All the selected contacts are included in the MVAR model used for the computation of ERC so that it is not computed for each brain modules separately. In general, the significant ERC is observed for pairs of contacts which are both active and correlated. The coefficients of the MVAR model used in this analysis are calculated using the correlation matrices for all observed contacts. Therefore, the correlation may influence the magnitude of the ERC but will not determine the directionality of the ERC effect. This additional information about directionality of influences is determined by the statistical analysis embedded in the ERC analysis.

All the programs for the ERC analysis were written in C language and developed in the Linux environment and run on a computer cluster implemented as one distributed system.

Statistical Testing

To identify the significant changes in the causal influences, a baseline statistical test was applied in this study, with the significant level set to $\alpha=0.05$. For each paired electrode combination, this test compared the causal influence in every frequency and time between baseline and the intervals of interest (i.e. pre and post stimulus intervals) using a semi-parametric regression model (Boatman-Reich et al., 2010; Korzeniewska et al., 2008). The baseline was taken from data immediate prior to the pre-stimulus interval and the durations for the baseline and intervals of interest were all set to 1 sec.

In addition, a formal bivariate smoothing model that accounted for both frequency and time was also used to reduce the effect of inherited noise in the recorded signals. The number of the knots used for the statistical testing in this study was set to 20 for both time and frequency, and was operationally chosen in order to obtain adequate time and frequency resolution. The significant causal influence in the interval of interest was declared if the causal influence was significantly greater than all those occurred in the baseline. In this study, the significant causal influences were color coded and shown in a time-frequency plot. The computer programs for the statistical test were written in R language and have been previously tested and used in similar multichannel human intracranial recordings (Boatman-Reich et al., 2010; Korzeniewska et al., 2008).

RESULTS

This study was carried out in four subjects (29 to 39 years old, one woman, three men) with medically intractable complex partial seizures, but without tonic clonic seizures. Scalp monitoring suggested the possibility of temporal lobe onsets in all subjects which led to further investigation by bilateral implantation of depth electrodes in the hippocampus, amygdala and frontal lobe. Preoperative evaluation by a neurologist and neurosurgeon, including standard somatic sensory testing, disclosed no neurological abnormality except epilepsy (Lenz et al., 1993). No subject had any medical or psychiatric condition other than epilepsy, or took medications other than anti-epileptic drugs. Laser stimulations evoked painful, pin-prick sensations in all four subjects. The VAS sensory and unpleasantness scores did not vary by number of causal pairs across patients and stages of conditioning (Table 1).

Figure 2 shows the grids of time and frequency plots of ERC during both the habituation and acquisition stages (as labeled) of the FL>AMY interactions located contralateral to the stimulus. Each of the AMY and HIP depth electrodes has five contacts, and each of the FL depth electrodes has six contacts. Therefore, in interpreting Figure 2, the highest possible number of distinct causal pairs for the connection FL>AMY (or FL>HIP, FL>FL) is 30, and in Figure 4 for the connection HIP>HIP this number is 20.

For each time-frequency plot within a grid, the x axis denotes the time from 1 sec before the US to 1 sec after the stimulus, while the Y axis denotes frequency from 0 to 50 Hz. The onset and offset of the response is not precisely interpretable because a sliding window technique is used in both ERC estimation and statistical testing of the data in this figure. It is clear from this figure that the peak-averaged significant ERC occurred within the alpha and beta frequency bands. Decreases for the causal influences were not taken into consideration because the physiological interpretation of decreases in ERC is unclear.

Overall, the results indicate that there are significant causal interactions during fear conditioning in the indirect pathway within the amygdala, between the dIPFC>AMY, and within the hippocampus.

FL>AMY, and dIPFC>AMY Interactions

Contacts in the frontal lobe, and particularly the dIPFC (see Figure 1), and its connections with the amygdala, are an important part of the systems regulating pain and fear (Lorenz et al., 2003) (Apkarian et al., 2005; Lenz et al., 2010; Delgado et al., 2008). Therefore, we first examined grids in Figure 2 for dIPFC>AMY interaction beginning with an analysis of causal interactions of FL>AMY overall.

Prestimulus FL>AMY, and dIPFC>AMY Interactions—The number of FL>AMY ERC pairs during the pre-stimulus interval (outlined in blue and yellow) was significantly less versus that for post-stimulus intervals (outlined in red or yellow)(48/240 vs 71/240, $P=0.0151$). The number of ERC interactions during the habituation stage was not significantly different from that during acquisition (62/240 vs 57/240, $P=0.59$). Examination of Figure 2 suggests that blue pre-stimulus ERC pairs are more common during habituation than acquisition while red post-stimulus ERC pairs are more common during acquisition. Therefore, we examined FL>AMY ERC pairs separately for the pre-stimulus and post-stimulus intervals.

The number of pre-stimulus FL>AMY ERC interactions (blue and yellow) was significantly greater for the habituation than the acquisition stage (31/120 vs 18/120; $P=0.037$, Chi-square). This result is shown in Figure 3A with the width of the arrow reflecting habituation (31/120, 26%). Within each of the four subjects the prestimulus FL>AMY ERC interaction was consistently greater for the habituation than acquisition stage, which is consistent with the test of proportions over all four combined.

The number of Prestimulus dIPFC>AMY interactions was not different between habituation and acquisition (7/20 vs 3/20, $P=0.27$, Fisher).

Poststimulus FL>AMY, and dIPFC>AMY Interactions—We next examined the post-stimulus FL>AMY modular interaction. The number of post-stimulus FL>AMY ERC pairs (outlined in red or yellow) was not significantly different between the habituation than the acquisition stage (32/120 vs 39/120; $P=0.32$, Chi-square).

The proportion of dIPFC>AMY ERC interactions was much greater than that for other FL>AMY interactions overall (24/40 vs 64/200, $P=0.0027$), and in the post-stimulus interval, (21/40 vs 49/200, $P=0.0004$) but not for the pre-stimulus interval (8/40 vs 28/200, $P=0.33$). This suggests that the post-stimulus dIPFC>AMY ERC interactions are greater than the other FL>AMY interactions.

The post-stimulus dIPFC>AMY interactions were not significantly different during the acquisition stage versus habituation (13/20 vs 9/20, $P=0.21$, Chi square), but were very common in both, as illustrated in Figure 3C (9/20, 45%) and Figure 3D (13/20, 65%), as indicated by *.

Prestimulus AMY>FL Interactions—We next examined AMY>FL Interactions since reciprocal relationships can be an important feature of forebrain ERC interaction related to pain (Liu et al unpublished)(Liu et al., 2011a). The number of AMY>FL pairs in the pre-stimulus interval was significantly less than that in the post-stimulus interval (28/240 vs 48/240, $P=0.018$, Chi square). The number of AMY>FL ERC interactions across both intervals was significantly less for the habituation stage than the acquisition stage (15/120 vs. 33/120; $p=0.006$, Chi square).

In the post-stimulus interval, the number of ERC pairs was less in habituation versus acquisition (17/120 vs 33/120, $P=0.011$, Chi Square), and the post-stimulus acquisition case

(33/120, 28%) is shown in Figure 3D. The AMY>FL interaction was consistently greater during the acquisition stage for each of the four subjects.

HIP>HIP and HIP1>HIP2–5 Interactions

We next examined the possibility that interactions between the hippocampus and associated structures are involved in fear conditioning protocols (Buchel et al., 1999; Knight et al., 2004). Among these HIP>HIP interactions no difference was found between pre-stimulus and post-stimulus intervals (29/160 vs 36/160, $P=0.33$, Chi square). The number of significant HIP>HIP ERC interactions was significantly higher for the acquisition stage versus the habituation stage overall (31/80 vs 18/80, $P=0.0258$; Chi-square). For the pre-stimulus period, the proportion of ERC pairs was less during habituation than acquisition (12/80 vs 24/80, $P=0.048$), as indicated in Figure 3B. The number of ERC pairs was consistently greater during acquisition in all four subjects.

Examination of Figure 4 suggests that along the electrodes in the hippocampus during acquisition a high proportion of ERC pairs were found from the deep contact to more superficial contacts (i.e. HIP1>HIP2–5). These contacts may be located in perihippocampal gyrus and hippocampus respectively (Liu et al., 2010; Liu et al., 2011b). During the post-stimulus period (red or yellow), the numbers of these ERC pairs was proportionately greater versus all other HIP>HIP ERC pairs (13/32 vs 23/128, $P=0.012$, Chi square). These interactions were no more common in the pre-stimulus interval versus the post-stimulus interval (9/32 vs 13/32, $P=0.43$, Chi square). Finally, the proportion of these ERC pairs ($n=14$) was greater during acquisition than habituation (10/14 vs 4/14, $P=0.05$, Fisher). The number of post-stimulus HIP1>HIP2–5 interactions is indicated in Figure 3B by the diameter of the small circle labeled HIP1>HIP2–5 interaction for both stages.

AMY>AMY and indirect pathway interactions

We next tested the hypothesis that interactions between structures within the amygdala, particularly the indirect pathway, are dependent upon intervals and stages of fear conditioning protocols. The proportions of AMY>AMY ERC pairs in the pre-stimulus interval were not significantly different from those in the post-stimulus period (Figure 4) (32/160 vs 37/160, $P=0.496$). The proportion of ERC pairs was not apparently different between the habituation versus the acquisition stage overall (AMY>AMY, 28/80 vs 26/80, $P=0.738$, Chi square).

In the pre-stimulus interval, the number of AMY>AMY ERC pairs during the habituation stage was greater than that in the acquisition stage (21/80 vs 11/80, $P=0.044$, Chi square), as shown by the large circle in Figure 3A. No such difference was seen between habituation and acquisition in the post-stimulus stage (21/80 vs 15/80, $P=0.238$, Chi square).

We then tested the possibility that these causal interactions within the amygdala were the result of connections within the putative indirect pathway through the AMY from ventral contacts (AMY123) to dorsal contacts (AMY4, 5). The putative indirect pathway AMY1–3>AMY4–5 includes a maximum of 6 causal pairs of the twenty in the AMY>AMY grid so that there are fourteen ERC pairs in AMY>AMY grid outside the indirect pathway (Figure 4).

The number of causal pairs in the indirect pathway was not different versus all others with inclusion of both stages and both intervals (15/48 versus 39/112, $P=0.66$, Chi square). The magnitude of these interactions overall is shown by the smaller circle (labeled Δ) and the percentages in Figure 3A (15/48, 31%).

The number of ERC pairs in the indirect pathway across the intervals was not different between habituation versus acquisition (5/24 vs 10/24, $P=0.212$, Fisher). Therefore, significant ERC interactions were common in the indirect pathway but were not proportionately more common than all other AMY>AMY ERC pairs and did not differ between intervals or stages.

Exploratory analysis: Pre-stimulus HIP>FL interactions

We next carried out exploratory statistical testing for all modular ERC interactions not considered above in an attempt to identify unanticipated connections. These results are shown in Table 2 which reveals that the number of ERC pairs in each modular interaction changes minimally between intervals and stages for most of these connections.

The exploratory statistical testing demonstrated that the proportion for the prestimulus HIP>FL ERC interaction was significantly greater for the habituation stage than the acquisition stage ((33/120 vs 15/120; $p=0.0061$, Fisher)(Figure 3A, see below). The proportion for the ERC HIP>FL interactions was not apparently different for the habituation stage versus the acquisition stage in the post-stimulus period (22/120 vs 19/120, $P=0.607$, Chi square).

Examination of the HIP>FL grids suggested that during the habituation stage of the intervals combined reveals that the ERC interaction was much greater for HIP5>FL than all other HIP>FL interactions (12/24 vs 15/96, $P=0.0008$). The HIP5>FL ERC interaction was also greater during habituation versus acquisition for intervals combined (12/24 vs 4/24, $P=0.015$, Fisher), and during the pre-stimulus interval (11/24 vs 3/24, $P=0.012$, Fisher) and the post-stimulus interval (10/24 vs 2/24, $P=0.0087$). The prestimulus result is shown in Figure 3A where the whole arrow represents the HIP>FL interaction and the smaller component of the arrow represents the HIP5>FL interaction. The poststimulus result is shown in Figure 3C.

Pre-Stimulus versus Post-Stimulus Interval

Overall, the number of ERC found in the direction from FL>AMY was significantly greater during the post-stimulus versus the pre-stimulus interval under the acquisition stage (10/90 vs 29/90; $p=0.0006$; Chi-square). The number of ERD found in the opposite direction, namely, AMY>FL, was significantly greater during the post-stimulus versus the pre-stimulus interval under the acquisition stage (10/90 vs 22/90; $p=0.019$, Chi-square). Furthermore, the number of ERD found for HIP>FL was significantly greater during the post-stimulus versus the pre-stimulus interval under the acquisition stage (4/90 vs 19/90; $p<0.001$, Chi-square).

Ipsilateral versus Contralateral

In our prior study, functional interactions within the temporal lobe ipsilateral to a painful stimulus were consistently less than those contralateral (Liu et al., 2011b). Similarly, in the present results the numbers of pairs of contacts with significant ERC found under acquisition stage were significantly less on ipsilateral than contralateral side for all three subjects ($p<0.0001$, $p=0.007$ and $p=0.003$ for subjects 2, 3 and 4, respectively, Chi-square). During habituation, the proportion of ERC pairs ipsilateral was less than contralateral in subject 3 ($p<0.001$), while there was no difference related to laterality for subjects 2 and 4. During habituation, the proportion of significant ERC ipsilateral to the stimulus was less versus that for acquisition for all subjects ($p<0.0001$, Chi square).

Therefore, significant causal pairs ipsilateral to the stimulus were less common than those contralateral and were more common during acquisition versus habituation. Therefore, most of this analysis has focused on activity in structures contralateral to the laser stimulus.

In the present study for contacts located ipsilateral to the side of stimulation, the number of AMY>FL ERC interactions was significantly greater under acquisition than habituation stage (19/90 vs 5/90; $p=0.002$, Chi-square).

DISCUSSION

The present results demonstrate that the number of interactions and their direction can change with the time interval and stage of fear conditioning. The change from a common, consistent FL>AMY interaction (Figure 3A) during habituation in the pre-stimulus interval to a common, consistent AMY>FL interaction during acquisition in the post-stimulus interval (Figure 3D) is strong evidence that these networks can change dynamically across time intervals and stages of conditioning.

These dynamic causal interactions also suggest that timing may be a critical factor in the networks subserving fear. The effect of timing on fear conditioning could be manifest through the time course of the stimuli (CS+ and US), the ongoing neural activity, and of electrical or magnetic stimulation for treatment of pathologic anxiety (see below). From the timing perspective, studies of the dynamics of emotion demonstrate that the timing of the reappearance of the inciting stimulus extends throughout the duration of the emotion (Verduyn et al., 2009). Furthermore, strategies of emotional regulation for the treatment of depression demonstrate markedly different time courses for the response to different therapeutic strategies. From an anatomical perspective, the hippocampus and related structures seem to be sensitive to the dynamics of the reversal of extinction (Bouton et al., 2006; McNaughton, 2006).

Cortical causal interactions change dynamically with the time interval and tasks incorporating the laser stimulus (Ohara et al., 2004; Ohara et al., 2006; Ohara et al., 2008). Modular interactions involving limbic structures or association cortex are more likely to be static. Prolonged or stable BOLD functional interactions have been observed during ratings of painful laser stimuli, although these results may be the result of the long intervals involved in the analysis of these interactions (Boly et al., 2007; Kong et al., 2006; Kong et al., 2010).

Methodological Considerations

The subjects in the studies described here all have epilepsy with associated clinical, radiological and electrical abnormalities related to seizure onsets, and with substantial blood levels of antiepileptic drugs (see Table 1 in (Liu et al., 2010), and (Williamson et al., 1993; Levy et al., 2002). As described previously, the occurrence of LEPs or event-related theta activity was not related to mesial temporal sclerosis (Liu et al., 2010), scarring of the medial temporal lobe, which is associated with epilepsy (Williamson et al., 1993). Therefore, differences in the side on which LEPs were recorded could not be explained by the presence of mesial temporal sclerosis or electrical seizure related activity. In the present study, the number of causal interactions was greater on the side contralateral to the stimulus, leading to the focus of the results upon the contralateral side.

There are limitations to ERC analysis which constrain the interpretation of causality. The observation of significant ERC between signals recorded from a pair of electrode contacts does not itself prove that neurons around one recording contact exert a direct causal influence over those at the other contact. It is important to consider the possibility that

causality may be the result of causal interactions involving other 'unobserved' modules, such as the intercalated cell group (see below).

In addition, causal interactions might be detected between signals that are both active and correlated, but not truly causal. However, the coefficients of the MVAR model used in this analysis are calculated using correlation matrices for all observed channels. Therefore, the correlation may influence the magnitude but not the directionality of causal connectivity.

A Hierarchical Distributed Network Subserving Fear

The presence of a significant difference in an ERC interaction across stages was found consistently in all for subjects only for FL>AMY, AMY>FL and HIP>HIP. The presence of differences of this type is emphasized by some of the grids. For example, under the habituation stage for Figure 2, red outlined plots are concentrated on the right for subjects 1 and 2 but not for the others. These differences may reflect individual variation which might be random or related to differences in the neuronal mechanisms by subject. Differences of this kind have been described in the literature of functional imaging (Davis et al., 1998; Chmielowska et al., 1998), but the presence of consistent patterns is additional evidence of the importance of these ERC interactions.

The tests of proportions and some consistent causal pairs demonstrate dense reciprocal relationships between AMY and FL, including dlPFC, (Figures 2A, C & D), which is consistent with the imaging studies of functional connectivity. Fear-related changes in connectivity of the human AMY and FL by fMRI have been reported during the awareness of signals of fear (Williams et al., 2006), or the presentation of objects which produce fear in phobic patients (Ahs et al., 2009). During extinction of fear conditioning, activations are found within the AMY (Buchel et al., 1999; LaBar et al., 1998; Delgado et al., 2008) which are associated with, and may be correlated with, activations in the orbital frontal and subgenual cortex (Milad et al., 2007; Kalisch et al., 2006; Sotres-Bayon et al., 2006).

In the present results, the dlPFC exerted an intense post-stimulus driver role upon all AMY contacts (dlPFC>AMY ERC), including 45 to 65% of all possible ERC interactions (Figure 3C&D). The dlPFC has been implicated in the modulation of fear by cognitive emotional regulation of the CR during fear conditioning (McRae et al., 2010; Delgado et al., 2008). fMRI-based pathway analysis has demonstrated connectivity between the prefrontal cortex and the amygdala during regulation of the response evoked by aversive images (Wager et al., 2008). This connectivity may be related to increased activity in the prefrontal cortex and decreased activity in the AMY during decreases in negative affect associated with emotional regulation by distraction or reappraisal (Ochsner and Gross, 2005; McRae et al., 2010).

Local Networks' Within a Hierarchical Distributed Network Subserving Fear

The pattern of distributed and local causal interactions during the pre-stimulus and post-stimulus periods may be a basic organizing principle of networks involved in fear conditioning. Specifically, these networks may be described as a hierarchical network of modules in which some modules are widely distributed while others are 'local networks' within the amygdala and hippocampus (see Figure 3A&B). These local networks are both found during the pre-stimulus period, AMY>AMY during habituation, and HIP>HIP during acquisition which may reflect the role of the hippocampus in our trace fear conditioning protocol (Buchel et al., 1999; Knight et al., 2004).

ERC interactions within modules (AMY>AMY, and HIP>HIP) were found during pre-stimulus intervals, rather than between modules in the network subserving fear. A significant HIP>HIP modular interaction is from ventral to dorsal may reflect the pathway from perihippocampal gyrus to hippocampus (Wilson et al., 1990; Ploghaus et al., 2001;

Blatt et al., 2003). The pre-stimulus AMY>AMY causal interaction during habituation may be related to vigilance for the CS+ and US which is a function of the amygdala (Davis and Whalen, 2001).

Lesions of modules in a hierarchical network lead to 'double dissociation' in which lesions of each module produce a distinct abnormality, as in the case of language networks (Bullinaria, 2002). In the present case, separate abnormalities of conditioning are found with lesions of AMY and the HIP (Bechara et al., 1995; Meunier et al., 1999; Baxter and Murray, 2000). In view of these reports, the present data from the AMY and HIP may represent 'local networks' which subserve conditioned fear through their involvement in a hierarchical network.

The putative 'local network' in the AMY may involve the direct and indirect pathways. LEP and ERC may indicate activity of the putative direct and indirect pathway respectively (Liu et al., 2011a). These results demonstrate that the indirect pathway apparently is not different across intervals and stages. It may be a pathway which is involved broadly enough to play a pivotal role in networks subserving fear conditioning overall.

The connection between the lateral and the central nucleus of the amygdala could be mediated via the inhibitory intercalated cell group, acting as an unobserved module (Bernard et al., 1996; Neugebauer, 2007; Pare et al., 2004). In that case, decreased input from the lateral nucleus to the intercalated cell group would lead to decreased inhibition of the central nucleus and so to increased firing, a mechanism known as disinhibition. Unobserved causal interactions of this kind could account for the interactions within the amygdala, which are not explained by the direct and indirect pathway.

This model of a hierarchical network composed, in part, of 'local networks', may lead to a better understanding of the anatomical and physiological bases of fear conditioning. This model may also suggest new targets which may optimize stimulation therapies for anxiety disorders like OCD (Nuttin et al., 2003; Rauch, 2003; Goodman et al., 2010). A range of conditions have been treated with stimulation techniques including: 1) transcranial magnetic stimulation (TMS) (Leo and Latif, 2007; Wassermann et al., 2010), and 2) electrical stimulation at sites including frontal (subgenual) cortex (Fontaine et al., 2008). The efficacy of these therapies may result from activation and disruption of a single module (Desmurget et al., 2009) (Sirigu et al., 2010), or from activation of a network by stimulation of either a single module (Karnath et al., 2010; Desmurget et al., 2009; Sirigu et al., 2010) or a subcortical white matter pathway (Karnath et al., 2010; De et al., 2007; Herbsman et al., 2009). In psychiatric disease, effective stimulation has been shown to disrupt widespread networks as measured by the extent of stimulation-evoked change on cognitive testing (Levit-Binnun et al., 2007), and of activation plus functional connectivity in imaging studies (Shajahan et al., 2002). Therefore, studies of the network involved in fear conditioning may predict frontal lobe stimulation sites based on their widespread causal influence upon other modules in the networks subserving fear.

Acknowledgments

This work was supported by the National Institutes of Health – National Institute of Neurological Disorders and Stroke (NS38493 to FAL). We thank L.H. Rowland and J. Winberry for excellent technical assistance.

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Highlights

- Human amygdala, hippocampus and frontal lobes process nociceptive inputs during fear conditioning
- Causal influences are exerted between amygdala, hippocampus and frontal lobes
- Causal influences are also exerted within the hippocampus and amygdala.
- Dorsolateral prefrontal cortex exerts causal influences upon amygdala
- These interactions change with time and stages of fear conditioning
- The network subserving fear includes dynamic distributed and local networks

Figure 1.
Location of stereotactic implant of the depth electrode in the frontal lobe. See text.

Figure 2.

The ERC between a pairs of electrodes between the FL and the AMY (FL>AMY). This is displayed in grids of plots of time (horizontal axis -1 to 1 sec) versus frequency (vertical axis 0 – 50 Hz) for contacts contralateral to the stimulus for all three subjects, as labeled. Time is also indicated by a tic on the horizontal time axis. The labels across the top of the grid indicate the driver module while those along the left side of the grid indicate the receiver role. These labels indicate side (R or L), module A, H and F, depth electrode (D) and number of the contact along he electrode; therefore, RFD1 is the first contact on the right frontal depth electrode, and RAD1 is the first contact on the right AMY depth electrode. Habituation versus acquisition stages of conditioning are in the left and right columns, as labeled. Time zero is indicated by a tic on the horizontal (time) axis. Significant ERC is indicted by hot colors so that increasingly dark colors indicate increasing causality above the significant level. The borders of the significant ERC time frequency plots between electrode contacts are colored blue, red and yellow to indicate the time intervals when ERC was found. Blue indicates the pre-stimulus, red denotes post-stimulus and yellow denotes both pre-stimulus and post-stimulus ERC.

Figure 3.

Diagram of the significant interactions between and within AMY, HIP and FL modules. In this Figure, columns (3A&C and 3B&D) are habituation and acquisition, while rows (3A&B and 3C&D) are pre-stimulus and post-stimulus intervals, respectively. The width of the arrow, the diameter of the circle, and the percentages correspond to the number and percentage of ERC pairs in that time interval and stage of fear conditioning as labeled. For example, 3C presents the results for the prestimulus interval and the acquisition stage. See text.

Figure 4.

The ERC between a pairs of electrodes within the hippocampus (HIP>HIP). RHD1 indicates the first contact on the right hippocampal electrode. Conventions are as in Figure 2.

Table 1

The ratings for the pain and unpleasantness. Within each column there are two stages of fear conditioning protocols, i.e. habituation, conditioning.

Subject	VAS		Unpleasantness	
	First run	Second run	First run	Second run
S2	3/10, 3/10	3/10, 3/10	NA, NA	NA, NA
S3	5/10, 5/10	5/10, 5/10	5/10, 5/10	5/10, 5/10
S4	6/10, 6–7/10	7/10, 7/10	2–3/10, 3/10	5/10, 8/10
S5	2/10, 2/10	4/10, 3/10	1/10, 2/10	4/10, 2/10

Table 2

Exploratory ERC interactions not suggested by prior evidence are listed by the interaction and the total possible of ERC interactions (left column). Numbers and percentages of observed ERC interactions between habituation and acquisition in the pres and poststimulus intervals as labeled.

	Pre-stimulus		Post-stimulus	
	Habituation	Acquisition	Habituation	Acquisition
AMY>HIP, 100	30 30%	18 18%	19 19%	19 17%
FL>FL, 120	31 26%	26 22%	41 34%	41 31%
FL>HIP, 120	22 18%	20 17%	26 22%	26 23%
HIP>AMY, 125	19 19%	16 16%	23 23%	23 17%
HIP>FL, 120	33 28%	15 13%	22 18%	19 16%