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Dynamic neural activity recorded from human amygdala during fear conditioning using magnetoencephalography

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Abstract

Magnetoencephalography (MEG) was used to record the dynamics of amygdala neuronal population activity during fear conditioning in human participants. Activation during conditioning training was compared to habituation and extinction sessions. Conditioned stimuli (CS) were visually presented geometric figures, and unconditioned stimuli (US) were aversive white-noise bursts. The CS+ was paired with the US on 50% of presentations and the CS− was never paired. The precise temporal resolution of MEG allowed us to address the issue of whether the amygdala responds to the onset or offset of the CS+, and/or the expectation of the initiation or offset of the an omitted auditory US. Fear conditioning elicited differential amygdala activation for the unpaired CS+ compared to the CS−, extinction and habituation. This was especially robust in the right hemisphere at CS onset. The strongest peaks of amygdala activity occurred at an average of 270 ms in the right and 306 ms in the left hemisphere following unpaired CS+ onset, and following offset at 21 ms in the left and 161 ms in the right (corresponding to an interval of 108 ms and 248 ms after the anticipated onset of the US, respectively). However, the earliest peaks in this epoch preceded US onset in most subjects. Thus, the activity dynamics suggest that the amygdala both differentially responds to stimuli and anticipates the arrival of stimuli based on prior learning of contingencies. The amygdala also shows stimulus omission-related activation that could potentially provide feedback about experienced stimulus contingencies to modify future responding during learning and extinction.

Keywords: Amygdala; Magnetoencephalography; Fear conditioning; Classical conditioning; Associative learning; Dynamic activity

1. Introduction

Fear conditioning involves the pairing of a conditioned stimulus (CS) with an aversive unconditioned stimulus (US), following which the CS serves to elicit a conditioned response (CR) normally elicited by the US. Converging evidence suggests that the amygdala is activated during fear conditioning. Damage to the amygdala leads to impaired fear conditioning in humans and non-human species [3,5,10,21,26,37,39,46]. Amygdala activation is found during fear conditioning in non-human invasive recording [2,27,28,40,41,44], as well as in humans during positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) [7–9,11,20,23,33,38]. However, little is known about the dynamics of neuronal responses in the human amygdala in the conditioned state.

In the current study, we used magnetoencephalography (MEG) to record human amygdala activity during fear conditioning. MEG permits identification of the dynamics of neuronal responses with temporal resolution not available with PET and fMRI. Importantly, this effort involved identification of the dynamics of responses in a subcortical region that has not been

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explored previously with scalp electroencephalography (EEG) methods.

In a previous report, we used MEG to characterize the dynamics of neuronal activity within the auditory cortex during fear conditioning [34]. The participants, paradigms and recorded MEG data were identical to those used in the present study. We found a differential "magnetocerebral" response (C50m) in the primary auditory cortex following the unpaired CS+ at approximately 50 ms after US omission. The C50m was stronger for the unpaired CS+ during training than for the CS−, and compared to habituation and extinction sessions. We also reported differential autonomic (pupil dilation) responses for the unpaired CS+ versus CS− in a follow-up study with identical stimuli. The observation of a differential autonomic response demonstrated that the presented stimuli could elicit a conditioned response. The differential C50m activity in the primary auditory cortex elicited by the same stimuli during the different phases of the study constituted a direct observation of associative neural plasticity within the human auditory cortex.

In the present analysis of these data, we expand our findings with an investigation of the contributions of the amygdala to associative learning and plasticity during fear conditioning. Human fMRI studies suggest that amygdala activation is closely related to CS presentation and production of the CR, rather than simply co-occurring along with CR expression [9]. The excellent temporal resolution of MEG allowed us to address the issue of whether the amygdala shows differential activation to the presentation of stimuli based on prior learning (to the onset or offset of the CS) or actually anticipates the arrival of stimuli based on knowledge of stimulus contingencies (to the omission of the US). Activation following US omission would suggest that the amygdala may provide feedback as to whether expected stimulus contingencies were realized, and could potentially participate in the generation of a conditioned response.

2. Methods

2.1. Participants and data acquisition

MEG data were recorded from four male and four female participants, aged 24–31, with no history of neurological or other diseases. This study was approved by the Institutional Review Boards of the University of New Mexico and Helsinki University Central Hospital. Informed consent was obtained from all participants. Data were collected at the BioMag Laboratory, Helsinki University Central Hospital with a 306-channel MEG array (VectorView™, Elekta Neuromag Ltd.). Participants were seated in a magnetically shielded room under a cryogenic dewar containing the MEG sensors. Electrodes were placed on participants to record eye movements and blinks, and cardiac activity. Four small coils of wire were attached to the participants' scalp and energized with minute currents to determine the location of the head with respect to the MEG array. Data were collected at the sampling rate of 600 Hz, with a band-pass filter of 0.3–200 Hz. Continuous and averaged data were stored for off-line analysis. Structural magnetic resonance images (MRI) were obtained for each subject using standard clinical procedures with a 1.5-T Siemens Magnetom system at the Helsinki University Central Hospital.

2.2. Stimuli and experimental procedures

Each conditioned stimulus (CS) was an achromatic square displayed for 1500 ms in the center of a back-projection screen on a black background (subtended visual angle 1.4°). The stimuli were all the same size, shape, colour, brightness, etc., and were quite similar in appearance, so that they would not elicit differences in visual activation. The unconditioned stimulus (US) was a 100-ms binaural white-noise burst presented via headphones, adjusted for each subject's hearing level so that it was loud enough to be aversive, but not painful (90–100 dB). The inter-trial interval (ITI) was 4 s. A delay conditioning paradigm was used, meaning that the presentation of the US overlapped the termination of the CS+. The CS+ had a diagonal striped pattern (stimulus 2), and the CS− had a checker-board pattern (stimulus 1; Fig. 1). The stimulus sequences in each phase are described below.

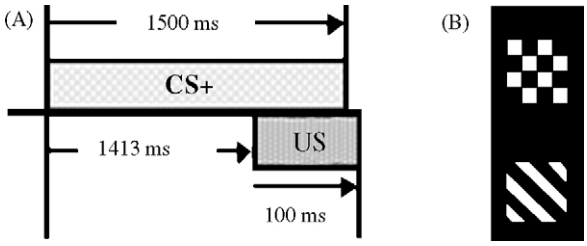

Fig. 1. Schematic of conditioning paradigm. (A) The US onset prior to the termination of the CS+ and (B) visual stimuli: checker-board stimulus (top: stimulus 1) presented during habituation and used as CS− during conditioning training and extinction, diagonally striped (bottom: stimulus 2) stimulus used as CS+ during conditioning training and extinction.

2.2.1. Habituation

Stimulus 1 was presented 100 times. A different stimulus, a square containing smaller squares, was used for two participants during the habituation phase in order to examine whether the subsequent differential responses to CS+ versus CS− during training were influenced by the presentation of Stimulus 1 during the habituation session. Since these participants did show the expected pattern of differential responses, their data were combined with that of the other participants for all analyses.

2.2.2. Training

The CS+ was paired with the US on 50% of presentations, and the CS− was never paired with the US. The US was presented 1413 ms following the onset of paired CS+. The CS−, the paired CS+, and the unpaired CS+ were presented 100 times each in random order (Fig. 1).

2.2.3. Extinction

The CS+ and the CS− were presented 100 times each in random order. The US was never presented.

2.2.4. Other sessions

A trace conditioning session was also run, following the end of delay conditioning extinction. However, the data from this session will not be discussed in the current manuscript, and is reported previously [34].

Short pilot sessions were run throughout the data collection in which ITIs were 10 s. Prior to the training phase, the participants viewed the paired CS+ and the CS− four times each. Subsequently, a test session was run in which the subject viewed the unpaired CS+ and the CS− four times each. This test was also run prior to and following the extinction phase, as well as following the training sessions. All participants were run on all of the experimental phases; however, due to a technical error the extinction data from one participant was lost. The participants also viewed the CS− four times prior to, and after, the habituation phase.

The purpose of the test sessions was to investigate whether conditioning-related heart rate changes could be measured given the relatively short ITI used in the present study. Observations of changes in human heart rate during conditioning typically use much longer ITI. The cardiac signals for these trials were examined; however, they were extremely variable between the six participants. We concluded that this indirect measure of change in brain activity during conditioning is unlikely to be practical or productive for MEG conditioning studies using short ITIs (data not shown).

In order to ensure that the short pilot sessions did not have a major effect on the results, two participants were run with the pilot session omitted. We also wanted to ensure that using the same stimulus for habituation and the CS− during training did not influence our findings, so we used a different stimulus for the habituation session for these two participants. Data collected from these two participants showed no detectable differences with data from the original six participants, and thus data from all eight participants were pooled for the final data analysis.

Thus, all participants underwent identical conditioning sessions. The only difference was prior to conditioning six participants passively viewed an achromatic square stimulus with a checker-board pattern, while two passively viewed a similar achromatic square stimulus (of the same size, brightness, etc.) containing successive smaller squares. No conditioning training occurred during this passive habituation session, and no noise was presented during this session. All subjects then underwent conditioning using identical stimuli and stimulus parameters, and the two that viewed the alternate (but very similar) stimulus showed the expected pattern of differential conditioned responses shown by the other six subjects. No detectable differences occurred between these two subjects and the other subjects upon visual inspection, and the amplitudes and latencies of their responses were within the range of the other six participants.

2.3. Data analysis

2.3.1. Preparation for source analysis

MEG data were averaged off-line over 2300-ms epochs, time-locked to the presentation of the different stimulus types (paired CS+, unpaired CS+, CS−) during the different experimental phases (habituation, training and extinction; see Fig. 2 for an example of averaged sensory data). The averages were low-pass filtered at 45 Hz. A baseline for measured responses was selected at 2100–2200 ms following CS onset. This post-stimulus baseline was selected in order to minimize possible pre-stimulus anticipatory activity. An L1 minimum-norm current estimate (MCE) [30,54] was used to estimate the primary current distribution in the brain from the MEG data. A realistically shaped boundary element model (BEM) for the conducting volume of the brain was extracted from individual participants' MRIs for magnetic field computations in the MCE analysis. This algorithm was implemented with signal-space projection to remove cardiac and blink artifacts as well as ambient noise [55].

2.3.2. Extraction of sources

The MCE algorithm permits detailed modeling of distributed activity, as well as approximations based on more discrete neuronal activation, with no a priori assumptions on the number of active regions. The MCE data inversion algorithm has been used previously to characterize regions of activation within the brain [24,42,54–57].

The following procedure was used to select specific source locations from the MCE inversion. (1) A threshold of 2 nA m was used to establish the current flow distributions. Using this threshold, inspection of the MCE inversions revealed that current flow in the amygdala appeared as disjoint volumes of activation (Fig. 3). (2) Sources were determined for disjoint volumes of activation in amygdala in the left and right hemispheres. Each such volume was approximated by an ellipse that encompassed all contiguous current sources with amplitudes >60% of the value at the center of the ellipse. Source locations that overlapped the left or right amygdala by 1 cm were retained for further analysis. (3) Each source defined an associated dipolar current approximate and waveform for the mean current orientation and amplitude from the MCE inversion. Current dipole approximates and the corresponding waveforms (depicting strength of activation within the source over time) were retained for further analysis.

2.3.3. Analysis of waveform amplitude peaks and latencies

Latencies and amplitudes of evoked-responses were determined from the waveforms for the first 600 ms, as well as 1300–1900 ms following the onset of the visual stimulus during the habituation, training and extinction sessions. This allowed for examination of brain activity during stimulus onset and offset. The single highest amplitude peak from each epoch was selected for analysis. If several peaks occurred in the waveform during these epochs, the largest was selected for analysis.

Peak amplitudes were determined for activity corresponding to amygdala sources following presentation of the paired and unpaired CS+. These peaks were subsequently examined for activity corresponding to the identical source locations for the responses to the unpaired CS+, to presentation of other stimulus types (CS−) and for other phases (extinction, habituation). The peak amplitude of the unpaired CS+ was compared to that in the other conditions within ±10 ms of the peak selected for the unpaired CS+ condition. If no peak occurred during this latency window, the amplitude of the activity at the exact latency of the unpaired CS+ peak was selected for analysis. These criteria for selecting activity peaks in MEG data for comparison across conditions are similar to those used in previous MEG studies of auditory activation [14,52,53].

2.4. Statistical analysis

Comparisons of the number of participants showing sources in the amygdala during the different stimulus types and phases were performed using Fischer's exact statistic. For analysis of peak amplitude, the strongest responses from each epoch were compared between stimulus types and phases for each brain hemisphere using repeated measures analysis of variance (ANOVA). For comparison

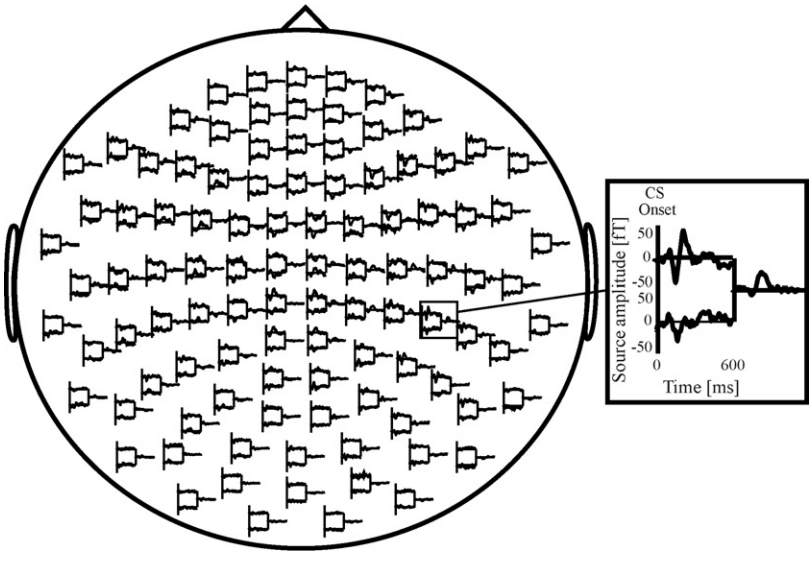

Fig. 2. MEG 306-channel sensor data for presentation of the conditioned stimulus with the US omitted (unpaired CS+) during delay conditioning (subject 1).

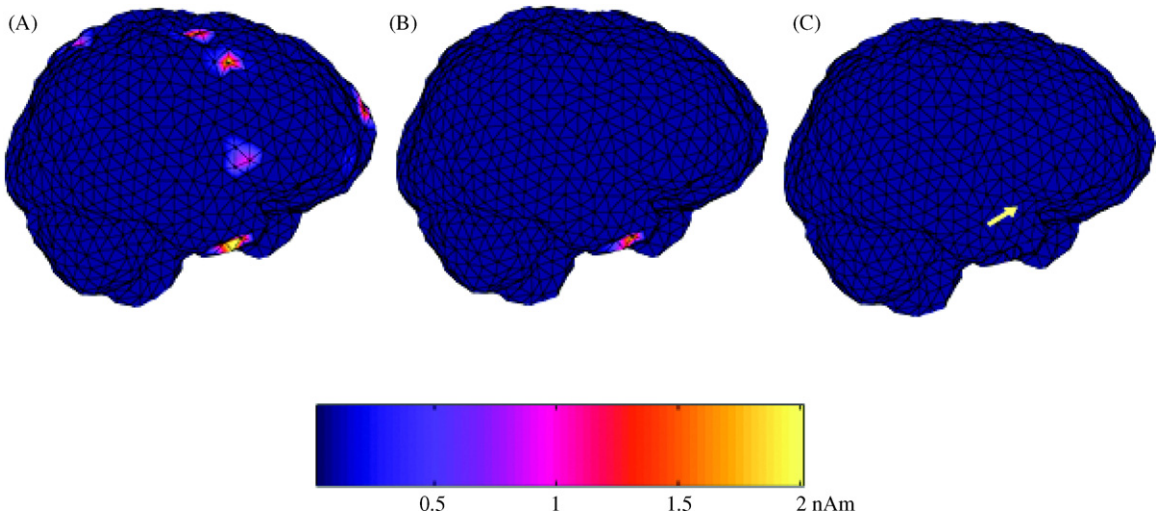

Fig. 3. Response to unpaired CS+ (subject 1, at 125 ms following stimulus onset). (A) Regions of activity obtained with the MCE inversion algorithm are projected onto the right surface of the brain; (B) selection of isolated sources within the right amygdala; (C) representation of current dipole approximates for activity within.

of responses to the different CS within the training sessions, factors included "stimulus" (CS+ and CS−) and "hemisphere". For comparison of responses to the CS across the different phases, factors included "phase" (habituation, training and extinction) and "hemisphere". Additionally, the analysis of the strongest peaks was repeated using the non-parametric "sign test", which does not assume normality.

3. Results

3.1. Amygdala source incidence

Comparisons revealed that during conditioning training, more participants showed amygdala activity for the unpaired CS+ during training than for the CS−, the unpaired CS+ during extinction, and compared to habituation (Table 1; Fig. 4). Every subject showed amygdala activity following presentation of the unpaired CS+ during training, collapsed across hemispheres and epochs. For the unpaired CS+ during training compared to habituation, significantly more participants showed sources in the right amygdala during the first 600 ms ($p < 0.001$), and during the 1300–1900 ms epoch ($p = 0.020$). Significantly more participants showed amygdala activity during training compared to habituation for the first 600 ms collapsed across hemispheres ($p = 0.003$) and collapsed across epochs ($p = 0.038$). Additionally, collapsed across hemispheres, significantly more participants showed amygdala activity during training compared to habituation for the 1300–1900 ms epoch ($p = 0.009$) and collapsed across epochs ($p = 0.013$). There was a tendency for more participants to show right amygdala activity during the 1300–1900 ms epoch during training compared to habituation ($p = 0.056$). Significantly more participants showed amygdala activity for the unpaired CS+ during training compared to the CS− in the right hemisphere during the first 600 ms ($p = 0.020$) and collapsed across hemispheres and epochs ($p = 0.038$). Significantly more participants showed right amygdala activity for the unpaired CS+ during training compared to extinction for the first 600 ms ($p = 0.009$) and during the

Table 1
Incidence of participants showing sources in the amygdala during conditioning training, habituation and extinction

Source incidence	Amygdala		
Stimulus	L	R	R or L
Unpaired CS+ (training)			
0–600 ms	5	7	8
1300–1900 ms	5	6	7
Either	6	7	8
CS− (training)			
0–600 ms	4	2	4
1300–1900 ms	3	2	4
Either	4	3	4
Habituation			
0–600 ms	2	0	2
1300–1900 ms	3	0	3
Either	2	0	4
Unpaired CS+ (extinction)			
0–600 ms	1	1	2
1300–1900 ms	1	1	1
Either	1	2	3

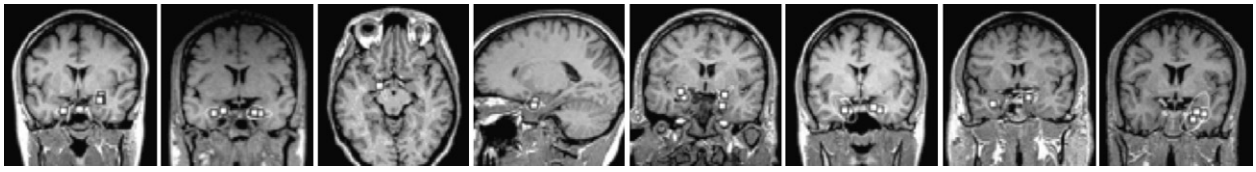

Fig. 4. Amygdala sources for all participants following presentation of unpaired CS+ during conditioning training.

1300–1900 ms epoch ($p = 0.030$). There was a tendency for more participants to show more left amygdala activity during training compared to extinction for each epoch, although this failed to reach significance ($p = 0.078$).

3.2. Peak latencies and amplitudes

During delay conditioning, the largest peak in the waveform corresponding to amygdala sources following the unpaired CS+ during training occurred during the first 600 ms at an average of 270 ms (S.D. 158) in the right hemisphere and 306 ms (S.D. 159) is the left hemisphere following CS+ onset. The earliest peaks, which were not the largest, in this epoch occurred at an average of 175 ms (S.D. 130) in the right and 225 ms (S.D. 168) in the left hemisphere following CS+ onset. During the 1300–1900 ms epoch the largest peak occurred at an average of 1521 ms (S.D. 168) in the left hemisphere and 1661 ms (S.D. 145) in the right hemisphere (21 and 161 ms following CS termination, respectively). This corresponds to 108 ms and 248 ms after the anticipated onset of the US, respectively. The earliest peaks in this epoch occurred at an average of 1415 ms (S.D. 203) in the right and 1494 ms (S.D. 245) in the left hemisphere, prior to CS termination. Note that for five of six subjects showing right amygdala activation during this epoch, and for four of five showing left activation, the earliest amygdala peaks preceded the onset of the US. Thus, amygdala activation occurred in response to both CS+ onset and offset, as well as in anticipation and in response to the omission of the US. Interestingly, although activation was found in anticipation of the US, the strongest peaks were found in response to US omission.

Table 2
Means of peak amplitude of CS+-evoked activity in amygdala

Stimulus	Left	Right
Unpaired CS+ (training)		
0–600 ms	17 (9)	12 (7)
1300–1900 ms	13 (7)	6 (3)
CS− (training)		
0–600 ms	3 (7)	2 (2)
1300–1900 ms	0 (0)	0 (0)
Habituation		
0–600 ms	1 (2)	−2 (3)
1300–1900 ms	1 (1)	0 (1)
Unpaired CS+ (extinction)		
0–600 ms	0 (0)	0 (1)
1300–1900 ms	0 (0)	0 (0)

Means for average waveform response amplitude (nA m) following presentation of the unpaired CS+ during delay and trace conditioning training. Standard deviations in parentheses.

Average amplitude of the strongest peak of amygdala activity was significantly larger for the unpaired CS+ during training compared to the CS− during the first 600 ms in the right hemisphere (ANOVA: $F(1, 6) = 23.0$, $p = 0.003$; sign test: $p = 0.016$; Figs. 5 and 6A; Table 2), and bilaterally during the 1300–1900 ms epoch (ANOVA, right—right: $F(1, 5) = 27.9$, $p = 0.003$, left: $F(1, 4) = 13.9$, $p = 0.020$; sign test, right: $p = 0.031$, left: $p = 0.063$ (trend); Fig. 6C). Bilateral amygdala activity was significantly stronger for unpaired CS+ during training compared to the CS+ during extinction and compared to habituation for the first 600 ms. This effect is sta-

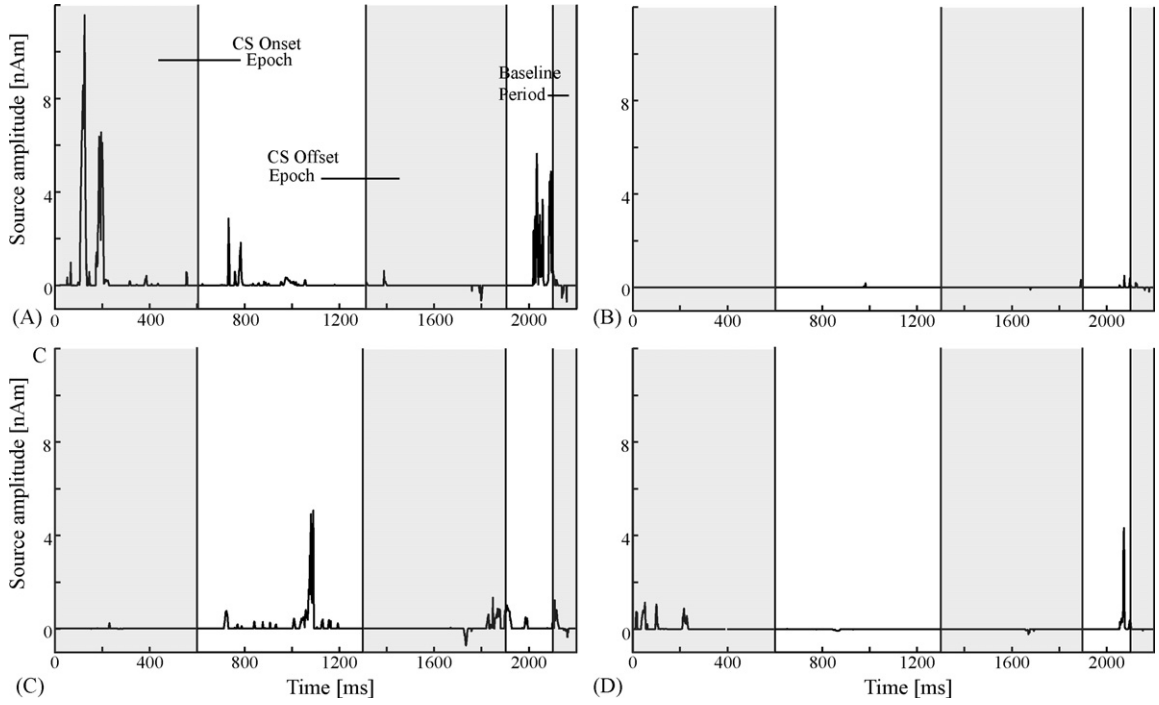

Fig. 5. Response to unpaired stimuli corresponding to a source in the right amygdala during conditioning following CS onset (subject 1). (A) Waveform during the training phase following presentation of the unpaired CS+; (B) waveform during the training phase following presentation of CS−; (C) waveform during the extinction phase for unpaired CS+; (D) waveform for stimulus during pre-training habituation phase.

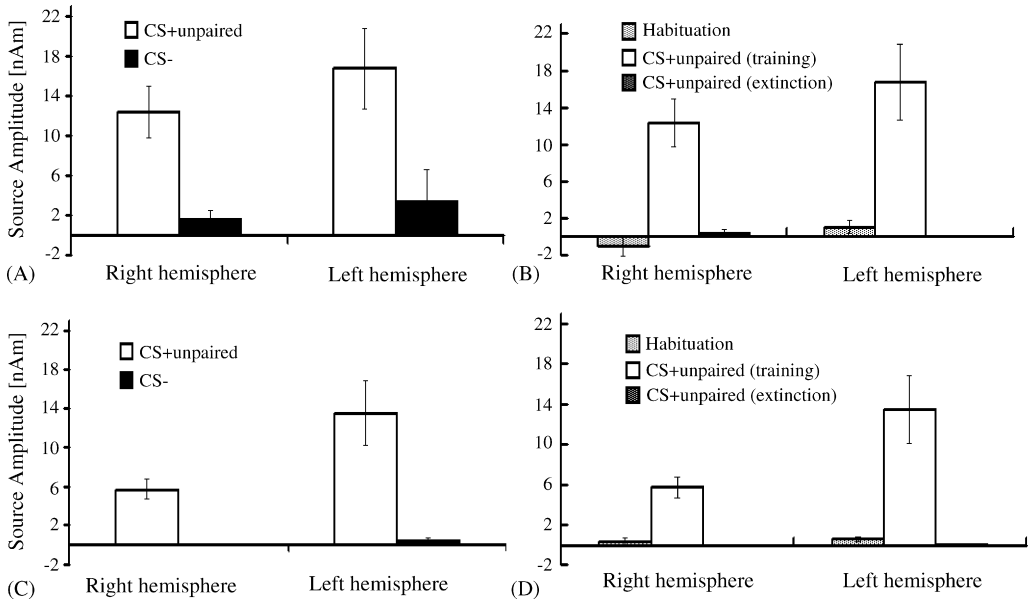

Fig. 6. Unpaired CS+ activity in amygdala vs. other conditions during conditioning training. (A) Activity was stronger for the unpaired CS+ during training compared to the CS− during the first 600 ms in the right hemisphere; (B) activity was stronger during the first 600 ms for the unpaired CS+ during training compared to extinction and habituation, with a similar trend in the left for habituation; (C) activity was stronger in the right hemisphere during the 1300–1900 ms epoch, with a similar trend in the left; (D) activity was stronger during the 1300–1900 ms epoch for the unpaired CS+ compared to habituation in the right hemisphere, and there was a similar trend compared to extinction.

tistically demonstrated with the ANOVA by a main effect of "phase" (left: $F(2, 6) = 16.6$, $p = 0.022$; right: $F(2, 10) = 15.6$, $p = 0.001$) and contrasts comparing unpaired CS+ to habituation and extinction ($F(1, 3) = 1.844$, $p = 0.0268$; right: $F(1, 5) = 20.0$, $p = 0.007$). The sign test revealed a similar pattern of results, however only right amygdala activity during the first 600 ms was significantly stronger for the unpaired CS+ during training compared to the CS+ during extinction ($p = 0.031$) and compared to habituation ($p = 0.016$, Fig. 6B). Although, there was a tendency for left amygdala activity to be stronger during the same epoch for the unpaired CS+ during training compared to habituation ($p = 0.063$). Bilateral amygdala activity was also stronger for the unpaired CS+ compared to other stimuli during the 1300–1900 ms epoch, demonstrated by a main effect of "phase" (left: $F(2, 6) = 12.9$, $p = 0.035$; right: $F(2, 8) = 19.5$, $p = 0.012$), and contrasts comparing unpaired CS+ to habituation and extinction ($F(1, 3) = 17.0$, $p = 0.026$; right: $F(1, 4) = 19.5$, $p = 0.012$; Table 2; Fig. 6D). Again the sign test revealed a similar pattern, but only right amygdala activity was significantly stronger during the 1300–1900 ms epoch for the unpaired CS+ compared to habituation ($p = 0.031$), and there was a tendency in this hemisphere compared to extinction ($p = 0.063$; Fig. 6D).

4. Discussion

We recorded the dynamics of human amygdala activity during fear conditioning with MEG. In a previous examination of the same MEG dataset we identified a differential magnetocerebral conditioned response in primary auditory cortex that occurred at approximately 50 ms following omission of the auditory US (the C50m). We also observed a differential autonomic conditioned response in a follow-up behavioral study using identical stimuli.

In the present study, we found differential activation of the amygdala in response to the unpaired CS+ compared to the CS− during the training phase, as well as the unpaired CS+ during the training phase compared to the unpaired CS+ during the extinction phase and the unpaired stimulus during the habituation phase. These findings demonstrate that the amygdala responds to the different stimuli based on prior learning.

Every participant showed activation of the amygdala within the first 600 ms following presentation of the unpaired CS+ during training. More participants showed activity for the unpaired CS+ during training than for CS−, extinction, and habituation. This effect was robust in the right, although bilateral differences were observed for the unpaired CS+ during training versus extinction. Activation of the amygdala observed in the present MEG study is consistent with previous reports of right amygdala involvement during fear conditioning [11]. Moreover, right amygdala activity was stronger for unpaired CS+ during training versus CS−, and versus extinction and habituation; similar trends occurred in the left hemisphere. These results are consistent with fMRI findings of differential amygdala activation elicited during fear conditioning [8,7,22,38], and with non-human electrophysiological findings that amygdala neurons show increased responding following CS+ presentation [2,12,28,31,40,41,43].

The number of participants showing amygdala activity was similar for extinction and habituation. Only three of the eight participants showed activation during extinction, in contrast to previous fMRI findings of amygdala activation during extinction [20,22]. In the present study 100 extinction trials without reinstatement were averaged in order to obtain an optimal

signal-to-noise ratio and to facilitate comparison with the habitation and training phases. Reinstatement trials may be necessary for observation of extinction-related brain activity with methods such as MEG that require fairly large numbers of trials. This suggestion is consistent with the findings of Knight et al. [20], who found rapidly habituating increased amygdala activation at the beginning of an extinction session. Similarly, Phelps et al. [38] found differential amygdala responding during day 1, but not day 2, of extinction.

The precise temporal resolution of MEG allowed us to examine the timing of the amygdala response, in order to address the issue of whether or not the amygdala responds to the initiation of the CS+, the offset of the CS+, and/or the expectation of the initiation or offset of an omitted auditory US.

The strongest peak of amygdala activation within the CS-onset epoch occurred at an average of 270 ms in the right and 306 ms in the left hemisphere following unpaired CS+ onset. This timing is consistent with previous MEG recordings of the latency of amygdala activation during facial recognition [25,45] and oddball detection [17]. Additionally, this timing is consistent with Halgren et al. [13], who found the most consistent amygdala peaks at 250–560 ms using human invasive recording during facial processing. The timing is also consistent with non-human electrophysiological reports that excitatory neuronal responses in the basolateral and central amygdala nuclei occurred approximately within the first second following stimulus onset [43]. Note that some non-human electrophysiological studies [2,12,28,40,41] report extremely early (within first 100 ms) amygdala responses during conditioning, although these responses are sustained for up to 5 s [2]. In the current study we focused our analyses on the strongest detected amygdala peaks. However, we did find that half of our subjects showed their earliest peaks within the first 100 ms. It is possible that the current methodology was not sensitive enough to detect the extremely early amygdala responses.

The strongest peak of amygdala activation within the CS-offset epoch occurred at an average of 21 ms following unpaired CS+ offset in the left hemisphere, and at an average of 161 ms following offset in the right hemisphere (corresponding to an interval of 108 ms and 248 ms after the anticipated onset of the US, respectively). The activation of the amygdala following omission of the US observed here suggests that recruitment of this structure may occur in response to omission of the US in the conditioned state. This may point to a possible feedback mechanism for responding to the realization of expected stimulus contingencies, and the potential for the amygdala to then modify future responding during associative learning and extinction. In addition, the earliest amygdala peaks within the CS-offset epoch were found to precede the anticipated arrival of the US in most participants, suggesting a possible anticipatory role for the amygdala in fear conditioning. The activity latency also supports the potential for amygdala participation in the generation of a conditioned response in auditory cortex.

Some question the capacity of MEG to accurately detect and localize signals from deep neural structures [4,32]. Individual MEG sensors are often optimized to detect activity represented locally on the scalp from sources within superficial fissural cortex. However, modern whole-scalp sensor arrays [1] capture magnetic flux signals represented across the entire array that are generated by sources deep in the brain [47]. MCE and other data analysis methods now exist that are used routinely to detect activity in MTL and other deep structures (cf. [15–19,29,35,36,47–51]). Importantly, the MEG array used for the present study (VectorView^{TM}, Elekta Neuromag Ltd.) included 102 single-loop "magnetometer" pick-up coils with excellent sensitivity to deep sources in the brain.

The amygdala is a collection of nuclei. Although most MEG studies report activity attributed to current flow in the dendrites of cortical pyramidal cells in neocortex, there is no reason to assume that MEG/EEG cannot detect activity from nuclei. Current flow in neuronal structures is the primary source of both MEG and EEG signals. There are reports of successful recording MEG signals from thalamic [6,47] and amygdalar nuclei [17,18,25,45].

MEG detection of evoked brain responses typically requires averaging over many trials, which may be counterproductive if the region of interest habituates rapidly to the stimuli. Some fMRI studies report that amygdala activation decreases rapidly [8,7,22]. Büchel et al. [7,8] found that differential amygdala activation, evaluated by a subtraction between activation for the unpaired CS+ and the CS−, was best characterized by a time-by-event interaction, suggesting that amygdala activation was greater during the beginning of the study compared to the end. Labar et al. [21] also analyzed a subtraction between activation for the CS+ and CS−, and found significant amygdala activation occurring during the first eight trials but not the last eight. However, a recent fMRI study suggests that these findings may be due to an experimental design in which a new stimulus contingency is learned only at the beginning of a conditioning session [20]. Knight et al. [20] found rapidly habituating increased amygdala activation following changes in stimulus contingencies, such as the onset of an extinction session. Nonetheless, they also found significant activation of amygdala averaged over the entire conditioning training session. This particular study did not find differences in levels of amygdala activation between subjects that experienced true conditioning sessions (CS paired with US), or those that experienced the CS and US in an explicitly unpaired manner. This finding may be due to the lack of sensitivity of the between-subjects design, and this issue may be addressed by conducting within-subjects comparisons of a CS+ and CS−. Studies comparing within-subject responses to CS+ and CS− report differential amygdala responding [7,8,22,38]. Thus it appears that amygdala activation is strongest during the beginning of a conditioning session, or following changes in stimulus contingencies, but may be present throughout the entire session. In the current MEG study, we incorporated random presentations of the paired CS+, unpaired CS+ and CS− stimuli, which may have contributed to our ability to reliably detect amygdala activation in data averaged over the entire conditioning session. Furthermore, non-human electrophysiological recordings demonstrate that amygdala responses to the CS are present even after extensive overtraining (75 trials) [28].

5. Conclusions and future directions

We examined the temporal dynamics of localized activity in the human amygdala during fear conditioning. This first detailed characterization of the timing of responses within the amygdala during fear conditioning was achieved through the use of MEG, which provides excellent temporal resolution. We found that fear conditioning elicited differential amygdala activation, which was especially robust in the right hemisphere at CS onset. Amygdala activation also occurred following CS termination, prior to the anticipated onset of the US (prior to CS termination), and following US omission. The dynamics of our data suggest that the amygdala is likely involved in responding to the onset and offset of visual stimuli based on previously acquired associative information. Moreover, the anticipatory nature of the earliest responses within the CS-offset epoch supports the potential for amygdalar participation in the generation of a conditioned response, such as the C50m response in auditory cortex. Finally, the finding that the largest peaks during the CS-offset epoch occurred following US omission points to a possible feedback mechanism by which the amygdala has the potential to modify future responding based on realized stimulus contingencies.

There are no previous EEG studies of conditioning that attribute signals recorded at the scalp to activity within the human amygdala. Thus, further MEG and/or EEG studies are desirable to confirm and expand the present results. A potentially interesting future study might examine a change in the dynamics of the amygdala response over time, as found using fMRI [8,7,20]. This was not investigated in the current preliminary investigation, since we used all available trials to obtain an optimal signal-to-noise ratio. However, this type of analysis could be undertaken with the collection of more trials and continually changing stimulus parameters.

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