Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety

Ekaterina Likhtik1, Joseph M Stujenske2, Mihir A Topiwala1,4, Alexander Z Harris1,3,4 & Joshua A Gordon1,4

Successfully differentiating safety from danger is an essential skill for survival. While decreased activity in the medial prefrontal cortex (mPFC) is associated with fear generalization in animals and humans, the circuit-level mechanisms used by the mPFC to discern safety are not clear. To answer this question, we recorded activity in the mPFC, basolateral amygdala (BLA) and dorsal and ventral hippocampus in mice during exposure to learned (differential fear conditioning) and innate (open field) anxiety. We found increased synchrony between the mPFC and BLA in the theta frequency range (4–12 Hz) only in animals that differentiated between averseness and safety. Moreover, during recognized safety across learned and innate protocols, BLA firing became entrained to theta input from the mPFC. These data suggest that selective tuning of BLA firing to mPFC input provides a safety-signaling mechanism whereby the mPFC taps into the microcircuitry of the amygdala to diminish fear.

Discriminating between aversive and safe cues is a necessary skill for survival. Fear generalization negatively affects the ability to compete for resources in animals and is associated with a range of anxiety disorders in humans. Whereas some generalization of aversive stimuli occurs in humans as part of a normal threat assessment response1–2, a tip in the balance toward fear generalization across a wide range of stimuli is a hallmark of learned and innate anxiety disorders, typified by post-traumatic stress disorder3 and generalized anxiety disorder, respectively4,5. Clarifying the neural mechanisms underlying fear discrimination and generalization is therefore key to understanding these disorders.

The mPFC has emerged as a principal candidate for top-down-regulation of fear responses6 and impulse control7. Indeed, a decrement in fear is associated with increased activity in the mPFC as measured by cell firing8, local field potentials9, activation of immediate-early genes10,11 and blood oxygenation levels12.

Nevertheless, the mPFC is also recruited in states of high fear and anxiety. For instance, the dense projection it receives from the BLA, a critical site for fear processing, likely activates the mPFC during fear expression. In keeping with this idea, it has been shown that mPFC cell firing to conditioned tones is significantly decreased after BLA inactivation13. The mPFC also receives a dense projection from the ventral hippocampus (vHPC)14, which is the likely source of mPFC recruitment during periods of increased innate anxiety15–19.

Thus the mPFC, through its widely distributed outputs to multiple levels of the fear and anxiety circuit20–22, is in a unique position to gate fear discrimination and threat assessment during both fear expression and suppression1.22. One mechanism the mPFC uses for long-range communication with its subcortical targets is the theta range (4–12 Hz) oscillation. Evidence shows that the mPFC, BLA and hippocampus use theta oscillations to communicate during and after fear conditioning23,24 as well as during extinction of conditioned fear9 and during innate fear states15. These findings leave open the question how these structures dynamically interact as a network to differentiate anxiogenic and safe states.

To address these issues, and to evaluate the function of this network during fear generalization and discrimination, we simultaneously recorded activity in the BLA, mPFC, vHPC and dorsal hippocampus (dHPC) during the recall phase of a differential fear conditioning task and in the open field test of innate anxiety. In support of previous findings9,23,24, theta-frequency power and synchrony in the mPFC-BLA circuit increased during high fear states. Intriguingly, synchrony in this circuit was associated with discrimination between aversive and safe cues in both tasks. Indeed, changing dynamics within the mPFC-BLA circuit accompanied successful discrimination, as captured by the directionality of theta-frequency synchrony: safety stimuli induced BLA entrainment to theta inputs from the mPFC in both tasks. We conclude that mPFC input into the BLA is a key factor governing discriminative fear learning and anxiolysis.

RESULTS

Theta-frequency responses to conditioned stimuli

To examine interactions between the BLA and mPFC in learned fear, we trained and tested animals in a fear discrimination task. Training consisted of three differential fear conditioning sessions. Auditory conditioning stimuli (CS, each consisting of 30 pure-tone or white noise pips, 50 ms in duration, delivered at 1 Hz for 30 s) were paired with a mild (0.4 mA) shock to the paws (CS+) or explicitly unpaired (CS−). Five CS+ and five CS− were delivered in a pseudorandom order daily over 3 successive days (Fig. 1a). Recall of the conditioned responses was tested in a novel context on the fourth day. During recall, mice consistently froze to the CS+, but they varied considerably in their freezing to the CS−. Some animals froze to the CS+ and

1Department of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, New York, USA. 2Department of Neuroscience, College of Physicians and Surgeons, Columbia University, New York, New York, USA. 3Department of Psychiatry, Weill Cornell Medical College, New York, New York, USA. 4Division of Integrative Neuroscience, New York State Psychiatric Institute, New York, New York, USA. Correspondence should be addressed to J.A.G. (jg343@columbia.edu).

Received 8 July; accepted 23 October; published online 17 November 2013; doi:10.1038/nn.3582
Figure 1 Individual variation in discrimination after differential fear conditioning. (a) Experimental protocol. Over 3 successive days, mice were exposed to five presentations each of a CS+ (red) or CS− (blue). Each stimulus consisted of 30 pips, 50 ms in duration, presented at 1 Hz. Each presentation of the CS+ was paired with a 1-s shock. On the fourth day, freezing responses to five more presentations each of the CS− and CS+ were assessed in the absence of shock. (b) Individual animals’ freezing to CS+ (red circles) and CS− (blue circles). (c) Histogram of DS values in the sample; vertical line, cutoff for discrimination (d) Freezing to CS+ and CS− for generalizers (left) and discriminators (right) (mean ± s.e.m.).

Figure 2 Pip-evoked responses in amygdala and mPFC are modulated by successful discrimination. (a) Example pip-evoked LFP in mPFC, BLA, vHPC and dHPC. Mean of 145 pip presentations from a single animal. Dashed line, pip offset. (b) Example spectrogram of BLA and mPFC pip responses. Dashed lines, pip onset. (c) Examples of averaged (light) and theta-filtered average (dark) traces ± s.e.m. (faded bands) of CS+ and CS− pip-evoked responses from generalizers and discriminators. Black line, 50-ms pip presentation. (d) Pip-induced change in theta power by CS type and area for generalizers (G) and discriminators (D). Mean ± s.e.m. Generalizers in BLA: n = 8; CS+, 1.14 ± 0.04; CS−, 1.17 ± 0.04; sign-rank, P = 0.06; discriminators in BLA: n = 13; CS+, 1.14 ± 0.04; CS−, 1.09 ± 0.04; sign-rank, P = 0.0052; generalizers in mPFC: n = 12; CS+, 1.12 ± 0.04; CS−, 1.12 ± 0.03; sign-rank, P = 0.73; discriminators in mPFC: n = 14; CS+, 1.26 ± 0.05; CS−, 1.20 ± 0.05; sign-rank, P = 0.013. (e) Subtractive spectrograms of pip-evoked power. The difference between power evoked by the CS+ and the CS− is shown as a function of frequency and time relative to each pip. Warm colors, CS+ − CS−; cool colors, CS− − CS+. Significant (P < 0.05) power differences between CS+ and CS− are circumscribed by white dashed lines. Fifty consecutive significant windows were required for significance. (f) Changes in pip-evoked theta power from the CS− to the CS+ correlate with DS (BLA, r = 0.56, P = 0.012; mPFC, r = 0.37, P = 0.06).

CS− equally, indicating generalization of fear, whereas others froze more to the CS+ than the CS−, suggesting that they discriminated the fear-associated CS+ from the neutral CS−. We used both continuous and dichotomous measures to classify the extent to which animals differentiated the CS+ and CS−. To quantify relative freezing to the two stimuli on a continuous basis, we used a discrimination score (DS), subtracting percentage freezing to the CS− from percentage freezing to the CS+. In our sample, animals that generalized across stimuli froze to the CS+ up to 10% more than the CS+. We therefore reasoned that freezing up to 10% more to the CS+ than the CS− (Supplementary Fig. 1a, DS = 10) was also within the range of fear generalization. A DS of 10 also represents a reasonable midpoint between roughly two distribution peaks (Fig. 1c). Thus, to conduct a dichotomous analysis where required, we classified those at DS ≤10 as generalizers (n = 12) and those at DS > 10 as discriminators (n = 17, Fig. 1b,c). Note that generalizers learned the cue-shock association, showing the same levels of elevated freezing to both stimuli during the recall session (generalizers CS+, 59 ± 0.063%; CS−, 58 ± 0.06% freezing), whereas discriminators froze significantly more to the CS+ (53.2 ± 0.05%) than the CS− (34.8 ± 0.04%) (Fig. 1d and Supplementary Fig. 1). To eliminate any artificial differences...
introduced by grouping animals, we perform both dichotomous and continuous-measure analyses.

During the recall test session on day 4, we recorded local field potentials (LFPs) from the mPFC, BLA, dHPC and vHPC, as well as multi- and single-unit activity from the BLA (Supplementary Fig. 2). Each pip evoked a field potential response in all the recorded structures with prominent components in the delta (1–4 Hz) and theta (4–12 Hz) frequencies (Fig. 2a,b). Previous work has shown an increase in theta power in the amygdala in anticipation of noxious stimuli20, as well as in the amygdala during presentations of a tone CS+21, and during sleep following fear conditioning24. Consistent with these findings, we found strong pip-evoked (50–300 ms) changes in theta power during both CS+ and CS− in all regions, but only in the BLA and mPFC of discriminators were the pip-evoked increases in theta power larger during the CS+ than the CS−. In generalizers, theta power increased in the BLA and mPFC equally during the CS+ and CS− (Fig. 2c–e, sign-rank, P > 0.05). Given that stimulus-dependent theta modulation in fear discrimination was limited to the BLA and mPFC (Supplementary Fig. 3), we concentrated further analyses on these two sites. Theta power fluctuations were not due purely to differences in locomotion between groups, because the same changes in theta power were found when the analysis was restricted to epochs of low speed (0–5 cm/s; Supplementary Fig. 4). Additionally, analyses of BLA and mPFC theta power and coherence by speed did not yield any significant correlations (Supplementary Fig. 4). On a continuous basis, differential (CS+ − CS−) pip-evoked theta correlated with DS across the entire sample in the BLA and trended toward significance in the mPFC (Fig. 2f; BLA, n = 23, Spearman correlation r = 0.56, P < 0.01; PFC, n = 27, r = 0.37, P = 0.06), suggesting that these increases in theta power were behaviorally relevant. The higher theta-power signal during the CS+ lasted for up to 200 ms after pip onset in the BLA and up to 300 ms after pip onset in the mPFC (Fig. 2e). Finally, pip-evoked increases in theta power in the BLA and mPFC were correlated with each other (Supplementary Fig. 4; r = 0.7, P < 0.01).

**Figure 3** Enhanced BLA-mPFC synchrony after successful fear discrimination. (a) Pip-evoked change in theta-frequency coherence in an exemplar discriminator, by stimulus type. Inset, same in an exemplar generalizer. (b) Medians and distribution of pip-evoked changes in theta-frequency coherence for all generalizers and discriminators, by stimulus type. (c) Changes in mPFC-BLA theta coherence correlate with DS (r = 0.5229, P < 0.05). (d) Subtractive coherograms of pip-evoked coherence. Conventions as in Figure 2e.
Bidirectional

Figure 5 Short-timescale fluctuations in mPFC lead are associated with discrimination. (a) Examples of BLA and mPFC theta-filtered recordings illustrating the power cross-correlation lag analysis in the CS+ (top) and CS− (bottom). Arrows are drawn from the leading area to the lagging area. (b) Example of a discriminator, showing that the proportion of time with an mPFC lead in the power correlation increases in CS− (average across 5 CS+ and 5 CS− trials). (c) Fine-scale switches in power lead/lag correlations. Within-trial example showing that an increased BLA lead is associated with freezing, whereas increased mPFC lead occurs during movement. (d) Discriminators show a negative correlation between the proportion of time the mPFC leads and percentage freezing on a given trial (CS+, r = −0.57, P < 0.001; CS−, r = −0.6, P < 0.001). (e) Correlation between the change (CS+ − CS−) in the probability of an mPFC lead and DS (r = −0.5783, P < 0.01). A larger mPFC lead in the CS− occurs at higher DS values.

$P = 2.8 \times 10^{-4}$, suggesting the possibility that these increases reflected an increase in functional connectivity between the two structures.

BLA-mPFC pathway theta synchrony and fear discrimination

Given the importance of theta-frequency oscillations in long-range synchrony within the HPC-BLA-mPFC circuit during fear and anxiety, we examined whether the pip-evoked increases in theta power were accompanied by increases in theta-frequency synchrony between the BLA and mPFC, and whether such synchrony was modulated by CS type. To this end, we calculated pip-evoked coherence to evaluate the moment-by-moment synchrony across LFPs recorded from the BLA and mPFC. Together these results pointed to a dynamic, behaviorally relevant modulation of theta synchrony between the BLA and mPFC.

Analyses of theta coherence in this circuit suggested a striking relationship between BLA-mPFC synchrony and the dynamic evaluation of threat. In generalizers, theta-frequency coherence between the BLA and mPFC was not significantly affected by either CS (Fig. 3a, n = 9, CS+ median, −0.008, CS− median, 0.001, sign-rank, P = 0.097). In discriminators, both CS types increased theta coherence (Fig. 3b, n = 13, sign-rank, P = 0.038), and the CS+ pips elicited higher theta coherence than the CS− pips (Fig. 3b, CS+ median, 0.038, CS− median, 0.016, sign-rank, P = 0.0012). This difference was not related to freezing per se, because the generalizers, a group that froze during the CS+, had no change in pip-evoked coherence above baseline for either stimulus type (Fig. 3b, c). Indeed, BLA-mPFC coherence increased as a function of discrimination ($r = 0.52$, $P < 0.05$, Fig. 3c).

Subtraction coherograms revealed increased theta coherence during the CS+ than during the CS− in discriminators for up to 300 ms after pip onset (Fig. 3d, sign-rank, $P < 0.05$), which is similar to the time course of stimulus-evoked theta power changes (Fig. 2e). Intriguingly, these data demonstrate a pip-evoked increase in theta-frequency synchrony between the BLA and mPFC during both the CS+ and CS−, but only when animals have successfully learned the distinction between the aversive CS+ and the neutral CS− (Supplementary Fig. 5). These findings suggest that the BLA-mPFC circuit is engaged after successful acquisition of differential fear conditioning and is involved in dynamically evaluating the behavioral significance of either conditioned stimulus.

LFP recordings are susceptible to volume conduction, raising questions as to the origin of theta-frequency oscillations, particularly in the BLA, which is relatively close to the hippocampus. To address this issue, we recorded multiunit-activity (MUA) in the BLA. In keeping with the critical role the BLA plays in processing fear conditioned stimuli, pips evoked a firing rate increase during both the CS+ and CS− (Supplementary Fig. 5). BLA spikes tend to occur more frequently near the trough of the mPFC theta oscillation (Fig. 4a, b).

The strength of this phase-locking was assessed using the mean resultant length (MRL) statistic, a measure of circular concentration (see Online Methods). In generalizers, phase-locking did not increase above pre-tone baseline during either stimulus (Fig. 4c). By contrast, discriminators showed significantly higher BLA phase-locking to mPFC theta during presentations of both CS types than at baseline (Fig. 4c). These findings are consistent with the coherence data, reinforcing the notion that the BLA and mPFC work together to dynamically evaluate threat signals.

Learned anxiety: mPFC leads the BLA during the CS−

Our results suggest that the BLA and mPFC use theta oscillations as a means of long-range communication for successful threat evaluation. However, the highly reciprocal connectivity between these areas precludes any anatomical inferences about the direction of information transfer between them. We therefore examined the temporal relationship of BLA phase-locking to mPFC theta in generalizers and discriminators during stimulus presentation. Directionality is inferred by determining the lag at which phase-locking of BLA MUA to mPFC theta tends to be maximal; if, for example, BLA activity is best phase-locked to the mPFC LFP of the past, we infer that the predominant directionality is from the mPFC to the BLA. In generalizers, BLA MUA did not have a preferential temporal relationship with mPFC theta during either stimulus, suggesting equal influence in both directions (Supplementary Fig. 5). In discriminators, directionality depended on CS type. During the aversive CS+, again no net directionality was found ($n = 24$, sign-rank, $P > 0.05$). During the neutral CS−, however, the BLA MUA had a statistically significant tendency to phase-lock best to the mPFC of the past (Fig. 4d, bottom; $n = 26$, $P < 0.01$).

ARTICLES

NATURE NEUROSCIENCE | VOLUME 17 | NUMBER 1 | JANUARY 2014

© 2014 Nature America, Inc. All rights reserved.
Figure 6 BLA synchronizes with mPFC in the periphery and increases firing in the center of the open field. (a) Fold increase in BLA theta power (compared to familiar environment). Insets, example maps of occupancy in the open field, linked (gray lines) to specific animals on the graph. Warmer colors indicate greater time spent in a given location. (b) Change in theta power correlation between BLA and mPFC as a function of center avoidance in the open field. * $P < 0.05$ for linear correlation (black fit line). (c) BLA firing rate as a function of distance from the center on the open field for anxious (z = –0.6344, ***$P < 0.001$) and non-anxious (z = –0.3294, $P > 0.05$) animals. (d) Fold change in BLA spike rate in center compared to periphery for multiunit recordings from anxious and non-anxious animals. Firing rate increased by 7.55 ± 3.53 Hz (paired t-test, $P < 0.05$) for the anxious animals (left inset), whereas it did not change for the non-anxious animals (right inset, 1.33 ± 1.23 Hz increase, paired t-test, $P > 0.05$). (e) In anxious animals, normalized (norm.) mean mPFC (black line) and BLA (blue line) theta power increased as animals traveled from the center to the periphery of the open field. (f) Anxious animals’ normalized BLA spike–mPFC field (blue line, left axis) and BLA field–mPFC field (green line, right axis) coherence increased with the distance from the center. Faded bands, ± s.e.m.

~27.5 ms, sign-rank, $P < 0.01$), suggesting a predominant mPFC-to-BLA directionality. Notably, discriminators showed a significant shift ($n = 22$, sign-rank, $P = 0.011$) in directionality from the CS+ (no net directionality) to the CS− (mPFC lead), whereas generalizers did not have such a switch ($n = 11$, sign-rank, $P = 0.4$).

To confirm these findings from the BLA MUA, we examined directionality using well-isolated BLA single units ($n = 25$, 8 mice; see Online Methods for inclusion criteria). The firing of one such BLA unit and a simultaneously occurring mPFC theta oscillation is shown in Figure 4e, showing a similar phase-locking profile to mPFC theta as in the MUA recordings. We did not obtain sufficient numbers of spikes to conduct a lag analysis on each of the 25 single units. However, because most of the single units tended to phase-lock best to similar theta phases (near the trough, or 180° phase), we were able to pool the single units and conduct an aggregate directionality analysis (Fig. 4f). The aggregate analysis revealed the same temporal pattern

Figure 7 BLA-mPFC activity predicts center-periphery transitions of anxious animals. (a,b) Top, mean (black line) ± s.e.m. (gray bands) animals’ positions during the transitions from the periphery to the center (left) and center to the periphery (right) as a function of time (transition occurs at zero; 5 s of data on both sides of the transition are shown). Red area, center of the open field; blue area, periphery of the open field. Bottom, mean (± s.e.m.; faded bands) BLA firing rate for anxious and non-anxious animals as they transition into (left) and out of (right) the center. Only the anxious animals show a significant increase in BLA firing as they are going toward the center, ±2 s around transition point were compared to 3 s of baseline (~5 to ~2 s). Bonferroni-corrected ($P < 0.00042$ (0.05/120)) significant bins were identified (darker significance line). All time bins adjacent to the point-wise significant bins were tested for global significance ($P < 0.05$, lighter significance lines); see Online Methods for statistical analysis details. (c,d) BLA-mPFC coherograms for anxious animals as they are entering the center (c) or the periphery (d). Black line, average theta coherence during the transitions; white contours, rank-sum $P < 0.05$, comparing ±2 s around transition point to baseline. (e,f) Mean theta power BLA and mPFC (± s.e.m.; faded bands) for anxious animals during transitions into (e) and out of (f) the center. Darker significance lines show point-wise significance (sign-rank, $P < 0.0039$) for at least two consecutive bins; lighter significance lines, globally significant (sign-rank, $P < 0.05$) bins adjacent to the point-wise significant bins (see Online Methods).
of activity as did the MUA analysis. During presentations of the CS− to the discriminator group, BLA single units (n = 12, 4 mice) were significantly more phase-locked to mPFC theta of the recent past than to mPFC theta of the near future (Fig. 4f; −200 to 0 ms, 0.28 ± 0.2; 0 to 200 ms, 0.24 ± 0.02; sign-rank, P = 0.018). These same units, however, did not exhibit a significant difference in phase-locking between past and future during presentations of the CS+ (Fig. 4f; −200 to 0 ms, −0.25 ± 0.01; 0 to 200 ms, 0.25 ± 0.02; sign-rank, P = 0.05). Single units (n = 13, 4 mice) recorded from generalizers did not demonstrate a preferred lag during either stimulus type (Fig. 4f; CS−, −200 to 0 ms, 0.27 ± 0.02; 0 to 200 ms, 0.28 ± 0.03; sign-rank, P > 0.05; CS+, −200 to 0 ms, 0.22 ± 0.01; 0 to 200 ms, 0.23 ± 0.01; sign-rank, P > 0.05).

To evaluate mPFC-BLA directionality on a continuous basis, we performed a trial-by-trial analysis of directionality28. Because unit data require large numbers of spikes to estimate directionality, we instead calculated mean lag times from the cross-correlation of theta power between LFPs recorded from the BLA and mPFC (Fig. 5a).

Specifically, we analyzed the probability of the mPFC leading as a function of percentage freezing on any given trial (Fig. 5b,c). This analysis showed that, whereas for discriminators the probability of an mPFC lead on a given trial was negatively correlated with percentage freezing (Fig. 5d; multiple linear regression; CS+, r = −0.67, P < 0.001; CS−, r = −0.60, P < 0.001), there was no such correlation for the generalizers (Supplementary Fig. 5; multiple linear regression; CS+ and CS−, P > 0.05). Indeed, on a continuous scale of discrimination, increased probability of the mPFC leading during the CS− (Fig. 5b) was associated with better discrimination (Fig. 5e, r = −0.57, P < 0.01). Together, these data argue strongly for a direct relationship between a predominant mPFC-to-BLA directionality and suppression of freezing behavior during successful fear discrimination.

**Innate anxiety: mPFC leads the BLA in safe zones**

Conditioned, anxiogenic stimuli functionally elevate and modulate the theta oscillation in the BLA-mPFC circuit. Neutral stimuli, when recognized as such, shift the directionality of mPFC-BLA communication toward an mPFC-to-BLA direction of information transfer. To test whether this shift in communication is task specific or a hallmark of a broader safety recognition mechanism, we turned to the open field (Supplementary Fig. 6), a classic test of innate anxiety29. In this task, we first exposed animals to a small, dark, familiar environment for 10-min sessions over 4 d. On the fourth day, we also placed the animals in a brightly lit (185 lb) open field (Supplementary Fig. 6a). This task taps into the innate avoidance of bright, open spaces displayed by rodents; the level of anxiety elicited by the open field is determined by the amount of time spent in the center of the field, with less center time indicating more anxiety. **Supplementary Figure 6a** shows an example of an animal’s movement in the open field. Notably, although the same animals were exposed to learned and innate anxiety tasks, there was no correlation between anxiety level in the open field and DS in fear conditioning (r = 0.183, P > 0.05).

In agreement with the aversive conditioned stimulus data shown above, theta power in the BLA increased with innate anxiety in the open field. We have previously demonstrated similar changes in mPFC and vHPC theta power in this task15. The more anxious an animal tended to be, the more BLA theta increased on the open field, especially in the relative safety of the periphery (Fig 6a, n = 14, r = −0.42, P < 0.05). We therefore reasoned that theta synchrony between the two regions might also increase in this task. Indeed, using theta power correlations, we found that BLA-mPFC theta synchrony increased with anxiety in the periphery of the open field (n = 14, r = −0.52, P < 0.05; Fig. 6b). Notably, increased BLA-mPFC synchrony in the open field was not due to novelty. BLA-mPFC power correlations, similarly to those in the mPFC-vHPC circuit15, were also higher in the open field than in the first familiar environment exposure (Supplementary Fig. 7).

BLA theta modulation by innate anxiety led us to examine whether BLA firing in the open field also differs with anxiety. To this end, we divided animals into two groups, anxious (n = 9, spending <10% of their time in the center, and non-anxious (n = 5), showing >10% center time in the open field; **Supplementary Fig. 6b**). It should be noted that whereas “non-anxious” refers to little evidence of anxiety, such that animals are not actively seeking the safety of the periphery, “generalizers” in learned fear refers to generalized defensive behavior to both CS types.

Notably, BLA firing rates of anxious animals increased as they moved away from the periphery and into the center of the open field, whereas in the non-anxious animals BLA firing rates did not change with radial distance (multiple linear regression; anxious, r = −0.6344, P < 0.001; non-anxious, r = −0.3294, P > 0.05; Fig. 6c,d). Intriguingly, in anxious animals, as BLA firing decreased in the periphery of the open field, mPFC theta power and mPFC-BLA coherence increased (Fig. 6e,f). Thus, when the anxious animals were in the periphery of the open field, BLA and mPFC theta power and synchrony increased whereas BLA neural firing decreased.

To investigate these relationships further, we analyzed the transitions as animals shuttled between the periphery and the center. In anxious animals, BLA firing rates increased only as they transitioned into the center from the periphery (Fig. 7a,b). Indeed, as anxious animals moved from the periphery into the center, BLA and mPFC theta power, as well as BLA-mPFC field-field and spike-field coherence, decreased (Fig. 7c,e) until the animals reentered the periphery, when mPFC power and mPFC-BLA synchrony increased again (Fig. 7d,f). In non-anxious animals, we did not observe such changes (Supplementary Fig. 8a,b). These data indicate that an innate anxiety component contributes to BLA spiking as anxious animals are going toward the center. Conversely, when an anxious animal is headed toward the relative safety of the periphery, the mPFC signal increases in power and synchrony with the BLA, and the BLA firing rate decreases.

Finally, we examined the effect of the open field on directionality in the circuit by analyzing power correlation lag28 and phase-locking lag16,27. An analysis of BLA MUA phase-locking to the mPFC LFP showed that, in accord with the findings in the fear conditioning task, when anxious animals were in the safety of the periphery,
spiking in the BLA was best phase-locked to the mPFC theta oscillation of the past (Fig. 8, \( n = 30, -15 \text{ ms}, \text{ sign-rank, } P < 0.01 \)). Notably, this relationship was absent when anxious animals were in the anxiogenic center of the open field (Fig. 8, \( n = 30, \text{ sign-rank, } P > 0.05 \)). BLA MUA from non-anxious animals did not show any net directionality in either location (Supplementary Fig. 8c, sign-rank, \( P > 0.05 \)). These findings demonstrate a similar relationship between BLA spiking and the mPFC theta oscillation in conditioned and innate anxiety: namely, when an animal evaluates a potentially anxiogenic situation and detects safety (be it a neutral CS or a safe zone in an aversive environment), BLA spiking follows theta input from the mPFC (Fig. 8).

**DISCUSSION**

We investigated the dynamic interactions of the mPFC-BLA-hippocampal network in learned fear and innate anxiety. While responses evoked by the CS− were found throughout the network, mPFC-BLA interactions were specifically enhanced during successful fear discrimination. Theta-frequency synchrony between the mPFC and BLA was enhanced by both CS types in animals that successfully discriminated between a CS+ and CS−; similarly, enhancements in mPFC-BLA synchrony correlated with center-avoidance in the open-field test. In both environments, safety was accompanied by a net mPFC-to-BLA directionality. These data demonstrate that the mPFC-BLA circuit is dynamically engaged in fear discrimination and suggest that the inputs from the mPFC to the BLA are involved in actively squelching behavioral responses to fear by entraining activity in the BLA to mPFC theta input.

The mPFC is widely accepted as a critical site for inhibition of fear in human anxiety and in animal models of post-traumatic stress disorder\(^{30}\) and generalized anxiety disorder\(^{6}\). The amygdala is a hub of fear expression and also a centralized site for prefrontal control of fear suppression. The anxiolytic role for the mPFC has been most widely studied using extinction of conditioned fear in animals and humans, for which findings converge to show that increased mPFC activity and cortical volume correspond with better and longer lasting extinction in experimental and clinical settings\(^{8,31}\). Indeed, extinction training is one of the most widely used techniques for overcoming fear in clinical practice\(^{32}\). Our findings are consistent with the notion that the mPFC-BLA circuit is a key player in diminishing fear and anxiety\(^{33,34}\), and they extend the role of the mPFC and the mPFC-BLA circuit from fear extinction to fear discrimination, showing that mPFC-BLA interactions partake in the appraisal of safety versus averseness.

**mPFC inputs and microcircuits of the amygdala**

Our data demonstrate a distinct mPFC-to-BLA directionality during fear discrimination. Our findings suggest that the mPFC relies on this projection to shape activity in the BLA during fear discrimination, possibly resulting in inhibition of amygdala output. The entrainment during fear discrimination of BLA cells to theta input from the mPFC is likely due to the interplay between this input and intrinsic currents of BLA neurons, which predispose them to oscillate in the theta range\(^{35}\). Anatomically, a robust mPFC-to-BLA projection has been described, with most mPFC axon terminals synapsing on dendritic spines of BLA projection neurons and only a few terminating on putative interneurons\(^{36}\). How this predominantly excitatory-to-excitatory projection\(^{37}\) results in a fear decrement (which presumably requires inhibition of amygdala output) is unclear. Given that GABAergic transmission in the BLA is key to reducing fear\(^{38-41}\), it may be that tuning into theta-encoded input from the mPFC allows the activation of a subset of local inhibitory networks in the amygdala. Indeed, \(Gad65^{+/−}\) mice, lacking the glutamic acid decarboxylase 2 gene, have been shown to generalize fear early in extinction training and show decreased theta communication between the mPFC and amygdala\(^{42}\).

Discrimination might rely on similar mechanisms as fear extinction. Indeed, prefrontal theta has been shown to increase during extinction training\(^{9}\), and there is mPFC-dependent inhibition of fear output cells in the central nucleus of the amygdala\(^{43}\). This is associated with increased efficacy of the mPFC-to-BLA synapse, which in turn may activate the central nucleus–projecting GABAergic cell clusters in the amygdala known as the intercalated cell masses\(^{43}\). Indeed, evidence in rats and mice shows that intercalated cell mass cells are active during and required for extinction\(^{44,45}\).

**Safety across protocols**

Our findings show that BLA firing is tuned to mPFC input in recognized safety across fear discrimination and innate anxiety, suggesting that this is a widely used mechanism for safety detection. This idea supports previous work showing that BLA cells active after extinction are responsive to stimulation of the mPFC\(^{34}\). Critically, this mechanism was only engaged in animals that identified safety as either a cue (CS−) or a location (periphery of open field) in an otherwise aversive setting. During CS+ presentations there was higher theta power and sharper theta resets (Supplementary Fig. 9) in the BLA and mPFC than during CS− presentations, suggesting that there is an additional fear-related input. During recognized safety, this common input is likely decreasing, diminishing theta power in these areas and allowing the mPFC to influence activity in the BLA. This mechanism was not engaged in animals that generalized fear across the two stimuli or were not anxious in the open field.

A growing literature suggests that it is the infralimbic cortex rather than the more dorsal prelimbic cortex of the mPFC that plays a role in fear decrement\(^{12,24,46,47}\). Most of our recordings were performed in the prelimbic cortex, with some on the prelimbic cortex/infralimbic cortex border. We did not see any differences in our results based on electrode placement. However, given that we were recording LFPs in two contiguous areas, we cannot rule out the possibility that the relatively slow theta oscillation of the mPFC was volume conducted from one subregion of the mPFC to another.

These data support a unified view of forebrain fear and anxiety circuitry in safety detection (Supplementary Fig. 10). In conditioned fear discrimination and innate anxiety, the mPFC and BLA appear to work together to evaluate behaviorally relevant stimuli: for safe stimuli, the mPFC drives BLA activity, inhibiting fear. In this way the dynamics of cooperation and competition in the mPFC-BLA circuit determine the expression of fear- and anxiety-related behaviors.

**METHODS**

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

**ACKNOWLEDGMENTS**

We would like to thank T. Spellman and other members of the Gordon laboratory for technical assistance and discussions. This work was supported by grants from the US NIMH to J.A.G. (R01 MH081968 and P50 MH086991) and E.L. (F32 MH088103), by the International Mental Health Research Organization (J.A.G.) and by the Charles H. Revson Foundation (E.L.). J.M.S. is supported through the Columbia University Medical Scientist Training Program.

**AUTHOR CONTRIBUTIONS**

E.L. designed and performed the experiments, analyzed the data and wrote the paper. J.M.S. analyzed the data. M.A.T. assisted in performing the experiments.
A.Z.H. assisted in analyzing the data. J.A.G. designed the experiments, supervised the performance of the experiments and data analysis, and wrote the paper.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Resnik, J., Sobel, N. & Paz, R. Auditory aversive learning increases discrimination thresholds. *Nature. Neurosci.* 14, 791–796 (2011).
2. Dunsmoor, J.E., Mitrnfl, S.R. & LaBar, K.S. Generalization of conditioned fear along a dimension of increasing fear intensity. *Learn. Mem.* 16, 460–469 (2009).
3. Jovanovic, T., Kazama, A., Bachevalier, J. & Davis, M. Impaired safety signal learning may be a biomarker of PTSD. *Neuropsychopharmacology* 62, 695–704 (2012).
4. Reinecke, A., Becker, E.S., Hoer, J. & Rinck, M. Generalized implicit fear associations in generalized anxiety disorder. *Depress. Anxiety* 27, 252–259 (2010).
5. Gazendam, F.J., Kamphuis, J.H. & Kindt, M. Deficient safety learning characterizes high trait anxiety individuals. *Biol. Psychol.* 92, 342–352 (2013).
6. Greenberg, T., Carlson, J.M., Cha, J., Hajcak, G. & Mujica-Parodi, L.R. Ventromedial prefrontal cortex reactivity is altered in generalized anxiety disorder during fear generalization. *Depress. Anxiety* 30, 242–250 (2013).
7. Narayanan, N.S., Horst, N.K. & Laubach, M. Reversible inactivations of rat medial prefrontal cortex impair the ability to wait for a stimulus. *Neuroscience* 139, 865–876 (2006).
8. Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E. & Quirk, G.J. Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron* 53, 871–880 (2007).
9. Lesting, J., Narayanan, R.T., Kluge, C., Sanga, S., Seidenbecher, T. & Pape, H.-C. Patterns of coupled theta activity in amygdala-hippocampal-prefrontal cortical circuits during fear extinction. *PLoS ONE* 6, e21714 (28 June 2011).
10. Knapska, E. & Marem, S. Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learn. Mem.* 16, 486–493 (2009).
11. Henry, C. & Mons, N. Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. *Eur. J. Neurosci.* 20, 781–790 (2004).
12. Phelps, E.A., Delgado, M.R., Nearing, K.I. & LeDoux, J.E. Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* 43, 897–905 (2004).
13. Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E. & Quirk, G.J. Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron* 76, 804–812 (2012).
14. Jay, T.M. & Witter, M.P. Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *J. Comp. Neurol.* 313, 574–586 (1991).
15. Adhikari, A., Topiwala, M.A. & Gordon, J.A. Synchronized activity between the prelimbic and infralimbic cortex during fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron* 76, 2956–2961 (2013).
16. Sangha, S. et al. Microstimulation of the infralimbic and prelimbic cortices of the rat: an anterograde tracing study with Phaseolus vulgaris leucoagglutinin. *J. Comp. Neurol.* 290, 213–242 (1989).
17. Vertes, R.P. Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse* 51, 32–58 (2004).
18. Carmichael, S.T. & Price, J.L. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *J. Comp. Neurol.* 363, 615–641 (1995).
19. Reinecke, A., Becker, E.S., Hoyer, J. & Rinck, M. Generalized implicit fear associations in generalized anxiety disorder. *Depress. Anxiety* 27, 252–259 (2010).
20. Siapas, A.G., Zilbou, E.V. & Wilson, M.A. Prefrontal phase locking to hippocampal theta oscillations. *Neuron* 46, 141–151 (2005).
21. Adhikari, A., Sigurdsson, T., Topiwala, M.A. & Gordon, J.A. Cross-correlations of instantaneous amplitudes of field potential oscillations: a straightforward method to estimate the directionality and lag between brain areas. *J. Neurosci. Methods* 191, 191–200 (2010).
22. Crawley, J.N. Exploratory behavior models of anxiety in mice. *Neurosci. Biobehav. Rev.* 39, 37–44 (1985).
23. Rauch, S.L., Shin, L.M. & Phelps, E.A. Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research-past, present, and future. *Biol. Psychiatry* 60, 376–382 (2006).
24. Milad, M.R. et al. Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proc. Natl. Acad. Sci. USA* 102, 10706–10711 (2005).
25. Hauker, K.K., Minek, E., Voss, J.L. & Paller, K.A. Exposure therapy triggers lasting reorganization of neural fear processing. *Proc. Natl. Acad. Sci. USA* 109, 2023–2028 (2012).
26. Pape, H.-C. & Paré, D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev.* 90, 419–463 (2010).
27. Henry, C. et al. Switching on and off fear by distinct neuronal circuits. *Nature* 454, 560–566 (2008).
28. Pape, H.C., Paré, D. & Driesang, R.B. Two types of intrinsic oscillations in neurons of the lateral and basolateral nuclei of the amygdala. *J. Neurophysiol.* 79, 205–216 (1998).
29. Brosch, M., Mascagni, F. & McDonald, A.I. Synaptology of prefrontal cortical projections to the basolateral amygdala: an electron microscopic study in the rat. *Neurosci. Lett.* 202, 45–48 (1995).
30. Likhtik, E., Pelletier, J.G., Paz, R. & Paré, D. Prefrontal control of the amygdala. *J. Neurosci.* 25, 7429–7437 (2005).
31. Truitt, W.A., Johnson, P.L., Dietrich, A.B., Fitz, S.D. & Shehkar, A. Anxiety-like behavior is modulated by a discrete subpopulation of interneurons in the basolateral amygdala. *Neurosci.* 160, 284–294 (2009).
32. Woodruff, A.R. & Sah, P. Inhibition and synchronization of basolateral amygdala principal neuron spiking by parvalbumin-positive interneurons. *J. Neurophysiol.* 98, 2956–2961 (2007).
33. Shaban, Y. et al. Generalization of amygdala LTD and conditioned fear in the absence of presynaptic inhibition. *Nat. Neurosci.* 9, 1028–1035 (2006).
34. Heldt, S.A., Mou, L. & Ressler, K.J. In vivo knockdown of GAD67 in the amygdala disrupts fear extinction and the anxiety-like effect of diazepam in mice. *Transl. Psychiatry* 2, e181 (13 November 2012).
35. Sangha, S. et al. Deficiency of the 65 kDa isofrom of glutamic acid decarboxylase impairs extinction of cued but not contextual fear memory. *J. Neurosci.* 29, 15713–15720 (2009).
36. Amano, T., Unal, C.T. & Paré, D. Synaptic correlates of fear extinction in the amygdala. *Nat. Neurosci.* 13, 489–494 (2010).
37. Busii, D. et al. Different fear states engage distinct networks within the icentralic cell clusters of the amygdala. *J. Neurosci.* 31, 5131–5144 (2011).
38. Likhtik, E., Popa, D., Apergis-Schoute, J., Fidacaro, G.A. & Paré, D. Amygdala intercalated neurons are required for expression of fear extinction. *Nature* 454, 642–645 (2008).
39. Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L. & Quirk, G.J. Microstimulation reveals opposing influences of prefrontal and infralimbic cortex on the expression of conditioned fear. *Learn. Mem.* 13, 728–733 (2006).
40. Laurent, V. & Westbrook, R.J. Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. *Learn. Mem.* 16, 520–529 (2009).
**ONLINE METHODS**

**Animal experiments.** The procedures described here were conducted in accordance with US National Institutes of Health regulations and approved by the Columbia University and New York State Psychiatric Institute Institutional Animal Care and Use Committees.

**Microdrive construction.** Custom microdrives were constructed using interface boards (EIB-16, Neuralynx, Bozeman, MT) fastened to machine screws (SHCXX-080-6, Small Parts, Inc, Miramar, FL). Stereotrodes (4 or 5 per animal) were constructed of 25-µm Formvar-coated tungsten micro wire (California Fine Wire, Grover Beach, CA), fastened to a cannula attached to the interface board, and implanted in the BLA. Single-wire, 76.2-µm tungsten electrodes were stereotactically placed into the dHPC, vHPC and mPFC and cemented directly to the skull during surgery.

**Surgery.** Three- to 6-month-old male 129SvEv wild-type mice (Taconic, Germantown, NY) were initially anesthetized with ketamine/xylazine (160 and 5.5 mg/kg, in saline), placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) and maintained on inhaled isoflurane (0.5–0.8%) in oxygen for the duration of the surgery. Temperature was monitored and maintained at 36.6 ± 0.5°C with a feedback-regulated heating pad. The skull was leveled using bregma and lambda landmarks and craniotomies were made using anterior-posterior (AP) coordinates from bregma, medio-lateral (ML) coordinates from the midline and dorso-ventral coordinates (DV) from brain surface. Stereotrodes were implanted in the BLA (−2.06 mm AP, 3.15 mm ML, −3.4 mm DV) and tungsten wires were placed into the dorsal CA1 of the hippocampus (dHPC: −1.85 mm AP, 1.25 mm ML, −1.15 mm DV), the medial prefrontal cortex (mPFC: +1.65 mm AP, 0.3 mm ML, −1.6 mm DV) and the ventral hippocampus (vHPC: −3.16 mm AP, 3.0 mm ML, −3.7 mm DV). Skull screws overlying the cerebellum and frontal cortex served as ground and reference, respectively. All wires were connected to a 16-channel interface and the BLA electrodes were anchored to a microdrive that made it possible to advance them along the DV axis. Postoperatively, animals were given analgesics (Carprofen, 5 mg/kg, s.c.) and monitored for comfort and weight gain. Following surgery, animals were housed individually on a 12-h light/dark cycle, with bedding squares provided for enrichment.

**Behavioral protocol.** **Innate anxiety.** Animals recovered for at least 1 week or until regaining presurgery body weight. Mice were then food-restricted to 85% body weight. During food restriction animals were familiarized with the recording setup and handling by being tethered to the head stage preamplifier in their home cages for two to three daily sessions of 15 min each. All behavioral experiments were performed between 2 and 5 p.m. Upon reaching their target weight, mice (n = 21) were exposed to a small rectangular box (familiar arena, 30 × 20 cm) in the dark in which they foraged for food once a day for 3 consecutive days (10 min per session). On the fourth day, to test innate anxiety, the animals were again exposed to the familiar arena for 10 min and, 1 h later, to a brightly lit (180 lx) open field (25 cm radius, 40 cm high; Fig. 6a).

**Differential fear conditioning.** After 3 d of rest, the same animals were exposed to differential fear conditioning (1 session a day for 3 d) in a dimly lit (30 lx) conditioning chamber with a grid floor of stainless steel bars for shock delivery (MedAssociates, St. Albans, VT). The animals were habituated to the context for 2–3 min before each training session and were then presented with 10 trials of tones (8 kHz, white noise, counterbalanced), 5 of which were the aversive conditioning stimulus (CS+) and coterminated with a footshock to the paws (0.4 mA, 1 s) and 5 of which were the neutral conditioning stimulus (CS−) and were not paired with anything. Therefore the animals received a total of 30 stimuli over 3 d, 15 CS+ and 15 CS−. The order of stimulus presentation was pseudorandom on all days of training and testing. Stimuli consisted of pure tone pips lasting 50 ms and delivered at 1 Hz for 30 s (inter-trial interval, 60–180 s). Stimuli were randomly assigned as the CS+ and CS− and were counterbalanced between animals (Fig. 1a). On the fourth day, the animals were placed in a new context (a wooden enclosure, 60 lx) for testing how well they had learned the differential associations with the two stimuli. The animals were connected to the recording equipment and, after 2–3 min of habituation, presented with the same CS+ and CS− stimuli as during training. An overhead video camera was used to monitor and record the behavior of the animals for offline analysis of freezing.

Freezing was manually scored twice by a trained observer blind to the valence of the tone. The two scores had to be less than 2 s apart to be averaged for a final score. To increase the number of discriminators and thereby the power of our tests, we also tested a second group of 8 animals in differential fear conditioning using 6 trials of CS+ and CS− session over the same 3-d period as in the previous group. Therefore, these animals received a total of 36 trials over 3 d, 18 CS+ and 18 CS−. Sample size–synchrony between areas by looking at whether BLA firing was modulated by, or phase-locked to, the mPFC theta oscillation by using custom Matlab scripts along with the circular statistics toolbox. The phase of each theta-filtered sample was extracted from the Hilbert transform and each spike was assigned the phase of its contemporaneous field potential sample. Phase-locking was quantified as the circular concentration of the resulting phase distribution, which was defined as the mean resultant length (MRL) of the phase angles. The MRL is the sum of the unit vectors representing the phases at which each spike
occurred, divided by the number of spikes. It therefore takes values between 0 (no phase-locking) and 1 (perfect phase-locking). Because the MRL statistic is sensitive to spike number, the number of spikes used for the analysis was fixed at 500, which is large enough to avoid overestimating phase-locking as a result of small spike numbers. Thus, only multiunits with at least 500 spikes for each pre-tone and each CS type were included for analysis. The MRL statistic was calculated using 500 randomly selected spikes, repeated 2,000 times, and the results averaged for each multiunit.

To analyze the directionality of BLA multiunit phase-locking to mPFC theta, multiunits with at least 100 spikes in each 30 s CS period were included because the MRL statistic can be highly variable for small spike numbers. The spike times were lagged relative to the theta filtered signal from −100 ms to 100 ms, stepping by 5 ms, and the time of the peak MRL value was determined for each multiunit. Multiunits were determined to be significantly phase-locked using a Bonferroni-corrected P-value for the Rayleigh z-test (P < 0.0012, or 0.05/41). The number of significantly phase-locked units did not significantly vary for different time windows or time steps, and the directionality of the mPFC-BLA interaction was consistent across different calculation parameters. The median of the peak MRL times was compared to the null hypothesis of a zero time lag using sign rank and determined to be significant for P < 0.05. For single units, cells were clustered using KlustaKwik (by K. Harris, https://github.com/klusta-team/klustakwik/), using the first three principal components for cluster isolation. Clusters were kept for analysis if two independent signal-to-noise ratios were ≥3 and their isolation distance was ≥10. MRL calculations with single units were performed only on units with a firing rate of at least 0.1 Hz. To analyze directionality of mPFC-BLA power-power correlations as a function of freezing, the raw LFP was filtered for theta (4–12 Hz, 400 sample FIR filter) and the power envelope was extracted with the Hilbert transform. Cross-correlation lag analysis was performed with 1 s windows, stepping by 5 ms. The probability of PFC, BLA, and no lead was quantified as the percentage of windows with a positive, negative, or zero lag at the peak, respectively.

For the open field analyses, theta power was calculated with Welch’s power spectral density, using 1,000 samples (528.262 ms), and stepping by 100 samples (52.82 ms). For the power correlations, first we calculated multitaper theta frequency spectrograms (2.6 s windows, NW of 2.5) across the 10 min exposure to the familiar environment and the open field. The linear correlation coefficient between summed BLA and mPFC theta power was calculated separately for each animal, multiple linear regression was performed in Matlab (regstats function), including categorical variables corresponding to animal identities to account for the center. Theta coherence and power at the transitions was calculated using multitaper spectral analysis (1,024 sample window size, 2,048 FFTs, stepping by 60 samples, NW of 2,3 tapers). For the power-power correlations directionality analysis, we used the same parameters as in the differential fear conditioning.

**Statistics.** Wilcoxon’s signed-rank test was used for comparisons involving measurements from the same animal across behavioral conditions, such as changes in theta power to the CS+ and the CS−. Wilcoxon’s rank-sum (equivalent to the Mann-Whitney U-test) nonparametric tests were used for unpaired, independent observations. Two-tailed tests were used throughout. A paired t-test was used when we had a sufficiently large sample size to adequately estimate normality.

To test for significance in firing rate around transition points (time 0) in the open field (Fig. 7a,d), we compared ±2 s around the transition point to a 3-s baseline before the transition (−5 to −2 s). To control for type I error, we first found only those time bins that were different from baseline at a significance level that was Bonferroni-corrected for the number of bins used in the analysis—point-wise significance (120 bins, 33 ms each, t-test, P < 0.00041 (0.05/120))39. The point-wise significance level is indicated by a darker line in Figure 7a. We then tested only those bins that were contiguous with the point-wise significant bins for ‘global’ significance (P < 0.05)39. The global significance is indicated by a lighter line in Figure 7a. Therefore, data marked globally significant only come from data that are also point-wise significant. Similarly, to test for significance in theta power changes around the transition point, the Wilcoxon sign-rank test was performed on the ±2 s around the transition and compared to 3 s of baseline. Point-wise significance (Fig. 7c.f, darker significance line) was achieved only when two bins in a row were P < 0.0039 different from baseline (maximal sign-rank significance, n = 9 (anxious animals)). To assess global significance (Fig. 7c.f, lighter significance colors), we took bins with P < 0.05 significance only if they were contiguous with bins that were point-wise significant.

We used nonparametric tests because we test ratios, which are not normally distributed; percentages, which have floor and ceiling effects; and circular statistics, such as the Rayleigh test, to assess significance of phase-locking. Analyses of means and/or medians ± standard errors of means were calculated and plotted to show the accuracy of the estimation of the mean of the population. Degrees of freedom are n−1 throughout. For the continuous analyses, Pearson’s correlation statistics were used unless otherwise stated. For correlations with multiple data points per animal, multiple linear regression was performed in Matlab (regstats function), including categorical variables corresponding to animal identities to account for within-animal dependence along with the explanatory variables of interest.

48. Mitra, P. & Bokil, H. Observed Brain Dynamics (Oxford Univ. Press, New York, 2008).
49. Rogan, M.T., Stäubli, U.V. & LeDoux, J.E. Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390, 604–607 (1997).
50. Fujisawa, S., Amarasingham, A., Harrison, M.T. & Buzsáki, G. Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex. Nat. Neurosci. 11, 823–833 (2008).