VIGILANCE-ASSOCIATED GAMMA OSCILLATIONS
COORDINATE THE ENSEMBLE ACTIVITY OF BASOLATERAL AMYGDALA NEURONS

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Abstract

Principal basolateral amygdala (BL) neurons profoundly influence motivated behaviors, yet few of them are activated by emotionally-valenced stimuli. Here we show that a likely explanation for this paradox is the synchronizing influence of the high-gamma rhythm. High-gamma (75–95 Hz) entrained BL firing more strongly than all other rhythms. It was most pronounced during states of increased vigilance, when rats were apprehensive. Relative to behavioral states, high-gamma produced minor changes in firing rates yet dramatic increases in synchrony. Moreover, connected pairs of cells showed similarly high levels of entrainment and synchronization. Unexpectedly, prefrontal- and accumbens-projecting cells respectively showed high and low entrainment by high-gamma, indicating that this rhythm differentially synchronizes the activity of BL neurons projecting to specific sites. Overall, our findings suggest that individual BL neurons not only encode information by changing their firing rates but also by synchronizing their collective activity, amplifying their impact on target structures.

Keywords

amygdala; oscillations; beta; theta; memory; fear; emotions

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A.A., D.B.H. and D.P. contributed to the design of the experiments as well as to the data and statistical analyses. A.A. conducted most of the experiments. S.C.L. and D.H. conducted some the experiments and contributed to the data analyses. D.P. wrote the first draft of the manuscript and made the figures. All authors contributed to refining the manuscript.

Declaration of Interests
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Like the cerebral cortex, the basolateral nucleus of the amygdala (BL) contains a dominant group of glutamatergic projection cells and diverse subtypes of GABAergic interneurons (Muller et al., 2003, 2005; Spampanato et al., 2011). Although BL exerts a profound influence over motivated behaviors (Amano et al., 2011; Ambroggi et al., 2008; Herry et al., 2008; Stuber et al., 2011; Tye et al., 2011), principal BL neurons fire at much lower rates than their cortical counterparts (Ambroggi et al., 2008; Jacobs and McGinty, 1971; Pare and Gaudreau, 1996; Steriade and Hobson, 1976), and very few of them are activated by emotionally-valenced stimuli (Lee et al., 2016). Thus, BL signaling may not depend solely on the firing rates of individual cells but on synchronizing their discharges, thereby enhancing their impact on target networks.

A potential mechanism for such synchronization are gamma oscillations (Fries et al., 2007; Fries, 2015). First identified as an EEG correlate of behavioral arousal (Magoun, 1958), gamma oscillations (30–100 Hz) have since been observed in local field potentials (LFPs) throughout the brain. In keeping with the diverse roles of the networks exhibiting them, gamma oscillations have been implicated in multiple functions (Engel and Singer, 2001). Depending on the brain region, state of vigilance, experimental context, and behavior, different gamma subtypes with distinct frequencies predominate (Ainsworth et al., 2011; Colgin et al., 2009; van der Meer et al., 2010).

In the human amygdala, emotional stimuli, particularly negative ones, elicit robust gamma oscillations (Luo et al., 2007; Oya et al., 2002), even when presented subliminally (Luo et al., 2009). In animals, gamma is enhanced during periods of arousal (Pagano and Gault, 1964). Moreover, stimuli that predict affective events can elicit coherent gamma in BL and related networks (Bauer et al., 2007; Popescu et al., 2009). Last, BL gamma also shows differential coupling to hippocampal or medial prefrontal theta during fear and safety (Stujenske et al., 2014).

The cellular basis of gamma oscillations has been most extensively characterized in cortical networks where they depend on reciprocal interactions between pyramidal and fast-spiking cells (Buzsaki and Wang, 2012). Indeed, of the various interneuron subtypes, fast-spiking cells show the greatest gamma entrainment (Bragin et al., 1995; Csicsvari et al., 2003; Gloveli et al., 2005; Otte et al., 2010). They receive strong excitatory inputs from pyramidal cells (Levy and Reyes, 2012), are interconnected by chemical and electrical synapses (Galaretta and Hestrin, 1999; Gibson et al., 1999) and have divergent axons, each contributing many inhibitory synapses in the perisomatic region of pyramidal cells (Buhl et al., 1994). These properties promote activity synchronization of large subsets of principal cells.

Although similar principles of connectivity have been reported in BL (Smith et al., 2000; Spampanato et al., 2011; Woodruff and Sah, 2007), far less data are available on the genesis of gamma. Gamma emerges in amygdala slices in the presence of glutamate (Randall et al., 2011) or cholinergic agonists (Sinfield and Collins, 2006). In vivo, gamma occurs spontaneously and entrains principal cell firing (Bauer et al., 2007; Popescu et al., 2009; Stujenske et al., 2014). However, no data is available regarding the gamma-related activity of...
fast-spiking cells. Nor is it clear whether gamma synchronizes amygdala neurons as in cortex.

At present, these and many aspects of gamma genesis and function remain uncharacterized in BL. The present study was undertaken to shed light on these questions.

RESULTS

Behavioral correlates

To characterize the behavioral dependence of gamma in BL, we obtained simultaneous unit and LFP recordings in rats engaged in two tasks characterized by different threat levels: a semi-naturalistic foraging task (Fig. 1A) and a food-seeking shuttle task (Fig. 1B).

Foraging task—In this task (Choi and Kim, 2010; Amir et al., 2015), hungry rats (n=5) were confronted with a mechanical predator when they left their nest to retrieve food pellets in a long and brightly lit arena (Fig. 1A1). The nest and predator were located at opposite ends of the apparatus and the food pellets at varying distances between them. When rats approached the predator to attain food, it surged forward, causing rats to flee to their nest. Rats were first trained to retrieve food in the absence of the predator. Trials started when the door to the foraging arena opened. Following a delay during which rats seemed to hesitate at the door threshold, they ran into the arena, took the food pellet in their mouth, and returned to the nest to consume it, at which point the door was shut. The next trial started ≥1 min later. On later days, alternating blocks of 10–20 trials were conducted with the predator absent or present.

We first summarize our earlier behavioral findings in the foraging task (Amir et al., 2015) and then describe task-related changes in gamma activity. Introduction of the predator changed the rats’ behavior in many ways, all consistent with a state of apprehension and increased vigilance. After door opening and waiting at the door threshold, rats sometimes retreated to the nest instead of initiating foraging. Such aborted trials (marked “A” in Fig. 1A2) were ~10 times more frequent in the presence than absence of predator. Also, on trials with foraging initiation, the proportion of successful trials (marked “S” in Fig. 1A2) decreased by up to 70% and the food retrieval interval almost doubled as rats foraged more tentatively. Last, behavior on a given predator trial depended on the outcome of the prior trial: rats spent twice longer retrieving the food on trials that followed failed trials (marked “F” in Fig. 1A2) than successful ones. See Supplementary table 1 for a breakdown of these various types of trials in each animal.

In BL, we observed task related variations of LFP power in the theta (6–10 Hz), beta (15–25 Hz), and three gamma bands: low- (35–55 Hz), mid- (55–75 Hz) and high-gamma (75–95 Hz) (repeated measures Friedman ANOVAs, $X^2$s=40.83 to 1564, df=3, p<0.001; see figure legend 1 for post-hoc tests). Figure 1C1 shows average z-scored LFP power fluctuations as a function of relative time for all available rats (n=5) and trials with foraging initiation (n=1238). Similar results were obtained with raw power and individual rats yielded consistent results (Supplementary Fig. 1). The spectrogram starts 30 s prior to door opening. When rats were in the nest (Baseline), beta-power was high while theta- and gamma-power
was low, except for a transient rise of mid-gamma when rats approached the door in anticipation of its opening, consistent with prior findings in the corticospinal system (Schoffelen et al., 2005, 2011). When rats started waiting at the door threshold, a weak elevation of low- and high-gamma occurred. Upon initiation of foraging, the power of low- and high-gamma increased further, in parallel with a rise in theta power. Upon escape, low- and high-gamma power abruptly decreased while theta power remained elevated. During escape, an increase in the power of frequencies above 100 Hz was also observed but this change likely resulted from movement artifacts.

The elevated low and high-gamma power observed during foraging are not simply related to movement since it was not seen during escape (Fig. 1C1), when rats ran the fastest. To analyze the behavioral correlates of these gamma bands, we compared different trial types. Figure 1C2 depicts gamma power on trials where rats, after waiting at the door, retreated back into the nest (134 trials) instead of initiating foraging (Fig. 1C1). A Kruskal-Wallis ANOVA comparing the baseline and waiting periods in the two trial types revealed a significant effect of trial type and task phase on low- ($\chi^2=96.37$, df=3, $p<0.001$) and high-gamma power ($\chi^2=124.97$, df=3, $p<0.001$). During the waiting period, when rats were immobile, low- and (particularly) high-gamma power was higher when rats eventually aborted (Fig. 1C2) rather than initiated foraging (Fig. 1C1; Dunn tests, $p<0.05$). Similarly, low- and high-gamma power during the baseline and waiting periods was higher on trials that followed failed (Fig. 1D1) than successful trials (Fig. 1D2; 231 and 992 trials, respectively; see figure legend 1 for statistics).

Since waiting periods prior to aborted foraging or after failed trials are devoid of movement, the associated enhancement of low- and high-gamma is likely related to an inner state akin to apprehension or a process associated with this state such as scanning vigilance. If so, why is low- and high-gamma power increased during foraging? To address this question, we next compared gamma power on trials with low or high foraging speeds as we previously found that low foraging velocities betray a state of apprehension in this task (Amir et al., 2015). Consistent with this interpretation, low- and, particularly high-gamma power during foraging was higher on trials when rats moved slowly (Fig. 2A1) than fast (Fig. 2A2) and the opposite relationship was seen in the theta band. Moreover, a separate analysis of 151 trials where rats showed signs of hesitation during foraging, pausing one or more times during approach of the food pellet, revealed that low- and high-gamma power were higher during foraging pauses than during forward movements (Fig. 2B1,2).

To dissociate the influence of movement speed, presence or absence of the predator, and whether the prior trial had been a success or failure, we computed a generalized linear model (GLM) on LFP power. The GLM confirmed a significant negative association between movement speed and low- and high-gamma as well as a positive association between movement speed and theta ($p$’s$<0.01$; Fig. 2C1–4). The predator and prior trial variables had a smaller, positive, influence on low- and high-gamma (see figure legend 2 for statistics).

**Food-seeking shuttle task**—The above indicates that different gamma bands with distinct behavioral correlates can be distinguished in BL. Mid-gamma is most prominent when rats anticipate door opening. In contrast, power in the low- and high-gamma bands is
higher during behaviors consistent with a state of apprehension and increased vigilance. However, the foraging task involves conflicting threats and rewards, complicating interpretation of the data. Thus, we next studied fluctuations in gamma activity in a task devoid of explicit threats where rats ran back-and-forth between two nest-like areas to retrieve food pellets. Retractable doors separated the nests from a dimly lit central compartment (Fig. 1B1). Prior to the recordings, rats were habituated extensively to the apparatus. During the recordings, rats were placed in one of the nests and a food pellet in the other. After opening of the doors, rats ran across the central compartment to the other nest to consume the food (Fig. 1B2). While rats ate the food, the doors were closed and another trial was initiated one minute later.

In contrast with the foraging task, there were no signs of apprehension in the shuttle task. Upon door opening, rats did not hesitate but initiated foraging at once. Also, they never skipped a trial and their foraging speed was uniformly high (~0.35 m/s). During the baseline period, power in the frequency bands of interest did not differ between the shuttle (277 trials) and foraging tasks (1238 trials; rank-sum test, p’s≥0.077). However, in contrast with the foraging task, high- and low-gamma power did not increase when rats sought the food pellets in the shuttle task (signed-rank tests, p≥0.64; Fig. 2D). Since there are no explicit threats in the shuttle task, these results again support the idea that low- and high-gamma are linked to a state of apprehension or a process associated with this state, such as scanning vigilance.

Entrainment of unit activity by various LFP rhythms

Next, we compared the entrainment of BL units by the rhythms described above. BL units (n=436) were classified as putative principal cells (n=381) or interneurons (n=50) on the basis of spike width and firing rates in the nest. Cells with firing rates <6 Hz and spike peak-to-through times >0.6 ms were classified as principal cells; units with firing rates ≥6 Hz and peak-to-through spike times ≤0.6 ms, as interneurons. Units that only met one of the two criteria (n=5) were not considered further. A subset of 56 units were positively identified as projection cells by antidromic invasion from the mPFC or nAc and they all met the above criteria for principal cells. Figure 1C,D (bottom panels) plots the average firing rates of principal cells (black) and interneurons (red) with significantly decreased (dashed lines) or increased (solid lines) firing rates during foraging (Kruskal-Wallis ANOVA followed by Dunn test, p<0.05). As evident in this figure, there was no systematic relation between firing rates and fluctuations in gamma power, a point investigated in detail below.

To avoid spectral leakage of spike waveforms into LFPs (Scheffer-Teixeira et al., 2013; Zanos et al., 2011), we referenced unit activity to LFPs recorded by a shank located 200 μm away from the unit under consideration. For each unit, the LFP was bandpass filtered for the frequency band of interest, the magnitude of entrainment (R) was ascertained and a Rayleigh test was performed to determine if the cell was significantly modulated. As detailed in the Methods, various approaches were used to bin the frequency space (4–250 Hz), but they all gave qualitatively identical results.

Although the physiological properties of principal cells and fast-spiking interneurons differ markedly (reviewed in Spampaenato et al., 2011), their frequency profile of firing...
entrainment was nearly identical. Figure 3 provides examples of peri-event histograms (PEHs) of spiking around high-gamma, low-gamma, beta and theta peaks ≥2.0 SD for a representative principal cell (Fig. 3A) and an interneuron (Fig. 3B). As in these examples, the average firing entrainment of both cell types varied significantly with frequency (ANOVA, $F_{(1,68)}=319.1, p<0.001$) and was maximal in the high-gamma range (Fig. 3C,D). As the distance between the reference LFP and the recorded unit increased from 200 to 800 μm, both the entrainment (Fig. 3C,D; ANOVA, $F_{(1,3)}=4736.2, p<0.001$) and the proportion of cells significantly entrained by high-gamma (Fig. 3E) decreased ($X^2=70.648, p<0.001$). Similar results were obtained with mid- and low-gamma (Fig. 3C–E).

Whereas there was no relationship between the firing rates of principal cells and their entrainment by high-gamma (Spearman $r=0.025, p=0.625$), a significant positive correlation was found in fast-spiking cells (Spearman $r=0.306, p=0.031$). Unexpectedly, frequency distributions of firing entrainment by high-gamma were skewed to the left in principal (Fig. 3F) and fast-spiking (Fig. 3G) cells, suggesting that high-gamma does not influence BL neurons homogenously, a point we will return to below. Among the significantly entrained units, principal and fast-spiking cells preferentially fired at different phases of high-gamma (Fig. 3H–J). Principal cells mainly fired during the trough of high-gamma (Fig. 3H,J) and fast-spiking cells, during the rise of its positive phase (Fig. 3I,J).

**Relation between behavioral states and entrainment by high-gamma**

In the previous section, we considered the entrainment of BL firing by high-gamma, irrespective of behavioral state. Here we examine the impact of this factor. We distinguished four different waking states, three of which occurred during foraging trials (baseline, waiting, foraging) and the last, in between trial blocks (quiet wakefulness). These states were associated with significant changes in the incidence of gamma bursts and in the firing rates of BL neurons (Supplementary table 2). Of note, the frequency of gamma bursts was higher than the average firing rate of principal cells in all states, but not of interneurons.

We first tested whether the neurons’ preferred firing phase changed as a function of behavioral state. Because principal cells generally have low firing rates (Supplementary table 2) and this analysis required data segmentation by states, to compensate for the loss of statistical power, we restricted our attention to a subset of 65 principal cells selected because they were more active ( ≥100 spikes in each of the conditions under consideration; see figure legend). A non-parametric multi-sample test for equal medians revealed that the preferred firing phase of 10% of principal cells (Fig. 4A1) and 25% of fast-spiking neurons (Fig. 4A2) differed significantly between states ($p<0.05$). However, even in these cells, the effect was minute (~6 degrees difference) and the direction of the change was not consistent.

Next, we studied the influence of behavioral states on firing entrainment by high-gamma (Fig. 4B–D). As a first step, we asked whether the relative magnitude of entrainment across our samples was consistent between states. To this end, we sorted the same subset of spontaneously active principal cells (Fig. 4C) and all interneurons (Fig. 4D) by their entrainment in quiet waking (Fig. 4C1, 4D1; warmer colors indicate stronger entrainment) and plotted the data obtained in the other states without changing the order of the cells (Fig. 4C2–4, 4D2–4). Also, to control for state differences in gamma levels, we stratified the
entrainment by gamma burst amplitude (y-axis; see Methods). These analyses revealed that cell-to-cell variations in firing entrainment by high-gamma are largely consistent across states (Fig. 4B; see figure legend for statistics).

To compare the influence of task-related behavioral states and gamma on firing rates, for each unit we used a GLM to fit the binned spiking (12 ms windows) with behavioral state (baseline, waiting, foraging) and z-scored gamma amplitude. Figure 5 plots the beta coefficients for each state with respect to the gamma amplitude beta, revealing that states have a much larger influence on firing rates than high-gamma but that this effect is much more pronounced in principal cells (Fig. 5, blue) than interneurons (Fig. 5, red). In this analysis, the absolute ratio between state and gamma beta coefficients indicates how many standard deviations in gamma amplitude are required to cause a change in firing rate similar to that induced by a change in task-related states. For instance, in principal cells, gamma amplitude would have to change by 21.6 standard deviations to cause the same change in firing rate induced by a switch from quiet waking to foraging (Fig. 5A). Changes from quiet waking to waiting (Fig. 5B) or baseline (Fig. 5C) had a similarly outsized impact on the firing rates of principal cells relative to gamma amplitude.

Yet, there was much heterogeneity in our samples of principal cells and interneurons (Fig. 6). In both cell types, entrainment by high-gamma (resultant vector – R) varied widely (principal cells, 0–0.36, Fig. 6A; interneurons, 0–0.3; Fig. 6B) and it correlated positively with the influence of high-gamma on firing rates (Fig. 6A5, B2; Supplementary Fig. 2A). Also, a minority of neurons (Fig. 6B4) showed little firing entrainment to high-gamma (R < 0.1), yet displayed a clear reduction in firing rate during high-gamma bursts, reminiscent of prior observations in cortex (Puig et al., 2008).

**Impact of high-gamma on firing synchrony in BL**

Even though high-gamma has minor effects on firing rates, it might still shape the ensemble activity of BL neurons. To test this possibility, we examined the cross-correlation in spiking between pairs of simultaneously recorded neurons during periods with low or high gamma power (see Methods and Supplementary Fig. 2B,C). We then compared the amount of synchrony in the two conditions, after normalizing for differences in baseline activity (Synchronization Index – SI, see Methods). These analyses were carried out for couples of principal cells, of fast-spiking interneurons, or of principal cells and interneurons (top to bottom rows of Fig. 7, respectively). In these three types of cell couples, normalized cross-correlograms (CCGs; Fig. 7A) and SIs (Fig. 7B) revealed that firing synchrony was higher when high-gamma power was elevated (blue) than low (red; inter-cell distance of 200 μm; signed-rank tests; the number of pairs and p values are indicated in Fig. 7). While this effect held irrespective of how far apart the cells were from each other, distance attenuated it significantly (Fig. 7C; P-P, ANOVA $F_{(2,3661)}=4.55$, $p=0.011$; INT-INT, ANOVA $F_{(2,305)}=10.3$, $p<0.001$; INT-P, ANOVA $F_{(2,3121)}=60.1$, $p<0.001$).

However, the above analyses did not take into account how much gamma entrained each cell in a couple to begin with. To address this question, we correlated SIs with the joint gamma entrainment of the cells in each couple. This was operationally defined as the product of the $R$ variable computed individually for each cell in a couple. The results of this analysis are...
shown in figure 7D where couples are stratified by percentile of their $R$ product. We found a significant correlation between $R$ product and SI, which was strongest for couples of interneurons (Spearman $r = 0.77$, $p<0.001$; Fig. 7D2) and mixed couples (Spearman $r = 0.53$, $p<0.001$; Fig. 7D3) but lower in pairs of principal cells (Spearman $r = 0.16$, $p<0.001$; Fig. 7D1), likely because of their low firing rates. Together, these findings suggest that while high-gamma exerts minor effects on firing rates, it is associated with a marked increase in neuronal synchrony, particularly among cells that are highly entrained by high-gamma.

In the foraging task, since high-gamma levels increased during foraging (Fig. 1C1), the above results predict that firing synchrony in the three types of cell couples should be elevated at that time relative to baseline. To test this idea, we computed CCGs of unit activity irrespective of high-gamma levels during the baseline period and, separately, foraging. We then computed SIs for both. Consistent with our prediction, these analyses revealed that firing synchrony was significantly enhanced during foraging for the three types of cell couples (Signed-rank tests; 559 couple of projection cells, $41.1\pm17.6\%$ increase; $p=0.005$; 84 couples of interneurons, $13.6\pm5.1\%$ increase, $p=0.03$; 587 mixed couples, $14.2\pm6.2\%$ increase, $p=0.01$).

Relation between connectivity and entrainment by high-gamma

BL contains many subsets of principal cells, as defined by their different cortical and subcortical projections (Pitkanen, 2000). High-gamma might similarly entrain all principal cells irrespective of their connectivity or selectively entrain particular subsets that belong to specific networks. Thus, we examined how gamma entrainment relates to the cells’ intrinsic (Fig. 8A) and extrinsic (Fig. 8B) connectivity.

To test whether high-gamma synchronizes monosynaptically connected cells more strongly than uncoupled neurons, we segregated cell pairs based on whether their CCGs displayed evidence of monosynaptic connections (see Methods for criteria). To avoid confounding monosynaptic connections and gamma-induced synchronization, we first determined the range of high-gamma power that caused a rise in synchrony and then restricted our CCG analyses to epochs with lower gamma power (bottom 50th percentile; Supplementary Fig. 3A). Examples of CCGs with putative monosynaptic connections are illustrated in supplementary Fig. 3B). We then compared the gamma entrainment of cells belonging to monosynaptically connected (red) or unconnected (blue) cell pairs (connections from principal cells to interneurons, Fig. 8A, Supplementary Fig. 4A; interneurons to principal cells, Supplementary Fig. 4B; between principal cells, Supplementary Fig. 5).

For couples of principal cells and interneurons, we found that high gamma entrainment was associated with an increased incidence of monosynaptic connections. That is, principal cells with a high resultant vector were more likely to be connected to interneurons with a high resultant vector (Fig. 8A1). However, this effect was marginal in the opposite direction (from interneurons to principal cells; Supplementary Fig. 4). To evaluate the statistical significance of this observation, we correlated the connection status of each cell couple (unconnected=0; connected=1) with the product of their resultant vector. The Spearman correlation coefficient was 0.49 ($p<0.001$) for connections from principal cells to interneurons and 0.17 ($p<0.001$) for interneurons to principal cells.
However, a potential confound in the latter analysis is the possibility that the higher synchronization by high gamma seen in monosynaptically coupled cells is due to the fact that these cells are more strongly modulated by gamma to begin with. To eliminate the influence of each cells’ gamma rhythmicity from synchronization estimates, we computed unit-unit coherence, an approach that controls for the autocorrelation structure of each cell’s activity (Supplementary Fig. 6). Comparing coherence estimates between connected and unconnected cell couples confirmed that relative to all other frequencies, high gamma synchronizes monosynaptically connected cells more strongly than non-connected neurons (Fig. 8A2; Supplementary Fig. 7; rank-sum test, p<0.001).

So far, we have considered the impact of intrinsic connectivity on firing synchrony among pairs of principal cells or couples of interneurons and principal cells. For couples of interneurons, monosynaptic connectivity could not be assessed because their CCGs often had a peak at time lag zero such that subsequent reductions in spike counts might have been caused by spike afterhyperpolarizations, synaptic inhibition, or both. Nevertheless, we noticed that there was a strong correlation between, on the one hand, the height of the peak at time zero in the absence of gamma and, on the other, their $R$ product ($r=0.76; p<0.0001$) or the increase in synchrony observed during high-gamma ($r=0.50; p<0.0001$).

Last, we examined whether gamma entrainment varies depending on the cells’ projection site(s) (Fig. 8B). To this end, we computed separate frequency distributions of gamma entrainment for the subsets of cells antidromically responsive to electrical stimulation of nAc (Fig. 8B3) or the mPFC (Fig. 8B4). For reference, the gamma entrainment of all putative principal cells as well as of all principal cells positively identified as such by antidromic invasion are depicted in figure 8B1,2 respectively. We found that gamma entrainment varied significantly as a function of the cells’ projections site(s). It was significantly lower among nAc-projecting cells (0.092 ± 0.032), than neurons projecting to mPFC (0.174 ± 0.033; rank-sum test, p<0.001). Some neurons projected to both sites but there were too few of them (n=6) for meaningful statistical comparisons with the other two subsets.

Potentially related to these results, we also found that adjacent pairs of principal cells, that is cells recorded from the same shank, had more similar levels of gamma entrainment compared to pairs recorded from different shanks. To test the statistical significance of this observation, we used two approaches (Supplementary figure 8). We first correlated the $R$ of cells in each pair of simultaneously recorded principal cells. The correlation between $Rs$ was significant ($r=0.52, p<0.0001$) for cell pairs recorded from the same shank (n=1615), but not for cell pairs recorded from different shanks (n=6247; $r=-0.003, p=0.82$). In the second approach, we compared the absolute difference in the $Rs$ for cell pairs recorded from the same or different shanks. A significant difference was observed (Rank Sum test, p<0.0001). This result likely reflects regional differences in the connectivity of BL neurons that impact on their gamma-related activity.
DISCUSSION

This study reports on the behavioral correlates and cellular mechanisms of gamma oscillations in BL. To address these questions, we recorded unit and LFP activity in rats performing tasks characterized by different threat levels. Our main findings are as follows. First, different gamma bands with distinct behavioral correlates occur in BL. Second, compared to all other frequencies, high-gamma most strongly entrains BL firing and it is particularly pronounced when the rats’ behavior betrays scanning vigilance. Third, principal cells and fast-spiking interneurons preferentially fire at different phases of high-gamma. Fourth, phase preference and entrainment of BL units by high-gamma do not change appreciably with behavioral states. Fifth, relative to behavioral states, high-gamma only produces minor changes in firing rates. Sixth, the entrainment of principal cells by high-gamma varies as a function of their projection site. Last, high-gamma dramatically increases firing synchrony, particularly among synaptically-coupled cells.

Behavioral correlates of different gamma bands

Many brain regions display multiple gamma bands with different input dependencies and behavioral or cognitive correlates (Ainsworth et al., 2011; Colgin et al., 2009; van der Meer et al., 2010). We found that a similar situation exists in BL. In the foraging and shuttle tasks, mid-gamma was most prominent when rats anticipated door opening. In contrast, power in the low- and, especially, high-gamma bands was generally higher when rats were apprehensive. For instance, in the foraging task, low- and high-gamma were pronounced during waiting periods that preceded aborted trials or followed a failed trial. On trials where rats initiated foraging, low- and high-gamma power increased more during waiting periods that followed failed than successful trials. Moreover, it was also higher on trials where rats foraged tentatively, hesitating as they approached the reward. Underscoring the importance of apprehension, no significant increase in low- and high-gamma power was observed in a shuttle task, which is devoid of explicit threats. While these results suggest that low- and high-gamma are linked to a state of apprehension, the negative correlation between low- and high-gamma power with movement speed, including when rats escaped to the nest in response to the charging predator, suggest that fear per se is not the critical correlate. An associated process such as scanning vigilance seems more likely. This interpretation is consistent with a recently revised model of amygdala function, which asserts that the amygdala mediates an evaluative process that regulates behavioral engagement (Pare and Quirk, 2017).

High-gamma strongly entrains BL firing

By comparing the entrainment of units to the LFP in different frequency bands, we found that BL cells were more strongly entrained by high-gamma than all other rhythms. Critically, this result does not mean that the main LFP rhythm in BL is high-gamma but that when high-gamma occurs, it entrains BL neurons more robustly. In principal cells, gamma-related activity occurred on a mostly silent background and consisted of occasional single spikes concentrated during negative gamma phases. By contrast, fast-spiking cells discharged tonically and gamma produced alternating periods of decreased and increased firing rates, the latter occurring mainly after the preferred firing phase of principal cells.
While the propensity of principal cells and interneurons to fire at different gamma phases is ubiquitous in cortical networks (Bragin et al., 1995; Atallah and Scanziani, 2009; Hasenstaub et al., 2005; Vinck et al., 2013; reviewed in Buzsaki and Wang, 2012), preferential entrainment by high-gamma is not (Newman and Hasselmo, 2014). At present, the origin of this property is unclear. One possibility is that coherent gamma inputs to BL preferentially occur in the high-gamma range. Arguing against this idea however, entrainment by high-gamma (magnitude and phase preference) did not vary as a function of behavioral states, even though such shifts are expected to correlate with changing input patterns to BL. The state-invariant entrainment of BL neurons by high-gamma raises a second possibility, namely that the intrinsic BL network is such that it gives rise to a preferential resonance at high-gamma. The length of the gamma cycle is determined by multiple interacting properties such as the kinetics and reversal potentials of synaptic currents as well as the neurons’ intrinsic properties and connectivity (Buzsaki and Wang, 2012). While these properties fall in a similar range in BL and cortical networks (Martina et al., 2001; Spampanato et al., 2011; Wang, 2010; Woodruff and Sah, 2007), this possibility cannot be excluded at this time.

Entrainment by high-gamma varies as a function of the cells’ projection site

Entrainment by high-gamma was not expressed homogeneously in projection cells. Whereas most were significantly entrained by high-gamma, a small subset was not, even though both groups had similar firing rates. By comparing how high-gamma entrained neurons backfired from the mPFC or nAc, we found that this heterogeneity was related to the neurons’ projection sites. Neurons projecting to mPFC were strongly entrained whereas most of those targeting nAc were weakly to negligibly so. While we cannot rule out that nAc-projecting neurons would be more strongly entrained by high-gamma under different circumstances, our observation that the entrainment of BL cells did not change appreciably across many different states and behaviors argues against this possibility. Rather, it seems more likely that BL cells projecting to distinct sites form different connections with fast-spiking interneurons.

Interestingly, the contrasting expression of gamma entrainment as a function of projection sites is paralleled by differences in the connectivity of mPFC neurons that target the amygdala or nAc. Indeed, McGarry and Carter (2016) reported that BL neurons excite corticoamygdala neurons more strongly than those targeting nAc. Moreover, they found that, via PV cells, BL inputs inhibit corticoamygdala neurons more strongly than corticostriatal neurons. In light of these findings, the target-specific expression of high-gamma by principal BL cells emerges as but one manifestation of a larger system design that segregates mPFC and amygdala influences on nAc from those exerted by the mPFC and amygdala on each other.

Impact of high-gamma on firing rates vs. synchrony

Whereas high-gamma and behavioral states caused similarly modest changes in the firing rates of interneurons, in projection cells the impact of behavioral states was much larger. Among the significantly entrained cells, the effect of high-gamma ranged from a ~25% increase in firing rate to a ~10% reduction in activity. While high-gamma had a minor
impact on discharge rates, it caused a major increase in firing synchrony, particularly among monosynaptically connected cells. This effect was not ubiquitous however. Gamma-related synchrony decreased rapidly as the distance between the recorded cells increased, much like entrainment by high-gamma decayed with distance between the recorded cell and reference LFP. Also, even with nearby cells, the effect of gamma on synchrony was large when both cells were highly entrained by gamma and low when one or both cells were poorly entrained by gamma.

Conclusions

Overall, our results indicate that high-gamma synchronizes particular subsets of monosynaptically connected principal cells with specific projection sites and preferential connectivity with fast-spiking interneurons. Critically, gamma-related changes in firing rates are much smaller than those associated with shifts in behavioral states. As a result, modulation of BL activity by high-gamma can easily go undetected unless multiple cells are recorded simultaneously. The synchronization of BL cells by high-gamma may explain how BL neurons impact motivated behaviors despite the fact that emotionally-valenced stimuli activate very few of them. Indeed, by clustering the discharges of multiple cells in short recurring time windows, high-gamma should enhance their impact on common postsynaptic targets (Fries et al., 2007; Fries, 2015). Also, depending on whether they coincide with the de- or hyperpolarizing phases of the BL driven gamma oscillation, convergent synaptic inputs should be biased to undergo associative potentiation or depression (Wespatat et al., 2004). An important challenge for future studies will be to test these hypotheses by selectively manipulating high-gamma in real time, which would require novel techniques such as closed-loop optogenetic manipulations.

STAR METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Denis Pare (pare@andromeda.rutgers.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Procedures were approved by the Institutional Animal Care and Use Committee of Rutgers University, in compliance with the Guide for the Care and Use of Laboratory Animals (DHHS). We used naïve male Sprague-Dawley rats (10–11 weeks old and 310–360 g at the beginning of experiments, Charles River Laboratories, New Field, NJ).

METHOD DETAILS

Overview of the experimental timeline—Rats were maintained on a 12 h light/dark cycle. Experiments were performed during the light phase. After habituation to the animal facility and handling, rats underwent a stereotaxic surgery during which silicon probes were inserted in the amygdala. Following the surgery, rats were housed individually with ad libitum access to water. The behavioral experiments began ≥7 days after the surgery.
Surgery—Rats were anesthetized with isoflurane and administered atropine sulfate (0.05 mg/kg, i.m.) to aid breathing. In aseptic conditions, rats were mounted in a stereotaxic apparatus. A local anaesthetic (bupivacaine, sc) was injected in the scalp and 15 min later, the scalp was incised and a craniotomy was performed above the amygdala. Then, silicon probes (Neuronexus, Ann Arbor, MI) attached to Buzsaki-style microdrives (Vandecasteele et al., 2012) were aimed at BL under stereotaxic guidance. Silicon probes consisted of either eight (3 rats) or four (3 rats) shanks (inter-shank distance of 200 μm), each with 8 recording leads (de-insulated area, 144 μm²) separated by ~20 μm dorsoventrally. One of the six rats had to be excluded because movements caused artifacts in local field potentials (LFPs). In three of the five remaining rats, a second craniotomy was performed above nAc and mPFC. Then, pairs of tungsten stimulating electrodes (inter-tip spacing, 1–1.7 mm) were inserted into these two structures. We used the following stereotaxic coordinates (all expressed in mm relative to bregma, and where AP, ML, and DV stand for anteroposterior, mediolateral, and dorsoventral, respectively). BL, AP −2.2 to −3.6, ML 5 to 5.3, DV 8.8; mPFC, AP 2.7 to 3.7, ML 0.5, DV 3.6 to 5.2; nAc, AP 1.5, ML 1.35, DV 6.7. Rats were allowed two to three weeks to recover from the surgery.

Foraging task—To ensure proper motivation in the behavioral tasks, daily access to food was restricted in time so that the rats’ bodyweight was maintained at about 90% of age-matched subjects with continuous access to food.

Foraging apparatus: The foraging apparatus was a long rectangular alley (length, 245 cm width, 60 cm) with high walls (60 cm) and no ceiling. A door (height, 50 cm; width, 10 cm) divided it into two compartments. One compartment (length, 30 cm) was dimly lit (10 Lux) and served as a nest area with a water bottle. The other compartment was a much longer (215 cm) and brighter (200 Lux) foraging arena.

Mechanical predator: On some trials, a mechanical predator (Mindstorms, LEGO systems, Billund, Denmark; length, 34 cm; width, 17 cm; height, 14 cm) on wheels was positioned at the end of the foraging arena, facing the nest. It was equipped with a sensor that detected the rats’ approach, triggering a sudden forward movement (80 cm at 60 cm/sec) and repeated jaw openings and closings, followed by return to its original position.

Habituation to nest (Days 0–1): Rats were first habituated to the nest for 2 daily consecutive sessions of 7 h with the door shut at all times. On habituation days, rats could consume up to 6 g of food (sweet cereal pellets).

Foraging in the absence of predator (Days 2–3): On the next two days, in the absence of the predator, rats were trained to retrieve sweetened food pellets (80–100 mg) in the foraging arena. No food was available in the nest. One each trial, a single food pellet was placed at various distances from the door. The door was then opened. After a period of hesitation at the doorway, rats retrieved the food pellet and returned to the nest to consume it. Upon return to the nest, the door was closed. Initially, the distance between the nest and food pellet was progressively increased in steps of 25 cm (from 25 to 150 cm), after three successful trials at each distance. Later on, the distance was varied randomly from trial to trial.
**Foraging in the presence of the predator (Days 4–5):** On the next two days, rats were again given the opportunity to retrieve food pellets in the foraging arena. However, ~60% of trials were conducted with the predator present. Alternating blocks of trials with (n=10–20) or without (n=10–15) the predator were conducted. All our analyses are based on activity recorded in Days 3–5.

**Shuttle task**—In this task, rats ran back and forth between two nest-like areas (length, 50 cm; width, 20 cm) through a central compartment (length, 50 cm) to retrieve food pellets at the end of the other nest. The apparatus was made of black Plexiglas, had walls 45 cm in height, and was dimly illuminated (10 Lux). Retractable doors separated the nests and central compartment. Prior to the recordings, rats received extensive habituation to the apparatus with the doors open. During the recordings, rats were placed in one of the nests and a food pellet in the other. After opening of the doors, rats immediately ran to the other nest to retrieve and consume the food. While rats consumed the food, the door was closed. The inter-trial interval was ≥1min. Three of the five rats used for the foraging task also performed the shuttle task. In these three rats, we alternated between the two tasks during different recording sessions.

**Analysis of behavior**—Behavior was recorded by an overhead videocamera at a frame rate of 29.97 Hz and analyzed using the following two approaches. First, the position and velocity of the rats was automatically detected using a matlab script that considered the shifting distribution of light intensity across frames. In addition, an experienced observer conducted a frame-by-frame analysis of the video file to ascertain when rats started waiting at the door threshold (defined as when the rat’s snout extended beyond the door into the foraging arena), when they initiated foraging (defined as the last frame of immobility prior to completely moving out of the nest), retrieved the food pellet, escaped, and retreated into the nest. The observer also noted the outcome of each trial (failure or success). Escape onset was defined as when rats, after approaching the food pellet, abruptly turned around and ran all the way to the nest.

When detecting transitions between immobility and movement, the following conditions had to be met. In order not to mistake waiting for interrupted foraging, rats had to be at least 30 cm away from the nest. Also, rats had to be completely immobile for ≥1 s, followed by ≥1 s of forward movement with a peak speed ≥5 cm/sec, followed by another pause for ≥1 s. In the second pause, rat had to be ≥30 cm from the food pellet. The last condition was imposed to avoid confusing hesitation with reaching the food. After automatically detecting potential transitions, we visually inspected each and eliminated cases where rats did not face the predator.

**Histology**—At the end of the experiments, the animals were deeply anesthetized. On each shank, one of the recording sites was marked with a small electrolytic lesion (10 μA between a channel and the animals’ tail for 10 sec). Rats were then perfused-fixed through the heart, their brains extracted, cut on a vibrating microtome and the sections counterstained with cresyl violet. We only considered neurons that were histologically-determined to have been recorded in BL.
QUANTIFICATION AND STATISTICAL ANALYSES

All grouped data are reported as averages ± SEM. All statistical tests were two-sided. When comparing only two conditions, we used rank-sum or signed-rank tests, as appropriate. To assess the statistical significance of differences in LFP power between different phases of the same trial type, we computed non-parametric repeated measures Friedman ANOVAs. For between trial type comparisons, we used a non-parametric Kruskal-Willis ANOVA for independent samples. In both cases, each trial was an observation of the mean power in a particular frequency band across the behavioral epoch of interest. Post-hoc comparisons were performed with Dunn tests using a p-value of 0.05. In a few instances, when assessing the statistical significance of multiple factors (e.g. frequency, distance between recording sites), we had to use a parametric ANOVA as there are no non-parametric alternatives. MATLAB was used for statistical analyses.

Behavioral states—Periods of slow-wave sleep were excluded from all the analyses reported in this study. States of vigilance were distinguished using spectral analyses of LFPs and behavioral observations. Spontaneous LFP activity was segmented in five-second windows and frequency distributions of LFP power in different frequency bands computed. Active waking could be distinguished from all other states because it was associated with a broadband increase in the power of high frequencies (200–240 Hz), reflecting electromyographic activity. After eliminating active waking, we could easily distinguish slow-wave sleep from quiet waking because total power at frequencies <20 Hz was distributed bimodally between the two states: epochs of high power at low frequencies corresponded to periods of slow-wave sleep.

LFP processing, unit recording and clustering—BL unit and LFP recordings were performed during all the phases of the behavioral protocols described above with the exception of habituation. However, the first training day of the foraging task was not included in the analyses because the rats’ performance was poor on that day. In rats that had been implanted with stimulating electrodes, at the end of each behavioral session, electrical stimuli (300–600 μA, 0.1 ms) were delivered at 1 Hz to determine whether recorded cells could be antidromically invaded from one or more of the stimulated sites. Then, the silicon probe was lowered ≥30 μm (40 ± 10 μm), ≥8 hours ahead of the next recording session, to ensure mechanical stability. In our experience, when recordings are performed shortly after moving the probes, spike shapes are not stable. Presumably, this is the case because the surrounding brain tissue has not completely adapted to the probe’s movement. In contrast, if we perform recordings ≥8 hours after moving the probes, spike shapes are stable. Note that it is possible that the same cells were sampled from on different days. However, the incidence of such recordings is impossible to assess because moving the probes changes spike shapes.

The signals were sampled at 25 kHz and stored on a hard drive. The data was first high-pass filtered using a median filter (window size of 1.1 ms), then thresholded to extract spikes. We then ran PCA on the spikes and the first three components were clustered using KlustaKwik (http://klustakwik.sourceforge.net/). Spike clusters were then refined manually using Klusters (Hazan et al., 2006). The reliability of cluster separation was verified by inspecting
auto- and cross-correlograms (CCGs). Autocorrelograms had to display a refractory period of at least 2 ms. CCGs should not show evidence of a refractory period, as this feature betrays overlap between clusters. Units with unstable spike shapes during a given recording session were excluded.

**Identification of principal cell and interneurons:** Cells with firing rates <6 Hz and spike peak-to-through times >0.6 ms were classified as principal cells; units with firing rates ≥6 Hz and peak-to-through spike times ≤0.6 ms, as interneurons. To determine spike duration, we first selected the channel where, for a given cell, action potentials had the largest peak to trough amplitude. We then measured spike duration as the time between spike trough and peak (Bartho et al., 2004). To test the reliability of our classification criteria, using CCGs, we looked for evidence of monosynaptic inhibition from putative principal cells to other cells (which would be an instance where an interneuron was misclassified as a projection cell) or of excitatory connections from putative interneurons to other cells (which would be an instance where a principal cell was misclassified as an interneuron). A total of 15,514 cell pairs were examined for the first case, and 2046 cell pairs for the second. Of these, respectively 5.82% (or 904) and 5.37% (or 110) formed a putative connection. Supporting the validity of our classification criteria, the incidence of misclassified connections was extremely low (Principal cells: 0.99% or 9 of 904; Interneurons: 3.6% or 4 of 110).

Furthermore, repeating the analyses shown in figures 3–8 on these restricted samples of BL cells yielded qualitatively identical results.

Antidromic action potentials were identified as such when they had a fixed latency (≤0.1 ms jitter) and collided with spontaneously occurring spikes.

**Nomenclature used for gamma:** Based on their different frequencies and contrasting behavioral correlates, we distinguished three different gamma bands, which we termed low- (35–55 Hz), mid- (55–75 Hz) and high-gamma (75–95 Hz). We wish to emphasize that high-gamma is not non-specific high frequency activity that shows up non-rhythmically in the higher frequencies, but a genuine LFP rhythm in the band identified.

**Processing of LFPs:** Shanks that were located outside BL were not considered. LFP analyses involved many steps. We first enumerate these steps and then discuss each step in detail. The LFP processing steps were (1) down-sampling, (2) extraction of one LFP per shank and recording session, (3) calculation of spectrogram for all foraging trials on each day and for each shank, (4) calculation of the average and standard deviation of the spectrogram for each day and shank, (5) normalization of each trial by the above average and standard deviation, (6) time normalization, (7) average of normalized spectrogram across shanks, and finally, (8) average across trials of a given type.

1. **Down-sampling:** signals were first down-sampled to 1250 Hz.

2. **Extraction of one LFP per shank and recording session:** We extracted a single LFP for each shank by taking the median LFP signals of the eight leads on the corresponding shank.
3. **Calculation of spectrogram for all foraging trials on each day and for each shank:**
   We used the Chronux spectral analysis MATLAB toolkit. We used the function `mtspecgramc` to calculate a multi-taper time-frequency spectrogram. The number of tapers was set to 5 and the time-bandwidth product to 3. To compensate for power attenuation with frequency, power was calculated as its natural logarithm. We used a 0.8 s window sliding in 0.2 s steps. The only exception to this rule is when we analyzed trials where rats showed signs of hesitation during foraging, in which case 0.1 s steps were used. For each shank on a given day, we calculated the power spectrogram during all available foraging trials, excluding the inter-trial periods. Below, we refer to this spectrogram as $S(\text{shank, freq, time})$.

4. **Calculation of the average and standard deviation of the spectrogram for each day and shank:**
   The average and SD was calculated as:
   \[
   \text{mean}S(\text{shank, freq, time}) = \frac{\sum_{\text{time}} S(\text{shank, freq, time})}{N}
   \]
   \[
   \text{std}S(\text{shank, freq}) = \sqrt{\frac{\sum_{\text{time}} (S(\text{shank, freq, time}) - \text{mean}S(\text{shank, freq}))^2}{N-1}}
   \]
   These averages and standard deviations were later used to normalize (or in other words z-score) all the trials, including the shuttle trials. This approach allowed us to compare different trial types using the same metric.

5. **Normalization of each trial by the above average and standard deviation:**
   To normalize each trial, we used the following equation
   \[
   z_{\text{trial}}(\text{shank, freq, time}) = \frac{S_{\text{trial}}(\text{shank, freq, time}) - \text{mean}S(\text{shank, freq})}{\text{std}(\text{shank, freq})}
   \]
   The rationale for z-scoring LFP power is the following. First, due to variations in the properties of electrodes, signal amplitudes can vary between shanks. As a result, not z-scoring would give more weight to data obtained with some shanks. Second, low frequencies have a much higher power than high frequencies. As a result, when power is not z-scored, it is difficult to visually detect changes in the power of high frequencies in raw spectrograms.

6. **Time normalization:**
   In order to average trials of different durations, we computed relative time, using the linear interpolation function `interp1` of MATLAB. Effectively, this function distributes a fixed number of power samples across the various phases (20 for baseline, waiting, and foraging; 5 for escape). So for example, if a given waiting period lasted 40 seconds, the power data was
pooled into 20 consecutive samples. When the duration of the phase under consideration was too short (<0.8 s), the trial was excluded. Of course, when using actual time, we skipped this step.

7. **Average of normalized spectrogram across shanks**: For each trial, we then computed the average z-scored spectrogram across shanks using the following formula.

\[
zs_{\text{trial}}(\text{freq, norm Time}) = \frac{\sum_{\text{shank}} zs_{\text{trial}}(\text{shank, freq, norm Time})}{N_s}
\]

8. **Average across trials of a given type**: Last, we computed a grand average of all trials of a given sub-type using the following equation.

\[
zs(\text{freq, norm Time}) = \frac{\sum_{\text{trial}} zs_{\text{trial}}(\text{freq, norm Time})}{N_{\text{trial}}}
\]

**Generalized linear model**: We computed a generalized linear model (GLM) relating LFP power in different frequency bands to movement speed, presence or absence of the predator, and outcome of the previous trials. After calculating the z-scored power spectrum of each individual trial across shanks, trials were labeled for the presence or absence of the predator (respectively 1 or 0) and whether the prior trial was a failure or success (respectively 1 or 0). In addition, instantaneous velocity was measured. Next, we combined the data from all available trials and z-scored the velocity, robot presence/absence and prior trial outcome. Last, we fit a GLM as follows:

\[
z\text{Spectrum}(\text{freq, time}) = \text{BetaVel}(\text{freq, time}) \ast \text{zVel}(\text{time}) + \text{BetaRobot}(\text{freq, time}) \ast \text{zRobot}(\text{time}) + \text{BetaTrialOutcome}(\text{freq, time}) \ast z\text{TrialOutcome}(\text{time}) + \text{constant}
\]

Similar results were obtained when the trial outcome and Robot variables were not z-scored.

**Relationship between unit activity and field potentials**: We referenced unit activity to LFPs picked up by a shank located 200, 400, 600 and 800 μm away from the unit of interest. When two shanks at the same distance from the recorded cell were available, we selected the shank nearest to the center of the silicon probe. For calculating the entrainment and preferred phase of cells, we first bandpass filtered the LFPs in the frequency band of interest using the 2 pole butterworth filter using the filtfilt MATLAB function and applied a Hilbert transform to the resulting signal to determine the phase and amplitude at each point. Then, each spike was assigned a phase. Last, using the MATLAB Toolbox for Circular Statistics, we computed the mean resultant vector length for circular data (circ_r), a measure of entrainment, the mean phase (circ_mean), and determined whether the cell was significantly entrained using a Rayleigh test. However, the resultant vector metric is positively biased with low spike counts. That is, even if cells are not entrained by an oscillation, the resultant
vector does not approach zero when few spikes are considered. Thus, before conducting the analyses shown in our paper, we examined the reliability of the resultant vector. Specifically, we randomly selected gradually increasing subsets of spikes from the same spike trains to determine the minimum number of spikes needed for the resultant vector to become reliable. These tests revealed that resultant vector values stabilize when the spike train includes >100 spikes. To place these figures in context, note that in our samples, the average spike count was 9803 for principal cells and 618,616 for interneurons. The principal with the lowest spike count we considered included 119 spikes and only 13 principal cells (out of 381) had trains of less than 500 spikes. We estimated that, on average, the R vector was biased by less than ~1% for principal cells and less than ~0.1% for interneurons. Note that only in figure 4 did we have to exclude cells because they had less than 100 spikes in some of the conditions considered.

When comparing preferred phase across states, statistical significance of the change was assessed using the non-parametric multi-sample test for equal medians (circ_cmtest) with a significance threshold of 0.05. Cells that fired <100 spikes in the period under consideration were excluded.

When computing peri-event histograms of neuronal discharges around oscillatory bursts, we used the following approach. The signal was first bandpass filtered in the frequency band of interest as described above. Next, the standard deviation of the signal was calculated and bursts of oscillations that included at least three consecutive peaks ≥2 standard deviations were detected. The largest trough was used as temporal reference of the peri-event histograms.

Two approaches were used to bin the frequency space (4–250 Hz). In all cases, the frequency space was divided in 69 bins that partially overlapped and shifted in different increments, as described below. The approach illustrated in the Results consisted of gradually increasing the bandwidth as the frequency under consideration (from 4 Hz in the theta range to 20 in the gamma range), generally matching the bandwidth of the oscillations that varied in a behavior-dependent manner in the foraging task. The specific binning scheme is detailed below, using the following abbreviations: Freq, center frequency; freq_high, high cutoff frequency; freq_low, low cutoff frequency; deltaFreq, bandwidth.

\[
\begin{align*}
\text{Freq}<8 & \quad \text{deltaFreq=4} \\
8<\text{Freq}<20 & \quad \text{deltaFreq=4+(Freq-8) * 1/2} \\
20<\text{Freq}<45 & \quad \text{deltaFreq=10+(Freq-20) * 2/5} \\
\text{Freq}>45 & \quad \text{deltaFreq=20}
\end{align*}
\]

The above method of frequency binning was modified in the following way. The bandwidth was always 10 Hz and the center frequencies shifted logarithmically in 0.2 Hz increments in the theta range to 15 Hz in the gamma range. All these binning methods gave qualitatively identical results.
Impact of behavioral states on the entrainment of unit activity by gamma: Because gamma power varied with behavior, when comparing entrainment of unit activity across states, we stratified the data by gamma magnitude in the baseline period and then applied the same stratification scheme to the data obtained in other states. As a result, the percent time spent in the different strata varied with states but the unit activity in each stratum can be compared directly across states.

Using a GLM to compare the influence of states vs. gamma on firing rates: To compare changes in firing rates associated with states versus gamma power, we used a GLM with a Poisson distribution. We used non-overlapping time windows of 12 ms, but longer windows (50 or 100 ms) yielded qualitatively identical results. For each cell and time window, the number of spikes, z-scored gamma amplitude, and a dummy-coded state vector (quiet waking, baseline, waiting, foraging) were tallied. We then computed a GLM, with the intercept set to the quiet waking period, to find for each cell, beta values associated with variations in task-related behavioral states and gamma power. The GLM fitted the number of spikes as follows:

\[ \text{NumSpike} = \text{NumSpike}_{\text{QW}} \exp(\beta_{\text{Foraging}} S_f + \beta_{\text{Waiting}} S_w + \beta_{\text{Baseline}} S_b + \beta_{\text{Gamma}} \text{ZscoreGamma}) \]

Where \( S_f = 1 \) during foraging state, otherwise \( S_f = 0 \); \( S_w = 1 \) during waiting state, otherwise \( S_w = 0 \); \( S_b = 1 \) during baseline state, otherwise \( S_b = 0 \).

Quantifying the influence of high-gamma on firing rates: To account for the variable duration of gamma cycles and the dependence of firing rates on gamma phase, we used the following approach. After detecting gamma bursts as described above, for each burst, the largest gamma cycle was identified and we counted the number of spikes generated during this cycle and \( \pm 55-80 \) ms from the center of the gamma burst. After dividing the number of spikes by duration of the windows, we then computed \( (\text{FR(gamma)}-\text{FR(no gamma)}) / \text{FR(no gamma)} \).

Quantifying the influence of gamma on the synchronization of unit activity: To quantify the impact of gamma on firing synchrony, we computed a synchronization index (SI) when gamma power was high or low. This was done for all available cell couples, using the LFP derived from the shank where the reference cell was located. After detecting high amplitude gamma bursts as described above, the highest gamma peak within each burst was located. In addition, for each gamma burst, we identified the lowest gamma peak within \( \pm 0.5 \) s of the highest peak. Then, we computed two CCGs, using the activity within \( \pm 18 \) ms of the highest and lowest gamma peaks, respectively. Last, in both CCGs, we measured the number of counts within \( \pm 3 \) ms of the CCG’s time zero (“on window”) as well as \( -6 \) to \(-3 \) ms and \( 3 \) to 6 ms from the CCG’s time zero (“off window”). For both the high- and low-amplitude gamma peaks, we computed a SI using the spike counts in the on and off windows as follows: \( (\text{on windows} - \text{off windows}) / (\text{on windows} + \text{off windows}) \). The rationale for taking into account spike counts in the off windows when computing the SI is to control for the influence of slow changes in firing rates on synchrony estimates. Importantly, the following changes in the definition of the on and off windows yielded qualitatively identical results:
we tested off windows of 47 to 50 and −47 to −50 ms as well as on windows of −1.5 to 1.5 ms and off windows of −3 to −1.5 and 1.5 to 3 ms.

In order to successfully calculate the SI of a cell pair, at least one pair of spikes in one of the windows had to be detected. To be included in the statistical analyses (signed-rank test), a cell couple had to satisfy this condition during both the high and low gamma periods. When carrying out this analysis for cell couples that included a principal cell and an interneuron, since principal cells generally fire ~2 ms before interneurons, the on and off windows were shifted by 2 ms. Finally, to test whether synchronization and entrainment by high gamma are related, for each cell couple, we computed the product of their resultant vectors. We then correlated these products to their SI during periods of high amplitude gamma (Spearman r).

**Comparing gamma-related unit synchrony as a function of behavior:** For each pair of cells separated by 200 μm, we calculated two SIs as above for the baseline and foraging periods, but regardless of gamma amplitude. We only considered cell pairs in which we could compute the two SIs (for the baseline and foraging periods) and used signed-rank tests to assess significance.

**Identifying putative monosynaptic connections:** The spikes of all simultaneously recorded cells were cross-correlated, separately considering couples that included a principal cell and an interneuron or two principal cells, and further stratifying the data as a function of the distance between the two cells (200, 400, or 600 μm). Each cross-correlogram (CCG) was computed with a bin size of 1 ms in windows of ±29.5 ms. To determine whether peaks or troughs in the CCGs were significant, we used the procedure described in Fujisawa et al. (2008).

First, for each couple, the spikes of the reference cell were jittered randomly within an interval of ±5 ms. Then, the jittered spike train from the reference cell and the original spike train from the target cell were cross-correlated. This procedure was repeated 1000 times to compute 1000 surrogate jittered CCGs. The minimum and maximum values (from −29.5 to 29.5 ms) of each of the surrogate CCGs were extracted to compute a distribution of 1000 global minimum and 1000 global maximum values. From these, the 99% of the maximum distribution was considered the global maximum band and the lower 1% of the minimum distribution was considered as the global minimum band. In order to be considered a significant excitatory monosynaptic connection, the peak of the actual CCG had to exceed the global maximum band. In addition, the peak of the actual CCG had to be located within 0.5 to 4.5 ms of the zero time. For inhibitory connections, the trough of the actual CCG had to be lower than the global minimum band for at least two consecutive bins. In addition, the trough of the actual CCG had to be located within 0.5 to 5.5 ms of the zero time. Finally, in order to be classified as a putative monosynaptic connection, the CCG at time 0 (~0.5 to 0.5 ms) should not exceed the global maximum band or be below the global minimum band. See Figure S3.

**Analyzing the frequency dependence of unit-unit coherence:** For a given pair of neurons, we extracted the autocorrelation of the reference cell (ACG1), the autocorrelation of the
target cell (ACG2) as well as their cross correlation (CCG). The coherence between these cells was derived using a Fast Fourier Transform (fft) as follows:

\[
\text{coherence spectrum of a given pair} = \text{fft}(\text{CCG})/\left((\text{fft}(\text{ACG1})\ast\text{fft}(\text{ACG2}))^{0.5}\right)
\]

For each type of cell couple, we then averaged absolute coherence values across all available pairs. To obtain CCGs corrected for the autocorrelation structure of each unit, we computed an inverse Fast Fourier Transform on the coherence spectrum.

DATA AND SOFTWARE AVAILABILITY

The full dataset will be made available upon request.

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**Deposited Data**

The full dataset and custom MATLAB code will be made available upon request.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References


HIGHLIGHTS

In BL, high-gamma is most pronounced when rats are vigilant.

High-gamma entrains BL firing more strongly than all other rhythms.

High-gamma causes minor changes in firing rates but large increases in synchrony.

The entrainment of principal cells varies depending on their projection site.
Fig. 1. Behavioral tasks and changes in theta, beta, and gamma power seen during the foraging task. (A,B) Behavioral tasks. (A1–2) Foraging task. (A1) Apparatus. (A2) Five example trials where red dots indicate rat position and the distance between the dots is proportional to the rat’s velocity. S, A, and F mark successful, aborted and failed trials, respectively. Empty black squares on the right indicate position of food pellets. Vertical dashed lines mark 25 cm intervals. (B) Shuttle task. (B1) Apparatus. (B2) Three example trials where red and blue dots indicate rat position and the distance between the dots is proportional to the rat’s velocity. (C,D) Spectrograms showing z-scored power fluctuations in various frequency bands as a function of relative time. All spectrograms shown here and in the following figures were normalized using the same data (see Methods) and thus can be directly
compared. Warmer colors indicate higher power. (C–D) Spectrograms (top) and average unit activity (bottom; normalized to baseline firing rates in C1) in various conditions (black, principal cells; red, interneurons; dashed and solid lines respectively represent cells with significantly decreased [Type-1] and increased [Type-2] firing rates during foraging (Kruskal-Wallis ANOVA followed by Dunn test, p<0.05). (C1) All trials with foraging (1238 trials). (C2) Subset of trials where rats, after hesitating at the door retreated in the nest (134 trials). Post-hoc Dunn tests on the data shown in C1 revealed that theta power was significantly (p<0.05) different in the four phases (baseline<waiting<foraging<escape). The same approach revealed that beta power was significantly different in the four phases (foraging<waiting<escape<baseline). For mid-gamma, power was higher during baseline relative to the other three phases with no difference between waiting, foraging, and escape. For low- and high-gamma, power differed significantly in the four phases (foraging>waiting=escape>baseline). (D) Subsets of trials that followed failed (D1) or successful (D2) trials. Kruskal-Wallis ANOVAs; df=5; low-gamma $\chi^2=430.8$; p<0.001; high-gamma $\chi^2=552.2$; p<0.001. Post-hoc Dunn tests revealed the following significant (p<0.05) differences. For low-gamma, (foraging[n-1,fail]=foraging[n-1,success]) > (baseline[n-1,fail]=waiting[n-1,fail]) > waiting[n-1,success] > baseline[n-1,success]. For high-gamma, (foraging[n-1,fail]=waiting[n-1,fail]= baseline[n-1,fail]) > foraging[n-1,success] > waiting[n-1,success] > baseline[n-1,success]. Number of units in the various panels: (C1, D1, D2: principal cells, 271 Type-1 and 25 Type-2; interneurons, 11 Type-1 and 35 Type-2; C2 principal cells, 226 Type-1 and 22 Type-2; interneurons, 8 Type-1 and 29 Type-2). Average baseline firing rates: Principal cells Type-1 (0.21 ± 0.03 Hz) and Type-2 (1.55±0.33 Hz); Interneurons Type-1 (27.47±3.45 Hz) and Type-2 (22.44±2.19 Hz). See also Figure S1 as well as Tables S1 and S2.
Fig. 2.
Relation between running speed and gamma power. (A,B) Spectrograms showing z-scored power fluctuations in various frequency bands as a function of when the rats’ velocity during foraging was low (A1; 30% trials with lowest velocity; n=371), when it was high (A2; top 30% trials; n=371), or when rats hesitated during foraging, exhibiting one or more starts (B1) and pauses (B2). (C1) Relation between rat velocity and LFP power of different frequencies as estimated by a generalized linear model. Beta velocity values are color coded (scale on left) during foraging. (C2) Average beta velocity values (y-axis) computed from entire foraging period for different power in different frequencies. (C3) Bins of C1 with statistically significant positive (red) and negative (blue) beta values during foraging (relative time, x-axis). (C4) Proportion of bins with significant beta velocity values during entire foraging period. Repeated measures Friedman ANOVAs relating absolute beta values associated to the speed, predator, and prior trial variables for low and high gamma: $X^2_s=417$ and 582, df=2, p<0.001. Post-hoc Dunn tests for GLM results revealed the following significant differences (p<0.05): absolute beta speed > absolute beta prior trial > absolute beta robot for both low- and high-gamma. (D) Spectrogram showing z-scored power fluctuations in various frequency bands as a function of relative time in the shuttle task (277 trials).
Fig. 3.
Entrainment of BL firing by different LFP rhythms. (A–B) Peri-event histograms of neuronal discharges for a representative principal cell (A) and fast-spiking interneuron (B) around large amplitude (≥2 SD) oscillatory peaks in different frequency bands (1, high-gamma; 2, low-gamma; 3, beta; 4, theta). In all cases, the bin width was set to 0.1 of the period under consideration. (C–D) Entrainment of unit activity (y-axis; median of the entire sample) as a function of frequency (x-axis) for principal cells (C) and fast-spiking interneurons (D). (E) Proportion of principal cells significantly entrained by different rhythms (y-axis) as a function of distance between the recorded cell and reference LFP (x-axis). (F–G) Frequency distributions of entrainment by high-gamma among principal cells (F) and interneurons (G). (H–I) Frequency distribution of preferred firing phase in relation to high-gamma for principal cells (H) and interneurons (I). (J) Average firing phase in relation to high-gamma for principal cells (blue) and interneurons (red).
Fig. 4.
Impact of behavioral states on entrainment of unit activity by high-gamma. Average ± SEM firing phase (A) and entrainment (B) by high-gamma for principal cells (A1, B1) and interneurons (A2, B2) during different behavioral states (x-axis; QW, quiet waking; B, baseline; W, waiting; F, foraging). Principal cells (C) and interneurons (D) were sorted by their entrainment in quiet waking (C1, D1) and high-gamma bursts were stratified by amplitude (y-axes). Data obtained in the other states (C2–4, D2–4) was plotted without changing the ordering of the cells. Warmer colors indicate stronger entrainment. Note that the number of cells included in panels A (54 principal cells and 45 interneurons) vs. B–D differs because panel A does not include cells that were not significantly entrained whereas panels B–D do (65 principal cells and 50 interneurons). Non-significantly entrained cells were excluded from A since this panel reports on phase, which is meaningless when cells are not entrained. Regarding firing entrainment, a repeated measures Friedman ANOVA revealed minor but significant differences in entrainment magnitude as a function of state in interneurons ($X^2(3,199) = 10.49; p = 0.015$) but not in principal cells ($X^2(3,259) = 4.99; p = 0.17$). The maximal significant difference in entrainment between states was 4% in interneurons (Post-hoc Dunn tests; $p < 0.05$).
Fig. 5.
State-related variations in firing rates are much larger than those produced by high-gamma. Comparisons of GLM beta coefficients for gamma amplitude and foraging (A), waiting (B), and baseline (C). Red, 50 interneurons; Blue, 381 principal cells. The data was analyzed in 12 ms windows but longer windows (50 or 100 ms) yielded qualitatively identical results. On the top right of each panel, we provide the average absolute ratio of the beta coefficients associated with state and gamma.
Fig. 6.
Impact of high-gamma on firing rates. (A) Principal cells. (B) Interneurons. Examples of PEHs of neuronal discharges computed around high gamma peaks (≥ 2 SD) for principal cells (A1–4, A6) and interneurons (B1,3–6). Firing entrainment (y-axis) as a function of change in firing rate (% of baseline) produced by high-gamma for principal cells (A5) and interneurons (B2). Firing rate (FR) changes are expressed as (FR(γ) – FR(noγ)) / FR(noγ). Related to Figure S2.
Fig. 7. Impact of high-gamma on firing synchrony. Couples of principal cells (1), interneurons (2), or principal cells and interneurons (3). (A) Crosscorrelation of unit activity when high-gamma power was high (blue) or low (red). Average ± SEM of normalized CCGs for all available cell couples. Note that in contrast with couples of principal cells (A1) and interneurons (A2), mixed couples generally fired with a time lag between them, interneuronal discharges following principal cells’ by ~2 ms (A3). Thick lines, averages; thin lines, SEM. (B) Synchronization indices for individual cell couples where both neurons generated at least one spike in at least one of the analyzed time windows. The n’s indicate the number of couples included in each analysis but we only show in B1 and B3, we only show the 200 most active cell pairs for clarity. Because principal cells fire rarely, only ~40% of cell couples met this criterion. In contrast, it was met by all interneuron pairs as well as most mixed couples. In B1–3, the black horizontal lines in the blue rectangles indicate the medians. The black vertical lines indicate the range of values including 95% of the observations. The top and bottom edges of the blue rectangles indicate the 75th and 25th percentile, respectively. In A and B, the distance between recorded units was 200 μm. (C) Relation between synchronization index (y-axis; average ± SEM) and distance between units (x-axis) when high-gamma power was high (blue) or low (red). In B and C, significance levels are indicated, based on signed-rank tests. Provided we controlled for gamma level, we found no effect of states (QW, baseline, waiting, foraging) on SIs in pairs of interneurons (ANOVA F(3,1223)=0.87, p=0.45), and couples of principal cells and interneurons (ANOVA F(3,6057)=1.0, p=0.39). This analysis could not be performed in pairs of principal cells, due to their low firing rates. (D) Relation between R product and gamma-related firing synchrony. Cell couples were stratified by their R product (numbers on the right are percentiles; thick lines are averages; thin lines are SEM). Related to Figures S2.
Fig. 8.
Relation between connectivity and entrainment by high-gamma. (A) Connections from principal cells to interneurons separated by 200 μm. (A1) Red and blue are used to represent mono-synaptically connected vs. non-connected cell couples, respectively. The x- and y-axes represent the resultant vector of the two cells in each couple. The graphs on the left and at the bottom plot the probability of connections as a function of the resultant vector. To generate these graphs, we computed the probability of connections in bins containing 20% of the cells. The black lines indicate the borders of the bins. (A2) Average unit-unit coherence (±SEM, dotted lines) plotted as a function of frequency for connected (red) and unconnected (blue) couples of principal cells and interneurons. (B) Entrainment of principal cells by high-gamma varies depending on the cells’ projection site(s). B shows frequency distributions of the resultant vector for all presumed principal cells (n = 381; B1), all cells that were positively identified as projection cells by antidromic invasion (n = 56; B2), cells that were backfired from nAc (n = 23; B3), and cells that were backfired from the mPFC (n = 27, B4). Six neurons that were backfired from nAc and mPFC are not included in B3,4. Related to Figure S4, S5, S6, S7, and S8.