HIGH FREQUENCY OSCILLATIONS ARE PROMINENT IN THE EXTENDED AMYGDALA

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Previously, it was reported that various cortical and subcortical structures display high frequency local field potential (LFP) oscillations in the 110-160 Hz range (HFOs), distinct from sharp-wave ripples. In the present study, we characterize HFOs in the extended amygdala. Rats were implanted with tetrode bundles in the bed nucleus of the stria terminalis (BNST), central amygdala (CeA), as well as adjacent regions (pallidum, caudate-putamen, lateral septum). At all recorded sites, HFO power showed a systematic dependence on behavioral state: highest during quiet wakefulness, intermediate during paradoxical sleep, and lowest during active waking or slow-wave sleep. CO2 asphyxiation as well as anesthesia with isoflurane or urethane abolished HFOs. HFOs stood out relative to all other fast frequency LFP components because they were highly coherent between distant sites of the extended amygdala, ipsi- and contralaterally. HFOs affected neuronal firing in two ways: firing rate could vary as a function of HFO power (rate modulation) or HFOs could entrain firing on a cycle-to-cycle basis (phase modulation). The incidence of phase-modulated neurons was about twice higher in BNST and CeA (20-40%) than in adjacent regions (≤8%). Among BNST and CeA neurons, many more were phase-modulated than rate-modulated, although about half the latter were also phase-modulated. Overall, these results indicate that HFOs entrain the activity of a high proportion of neurons in the extended amygdala. A major challenge for future studies will be to identify the mechanisms supporting the high coherence of HFOs within and across hemispheres.

Keywords: bed nucleus of the stria terminalis; amygdala; fast oscillation; gamma.
Even in the absence of sensory stimulation, neuronal networks generate rhythmic population events, measurable in local field potentials (LFPs) as oscillations of various frequencies (Buzsaki, 2006). Rhythms of different frequencies predominate depending on the brain region and these oscillations vary markedly as a function of the subject’s behavioral state. Understanding these oscillations is important because neuronal events underlying cognitive activity are embedded in these endogenous population rhythms.

Recently, a novel form of oscillatory activity (110-160 Hz) termed high frequency oscillations (HFOs) was observed in the neocortex, hippocampus (reviewed in Tort et al. 2013), and many subcortical structures (reviewed in Olszewski et al. 2013). While the HFO frequency band overlaps with that of sharp wave ripples (Buzsaki et al. 1992; Ylinen et al. 1995), the two oscillations are undoubtedly distinct because they predominate in different behavioral states. Indeed, HFOs were initially identified in studies of phase-amplitude coupling between oscillations of different frequencies, revealing a modulation of HFO amplitude as a function of theta phase (Tort et al. 2008). Subsequently, HFOs were demonstrated using power density analysis and observed in unfiltered LFPs (Scheffzuk et al. 2011; Scheffer-Teixeira et al. 2012), with a phase reversal between superficial and deep hippocampal layers (Tort et al. 2008).

Although little is known about the cellular mechanisms generating HFOs, the available evidence suggests that unlike gamma oscillations, HFOs are not dependent on rhythmic interactions between glutamatergic and GABAergic neurons (Fisahn et al. 1998; Whittington et al. 2000). Indeed, hippocampal HFOs resist blockade of AMPA/kainate receptors, but are abolished by GABA-A receptor antagonists (Jackson et al. 2011). Moreover, in many subcortical structures (basal ganglia, septum, and thalamus), HFO amplitude increases markedly following systemic administration of NMDA receptor antagonists (Hunt et al. 2011; Hunt and Kasicki 2011).
In the case of nucleus accumbens at least, HFOs are generated locally and/or in a nearby structure because they disappear or are largely reduced following local infusions of tetrodotoxin or muscimol (Olszewski et al. 2013). In contrast, TTX infusions in the frontal or parietal cortices as well as in the caudate nucleus had no effect on the power of HFOs recorded locally at these sites (Olszewski et al. 2013), suggesting that they are volume conducted from a distant source. Although studying the relation between unit activity and HFOs might yield useful insights into the underlying network interactions, this question has received little attention so far. In part, this results from the difficulty of distinguishing genuine entrainment of unit firing by HFOs from spurious correlations resulting from spectral leakage of spike waveforms into LFPs (Scheffer-Teixeira et al. 2013). In the present study, we circumvent this confound by taking advantage of the high spatial coherence of HFOs. Because HFOs depend on interactions between GABAergic neurons (Jackson et al. 2011), we focused on structures of the extended amygdala, particularly the bed nucleus of the stria terminalis (BNST) and central amygdala, where GABAergic neurons prevail (Poulin et al. 2009; Swanson and Petrovich 1998).
MATERIALS AND METHODS

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). Our subjects were male Lewis rats (310-360 g, Charles River Laboratories, Wilmington, MA) maintained on a 12 h light/dark cycle. They were housed individually with ad libitum access to food and water. Prior to the experiments, they were habituated to the animal facility and handling for one week.

Surgery

Eleven rats were anesthetized with a mixture of isoflurane and O₂, and administered atropine sulfate (0.05 mg/kg, i.m.) to aid breathing. In aseptic conditions, rats were mounted in a stereotaxic apparatus with nonpuncture ear bars. A local anaesthetic (bupivacaine, sc) was injected in the region of the scalp to be incised. Fifteen minutes later, the scalp was incised and a craniotomy was performed above the regions of interest. Then, movable bundles of four tetrodes (nichrome microwires, 20-μm inner diameter, impedance 100-300kΩ following gold plating) were stereotaxically aimed to the BNST and, in a subset of rats, CeA and immediately adjacent components of the extended amygdala (Heimer et al. 2008). Tetrode tip positions within a bundle were staggered to facilitate histological reconstructions. The reference electrode was a stainless steel screw anchored over the cerebellum. In two rats, we also compared HFOs referenced to the cerebellum vs. the bone overlying the olfactory bulbs. However, the same results were obtained with the two references in terms of HFO power, coherence, and unit entrainment.
Data acquisition and analysis

Rats were allowed one week to recover from the surgery and then acclimated to handling for two additional days. In each rat, spontaneous neuronal activity was then recorded during prolonged (≥3 hours) daily recording sessions so that sufficient data could be obtained during different states of vigilance. Behavior was recorded by an overhead video-camera. During the recording sessions, rats were placed in a dimly lit (20 lux) standard plastic rat cage with bedding at the bottom. Electrodes were not moved during the experiments unless all units were lost overnight across all tetrodes within a bundle. In such rare cases (<5%), the tetrode bundle was lowered 60 µm and recordings resumed the following day.

Different behavioral states of vigilance were identified using a combination of spectral analyses of BNST LFPs and behavioral observations. Long epochs of spontaneous LFP activity were segmented in two-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). This broadband increase at high frequencies likely reflects electromyographic activity. After eliminating active waking, we could easily distinguish slow-wave sleep from quiet waking and REM sleep because total power at frequencies <20 Hz was distributed bimodally among the three states: epochs of high power at low frequencies corresponded to periods of slow-wave sleep. We then defined quiet waking as movement-free periods that followed active waking whereas REM sleep was defined as epochs that followed slow-wave sleep and were characterized by a high ratio of theta (6-10 Hz) to delta (1-5 Hz) power. These inferences were repeatedly confirmed by correlating LFP-based state scoring with off-line analyses of the rats’ behavior, recorded on video. Invariably, as the broadband 200-240 Hz activity diminished and
the rats transitioned from active to quiet waking, there was a sharp and sustained drop in movement. In contrast, analysis of behavior during electrophysiologically-identified REM sleep epochs revealed punctual movements (muscle twitches).

The signals were sampled at 40-kHz and stored on a hard drive. For spike extraction, the data was first high-passed filtered using a median-based filter and then thresholded to extract spikes. Next, we 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. Units with unstable spike shapes during a given recording session were excluded from the analyses.

For LFP analyses, we first down-sampled the data to 1250 Hz with sinc interpolation. Analyses were performed in Matlab. Spectral and phase analyses were done using Chronux (http://chronux.org/) and the Circular Statistics Toolbox for Matlab (Berens 2009), respectively. For spectral LFP analyses, the magnitude of each frequency in a given behavioral state was expressed as the deviation from the power law fit to the power spectrum of the data obtained during the entire recording period. When examining time-dependent changes in the spectral composition of LFPs, we used non-overlapping windows of 2 sec and multitaper spectral estimates with 5 tapers and a time-bandwidth product of three. Bandpassed LFP signals were obtained using forward and reverse filtering with a fifth order Butterworth filter over the frequency range indicated, and LFP phase and envelope were obtained using the Hilbert transform of the bandpassed signal.

For phase analyses of unit firing, the reference LFP was obtained using a microwire from a different tetrode than the one used to record the unit of interest and performed a Rayleigh test.
with a significance threshold of \( p < 0.01 \). The lack of spike waveform in the reference LFP excludes the possibility that spectral leakage of the spike waveform is responsible for significant unit-HFO relationships. To assess whether high amplitude HFO spindles were associated with significant firing rate modulations, we first computed spike counts in windows of \( \pm 1 \) s centered on HFO spindles of high amplitude (\( \geq 2 \) SD of averaged peak values). To assess significance, we performed a Kilmogorov-Smirnov test comparing the actual distribution of spike counts to a uniform distribution, for each cell independently. The significance threshold we used was \( p \leq 0.01 \). In addition to performing the K-S test, we excluded spurious positive results due to baseline modulations in firing rate using a binomial test comparing spike counts between the two 500 - 1000ms intervals flanking HFOs (with the null hypothesis that total spike counts in these two intervals are equal). For unit analyses, we ignored neurons that fired less than 200 spikes in the epoch under consideration.

**Histology**

At the end of the experiments, the animals were deeply anesthetized and recording sites marked with small electrolytic lesions (20 \( \mu A \) between a tetrode channel and the animals’ tail for 15 sec). One day later, the rats were perfused-fixed through the heart, their brains extracted, cut on a vibrating microtome and the sections counterstained with cresyl violet, as shown in figure 1.
RESULTS

All rats included in this study (n=11) had tetrode bundles implanted in the anterior part of BNST, many bilaterally (n=6). BNST recording sites were subdivided in lateral (BNST-AL) and medial (BNST-AM) sites depending on whether the electrodes were positioned laterally or medially to the intra-BNST component of the stria terminalis. In a subset of rats (n = 4), we also aimed tetrode bundles to CeA. In some cases, tetrode bundles did not reach their intended targets because of tetrode bending. As a result, we also obtained data about HFOs in a number of additional structures including the lateral septum (5 rats), striatum (4 rats), and globus pallidus (2 rats). Figure 1 shows examples of histologically identified recording sites.

Relation between HFO amplitudes and behavioral states of vigilance

To study the dependence of HFO amplitudes on the states of vigilance, we conducted LFP power analyses (Fig. 2A1) on prolonged (≥ 3 hours) epochs of spontaneous activity (Fig. 2A2) during which the rats cycled through different behavioral states multiple times. The magnitude of each frequency in a given 2 sec window (non-overlapping) was expressed as the deviation from the power law fit to the power spectrum of the data obtained during the entire recording period (Fig. 2A1; black, averaged data; red, fit). At all recording sites, we observed consistent variations in HFO amplitudes depending on the rats’ behavioral state.

Figure 2A2 and C illustrates this point using LFPs recorded in BNST-AM. Although there were marked moment-to-moment variations in HFO amplitudes, they were generally higher during quiet waking (green) and REM sleep (red). In contrast, HFO amplitudes were generally lower during SWS (blue) and active waking (yellow). This is also shown in figure 2B where we plot the average (line) ± SEM (shading) of the power spectra of 32 BNST LFPs recorded in 7 rats.
in active (yellow) and quiet (green) waking as well as in SWS (blue) and REM sleep (red). Note that for this analysis, HFO amplitude was defined as the difference between the power spectrum peak and the linear fit to flanking frequencies.

An ANOVA on HFO amplitudes revealed a main effect of behavioral state (F=149, p<0.0001). Bonferroni post-hoc t tests (p<0.01) revealed that the ranking of states by HFO power was quiet waking > REM > active waking ≈ SWS, with no significant difference between the latter two states. However, it should be stressed that active waking and SWS were not devoid of HFOs. Although HFO amplitudes were generally lower in these two states, high amplitude HFO spindles also occurred (Fig. 2C).

In a few rats, we also examined the impact of isoflurane (0.2% inhaled; n=3) or urethane (1.8 g/kg, ip; n=2) anesthesia on HFO amplitudes. In addition, to examine the possibility that HFOs constitute an electronic artifact independent of neuronal activity, we also recorded LFPs after CO₂ asphyxiation (n=2). As shown in figure 2B (black lines), no HFO peak could be detected under anesthesia or after death.

Importantly, not all recording sites displayed HFOs. For instance, figure 2D illustrates examples of simultaneously recorded LFPs in the endopiriform nucleus and basolateral amygdala (BLA) as well as in BNST and CeA, all in the same hemisphere. HFOs were nearly absent from the endopiriform nucleus (Fig. 2D1) and of consistently lower amplitude in the BLA relative to those seen in BNST and CeA (Fig. 2D1). Moreover, whereas moment-to-moment variations HFO amplitudes in BNST and CeA generally coincided (see below), those seen in BLA showed much more independence (red asterisks mark BLA HFO spindles with no counterparts in BNST and CeA). The lower power of HFOs in the endopiriform nucleus and BLA relative to BNST and
CeA is also documented in the power spectra depicted in figure 2D2 (t-tests, p<0.0001). Together, these results indicate that HFOs are not electronic artifacts of our recording equipment.

**HFO coherence within and across hemispheres**

Previously, it was observed that LFP coherence decreases with distance and that this effect is more pronounced for high frequency components (Collins et al. 1999, 2001; Steriade et al. 1993, 1996). Although it was reported that the coherence of fast rhythms between distant cortical sites could be high during some stimulation paradigms (Desmedt and Tomberg 1994; Gray et al. 1989), this is generally not the case in spontaneous conditions (Bullock and McClune 1989; Murthy and Fetz 1992; Steriade et al. 1996).

In contrast, spontaneous HFOs were highly coherent in the extended amygdala, within and across hemispheres. Indeed, in all rats, superimposition of LFPs recorded in the same or different hemispheres during waking or sleep states revealed that HFOs occurred nearly synchronously. For instance, figure 3 shows band-passed filtered LFPs (black, 125-150Hz; red, 225-250 Hz) recorded in different sub-regions of the right BNST and CeA, as well as in the left BNST during quiet waking (Fig. 3A) and REM sleep (Fig. 3B). The twenty HFO spindles with the highest amplitude seen in the right BNST-AM (panels 1; blue circles) are shown with an expanded time-base in panels 3, superimposed on LFPs from the other ipsi- and contralateral recording sites. Note the remarkable cycle-to-cycle synchrony of high amplitude HFOs within and across hemispheres for quiet waking (Fig. 3A) and REM sleep (Fig. 3B). Similar results were obtained in active waking and SWS.

To further characterize this phenomenon, we compared the coherence of HFOs to that of other high frequency LFP components. In particular, in all cases of tetrodes in the CeA of one
hemisphere and BNST bilaterally, we compared coherence between all available pairs of recording sites located in different parts of BNST ipsilaterally (24 pairs of recording sites in 6 rats), BNST bilaterally (36 pairs of recording sites in 6 rats), or between BNST and CeA ipsilaterally (16 pairs of recording sites in 4 rats) (Fig. 4). Because movement artifacts occurring during active waking yielded artificially high coherence values in all frequencies (yellow period in figure 4), we focused on behavioral states associated with immobility: quiet waking, SWS, and REM sleep.

An ANOVA on HFO coherence values revealed a significant dependence on recording configuration in the two behavioral states associated with high amplitude HFOs: quiet waking (Fig. 5A) and REM sleep (Fig. 5B; F's ≥ 37.8, p's < 0.0001). Bonferroni-corrected post-hoc t-tests revealed that ipsilateral intra-BNST HFO coherence was the highest (p<0.0001), as expected given the short distance between these recording sites (0.4-0.6 mm). Although distance between bilateral BNST recording sites was shorter (2-3 mm) than between ipsilateral BNST-CeA tetrode pairs (3.5-4.5 mm), HFO coherence across hemispheres was significantly lower (p < 0.0001). Of particular interest, for all recording configurations except cases where electrodes were within 0.6 mm of each other in BNST ipsilaterally, the coherence of HFOs was significantly higher than that of high (75-100 Hz) and low gamma (30-55 Hz, p’s < 0.0001) as well as for the 100-125 Hz and 150-175 Hz frequency bands (p’s < 0.0001). Overall, these results indicate that HFOs stand out, relative to all other fast LFP frequency components, for their remarkably high coherence.
Entrainment of unit firing by HFOs

A possible explanation for the high HFO coherence is volume conduction. Indeed, one could conceive of a situation where a densely interconnected group of neurons would generate open fields that are instantaneously conducted over large distances. This phenomenon would generate spurious LFP correlations, with no actual HFO-related firing or synaptic activity. However, if this were the case, one would not expect to find entrainment of unit activity by HFOs at many of the sites displaying them.

One problem when testing this prediction however is spectral leakage of spike waveforms into LFPs (Scheffer-Teixeira et al. 2013). Indeed, spike shapes can leak into several LFP bands, causing spurious unit-field correlations. To circumvent this difficulty, for all available units, we used spike-less reference LFPs (Fig. 6A, red; down-sampled to 1250 Hz) from a different tetrode than the one used to record the unit of interest (Fig. 6A, black; and performed Rayleigh tests (significance threshold of p<0.01). The lack of spike waveforms in the reference LFP excludes the possibility that spectral leakage of the spike shape into the LFP is responsible for significant unit-HFO relationships.

This analysis revealed that the incidence of units with significant HFO entrainment varied markedly depending on the recording sites (Fig. 6B1). Indeed, as many as 18-39% of BNST and CeA cells exhibited significant firing phase modulation by HFOs (Fig. 6C,D), indicating that HFOs are not volume conducted to the extended amygdala. In contrast, in the lateral septum, globus pallidus, and caudate-putamen, a significantly lower proportion of cells were entrained by HFOs (≤8%, chi-square test, p=0.016, units in BNST and CeA vs. all other sites). The higher incidence of phase modulated neurons in BNST and CeA relative to the other three recording sites was observed in all behavioral states (see colored symbols in Fig. 6B1). Although there
were cell-to-cell variations in the preferred HFO firing phase of BNST and CeA neurons (compare Fig. 6C-E), most discharged preferentially during the negative phase of HFOs, irrespective of the behavioral state (Fig. 7).

It should be noted that a significant entrainment of spiking by HFOs does not imply that units fired at the HFO frequency, even transiently. In fact, the majority of BNST and CeA cells with significant phase modulation had low firing rates, with 60, 68, and 79% of them firing below 5 Hz in quiet waking, REM sleep, and slow-wave sleep, respectively. However, the occasional spikes they generated tended to cluster at a consistent phase of the HFOs.

As mentioned above, relatively few neurons in the lateral septum, globus pallidus, and caudate-putamen showed a significant firing phase modulation by HFOs. Yet, a proportion displayed a significant firing rate modulation as a function of HFO power (Fig. 6B2). To assess this, we computed spike counts in windows of ± 1 s centered on high amplitude HFO spindles (≥2 SD of averaged peak values). Then, to assess significance, we performed a Kolmogorov-Smirnov test comparing the actual distribution of spike counts to a uniform distribution, for each cell independently (significance threshold of p ≤ 0.01). In addition, we excluded spurious positive results due to baseline modulations in firing rate using a binomial test comparing spike counts between the two 500 – 1000 ms intervals flanking HFOs.

Using this approach, we observed that at all recording sites and in most behavioral states, a small proportion of neurons showed significant firing rate modulations in relation to HFOs (Fig. 6B2). As shown in the representative examples of figure 6F, the vast majority of these cells (≈ 90%) showed increases in firing rates in relation to HFOs with the remaining 10% showing either inhibitory or biphasic (excitation followed by inhibition) modulations. Importantly, the incidence of phase-modulated cells among BNST and CeA neurons was much
higher (≥18%) than that of rate-modulated cells (≤7%; compare Fig. 6B1 and B2; note different y-axes) and only about half the latter were also phase-modulated (range 43-60%; Fig. 6B3). The highest incidence of rate-modulated cells was observed among striatal units in REM sleep and slow-wave sleep (Fig. 6B2), even though very few of these cells showed a significant phase modulation by HFOs (Fig. 6B1).

**DISCUSSION**

The present study was undertaken to characterize HFOs in structures of the extended amygdala, particularly, BNST, CeA, and immediately adjacent structures. We focused on the extended amygdala because work in other brain regions indicated that HFOs arise from interactions between GABAergic neurons (Jackson et al. 2011), the main cell type found in BNST and CeA (Poulin et al. 2009; Swanson and Petrovich 1998). Our experiments revealed that HFOs are prominent in the extended amygdala, they show systematic variations in amplitude as a function of behavioral states, they are coherent within and across hemispheres, and they entrain unit activity in a high proportion of neurons. The significance of these findings is considered below.

**HFOs vary in a state-dependent manner and are highly coherent within and across hemispheres**

In addition to the entrainment of extended amygdala neurons by HFOs (see below), several observations suggest that HFOs are not artifacts of our recording or filtering methods. First, periods of high-amplitude HFOs could be observed in the raw LFPs throughout the sleep-waking cycle. Second, HFOs were abolished by common anesthetics (isoflurane and urethane).
and could not be detected after CO₂ asphyxiation. Third, HFO amplitudes varied as a function of
the animals’ behavioral states. Indeed, they were significantly more pronounced during quiet
wakefulness and REM sleep than during active waking or SWS.

An unusual property of HFOs relative to all other fast frequency LFP components was
their high coherence. This is remarkable because LFP coherence generally decreases with
distance and this reduction is particularly steep for high frequency components (Collins et al.
1999, 2001; Steriade et al. 1993, 1996). In contrast, HFO coherence was high within and between
ipsilateral structures of the extended amygdala whereas that of other high frequency components
was generally lower. Interestingly, for contralateral recording sites, HFO coherence although
still very high, was significantly lower than between ipsilateral but more distant recording sites in
the extended amygdala, again arguing against volume conduction.

HFOs are generated locally within the extended amygdala

Previously, HFOs were observed in a variety of cortical and subcortical structures (de
Olszewski et al. 2013; Tort et al. 2013). In some cases, such as the hippocampus (Tort et al.
2013) and nucleus accumbens (Olszewski et al. 2013), HFOs appear to be generated locally.
However in others, such as the caudate nucleus as well as the frontal and parietal cortices, local
tetrodotoxin or muscimol infusions did not affect HFO power (Olszewski et al. 2013), suggesting
that they are volume conducted from a distant source.

Consistent with this, we found that the incidence of neurons whose firing were modulated
by the phase of HFOs varied markedly depending on the recording sites. In all behavioral states,
it was highest in the extended amygdala (BNST ≥ 18%; CeA ≥ 25%) and lowest in the lateral
septum, globus pallidus, and caudate-putamen (≤ 8%). These findings support the view that the
HFOs seen in the extended amygdala are not volume conducted from a distant source, but are generated locally. The scarcity of significantly entrained units in the globus pallidus and caudate-putamen is consistent with earlier findings suggesting that the HFOs seen in the caudate nucleus are not generated locally (Olszewski et al. 2013).

**Origin of HFOs**

It was reported that hippocampal HFOs resist blockade of AMPA/kainate receptors, but are abolished by GABA-A receptor antagonists (Jackson et al. 2011). Therefore, these results suggest that HFOs are not dependent on rhythmic interactions between glutamatergic and GABAergic neurons, in contrast with gamma oscillations (Fisahn et al. 1998; Whittington et al. 2000). Given their high frequency and independence from fast glutamatergic transmission, the high HFO coherence between distant recording sites raises a major explanatory challenge: what mechanisms could support the long-range synchronization of HFOs? Unfortunately, the study of HFOs is just beginning and we are reduced to conjectures.

One possibility, put forward by Traub and colleagues (Traub et al. 2011), is that HFOs arise from electrical coupling between axons. Although computational modeling supports this possibility (for instance see Maex and De Schutter 2007; Traub et al. 2008), there is little if any electron microscopic evidence of gap junctions between axons in the hippocampus, amygdala, or BNST. A related possibility is that ephaptic coupling among unmyelinated axons is sufficient to synchronize the firing of many closely spaced axons. Ephaptic interactions are maximized when the extracellular conductance is low (Barr and Plonsey 1992), a condition met in bundles of tightly packed unmyelinated bundles of axons such as found in the olfactory nerve, stria
terminalis, and anterior commissure (Lamantia and Rakic 1990; Blinder et al. 2003). A computational modeling study of the olfactory nerve provided support for this possibility (Bokil et al. 2001). Furthermore, actual demonstration of such ephaptic interactions was obtained in crab by Katz and Schmitt (1940, 1942). Of particular relevance to HFOs is the fact that ephaptic interactions can minimize differences in conduction velocity between neighboring axons with slightly different condition speeds (reviewed in Debanne et al. 2011). By synchronizing activity in bundles of unmyelinated axons, this effect might contribute to the high coherence of HFOs. However, this hypothetical model awaits experimental scrutiny.

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The authors declare that they have no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS:
Darrell Haufler carried out the electrophysiological experiments, analyzed most of the data, and contributed to the design of the experiments.
Denis Pare analyzed some of the data, wrote the manuscript and contributed to the design of the experiments.
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FIGURE LEGENDS

**Figure 1.** Histological identification of recording sites. (A-D) Coronal sections counterstained with cresyl violet. Recording sites are marked with small electrolytic lesions (arrows). (A) Electrolytic lesions in the lateral septal nucleus (LSN) and BNST-AM (AM). (B) Two recording sites in BNST-AL (AL). (C) Recording sites in the piriform (Pir) cortex, basolateral complex of the amygdala (BLA), central amygdala (CeA), and sublenticular extended amygdala (SLEA). Too few BLA and Pir recordings were obtained for meaningful analyses. Data obtained in CeA and SLEA were pooled since they are thought to constitute an anatomical entity. (D) Two recording sites in the left and right BNST-AM (AM). Other abbreviations: AC, anterior commissure; DBB, diagonal band of Broca; DG, dentate gyrus; Hab, habenula; OT, optic tract; Str, striatum; Thal, thalamus.

**Figure 2.** State-dependent changes in the spectral composition of LFPs recorded in BNST. (A1) Mean spectral power across frequencies for the entire session (black) is fit by a power law (red). (A2) Deviations from power law fit (gray scale) for different frequencies (y-axis) as a function of time (x-axis). The behavioral state of the rat is color coded (see legend at the top of the figure panel). (B) Average ± SEM power spectra in different behavioral states. A dashed red rectangle marks the HFO frequency band. For active waking, quiet waking, slow-wave sleep, and REM, the data shown is the average ± SEM of the power spectra of 32 BNST LFPs recorded in 7 rats. For death and anesthesia, we show the average of nine LFP recordings obtained from three rats. (C) Examples of LFPs recorded in BNST in different behavioral states (band-pass filtered 125-150 Hz). (D1) Simultaneously recorded LFPs in BNST, CeA, BLA, and endopyriform nucleus during quiet waking. LFPs were band-passed filtered at the HFO frequency (125-150 Hz).
near complete lack of HFOs in endopiriform nucleus, and generally lower HFO amplitude in BLA relative to CeA and BNST. Red asterisks mark BLA HFO spindles that had no counterparts in CeA or BNST. (D2) Power spectra of the LFPs shown in D1. Dashed red rectangle marks the HFO frequency band and immediately adjacent regions. To compute these power spectra, a long epoch of quiet waking was segmented in 2 s windows and the data averaged across windows. Shading represents SEM.

**Figure 3.** Synchrony of HFOs in BNST and the amygdala during different behavioral states. (A) Quiet Waking. (B) REM sleep. (1) Band-pass filtered LFPs from BNST and the central amygdala (red traces, 225-250 Hz; black traces, 125-150 Hz) shown with a slow time base. Blue circles mark the 20 largest HFO envelope peaks, which are depicted in panels 3 with an expanded time base. Note that high amplitude HFO spindles (black) were not associated with parallel increases in the power of the 225-250 Hz band (red). Thus, HFOs do not reflect a non-specific increase in the power of all high frequency components. To facilitate within-state comparisons, all signals are normalized by their standard deviation. (2) Raw LFPs with a fast time base. They correspond to the HFO epoch with the highest peak in panel 1. (3) Twenty HFO envelopes marked by blue circles in panels 1, rank-ordered by amplitude (highest to lowest from top to bottom). The signals in each row are normalized by the maximal value found across the four traces.

**Figure 4.** HFOs are highly coherent ipsilaterally but less so across hemispheres. (A) State-dependent changes in spectral composition of LFPs recorded in BNST-AM. (B) Time and frequency-dependent changes in coherence between LFPs recorded in BNST-AL and AM
ipsilaterally (B1), BNST-AM in both hemispheres (B2) as well as in BNST-AM and CEA (B3).

**Figure 5.** LFP coherence as a function of frequency. (A) Quiet waking (QW). (B) REM sleep. Group analyses of LFP coherence for three recording configurations depicted with different colors. **Green:** when the two electrodes were positioned in BNST of the same hemisphere. **Red:** when the two electrodes were positioned in BNST-AM of different hemispheres. **Black:** with one electrode in BNST-AM and the other in CeA ipsilaterally. Colored shading represents SEM.

**Figure 6.** (A) Approach used to study entrainment of unit activity by HFOs. The band-passed (125-150 Hz) LFP recorded by a BNST reference channel (red) devoid of unit activity was used to assess the timing of unit activity in a different channel (CeA, black). From top to bottom: CeA unit activity (high-pass filtered), BNST reference signal band-pass filtered (125-150 Hz, middle) and same signal high-pass filtered (bottom), revealing lack of unit activity. Left, slow time base; right, expanded time base. Note that the gain on reference HFOs was doubled from left to right panel of A to facilitate inspection of HFO-spike phase relationships. (B) Incidence of units with significant phase- and rate-modulation by HFOs in different brain structures and behavioral states. (B1) Phase modulation. (B2) Rate modulation. (B3) Venn diagrams showing overlap between cells (BNST and CeA combined) with significant phase (dashed lines) and/or rate (continuous lines) modulation by HFOs in quiet waking (QW, left), slow-wave sleep (SWS, center) and REM sleep (right). Numbers adjacent to areas of overlap indicate proportion of rate-modulated units that are also phase-modulated. (C-E) Examples of units that were significantly phase-modulated by HFOs. (C1, D1, E1) Peri-HFO histogram of neuronal discharges. To compute these histograms, HFO spindles of high amplitude (top half of the overall distribution)
were detected and centered on the positive cycle with the highest amplitude. Unit activity was then referenced to the corresponding HFO spindle. (C2, D2, E2) Rose plots of the cells shown in B1, C1, and D1, respectively. Unit in B was recorded in CeA during QW. Unit in C was recorded in BNST in REM sleep. Unit in D was recorded in BNST during QW. (F) Examples of cells recorded in BNST (F1) or CeA (F2) with significant firing rate modulation by HFOs. To construct these histograms, we computed spike counts (left y-axis; 20 ms bins) in windows (± 1 s, x-axis) centered on high amplitude HFO spindles (≥ 2 SD of averaged peak values). Right y-axis represents HFO power. To assess entrainment, we referenced unit activity to HFOs from a different tetrode than the one used to record the unit and performed a Rayleigh test with a significance threshold of p< 0.01. To assess dependence of firing rate on HFO power, we computed spike counts in windows of ± 1 s centered on high amplitude HFO spindles (≥ 2 SD of averaged peak values). Then, to assess significance, we performed a Kilmogorov-Smirnov test comparing the actual distribution of spike counts to a uniform distribution, for each cell independently (significance threshold of p ≤ 0.01). In addition, we excluded spurious positive results due to baseline modulations in firing rate using a binomial test comparing spike counts between the two 500 - 1000ms intervals flanking HFOs.

**Figure 7.** Preferred firing phase of significantly modulated BNST and CeA units. Colored symbols indicate behavioral state of epoch analyzed (red, REM; blue, SWS; green, quiet waking). Y-axis indicates depth of modulation. Data is replicated over two cycles for clarity.
A  Spectral Composition of BNST-AM LFP

B1  Coherence BNST-AM vs. BNST-AL (ipsi)

B2  Coherence BNST-AM vs. AM (contra)

B3  Coherence BNST-AM vs. CEA (ipsi)
(A) QW

(B) REM

Coherence vs Frequency (Hz)

- BNST-BNST (ipsi)
- BNST-BNST (Contra)
- BNST-CeA (ipsi)

HFOs