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NMDA receptor antagonist-enhanced high frequency oscillations: Are they generated broadly or regionally specific?

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Abstract
Systemic administration of NMDA receptor antagonists, used to model schizophrenia, increase the power of high-frequency oscillations (130–180 Hz, HFO) in a variety of neuroanatomical and functionally distinct brain regions. However, it is unclear whether HFO are independently and locally generated or instead spread from a distant source. To address this issue, we used local infusion of tetrodotoxin (TTX) to distinct brain areas to determine how accurately HFO recorded after injection of NMDAR antagonists reflect the activity actually generated at the electrode tip. Changes in power were evaluated in local field potentials (LFPs) recorded from the nucleus accumbens (NAc), prefrontal cortex and caudate and in electrocorticograms (ECoGs) from visual and frontal areas. HFO recorded in frontal and visual cortices (ECoGs) or in the prefrontal cortex, caudate (LFPs) co-varied in power and frequency with observed changes in the NAc. TTX infusion to the NAc immediately and profoundly reduced the power of accumbal HFO which correlated with changes in HFO recorded in distant cortical sites. In contrast, TTX infusion to the prefrontal cortex did not change HFO power recorded locally, although gamma power was reduced. A very similar result was found after TTX infusion to the caudate. These findings raise the possibility that the NAc is an important neural generator. Our data also support existing studies challenging the idea that high frequencies recorded in LFPs are necessarily generated at the recording site.

1. Introduction

There is good evidence from clinical, preclinical and theoretical studies that the NMDAR complex is involved in the pathophysiology of several psychiatric diseases, such as schizophrenia, bipolar depression and drug addiction (Paul and Skolnick, 2003; Krystal et al., 2003). NMDAR antagonists have long been used to model some of the psychiatric symptoms of schizophrenia (Abi-Saab et al., 1998) and over the last decade this class of compounds has been found clinically useful in the treatment of bipolar depression (Mathew et al., 2012). Therefore, understanding how

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NMDAR antagonists influence brain activity may shed important light on the possible mechanisms underlying these diseases.

Local field potential (LFP) oscillations, part of the extracellular potential encompassing frequencies below 300 Hz, are receiving increased attention for the role they may play in health and disease. Previously, we showed in freely moving rats that subanesthetic doses of ketamine produce rapid (within tens of seconds) and substantial increases in the power of high-frequency oscillations (130–180 Hz, HFO) (Hunt et al., 2006). Spontaneous HFO occur in the nucleus accumben NAc during waking and rapid-eye movement sleep, but are reduced during slow-wave sleep and attenuated by anesthesia (Hunt et al., 2009). Notably, large increases in power also occur during emergence from ketamine anesthesia (Hunt et al., 2006) which may be related to emergence reactions known to occur in humans. We have also found HFO in the NAc can be modulated by antipsychotic compounds (Olszewski et al., 2012). NMDAR antagonists have also been reported to increase the power of HFO in many other structurally and functionally distinct brain regions, including the caudate nucleus, motor cortex, visual cortex, hippocampus, medial septum, substantia nigra pars reticulata, subthalamic nucleus and posterior thalamus (Nicolas et al., 2011; Phillips et al., 2012; Hunt et al., 2011). Small but significant increases in the power of HFO in electroencephalograms have been recorded in the methylazoxymethanol developmental rat model, and these animals also display an exaggerated response post NMDAR antagonist injection (Phillips et al., 2012). Together, these findings indicate the neural networks mediating the generation of HFO may have relevance to the pathophysiology of schizophrenia.

A number of studies, mostly based on recordings from cortical areas, have presented compelling evidence that LFP oscillations >40 Hz are highly localized to the electrode tip (Gray and Singer, 1989; Liu and Newsome, 2006). This would indicate that NMDAR antagonist-enhanced HFO are a spatially well localized neuronal signal that is generated in structurally and functionally diverse areas. As such synchronous HFO may represent a mechanism whereby altered brain functioning occurs through the formation of aberrant neuronal networks and loops. In support of this Nicolas et al. (2011) provided evidence, from imaginary coherence studies, that HFO, at least for basal ganglia areas, are likely to be generated locally. However, such an interpretation is at odds with our current source and sink density analyses, which indicate good localization to the ventral but not dorsal striatum (Hunt et al., 2011). Recently, a series of studies have begun to challenge the notion that the generation of high frequency LFP oscillations is necessarily localized to the vicinity of the recording electrode. For example, cortical tissue can transmit all frequencies comparably well (Logothetis et al., 2007) and the spread of LFP may reach a centimeter and is chiefly a function of amplitude, rather than intrinsic frequency (Kajikawa and Schroeder, 2011). Understood in this way, the widespread nature of NMDAR antagonist-enhanced HFO may instead represent spread from more distant generator(s).

As emphasized by many, distinguishing the authentic LFP is pivotal for correct understanding of the nature of LFP recordings and what they functionally mean. To address this issue, we used local infusion of TTX to distinct brain areas to determine how accurately HFO recorded in LFPs in distinct brain areas could be explained based on local neural activity occurring at the recording site.

2. Experimental procedures

2.1. Surgery

Male Wistar rats (250–350 g) were assigned to three experimental groups. The NAc group consisted of 12 rats implanted bilaterally with 22 gauge stainless steel guides (Bilaney, Germany) [AP 1.6, ML 0.8, DV 7 mm] (Paxinos and Watson, 1986) with a pair of twisted stainless steel electrodes (125 μm, Science Products, Germany) placed along one guide (to permit monopolar and derived-bipolar analyses). For simultaneous NAc and ECoG recordings, 6 rats were implanted as the NAc group but with additional stainless steel screws above the frontal [AP 5.0, ML 2.0] and visual [AP −6.0, ML 5.5] cortices. In the prefrontal group, 7 rats were implanted with guides and electrodes in the dorsal prelimbic area of the PFC [AP 3.2, ML 0.5, DV 2–3 mm] and electrodes in the NAc. In the caudate putamen group, 8 rats were implanted with guides and electrodes in the caudate [AP 0.5, ML 3.5, DV 4 mm] and electrodes in the NAc. In all cases, a silver wire was used as the ground/reference electrode connected to a screw above the olfactory bulb. Rats were housed with access to water and food ad libitum. All experiments were conducted in accordance with the European Community guidelines on the Care and Use of Laboratory Animals (86/609/EEC) and approved by a local ethics committee.

2.2. Recording

Animals were placed in a recording chamber 35 cm wide, 35 cm long, and 42 cm high. LFPs and ECoGs were recorded through a JFET preamplifier. The signal was relayed through a commutator (Crist Instruments, USA) amplified $\times 1000$, filtered 0.1–1 kHz (A-M Systems, USA), digitized 4 kHz (Micro1401, CED, Cambridge, UK), allowing the rat free movement inside the recording chamber, and data stored on a PC for offline analysis.

LFPs were recorded for 20 min, followed by i.p. injection of 0.1 mg/kg MK801, 30 min later followed by infusion of TTX (10 ng/side) or saline to the NAc (N=12), PFC (N=7) or caudate (N=8). In a separate study, muscimol (1 μg/side) was infused, followed 10 min later by i.p. injection of 25 mg/kg ketamine (N=6 rats). For infusion, cannulae (28 gauge, Bilaney) that extended 2 mm below the tip of the guide were inserted for 60 s, followed by infusion (1 μl, 0.5 μl/min) and left in place for a further 60 s. Experiments were conducted in a Latin square design, whereby each rat received TTX/saline or muscimol/saline infusions in a randomised order separated by at least 72 h. The location of tips of cannulae and electrode (electrolytic lesion) was determined on 40 μm Cresyl violet stained sections.

2.3. Data analysis

Mean power spectra of the raw monopolar LFP and derived-bipolar signal (difference between the two monopolar recordings) were carried out on successive 60-s data blocks using a fast Fourier transform of 4096 points. Total power (130–180 Hz) and power of dominant frequency (maximal power at the dominant frequency in the spectra) were calculated. Total power of the gamma band (30–90 Hz) was also computed for the same 60-s data blocks. Coherence between pairs of raw monopolar LFP signals was calculated using a script available from the CED website http://www.ced.co.uk/upu.shtml. Coherence analyses (4096 points) were done for 300 s periods at the end of baseline, immediately prior to

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and after infusion of TTX. A coherence value which corresponded to the peak of the HFO band was obtained from the coherence spectra. For further anaylses the raw LFP was digitally filtered 130–180 Hz and HFO events were extracted in the following manner. The mean amplitude and standard deviation (SD) of the filtered signal were calculated at baseline for each rat. Peaks at or exceeding a threshold of 6 SD of baseline were marked as events. Using built-in functions (Spike 2) cross-correlograms and phase-histograms for HFO events extracted from the NAc and other sites were calculated for a period of 300 s after infusion. We also calculated the waveform correlation (Spike 2) between pairs of filtered signals.

2.4. Statistics

Changes in power were analyzed using a two-way ANOVA with time as the repeated measure. The relationship between changes in power in different brain regions was calculated using Pearson R correlation. Values were considered significant when $p<0.05$.

3. Results

3.1. Reversible inactivation of the accumbens reduces the power of pharmacologically-enhanced HFO

Systemic injection of 0.1 mg/kg MK801 led to an increase in the power of HFO in both mono- and bi-polar LFP recordings in the NAc. Thirty min post injection of MK801 (when the power of HFO had reached plateau) rats received bilateral infusion of 10 ng TTX or saline. This dose has been used by many to reversibly inactivate different brain regions (Sacchetti et al., 2002; Gao et al., 2007; Sierra-Mercado et al., 2006; Freund et al., 2010). Bilateral local infusion of 10 ng TTX to the NAc produced an immediate and almost complete reduction in HFO power ($F_{(79,790)}=4.62$, group $\times$ time $p<0.0001$, Figure 1A and B), whereas infusion of saline had little effect. Significant reductions were also found in the bipolar signal ($F_{(79,790)}=4.56$, group $\times$ time $p<0.0001$). Deriving the bipolar signal should remove common-mode input and thus reflect more localized activity. The presence of the HFO band in the bipolar signal and the dramatic effect following TTX infusion further indicates HFO recorded in the NAc are locally generated.

Bursts of HFO could be clearly seen in raw and 130–180 Hz band-pass filtered signal (Figure 1C1). Analysis of the first 5 min post TTX infusion revealed a significant reduction in the number of HFO oscillatory events ($\leq$6 SD above baseline) and the root mean square amplitude of the filtered signal ($p<0.05$, for both) (Figure 1C2). Post infusion of TTX we also observed an immediate reduction in the power of gamma ($F_{(79,790)}=4.87$, group $\times$ time $p<0.0001$), but no reduction in the power of delta ($F_{(79,790)}=1.19$, group $\times$ time $p=0.13$) (Figure 1B).

Muscimol is a GABA-A receptor agonist that acts on postsynaptic receptors which leads to hyperpolarisation of neurons. Inactivation using muscimol does not affect fiber passage and thus represents axon-sparing local inactivation (Majchrzak and Di, 2000). Rats were bilaterally infused with 1 μg muscimol, to produce regional inactivation (Zhang et al., 2002; Hope et al., 2006), followed 10 min later with injection of ketamine 25 mg/kg. Muscimol reduced the power of HFO after injection of ketamine compared to saline-infused rats ($F_{(78,780)}=2.99$, group $\times$ time $p<0.0001$, Figure 1D). Reductions in gamma power were also found ($F_{(78,780)}=1.39$, group $\times$ time $p<0.0001$).

Based on observations, MK801 produced a weak locomotor stimulatory effect, characterized by bouts of increased rearing and stereotypic sniffing. Post infusion of TTX we observed a change in rodent behavior with what can be best described as aggressive-like behavior and posturing. This was apparent almost immediately from the moment of infusion. Post infusion the rats remained alert for most of the remainder of the study and we observed a general reduction in movement. On several occasions we observed low grade seizure-like activity. We found no obvious relation between changes in overt behavior and online changes in HFO.

3.2. Correlation between pharmacologically-enhanced accumbal HFO and occurrence of cortical HFO

HFO have been observed in several cortical areas after injection of NMDAR antagonist (Nicolas et al., 2011; Phillips et al., 2012). We therefore wanted to examine the relationship between HFO recorded in the NAc and frontal (FCx) and visual cortices (VCx). Prior to MK801 injection, spontaneous HFO were visible as a small peak in the power spectra of accumbal activity, while practically no HFO were observed in the power spectra of cortical signals. After injection of MK801 the power of HFO was clearly dominant in the NAc and much weaker in cortical areas (NAc > FCx > VCx, see Figure 2A and B). Analysis of the mean power of dominant frequency for the first 30 min revealed that the maximal power was around 25 times smaller in the FCx compared to the NAc and 80 times smaller in the VCx. Our data are in line with Phillips et al. (2012) who observed greater HFO power in the motor cortex, compared to the VCx.

The degree of association between HFO power in these regions was estimated using the Pearson correlation coefficient. In all saline-infused rats min-by-min analysis post injection of MK801 revealed strong positive correlations between the NAc and ECoG recordings from the FCx (R range 0.76–0.93, $p<0.001$) and VCx (R range 0.66–0.96, $p<0.001$, all rats) (Supplementary Figure S1).

To examine whether HFO recorded in cortical regions were associated with accumbal HFO, we reversibly inhibited the NAc using TTX. Post infusion of TTX, we observed an immediate and sustained reduction of HFO power in all three structures. Repeated measures ANOVA for change in power over time revealed significant effect of TTX infusion to the NAc, compared to saline (NAc, $F_{(75,750)}=4.23$, $p<0.0001$; FCx $F_{(75,750)}=3.95$, $p<0.0001$; VCx $F_{(75,750)}=10.59$, $p<0.0001$). In all rats, post infusion of TTX changes in accumbal HFO power positively correlated with changes in power recorded in the FCx (R range 0.76–0.98, $p<0.001$) and in the VCx (R range 0.78–0.97, $p<0.001$, all rats) (Supplementary Figure S2).

Coherence is an important index of frequency coupling between two simultaneously recorded LFP oscillations. After injection of MK801, a dominant peak in the HFO range was present in the coherence spectra calculated on the raw LFPs for NAc–FCx and NAc–VCx pairs, with weaker coherence for other frequencies (Figure 2C). We next examined the 130–180 Hz band-pass filtered signals to

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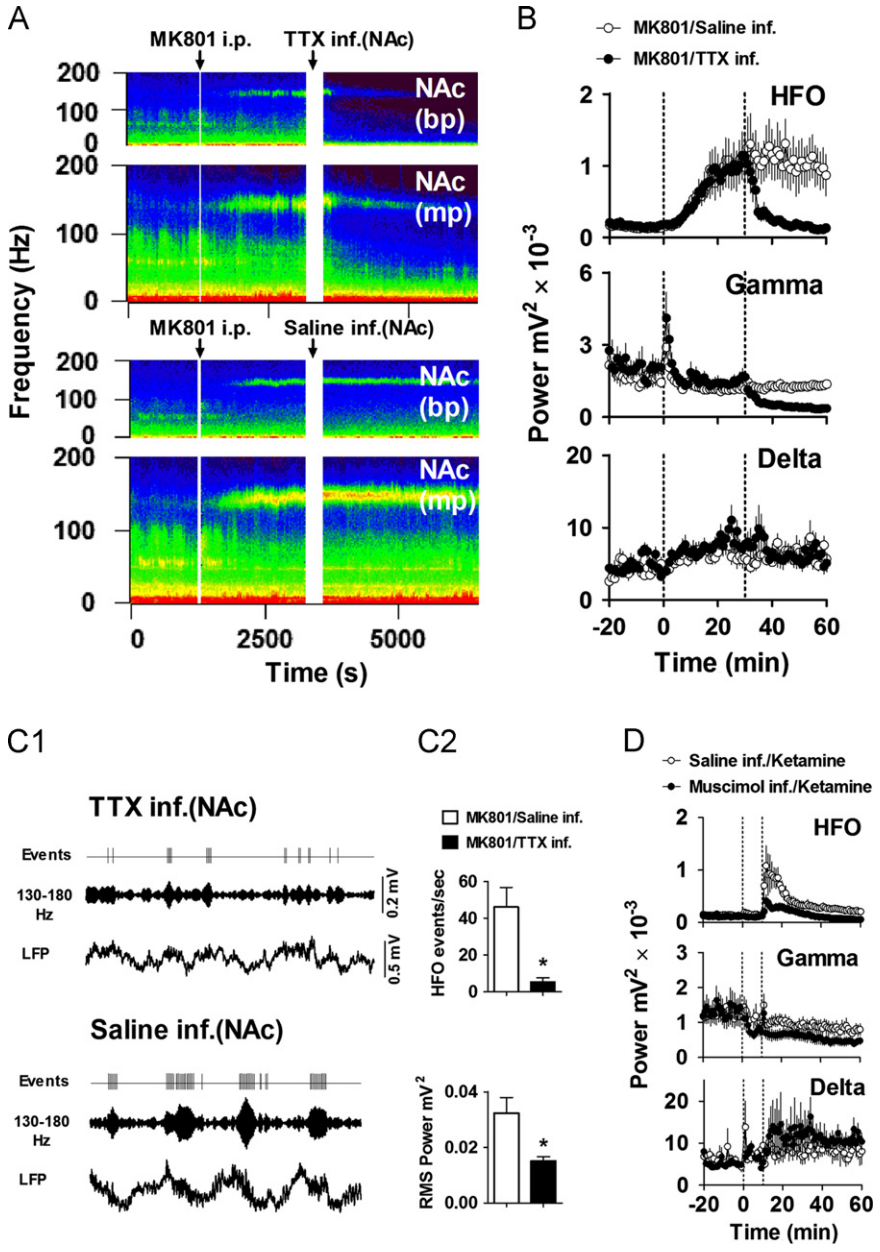

Figure 1 Reversible inactivation of the NAc reduces pharmacologically-enhanced accumbal HFO. (A) Accumbal LFPs expressed as spectrograms of monopolar (mp) and bipolar (bp) recordings showing the effect of systemic injection of 0.1 mg/kg MK801 followed 30 min later by bilateral intraNAc infusion of saline or 10 ng TTX. Note, MK801-enhanced HFO are clearly visible in both mono- and bi-polar recordings and are attenuated by infusion of TTX. **(B)** Time-courses showing the group effect for HFO, gamma and delta frequency bands ($N=6$ rats). First dotted line indicates systemic injection of MK801, and the second line intraNAc infusion of saline or TTX. IntraNAc infusion of TTX reduced the power of MK801-enhanced HFO. We also found reductions in the power of gamma, but not delta. **(C1)** Examples of raw LFPs and the 130–180 Hz band-pass filtered signal, a few minutes after infusion of TTX or saline. In each case, individual HFO oscillatory events ($\geq$6 SD baseline) are shown. **(C2)** TTX significantly reduced the number of events and the RMS power (paired t-test, $^{*}p<0.05$). **(D)** The effect of pre-infusion of 1 μg muscimol on oscillatory activity after systemic injection of 25 mg/kg ketamine. We observed significant group × time effects for HFO and gamma bands, but not for delta ($N=6$ rats). First dotted line indicates intraNAc infusion of saline or muscimol; second line indicates systemic injection of ketamine. Values are mean ± SEM.

precisely determine the temporal relationship between HFO recorded in these structures. Bursts of HFO could clearly be seen in the filtered NAc LFP but were harder to discriminate in ECoGs due to their much smaller amplitude (Figure 2D). For all rats, cross-correlograms of HFO events ($\leq$6 SD amplitude at baseline) in the ECoGs and NAc were coherent for multiple cycles (an example is shown in Figure 2E1). Closer inspection revealed that the individual peaks were not perfectly in-phase for the NAc–FCx, typically occurring around 0.5 ms earlier in the FCx. In contrast, with exception to a small amount of jitter HFO were in-phase for NAc–VCX signals (Figure 2E2). These

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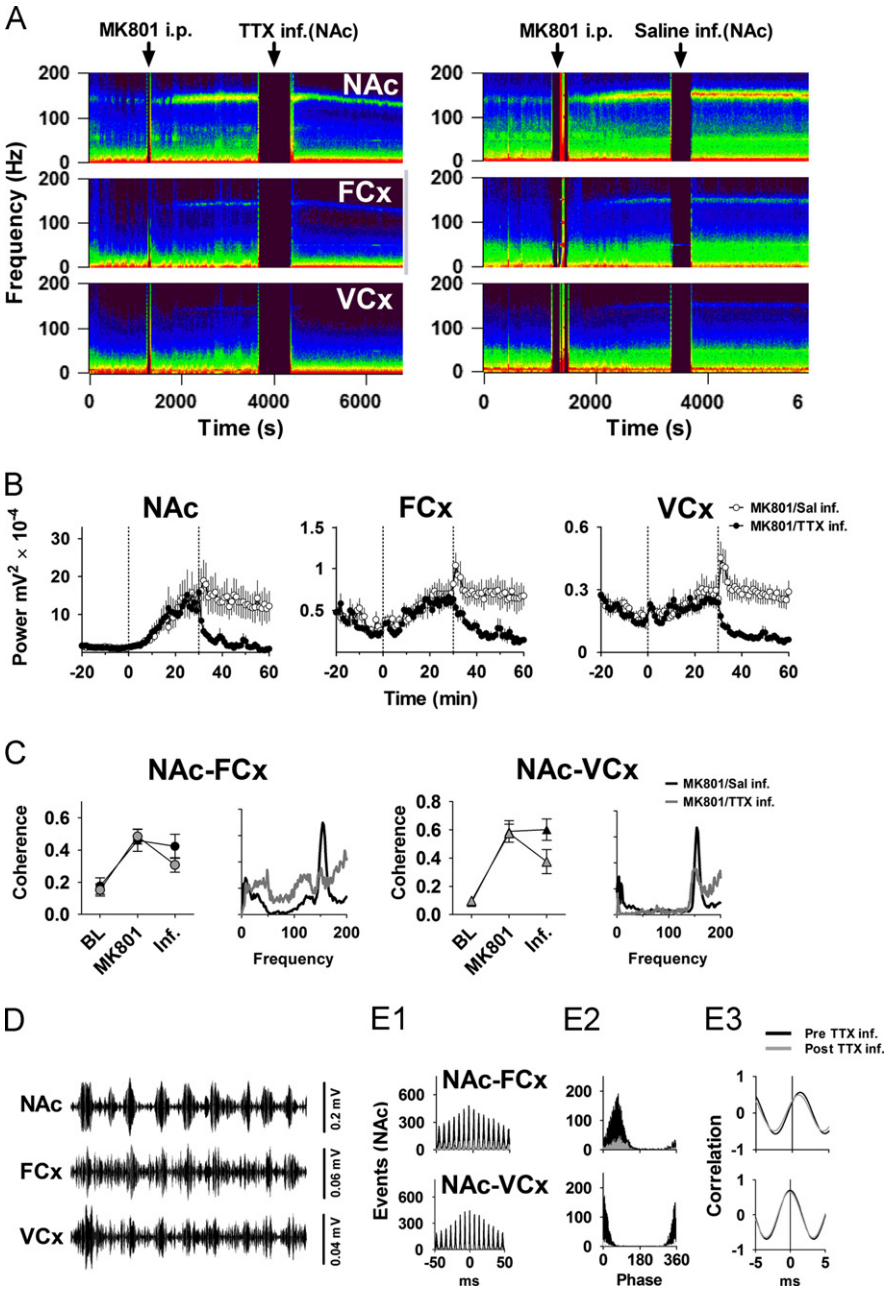

Figure 2 Reversible inactivation of the NAc reduces pharmacologically-enhanced HFO in frontal and visual cortices. (A) LFPs from the NAc and ECoGs from the frontal (FCx) and visual (VCx) cortices recorded simultaneously showing the effect of systemic injection of 0.1 mg/kg MK801 and intraNAc infusion of 10 ng TTX or saline. HFO covary in power and frequency in all three regions with the power of HFO being dominant in the NAc. Parallel reductions in the power of HFO after intraNAc infusion of TTX are observed in all regions. **(B)** Significant group × time interactions were observed for all regions. The dotted lines indicate systemic injection of MK801, followed 30 min later by TTX or saline infusion ($N=6$ rats). **(C)** Systemic injection of MK801 induces oscillatory activity that is coherent for the HFO band for NAc–FCx and NAc–VCx pairs of signals. X–Y plots show peak coherence in the 130–180 Hz range (mean ± SEM) calculated for the last 300 s at the end of baseline and MK801 injection and first 300 s after infusion of TTX or saline. Examples of coherence spectra after infusion of saline (black) or TTX (grey) are shown adjacent to each plot. **(D)** Example of 130–180 Hz band pass filtered LFP recorded after injection of MK801. Note the different scales. **(E1)** Cross correlation analysis showing HFO oscillatory events were coherent on a cycle-by-cycle basis between the cortical areas and NAc. Note the smaller number of HFO events after intraccumbal infusion of TTX (grey) compared prior to infusion (black); data are from a representative rat. **(E2)** Phase histograms showing the earlier occurrence of HFO in the FCx, but not in the VCx, with respect to the NAc. **(E3)** Waveform correlations for cortical and NAc 130–180 Hz, filtered signals, prior to and immediately after infusion of TTX. Note that HFO are slightly shifted to the right in the NAc–FCx, but not in the NAc–VCx pair of waveforms.

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observations were confirmed using waveform correlation (Figure 2E3). Although a substantial reduction in the number of HFO events was found after TTX infusion, this did not affect oscillatory phase.

3.3. Reversible inactivation of the PFC does not affect the power of NMDAR antagonist-enhanced HFO recorded locally

Post injection of MK801, HFO were clearly recordable in the cortical areas. We next examined the effect of local TTX infusion on cortical oscillations. To achieve this we implanted rats with guides bilaterally in the PFC and recording electrodes adjacent to one of the guides and also in the NAc. This study also permitted direct comparison of the signals obtained using the same type of electrode.

After MK801 injection HFO were clearly visible in the LFPs recorded in the PFC and NAc. The power of HFO was larger in the NAc compared to PFC ($F_{(76,912)}=3.84$, group $\times$ time, $p<0.0001$). The relationship between HFO in these areas was markedly similar to our findings for ECoG recordings (Figure 3)—if anything, the similarity between the signals was stronger in LFP recordings. Examples of time-course spectrograms are shown in Figure 3A. Reversible inactivation of the PFC did not influence the power of HFO recorded locally ($F_{(76,912)}=0.13$, group $\times$ time, $p=1.0$), nor did it influence the power of HFO recorded from the NAc ($F_{(76,912)}=0.27$, $p=1.0$) (Figure 3B). Infusion of TTX did, however, significantly reduce gamma band power recorded in the PFC ($F_{(76,912)}=1.5$, group $\times$ time $p=0.0053$), but not in the NAc ($F_{(76,912)}=0.48$, $p=0.99$, Figure 3C). Evidence of the effectiveness of TTX infusion on LFPs recorded from the PFC was also clearly visible in the bipolar recordings, where we found abrupt effects on power, similar to that observed for NAc infusions (Figure 3A, compare with Figure 1A).

Analysis of minute-by-minute changes in HFO power, after injection of MK801, revealed strong positive correlations for the PFC and NAc (Pearson R range 0.94–0.99, all rats $p<0.001$). Also, consistent with our findings described for the ECoG recordings, post injection of MK801 we observed strong coherence for the HFO band in the raw monopolar LFPs in the PFC and NAc (Figure 3D).

In the 130–180 Hz band-pass filtered signal, individual bursts of HFO typically co-occurred in the PFC and NAc (Figure 3E). For all rats, cross-correlograms of HFO events in the PFC triggered on HFO events from the NAc appeared coherent for multiple cycles of the HFO. Close inspection of the filtered signal and cross correlagrams revealed there was a small phase-lag for HFO peaks in the NAc channel. This was confirmed in the phase histograms and waveform correlation of the band pass filtered signals (Figure 3F). Although the signals were not perfectly in-phase, in all cases the correlation value was close to 0.9.

Based on observation of rat behavior, MK801 provoked a comparable mild increase in locomotor activity as observed for the NAc infusion study. Infusion of TTX was associated with a general reduction in locomotor activity and no other overt changes were noted.

3.4. TTX inactivation of the caudate nucleus weakly reduces the power of HFO recorded in the NAc

We also examined the effect of TTX infusion to the caudate nucleus, a region neuroanatomically related to the NAc, that also displays increased HFO power after ketamine (Hunt et al., 2011; Nicolas et al., 2011). Examples of spectrograms are shown in Figure 4A. We found no significant effect of TTX infusion on the power of HFO recorded in the caudate ($F_{(76,1064)}=1.2$ $p=0.102$, group $\times$ time) or NAc ($F_{(76,1064)}=1.28$ $p=0.057$, group $\times$ time). There was however a trend for a reduction in HFO power which occurred more tardily, often becoming visible 15–20 min after infusion (Figure 4B). Similar to our findings for the PFC, systemic injection of MK801 increased gamma power. Local infusion of TTX produced a reduction in caudate gamma power, with values rapidly returning to baseline value ($F_{(76,1064)}=6.16$, $p<0.0001$ group $\times$ time). A small but significant reduction in gamma power was also found in the NAc ($F_{(76,1064)}=1.36$, $p=0.024$, group $\times$ time Figure 4C). Analysis of the mean change for the 30 min post infusion period also revealed significant effects. In all rats, coherence analysis for the raw NAc and caudate nucleus LFPs revealed strong coherence for the HFO band after injection of MK801 (Figure 4D). Also HFO power recorded in the caudate nucleus strongly correlated with NAc activity (Pearson R range 0.89–0.99, all rats $p<0.001$).

In the 130–180 Hz band-pass filtered signal, individual bursts of HFO typically co-occurred in the PFC and NAc (Figure 4E). For all rats, cross-correlograms of HFO events revealed they were coherent for multiple cycles of the HFO. No obvious phase lag was visible. In-phase activity was confirmed by phase analyses and waveform correlation between the signals (Figure 3F).

Based on observation of rat behavior, MK801 provoked a comparable mild increase in locomotor activity as observed for the earlier studies. Infusion of TTX was associated with a general reduction in locomotor activity and some mild aggressive-like changes, such as postural changes observed.

4. Discussion

4.1. HFO recorded in the NAc after injection of MK801 are locally generated

Our major findings are as follows. (1) The power of HFO after injection of MK801 was consistently larger in the NAc compared to the other regions we investigated and remote HFO covaried in power and frequency with the activity recorded from the NAc. As can be seen in our ECoG recordings, the power of HFO diminished with distance from the NAc, and is consistent with findings from Phillips et al. (2012), who also reported smaller HFO power in VCX versus motor cortex. (2) TTX infused locally to the NAc produced an immediate reduction in the power of HFO which positively correlated with decreases in power of HFO recorded in distant cortical areas. Our experiments with muscimol, a GABA-A receptor agonist that hyperpolarizes the membrane of neurons, also significantly reduced NMDAR antagonist-enhanced HFO. This showed the reduction we observed

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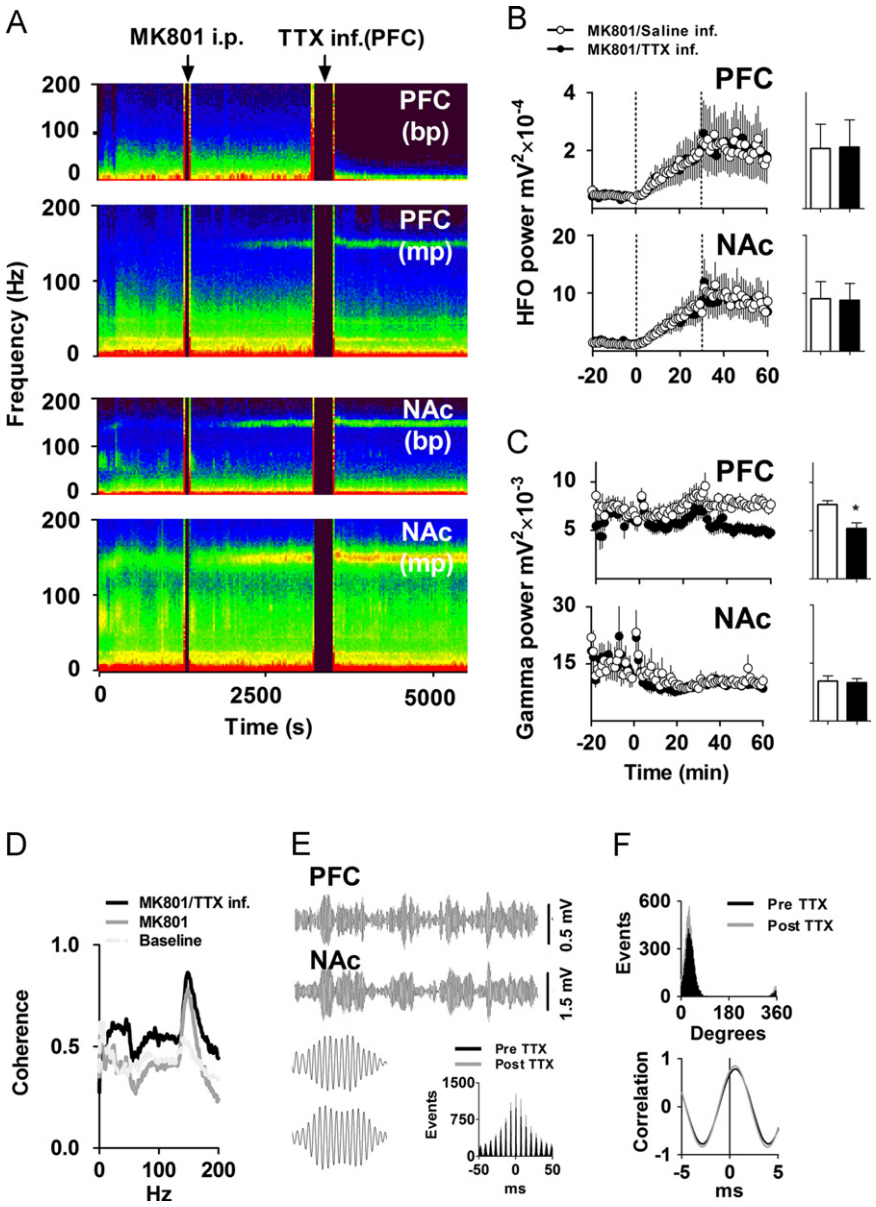

Figure 3 Reversible inactivation of the prefrontal cortex does not reduce the power of pharmacologically-enhanced HFO recorded locally. (A) Spectrograms of monopolar (mp) and bipolar (bp) LFPs, recorded in the PFC and NAc showing the effect of systemic injection of MK801 followed 30 min later by infusion of TTX in the PFC. Note HFO are absent from the bipolar recording in the PFC. (B) Time-courses showing changes in HFO power recorded in the PFC and NAc after intraPFC infusion of TTX or saline. No significant effects were found for the power of HFO recorded locally in the PFC or in the NAc ($N=7$ rats). First dotted line indicates injection of MK801 (i.p.), and the second dotted line infusion of saline or TTX. Histograms show the average change in power for the 30 min time period after infusion of TTX or saline. (C) Time-courses showing changes in gamma power. TTX significantly reduced gamma power recorded in the PFC, but not in the NAc. Histograms show the average change in power for the 30 min time period after infusion of TTX or saline (paired t-test, $^{*}p<0.05$). (D) Coherence spectra (300 s) of the raw PFC and NAc LFPs calculated at baseline, after systemic injection of MK801 and TTX infusion. (E) 130–180 Hz band pass filtered LFPs (1.5 s) after injection of MK801 from a representative rat. The first HFO burst is expanded, and a cross correlogram showing HFO events ($\geq$6 SD) in the PFC and NAc are coherent for multiple cycles immediately before and after infusion of TTX (300 s) is shown. (F) Phase histogram for the same time shows a phase-shift between PFC and NAc channels (top); note the phase was not influenced by TTX infusion. This was confirmed in the waveform correlation of the filtered signal, which showed high correlation, but a small delay in the NAc channel.

after TTX could not be attributed to blockade of crossing fibers. (3) TTX infused to the cortex and caudate did not significantly influence the power of NMDAR antagonist-enhanced HFO recorded at the infused site, but did reduce the power of gamma which has been shown to be generated locally. (4) Consistent with our earlier study, NMDAR antagonist-enhanced HFO were also recorded in the NAc using a local reference (which would be expected to

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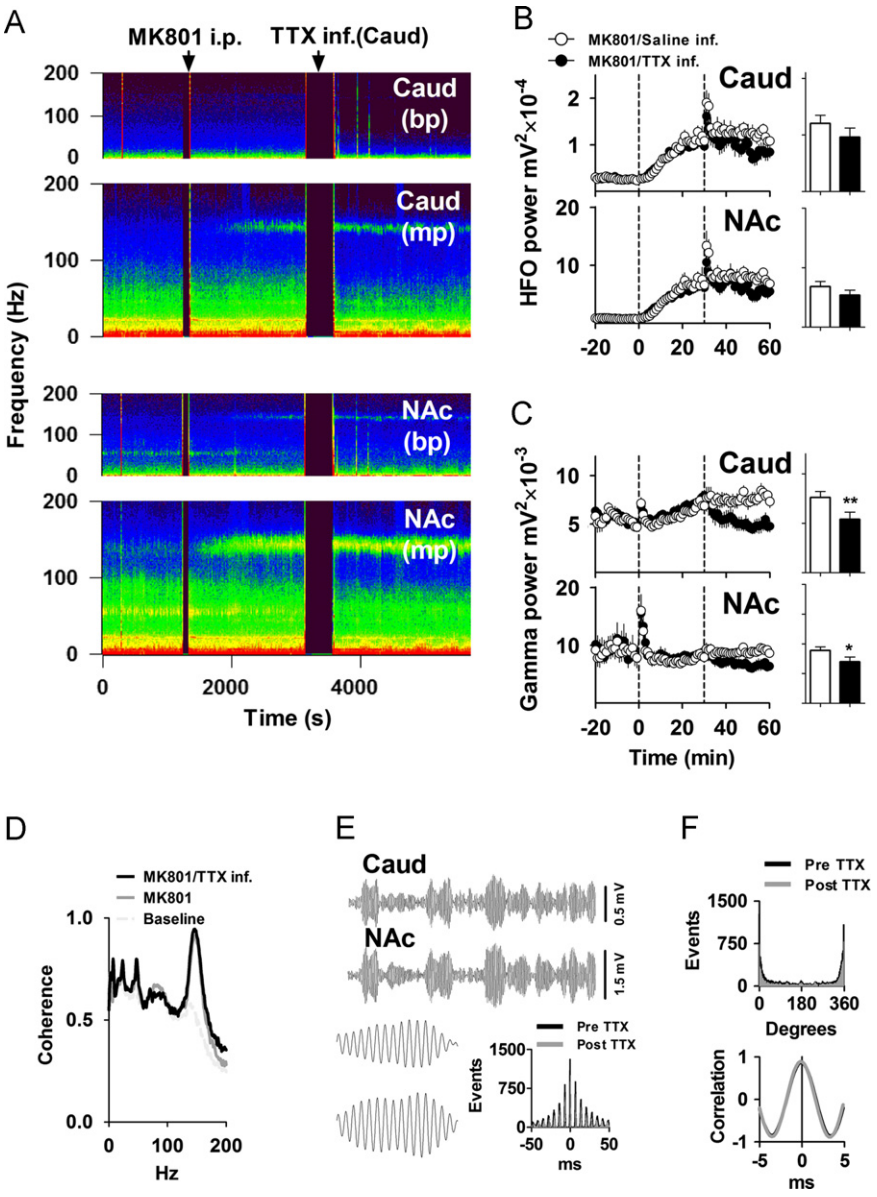

Figure 4 Reversible inactivation of the caudate does not reduce the power of pharmacologically-enhanced HFO recorded locally. (A) Spectrograms of monopolar (mp) and bipolar (bp) LFPs, recorded in the caudate (Caud) and NAc showing the effect of systemic injection of 0.1 mg/kg MK801 followed 30-min later by infusion of 10 ng TTX in the Caud. **(B)** Time-courses showing changes in HFO power recorded in the Caud and NAc after intraCaud infusion of TTX or saline ($N=8$ rats). First dotted line indicates injection of MK801 (i.p.), and the second dotted line infusion of saline or TTX. Histograms show the average change in power for the 30 min time period after infusion of TTX or saline, and no significant effect was found (paired t-test). **(C)** Time-courses showing changes in gamma power. TTX significantly reduced gamma power recorded in the Caud and NAc. Histograms show the average change in power for the 30 min time period after infusion of TTX or saline (paired t-test, $^{*}p<0.05$, $^{**}p<0.01$). **(D)** Coherence spectra (300 s) of the raw Caud and NAc LFPs calculated at baseline, after systemic injection of MK801 and after TTX infusion. Data are from a representative rat. **(E)** 130–180 Hz band pass filtered LFPs (1.5 s) after injection of MK801. The first HFO burst is expanded, and a cross correlogram showing HFO events ($\geq$6 SD) in the Caud and NAc are coherent for multiple cycles immediately before and after infusion of TTX (each 300 s) is shown. **(F)** Phase histogram shows little difference in phase between HFO recorded in the Caud and NAc (top). Waveform correlation of the filtered signal shows a high correlation zero-phase delay between HFO recorded in the two channels.

minimize conducted currents) but were absent in all bipolar recordings from the prefrontal cortex and the majority from the caudate (Hunt et al., 2011).

The finding that HFO can be recorded in cortical and subcortical regions after systemic injection of NMDAR antagonist is in line with studies published by other groups as well as ourselves (Phillips et al., 2012; Nicolas et al., 2011; Kulikova et al., 2012; Hunt et al., 2011). Anatomical coupling between structures can permit the emergence of coherent oscillatory activity; however, to date, there does

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not appear to be a neurobiological foundation at the network level to explain the near-synchronous activity in the many regions where HFO have been detected. Critically, our data call into question the idea that HFO after NMDAR antagonist injection are generated by all these regions. Spread by volume conduction, from source(s) generating HFO seems to offer the most parsimonious explanation for why HFO are detected in the caudate and cortical areas we investigated. Recent findings have shown that the spread of the LFP may be independent of frequency (Logothetis et al., 2007; Kajikawa and Schroeder, 2011). This interpretation has a lot of explanatory power, and is consistent with most of the main findings of our data (see first paragraph of discussion). However, based on the current understanding of volume conduction, spread of HFO from the NAc alone cannot satisfactorily explain all our experimental results. Volume conducted signals should spread close to the speed of light and therefore occur almost perfectly in phase. Whilst a certain amount of jitter may be expected, we observed reproducible negative phase-shifts for both the frontal ECoG and PFC LFP recordings with respect to the NAc. This is unexpected, in that the peaks of HFO fractionally preceded those occurring in the NAc. In contrast, data obtained from the visual cortex and caudate almost always occurred with zero phase lag. This is hard to explain; however, we speculate that given the EEG electrodes record the difference of potentials for the entire area between their site and the reference electrode (above the olfactory bulb). It is possible, therefore, that this differential recording may have introduced some distortions in the phase. That is to say, assuming HFO do in fact spread, then subtracting the signal seen by the EEG electrodes from the reference electrode may distort the original phase relation of the signal. Considering the relatively closer distance of the frontal EEG and PFC LFP to the reference greater distortions may be expected to be observed. Notwithstanding, whilst we show that HFO in the NAc are critical for detection of HFO in the areas we investigated, volume-conduction from the NAc may not fully account for this phenomenon.

Our experimental evidence supports our conclusion that the NAc is a powerful generator of HFO after injection of NMDAR antagonist; however, we do not claim it is the exclusive source. Indeed, the generation of HFO in other regions may help explain differences in the HFO phase-delay we found between the NAc and other regions. Nicolas et al. (2011) reported the widespread occurrence of ketamine-induced HFO in motor regions which were coherent for the imaginary part of coherence, indicating they were not caused by volume-conducted currents. It is hard to reconcile this result with our present study. However, in their study TTX was not applied to demonstrate local generation of HFO, and methodological differences such as the use of asymmetric bipolar concentric electrode which may sample from an unbalanced area of tissue may account for this discrepancy. Further studies are warranted to examine the neuroanatomical specificity of HFO after NMDAR antagonist injection which is an essential step in order to address more fully the potential mechanisms and behavioral end-point of this activity.

It is important to note that spontaneous HFO are known to be generated in cortical areas and the caudate (Jones and Barth, 1999; Tort et al., 2008). Our analysis does not address the relationship between accumbal HFO and spontaneous HFO in these areas; indeed in most cases we found little spontaneous activity during the baseline recordings or after saline injection. However, in an earlier study we showed hippocampal sharp-wave ripples and accumbal HFO differ in several respects, such as frequency and state (Hunt et al., 2011) suggesting they are not closely related.

4.2. Possible neural bases of MK801 enhanced HFO

Phillips et al. (2012) recently demonstrated HFO recorded from cortical regions could be measured after systemic injection of MK801, phencyclidine, ketamine and the competitive NMDAR antagonist SDZ 220,581. We have found that local infusion of CPP, a selective and competitive NMDAR antagonist, also induces HFO in the NAc (unpublished findings). Therefore, we consider the increases in HFO power to be related to the NMDAR antagonist properties of these drugs, rather than caused by activity on other receptor systems, such as nAChR (Ramoa et al., 1990) and dopamine D2 receptors (Seeman et al., 2005). In support of this we have shown that intraccumbal infusion of the dopamine D2 antagonist raclopride does not influence the ketamine-enhanced HFO power (Matulewicz et al., 2010).

LFPs are created by the sum of transmembrane currents, which arise predominantly from synaptic activity, generating sinks and sources. Currents that are synchronized in time and space significantly influence the LFP. In non-laminar regions, such as striatal tissue, the origin of LFPs is not well understood and volume conducted currents may contribute quite substantially to the signal (Boraud et al., 2005). LFPs in the striatum are largely composed of subthreshold currents generated at the soma and dendrites of neurons (Boraud et al., 2005). At present we can only speculate on the neural origin of HFO in the NAc. The presence of large amplitude HFO recorded after NMDAR antagonist injection indicates that rapid fluctuations in the membrane potential occur in either a large population of accumbal neurons or they result from profound intrinsic membrane changes in much smaller neuronal clusters. The finding that local infusion of MK801 to the NAc dose-dependently enhances HFO suggests that direct effects on the intrinsic circuitry of the NAc are sufficient to generate HFO (Hunt et al., 2010). However, local blockade of NMDAR would also be expected to change the synaptic weight of afferent signals. TTX infusion to the dorsal prelimbic region of the PFC (which is known to project to the NAc) did not significantly influence the power of HFO in the NAc. However, the more ventral, infralimbic area of the PFC also projects to the NAc and our cortical TTX infusion study is unlikely to have affected this area. Further studies using a combination of techniques are required to address the mechanism responsible for the generation of HFO after NMDAR antagonist injection.

Modeling studies have shown that the ratio of NMDA/AMPA receptors in the NAc plays an important part in how medium spiny neurons respond to afferent input and subsequent cellular output (Wolf et al., 2005). For example, changing this ratio can lead to compensatory changes in synaptic weight in the model cell. In the NAc, NMDARs are critically important for triggering homeostatic synapse-driven membrane plasticity, which provides a compensatory mechanism adjusting membrane excitability to shifts in

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excitatory synaptic input (Ishikawa et al., 2009). Blockade of NMDAR would be predicted to interfere with these homeostatic processes, which in turn may set in motion a series of compensatory responses, such as changing the responsiveness of ion channels. Large amplitude HFO may reflect these transient changes which bring cells closer to (or further away from) their firing threshold and lead to disorganized NAc output. Single-unit studies, which were beyond the scope of the present work, are required to address this issue.

In summary, we demonstrate that the NAc is a generator of HFO after injection of MK801. We show that, the HFO recorded in the extraaccumbal areas, at least those investigated here, although detectable are not generated at the recording site. Instead their power is correlated with the HFO occurring in the NAc. These findings add to a growing body of evidence indicating that altered activity in the NAc is a fundamental effect produced by NMDAR antagonists.

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Contributors

MO managed the experiments and analyses, WD performed analyses, MP performed experiments, SK designed the study and wrote the manuscript, MJH designed the study and wrote the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare we have no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2013.01.012.

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