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BRIEF COMMUNICATION

Oscillatory Local Field Potentials Recorded from the Subthalamic Nucleus of the Alert Rat

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Hitherto, high-frequency local field potential oscillations in the upper γ frequency band (40–80 Hz) have been recorded only from the region of subthalamic nucleus (STN) in parkinsonian patients treated with levodopa. Here we show that local field potentials recorded from the STN in the healthy alert rat also have a spectral peak in the upper γ band (mean 53 Hz, range 46–70 Hz). The power of this high-frequency oscillatory activity was increased by 30 ± 4% (±SEM) during motor activity compared to periods of alert immobility. It was also increased by 86 ± 36% by systemic injection of the D2 dopamine receptor agonist quinpirole. The similarities between the high-frequency activities in the STN of the healthy rat and in the levodopa-treated parkinsonian human argue that this oscillatory activity may be physiological in nature and not a consequence of the parkinsonian state.

We have recently shown that population activity, as evinced by local field potentials (LFPs), in the region of the human subthalamic nucleus (STN) is coherent with that in the globus pallidus interna and cerebral cortex at frequencies of 60–80 Hz in alert parkinsonian patients treated with levodopa (4, 20). When the same patients were withdrawn from levodopa or dopaminergic agonists, the predominant frequency of coherent LFP oscillations in the STN shifted from the high γ band to below 30 Hz (4, 20). Furthermore, in a separate study, we demonstrated that activity at 60–80 Hz is increased by movement (5). Taken together, these data suggest that this oscillatory coupling in the high γ band is physiological, requires adequate dopaminergic activity, and is relevant for movement.

However, these studies in humans have their limitations. First and foremost is the fact that all basal ganglia recordings are performed in patients with disease, most commonly Parkinson's disease. Thus it is difficult to be sure whether a given activity is primarily physiological or pathological in its nature. Second, histological confirmation of the recording site is lacking in these human studies. Evidence supportive of a localization in the STN can be derived from stereotactic coordinates, intraoperative microelectrode recordings and stimulation effects, postoperative magnetic resonance imaging, and functional outcome, but unfortunately these measures do not provide absolute proof of localization within STN. Third, recordings from the basal ganglia in humans are made in the period immediately following implantation of the electrode and prior to connection to the subcutaneous stimulator. This period lasts at most a few days and possible pharmacological manipulations are therefore limited.

With these limitations of human studies in mind, we sought to characterize any oscillatory activity in the LFPs recorded from the STN of the healthy alert rat. Using this experimental approach, histological confirmation of recording site can be reliably made. Furthermore, pharmacological manipulation of STN activity is not only feasible, but is aided by the considerable knowledge about the pharmacology of the rat basal ganglia. In the first instance, we reasoned that oscillatory activity in the high γ band in rat STN, if present, should be altered by D2 receptor agonists, in line with the evidence that high γ activity in the parkinsonian human is critically dependent on levodopa supplementation (4) and consistent with the evidence that functional D2 receptors are present at both pre- and postsynaptic loci in the STN (7, 11, 19).

The study was carried out on 16 naive male Wistar rats (Harlan–Winkelmann, Germany), weighing 300–400 g during the experiment. Animals were housed under a 12-h light–dark cycle with free access to chow and water. Rats were anesthetized with chloral hy-

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drate (400 mg/kg ip) and placed in a stereotaxic frame (David Kopf Instruments, U.S.A.). Body temperature was maintained at 37°C by a rectal probe connected to a heating device (CMA 150; Carnegie, Sweden). A concentric bipolar "semimicroelectrode" (SNE-100; Rhodes Medical Instruments, U.S.A.) was placed into the left STN under stereotaxic conditions (A −3.8 mm, L 2.5 mm, V −7.6 mm (13)). A reference/ground wire was also fixed to the skull via a screw anterior to the bregma. Thereafter, microelectrode and wire were mounted to the skull with dental cement (Technovit; Kulzer GmbH, Wehrheim, Germany). In three animals, the wire was inserted subcutaneously in the scalp, rather than fixed to a skull screw, and in one rat a reference was clipped to the tail. Animals were allowed at least 48 h to recover from the surgery before recordings and injections.

Animals were placed in a Plexiglas bowl within a Faraday cage. Mains artifact (50 Hz) was eliminated by the use of a "humbug" (Quest Scientific, North Vancouver, Canada). The latter constructs a noise replica in real time and continuously subtracts this replica from the input signal. The microelectrode was connected to a Neurolog 100AK head stage and then to a Neurolog 104A preamplifier (Digitimer, Welwyn Garden City, Hertfordshire, UK). The −3 dB limits of the intrinsic band-pass filter were 0.1 and 100 Hz and the sampling rate was 256 Hz. Recordings were made from the concentric bipolar microelectrode in one of two modes: (1) bipolar recordings were taken from the microelectrode and the skull screw used as ground or (2) the central core was used as a monopolar electrode and referenced to the skull screw or a subcutaneous wire in the scalp ($n = 3$) or wire attached to the tail ($n = 1$) and the outer shell of the concentric needle electrode was used as ground. Five rats were systemically injected with the D2 receptor agonist quinpirole (0.5 mg/kg, ip; RBI, Natick, MA). The same five rats served as their own controls, being injected with vehicle (identical volume of 0.9% saline adjusted to a pH of 6.0–7.2) in a crossover design, with each injection performed on a separate day. In rats treated with drug or vehicle, LFPs were recorded both immediately before and 20 min after systemic injections.

After the experiments, animals were transcardially perfused under deep anesthesia with 30 ml 0.1 M phosphate-buffered saline followed by 100 ml 4% paraformaldehyde and decapitated. Brains were removed, postfixed in 4% paraformaldehyde for at least 24 h, and then processed for Nissl staining on coronal sections (30 μm thick). The location of the recording site was verified by light microscopy (Axioskop, Zeiss, Germany).

Motor activity (defined as walking, rearing, and grooming) was scored as present (1) or absent (0) during sequential 30-s recording periods and the total score over 300 s expressed as a percentage of the total possible score of 10 over the 300 s. To characterize LFP oscillations, autospectra (with 1 Hz resolution) of LFP power and frequency were derived using Spike 2 (Cambridge Electronic Design, Cambridge, UK), having excluded sections with large spike artifact due to excessive movement. The presence of an autospectral peak in the 40–80 Hz γ band was defined as four or more contiguous 1-Hz bins, each with power greater than 150% of the mean power in the three bins flanking each side of the peak. Whether such peaks were selectively picked up from semimicroelectrodes implanted in the STN was tested by χ^2 test. In pharmacological experiments, the frequency band of the peak was defined as above, and then the mean power over this band was measured before and after injection of drug or vehicle. Both γ power and motor scores were then expressed as a percentage of the respective preinjection value and analyzed by separate single-factor ANOVAs. Mean ± standard error of the mean are shown in the text. All electrophysiological recordings and determinations of spectral peaks were performed blind to the results of histology.

Figures 1A and 1B are representative examples of the LFP recorded by a semimicroelectrode in the STN and its autospectrum, respectively. The autospectrum shows peaks at around 8 and 60 Hz (arrowed). For comparison, Figs. 1C and 1D show the signal and autospectrum of the LFP recorded between contacts 1 and 2 of a depth macroelectrode implanted in an alert patient with Parkinson's disease following treatment with levodopa. The patient was recorded while at rest but dyskinetic. Contacts 1 and 2 were believed to lie in STN on the basis of stereotactic coordinates, intraoperative microelectrode recordings and stimulation effects, postoperative magnetic resonance imaging, and functional outcome. Note that the autospectrum in Fig. 1D also shows a clear peak near 60 Hz (arrowed). Figure 1E confirms that the semimicroelectrode was placed in the STN of the rat.

With monopolar recordings, spectral peaks in the upper γ band were absent in 1 rat and present in another 11 rats in which the semimicroelectrode was found to lie within STN. The mean peak frequency of the peak in the upper γ band was 53 Hz (range 46 to 70 Hz, $n = 11$). Conversely, no peaks at high frequency were present in the 4 animals in which the semimicroelectrode was found outside of the STN. Thus peaks in LFP activity in the upper γ frequency band were selectively localized in the STN of the rats ($P = 0.005$, χ^2 test). This was regardless of whether monopolar electrodes referenced to skull, scalp, or tail were used to pick up fast activity. In contrast, when the same activity was recorded bipolarly rather than monopolarly in 8 of the rats with STN implantations, it formed a discrete peak in only 2, perhaps because the smaller difference between the potentials picked up by the

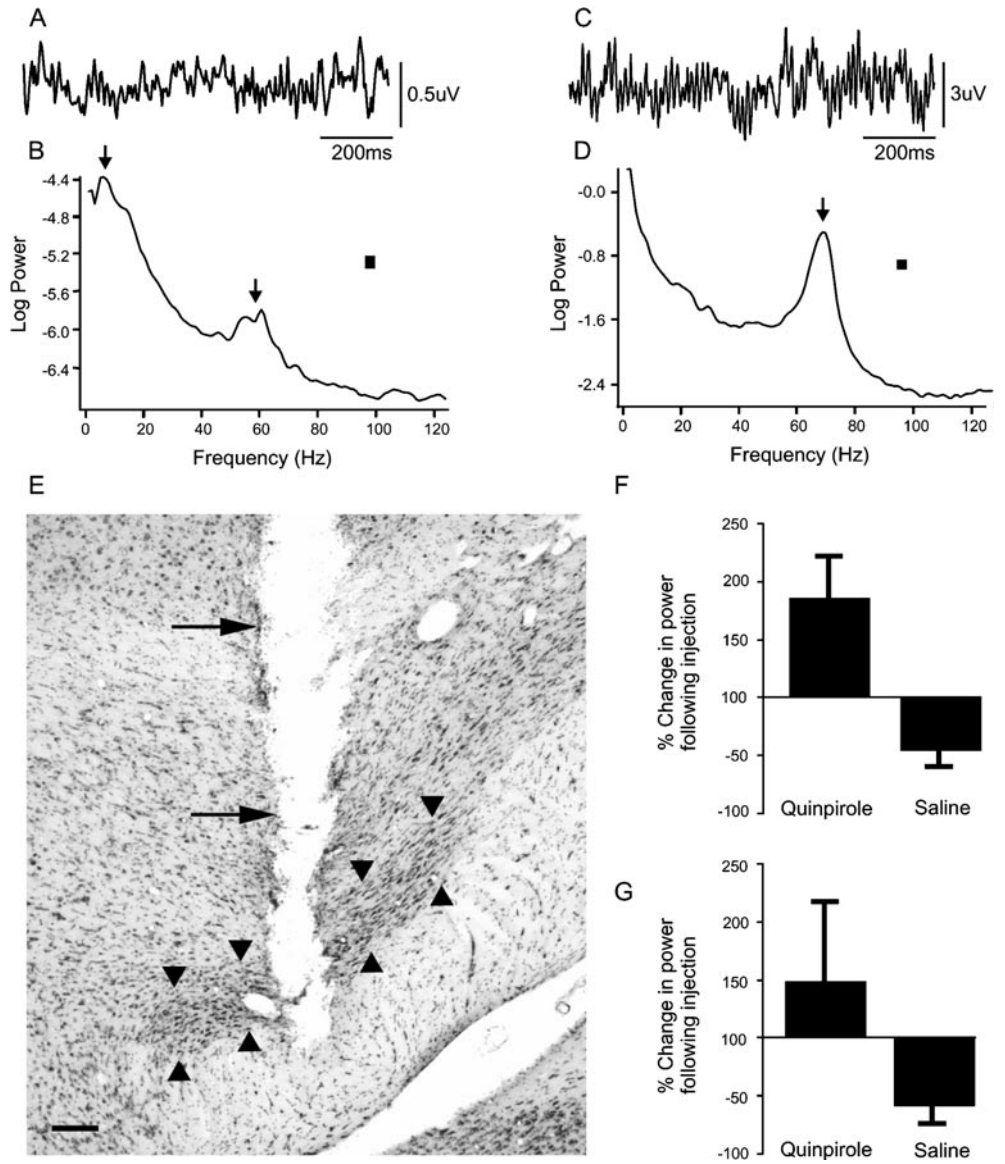

FIG. 1. Oscillatory activities in the local field potentials recorded from the STN of an alert healthy rat and a levodopa-treated parkinsonian human. (A) Raw signal (monopolar recording) from STN of rat. (B) Autospectrum of 300 s of LFP data in A. (C) Raw signal (bipolar recording) and (D) autospectrum of 240 s of LFP data recorded between contacts 1 and 2 of a macroelectrode believed to be in STN in an alert patient with Parkinson's disease following treatment with levodopa. The patient was a 49-year-old female with a 17-year history of Parkinson's disease, who has been previously reported (4). The patient was recorded while at rest but dyskinetic. Note that the autospectra in B and D show peaks near 60 Hz. The vertical calibration lines in the spectra are 95% confidence limits. (E) Histological confirmation of the recording site in the same rat as in A and B showing the tract of the semimicroelectrode (black arrows) reaching STN. The ventral and dorsal borders of STN are indicated by black triangles. Bar, 300 μm. (F) Mean effects of systemic injections of quinpirole (0.5 mg/kg, ip) or vehicle (physiological saline) on activity in the upper γ frequency band. (G) Mean effects of same on motor activity score. Means $\pm$ standard errors of means are illustrated. Note that there was a significant increase in high-frequency LFP activity following quinpirole compared to saline (single-factor ANOVA, F 11.28, df 1, $P = 0.01$). This occurred in the setting of a trend toward increased motor activity that did not reach significance (F 2.32, df 1, 4, nonsignificant).

microelectrode tip and nearby concentric shell gave a poorer signal-to-noise ratio.

The total power in the peak was increased by 30 $\pm$ 4% (paired t test, $P = 0.01$, $n = 5$) when periods scored as motor activity (as defined in methods) were compared to periods of alert immobility in the same animal.

The effects of intraperitoneal injection of the D2 agonist quinpirole in five rats are shown in Figs. 1F and 1G. The D2 agonist selectively increased the high γ activity recorded in STN by 86 $\pm$ 36% (single-factor ANOVA, F 11.28, df 1, 4, $P = 0.01$). This occurred in the setting of a trend toward increased motor activity (did not reach significance). In particular, yawning and

stereotyped chewing activity appeared to be increased in three rats following quinpirole. Note that intraperitoneal injection of saline alone tended to cause reduced motor activity, perhaps due to the mild trauma of handling and injection.

An additional peak at approximately 8 Hz was often evident in autospectra regardless of whether drawn from periods of activity or alert immobility. This peak was often accompanied by a second smaller peak at double this frequency, likely to be a harmonic due to the tendency of the 8-Hz oscillation to be angular in shape. This activity tended to occur in discontinuous runs of 1–3 s in duration. The 8-Hz peak was evident in all monopolar recordings, despite the varied location of microelectrodes, and was also present when bipolar recordings were made from the concentric depth electrodes. This 8-Hz activity was, therefore, likely to originate from both the STN and the surrounding structures.

Our results show that local field potentials recorded from the STN of healthy and alert rats contain a high-frequency oscillatory component in the range of 46–70 Hz. This activity in the upper γ frequency band resembles that recorded in the human in terms of its overlapping frequency range, localization, and potentiation by movement. In addition, the upper γ activity in the rat is increased by dopaminergic stimulation, just as dopaminergic stimulation restores upper γ activity in the STN of parkinsonian patients (4, 5, 20). These similarities suggest that the two activities are homologous in the two species, although the mean frequency of upper γ activity in the rat was slightly lower than that seen in the human (personal observations).

Peaks in the autospectra of LFPs recorded from rat STN were not found in the 15–30 Hz band, whereas these have been reported in recordings from the region of STN in untreated parkinsonian patients (4, 5, 20). Thus synchronization within this frequency range may be primarily pathological and related to a chronic state of dopaminergic underactivity. It remains to be seen whether LFPs recorded from the STN in rodent models of Parkinson's disease, such as the 6-hydroxydopamine-lesioned rat, contain synchronized activity in the 15–30 Hz band.

A spectral peak at approximately 8 Hz was also found and was more widespread than activity in the upper γ band. This lower frequency oscillation may relate to periodic whisker movements (so-called "whiskering" or "whisking"; 10, 17) and/or α activity (18). In particular, a preliminary report suggests that synchronized LFP activity in the α band can be detected in the rat striatum and globus pallidus and is highly correlated with the cortical α rhythm (2). Note also that there was considerable power, but no discrete peak, at frequencies below 2 Hz. Oscillatory activity at such low frequencies is well described in the rat STN, both in organotypic cocultures (14) and in anesthetized *in vivo* preparations (12).

The finding of high γ activity in the rat strongly suggests that similar activity found in the levodopa-treated parkinsonian human is also likely to arise in STN and to be physiological rather than a consequence of, or adaptation to, the parkinsonian state. This activity was increased by D2 receptor stimulation, in keeping with the importance of D2 receptors in the so-called "indirect pathway" of the basal ganglia, in which the STN lies (1, 6). However, before accepting that the effect of quinpirole upon high-frequency activity was exerted at the level of the indirect pathway, we must consider an alternative suggestion. The systemic administration of quinpirole was accompanied by a trend toward increased motor activity and it may have been this altered behavior that led indirectly to an increase in high-frequency LFP activity. Nevertheless, we would consider this an unlikely explanation. Indeed, the increase in motor activity after quinpirole injection was not significant, while the increase in upper γ band activity was such that there was almost three times more power in this frequency range compared to that seen during sustained motor activity in the absence of drug. In any case, it is possible that the increase in high-frequency activity occurring in the setting of movement is itself caused by the increased release of endogenous dopamine under these circumstances (8, 15, 16).

Peaks in the power spectra of LFPs recorded from STN most likely reflect the tendency for synaptic inputs and/or STN discharges to synchronize in certain frequency bands (4). A more detailed understanding of the nature of the LFP must await correlations of local spike activity with LFP oscillations. Such correlations would also provide further evidence that oscillatory activity is locally generated rather than volume conducted, but were beyond the scope of the present study.

The finding of upper γ band activity sufficiently synchronized to be picked up through local field potential recordings made with semimicroelectrodes and macroelectrodes in STN in the rat and human, respectively, suggests that this activity is likely to subserve some basic function as it appears conserved across very different species. Synchronization increases postsynaptic efficacy at subsequent projection targets, while nonlinearities in the frequency–current relationship of basal ganglia neurons may further increase the saliency of inputs in particular frequency bands (3). One possibility is that synchronous high-frequency oscillations may act to temporally coordinate circuits in the production of a motor act in a way analogous to that posited in perceptual binding (9). The loss of this high-frequency activity in parkinsonian humans would support the hypothesis that it is necessary for, or at least strongly associated with, voluntary movement. Thus, the alert rat provides a useful model for testing the neurophys-

iological and pharmacological bases of these rhythms, through systemic and local injections of dopamine agonists, antagonists, and other modulatory agents.

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