Independent Neuronal Oscillators of the Rat Globus Pallidus

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In vivo, neurons of the globus pallidus (GP) and subthalamus nucleus (STN) resonate independently around 70 Hz. However, on the loss of dopamine as in Parkinson’s disease, there is a switch to a lower frequency of firing with increased bursting and synchronization of activity. In vitro, type A neurons of the GP, identified by the presence of I_h and rebound depolarizations, fire at frequencies (≤80 Hz) in response to glutamate pressure ejection, designed to mimic STN input. The profile of this frequency response was unaltered by bath application of the GABA_A antagonist bicuculline (10 μM), indicating the lack of involvement of a local GABA neuronal network, while cross-correlations of neuronal pairs revealed uncorrelated activity or phase-locked activity with a variable phase delay, consistent with each GP neuron acting as an independent oscillator. This autonomy of firing appears to arise due to the presence of intrinsic voltage- and sodium-dependent subthreshold membrane oscillations. GABA_A inhibitory postsynaptic potentials are able to disrupt this tonic activity while promoting a rebound depolarization and action potential firing. This rebound is able to reset the phase of the intrinsic oscillation and provides a mechanism for promoting coherent firing activity in ensembles of GP neurons that may ultimately lead to abnormal and pathological disorders of movement.

INTRODUCTION

In the presence of normal dopamine drive, neurons of the basal ganglia (BG) oscillate independently at gamma frequencies (around 70 Hz) (Brown et al. 2001). However, on loss of dopamine in idiopathic and experimental models of Parkinson’s disease, neurons of the globus pallidus (GP) and subthalamus nucleus (STN), among others in the BG, lose their independence and show increases in burst firing and synchronization of activity (Filion and Tremblay 1991; Nini et al. 1995). This neuronal activity favors frequencies either in the β range (approximately 20 Hz), a frequency that exacerbates Parkinson’s symptoms and contribute to abnormal motor patterns (Brown and Marsden 1998; Levy et al. 2000) or bursting activity in the theta range (4–12 Hz), which appears phase related to resting tremor (Bergman et al. 1994; Magnin et al. 2002). This switch in the pattern and frequency of neuronal activity may be reversed on administration of levodopa or by initiation of movement, a finding that has led to the proposal that 70 Hz is the optimum frequency for carrying motor commands (Brown et al. 2001).

It has previously postulated that coupled GP–STN network constitutes a central pattern generator that is able to maintain synchronized burst discharges (Plenz et al. 1999). In vivo, the cortex appears to play a fundamental role in driving the GP–STN neurons (Magill et al. 2000) dopamine depletion rendering the system more sensitive to rhythmic cortical input (Magill et al. 2001). Nevertheless, a small proportion of STN and GP cells does oscillate in the absence of cortical input, indicating that either a local network of GP–STN neurons or intrinsic neuronal properties are able to sustain such activity (Magill et al. 2001).

Consequently, burst firing, oscillations, and neuronal synchronization could arise through the properties of individual cells and/or network properties of neuronal ensembles. Whole cell patch-clamp techniques were used to record from single and pairs of GP in vitro in a study of the interplay between intrinsic oscillations and synaptic conductances and to test the hypothesis that the intrinsic properties of an individual neuron may confer independent activity while enhanced synaptic efficacy or connectivity leads to coherent activity in neuronal networks. We show that, in response to excitatory agents, type A GP neurons (see Cooper and Stanford 2000, equivalent to type II GP neurons of Nambu and Llinas 1994) preferentially fire at a frequency that is determined by intrinsic subthreshold membrane oscillations, while GABA inhibitory postsynaptic potentials (IPSPs) are able to produce rebound spiking that promotes coherent firing in multiple GP neurons through a phase realignment of this intrinsic neuronal activity.

METHODS

In vitro slice preparation

Slices of GP/striatum (300-μm thick) were obtained from 9- to 16-day-old Wistar rats as previously outlined (Stanford and Cooper 1999). Briefly, animals were first anesthetized with fluorothane and killed by decapitation in accordance with the Animals (Scientific Procedures) Act 1986, UK. The brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (aCSF) containing (in mM) 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.3 MgCl_2, 2.4 CaCl_2, and 10 glucose, buffered to pH 7.4 with 26 NaHCO_3 saturated with 95% O_2–5% CO_2. Slices were cut using a DTK-1000 Microslicer (Dosaka, Japan) and transferred to a holding chamber or recording chamber at 32–34°C where they were perfused continuously at 2–3 ml/min with aCSF.

Electrophysiological recording

Whole-cell recordings were made using borosilicate glass pipettes of 3–6 MΩ resistance containing (in mM) 125 K-glucuronate, 10 NaCl, 1 CaCl_2, 2 MgCl_2, 10 BAPTA, 10 HEPES, 0.3 GTP, and 2 Mg-ATP, adjusted to pH 7.25 with KOH. Individual neurons were visualized (40× water immersion objective) using differential interference con-
contrast infrared microscopy (Olympus BX 501, Japan) with CCD camera (Hitachi KP-M1, Japan) linked to a contrast enhancement system (ADV-2, Brian Reece Scientific Ltd, UK).

Membrane current and potentials were recorded using an Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA). All current clamp recordings were made in Axopatch 200B fast mode; data were amplified and low-pass filtered at 5 kHz and digitized at a frequency of 10 kHz using CED micro-1401 SIGAVG (Cambridge Electronic Design). Auto- and cross-correlation analysis was conducted using the waveform correlation of SPIKE3 (CED, UK).

Drugs were applied by pressure ejection (4 – 40 kPa) from a glass micropipette using a Picospritzer III (Parker Hannifin) or by additions to the superfusate by exchanging the aCSF in the recording chamber for one differing only by the addition of a known concentration of drug, with the exchange beginning after a dead time of approximately 20 s. Drugs used were N-(2,6-dimethylphenylcarbamoylmethyl) triethyl-ammonium bromide (QX-314), bicuculline methiodide, (RS)-3,5-dihydroxyphenylglycine (DHPG), ZD 7288 (all from Tocris) and TTX (Alomone).

RESULTS

Type A GP neurons, which constitute 63% of the neuronal GP population, have been previously identified by the presence of anomalous inward rectifier ($I_h$) and the low threshold calcium conductance ($I_t$), which gives rise to a rebound depolarizations (see Fig. 1A and Cooper and Stanford 2000).

Local pressure ejection of glutamate (100 mM, $n = 23$), potassium (100 mM, $n = 4$), or the mGluR1 agonist DHPG (20 mM, $n = 5$) was used to initiate activity in populations of type A GP neurons simultaneously. In a single cell, this produced a depolarizing envelope (1–8 s in duration, 4–20 mV amplitude) and a burst of action potentials (Fig. 1B). Autocorrelation analyses of the evoked activity revealed rhythmic oscillatory activity in the frequency range 8–80 Hz. All spiking activity was abolished by bath application of TTX (1 μM, $n = 4$, Fig. 1Ci), showing dependence on sodium ions, and eliminated when recording with intracellular solutions containing the sodium channel blocker QX-314 (5 mM, $n = 6$, Fig. 1Cii), indicating that the oscillatory activity is promoted by the intrinsic properties and not by the extrinsic synaptic or electrotonic activity. Increasing the duration of the pressure ejection enhanced the depolarizing envelope and the number and frequency of action potential firing (Fig. 1D) while bath application of the GABA$_A$ antagonist bicuculline (10–20 μM, $n = 4$)

![Fig. 1](http://jn.physiology.org/)

Pressure ejection of glutamate evokes burst activity, which is independent of a GABA synaptic activity. A: typical records of membrane potential of a quiescent type A globus pallidus (GP) neuron in response to 300-ms current steps (25-pA increments) from resting membrane potential. Type A neurons were characterized by a time- and voltage-dependent sag in the membrane potential indicative of $I_h$, the rebound depolarization and action potential firing on repolarization of the membrane. Bi: pressure ejection of glutamate (100 mM, arrow) in the vicinity of the type A GP neuron produces membrane depolarization and a burst of action potentials. Arrows at the end of the trace highlight membrane oscillations. Bii: autocorrelation analysis (Waveform correlation - SPIKE3) of the activity presented in Bi indicates a peak interspike interval of 17 ms (vertical dashed line) corresponding to a firing frequency of 62 Hz. The analysis window is shown by dotted lines. Ci: bath application of 1 μM TTX abolishes membrane oscillations and firing activity. Cii: when recording with 5 mM QX-314 in the patch pipette, pressure pulses of glutamate elicited a depolarizing envelope but no membrane oscillations or action potentials. D: increasing the duration of the glutamate pulse enhances the depolarizing envelope and the number and frequency (as determined by autocorrelation analysis) of evoked spikes. Bath application of the GABA$_A$ antagonist bicuculline (10 μM) was without effect on the response profile.
was without effect on the evoked activity, indicating a lack of involvement of a local GABAergic network.

Recording from two type A GP cells simultaneously, pressure ejection of glutamate (100 mM, from a single source) onto the pair evoked a burst of activity in each cell. Usually, each cell fired at markedly different frequencies resulting in insignificant cross-correlations (Fig. 2C), indicating that each type A GP cell acts as an independent oscillator. Indeed, when each cell fired action potentials at similar frequencies resulting in positive cross-correlations, the observed coherent activity was always accompanied by a variable phase difference, such that cell 1 may lead cell 2 or vice versa in response to a single glutamate application.

At resting membrane potential or in response to small depolarizing current injections, 65% (28/43 cells) of type A neurons displayed prominent subthreshold membrane oscillations (Fig. 3A). The frequency of these oscillations could be further increased by membrane depolarization, although they were often masked by action potential firing on the crest of the oscillation.

As with glutamate-evoked activity, subthreshold membrane oscillations were not observed when recording with QX-314 in the pipette (5 mM, \( n = 3 \)) and were abolished by bath application of TTX (1 \( \mu M, \ n = 5 \), Fig. 3B), indicating their dependence on sodium ions and most probably the voltage-dependent, persistent sodium current. The oscillations were also unaffected by bath application of the specific \( I_h \) blocker.

**FIG. 2.** Type A GP cells act as independent oscillators. A: records of membrane potential from two cells recorded simultaneously. Application of glutamate (100 mM, from a single source) onto the pair evoked a burst of activity in each cell. B: autocorrelations show pronounced peaks at 25 and 31 ms, respectively, (dashed lines) corresponding to firing frequencies of 40 and 32 Hz. C: cross-correlation analysis of the two cells in B show no correlated activity.

**FIG. 3.** Type II GP cells exhibit TTX-sensitive subthreshold membrane oscillations. A: records of membrane potential from a type A GP cell of resting membrane potential -63 mV. Depolarizing current injections revealed subthreshold membrane oscillations prior to action potential firing. Further membrane depolarization induced a cessation of action potential firing presumably as a result of fast sodium channel inactivation. B: sample record highlighting membrane oscillations, which were abolished by bath application of TTX (1 \( \mu M \)). Records were taken at a membrane potential of -30 mV and have been low-pass filtered at 500 Hz. C: autocorrelation analysis of control trace shown in B, revealed a peak at 29 ms, corresponding to frequency of oscillation of 34 Hz.
During a glutamate-induced burst, single IPSPs were evoked by electrical stimulation GABA afferent input, within 500 μm of the recorded cell. Each IPSP was followed by a rebound depolarization, thus resetting the phase of the oscillation. On the crest of the rebound depolarization, action potentials were observed to fire in a temporal “window” (Fig. 4A). The time frame of this temporal window appeared reduced on membrane depolarization. Thus, when a cell is depolarized, the action potentials following an IPSP show enhanced temporal alignment over the next 75–100 ms (Fig. 4B, 12 cells). An evoked train of IPSPs disrupted the tonic firing, and spike clustering developed (Fig. 4C). Indeed, spike clustering was observed on presentation of multiple hyperpolarizing current pulses, indicating the probable interaction between the hyperpolarizing IPSPs and intrinsic membrane conductances. When recording from two cells simultaneously, single-shock electrical stimulation produces IPSPs followed by rebound depolarization and action potential firing in each cell. Although precise synchrony was not observed, rebound action potentials fired within a time window following each IPSP (Fig. 4D).

**DISCUSSION**

Type A cells constitute the major population of neurons in the GP (Cooper and Stanford 2000) (analogous to type II projection neurons of Nambu and Llinás 1994–1997). Although these cells can fire in excess of 300 Hz when driven by current injections, when exposed to agents that mimic excitatory STN synaptic input, they preferentially fire a burst of action potentials at frequencies similar to those of intrinsic subthreshold membrane oscillations. This type of oscillation has been shown to play an active role in determining firing threshold and pace population rhythms in a variety of neurons, when suitably coupled with chemical or electrical synapses (Cobb et al. 1995; Llinás 1988). In many cell types these oscillations are generated by one or more mechanisms involving the interaction of a
depolarizing and slow hyperpolarizing currents (Llinás 1988). In the GP, the depolarizing phase of the oscillation is abolished by extracellular TTX or intracellular QX-314, indicating involvement of a voltage-dependent sodium conductance. Hence, they may be dependent on the interplay of intrinsic persistent sodium current and another slow outward current, such as leak potassium current.

Evoked GABA IPSPs are able to produce rebound spiking that promotes coherent firing in multiple GP neurons through a phase realignment of the intrinsic oscillations. This temporal realignment of the membrane oscillation, following an IPSP, promotes a “window of opportunity” for cell firing of duration <10 ms. Our slice preparation and stimulation protocol does not allow us to define the specific GABA input responsible for resetting the phase of the oscillation. Indeed, the evoked IPSPs may be striatopallidal or pallido–pallidal in origin.

There is extensive anatomical evidence for local GP axon collaterals (Kita and Kitai 1994, Nambu and Linas 1997) innervating the perisomatic region of pallidal neurons (Kita 1994), which would undoubtedly promote large-scale local inhibition. In the slice preparation, spontaneous IPSPs presumably arising from active GP cells in the slice (Cooper and Stanford 2000) can be observed. Moreover, type A–type A GP synaptic connectivity has been observed in 1 of 40 recorded pairs (I. M. Stanford, unpublished observations). This relatively low ratio may reflect the need for optimization of slice thickness and orientation (P. Magill, personal communication).

Although striatal afferents principally innervate the dendritic shafts of GP neurons (Falls et al. 1983, Okayama et al. 1987), their numbers and climbing fiber arrangement suggests that IPSPs originating from this source are powerful enough to reset the phase of intrinsic oscillations. At present it is unknown whether one axon innervates several GP cells, which would have to be the case for phase resetting in multiple GP cells. Alternatively, the synchronized activity of striatopallidal inputs is required. Although some correlation between the up and down states of membrane potential in individual striatal cells has been observed (Stern et al. 1998), the evidence for precise timing of striatal activity is scant. Nevertheless, an increase in the synchronization of striatal neuron membrane potential and general neuronal excitability and has been reported in 6-hydroxydopamine–lesioned animals (Tseng et al. 2001). Furthermore, recent computer modeling studies have indicated that correlated oscillatory activity within the STN–GP network is enhanced by the increases in striatopallidal input (Terman et al. 2002).

In conclusion, intrinsic subthreshold membrane oscillations permit individual GP neurons to express independent activity, while GABA synaptic activity is able to optimize the activity into structured patterns. This activity is then relayed to the STN, which appears to be a fundamental requirement for the recruitment of the STN in such activity via rebound excitation (Bevan et al. 2000; Plenz et al. 1999). Thus, GABA phasing of intrinsic oscillations in GP cells may play a central role in the breakdown of independent neuronal activity and parallel processing required for normal function, the disruption of which leads to abnormal and pathological disorders of movement.

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