Firing rate and pattern heterogeneity in the globus pallidus arise from a single neuronal population

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Deister CA, Dodla R, Barraza D, Kita H, Wilson CJ. Firing rate and pattern heterogeneity in the globus pallidus arise from a single neuronal population. J Neurophysiol 109: 497–506, 2013. First published October 31, 2012; doi:10.1152/jn.00677.2012.—Intrinsic heterogeneity in networks of interconnected cells has profound effects on synchrony and spike-time reliability of network responses. Projection neurons of the globus pallidus (GPe) are interconnected by GABAergic inhibitory synapses and in vivo fire continuously but display significant rate and firing pattern heterogeneity. Despite being deprived of most of their synaptic inputs, GPe neurons in slices also fire continuously and vary greatly in their firing rate (1–70 spikes/s) and in regularity of their firing. We asked if this rate and pattern heterogeneity arises from separate cell types differing in rate, local synaptic interconnections, or variability of intrinsic properties. We recorded the resting discharge of GPe neurons using extracellular methods both in vivo and in vitro. Spike-to-spike variability (jitter) was measured as the standard deviation of interspike intervals. Firing rate and jitter covaried continuously, with slow firing being associated with higher variability than faster firing, as would be expected from heterogeneity arising from a single physiologically distinct cell type. The relationship between rate and jitter was unaffected by blockade of GABA and glutamate receptors. When the firing rate of individual neurons was altered with constant current, jitter changed to maintain the rate-jitter relationship seen across neurons. Long duration (30–60 min) recordings showed slow and spontaneous bidirectional drift in rate similar to the across-cell heterogeneity. Paired recordings in vivo and in vitro showed that individual cells wandered in rate independently of each other. Input conductance and rate wandered together, in a manner suggestive that both were due to fluctuations of an inward current.

globus pallidus; basal ganglia; patch-clamp recording; slice; IH; synchrony

ALTHOUGH CELLS OF A SPECIFIC morphological and physiological type are often treated as identical, any one quantity measured in a sample of them often exhibits a wide range of variation. Some kinds of heterogeneity among cells may be of small functional importance, or offset by some other parameter, but some are fundamental to the function of the cell. Spontaneous firing rate heterogeneity is a critical factor for many network properties. For example, the amount of heterogeneity in spontaneous firing rate and regularity of neurons determine whether those neurons will synchronize in response to common input or to mutual interconnections (Wang and Buzsáki 1996; White et al. 1998; Mancilla et al. 2007; Padmanabhan and Urban 2010). The globus pallidus (GPe) consists of autonomously active GABAergic neurons (Chan et al. 2004) that share common inputs and connect to each other via local axon collaterals (Kita and Kita 1994; Sadek et al. 2007). However, the GPe does not display synchronous rhythms and there are no clear correlations between its neurons in healthy mammals (Bar-Gad et al. 2003; Nini et al. 1995; Raz et al. 2000; Mallet et al. 2008) or even among neurons in slices (Stanford 2003). In contrast, strong correlations between GPe neurons emerge in Parkinson’s disease patients and MPTP-treated primates (Levy et al. 2002; Nini et al. 1995; Raz et al. 2000). Bar-Gad et al. (2003) suggested that there must be some mechanism that decorrelates GPe cells under normal circumstances and that breaks down in Parkinson’s disease. One possible decorrelator of GPe cells activity is rate heterogeneity. For example, correlations among spontaneously active neurons persist for long periods after the stimulus, if the cells are highly regular and firing at the same rate, but decay rapidly if the cells vary widely in natural frequency.

Studies in behaving animals have revealed the existence of two GPe cell types based on firing pattern (e.g., DeLong 1971). There are also two distinctly different types of GPe cell based on cytochemical markers and axonal targets, and these differ in firing rate when recorded in vivo (Mallet et al. 2012). Initial in vitro studies reported the presence of two distinct cell populations in GPe (Nambu and Linas 1994, 1997; Cooper and Stanford 2000) based on their membrane potential responses to injected current pulses. The relationships between these three different measures of cell types have not been determined. However, the fact that GPe cells fall into two different groups with different properties by itself cannot account for the general state of decorrelation in the GPe. Instead it might suggest the possibility for a clustered state, with correlations among cells of the same type and perhaps a different kind of correlation between types. It also does not help to understand how synchrony could re-emerge in the disease state.

Unlike the earlier reports, the most recent studies in slices have not revealed discrete cell types differing qualitatively in intrinsic properties. These studies suggested that a single spontaneously active cell population accounts for the majority of GPe neurons, but these neurons exhibited a wide range of firing rates (Chan et al. 2004, 2011; Hashimoto and Kita 2006; Günay et al. 2008). If the GPe cell types do not differ qualitatively in their composition of ion channels, they may differ quantitatively, and that might account for the broad variation in rate within the population of spontaneously active cells. This explanation is suggested in the modeling study by Günay et al. (2008). Their study suggests that if cells of all types in the GPe exhibit continuous variations in ion channel composition, it could account for the entire range of firing rates and patterns observed in the nucleus. Intrinsic heterogeneity in this popu-
loration that results could account for the lack of synchrony in healthy animals and a mechanism for controlling synchrony.

We determined the degree of heterogeneity of GPe neurons in vivo and in vitro and investigated its cellular basis in synaptically isolated GPe neurons. We show that there is significant heterogeneity in GPe, which is not accounted for by variations in synaptic input or different cell populations but is instead the result of intrinsic factors. We also show that these variations arise from continuous drift in cellular properties over a time frame of a few minutes, rather than from permanent variation across cells. The firing of any neuron can, over the course of minutes to hours, spontaneously wander through all the states of firing seen in the population.

**METHODS**

**Animal handling and slice preparation.** For all the in vitro studies, Sprague-Dawley rats (Charles River) aged 15–25 days were used. Animal handling and all procedures were approved by the University of Texas, San Antonio and University of Tennessee Health Science Center Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines. We made all efforts to minimize the discomfort to the animals and the number used. Animals were anesthetized with either a lethal dose of ketamine (160 mg/kg) and xylazine (24 mg/kg), or isoflurane (≥2.5%), and then rapidly decapitated. Brains were quickly, but carefully, removed and placed into ice-cold artificial cerebrospinal fluid (aCSF) comprised of the following (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2.0 MgSO₄, 10 dextrose, and 2.0 CaCl₂ continuously bubbled with 95% O₂-5% CO₂ (carbogen). Tissue blocks containing the GPe and its surrounding structures were prepared and sliced at 300-μm thickness in either the sagittal or coronal plane using a vibrating tissue slicer (Leica VT-100us). After the initial preparation, slices were allowed to equilibrate at either 37°C for 30 min or at room temperature for 1 h before they were used.

**Electrophysiological recordings.** Slices were transferred to a recording chamber that was continuously perfused with oxygenated aCSF at a rate of 2 to 3 ml/min. The temperature was maintained at 33–35°C. Infrared differential interference contrast (IR-DIC) video microscopy was used to visualize slices through a ×40 water-immersion lens equipped to either a Zeiss Axioscope or an Olympus BX51 upright microscope. Patch pipettes were pulled from thin-walled borosilicate glass (outer diameter = 1.5 mm and inner diameter = 1.17 mm) using either a P-97 or P-87 Flaming-Brown electrode puller (Sutter Instruments). For perforated-patch recordings, a “whole cell” solution containing the following (in mM): 140.5 K₂MeSO₄, 0.2 EGTA, 7.5 NaCl, 10 HEPES, 2 NaATP, and 0.2 NaGTP, adjusted to a pH of 7.3 with KOH was used. Gramicidin-D (Sigma) was added to this solution at a final concentration of 100 μg/ml and sonicated 15 to 30 min before recording. Pipette tips were most often filled with the same whole cell solution that was free of gramicidin, but in practice this appeared unnecessary for successful seal formation or perforation. A large and rapid decrease in series resistance, or abrupt increase in spike height, during a perforated-patch recording was interpreted as a rupture of the seal; unit isolation was confirmed by the existence of an absolute refractory period. The locations of the electrode tips were monitored periodically by X-ray imaging. On the last day of recordings, recording sites were marked by electrolytic lesion and locations were verified histologically.

**Data analysis and statistics.** In vitro recordings were made using an Axopatch 200B (Molecular Devices). Signals were digitized at 10–20 kHz and low-pass filtered with a corner frequency of 5–10 kHz.

![Fig. 1. Heterogeneity of globus pallidus (GPe) neuron firing rate in vivo.](https://www.jn.org)

**In vivo recordings.** In vivo recordings were performed in two 350- to 400-g male Sprague-Dawley rats. In a preparatory surgery, rats were anesthetized with an intraperitoneal injection of ketamine (85 mg/kg) and xylazine (15 mg/kg) and placed in a stereotaxic frame. A recording chamber and two stainless steel head-holding tubes were fixed to the skull with dental acrylic. For recording, rats were anesthetized with 2.5% isoflurane, mounted in a stereotaxic device, and subsequently maintained with 1.0–1.5% isoflurane. Glass-coated elgiloy alloy microelectrodes (1.5–2.5 MΩ at 1 kHz) were advanced into the GPe and signals were amplified, band-pass filtered at 0.7–2 kHz (model 1800 AC amplifier; A-M Systems, Sequim, WA), digitized, and recorded. Spikes were detected with a window discriminator; unit isolation was confirmed by the existence of an absolute refractory period. The locations of the electrode tips were monitored periodically by X-ray imaging. On the last day of recordings, recording sites were marked by electrolytic lesion and locations were verified histologically.

![Fig. 1. Heterogeneity of globus pallidus (GPe) neuron firing rate in vivo.](https://www.jn.org)
Digitized data were acquired for subsequent off-line analysis using PCclamp 8.1 software (Molecular Devices) or Igor Pro 6 (WaveMetrics, Lake Oswego, OR). Data analysis was performed with Mathematica (Wolfram Research, Champaign, IL), and statistical tests were performed with the software package R (R Foundation for Statistical Computing, Vienna, Austria). Wilcoxon signed rank test for repeated measures, or Mann-Whitney U-test (2 groups) and Kruskal-Wallis test (>2 groups) for unpaired samples, were used to evaluate whether sample groups differed significantly. A P value of < 0.05 was used as our significance level for all tests. All numerical data are expressed as the value ± the sample-corrected SE.

RESULTS

Heterogeneity of firing rate and regularity. To characterize the nature of heterogeneity seen among rodent GPe neurons in vivo, we made single-unit extracellular recordings from GPe neurons in anesthetized (1.0–1.5% isoflurane) rats. We analyzed each cell firing as a series of 1-min long records of spontaneous firing for 25 single units. Average firing rate ranged from 15.5 to 114.6 spikes/s (mean = 42.8 ± 4.8 spikes/s; n = 25). Firing patterns varied substantially among cells, as indicated by the examination of their interspike interval histograms (Figs. 1, A and B). However, both rate and the variability in rate, as estimated using the standard deviation of interspike-intervals (ISIs), varied continuously, and there was a very consistent relationship between ISI variability and average firing rate. This relationship was well described by a simple hyperbolic function (Fig 1C). The range of ISI coefficient of variations measured was 0.36–1.32. Firing rates and patterns showed a similar range to recordings made in primates under similar conditions (Kita et al. 2004, 2005), although with a slower average firing rate. In intact primates, blockade of local synaptic transmission increases the regularity of firing of individual cells but has less effect on firing rate, suggesting that rate heterogeneity has an intrinsic basis (Kita et al. 2004, 2005).

To determine how much of the heterogeneity in firing rates could be of intrinsic origin, we obtained cell-attached recordings of the spontaneous firing pattern of GPe neurons in slices. GPe neurons were selected for recording based solely on their apparent health, which was assessed by standard criteria in the IR-DIC image (Stuart et al. 1993). We chose the cell-attached technique due to its relatively noninvasive nature, allowing us to study the firing pattern without washout of critical currents or cellular processes. Neurons displayed a range of autonomous firing rates and patterns, from a relatively regular pattern (Fig. 2A), to a highly irregular pattern (Fig. 2C). The range of firing rates (2.3–40.6 spikes/s) overlapped that for the in vivo recordings, but they were on average slower (mean = 11.6 ± 1.97 spikes/s) and more regular. The recordings included for this analysis had durations between 2 and 5 min. Neurons displaying the most regular firing patterns were characterized by a relatively high firing rate and narrow ISI histograms (Fig. 2B). Neurons that displayed irregular patterns showed sponta-

Fig. 2. Firing patterns of GPe neurons from in vitro cell-attached recordings. A: portion of a spike train recorded from a neuron firing in a regular fashion. B: example ISI histogram from the same neuron in A. C: portion of a spike train recorded from a more irregularly firing neuron. D: ISI histogram of the irregular neuron shown in C. E: lot of the standard deviation (σ) of ISIs against mean firing rate for each neuron in our sample. Distribution was well described at rates >1 Hz, by a simple hyperbolic function. F: scatter-plot of average firing rate plotted against the age of the animal from which the neuron was recorded from.
neous firing interrupted by variable periods of silence (Fig. 2C). The ISI histograms of the irregular neurons displayed a central mode with a long tail skewed towards long intervals (Fig. 2D). The relationship between mean firing rate and the SD for all neurons was well approximated by a simple hyperbolic function that was qualitatively similar to our in vivo recordings but with less variability. At rates well in excess of 1 spikes/s, the variability of ISIs was ~20% of the ISI (Fig. 2E), as opposed to 80% in vivo. Near 1 spike/s, variability increased dramatically so that firing was no longer periodic, and cells firing <1 spike/s were not observed. The mean SD of the ISIs was 0.11 ± 0.05 s, with a range of 0.002 to 1.19 s. The range of ISI coefficient of variation measured was 0.06–2.71 (data not shown). There was no clear relationship between the age of animal used in our sample and mean firing rate (Fig. 2F) in our sample (r = 0.16; P > 0.4) or between age and SD of the ISIs (r = −0.10; P > 0.6).

Much of the irregularity of firing seen in vivo is not present in slices, as expected if it is due to perturbations of the autonomous firing pattern by ongoing synaptic input. To test the possibility that irregularly arriving synaptic inputs may contribute to the remaining component of firing irregularity seen in slices, we recorded the spontaneous firing pattern in the cell-attached configuration and pharmacologically blocked synaptically activated currents. GPe projection neurons receive inhibitory synaptic input that is both GABA_A and GABA_B mediated (Kaneda and Kita 2005), in addition to excitatory input that is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. In our preparation, the most likely source of spontaneous synaptic input is from other GPe neurons, which is GABAergic (Sadek et al. 2007). The main source of excitatory input is from axons of neurons in the subthalamic nucleus, which are severed in our parasagittal slices (Hallworth and Bevan 2005; Loucif et al. 2005). Bath application of the GABA_B receptor antagonist CGP-55845 (1 μM) had no significant effect on firing rate (Fig. 3A) and neither did subsequent application of the GABA_A receptor antagonist SR-95531 (gabazine; 15–20 μM) along with MK-801 (50 μM), an NMDA receptor antagonist and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; 10 μM), an AMPA receptor antagonist (mean rate = 12.8 ± 3.01 spikes/s for control, 13.32 ± 3.37 spikes/s for CGP-55945, and 16.77 ± 4.27 for gabazine, MK801, and NBQX; P > 0.4, Kruskal-Wallis rank sum test). The variability of firing was also unaffected by blocking synaptic inputs (Fig. 3B; mean SD = 0.15 ± 0.08 s for control, 0.11 ± 0.06 s for CGP-55845, and 0.07 ± 0.04; P > 0.4, Kruskal-Wallis rank sum test). In addition, the relationship between SD and firing rate, in each condition, was well described by the same hyperbolic relationship as the control cells (Fig. 3C). These results indicated that the remaining variability observed in slices arises primarily from sources intrinsic to the neuron and not from synaptic input from other GPe cells or other sources. Neither rate nor

![Fig. 3. Synaptic inputs do not contribute to the distribution of GPe neuron firing rates and patterns. A: plot of SD of ISIs plotted as a function of mean firing rate for cells recorded in control artificial cerebrospinal fluid (black dots), CGP-55845 (blue dots), and a cocktail of gabazine (GBZ), MK-801, and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; red dots). Solid continuous line is the same hyperbolic function plotted in Fig. 1. B: sample distributions of mean firing rates across conditions. C: sample distributions of standard deviation in ISIs across conditions.](image-url)
variability was distributed discontinuously, suggesting variations within a single set of intrinsic cell properties.

Individual GPe neurons can fire at all observed rates and patterns. If the observed heterogeneity of rate and regularity all arise from variations within a single physiological cell type, a change in the firing rate of any neuron might cause a change in variability that maintains their relationship as seen in our cell-attached sample. To test this, we used perforated-patch clamp recordings from visually identified GPe neurons at 34–35°C. We chose the perforated-patch clamp method because it gives electrical access to the neuron without washout of key currents or intracellular processes. Once the perforation process had stabilized, we recorded the spontaneous discharge of GPe neurons and found the firing rate ranged from 3.8 to 35.1 spikes/s with an average firing rate of 13.1 ± 2.2 spikes/s (n = 13), similar to our cell-attached sample. We then applied constant hyperpolarizing currents to slow the rate of firing. We found that as the firing rate decreased so did the regularity (Fig. 4A). All GPe neurons examined were capable of highly irregular firing upon hyperpolarization as seen also in whole cell recordings (Hashimoto and Kita 2006). All cells were also capable of firing at low mean rates (Fig. 4, B and C). The relationship between rate and regularity across all neurons in our sample was the same as the relationship our cell-attached population displayed (Fig. 4C). This result suggested that the variations in firing pattern seen among the sample of GPe cells could arise from gradual changes in firing rate of each cell in the population.

We extended our cell-attached recordings for longer recording periods, which ranged between 35 and 90 min (n = 14). During these long-duration cell-attached recordings, we observed spontaneous fluctuations in baseline firing rate (Fig. 5A). One consequence of these fluctuations in baseline firing was to skew the distribution of ISIs, which can be seen in three example ISI histograms show in Fig. 5B. We observed that neurons with slower mean firing rates tended to be more skewed than those firing at higher mean rates (data not shown).

These results suggested that the distribution of intervals was distorted by nonstationarity of firing rate over the long recordings. We partitioned the long-duration recordings into groups of 100 adjacent spikes and plotted the SD within each group and the corresponding mean instantaneous firing rate. For data segments partitioned in that way, the relationship between rate and regularity was restored (Fig. 5C). Moreover, the slow changes in rate within cells measured in this way recreated the rate-variability relationship seen for cells made to fire at various rates. Breaking up a long record from an individual neuron into smaller segments could recreate all, or nearly all, of the rate and variability heterogeneity seen in across-cell samples of spontaneous activity. This result showed that the firing rates and patterns of individual neurons may wander, so that over a few minutes, each cell could visit all the firing rates and patterns seen in the population. If the cell wandered independently of each other, these changes could account for the variations seen within the population at any one moment.

Nearby cells fluctuate in rate independently, both in vivo and in vitro. To determine if slow spontaneous rate fluctuations in GPe neurons occurred asynchronously, we analyzed long (30–90 min) records of GPe neuron firing from simultaneously recorded neurons in vivo and in vitro. The distance between cells in vivo was <1 mm (mediolateral distance was 0.2 mm, and the dorsoventral distance was <0.5 mm). In vitro, the distance between recorded cells was ~150–600 μm. The firing rate of neuronal pairs consistently differed and showed varying degrees of slow drift (Fig. 6, A and B). Correlations between rate in pairs recorded in vivo and in vitro were weak or nonexistent (Fig. 6C). This was true either when an equal number of individual ISIs were correlated with each other (in vivo mean r = 0.006 ± 0.004, Pearson’s product-moment correlation; n = 18; in vitro mean r = −0.001 ± 0.08; n = 12), which would emphasize fast time-scale correlations, or when rate was averaged for 100 ISIs (mean in vivo r = 0.02 ± 0.03, Pearson’s product-moment correlation; n = 18; mean in vitro r = 0.07 ± 0.12; n = 12), which emphasizes slower correla-

Fig. 4. Individual GPe neurons can fire over the entire continuum of rate and pattern. A: portions of spike trains recorded from an example GPe neuron recorded in the perforated patch configuration with varying amplitudes of hyperpolarizing current injections. Note the significantly different time-scale for the −48 pA current injection. B: frequency-current relationship for the neuron in A. C: plot of r of intervals against mean firing rate, along with the same hyperbolic function shown in Figs. 1 and 2, for the neuron shown in A. Current injection amplitude is denoted above each symbol. Inset: compiled results from 13 neurons subjected to hyperpolarizing current injections.
tions. Linear correlations determined for fast and slow time scales were not significant, and the timescales were not significantly different (in vivo $P > 0.05$, Wilcoxon signed-rank test; $V = 69; n = 18$; in vitro $P > 0.05; V = 28; n = 12$). Similar to previous in vivo monkey (Raz et al. 2000; Bar-Gad et al. 2003) and rodent work (Mallet et al. 2008), we found that cross correlograms computed for 10000 spikes showed no correlations (Fig. 6B). Thus slow and fast fluctuations in basal firing rate are independently governed both in vivo and in vitro.

Consistent with the notion that GPe neurons comprise a heterogeneous network, we found that mean firing rate differed for neurons within a pair as much as for neurons recorded at different times. On average the difference in mean firing rate between neurons was 17.5 ± 3.6 spikes/s in vivo ($n = 18$ pairs), and 9.6 ± 1.6 spikes/s in vitro ($n = 12$ pairs; Fig. 6D). The rate differences among pairs was not significantly different for in vivo and in vitro conditions ($P > 0.05$, Mann-Whitney $U$-test; $W = 81$). Thus, at any moment, there will be significant rate heterogeneity among the GPe neuron population, and the mechanism responsible for its maintenance appears to have an intrinsic basis.

Intrinsic signatures of autonomous rate heterogeneity. Our data suggest that individual GPe neurons fire within a continuum of rate and pattern and are each capable of occupying any part of that continuum in a time frame inconsistent with changes in ion channel expression. Therefore, fluctuations in intrinsic conductances should determine at any moment a GPe neuron’s background firing state. We sought to determine the nature of the intrinsic conductance that underlies transitions in autonomous firing rate. To accomplish this, we measured the input resistance periodically as we recorded spontaneous firing in current clamp by delivering 1.5-s long hyperpolarizing current injections (~90 to ~160 pA), every 30–40 s (Fig. 7A) in the perforated patch configuration. We then averaged the mean firing rate for 100 spikes preceding each current injection. We were interested in the input resistance during firing, not conductances activated by our hyperpolarizing pulse, so we used the peak hyperpolarization achieved at the onset of the pulse (minimum $V_m$). Because GPe neurons do not have a resting membrane potential, input resistance was measured as the difference between the minimum $V_m$ reached during the pulse and the average subthreshold membrane potential during the preceding 100 spikes used to calculate rate (subthreshold $V_m$). This distance was normalized by the amplitude of the current injection (Figs. 7, B and C). A statistically significant correlation between input resistance and rate was found in five out of six neurons. The average correlation coefficient was $r = 0.5$ ± 0.1 ($n = 6$); the range was $-0.5$ to 0.87. These results provide evidence that an intrinsic conductance does vary with rate. The association of an increase in conductance with an increase in rate suggested that the variation with rate was an increase in an inward current, not a decrease in an outward one. There are two key inward currents that influence pacemaking in GPe neurons: persistent $Na^+$ and those contributed by the hyperpolarization-activated cyclic-nucleotide gated channel (HCN; Chan et al. 2004; Mercer et al. 2007). These currents differ greatly in reversal potential.

Fig. 5. Spontaneous rate fluctuations in GPe neurons. A: ISI plotted as a function of spike time for 3 examples cell-attached recordings from long duration (35–90 min) recordings. Examples were chosen to represent the range of firing rates observed. B: ISI histograms for the 3 examples shown in A and color coded in the same way. C: plot of SD against mean firing rate for the 3 examples shown in A. Each point represents the SD and mean firing rate calculated in a bin of 100 adjacent spikes.
To estimate the reversal potential for the current that was covarying with rate, we measured the rate-related changes in voltage during the relatively depolarized ISI trajectories of the subthreshold $V_m$, and compared that to the rate dependence of the membrane potential during the hyperpolarizing pulse. These were measured as the slope of the rate vs. $V_m$ plot for the two cases (Fig. 7C, right). These slopes give the degree to which the membrane potential is altered by the membrane current or currents that are changing with firing rate. Because those currents should still be present during the hyperpolarizing current pulse, the difference in slopes of the regression lines should be proportional to the current responsible for the rate changes. The slope of the regression line was significantly larger for minimum $V_m$ than subthreshold $V_m$ ($P < 0.05$, Wilcoxon signed-rank test, $V = 21; n = 6$; Fig. 7D). When we plotted the average regression slope values as a function of $V_m$, we found that the $x$-intercept of the best fit line was $-29$ mV (Fig. 7D). This indicates that the conductance that primarily covaries with rate should reverse at $-29$ mV during the subthreshold $V_m$, in line with nonspecific cation channels such as the known pacemaking current contributed by HCN channels (Santoro and Tibbs 1999).

DISCUSSION

Spontaneous firing in synaptically isolated GPe neurons is rhythmic, but there is substantial variability in ISI, presumably due to the stochastic nature of ion channel opening (White et al. 2000; Diba et al. 2004; Dudman and Nolan 2004). When cells are firing slowly, the effects of small fluctuations in ionic currents can accumulate during the ISI, and so it is expected that the standard deviation of ISIs would increase roughly in proportion to the period (e.g., Geisler and Goldberg 1966). Consistent with this view, in GPe neurons firing faster than $5$ spikes/s, the standard deviation of ISIs scaled linearly with period, being about one-fifth of the average interval. When GPe cells fired more slowly, the variation in ISIs increased dramatically, becoming unmeasurably large at rates around $1$ spike/s. This occurred because GPe cells could not maintain repetitive firing at these rates and instead fired repetitively at a higher rate for brief periods, which were interrupted by pauses of variable duration. This transition to episodic firing occurred regardless of whether firing rate was slowed spontaneously or experimentally by passage of constant hyperpolarizing current. In this firing pattern, neurons reside close to the boundary between firing and silence and spontaneously shift between repetitive firing and pausing under the influence of very small slow changes in excitability and their intrinsic noise. Although the average firing rate of GPe neurons appears to smoothly decrease to arbitrarily small rates with hyperpolarization, which is referred to as class 1 excitability (e.g., Izhikevich 2007), in fact the cells have a minimum repetitive firing rate between one and five spikes per second. Below this rate they...
shift spontaneously between episodes of silence and low-frequency firing.

**Origin of rate heterogeneity.** Early studies indicated that there may be as many as three electrophysiologically distinct types of neurons in the rodent GPe (Nambu and Llinas 1994, 1997, Stanford and Cooper 1999; Cooper and Stanford 2000). Later authors have suggested that there is one predominant subtype that is autonomously active and fires in a tonic fashion (Chan et al. 2004; Günay et al. 2008; Deister et al. 2009). One source of confusion is that quiescent cholinergic neurons from the basal forebrain appear to be intermingled with GPe (Chan et al. 2004). Of the two groups of GABAergic, nonquiescent, neurons characterized by Stanford and Cooper (1999), one appears to be more depolarized and tonically firing, while the other displays pauses and tends to fire at lower rates. Our results do not explain all the differences between these studies, but they do suggest that these different cell types may be extremes in the rate heterogeneity of a single cell type, differing in their momentary availability of pacemaking currents. A similar conclusion was made by Günay et al. (2008), who showed that they could explain the multidimensional variability in a large sample of cell recordings with a family of compartmental models that differed in the relative availability of pacemaking currents. However, there is an important mechanistic distinction between our results and those of Günay et al. (2008), in that our data suggest there is little variation in the distribution and composition of ion channels within GPe neurons and that it is the functional contributions of a relatively fixed set of ion channels that vary on a moment-to-moment basis. This moment-to-moment variability within a constant set of ion channels may also explain the results of Bugaysen et al. (2010), who also observed that GPe neurons exhibit a wide range of firing rates in slices. They showed that GPe neurons varied along a number of dimensions, including the action potential durations, spike frequency adaptation, afterhyperpolarization amplitude, maximal firing frequency, and the effectiveness of HCN currents. They separated the cells into three groups on the basis of the combination of these characteristics and reported a difference in average firing rates and variability among the groups, with cells firing faster also firing more regularly. However, the groups overlapped greatly along all the measured parameters, including firing rate.

The present results suggest that the relative availabilities of faster spike-generating pacemaking currents are a function of how much tonic depolarizing current GPe neurons are subject to, and this is ultimately what determines the amount of heterogeneity among pallidal neurons. Our results do not definitively implicate variations in HCN current as regulating the overall amount of tonic depolarization but do suggest they play a role. HCN currents are slow, and their availability is modulated directly by intracellular cAMP, which binds directly to the HCN channel increasing its open probability (Wainger et al. 2001; Wang et al. 2002), providing a potential substrate for slow time-scale modulation. In addition, downregulation of HCN expression and altered autonomous firing accompanies chemically induced dopamine depletion (Chan et al. 2011). Whether this leads to more correlated population discharge is still unknown, but we would predict that it would reduce rate heterogeneity and make the GPe more vulnerable to synchronizing inputs. Spontaneous firing rate distributions in 6-OHDA-
lesioned rats show an increase in mean but still show a broad range (Miguelez et al. 2012), suggesting that channels other than HCN may contribute to rate heterogeneity. It is unclear if the presently reported slow rate fluctuations persist in dopamine-depleted animals, or if heterogeneity is impacted in vivo, but this should be an interesting target for future investigations. Edgerton and Jaeger (2011) have shown that dendritic Na+ channels may contribute to an active decorrelation mechanism in the GPe by reducing the propensity to synchronize to rhythmic input extrinsic to the GPe. It is still unclear how pathologic synchrony in the basal ganglia, including the GPe, develop, but it is likely that a variety of mechanisms contribute. Heterogeneity could both decrease the propensity to inherit correlations extrinsic to the GPe and decrease the propensity for the GPe itself to synchronize.

Immunocytochemical studies have revealed at least two separate subpopulations of pallidal neurons (Hoover and Marshall 1999; Kita and Kita 2001; Mallet et al. 2012). The majority of GPe neurons are parvalbumin positive, with a portion of the population expressing enkephalin. There are also subpopulations of GPe cells distinguished by their axonal projections, as some GPe neurons project primarily caudally to subthalamic nucleus and globus pallidus internus, whereas others project to the striatum (Kita and Kita 2001; Sadek et al. 2007; Mallet et al. 2012). Because we did not identify our cells according to this classification, we cannot be certain that we sampled both sets of neurons. We did, however, record from GPe neurons whose firing rate covered the entire range described in those studies. The intrinsic firing patterns of neurons firing over the entire range of spontaneous rates exhibited a common relationship between firing rate and regularity, and all cells could be made to fire in all patterns as their rates were adjusted using intracellular current. This indicates that a common set of physiological properties govern the firing of at least the most common type of GPe cells over the entire range of firing rates seen in the nucleus. Our results do not rule out the possibility that the GPe cell subpopulations may vary more subtly in their intrinsic electrophysiological properties, but many of their physiological differences seen in vivo might also arise from various network-level mechanisms such as differences in synaptic connections, including their innervation by striatal or subthalamic afferents.

Functionally important heterogeneity in cell properties is usually assumed to arise either from discrete cell classes or from static (or slowly changing) differences in expression of ion channels. Our results suggest another kind of heterogeneity, that results from neurons wandering through the parameter space defined by their ion channels. Slow modulation of ion channels is a well-established mechanism for changing the intrinsic properties of neurons, but neurons subjected to an extrinsic modulator should shift their properties together. In vivo, extrinsic modulators might coordinate the parametric wander of GPe cells. Slow multisecond oscillations of rate have been observed in globus pallidus neurons in vivo, and their frequency and strength are sensitive to dopamine agonists and antagonists (Ruskin et al. 1999); any potential relationship between this slow oscillation and drift in intrinsic parameter space is still yet to be determined.

**Heterogeneity and synchrony.** Activity among pallidal neurons is normally desynchronized, whereas synchrony in the beta-frequency band accompanies dopamine depletion in rodents, monkeys, and human Parkinson’s disease patients (Nini et al. 1995; Raz et al. 2000; Mallet et al. 2008; Levy et al. 2002). The generator (or generators) of beta-frequency activity is not known, but sharing of synaptic inputs among GPe cells is a likely source of synchronization. The ratio of striatal projection neurons to GPe neurons is ~60 to 1 (Oorschot 1996), and the striatopallidal neuron axonal arbor is large and can influence many pallidal neurons (Chang et al. 1981; Kawaguchi et al. 1990). Despite this, correlations between neurons are rarely observed in healthy animals (Raz et al. 2000). This unexpected phenomenon has led to the speculation that the GPe may actively decorrelate itself for functional reasons (Bar-Gad et al. 2003; Nini et al. 1995). For oscillating neurons, rate heterogeneity is a powerful decorrelator. Neurons firing at similar rates approach each other in phase a little (or a lot) with each shared input, and this influence accumulates over repeated shared signals. Neurons firing at widely different rates do not accumulate synchronization. Rate heterogeneity created by autonomous independent slow fluctuations in rate may contribute to decorrelation of activity in the healthy GPe.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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