Neurons in Both Pallidal Segments Change Their Firing Properties Similarly Prior to Closure of the Eyes

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INTRODUCTION

The basal ganglia (BG) are usually defined in terms of the cortico–BG neural network architecture. In this network (Albin et al. 1989; DeLong 1990) projections from all cortical areas are integrated within the striatum and projected to the BG output nuclei—i.e., the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). Transmission of information within the BG occurs both directly from the striatum to the GPi/SNr and indirectly through the external segment of the globus pallidus (GPe) and subthalamic nucleus (STN) (Haber and Gdowski 2004). Since the striatal origins of the direct and indirect pathways are oppositely affected by D1 and D2 dopamine receptors (Gerfen et al. 1990; Surmeier et al. 2007), this implies reciprocal discharge patterns between GPe and GPi neurons. Another mechanism that supports GPe–GPi reciprocal activity is the recent anatomical findings showing inhibitory connections from the GPe to the GPi. These connections are predicted to play a powerful role in the regulation of GPi discharge since they are often in close proximity to the soma and proximal dendrites of these neurons (Harratzi et al. 1990; Kita 2001, 2007; Sato et al. 2000).

The direct/indirect view of the BG network has been reinforced by evidence from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–treated monkeys showing an increase in GPi discharge rates but a decrease in the GPe (Filion and Tremblay 1991; Miller and DeLong 1987). Moreover, in response to dopamine replacement therapy, both in human patients with Parkinson’s disease (PD) (Hutchinson et al. 1997; Levy et al. 2001; Merello et al. 1999) and in MPTP-treated primates (Filion et al. 1991; Heimer et al. 2002; Papa et al. 1999), the opposing changes in discharge rates shifted back. However, physiological studies conducted on normal animals have not supported this view (Elias et al. 2008), in that the discharge of neurons in both pallidal segments is similarly, rather than oppositely, modulated during the execution of movements (Georgopoulos et al. 1983; Nambu et al. 1990; Turner and Anderson 1997) and reward-related events (Arkadir et al. 2004; Gdowski et al. 2001; Joshua et al. 2009). Thus the similarity in behaviorally triggered responses (Mitchell et al. 1987; Turner and Anderson 1997, 2005) between the GPe and GPi neurons challenges the current models of BG connectivity. We sought to shed light on these conflicting results by further investigating the spontaneous activity of GPe and GPi neurons in different brain states rather than by studying their responses to phasic behavioral events.

The cortex exhibits changes in its patterns of activation in different brain states, such as during slow-wave sleep and under anesthesia (Contreras and Steriade 1997; Evarts 1964; Hobson and Pace-Schott 2002; Steriade et al. 1993; Webb 1976). Recent studies have even reported changes in cortical activation that were associated with “resting” states (Poulet and Petersen 2008) and with the eye state (McAvoy et al. 2008). The cortex has a strong influence on the spontaneous discharge properties of neurons in the BG (Aldridge et al. 1990; Nambu et al. 2000, 2002a; Yoshida et al. 1993) and in fact the cortico–BG loop architecture suggests that discharge modulation of basal ganglia neurons should follow the cortical changes. However, subcortical loops from the midbrain via the thalamus have the capacity to influence activity in the BG (McHaffie et al. 2005). To provide further insights into the relative role of subcortical versus cortical drive of the BG, we

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compared the spontaneous discharge properties of neurons in the cortex (primary motor cortex [M1]) and both pallidal segments in the transition between different brain states—upon closing and opening of the eyes.

METHODS

Animals

Two monkeys (W; Vervet, Cercopithecus aethiops, female 4.5 kg; and L; Macaque fascicularis, female 4 kg) were used in this study. Monkey L was trained on a probabilistic classical conditioning task (Joshua et al. 2008, 2009). At the end of the experiment after a recovery period, monkey L was sent to a primate sanctuary (http://www.ipsf.org.il). Monkey W was not engaged in a behavioral task and was trained only to sit quietly in the primate chair. Monkey W was systematically treated with MPTP after recording in the normal state (Rivlin-Etzion et al. 2008) and its neural activity was also recorded after development of the clinical signs of PD. It was perfused through the heart with saline followed by a 4% paraformaldehyde solution within 30 min of its death, which occurred 13 days from the first MPTP injection. The MPTP data are not included in this report.

All experimental protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Hebrew University guidelines for the use and care of laboratory animals in research. The experimental protocols were approved and supervised by the Institutional Animal Care and Use Committee of the Hebrew University and Hadassah Medical Center. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care International accredited institute. Materials and methods were given in detail in previous studies (Joshua et al. 2008; Rivlin-Etzion et al. 2008). Here we give a brief summary of these methods and provide information on the methods not used in the previous reports.

Recording and data acquisition

During recording sessions, the monkeys’ heads were immobilized and eight glass-coated tungsten microelectrodes were advanced separately (EPS, Alpha-Omega Engineering, Nazareth, Israel) into the arm-related area of the motor cortex (monkey W) and into the GPi or the GPe (monkeys W and L). Pallidal recordings were not restricted to specific areas within the GPe and GPi, but spanned the entire nuclei. Two experimenters controlled the position of the eight electrodes and real-time spike sorting (AlphaMap, ASD, Alpha-Omega).

A titanium screw was implanted above the frontal area of the skull for electroencephalograhic (EEG) recordings in monkey L. The recorded EEG was amplified with a gain of 500 and band-pass filtered with a 1-100 Hz four-pole Butterworth filter and sampled at 1.56 kHz.

Near-infrared digital video cameras were used to record the monkeys’ facial movements (monkey W: AVC 307R B/W, Avtech systems, Taipei, Taiwan; monkey L: AVer-s 2.54, AVerMedia Systems, Taipei, Taiwan). The recordings were made at a sampling rate of 9–13 and 50 frames/s (monkey W and monkey L, respectively). Video analysis was carried out on custom software to identify periods when the monkeys’ eyes were closed (Mittelman et al. 2009). We divided the recording times into two distinct periods, depending on the state of the monkey’s eyes. Periods when the monkey’s eye was closed continuously for >1 s (which is longer than the typical duration of monkey blinks, 50-ms down phase; Baker et al. 2002) were considered “eye closed” states. Other periods (including short, <1 s periods with closed eyes, i.e., suspected blinks) were considered as “eye open” states.

EEG data analysis

EEG data from all recording days were visually inspected and we discarded any day in which the signal was contaminated with artifacts (mainly due to cross talk between recording channels). The final analysis was run on 18 of 26 recording days. The recorded EEG signal was digitally low-pass filtered at 50 Hz using an 11th-order Butterworth zero-shift filter. Subsequently, the EEG signal was divided into segments according to the distinction between “eye open” and “eye closed” states. To compare the amplitude of the EEG waves between states we calculated the root mean square (RMS) of the signal in each state for each day. We compared the RMS in the two states using a paired Wilcoxon signed-rank test. The power spectrum of the EEG signal was calculated separately in each state using a fast Fourier transform with a window size of 5%, 50% overlap, and a frequency resolution of 0.2 Hz. The power spectral density in each state was normalized (divided by the mean power of all 1- to 50-Hz frequencies) and averaged across days.

Neuronal data analysis

Recorded spike trains were subjected to an off-line quality analysis, which included tests for discharge rate stability, refractory period, spike waveform isolation, and recording time. As a first step, the firing rate was graphically displayed and visually inspected. The largest continuous segment of data with a stable discharge rate was selected for further analysis. To rule out the possibility that cells with very large modulations in discharge rate due to transitions between the “eye open” and “eye closed” states had been excluded, we repeated the analysis using the cells’ entire recording durations and obtained similar results (data not shown). Second, cells in which >0.02 of the total interspike intervals (ISIs) were <2 ms were excluded from the database (Fee et al. 1996). Third, only pallidal and M1 cells with an isolation score (Joshua et al. 2007) >0.8 and 0.7, respectively, were used. We repeated our analysis on the subpopulation of M1 neurons that had isolation scores >0.8 (n = 68 cells) and obtained similar results to those reported in the following text (data not shown). Fourth, for the pallidal population of cells, only high-frequency discharge neurons (i.e., discharge rate in the “eye open” state >20 Hz) were included in the database. This was done to exclude GPe low-frequency discharge bursters (DeLong 1971), which may represent a different population of GPe neurons (Francois et al. 1984; Kita and Kita 1994). Finally, the recording time (after off-line quality analysis) of each cell was segmented into “eye open” versus “eye closed” states; thus each cell had multiple distinct recording segments in each state. Only cells that fulfilled the aforementioned inclusion criteria (discharge stability, isolation quality, and refractory period) for >240 s and had ≥5 s (not necessarily continuous) of recording in each state (“eye open” and “eye closed”) were included in the database. Monkey L was engaged in a behavioral task during recording sessions. However, even during behavioral sessions, the monkey paused in its task from time to time and closed its eyes. These periods were used for the analysis in this report. Thus in monkey L, the average recording duration of neurons was 3,162 and 256 s in the “eye open” and “eye closed” states, respectively. In monkey W, which was not engaged in a behavioral task, the average recording duration of the neurons was similar in the two states and was 267 and 342 s in the “eye open” and “eye closed” states, respectively (see details in Table 1).

Discharge rate analysis

We calculated the mean discharge rate for each neuron in the “eye open” and “eye closed” states and tested the difference in discharge rate between the two states at both the single-cell and the population level.

At the single-cell level, for each neuron we first computed the discharge rates in 1-s bins separately for the “eye open” and “eye closed” states and then found the absolute difference between the means of these
Recording statistics were calculated separately for each monkey (W and L) and for each neural population. Values are means ± SD, with confidence intervals in parentheses. The isolation score is from zero to one. Fraction ISI < 2 ms is the fraction of ISIs shorter than 2 ms out of all ISIs of a cell. Recorded time is the total time that satisfied the inclusion criteria of the analysis database. Recorded time “eye open”/“eye closed” states was significant at both the single-cell level and the population level. At the single-cell level we used a resampling (bootstrap) method to test the significance of the “discharge rate difference” (Efron and Tibshirani 1993). The discharge rates in 1-s bins for both states (closed and open) were shuffled and resampled repeatedly into two groups (of the same size as in the original groups) and the “discharge rate difference” was calculated for each replication. This process was repeated 2,000 times and the P value of the discharge rate difference was estimated as the fraction of replications for which the “discharge rate difference” calculated on the original data were larger than that calculated on the shuffled data.

At the population level we tested the difference in discharge rate using a paired Student’s t-test comparing the mean discharge rates in the “eye open” state with those in the “eye closed” state. Data in M1 are displayed in logarithmic scale for clarity. Finally, since monkey L (and not monkey W) was engaged in a behavioral task we also performed this analysis on neurons not recorded during task performance and obtained similar results (data not shown).

We characterized the time course of the changes in discharge rates between the “eye open” and “eye closed” states by peristimulus time histograms (PSTHs) aligned to the closing and opening of the eyes. PSTHs were constructed for two time periods: 1) 60 s prior to 10 s following eye closing and 2) 10 s prior to 60 s following eye opening. These PSTHs were calculated in 1-ms bins and smoothed with a Gaussian window with SD of 300 ms. To deal with edge effects we padded the PSTHs with a mirror image of the PSTH edge for half the length of the smoothing window. We then smoothed the padded PSTH with the Gaussian window and removed the padded edges. Since “eye open” and “eye closed” time segments were continuous and had different durations, there could be a temporal overlap between the two constructed PSTHs. Furthermore, some of the segments were not the full length of the PSTH. These short segments were included in constructing the single-cell PSTH; however, a cell had to have at least one full-length segment to be included in the average population PSTH. For each cell, we denoted the average discharge rate in the “eye open” state as the baseline discharge rate. The population PSTH was calculated as the average deviation of the single cells’ PSTHs from their baseline discharge rates.

Discharge pattern analysis

We used the coefficient of variation (CV: SD to the mean) of the ISI distributions in the “eye open” and “eye closed” states to assess the differences in discharge patterns. As described earlier, each cell had multiple distinct recording segments in each state with different durations. In the motor cortex where cells can have low (<1 Hz) discharge rates (Abeles 1991), short recording segments can bias the ISI distribution and the CV values by excluding long ISIs. This could be especially problematic since the recording segments in the two eye states (open and closed) may have had different durations. To overcome this bias, another inclusion criterion for the discharge pattern analysis was added. For each cell we examined the ISI distribution of the entire recording time (without segmentation into “eye open” and “eye closed” states) and found its 99th percentile. Only recording segments, in both states, whose duration exceeded the 99th percentile time threshold were used in comprising the ISI distributions. Furthermore, only cells that had in total at least five ISIs in each state (Prut and Pelmutter 2003) were used in calculating the CV values. In addition, we calculated the CV values using ISI distributions that were constructed from recording segments that exceeded an absolute time threshold of 10 and 20 s and obtained similar results (data not shown).

In all three populations (M1, GPe and Gpi), we tested whether the difference in CV values between the “eye open” and “eye closed” states was significant at both the single-cell level and the population level. At the single-cell level we used a resampling (bootstrap) method. The ISIs of both states (closed and open) were shuffled and resampled repeatedly into two groups and the test statistic (T) was calculated for each replication as follows

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\[
\text{CV} = \frac{\text{SD}}{\text{Mean}}
\]

where SD is the standard deviation and Mean is the mean of the ISI distribution. This statistic was calculated for each replication. This process was repeated 2,000 times and the P value was calculated as the fraction of replications for which the “discharge rate difference” calculated on the original data were larger than that calculated on the shuffled data.
Arousal Modulation of Pallidal Discharge

The subjects were closing their eyes and relaxing. These large EEG waves were absent when the subjects opened their eyes (Buzsáki 2006; Nunez and Srinivasan 2006). Indeed we found a difference in the EEG signal between the two states. Figure 1A shows an example of the EEG recorded in the two states (third row), as well as its corresponding root mean square (RMS) values (fourth row) and spectral density (fifth row). In the “eye closed” state the EEG signal was characterized by high-amplitude waves reflected in larger RMS values. We averaged the RMS values in the open/closed states separately for each recording day (Fig. 1B). The difference between the averaged RMS values was highly significant ($P < 0.001$, Wilcoxon signed-rank test). In addition, the EEG power distribution averaged across days (Fig. 1C) was concentrated in different frequency bands in the two states. These differences in the EEG signal suggest that the distinction between “eye closed” and “eye open” states is indicative of different brain states. We tend to assume that these different brain states reflect different arousal levels. However, since we did not record neck EMG, multiple-contacts EEG, or other physiological parameters required for a definitive assessment of arousal level (Schulz 2008; Silber et al. 2007), we restricted ourselves to the more conservative definition of brain states.

Neuronal database

Out of 732 cells recorded, 174 neurons from the GPe, 75 neurons from the GPi, and 102 neurons from M1 met the quality and inclusion criteria and were studied in both states (Table 1; for details see METHODS).

Figure 2 depicts three examples of neurons recorded in the “eye open” (first row) and “eye closed” (second row) states, one for each area (column). The GPe neuron in Fig. 2A showed a decrease in discharge rate in the “eye closed” state (second row) compared with the “eye open” state (first row). This change was accompanied by an increase in the frequency of pauses and by a heavier tail of the ISI distribution (third row). Figure 2B shows an example of a GPi neuron, which like the GPe neuron decreased its discharge rate in the “eye closed” state (second row) and had a higher frequency of long ISIs (third row). Finally, the M1 neuron in Fig. 2C, unlike the two pallidal neurons, increased its discharge rate in the “eye closed” state and exhibited a burstier discharge pattern (compare second row with first row).

To summarize, these illustrative cells exhibited differences in discharge rates and in discharge patterns between the eye open/closed states; in the following text we give a detailed description of the changes found in discharge variables both at the single-cell and at the population level of all neurons recorded. We do not present the cross-correlation functions of the recorded neurons since the typically short duration of each segment in the “eye closed” state (Table 1) did not enable us to reliably assess the changes in the cross-correlation functions of simultaneously recorded pairs of neurons (especially for M1 low-discharge-rate neurons).

Opposite rate modulations of pallidal and cortical neurons in the transition to the “eye closed” state

Figure 3 shows the mean discharge rates in the “eye open” and “eye closed” states of all recorded neurons in the three neuronal...
populations. High-frequency discharge (HFD) neurons in the GPe and GPi displayed similar rate modulations in the transition from the “eye open” to “eye closed” state. Most of the neurons in the GPe and GPi showed significantly lower mean discharge rates in the “eye closed” state compared with those in the “eye open” state and only a few cells showed the reverse (Fig. 3, A, B, and D). Our pallidal recordings were not restricted to specific areas within the GPe and GPi, but spanned the entire nuclei, and this effect size was uniformly distributed. At the population level the mean discharge rate was also significantly lower in the “eye closed” state in both areas (Table 2 and Fig. 3, D and E).

M1 neurons displayed a less profound change compared with pallidal neurons in their mean discharge rates on closure of the eyes. Moreover, this change was in the opposite direction from the pallidal decrease in discharge rate. At the single-cell level, many M1 neurons showed a significant increase in their mean discharge rate in the transition from the “eye open” to the “eye closed” state, whereas only a few showed a decrease (Fig. 3, C and D). This difference was also significant at the population level (Table 2 and Fig. 3, D and E). M1 neurons showed more diverse discharge rate changes compared with the pallidum (Fig. 4) and thus the difference between the two states, at the population level, was weaker (Fig. 3E). However, at the single-cell level, the relatively small absolute changes in M1 discharge rates constituted a substantial relative change (due to M1 low basic discharge rates). Thus on average M1 neurons increased their firing rates on closure of the eyes by 0.46 spike/s and 61.83%, whereas GPe and GPi neurons decreased their average discharge rates by 8.02 and 8.34 spikes/s and 10.28 and 9.64%, respectively.

To appreciate the time course of the changes in discharge rates we examined the averaged PSTHs aligned at closing and opening of the eye. In both the GPe and the GPi the discharge rate changes were gradual, starting (10–25 s) prior to eye closing and continuing after eye opening (Fig. 5, A and B and Supplemental Fig. S1 for continuously recorded neurons). However, the increase in discharge rates after eye opening was steeper, especially in the GPe (Fig. 5A). The gradual decrease and increase in pallidal discharge rate strengthen the assumption that the differences in the neuronal discharge between the “eye open” and “eye closed” states were not merely due to the change in visual inputs (Linden et al. 2009), but rather reflect a more global process of changes in the monkeys’ arousal levels. Unlike the pallidal rate modulation, the dynamics of discharge rate changes in M1 neurons was not gradual (Fig. 5C and Supplemental Fig. S1), corresponding to the time course of changes in EEG (Fig. 5D). The analysis performed earlier used a population average; therefore it is possible that individual pallidal cells had abrupt discharge rate transitions (e.g., from high to low firing rates) whose timing differed between cells, thus leading to a population average with a smooth transition. We therefore examined and analyzed (change point analysis;
FIG. 2. Pallidal and M1 cortical neurons have different discharge properties in the “eye open” state compared with the “eye closed” state. A: example of neuronal activity from a single GPe cell from monkey L. Top row: raster in the “eye open” state. Middle row: raster in the “eye closed” state. Each raster contains 15 traces of 5 s each. Traces are not necessarily consecutive. Below each raster is an example of a 1-s analog trace of extracellular recording filtered between 300 and 6,000 Hz. An asterisk marks the trace in the raster from which the analog example is taken. Below the 1-s analog trace are examples of spike waveforms. The spike waveform plot includes 100 superimposed 1.5-ms waveforms selected randomly from the whole recording time of the cell. Last row: histogram of the logarithm of the interspike intervals (ISIs) in the 2 states (bin width 0.1 ms). B: example of neuronal activity from a single GPi cell from monkey L. Same conventions as in A. C: example of neuronal activity from a single cell in the arm related area of the primary motor cortex of monkey W. Same conventions as in A, spike waveform duration = 2 ms. GPe and GPi, external and internal segments of the globus pallidus.
Gallistel et al. (2004) the time course of discharge rate changes at the level of single trials/cells and found that the population mean reflected the single elements in the network (data not shown). However, the strong variability in the duration of single trials did not enable us to reliably assess the time course at the single-cell level and this possible confounding effect should be further examined in future studies.

**Pallidal and cortical neurons have increased discharge variability in the “eye closed” state**

We further looked at the discharge patterns of the neurons in the “eye open” and “eye closed” states using the coefficient of variation (CV) of the ISI histogram. The CV is a measure of the regularity of firing (CV = 1 for a Poisson process, < 1 for a more regular process, and > 1 for a bursty firing pattern).

In M1, sufficient data (see METHODS) to calculate the CV were available for 88 cells. These M1 cells displayed diverse CV values, ranging from 0.68 to 4.13. Many neurons showed a significant increase in their CV value in the transition from the “eye open” to the “eye closed” state, whereas only a few showed a significant decrease (Fig. 6, C and D). This difference was highly significant at the population level of the CV (Table 3 and Fig. 6, D and E) and implied a more bursty discharge pattern in the “eye closed” state. In addition, the ISI distributions showed a more predominant occurrence of shorter ISIs (<10 ms) in the “eye closed” state (see example of ISI distributions in Fig. 2C, last row) as well as less negative skewness values in accordance with higher discharge rates and more bursty firing patterns (Supplemental Fig. S2).

As in the cortex, in both pallidal areas CV values were significantly larger in the “eye closed” state (Fig. 6, A and B) compared with the “eye open” state both at the single-cell level (Fig. 6D) and at the population level (Table 3 and Fig. 6, D and E). We examined the difference in skewness and kurtosis values of the ISI distributions between the “eye open” and “eye closed” states (Supplemental Fig. S2). In the GPe and in the GPi, both parameters were significantly larger (Student’s%

**FIG. 3.** Pallidal and cortical neurons have opposite rate modulations in the “eye closed” state. A: discharge rates (in Hz) of GPe neurons (n = 174). Each point represents the average discharge rate of a single neuron in both states. Abscissa: average discharge rate in the “eye closed” state; ordinate: average discharge rate in the “eye open” state. Black diagonal line is the equality line, i.e., points above this line represent cells for which the discharge rate in the “eye open” state > “eye closed” state. Color code: red is for cells where the discharge rate was significantly higher in the “eye open” state (P < 0.05, bootstrap). Blue is for cells where the discharge rate was significantly lower in the “eye open” state. Gray is for cells where the difference was not significant. Small black arrow points to the GPe cell in Fig. 2A. B: discharge rates of GPi neurons (n = 75). Same conventions as in A. Black arrow points to the GPi cell in Fig. 2B. C: discharge rates of motor cortex neurons (n = 102) in logarithmic scale. Same conventions as in A. Black arrow points to the M1 cell in Fig. 2C. D: summary of percentage of cells with significant differences in discharge rate between “eye open” and “eye closed” states for all 3 neuronal areas. Color code is the same as in A. E: surprise value (minus the logarithm of the statistical P value, Student’s paired t-test) of the differences in discharge rates between “eye open” and “eye closed” states in each neuronal area. The surprise of a P value of 0.05 is 2.996; note the value for all neuronal areas is far greater.

**TABLE 2. Discharge rate statistics**

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of Cells</th>
<th>“Eye Open”</th>
<th>“Eye Closed”</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPe</td>
<td>174</td>
<td>75.63 ± 24.08 (22.8–143.86)</td>
<td>67.61 ± 22.43*** (18–130.55)</td>
</tr>
<tr>
<td>GPi</td>
<td>75</td>
<td>82.02 ± 23.66 (38.28–156.45)</td>
<td>73.68 ± 21.15*** (34.41–134.45)</td>
</tr>
<tr>
<td>CTX</td>
<td>102</td>
<td>3.18 ± 3.79 (0.1–15.68)</td>
<td>3.65 ± 4.21*** (0.11–18.32)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with ranges in parentheses. Discharge rate statistics were calculated separately for each neural population. ***P < 0.001, paired t-test. GPe and GPi, external and internal segments of the globus pallidus, respectively; CTX, cortex.
paired t-test, $P < 0.001$) in the “eye closed” state. These changes in the higher moments of the ISI distributions indicate that the ISI distributions were less symmetric in the “eye closed” state and more positively skewed (see examples of ISI distributions in Fig. 2, A and B, last row; and population means of skewness and kurtosis values in Supplemental Fig. S2).

Nonetheless, the higher moments of the ISI distributions as well as the CV values were in different ranges in the GPe compared with those in the GPi. CV values in the GPe ranged from 0.38 to 1.92, whereas in the GPe they showed higher average values and a higher degree of divergence ranging from 0.22 to 5.71 (Table 3). Furthermore, in the GPi skewness values were close to zero, which is the value describing a symmetrical distribution, and kurtosis values were near 3, which is the kurtosis of a normal distribution. In the GPe, however, skewness values were more positive and kurtosis values suggested the distributions were not normal (Supplemental Fig. S2). These differences are concordant with the basic “pauser” characteristic and heavy-tailed ISI distributions of the GPe neurons as opposed to the GPi (DeLong 1971; Elias et al. 2007).

We therefore looked at specific parameters of the pauses, in particular the percentage of the pauses of the total ISIs and their mean duration in the “eye open” and “eye closed” states. To identify the pauses we used three different definitions of pauses: two absolute thresholds that are frequently used as definitions of GPe pauses (ISIs >300, 500 ms; DeLong 1971) and an algorithm that maximizes the surprise function to detect pauses (“surprise” method; Elias et al. 2007). We also introduced a short absolute threshold of 150 ms (to include the long ISIs of the GPi). In the GPe, according to all four definitions, the percentage of pauses of the total ISIs was significantly larger in the “eye closed” state (Fig. 7A). The mean duration of the pauses was also significantly longer (in three of the four definitions; Fig. 7B), although the difference was small. In the GPi the percentage of pauses of the total ISIs was significantly larger in the “eye closed” state, only according to the 150-ms absolute threshold definition, and marginally significant ($P = 0.0568$) according to the “surprise” method (Fig. 7C). The mean duration of the GPi long ISIs in the “eye open” state did not differ significantly from the “eye closed” state. In conclusion, on the one hand, our “pause” analysis further revealed the differences between GPe and GPi neurons, where the duration of the long silent intervals was much longer in the GPe compared with that in the GPi, despite their similar discharge rates (68 and 76 vs. 74 and 82 spikes/s in GPe and GPi, respectively, in the eye closed/open states, Table 2). On the other hand, the “pause” analysis suggests that the changes in discharge pattern observed in the transition between the “eye open” and “eye closed” states in both pallidal segments can be attributed primarily to the rise in the frequency of pauses (or long silent intervals) and not to the duration of these pauses.

Finally, we tested the time course of the changes in the pallidal discharge pattern by examining the dynamics of the changes in CV values (Fig. 8 and Supplemental Fig. S3). Estimates of CV values and their dynamics were affected by the size of the analysis moving window (thus the low discharge rate of M1 neurons did not enable a reliable estimation of fast changes in their discharge pattern). The discharge of pallidal cells was characterized by rare (<1%, Fig. 7) long ISIs. Therefore CV values calculated using short windows mainly represent common short ISIs, whereas larger analysis windows enable detection of effects created by the long ISIs. Changes in the pallidal CV values calculated using a large analysis window (50 ISIs, ~1 s; Fig. 8) were gradual, exhibiting kinetics similar to that observed in the discharge rate modulations (Fig. 5). The changes in the pallidal CV values calculated using the shorter window size (5 ISIs, Supplemental Fig. S3), however, were less profound and displayed a steeper dynamics. These results suggest that changes in the pallidal discharge pattern might reflect two processes, differentially affecting the pallidal short and long ISIs. We examined and analyzed (change point analysis; Gallistel et al. 2004) the time course of pallidal discharge pattern changes at the single-cell level and found that the population mean, most probably, reflected the single elements in the network (data not shown). However, as in the discharge rate analysis, the strong variability in the single trial durations did not enable a robust analysis of the time course at the single-cell level and thus we defer this analysis for the future.

**DISCUSSION**

Herein we explored the discharge properties of neurons in the GPe, GPi, and the motor cortex in two brain states: “eye open” versus “eye closed.” We found that the changes in GPe and GPi discharge properties were positively correlated. Pallidal neurons showed a gradual decrease in discharge rate in the “eye closed” state starting prior to closing of the eyes. In addition, the CV of ISI distributions of both GPe and GPi neurons increased in the “eye closed” state. Increased pallidal CV values were associated...
with a larger fraction of longer ISIs (>150 ms) in the GPi and with a larger fraction of pauses (e.g., ISIs >300 ms) in the GPe. In the motor cortex the changes in discharge properties were less profound and lagged behind the changes observed in the pallidum. Unlike pallidal neurons, cortical neurons increased their discharge rates steeply in the transition to “eye closed” state. These rapid changes in M1 probably reflect distributed cortical activity, since similar kinetics was found in the frontal EEG recording. Finally, as in the pallidum, the CV of ISI distributions of cortical neurons increased in the “eye closed” state. Although assuming that the eye open/closed states reflected transitions in the monkeys’ arousal states (as supported by the EEG analysis), we limited our observation to different brain states, since the study was not designed specifically to test for arousal levels.

Cortex and basal ganglia exhibit different dynamics in their discharge rate modulations

Models of the BG commonly emphasize the cortico–BG functional connectivity and characterize the BG as part of a closed loop circuit connecting all cortical areas through the striatum, pallidum, and thalamus with the frontal cortex (Leblois et al. 2006; Rivlin-Etzion et al. 2006). In our study M1 neurons showed increased firing rates and increased irregularity in their spike trains in the “eye closed” state. These changes could be expected to influence the BG network, specifically on the changes observed in the pallidal discharge variables. However, the dynamics of modulations in discharge rates, as observed at the population level, suggest otherwise. The changes not only in cortical discharge rates but also in the amplitude (RMS values) of the EEG waves were steep and followed the pallidal rate modulations. Since our cortical recordings were...
restricted to the arm-related area of M1 and our EEG recording was of a single contact we cannot exclude other cortical areas as the source for the observed pallidal changes. However, we can conclude that the (motor) cortex probably did not drive the changes observed in the pallidum. Subcortical closed loops through the BG, which have a thalamic relay on the input rather than on the return link of the circuit (McHaffie et al. 2005), could better explain our results. The striatum and the STN are widely innervated by the intralaminar nuclei of the thalamus (Smith et al. 2004). These afferents presumably carry information on sensory events of behavioral significance and are likely to transmit information regarding arousal states to the BG network (Coizet et al. 2007; Kimura et al. 2004; Smith et al. 2009). Thus the pallidal changes in activity could be the drivers of the cortical discharge changes in the transitions between “eye open” and “eye closed” states (likely reflecting transitions in arousal levels).

### TABLE 3. Discharge pattern statistics

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of Cells</th>
<th>“Eye Open”</th>
<th>“Eye Closed”</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPe</td>
<td>174</td>
<td>1.73 ± 1.03 (0.22–5.52)</td>
<td>2.06 ± 1.08*** (0.34–5.71)</td>
</tr>
<tr>
<td>GPi</td>
<td>75</td>
<td>0.74 ± 0.15 (0.46–1.11)</td>
<td>0.98 ± 0.33*** (0.38–1.92)</td>
</tr>
<tr>
<td>CTX</td>
<td>88</td>
<td>1.52 ± 0.50 (0.68–3.77)</td>
<td>1.86 ± 0.53*** (0.92–4.13)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with ranges in parentheses. Coefficient of variation statistics were calculated separately for each neural population. ***P < 0.001, paired t-test. GPe and GPi, external and internal segments of the globus pallidus, respectively; CTX, cortex.

**Potential source for GPe and GPi changes in activity in the “eye closed” state**

A possible explanation for the similar changes in both pallidal segments could be a common pathway of innervation to both areas. Striatal medium spiny neuron (MSN) inhibitory projections ( Tremblay and Filion 1989) can serve as plausible

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**FIG. 6.** Pallidal and cortical neurons have increased coefficient of variation (CV) values in the “eye closed” state. Same conventions as in Fig. 3 for the CV of the ISI distribution. Points below the equality line represent cells for which the CV value in the “eye closed” state > “eye open” state. A: CV values of GPe neurons (n = 174). B: CV values of GPi neurons (n = 75). Inset: enlargement of the range 0.4–2. C: CV values of M1 cortical neurons (n = 88). D: summary of percentage of cells with significant differences in CV values between “eye open” and “eye closed” states for all 3 neuronal areas. Color code is the same as in C. E: surprise value (minus the logarithm of the statistical P value, Student’s paired t-test) of the differences in discharge rates between the 2 states in each neuronal area. The surprise of a P value of 0.05 is 2.996; note that the value for all neuronal areas is far greater.

**Closure of the eyes probably does not significantly affect dopaminergic input to the striatum**

According to the classic model of the basal ganglia (Albin et al. 1989) information is transmitted through direct and indirect pathways from the striatum and into the output nuclei of the BG. Dopamine increases the excitability of striatal D1 direct pathway projecting neurons and decreases the excitability of D2 indirect projecting neurons (Gerfen et al. 1990; Surmeier et al. 2007). However, previous studies (Brown et al. 2009; Monti and Monti 2007; Steinfels et al. 1981, 1983; Trulson 1985) and our unpublished observations suggest that there are no major changes in the discharge rate of dopaminergic neurons or striatal cholinergic tonically active interneurons at different arousal levels (although our study was not designed to specifically test for arousal levels). This is in line with our current results, which show that the vast majority of GPe and GPi neurons exhibit similar changes in discharge rate and pattern in the transition to the “eye closed” state, thus pointing to other features of the basal ganglia functional network, rather than a major modification of the dopamine tonus in the striatum.
In vivo studies have shown that under anesthesia there are fluctuations in the MSN membrane potential between depolarized “up” states and hyperpolarized “down” states (Mahon et al. 2001; Stern et al. 1998; Wilson 1993; Wilson and Kawaguchi 1996). The latter group also reported fluctuations in membrane potential during slow-wave sleep that were correlated with cortical field potentials (Mahon et al. 2006). These results suggest that there are indeed distinct patterns of MSN activity in different brain states. Increased striatal activity in the “eye closed” state (e.g., due to increased thalamic excitation) could explain the modulation observed in both the GPe and GPi. However, recent results (Berke et al. 2004; Mahon
et al. 2006) and our unpublished observations do not show clear changes in striatal projection neurons’ discharge rates associated with different arousal levels.

Another candidate for a common pathway that innervates both segments of the pallidum could be STN excitatory projections (Hazzard and Parent 1992; Shink and Smith 1995). Evidence from both animal and human patient studies suggests that STN activity is affected by anesthesia and by the sleep–wake cycle (Magill et al. 2000; Stefani et al. 2006; Urbain et al. 2000; Urrestarazu et al. 2009). In anesthetized animals, the discharge properties of STN neurons were found to be strictly related to cortical activity and thus to the sleep–wake cycle (Magill et al. 2000). In addition, evidence from human patients undergoing deep brain stimulation surgery points toward reduced spontaneous activity of STN neurons when the patients’ arousal levels are reduced (Stefani et al. 2006). In fact, blockage of STN neuronal activity has been shown to produce decreased discharge rates and increased frequency of long ISIs in both pallidal segments (Hamada and DeLong 1992; Nambu et al. 2000). However, the mechanism behind this reduction in STN discharge on reduced arousal levels is not clear. One candidate is the cortical drive to the STN. The STN discharge is strongly affected by cortical excitatory inputs (Nambu et al. 2002b); however, the direction and dynamics of the cortical discharge rate modulation in our M1 cortical (and EEG) recordings do not support this reasoning. On the other hand, the STN is innervated by subcortical regions such as the mesopontine tegmentum (Bevan and Bolam 1995) and the intralaminar nuclei of the thalamus (Orieux et al. 2000; Smith et al. 2004), which may have led to decreased firing rates in the STN and in the pallidum.

Finally, another factor that could explain the similar changes in both pallidal segments relates to the intrinsic circuitry of the BG. Recent studies have shown a more complex organization of the internal network of connections than originally thought, with closed feedback loops between the GPe, striatum, and STN, as well as other levels of the basal ganglia–cortical circuits (Bolam et al. 2000; Leblois et al. 2006).

Concluding remarks

The classic view of the cortico–BG network is based on the concept of D1/direct and D2/indirect pathways and predicts a reciprocal relationship between GPe and GPi. However, anatomical studies have revealed a more complex network of connection whereby the BG network is characterized by a closed-loop architecture at many different levels. In addition, previous physiological studies conducted on behaving animals have shown that GPe and GPi neurons display similar transient responses to behavioral and motor events. Our study extends these findings and describes the spontaneous activity of pallidal neurons in two distinct brain states. We observed similar changes in GPe and GPi firing rates, strengthening the evidence against GPe–GPi anatomical reciprocity and revealing that the functional efficacy of the GPe to GPi inhibitory connections is weak. The minimal changes in discharge rates of basal ganglia neuromodulators under different arousal states enable striatal and/or STN common inputs to similarly modify GPe and GPi firing rates. In addition, we observed early changes in pallidal compared with cortical activity on the transition from the “eye open” to the “eye closed” state. These unexpected early changes in the pallidum are better explained by a subcortical rather than a cortical loop through the BG. Nevertheless, the efficacy of the projections between two neural structures is not affected solely by the discharge rates of the neurons under study. Depending on the intrinsic properties of spatial and temporal summation, as well as on the convergence/divergence properties of the network, firing patterns, and neural synchronization may modify the efficacy of neural transmission. Future studies of the cortex–basal ganglia network may shed light on these intriguing questions.

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