Neurons in both pallidal segments change their firing properties similarly prior to closure of the eyes.

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Abstract

Current anatomical models of the cortico-basal ganglia (BG) network predict reciprocal discharge patterns between the external and internal segments of the globus pallidus (GPe and GPi respectively), as well as cortical driving of BG activity. However, physiological studies revealing similarity in the transient responses of GPe and GPi neurons cast doubts on these predictions. Here, we studied the discharge properties of GPe, GPi and primary motor cortex neurons of two monkeys in two distinct states: when eyes are open vs. when they are closed. Both pallidal populations exhibited decreased discharge rates in the "eye closed" state accompanied by elevated values of the coefficient variation (CV) of their inter-spike interval (ISI) distributions. The pallidal modulations in discharge patterns were partially attributable to larger fractions of longer ISIs in the "eye closed" state. In addition, the pallidal discharge modulations were gradual, starting prior to closing of the eyes. Cortical neurons, as opposed to pallidal neurons, increased their discharge rates steeply upon closure of the eyes. Surprisingly, the cortical rate modulations occurred after pallidal modulations. However, as in the pallidum, the CV values of cortical ISI distributions increased in the "eye closed" state, indicating a more bursty discharge pattern in that state. Thus, changes in GPe and GPi discharge properties were positively correlated suggesting that the sub-thalamic nucleus and/or the striatum are the main common driving force for both pallidal segments. Furthermore, the early, unexpected changes in the pallidum are better explained by a subcortical rather than a cortical loop through the BG.

Key words: primate, motor cortex, basal ganglia, firing pattern, brain states
Introduction

The 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 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 GPe and 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 to reciprocal discharge patterns between GPe and GPi neurons. Another mechanism which supports GPe-GPi reciprocity arises from 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 (Hazrati et al. 1990; Sato et al. 2000; Kita 2001; Kita 2007).

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 (Miller and DeLong 1987; Filion and Tremblay 1991). Moreover, in response to dopamine replacement therapy, both in human PD patients (Hutchinson et al. 1997; Merello et al. 1999; Levy et al. 2001) and in MPTP treated primates (Filion et al. 1991; Papa et al. 1999; Heimer et al. 2002), 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 (Nambu et al. 1990; Georgopoulos et al. 1983; Turner and Anderson 1997) and reward related events (Gdowski et al. 2001; Arkadir et al. 2004; Joshua et al. 2009). Thus, the similarity in behaviorally triggered responses (Mitchell et al. 1987; Turner and Anderson 1997; Turner and Anderson 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 (Evarts 1964; Steriade et al. 1993; Contreras and Steriade 1997; Hobson and Pace-Schott 2002; 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; Yoshida et al. 1993; Nambu et al. 2000; Nambu et al. 2002a) 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 vs. cortical drive of the BG, we 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, namely upon closing and opening of the eyes.
Materials and 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; Joshua et al. 2009). At the end of the experiment after a recovery period, monkey L was sent to a primate sanctuary (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 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (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 Parkinson’s disease. It was perfused through the heart with saline followed by a 4% paraformaldehyde solution within 30 minutes 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 (IACUC) of the Hebrew University and Hadassah Medical Center. The Hebrew University is an AAALAC International accredited institute. Materials and methods were given in detail in previous manuscripts (Rivlin-Etzion et al. 2008; Joshua et al. 2008). Here we give a brief summary of these methods and provide information on the methods not used in the previous manuscripts.

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 GPe or the GPi (monkey 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, AlphaOmega).

A titanium screw was implanted above the frontal area of the skull for 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 per second (monkey W and monkey L respectively). Video analysis was carried out on custom software to identify periods when the monkeys' eyes were closed (Mitelman 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 more than one second (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 second, 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 out 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. In order 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 2 seconds, 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 1-50 Hz frequencies) and averaged across days.

Neuronal data analysis

Recorded spike trains were subjected to an offline quality analysis which included tests for discharge rate stability, refractory period, spike waveform isolation and recording time. As a first step, firing rate was graphically displayed and visually inspected. The largest continuous segment of data with a stable discharge rate was selected for further analysis. In order 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 more than 0.02 of the total inter-spike intervals were shorter than 2 ms were excluded from the database (Fee et al. 1996). Third, only pallidal and MI cells with an isolation score (Joshua et al. 2007) above 0.8 and 0.7, respectively, were used. We repeated our analysis on the subpopulation of M1 neurons which had isolation scores above 0.8 (n=68 cells) and obtained similar results to those reported below (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 Kitai 1994). Finally, the recording time (after offline quality analysis) of each cell was segmented into "eye open" vs. "eye closed" states; thus each cell had multiple distinct recording segments in each state. Only cells that fulfilled the above inclusion criteria (discharge stability, isolation quality and refractory period) for more than 240 seconds and had at least five, not necessarily continuous, seconds 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 could close its eyes. These periods were used for the analysis in this paper. Thus, in monkey L the average recording duration of
neurons was 3162 and 256 seconds 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 seconds 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 bins of one second separately for the "eye open" and "eye closed" states and then found the absolute difference between the means of these two vectors which we denoted as the "discharge rate difference". Next we used a re-sampling (bootstrap) method to test the significance of the "discharge rate difference" (Efron and Tibshirani 1993). The discharge rates in one second 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 2000 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 was larger than the one 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 PSTHs aligned to the closing and opening of the eyes. PSTHs were constructed for two time periods: 1) 60 seconds prior to 10 seconds following eye closing, 2) 10 seconds prior to 60
seconds following eye opening. These PSTHs were calculated in 1 ms bins and smoothed with a Gaussian window with a standard deviation (SD) of 300 ms. In order to deal with data edges 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 variation (CV: standard deviation to the mean) of the ISI distributions in the "eye open" and "eye closed" states to assess the differences in discharge patterns. As described above, 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. In order 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 which had in total at least 5 ISIs in each state (Prut and Perlmutter 2003) were used in calculating the CV values. In addition, we calculated the CV values using ISI distributions that were constructed from recording segments which exceeded an absolute time
In all three populations (M1, GPe, GPi), we tested whether the difference in CV values between the "eye open" and "eye closed" states was significant at the single cell and at the population levels. At the single cell level we used a re-sampling (bootstrap) method. The ISIs of both states (closed and open) were shuffled and re-sampled repeatedly into two groups and the test statistic (T) was calculated for each replication as follows:

\[
T = \frac{\text{abs}(CV_o - CV_c)}{\sqrt{\frac{CV_o^2}{2N_o} + \frac{CV_c^2}{2N_c}}}
\]

where \(CV_o/c\) is the CV of the re-sampled data and \(N_o/c\) is the number of ISIs in the original open/closed data. This process was repeated 2000 times and the \(p\)-value of the difference in discharge pattern was estimated as the fraction of replications for which the statistic (T) calculated on the original data was larger than the test statistic. We also tested the difference in CV values between the "eye open" and "eye closed" state at the population level using a paired student's \(t\)-test. In monkey L, we also ran the analysis solely on spontaneously recorded neurons (not during task performance) and obtained similar results (data not shown).

In addition, to better characterize the differences in discharge pattern between the two states, we looked at the skewness and kurtosis values of the ISI distributions. We used the logarithm of the ISI to calculate the skewness and kurtosis values since the logarithmic transformation makes the ISI distributions independent of time scale and more symmetric (Bhumbra and Dyball 2004) and therefore compensates for the different durations of recording segments in each state. We used the same inclusion criteria as for the CV calculation. In addition, in all three populations (M1, GPe, GPi), we corrected for the bias in skewness and kurtosis estimation by random shuffling (bootstrap) with 2000 random replications (Efron and Tibshirani 1993). We tested the difference in skewness and kurtosis values between the "eye open" and "eye closed" state at the population level using a paired student's \(t\)-test.
We evaluated the time course of the changes in pallidal discharge pattern between the "eye open" and "eye closed" states by calculating the CV for each neuron over a fixed number of intervals around closing and opening of the eyes. For each neuron, each spike train was divided into a series of 50 ISIs (~1 second for the pallidal cells). CV values were computed on the 50-ISIs moving window with an overlap of 40 ISIs (80% window size) and assigned to the time of the first spike creating the 50-ISIs window. The CV values were then interpolated to create a continuous time series with a resolution of 100 ms and averaged across trains. Next, in order to obtain a population time course of the changes in CV values we averaged the CV time course across cells. Only cells that had at least one full CV time course were included in the population average (as in the PSTH analysis). We repeated this analysis for all neural populations (M1, GPe and GPi) using a 5-ISIs moving window with an overlap of 4 ISIs to compute the CV.

We did not detect any significant difference between the individual monkeys; therefore we grouped their results according to structure and analysis type. Significance level was set to 5% in all statistical tests. Data analysis was carried out on custom software using MATLAB V7 (Mathworks, Natick, MA, USA).
Results

Segmentation of recording times into "eye open" and "eye closed" states is reflected in EEG

We recorded neuronal activity in three areas of the cortex-BG network: the primary motor cortex (M1) and the external and internal segments of the pallidum (GPe and GPi respectively). In addition video cameras were used to record the monkeys' facial movements. We divided the recording times into two distinct periods depending on the state of the monkey's eyes, "eye open" vs. "eye closed" states (for details see Materials and Methods). Figure 1A shows an example of the raw eye state and the segmentation into "eye open" and "eye closed" states (first and second rows).

Early electroencephalogram (EEG) recordings from human subjects showed slow large amplitude EEG waves when the subjects were closing their eyes and relaxing. These large EEG waves were absent when the subjects opened their eyes (Buzsaki 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 which are required for a definitive assessment of arousal level (Silber et al. 2007; Schulz 2008), we restricted ourselves to the more conservative definition of brain states.
The 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 Materials and 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 Figure 2A showed a decrease in discharge rate in the "eye closed" state (second row) compared to 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 inter-spike interval (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 Figure 2C, unlike the two pallidal neurons increased its discharge rate in the "eye closed" state and exhibited a burstier discharge pattern (compare second to first row).

To summarize, these illustrative cells exhibited differences in discharge rates and in discharge patterns between the eye open/closed states; below 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 to the "eye open" state.
and only a few cells showed the reverse (Fig. 3A,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. 3D,E).

M1 neurons displayed a less profound change compared with pallidal neurons in their mean discharge rates upon 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. 3C,D). This difference was also significant at the population level (Table 2 and Fig. 3D,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 upon closure of the eyes by 0.46 spikes/s and 61.83 percent, whereas GPe and GPi neurons decreased their average discharge rates by 8.02 and 8.34 spikes/s and 10.28 and 9.64 percent respectively.

To appreciate the time course of the changes in discharge rates we examined the averaged peri stimulus time histograms (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 seconds) prior to eye closing and continuing after eye opening (Fig. 5A,B and Supplemental Fig. 1 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 strengthens the assumption that the differences in the neuronal discharge between the "eye open" and "eye closed" states was 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. 1), corresponding to the time course of changes in EEG (Fig. 5D). The analysis performed above 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, Gallistel et al. 2004) the time course of discharge rate changes at single trials/cells level 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 by 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 variation (CV) of the ISI histogram. The CV is a measure of the regularity of firing (CV equals one for a Poisson process, less than one for a more regular process, and more than one for a bursty firing pattern).

In M1, sufficient data (see Materials and 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. 6C,D). This difference was highly significant at the population level of the CV (Table 3 and Fig. 6D,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 (shorter than 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. 2).

As in the cortex, in both pallidal areas CV values were significantly larger in the "eye closed" state (Fig. 6A,B) compared to the "eye open" state both at
the single cell level (Fig. 6D) and at the population level (Table 3 and Fig.
6D,E). We examined the difference in skewness and kurtosis values of the
ISI distributions between the "eye open" and "eye closed" states
(Supplemental Fig. 2). In the GPe and in the GPi, both parameters were
significantly larger (Student's 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. 2A,B, last
row and population means of skewness and kurtosis values in Supplemental
Fig. 2).

Nonetheless, the higher moments of the ISI distributions as well as the CV
values were in different ranges in the GPe compared to the GPi. CV values
in the GPi 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. 2). 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 out 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 which 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 out 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 out of the four
definitions, Fig. 7B); however the difference was small. In the GPi the
percentage of pauses out 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 to 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 change in discharge pattern observed in the transition between the "eye open" and "eye closed" states in both pallidal segments can mainly be attributed 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. 3). The estimate of CV values and their dynamics was affected by the size of the analysis moving window (thus, the low discharge rate of MI 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. The changes in the pallidal CV values calculated using a large analysis window (50 ISIs, ~ 1 second, Fig. 8) were gradual, exhibiting similar kinetics to the one 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. 3) were, however, less profound and displayed a steeper dynamics. These results suggest that the 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 single cells 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 we
defer this analysis for the future.
Discussion

In this manuscript we explored the discharge properties of neurons in the GPe, GPi and the motor cortex in two brain states: "eye open" vs. "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 the 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 (longer than 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 the ISI distributions of cortical neurons increased in the "eye closed" state. Although assume 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 (Rivlin-Etzion et al. 2006; Leblois 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 impact on 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 in cortical discharge rates, as well as the change 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 can not 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 (Smith et al. 2008; Kimura et al. 2004; Coizet et al. 2007). 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 (which likely reflect transitions in arousal levels).

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 (Surmeier et al. 2007; Gerfen et al. 1990). However, previous studies (Steinfels et al. 1983; Trulson 1985; Monti and Monti 2007; Steinfels et al. 1981; Brown et al. 2009) 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 (though 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, and therefore points to other features of
the basal ganglia functional network, rather than a major modification of the dopamine tonus in the striatum.

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 candidates. In-vivo studies have shown that under anesthesia there are fluctuations in the MSN membrane potential between depolarized "up" states and hyperpolarized "down" states (Wilson 1993; Wilson and Kawaguchi 1996; Stern et al. 1998; Mahon et al. 2001). The latter group also reported fluctuations in membrane potential during slow wave sleep which 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 (Mahon et al. 2006; Berke et al. 2004) 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 (Hazrati 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 (Urbain et al. 2000; Urrestarazu et al. 2008; Magill et al. 2000; Stefani et al. 2006). In anesthetized animals, the discharge properties of STN neurons were found to be strictly related to cortical activity and hence to the sleep-wake cycle (Magill et al. 2000). In addition, evidence from human patients undergoing deep brain stimulation surgery points towards 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 (Nambu et al. 2000;
However the mechanism behind this reduction in STN discharge upon 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 (Smith et al. 2004; Orieux et al. 2000) which may have led to decreased firing rates in the STN and in the pallidum.

Finally, another factor which 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 reciprocality 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 enables 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 upon 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.

Acknowledgments
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Francois C, Percheron G, Yelnik J, and Heyner S. A Golgi analysis of
the primate globus pallidus. I. Inconstant processes of large neurons, other

Gallistel CR, Fairhurst S, and Balsam P. The learning curve: implications
of a quantitative analysis. *Proc Natl Acad Sci U S A* 101, 13124-13131,
2004.

Gdowski MJ, Miller LE, Parrish T, Nenonene EK, and Houk JC.
Context dependency in the globus pallidus internal segment during targeted

Georgopoulos AP, DeLong MR, and Crutcher MD. Relations between
parameters of step-tracking movements and single cell discharge in the
globus pallidus and subthalamic nucleus of the behaving monkey. *J
Neurosci* 3, 1586-1598, 1983.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma Jr.
FJ, and Sibley DR. D1 and D2 dopamine receptor-regulated gene
expression of striatonigral and striatopallidal neurons. *Science* 250, 1429-
1432, 1990.

Haber SN & Gdowski MJ. The Basal Ganglia. In: *The Human Nervous

Hamada I and DeLong MR. Excitotoxic acid lesions of the primate
subthalamic nucleus result in reduced pallidal neuronal activity during

Hazrati LN and Parent A. Differential patterns of arborization of striatal
and subthalamic fibers in the two pallidal segments in primates. *Brain Res*

Hazrati LN, Parent A, Mitchell S, and Haber SN. Evidence for
interconnections between the two segments of the globus pallidus in
primates: a PHA-L anterograde tracing study. *Brain Res* 533, 171-175,
1990.

Heimer G, Bar-Gad I, Goldberg JA, and Bergman H. Dopamine
replacement therapy reverses abnormal synchronization of pallidal neurons
in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of


Levy R, Dostrovsky JO, Lang AE, Sime E, Hutchinson WD, and Lozano AM. Effects of apomorphine on subthalamic nucleus and globus pallidus


Table 1 – The neural database

Recording statistics were calculated separately for each monkey (W and L) and for each neural population. In each entry of the table is the mean, the standard deviation and the range in brackets. The isolation score range 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 which satisfied the inclusion criteria of the analysis database. Recorded time "eye open"/"eye closed" is the total time (out of recorded time) the cell was in one period or the other. Average (Avg') recorded time/segment "eye open"/"eye closed" is the average time in each distinct segment. Abbreviations: CTX – Cortex, GPe and GPi – external and internal segments of the globus pallidus, respectively.

Table 2 – Discharge rate statistics

Discharge rate statistics were calculated separately for each neural population. In each entry of the table is the mean, the standard deviation and the range in brackets. ***p<0.001 paired t-test. Abbreviations: CTX – Cortex, GPe and GPi – external and internal segments of the globus pallidus, respectively.

Table 3 – Discharge pattern statistics

Coefficient variation statistics were calculated separately for each neural population. In each entry of the table is the mean, the standard deviation and the range in brackets. ***p<0.001 paired t-test. Abbreviations: CTX – Cortex, GPe and GPi – external and internal segments of the globus pallidus, respectively.

Figure 1: EEG signal reflects segmentation of eye states

A. First row: An example of the results from the video analysis in a 20 second time period detecting the raw eye state, O indicates times when the eye was open, C indicates times when the eye was closed. Second row: Eye state, O for “eye open” state and C for “eye closed” state. Short periods with closed eye (e.g., blinks) are included in the "eye open" state. Third row: corresponding EEG signal showing larger
amplitude waves when the eyes are closed. Fourth row: RMS values
calculated for the EEG signal (bin size one second). Last row:
spectrogram of the EEG signal. Ordinate: frequency in logarithmic scale
(maximal frequency is 50 Hz). Hot (red) colors stand for stronger power.

B. Comparison of RMS of the EEG signal in the two states. Each point
represents the average RMS calculated for a single recording day.
Abscissa: values in the “eye closed” state. Ordinate: values in the “eye
open” state. Gray diagonal line is the equality line. Note that all points
are below the equality line (i.e., RMS "eye closed">"eye open"). RMS
values in the "eye closed" state were significantly larger than in the "eye
open" state ($P<0.001$, paired Wilcoxon signed rank test).

C. Normalized power spectrum (divided by the mean power of all, 1-50 Hz,
frequencies) and standard error of the mean (bars) of the EEG signal
calculated separately for each state and averaged across days. Black, for
“eye open” state. Gray, for “eye closed” state.

Figure 2: Pallidal and MI cortical neurons have different discharge
properties in the "eye open" compared to the "eye closed" states

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 seconds each.
Traces are not necessarily consecutive. Below each raster is an
example of a 1 second analog trace of extracellular recording filtered
between 300 and 6000 Hz. An asterisk marks the trace in the raster
from which the analog example is taken. Below the one-second
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 inter-spike intervals in the two
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 = 2ms.

Figure 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 figure 2A.

B. Discharge rates of GPi neurons (n=75). Same conventions as in A. Black arrow points to the GPi cell in figure 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 figure 2C.

D. Summary of percentage of cells with significant differences in discharge rate between "eye open" and "eye closed" states for all three 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.
Figure 4: Pallidal and cortical discharge rate modulations in the “eye closed” state as percentage of their “eye open” baseline discharge rates
A. Percent of discharge rate changes of GPe neurons (N=174). Histogram depicting the average changes in discharge rates between the "eye open" and "eye closed" states as percentage of discharge rates in the "eye open" state. Abscissa: percent of discharge rate change. Ordinate: Fraction of cells. Gray horizontal line is in zero percent change. Arrow points to the average percent change. Note the mass distribution is to the left of the gray line, i.e. most cells have higher discharge rates in the "eye open" state.

B. Same as A for GPi neurons (N=75).

C. Same as A for cortical neurons (N=102). Most cells are to the right of the gray (zero) line, i.e. most cells have higher discharge rates in the "eye closed" state. The distribution is wider compared with the distributions in A and B (note the different scales of the X-axis).

Figure 5: Pallidal but not cortical neurons modify their discharge rate prior to changes in the eye state
A. GPe population PSTH averaged across cells and aligned at eye closing (time zero, left column) and at eye opening (time zero, right column). Abscissa: time in seconds. Ordinate: deviation from mean discharge rate in Hz. Error bars are standard error of the mean values. Discharge rates were calculated in 1 ms bins, smoothed with a Gaussian window with a SD of 300 ms and averaged across recording days. Number of cells is 143, 135 and 147 in the time epochs: prior to eye closing, during eye closing and following eye opening respectively.

B. Same as in A for the GPi population. Number of cells is 71, 40 and 68.

C. Same as in A for M1 population. Number of cells is 75, 94 and 70.

D. Time course of the changes in the amplitude (RMS) of the EEG waves. EEG was aligned at eye closing and at eye opening. RMS values of the signal were calculated in non-overlapping one second bins and averaged across recording days. Abscissa: time in seconds.
Ordinate: average RMS values. The deflection in RMS values at time zero in the right column probably reflects the EMG transients at opening of the eyes.

Figure 6: Pallidal and cortical neurons have increased CV values in the "eye closed" state

Same conventions as in Figure 3 for the coefficient variation (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 three neuronal areas. Color code is the same as in figure 6C.

E. Surprise value (minus the logarithm of the statistical p-value, Student’s paired $t$-test) of the differences in discharge rates between the two 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.

Figure 7: GPe and GPi neurons exhibit a larger fraction of long silent intervals ("pauses") in the "eye closed" state

A. Average percentage of pauses out of the total ISIs across all GPe cells in the two states. Color code: black, for “eye open” state, gray, for “eye closed” state. Each pair of bars is calculated according to a different definition of pauses: above 150, 300, and 500 ms and according to "surprise" method (denoted as "S"). **, *** indicate significant ($p<0.01$, $p<0.001$) difference between states (paired $t$-test). Error bars stand for 95% confidence interval.

B. Average duration of pauses across all GPe cells in the eye open/closed states. Same conventions as in A.
C. Same as in A for the GPi population. Note the different scale of the Y-axis. Except for ISIs above 150 ms, the values are small or absent due to paucity of GPi cells with ISIs fulfilling these definitions.

D. Same as in B for the GPi population.

Figure 8: Time course of the changes in pallidal discharge pattern in the transition between "eye open" and "eye closed" states

A. GPe population CV time course aligned at eye closing (time zero, left column) and at eye opening (time zero, right column). CV values were computed on a 50 ISIs moving window with an overlap of 40 ISIs and were averaged across cells (N is the number of cells averaged). Abscissa: time in seconds. Ordinate: CV values. Error bars are standard error of the mean values.

B. Same as in A for the GPi population.
A

GPe

rate (spk/s)

-12

-60 -50 -40 -30 -20 -10 0 10 20 30 40 50

B

GPi

rate (spk/s)

-12

-60 -50 -40 -30 -20 -10 0 10 20 30 40 50

C

CTX

rate (spk/s)

-4

-60 -50 -40 -30 -20 -10 0 10 20 30 40 50

D

EEG

RMS (uV)

35

15 20 25 30 35

-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60

Time (s)
<table>
<thead>
<tr>
<th>Population</th>
<th>Number of cells</th>
<th>Isolation score</th>
<th>Fraction ISI &lt; 2 ms</th>
<th>Recorded time (s)</th>
<th>Recorded time &quot;eye open&quot; (s)</th>
<th>Recorded time &quot;eye closed&quot; (s)</th>
<th>Avg' recorded time/segment &quot;eye open&quot; (s)</th>
<th>Avg' recorded time/segment &quot;eye closed&quot; (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPe W: 56</td>
<td>0.95 ± 0.05</td>
<td>0.0021 ± 0.0036</td>
<td>636 ± 136</td>
<td>256 ± 200</td>
<td>380 ± 224</td>
<td>19 ± 22</td>
<td>21 ± 18</td>
<td>[3 - 98]</td>
</tr>
<tr>
<td>L: 118</td>
<td>0.95 ± 0.04</td>
<td>0.0016 ± 0.0046</td>
<td>3,562 ± 2217</td>
<td>3,244 ± 2010</td>
<td>318 ± 390</td>
<td>115 ± 180</td>
<td>4 ± 1</td>
<td>[1 - 14]</td>
</tr>
<tr>
<td>GPi W: 11</td>
<td>0.94 ± 0.06</td>
<td>0.0024 ± 0.0034</td>
<td>471 ± 147</td>
<td>213 ± 161</td>
<td>258 ± 167</td>
<td>19 ± 19</td>
<td>7 ± 1</td>
<td>[4 - 45]</td>
</tr>
<tr>
<td>L: 64</td>
<td>0.95 ± 0.04</td>
<td>0.0009 ± 0.0034</td>
<td>3,152 ± 1,609</td>
<td>3,010 ± 1,568</td>
<td>142 ± 138</td>
<td>142 ± 143</td>
<td>4 ± 1</td>
<td>[1 - 7]</td>
</tr>
<tr>
<td>CTX W: 102</td>
<td>0.85 ± 0.08</td>
<td>0.0041 ± 0.0051</td>
<td>610 ± 142</td>
<td>279 ± 166</td>
<td>331 ± 201</td>
<td>35 ± 44</td>
<td>27 ± 33</td>
<td>[3 - 220]</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Population</th>
<th>Number of cells</th>
<th>Discharge rate (spk/s) &quot;eye open&quot;</th>
<th>Discharge rate (spk/s) &quot;eye closed&quot;</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>

**Table 2 – Discharge rate statistics**

Discharge rate statistics were calculated separately for each neural population. In each entry of the table is the mean, the standard deviation and the range in brackets. ***p<0.001 paired t-test. Abbreviations: CTX – Cortex, GPe and GPi – external and internal segments of the globus pallidus, respectively.
<table>
<thead>
<tr>
<th>Population</th>
<th>Number of cells</th>
<th>Coefficient variation &quot;eye open&quot;</th>
<th>Coefficient variation &quot;eye closed&quot;</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 ± 0.5, [0.68 – 3.77]</td>
<td>1.86 ± 0.53, [0.92 – 4.13]</td>
</tr>
</tbody>
</table>

Table 3 – Discharge pattern statistics

Coefficient variation statistics were calculated separately for each neural population. In each entry of the table is the mean, the standard deviation and the range in brackets. ***p<0.001 paired t-test. Abbreviations: CTX – Cortex, GPe and GPi – external and internal segments of the globus pallidus, respectively.