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Changes in basal ganglia processing of cortical input following magnetic stimulation in Parkinsonism

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ABSTRACT

Parkinsonism is associated with major changes in neuronal activity throughout the cortico-basal ganglia loop. Current measures quantify changes in baseline neuronal and network activity but do not capture alterations in information propagation throughout the system. Here, we applied a novel non-invasive magnetic stimulation approach using a custom-made mini-coil that enabled us to study transmission of neuronal activity throughout the cortico-basal ganglia loop in both normal and parkinsonian primates. By magnetically perturbing cortical activity while simultaneously recording neuronal responses along the cortico-basal ganglia loop, we were able to directly investigate modifications in descending cortical activity transmission. We found that in both the normal and parkinsonian states, cortical neurons displayed similar multi-phase firing rate modulations in response to magnetic stimulation. However, in the basal ganglia, large synaptically driven stereotypic neuronal modulation was present in the parkinsonian state that was mostly absent in the normal state. The stimulation-induced neuronal activity pattern highlights the change in information propagation along the cortico-basal ganglia loop. Our findings thus point to the role of abnormal dynamic activity transmission rather than changes in baseline activity as a major component in parkinsonian pathophysiology. Moreover, our results hint that the application of transcranial magnetic stimulation (TMS) in human patients of different disorders may result in different neuronal effects than the one induced in normal subjects.

Introduction

Parkinsonism is associated with altered neurophysiological activity throughout the cortico-basal ganglia (CBG) loop. Initially, the study of these changes focused on the baseline firing rate of the entire neuronal population of specific nuclei (Albin et al., 1989; DeLong, 1990). Later studies pointed to ongoing changes in neuronal activity patterns such as oscillations and in neuronal interactions such as increased coherence (Bergman et al., 1994; Brown et al., 2001; Filion and Tremblay, 1991; Hutchison et al., 1997; Nini et al., 1995). Studies of functional changes in the CBG loop neurophysiology have revealed a loss of segregation in the neuronal encoding of movement which leads to increased correlations and reduced specificity of individual neurons during behavior (Boraud et al., 2000; Bronfeld and Bar-Gad, 2011; Filion et al., 1988; Goldberg et al., 2002; Pessiglione et al., 2005; Raz et al., 2000). These measures all assess the modulation of neuronal activity in the CBG loop in a static manner that reflects baseline changes arising from the dopamine depleted state. However, the changes in information transmission along the loop, reflected as dynamic changes to the neuronal activity, have largely been overlooked. A recent study utilizing local electrical cortical stimulation in normal and parkinsonian anesthetized rats reported different response patterns in sub-cortical areas in the two states (Kita and Kita, 2011), hinting at differential information transmission along the pathway during dopamine depletion. The input to the basal ganglia is diffused in nature arriving from most cortical areas (Kemp and Powell, 1970), thus, studies of neuronal CBG activity propagation may benefit from techniques which simultaneously activate large distributed cortical areas that form the complex input to the basal ganglia. This type of descending activation can be induced by magnetic stimulation of the cortical tissue.

Transcranial magnetic stimulation (TMS) is a non-invasive method of manipulating behavior by altering neuronal activity (Barker et al., 1985). This technique is used extensively to study cognitive processes (Walsh and Cowey, 2000) and is being tested as a potential treatment for different neural disorders including Parkinson's disease (PD) (Edwards et al., 2008). TMS induces large scale (multiple centimeters), spatially diffuse activation of the underlying neuronal tissue (Cohen et al., 1990), making it a potential tool for studying the dynamic processing of converging cortical input patterns by the basal

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ganglia. Recent studies have demonstrated that the effects of magnetic stimulation are highly dependent on the underlying brain state (Pasley et al., 2009). This dependence may lead to substantial differences in the stimulation effect on normal brain activity relative to its pathological states and can potentially provide information on the changes in neural processing occurring during these disorders.

Here, we employed a novel configuration for magnetic stimulation based on a custom-built magnetic mini-coil developed in our lab (Tischler et al., 2011) that enables simultaneous multi-electrode extracellular recordings of neuronal activity from multiple brain regions during stimulation. We used this design to study differences in activity propagation along the CBG pathway between the normal and the parkinsonian states. We magnetically stimulated the cortex of normal and MPTP-treated monkeys and recorded neuronal activity both in a brain region directly affected by the stimulation (primary motor cortex — M1) as well as in downstream synaptically activated BG targets.

Materials and methods

Animals

Two male *Macaca fascicularis* male monkeys were used in this study (E—5.2 kg; P—4.5 kg). The monkeys' water and food consumption and weight were assessed daily and their health was monitored by a veterinarian. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Bar-Ilan University guidelines for the use and care of laboratory animals in research, and were approved and supervised by the Institutional Animal Care and Use Committee.

Surgery

The monkeys underwent a surgical procedure to attach a 27 mm square Cilux recording chamber (Alpha-Omega Engineering, Nazareth, Israel) to the skull allowing access to the primary motor cortex (M1) and the globus pallidus external (GPe) and internal (GPi) segments. The chambers were tilted 35° in the sagittal plane, with their center targeted at stereotaxic coordinates A5-L6-H2 of monkey E's right hemisphere, and A5-L8-H2 of monkey P's left hemisphere (Szabo and Cowan, 1984). The surgical procedure was performed under isoflurane and N_2O general anesthesia and aseptic conditions.

Experimental procedure

Following recovery from surgery, each animal underwent microelectrode guided mapping of M1, GPe and GPi, which was followed by the recording sessions. During all sessions, the monkey's head was fixed. Eight glass or Narylene coated tungsten microelectrodes (impedance 0.2–0.5 MΩ at 1 kHz) (We-Sense, Nazareth, Israel) were advanced separately (EPS 4.10, Alpha–Omega Engineering) to the M1 and GP. The somatotopic areas of M1 were identified at the beginning of every recording day using intracortical electrical microstimulation (20 biphasic pulses, 5–20 μA, at 300 Hz). Both segments of the globus pallidus were identified by their characteristic neuronal activity and their relation to known anatomical boundaries. The distinction between the external (GPe) and internal (GPi) segments was determined online based on recording depth, characteristics of neuronal activity (oscillations, pausing, background noise, etc.), and the existence of border cells and white matter fibers between the two segments. The electrodes' signals were continuously sampled at 40 kHz (Alphamap 10.10, Alpha-Omega Engineering), amplified (∗1000) and wide bandpass filtered (2–8000 Hz four-pole Butterworth filter) (MCP-Plus 4.10, Alpha-Omega Engineering).

Magnetic stimulation

A detailed description of the mini-coil we developed for magnetic stimulation appears elsewhere (Tischler et al., 2011). Briefly, the mini-coil was designed to provide magnetic pulses with a field intensity comparable to commercial TMS coils, namely on the order of 1 T with 400 μs pulse duration. The optimized coil configuration was selected to be a short circular solenoid shape with the following parameters: outer diameter 26.5 mm, inner diameter 16 mm, coil height 10 mm, wire diameter 1.2 mm, and number of turns 32. The circular coil was attached to the electrodes' microdriving terminal which enabled separate insertion of multiple micro-electrodes into different brain regions (Fig. 1). The whole complex was then attached to the recording chamber, with the coil situated completely within the chamber. A Pt100 temperature sensor was attached to the coil itself to verify that the coil temperature was within a safe physiological range throughout the experiment.

Motor threshold intensity was defined daily as the lowest intensity of a single magnetic pulse stimulation that produced movement in the contralateral upper limb. The motor threshold was determined with the animal at rest and was stable for each monkey during each day and only varied slightly over recording days. The stimulus trains (either 60 or 100 pulses) were applied at different intensities and frequencies. Low frequency (0.2, 0.5 and 1 Hz — pooled together across frequencies) and supra-motor threshold amplitude (mean ± SD: 500 ± 87 V, corresponding to 111 ± 12% of the identified motor threshold) were used in all sessions. Supra-motor threshold stimulation was used for this study to ensure direct activation of cortical neurons by the magnetic pulse.

Induction of Parkinsonism

After completion of the recording sessions in the normal state, Parkinsonism was induced and subsequent recording sessions were performed in that state. Parkinsonism was induced by five intramuscular injections of 0.4 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-HCl (Sigma-Aldrich, Rehovot, Israel) neurotoxin. MPTP injections were given under intramuscular Ketamine-HCl (10 mg/kg) anesthesia over a period of 4 days, after which the monkeys developed severe Parkinsonism. Additionally, both monkeys had minor dystonia, primarily in the lower limbs, a common effect of MPTP treatment (Perlmutter et al., 1997). The monkeys' parkinsonian state was assessed daily using the Schneider scale (Schneider et al., 2003) and was severe and stable throughout the recording period [mean ± SD: monkey E: 42.3 ± 5.4, monkey P: 44.0 ±

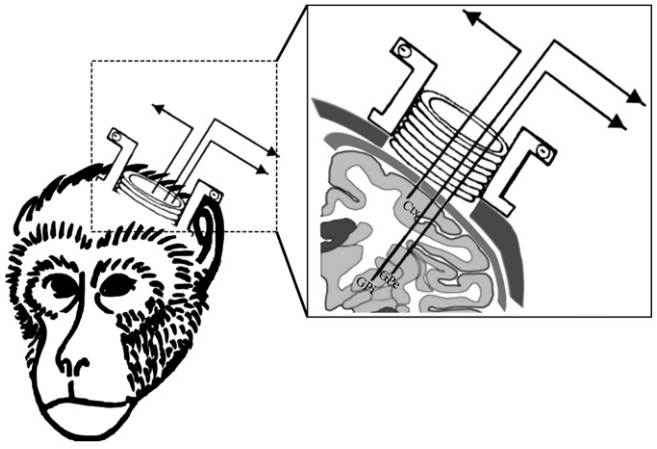


Fig. 1. The recording setup. A sketch of the chamber connected to the monkey's scalp. The mini-coil is positioned within the chamber on top of the dura. The electrodes enter perpendicularly through the inner space of the mini-coil and record simultaneously from the M1, GPe, and GPi.

2.7, scale of 0 (asymptomatic) to 53 (maximal symptoms)]. The recordings were resumed 8 days after the first MPTP injection. Following the final recording sessions in the parkinsonian state, the beneficial effects of dopaminergic agents (Dopicar, Teva, Israel) were tested, and showed a reduction in both rigidity and akinesia.

Histology

Following completion of the experiment, the animals were anesthetized using Ketamine 10 mg/kg and stereotactic marking micro-lesions (DC current 60 μA for 30 s) were made. The animals were then deeply anesthetized using sodium pentobarbital 50 mg/kg and transcardially perfused with 1 l of physiological saline, followed by 1 l of 4% paraformaldehyde. The whole brain was removed and buffered in graded sucrose solution 10–30% over seven days. The brain was then frozen to −25 °C and cut in the coronal plane using a cryostat (Leica Microsystems). Each section was digitized using a 10 Mpixel digital camera and sections of interest were mounted onto glass slides and Nissl stained. Contours of brain structures were traced using the digitized images and the position of each injection site was plotted on coronal planes, taking the anterior commissure (AC0) as the origin of the system axes.

Data pre-processing

Magnetic stimulation causes a significant electrical artifact on the recording electrodes. Minimizing the duration in which the artifact masks the neuronal activity is crucial for successfully identifying rapid (short-latency) neuronal responses to the stimulation. This was done by making hardware changes such as advanced grounding, wide band filtering combined with low amplification, and applying software based offline artifact removal. Artifact removal was done using the Stimulus Artifact Removal Graphical Environment (SARGE) (Erez et al., 2010). This framework allows high flexibility and reliability in dealing with artifacts which vary greatly in their characteristics, as is typical of magnetic stimulation. Using this framework, neuronal activity is lost only during the brief periods of complete stimulus-related saturation, but neuronal activity following that period can be reconstructed. Periods of stable artifacts following the magnetic stimulation were typically removed using a moving mean shape subtraction whereas unstable responses were handled using a single pulse polynomial fit of the artifact. The non-usable period (NP) in both the motor cortex and GP was short (median: M1 = 2.0 ms, GP = 0.8 ms) and enabled the detection of rapid responses to the stimulation pulses (Supplementary Fig. 1). The signal was then offline sorted (OFS-2.8.4, Plexon, Dallas, TX) to generate spike trains of individual neurons. The spike train quality met the selection criterion when there were less than 0.1% of short (<1 ms) inter-spike-intervals and the stability was verified by checking that the spike shape was not significantly altered throughout the session. All the neurons used in this study were chosen based on their spike quality relative to the background noise and not according to their responsiveness to the stimulation.

Data analysis

Changes in neuronal activity in response to magnetic stimulation were assessed using peristimulus time histograms (PSTHs). All the PSTHs were constructed using 1 ms bins and smoothed by a Gaussian window (SD = 4 ms). The confidence limits for the PSTHs were defined using the distribution of the expected PSTH values which were calculated using control PSTHs that were generated by selecting random times prior to the stimulation period. The SD of the each control PSTH was used to construct the confidence lines under the assumption of a normal distribution of the PSTH values. The population peristimulus time histograms (PPSTHs) were calculated by aggregating the normalized PSTHs of individual neurons and taking the median value within the population for each of the latencies. Each PSTH was normalized by dividing it by the baseline firing rate (mean value of the control PSTH). The confidence limits for the PPSTHs were defined using the distribution of values at the 100 ms period immediately preceding the stimulation.

The stimulation-induced correlation between the responses of neuronal pairs was assessed using the median correlation coefficient calculated over the time-lag ±20 ms between the activities of the two neurons. The definition of the correlation coefficient over this range, rather than only for the 0 offset, made it possible to overcome the jitter in the response latency between different experimental sessions, thus enabling an equivalent calculation within and between recording sessions.

Stimulation-dependent changes in the levels of correlated activity were studied using covariograms (Brody, 1999) and normalized joint peristimulus time histograms (JPSTHs) (Aertsen et al., 1989). The normalized JPSTHs were defined by the removal of the shift predictor from the raw JPSTHs. This was done in order to remove the direct effects of stimulation on the firing rate of each individual neuron. All JPSTH calculations were done using bins of 5 ms and smoothed with a 25 ms square window. The covariogram was defined accordingly using the formulation by Brody (1999) as the raw cross-correlation function after the removal of the shuffle corrector (similar to the shift predictor in this case) used to remove the effects of the stimulation on individual neuron activity. Only pairs of neurons recorded on different electrodes were used in both of these analyses to avoid artificial correlations derived by spike sorting limitations (Bar-Gad et al., 2001).

Results

Magnetic stimulation was applied over the cortex using the mini-coil while simultaneously recording from multiple extracellular electrodes (Fig. 1).

Cortical modulation following magnetic pulses

The activity of cortical neurons (36 neurons in the normal state, 43 neurons in the parkinsonian state) was recorded in the primary motor cortex (M1) during magnetic stimulation. The baseline firing rate of the neurons did not differ significantly between the two states (normal: 7.93 ± 1.38 spikes/s, parkinsonian 6.88 ± 1.18 spikes/s, mean ± SEM). The neurons displayed a stereotypic activity modulation in both the normal and parkinsonian states (Fig. 2). As expected from the low stimulation frequency (≤1 Hz), the neuronal response to the stimulation did not undergo dynamic changes during the stimulation sessions (Figs. 2A–B). Following a brief non-usable period (median 2.0 ms) induced by the magnetic stimulation, the response consisted of a rapid brief excitation following the stimulation pulse, which was followed by a prolonged inhibition ("silent period"). The duration of the silent period was similar in both states (normal: 94.4 ± 6.0 ms, parkinsonian: 94.2 ± 5.8 ms, mean ± SEM). Some of the neurons in both states displayed rebound excitation in the later (>100 ms) phase of the response. Most of the neurons (58%, 21/36) in the normal state expressed a rebound excitation following the silent period before returning to baseline firing rate, while a smaller fraction of neurons (42%, 18/43) expressed a similar late excitation in the parkinsonian state (non significant, $\chi^2 = 2.1$, DF = 1, $p > 0.1$). The rebound excitation was evident in the PPSTH reflecting the mean population response during the normal state but was not significant in the parkinsonian state (Figs. 2C–D).

Pallidal modulation following magnetic pulses

In the normal state, neurons in both pallidal segments (39 GPe and 14 GPi neurons) did not display significant stimulation-induced phasic changes in firing rate. This was evident both in the PSTH of single

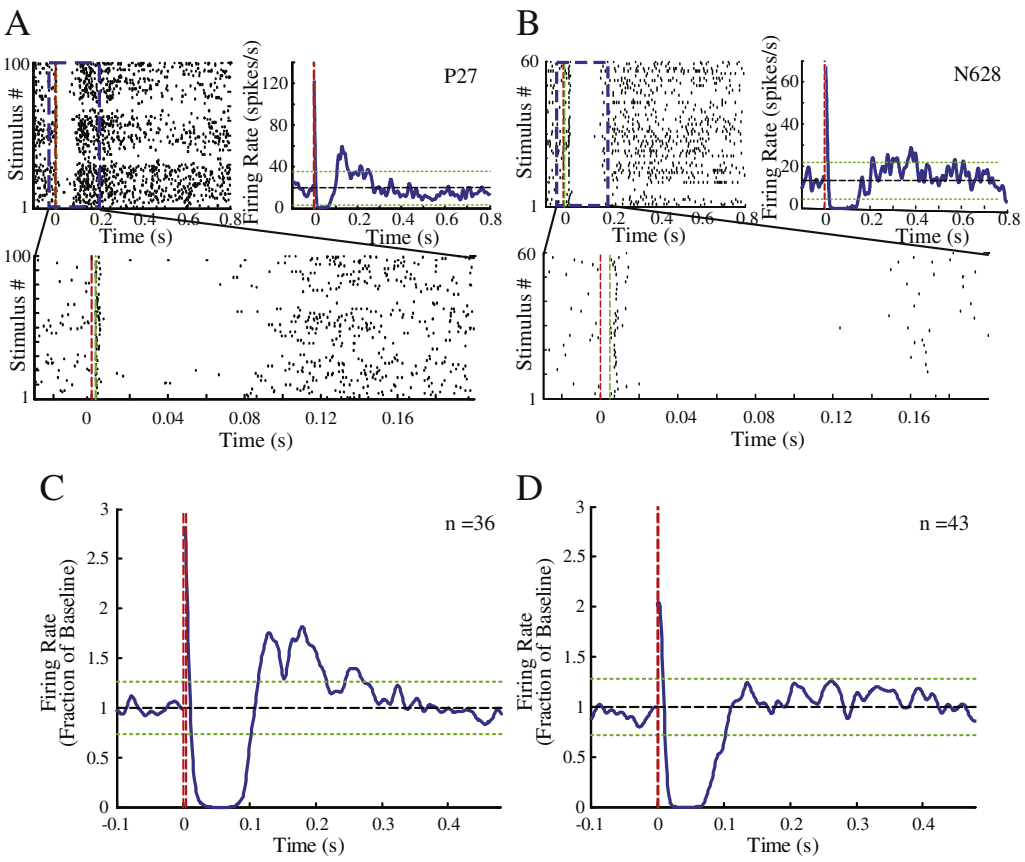
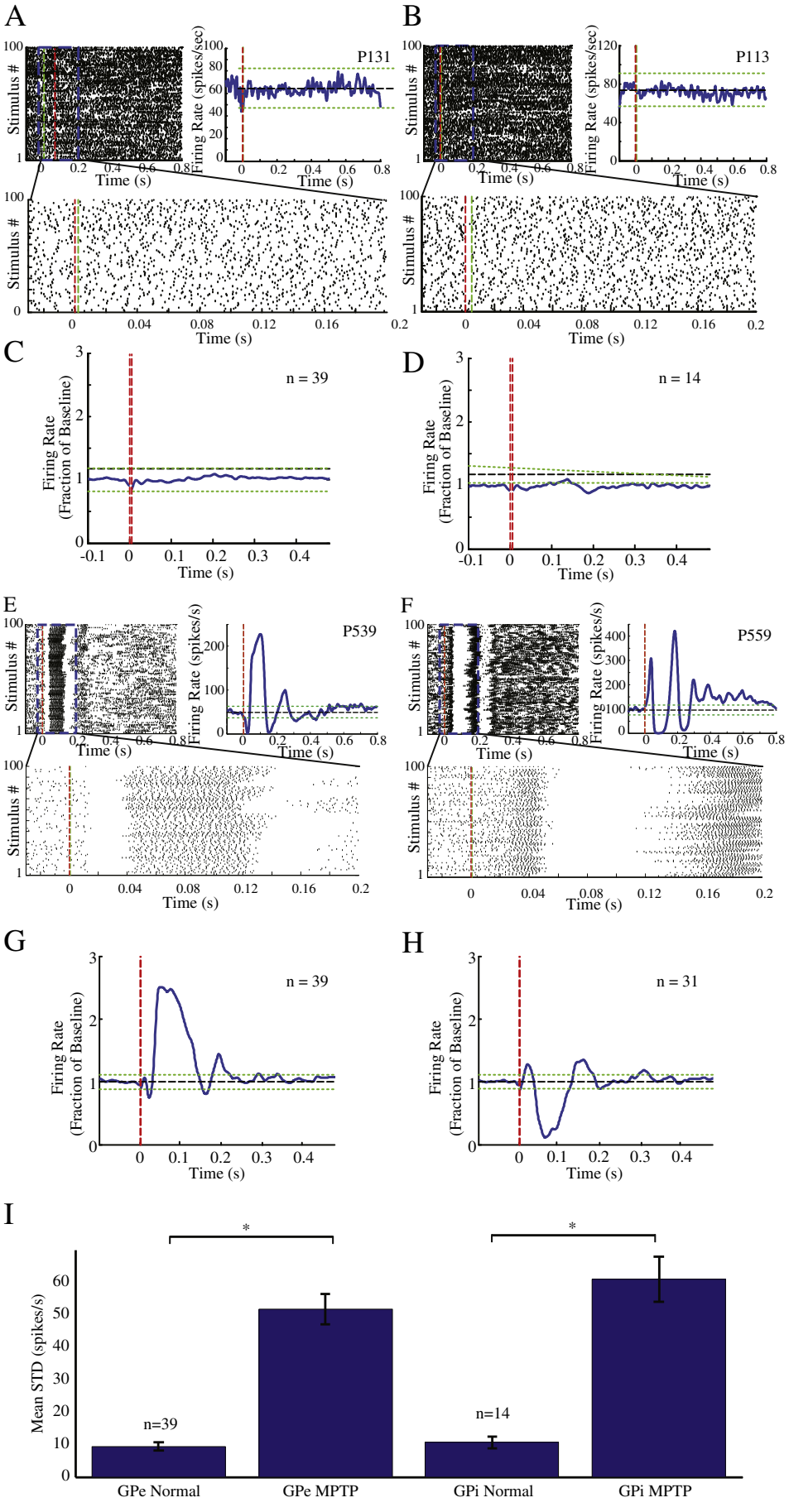


Fig. 2. Cortical response to magnetic stimulation. (A, B) Response of an M1 neuron in the (A) normal and (B) parkinsonian states to magnetic stimulation shown by a peri-event raster (top-left), a "zoomed-in" peri-event raster (bottom) and peri-stimulus time histogram (PSTH; top-right). The red dashed lines indicate stimulus onset; the green dashed lines indicate the end of the "non-usable period" (NP). (C, D) Median PPSTH of normalized PSTHs of (C) normal and (D) parkinsonian neurons. The area between the two vertical red dashed lines is the maximal NP for each neuronal population. The horizontal black dashed lines mark the averaged normalized firing rate; the horizontal green dashed lines indicate the 99% confidence limits. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

neurons (Figs. 3A–B) and in the population response to the stimulation (Figs. 3C–D) which did not cross the significance level ($p<0.01$) at any time. A very different pattern of stimulation-induced activity was apparent in pallidal neurons recorded in the parkinsonian state (39 GPe and 31 GPi neurons). In this state, neurons recorded in both pallidal segments displayed large changes in firing rate over a prolonged period following the stimulation (Figs. 3E–F). The neuronal response following the stimulation was highly stereotypic within each pallidal segment, but differed, displaying a mirror image, between the two segments (Figs. 3G–H). The stimulus derived modulation, as expressed by the average standard deviation of the PSTH over the first 200 ms, was significantly larger in the parkinsonian state than in the normal state for both pallidal segments (Fig. 3I, two-tailed *t*-test, $p \ll 0.01$. 8.9 ± 1.1 in normal GPe, 44.8 ± 3.9 in MPTP GPe, 8.5 ± 1.3 in normal GPi, and 49 ± 5.5 in MPTP GPi).

The stereotypic change in neuronal activity varied slightly in the latency and duration of the excitation and inhibition phases. Consequently, a relatively short portion of the response which was invariant to the jitter was chosen to classify the changes. The most prominent change that was stable across neurons in both pallidal segments occurred between 60 and 100 ms following each stimulation pulse. This short (40 ms) period which is central to a prolonged change (100 ms) in the firing rate, was not prone to changes due to latency jitters and enabled a quantification of the maximal firing rate changes. Comparison to control values calculated by rate calculation at random times revealed that assessing the changes during this period revealed that only a small fraction (6/53 11.3%) of the normal state pallidal neurons displayed a significant change in the firing rate during this period (Figs. 4A and C). In the parkinsonian state, a large majority (63/70 90%) of the GP neuronal responses to the stimulation displayed significant changes in firing rate during the 60–100 ms period relative to the control (Figs. 4B and D). In the parkinsonian state two typical responses to the stimulation were evident, with one pattern of activity typical of the GPe, and a second reversed pattern which was typical of the GPi (Supplementary Fig. 2). Type I responses were characterized by a long phase of excitation between 60 and 100 ms, whereas Type II responses were characterized by a long inhibition during the same time period. GPe neurons typically displayed Type I responses (37/38, 97% of the responding neurons in the parkinsonian state) and GPi neurons typically displayed Type 2 responses (24/26, 92%). Neurons in the normal state displayed the same types

Fig. 3. Pallidal response to magnetic stimulation. (A–B) Response of (A) GPe and (B) GPi neurons in the normal state to stimulation shown by a peri-event raster (top-left), a "zoomed-in" peri-event raster (bottom) and PSTH (top-right). The red dashed lines indicate stimulus onset time; the green dashed line indicates the end of NP. (C, D) Median of all normalized PSTHs of (C) GPe neurons and (D) GPi neurons. The area between the two vertical red dashed lines is the maximal NP for each neuronal population. The horizontal black dashed lines mark the average normalized firing rates; the horizontal green dashed lines indicate the 99% confidence limits. (E, F) Response of (E) GPe and (F) GPi neurons in the parkinsonian state using the same conventions as (A, B). (G, H) Median of all normalized PSTHs of (G) GPe neurons and (D) GPi neurons using the same conventions as (C, D). (I) The mean response amplitude, defined by the standard deviations of the neurons' PSTH during the period 0–200 ms in each population. Error bars indicate SEM. Significant differences between population are marked by asterisks (*t*-test, $p<0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)


A
B
P131
P113
Stimulus #
Firing Rate (spikes/sec)
Time (s)
C
D
n = 39
n = 14
Firing Rate (Fraction of Baseline)
E
F
P539
P559
G
H
n = 39
n = 31
I
Mean STD (spikes/s)
n=39
n=14
GPe Normal
GPe MPTP
GPi Normal
GPi MPTP
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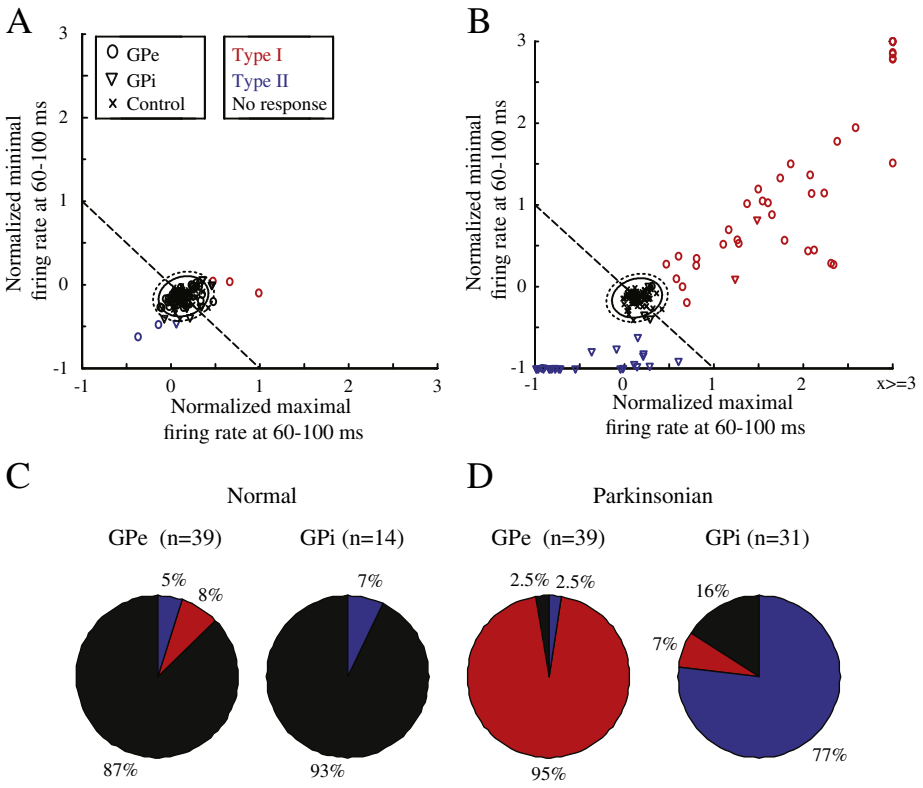

Fig. 4. Pallidal response types. (A–B) Scatter plot of responses to stimulation in the (A) normal and (B) parkinsonian states, defined by the maximum and minimum rates at 60–100 ms after stimulus. GPe (circles), GPi (triangles) and control (crosses) neurons are presented. The colors indicate the response type: red for excitation, blue for inhibition, and black for non-significant response. Confidence ellipse, solid line: $p<0.01$, dashed line: $p<0.001$. (C–D) Fraction of pallidal neurons responding in Type I (red), Type II (blue) or no response (black) in the (C) normal and (D) parkinsonian states. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

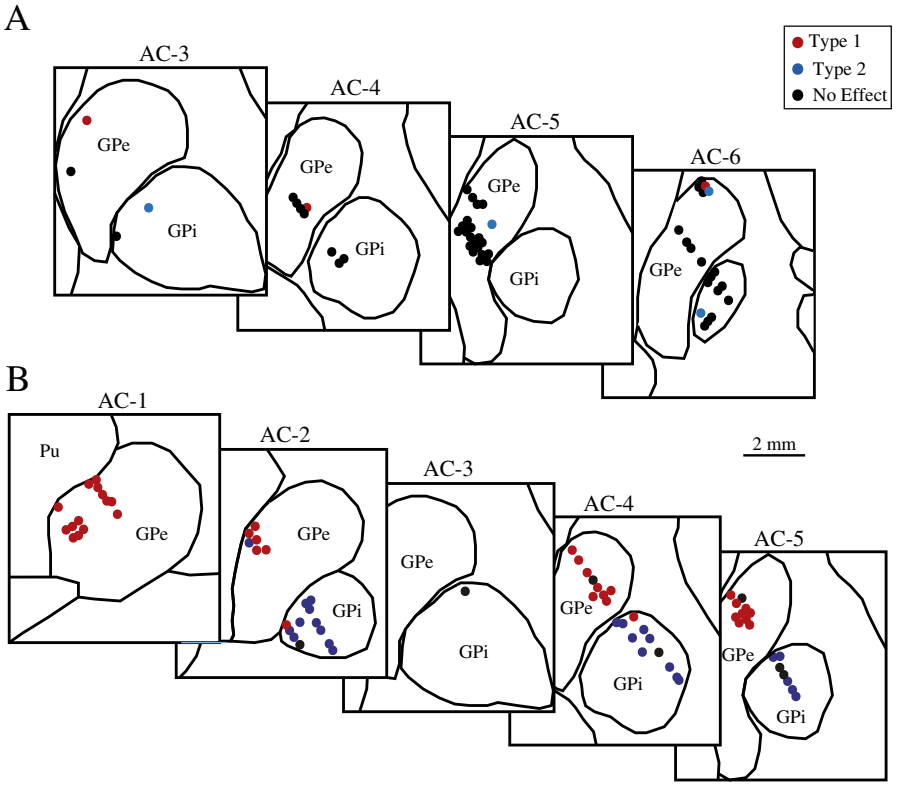

Fig. 5. Anatomical reconstruction. Anatomical reconstruction of the recorded neurons imposed on coronal sections corresponding to AC-1 to AC-6 of the left hemisphere of monkey P. The neurons are color coded (red — Type I, blue — Type II and black — no response). The neurons were recorded in the (A) normal and (B) parkinsonian states. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of responses and nucleus based division but the number of neurons and the magnitude of the response were drastically lower (Figs. 4C–D).

Anatomical reconstruction of the locations of the recorded neurons during the normal and parkinsonian states revealed that the majority of the neurons were recorded in the sensorimotor domain and a minority in the neighboring associative/executive domain (Fig. 5). Overall, most of the neurons recorded in the normal state did not respond to the stimulation and the spatial distribution of

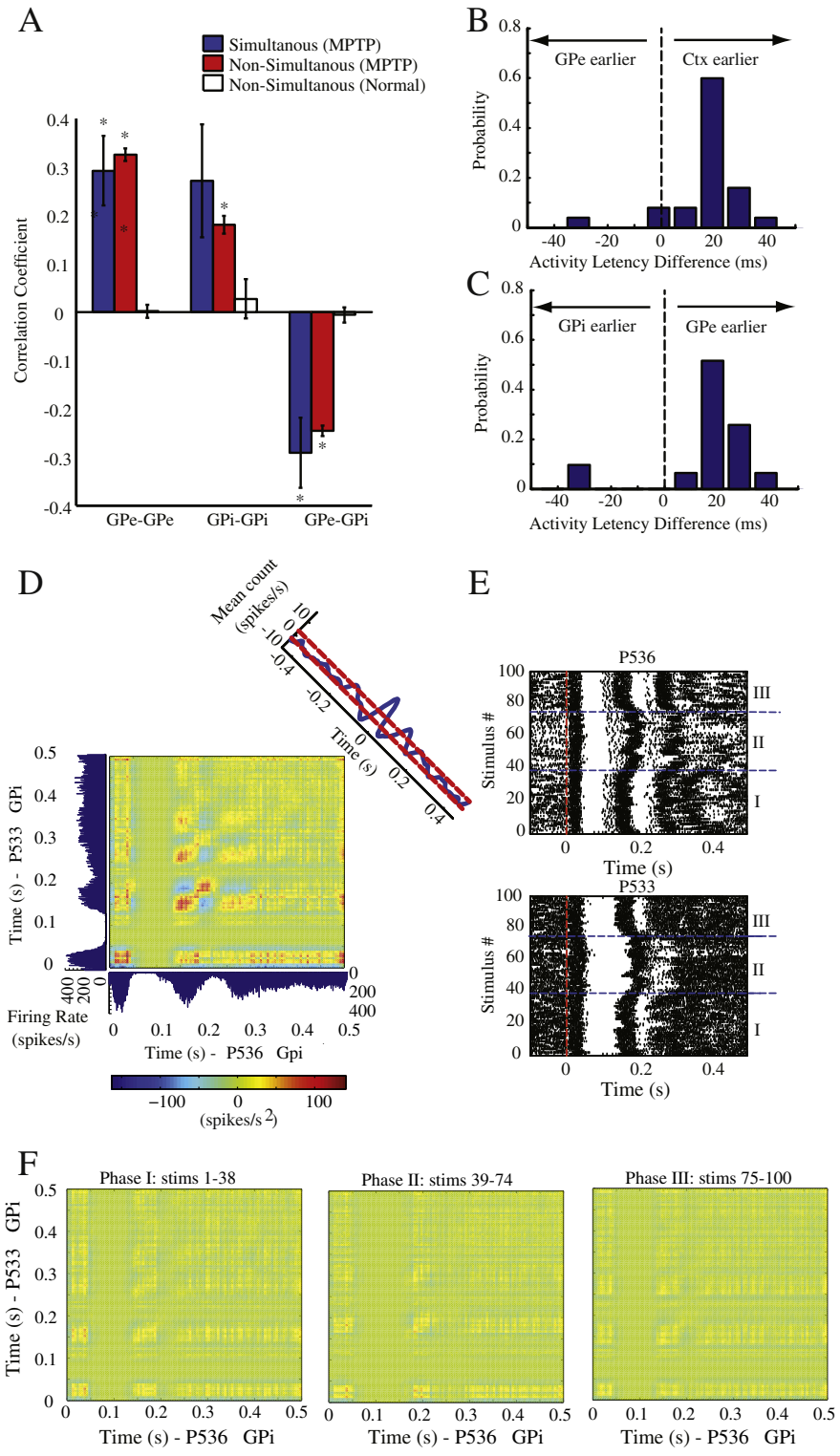

Fig. 6. Correlations in the pallidal response to stimulation. (A) Correlation coefficient between MPTP neurons recorded simultaneously (blue) vs. different trials (red) and normal neurons from different trials (white). Error bars represent SEM. Significant differences between populations are marked by asterisks (one-way ANOVA, $p<0.01$). (B–C) Distribution of the latency response time difference of (B) M1–GPe and (C) GPe–GPi pairs of neurons recorded simultaneously. (D) JPSTH of GPi–GPi pairs recorded simultaneously in the parkinsonian state, the PSTHs of each neuron appear to the left and bottom of the JPSTH and the covariogram appears over the diagonal. (E) Peri-event raster of the two neurons demonstrating a variation of the response over 3 time periods. The red dashed lines indicate stimulus onset time and the blue dashed lines indicate the three phases in the response. (F) JPSTH between the two neurons during each phase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the few responding neurons did not display any clear spatial organization within each nucleus (e.g. in relation to the sensorimotor vs. associative domain or to a somatotopic sub-division within the motor domain) (Fig. 5A). In the parkinsonian states, neurons in the GPe primarily responded by a Type 1 response without any clear organization between or within functional domains and GPi neurons responded with a Type 2 response with a similar diffuse spatial activity pattern (Fig. 5B). The responses in the parkinsonian state did not follow a spatial organization although the neurons were spread throughout most of the anterior–posterior span of the nuclei.

Neuronal interaction

In order to assess the co-activation of neurons within the GP in response to the magnetic stimulation we analyzed the correlation coefficient of pairs of neurons recorded either simultaneously or in different sessions to the stimulation. This made it possible to separate co-activation that resulted solely from the stimulation (different sessions) from co-activation that involved neuronal interaction (same session). The correlation coefficient of pallidal neuronal pairs recorded in the parkinsonian state simultaneously (31 GPe–GPe, 11 GPi–GPi, and 31 GPi–GPe pairs) and on different sessions (710 GPe–GPe, 454 GPi–GPi, and 1178 GPi–GPe pairs), and pallidal pairs of neurons recorded in the normal state in different sessions (721 GPe–GPe, 85 GPi–GPi, and 544 GPe–GPi) revealed a stereotypy of response patterns across neurons in the parkinsonian state (Fig. 6A). The correlation was similar between neurons within and between sessions, implying the presence of correlations that were dependent solely on the stimulation induced activity. GPe neurons in the parkinsonian state tended to respond in a similar way during all sessions, which was reflected by the significantly larger average correlation coefficient of these neurons compared to GPe–GPe pairs recorded in the normal state (0.3 ± 0.07, 0.3 ± 0.01, 0 ± 0.01 mean $\pm$ SEM for MPTP simultaneous, non-simultaneous and normal pairs, respectively, $p<0.01$, one-way ANOVA, Tukey's HSD post-hoc). The difference between the MPTP GPi non-simultaneous pairs and the normal pairs was significant (0.18 ± 0.02 for MPTP non-simultaneous vs. 0.02 ± 0.04 for normal pairs. $p<0.01$, one-way ANOVA, Tukey's HSD post-hoc). The considerable difference between the MPTP GPi simultaneous pairs and the normal GPi pairs was not significant (0.28 ± 0.12 for MPTP simultaneous; Tukey's HSD post-hoc, n.s.) probably due to the small neuronal pair population. The correlation between the GPe and GPi was negative since the responses to magnetic stimulation were in opposite phase to each other. Both MPTP GPe–GPi groups (simultaneously and non-simultaneously recorded) displayed significantly larger levels of correlation compared to the normal GPe–GPi pairs (-0.3 ± 0.07, -0.25 ± 0.01, 0 ± 0.01 for MPTP simultaneous, non-simultaneous and normal pairs, respectively. $p<0.01$, one-way ANOVA, Tukey's HSD post-hoc).

The timing of the response in different parts of the CBG pathways could be compared to identify the temporal order of activation. This difference was assessed by comparing the shift in the response phases (or their reversal) between pairs of neurons recorded simultaneously in different brain areas. The cross-correlation between the simultaneously recorded MPTP cortical and the GPe cells (25 M1–GPe pairs) indicated that the response in the GPe started significantly later than the response in M1 (Fig. 6B, mean latency 20 ± 14 ms, mean $\pm$ SD, two-tailed *t*-test, $p<0.01$). In addition, the response in the GPi (31 GPe–GPi pairs) started significantly later than the response in the GPe (Fig. 6C, mean latency 20 ± 18 ms, mean $\pm$ SD, two-tailed *t*-test, $p<0.01$).

The co-activation of neurons in the parkinsonian state leads to the formation of directly induced correlations between neuronal pairs (11 GPi–GPi, 31 GPi–GPe and 29 GPe–GPe pairs). The separation of direct neuronal interaction from the immediate stimulation effect required the usage of covariograms (Brody, 1999) and joint peri-stimulus histograms (JPSTHs) (Aertsen et al., 1989) instead of the more common cross-correlation functions. The covariogram of two neurons is the raw cross-correlation function after the subtraction of the shuffle corrector removes the direct effect of the stimulation. A large fraction (32/71 pairs 45%) of the covariograms displayed a significant peak even after this correction, suggesting a potential direct interaction between neuronal pairs (Fig. 6D). Close inspection of the peri-stimulus rasters revealed latency covariations in the response, i.e. similar changes in the time of the response to individual stimulation pulses in both neurons (Fig. 6E). In the example, the raster plots show that both neurons evolved through three response phases. During each phase the response was stable and changed primarily in the transitions between the phases. The JPSTHs for each time range did not show significant direct interaction between the pairs (Fig. 6F) whereas the JPSTH and the covariogram for the whole period indicated synchronization between neurons (Fig. 6D). This difference implies that the observed correlations in neural activity were not derived from synaptic-based neuronal interactions or common stimulation-derived interactions but rather were mostly induced by common, global network level, changes in activation.

Discussion

In this study, we used magnetic stimulation to investigate the propagation of neuronal activity along the CBG pathway in the normal and parkinsonian primate. Two recent advances aided this study: a novel custom made magnetic mini-coil configuration enabling concurrent recording in the behaving primate (Tischler et al., 2011) and a stimulus artifact removal graphical environment (SARGE) (Erez et al., 2010) minimizing the loss of recorded data caused by the large stimulation artifacts. This unique experimental configuration enabled, for the first time, simultaneous multi-electrode recordings during magnetic stimulation and the reconstruction of the resulting multiple spike trains. Moreover, the magnetic field decays by a factor of 2 over a 5 mm distance (Tischler et al., 2011) leading to direct cortical activation without non-synaptic effects on subcortical areas. Our results indicate that the cortex responds to stimulation during both states by a stereotypic activity pattern: a brief excitation followed by prolonged inhibition. However, basal ganglia neurons display a state-dependent synaptic response. In the normal state, only minor changes in activity patterns were observed, whereas the same stimulation evoked strong pallidal responses in the parkinsonian state. In particular, in the parkinsonian state the pallidal response tracked the pattern of activity in the cortex, with an identical phase in the GPi and an inverted phase in the GPe. A delay was observed between the cortical activation and the pallidal response, indicating that the pallidal effects can be attributed to synaptic activation from the cortex, rather than to a direct deep brain effect of the magnetic stimulation. The responses were highly stereotypic within each nucleus and displayed high signal correlation, indicating a network-wide activation without direct synaptic interaction between the recorded neurons. The recorded neurons in both states were mostly located in the sensorimotor domain of the nuclei with a minority situated in the classic associative domain. The response of the neurons in the parkinsonian state and the lack of response in the normal state did not follow any organization between the two domains or a somatotopic organization within the motor domain, hinting at a large network wide change in the pattern of activation.

The pattern of cortical activation evoked by stimulation is consistent with earlier studies of cortical activation by electrical and magnetic stimulation. Early studies of cortical activation using electrical stimulation revealed a short term excitation followed by a prolonged period of inhibition — the “silent period” (Krnjevic et al., 1966). Later, multiple studies performed on human subjects that combined electrical and magnetic stimulation with primarily peripheral recording measures such as motor evoked potentials (MEPs) identified a similar activation pattern (Inghilleri et al., 1993; Wilson et al., 1993) with comparable activation patterns in PD patients (Berardelli et al., 1996; Kleine et al., 2001; Priori et al., 1994). These studies reported

the same basic response to stimulation in PD patients and healthy controls, although there were some changes in the specific properties of different phases of the response. Similarly in our study, the cortical response in the two states did not reveal major differences in the initial phases of the response; i.e., an initial short excitation was followed by a prolonged inhibition.

Electrical microstimulation in different cortical areas revealed a stereotypic multi-phase response in both segments of the globus pallidus comprised of excitation and inhibition phases lasting a few tens of milliseconds (Kita and Kita, 2011; Nambu et al., 1990, 2000; Nishibayashi et al., 2011). The different phases of the response were associated with the known excitatory and inhibitory pathways from the cortex to the globus pallidus (Kita and Kita, 2011; Nambu et al., 2000; Tachibana et al., 2008). In previous studies, the early excitatory activity was shown to be mediated by the STN hyperdirect pathway (Nambu et al., 2000) whereas the later phases were mediated by the striatum and associated with the direct and indirect pathways (Tachibana et al., 2008). In studies of the normal primate (Nambu et al., 2000) and rat (Kita and Kita, 2011) there were no long term (more than a few tens of milliseconds) changes in pallidal activity following stimulation. Long duration responses were detected in parkinsonian (6-OHDA treated) rats, which were not present in normal rats, and were comprised of excitation in the GPe and inhibition in the STN and GPi (Kita and Kita, 2011). This response, although different in precise latency and duration, is consistent with the activation that we observed in the parkinsonian (MPTP treated) primate. Our results demonstrating the minimal responses of a few neurons to the stimulation during the normal state differ dramatically from other primate studies showing large and seemingly common neuronal responses during this state. This discrepancy may be attributed to the severe sampling bias in other studies where neurons were chosen for recording and analysis on the basis of their response to stimulation; i.e., all the neurons responded to the stimulus and they varied only in their response type (Nambu et al., 1990). In the current study we recorded and analyzed all the well-isolated neurons along the electrode trajectories without testing their stimulation responsiveness a-priori; thus the majority of our neurons in the normal state did not respond to the stimulation whereas in the parkinsonian state, there was a significant change in the fraction of the responding neuronal population between the two states.

The inverted response patterns of the GPe relative to the GPi observed in our study but not in earlier electrical stimulation studies (Nambu et al., 2000) are consistent with the long duration GPe/GPi differences observed in the parkinsonian rat (Kita and Kita, 2011) and may thus be an important property of the dopamine depleted state. Moreover, these changes may be a property of the widespread cortical activation in magnetic stimulation not observed in the localized (focal) cortical activation produced by electrical stimulation. The magnetically induced electric field affects multiple large cortical areas beneath the stimulation coil (for a detailed computational and experimental analysis of the field see Pashut et al., 2011; Tischler et al., 2011) whereas the electrical stimulation generates fields which are maximal near the tip and decay rapidly with the distance from the electrode. This argument is supported by a pioneering study that recorded single neuron activity in the human subthalamic nucleus following magnetic stimulation in PD patients undergoing deep brain stimulation (DBS) electrode implantation surgery (Strafella et al., 2004). This study reported STN temporal activation patterns similar to the cortex and GPi activation in our study.

Neuronal activity in the normal basal ganglia is characterized by independent firing in both the temporal domain (Poissonian-like firing of single neurons) and the spatial domain (no correlations between neurons) (Bar-Gad et al., 2003). This basic property declines during Parkinsonism and is reflected in a loss of independence in both domains: the appearance of bursting and oscillations on the single neuron level, and an increase in correlations and coherence at the network level (Raz et al., 2000). Moreover, neurons throughout the basal ganglia lose their highly specific response to behavioral events in favor of a non-specific broadly tuned response (Baker et al., 2010; Bronfeld and Bar-Gad, 2011; Filion et al., 1988). This loss of specificity and increased inter-neuronal correlation may underlie the stereotypic population-level responses we observed following cortical stimulation. A similar pattern of large population-level neuronal responses was reported in studies of cortical stimulation in the mouse model of dystonia (Chiken et al., 2008) and in human dystonia patients (Nishibayashi et al., 2011). These widespread correlated neuronal activity patterns during Parkinsonism and dystonia are in line with the breakdown of CBG computation thought to underlie involuntary movement (Mink, 2003). This is further supported by computational models implementing action selection (Mink, 1996) or information extraction (Bar-Gad et al., 2003) of basal ganglia input which require a sparse representation of information within the basal ganglia and break down upon the formation of correlated activity patterns.

The assessment of the changing functionality of different brain areas requires testing their response to different activation patterns. This approach involves identifying the dynamic changes that can only be revealed through input to the system and is in many ways reminiscent of studying the properties of a communication system by analyzing its transfer function for converting input signals into outputs. By utilizing a novel paradigm of magnetic stimulation combined with simultaneous recording along the pathway we were able to study the processing of the CBG system during the normal and parkinsonian states. Our results demonstrate a change in information transmission within the system that leads to stereotypic system-wide responses. The pronounced response of the GPi to incoming activation signals may lead to abnormal modulation of cortical activity, resulting in the clinical manifestation of PD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nbd.2012.07.021.

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