Bicuculline induced chorea manifests in focal rather than globalized abnormalities in the activation of the external and internal globus pallidus.

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Abstract

Chorea is a basal-ganglia (BG) related hyperkinetic movement disorder characterized by irregular continuous involuntary movements. Chorea and related hyper-behavioral disorders may be induced in behaving primates by local microinjections of the GABA-A antagonist bicuculline in the globus pallidus externus (GPe). We performed multi-electrode extracellular recordings in the GPe and in the globus pallidus internus (GPi) before, during and after bicuculline microinjections. Bicuculline led to an increase in the firing rate and a change in the firing pattern of GPe neurons. Two types of abnormal neuronal firing patterns were detected in GPe neurons close to the bicuculline microinjection site: continuous high-frequency activity, and bistable activity in which neurons transitioned between high-frequency and complete cessation of firing. Neuronal activity remained uncorrelated within and between the GPe and the GPi with no evidence of propagation of the focal GPe abnormal activity downstream to the GPi. Despite reduction in the information capacity of bicuculline affected GPe neurons, the ability to encode behavioral events was maintained. We found similar responses of GPe neurons to bicuculline in-vitro in the rat, suggesting a basic cellular mechanism underlying these abnormal firing patterns. These results demonstrate that chorea is associated with focal neuronal changes that are not complemented by global changes in the BG internal and output nuclei. This suggests a mechanism of stochastic phasic alteration of BG control leading to the chaotic nature of chorea. Thus, rather than imposing a globalized state of cortical excitability, chorea might be associated with changes in internal information processing within the BG.
Introduction

Chorea is a hyperkinetic movement disorder characterized by spontaneous involuntary movements that are continuous, irregularly timed and randomly distributed (Mark, 2004; Wild and Tabrizi, 2007). Chorea appears as a symptom in multiple basal ganglia (BG) related movement disorders such as Huntington’s disease (HD), L-Dopa induced dyskinesia in Parkinson’s disease and hemiballism (Wild and Tabrizi, 2007; Schrag and Quinn, 2000; Crossman, 1987). The BG are a group of interconnected subcortical nuclei which play a major role in control of movement and behavior through their reciprocal connections with the cortex (Alexander et al., 1986). Input to the BG is sent to the striatum and the subthalamic nucleus (STN) and is conveyed to the thalamus and cortex through the output nuclei – the globus pallidus internus (GPi) and the substantia nigra pars reticulata. Within the BG, information is processed through the interplay of two opposing pathways, the direct path from the striatum to the output nuclei and the indirect pathway which also involves the globus pallidus externus (GPe) and STN (Albin et al., 1989; DeLong, 1990).

Chorea seems to be specifically related to dysfunction in the indirect pathway of the BG circuitry. In HD patients the appearance of chorea is temporally correlated with a selective loss of striatal neurons projecting to the indirect pathway (Glass et al., 2000; Deng et al., 2004; Allen et al., 2009), and damage to the STN was found to be directly related to the appearance of hemiballism in both human patients (Martin, 1927; Martin and Alcock, 1934) and non-human primates (Carpenter et al., 1950; Hamada and DeLong, 1992b). Chorea and other behavioral abnormalities may be induced by local disruptions of GABA transmission in the indirect pathway. Microinjections of GABA-A antagonists into the motor territory of the primate GPe induce chorea (Matsumura et al., 1995; Crossman et al., 1988), while similar injections into the limbic and associative GPe territories induce other forms of excessive abnormal behaviors (hyper-behavioral symptoms), such as hyperactivity and stereotypic behaviors (Grabli et al., 2004). GABA plays a major role in information processing in the GPe as roughly 80% of all synapses within this nucleus are GABAergic (Kita, 2007). GABAergic inputs to GPe neurons originate from striatal projections and from GPe collaterals, and are mostly mediated by GABA-A ionotrophic receptors (Smith et al., 2001; Charara et al., 2005; Charara et al., 2000; Kita et al., 2006).

Some theoretical models of the BG hypothesize that chorea may result from global reduction of BG output leading to cortical disinhibition manifesting in the expression of excessive unwanted movements (Albin et al., 1989). Alternative models maintain that the movements may rather be displayed due to focal activation of small neuronal populations in
The BG output (Mink, 2003). Previous studies have found that chorea induced by GABA-A antagonists was associated with increased activity in the GPe (Mitchell et al., 1989; Matsumura et al., 1995), accompanied by an augmentation of pauses in some neurons (Matsumura et al., 1995). However, in the GPi, which is the main BG motor output nucleus, more complex changes in activity have been observed, consisting of both increases and decreases in firing rate (Matsumura et al., 1995).

The aim of this study was to explore the neuronal correlates of chorea induced by microinjections of the GABA-A antagonist bicuculline in the GPe of nonhuman primates. We used multi-electrode recordings of GPe and GPi neurons before and during the expression of chorea to identify changes in the activity patterns of single neurons and the interactions between them. In-vitro intracellular recordings from the rat GP were used to gain further insights into the effects of bicuculline on these neurons.

Methods

Animals

Two *Macaca fascicularis* monkeys (Monkeys M & I; male; weight: 4-5 kg) were used in this study. Data from two additional *Macaca fascicularis* monkeys (Monkeys A & N) were used as controls for neuronal activity in the normal macaque (details in (Erez et al., 2009)). The monkeys’ health was monitored by a veterinarian, and their fluid consumption, diet and weight were monitored daily. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and Bar-Ilan University guidelines for the Use and Care of Laboratory Animals in Research. The experiments were approved and supervised by the Institutional Animal Care and Use Committee (IACUC).

Surgical and experimental procedures

The surgical procedures for cranial implantation are described in detail in previous work from our lab (McCairn et al., 2009). Briefly, two square cilux chambers (27 mm x 27 mm) (Alpha-Omega Engineering, Nazareth, Israel) were implanted stereotaxically to allow bilateral access to the basal ganglia using a coronal approach. The recording chambers were tilted at 40° (monkey M) or 35° (monkey I) in the coronal plane with their center targeted to the center of the GPi: stereotactic coordinates A13, L8 and H3 (Szabo and Cowan, 1984).

The animals were trained to sit in a primate chair and perform a simple sensorimotor task consisting of pressing a central button followed by a press of one of two possible side buttons, depending on a visual cue, for delivery of a liquid reward. Spontaneous behaviors and
task execution were observed and recorded during experimental sessions using a multi-channel video system (GV-800, Geovision, Taiwan) and digital feedback from the behavioral system. The animal's behavior was monitored 20-40 min prior to injection and 40-120 min after injection. Detailed behavioral analyses were performed offline using the recorded video based on previously described methods used in this model (Grabli et al., 2004), and by automatic detection of events related to performance of the behavioral task.

Electrophysiological recording

Following recovery from surgery each animal underwent microelectrode guided mapping of the GPe and GPi. The preliminary mapping process was followed by the experimental sessions. The recording and injection setup are described in detail in supplementary material 1. Briefly, the injectrode was comprised of a 28-gauge stainless steel cannula with a straight tip which was partially encapsulated inside a piercing cannula (25 gauge outer diameter stainless steel cannula with a beveled tip). During each experimental session up to 11 glass-coated tungsten microelectrodes (impedance 250-750 KΩ at 1 kHz) and the injectrode were introduced using two separate manipulating towers that could move each electrode and the injection cannula independently with 2µm resolution (DMT and EPS, Alpha-Omega Engineering). The tip of the injection cannula was aligned to the tip of the recording electrodes mounted on the same manipulating tower, and depth of injection was determined by recordings made through these electrodes. Extracellular activity was filtered by a wide band pass filter (5-8000 Hz; 4 pole Butterworth filter), amplified by 2000 (MCP-Plus, Alpha Omega Engineering) and continuously sampled at 40 kHz (AlphaMap, Alpha-Omega Engineering).

Microinjections

The injection cannula was connected via a Delrin manifold to a 10 µl syringe (Hamilton Company, Reno, NV, USA), and filled with the injected solution. We used bicuculline-methiodide (Sigma-Aldrich, Israel) dissolved in physiological saline at a concentration of 15 µg/µl (29.5 mmol/l), or saline for control injections. At the beginning of each recording day electrodes were lowered into the brain and the GPe and GPi were identified. Subsequently the tip of the injection cannula was positioned at the chosen site within the GPe and was left in place during the entire experimental session. Once a sufficient number of separable neurons had been identified on the electrodes and were stable for a minimum of three minutes, bicuculline (1-3.5 µl) or saline (1.5-2 µl) was injected manually at a rate of approximately 2
µl/minute. Additional microinjections into the sensorimotor striatum were administered using the same apparatus; results were described in detail in a previous work (McCairn et al., 2009).

**Analysis of neuronal activity**

Action potentials of individual neurons were sorted offline (OfflineSorter V2.8.7, Plexon, Dallas, TX, USA), enabling high fidelity neuronal identification. Neurons were retained for analysis if they met the following criteria: (1) the recording was from a location within GPe or GPi. (2) The acquired neurons' action potentials were of a consistent distinct shape that could be fully separated with a high degree of certainty from the spike waveforms of other neurons and background noise. (3) The neurons' inter-spike intervals (ISI) were confirmed to have a minimum refractory period of 1.5 milliseconds (<0.1% of spikes within the period). (4) Stable neuronal recording was available for at least 35 seconds. Some neurons were recorded continuously before and after bicuculline microinjection and the subsequent appearance of abnormal behaviors. The firing rates of these neurons were calculated in 2 s bins for the entire recording period.

All measures in the Results section are described as mean ± standard deviation (STD) unless stated otherwise.

**Single unit analyses:** Firing patterns of the neurons following bicuculline injection displayed changes on multiple timescales ranging from a few milliseconds to multiple seconds. Thus, analyses were typically performed in multiple timescales to accommodate the different orders of magnitude in the temporal domain. The ISI distribution was calculated for all neurons using 2, 10, and 100 ms bin sizes. Autocorrelation functions were calculated to evaluate both short and long term regularity, using ±50 ms and ±10 s offsets in 0.1 ms and 1 ms bins, respectively. The firing patterns of GPe neurons were first evaluated by an expert human observer, and subsequently several parameters were tested to quantitatively group the neurons according to their firing patterns. The parameters that gave the best clustering results were: (i) the inverse of the median inter spike interval \([1/\text{median(ISI)}]\) and (ii) the mean length of the longest 1% of ISIs. Thresholds defining the different types of firing patterns were set based on the characteristics of data from normal untreated GPe controls.

**Distance from injection site:** The Euclidean 3D distance from the injection site was calculated for each recorded neuron, as given by: 
\[
d = \sqrt{(x_{inj} - x)^2 + (y_{inj} - y)^2 + (z_{inj} - z)^2},
\]
where \(d\) is the distance (in millimeters), \(x\) is the anterior-posterior coordinate, \(y\) is the medio-
lateral coordinate and $z$ is the ventro-dorsal coordinate and $inj$ denotes coordinates of the injection site (defined as the tip of the injection cannula).

**Information capacity:** The upper bound of the neurons' ability to transmit information can be defined as the total entropy of the ISI distribution (Stevens and Zador, 1996). For each neuron the ISI distribution in 0.5 ms bins was constructed and the entropy was calculated by: $H_{ISI} = -\sum_{bin=1}^{n} p_{bin} \cdot \log(p_{bin})$, where $p_{bin}$ is the probability of each bin, and $n$ is the total number of bins with a non-zero probability.

**Spectral analyses:** Oscillations were evaluated using the autocorrelation and the power spectral density (PSD) functions of the spike train downsampled to 1 kHz. The PSD was calculated with Welch’s method using multiple scales. High-frequency (HF) oscillations were evaluated by using a 4096 bin Hanning window that produced ~0.25 Hz spectral resolution. The significance level was set at $p=0.01$ (normalized to the number of bins), calculated based on the power in the 10-500 Hz frequency band. Low-frequency oscillations were evaluated by using a Hanning window of 16384 bins that produced ~0.06 Hz spectral resolution. The significance level was set at $p=0.01$ (normalized to the number of bins), calculated based on the power in the 0-10 Hz frequency band. For each spike train the ‘main oscillation frequency’ was defined as the frequency with the maximal power of all the frequencies which exceeded the threshold, within the frequency band of interest (10-500 or 0-10 Hz for high and low frequencies, respectively). Coherence was used to study the degree of interaction, as function of frequency, between pairs of neurons. The coherence function is the cross spectrum of the two traces normalized by their auto-spectrums, and yields values between zero (no coherence) and one (maximal phase correlation). The parameters used for coherence calculation were the same as for the PSD calculations. Coherence was considered significant ($p<0.01$) if it crossed the value given by: $\text{limit} = 1 - (1 - \alpha)^{\frac{1}{N-1}}$, where $\alpha = 0.99$ and $N$ is the number of consecutive windows used for coherence calculation (Rosenberg et al., 1989).

**Correlation analysis:** Spike-to-spike cross-correlations were calculated for pairs of neurons with overlapping periods of stable recording. Only neuronal pairs that were recorded by different electrodes were included to avoid possible artifacts in the cross-correlation functions (Bar-Gad et al., 2001). The cross-correlation functions were calculated for ±4 s offset, using 1 ms bins. Significance tests were performed by calculating the mean correlation values and STD of the first and last seconds of the cross-correlation function. Confidence levels were calculated based on these parameters at a threshold of $p=0.01$, normalized to the
number of bins. A peak or trough was considered significant if it crossed these thresholds and was within an offset of ±300 ms from zero offset.

**Analysis of peri-event neuronal activity:** Time stamps of events of interest were used to construct peri-event histograms (using 10 ms bins) of the neuronal data. Significance testing of neuronal activity in peri-event histograms was determined by constructing 99% confidence limits based on the mean and STD of the activity within the tail of the histogram; i.e., activity at 5 to 1 s prior to the task-related event.

**Histology**

Following completion of the experiment, animals were anaesthetized using Ketamine 10 mg/kg and stereotactic marking micro-lesions (DC current 60 µA for 30 s) were made. The lesions were targeted to dorsal white matter tracts at the anatomical plane that was derived from electrophysiological mapping to be consistent with the position of the anterior commissure (AC0) (McCairn et al., 2009). The animals were then deeply anaesthetized using sodium pentobarbital 50 mg/kg and transcardially perfused with 1 liter of physiological saline, followed by 1 liter of 4% paraformaldehyde. The whole brain was removed and buffered in graded sucrose solution 10-30% over seven days. The brain was then frozen at -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 anterioposterior position of each injection site was plotted on coronal planes, taking AC0 as the origin of the system axes.

**In-vitro slice preparation and recording**

Thick sagittal slices of 300 µm were obtained from the somatosensory cortex, the striatum and the GP of 12-21 days old Wistar rats killed by rapid decapitation in accordance with the guidelines of the Bar-Ilan University animal welfare committee, using previously described techniques (Stuart et al., 1993; Bar-Yehuda et al., 2008). Slices were maintained in artificial cerebrospinal fluid containing (in mM): 125 NaCl, 2.5 KCl, 25 NaHCO3, 1.25 NaHPO4, 2 CaCl2, 1 MgCl2, 25 glucose and 0.5 Na-Ascorbat (pH 7.4 with 95% O2/ 5% CO2, 310 mosmol/kg). Bicuculline-methiodide (50 µM) was added to the bath solution via the perfusion system. Complete substitution of the bath medium took less than 5 minutes. The experiments reported here were carried out at 34°C. The GP nucleus and individual GP neurons were visualized using infrared differential interference contrast (IR-DIC) microscopy. The
standard pipette solution contained (in mM): 130 K-gluconate, 10 KCl, 10 HEPES, 4 MgATP, 10 Na-phosphocreatin, 0.5 EGTA and 0.3 GTP (Sigma) (pH 7.2 with KOH, 312 mosmol/kg). Whole-cell current clamp recordings were performed from the soma of GP neurons with the Axopatch-200B (Axon Instruments) amplifier. Voltage was filtered at 10 kHz and sampled at 20 kHz using Patch pipettes (4-8 MΩ) pulled from thick walled borosilicate glass capillaries (2.0 mm outer diameter, 0.5 mm wall thickness, Hilgenberg, Malsfeld, Germany). Some excitatory input to the GP may have been severed by the slicing procedure. To offset the reduced excitation a constant current of 20 pA was injected via the patch pipette throughout the experiment.

The rodent GP is functionally and anatomically homologous to the primate and human GPe (Gerfen and Wilson, 1996). Therefore, for greater clarity the rodent GP will also be referred to as the GPe.

Software:
The MATLAB (MATLAB 2007B, Mathworks, Matick, MA, USA) software used for the data analysis in this article can be found at: http://neurint.ls.biu.ac.il/software/Chorea

Results
A total of 20 bicuculline microinjections (Table 1) and 6 saline (Table 2) microinjections were administered targeting the GPe in two monkeys (10 bicuculline and 3 saline injections in each monkey). Localization of the injection sites was done using microlesion markings and electrophysiological mapping and the sites were overlaid on the postmortem anatomical reconstruction of the brain (detailed in (McCairn et al., 2009)). The injection sites were all within the GPe, spanning the anterior-posterior levels AC0 to AC-5 (Fig. 1).

Behavioral effects
Normal behavioral patterns of the animals were characterized based on observations made prior to microinjection during each recording day. Abnormal behaviors were observed following 17/20 bicuculline microinjections, and were categorized into three previously defined hyper-behavioral states (Grabli et al., 2004) based on the observed behaviors: motor dysfunction, associative dysfunction and limbic dysfunction (Supplementary video). Behaviors classified as belonging to a primarily motor dysfunction were abnormal choreic movements of the limbs contralateral to the injection site. Choreic movements were irregular and complex,
usually characterized by internal and external rotation or flexion and extension movements involving either a single or a few contiguous joints or an entire limb. Chorea involved the contralateral lower limb, the upper limb or both, without any clear somatotopy. A detailed description of associative and limbic dysfunctions can be found in supplementary material 2.

Twelve microinjections resulted in chorea, presenting either as the only effect (8/12 injections), or mixed with another effect (4/12 injections). Locations of microinjection eliciting the different types of abnormal behaviors generally followed the previously described (Francois et al., 2004; Grabli et al., 2004; Haber et al., 1993) functional organization of the GPe: microinjections inducing limbic abnormalities were located at the anterior-ventral part of GPe; associative abnormalities were located at anterior-dorsal parts of GPe, and microinjections eliciting chorea were mostly located at posterior parts (Fig. 1). No abnormal behaviors were observed following any of the saline microinjections (Fig. 1, black markers). The first signs of abnormal movements were usually apparent within minutes following bicuculline microinjection (mean latency to first effect 7.3±8.2 min, Table 1). Chorea typically appeared gradually, with initial mild effects increasing in severity over several minutes. The effect would usually wax and wane over time, with the animal displaying alternating periods of severely abnormal and seemingly normal or mildly abnormal behavior. The abnormal movements usually dissipated towards the end of recording sessions, lasting 30-120 min.

The abnormal behaviors following bicuculline microinjection hindered the animal's performance of the behavioral task, but did not completely abolish it. Choreic movements affecting only the lower limbs mostly did not interfere with upper limb movements required for task execution. During sessions in which chorea appeared in the upper limb, the animals seemed able to temporarily suppress the choreic movements for completion of successful trials, while the abnormal movements were still present during the inter-trial intervals. Notably, this was true for periods of mild to medium behavioral effects, whereas during periods of very severe chorea the animals were unwilling or unable to engage in the behavioral task.

All subsequent analyses of neurons recorded after bicuculline microinjections were performed only on neurons recorded during sessions in which the injection led to chorea. The neurons used for analyses were recorded during the time periods after the bicuculline microinjection and the ensuing appearance of choreic movements, prior to the end of choreic symptoms. Some neurons were recorded continuously before and after bicuculline microinjection and were analyzed separately. No significant differences were found for any of the neuronal characteristics between animals; thus we pooled the data from both monkeys.
Changes in GPe neuronal activity

The GPe is known to include two main subpopulations of neurons: neurons displaying HF activity with a varying degree of pauses (HFP) and neurons with low-frequency bursting (LFB) activity (DeLong, 1971; Elias et al., 2007). In this study we analyzed GPe neurons from each group separately. Seventy-nine stable, well isolated HF GPe neurons (firing rate >40 spikes/s) recorded after chorea-inducing bicuculline microinjections (65 neurons monkey I, 14 neurons monkey M), 71 HF GPe neurons recorded after saline microinjections (62 neurons monkey I, 9 neurons monkey M) and 80 neurons recorded from the normal untreated GPe (21 neurons monkey A, 59 neurons monkey N) were used for the analyses. The firing rate of neurons recorded after saline microinjection was similar to the firing rate of neurons recorded from the untreated GPe (Fig. 2A & 2B). Bicuculline microinjection caused a marked increase in the overall mean firing rate of GPe neurons (normal 84.6±23.2 spikes/s, saline 83.9±35.5 spikes/s, bicuculline 113±35.9 spikes/s, post-hoc Tukey's HSD test, p<0.001, Fig 2B), with many individual neurons maintaining extremely high firing rates (120-230 spikes/s) over extended periods of time (Fig. 2A). 17 GPe neurons were recorded continuously before and after bicuculline microinjection and the appearance of chorea. Following microinjection 10/17 of these neurons showed significant increases in firing rate (Fig. 2C), which was accompanied by changes in firing pattern in 6/10 neurons (Fig. 2D).

The firing pattern of most control (untreated and saline injected) HF GPe neurons followed a Poisson process and had a variable degree of pauses (Elias et al., 2007; DeLong, 1971). GPe neurons displaying this kind of normal firing pattern were termed high-frequency pausers (HFP) (Fig. 3A). After bicuculline microinjection abnormal firing patterns were observed in many of the recorded neurons. Two types of abnormal patterns were observed, and were termed "bistable high-frequency" (BHF) and "continuous high-frequency" (CHF). In BHF neurons, pauses were long and pronounced while the overall firing rate increased, resulting in long trains of very high-frequency firing interspersed with periods of complete cessation of spiking activity (Fig. 3B). CHF neurons displayed a very high firing rate, firing either continuously or with very few short sparse pauses. Most CHF neurons displayed a regular firing pattern, with narrowly distributed ISIs (Fig. 3C). These changes in firing pattern were quantified using two parameters: the inverse of the median ISI (1/median[ISI]), and the mean length of the longest 1% of ISI. The first parameter gives an estimate of the neuron’s mean firing rate during the active periods, but unlike the mean it is not skewed by long pauses. The second parameter identifies neurons with long pauses, thus differentiating between CHF and BHF neurons. Threshold values used for classification of normal and abnormal activity
were determined by the distribution of data from the untreated control GPe (Fig 4A, open diamonds). Neurons with an inverse median ISI larger than 145 were classified as having an abnormal firing pattern (bicuculline-affected neurons). Of these affected neurons, neurons with a mean long ISI value of less than 90 ms were classified as CHF, and neurons with larger values were classified as BHF (Fig. 4A). This analysis revealed that 44% of GPe neurons recorded after bicuculline injection displayed abnormal activity (20% BHF, 24% CHF), whereas 56% were unaffected (Fig. 4B). BHF and CHF neurons were also recorded following control saline injections, but they were less frequent than after bicuculline microinjections ($\chi^2=14.4$, df=1, p<0.001): only 20% of the neurons recorded after saline injection were classified as displaying abnormal activity (7% BHF, 13% CHF) (Fig. 4B).

Analysis of the distances from injection site revealed a focal spatial distribution of abnormal firing patterns (Fig 4C). The average distance of unaffected neurons from the bicuculline injection site (2.78±1.2 mm, median 3.3) was significantly larger than the distance of neurons classified as BHF (1.49±0.8 mm, median 1.55, post-hoc Tukey's HSD test, p<0.01) or CHF (1.77±1.2 mm, median 1.38, post-hoc Tukey's HSD test, p<0.05). There was no significant difference between the distances of BHF and CHF neurons. Thus, bicuculline-affected neurons were mostly found closer to the injection site compared to unaffected neurons.

The abnormal post-bicuculline firing patterns also affected the ability of GPe neurons to transmit information. The information capacity of BHF and CHF neurons was significantly smaller than that of HFP neurons (post-hoc Tukey's HSD test, p<0.001), and the information capacity of CHF neurons was smaller than that of BHF neurons (post-hoc Tukey's HSD test, p<0.01) (Fig. 4D).

Neurons displaying low-frequency bursting activity (LFB) were recorded in the GPe after bicuculline (n=14) and saline microinjections (n=17). The mean firing rate of these neurons did not differ (saline 23.5±13 spikes/s, bicuculline 21±11.7 spikes/s, t-test p>0.1). However, LFB neurons recorded after bicuculline injection had more pronounced bursting activity, with a smaller incidence of single spikes fired between bursts. There was also a difference in the inter-burst intervals between the two population, with bursts of LFB neurons recorded after bicuculline microinjection appearing more regularly (Fig. 3D).

Oscillations

High-frequency oscillations: In some bicuculline-affected GPe neurons we observed HF (>100 Hz) oscillatory activity, reflecting the regularity of the spikes (Fig. 5A). This
regularity was evident in oscillatory peaks in the autocorrelation function calculated with a small offset (<100 ms) (Fig. 3C) and peaks in the PSD (Fig. 5B). After bicuculline microinjection, regular spiking activity was detected in most CHF (60%, 15/27), but not BHF (4%, 1/24) or HFP (2%, 1/56) neurons. After saline microinjections, regular spiking activity was detected in a small minority of the neurons (1/9 CHF, 0/5 BHF, 6/57 HFP). The mean frequency of the HF oscillations was higher for neurons recorded after bicuculline microinjection compared to neurons recorded after saline microinjection (saline 122.6±45 Hz, bicuculline 195.1±41.3 Hz, t-test p<0.001). Simultaneously recorded neurons displaying regular spiking activity did not oscillate at the same frequency (Fig. 5B), and did not show temporally correlated (Fig. 5C) or spectrally coherent (Fig. 5D) activity.

**Low-frequency oscillations:** After bicuculline microinjections very slow oscillations were detected in the activity of some BHF and LFB neurons. This was reflected by oscillatory peaks in the autocorrelation functions calculated with an offset of several seconds (Fig. 3B and 3D) and by peaks in the PSD (Fig. 5B'). In BHF neurons these slow oscillations reflected the regularity of changes in the bistable state (firing/pausing transitions), and in LFB neurons they reflected the regularity of the bursts. 9/24 (37.5%) BHF neurons recorded after bicuculline microinjections and 2/5 (40%) BHF neurons recorded after saline microinjections displayed slow oscillations. The mean oscillation frequency for bicuculline BHF neurons was 0.54±0.13 Hz. 10/14 (71%) of the LFB neurons recorded after bicuculline microinjections displayed regular bursting, compared to only 1/17 (6%) LFB recorded after saline microinjections. After bicuculline microinjection the mean frequency of the LFB oscillations was 2.2±1.1 Hz. No slow oscillations were detected in other types of GPe neurons, either after bicuculline or saline microinjections. Simultaneously recorded neurons with significant slow oscillations did not oscillate at the same frequency (Fig. 5B'), and did not show temporally correlated (Fig. 5C') or spectrally coherent (Fig. 5D') activity.

**Changes in GPi neuronal activity**

64 stable, well isolated GPi neurons recorded after chorea-inducing bicuculline microinjections (55 neurons monkey I, 9 neurons monkey M), 67 GPi neurons recorded after saline microinjections (59 neurons monkey I, 8 neurons monkey M) and 64 neurons recorded from normal untreated GPi (54 neurons monkey N, 10 neurons monkey A) were used for the following analyses. There were no significant differences in the mean firing rate of GPi neurons recorded under the different conditions (untreated 87.3±19.2 spikes/s, saline 78.3±22.5 spikes/s, bicuculline 82±27.3 spikes/s, one-way ANOVA, p>0.05) (Fig. 6A&6B). GPi neurons
from all conditions displayed the typical continuous random firing pattern (DeLong, 1971), with no abnormal firing patterns detected, and no oscillatory activity in either the high or low frequency ranges.

Correlations

Three types of correlations were examined, based on the location of the simultaneously recorded neurons: GPe-GPe, GPi-GPi and GPi-GPe correlations (supplementary material 3). Very little correlated activity was detected for any of the types of pairs (Fig. 7). There were no significant differences in the fraction of correlated pairs or in the direction of correlation (positive or negative) recorded from normal, saline-injected or bicuculline-injected GPe and GPi (all $\chi^2$ tests yielded $p>0.01$). Notably, even significantly correlated pairs showed only weakly correlated activity; $<10\%$ maximal change compared to baseline level (Fig. 7).

Behavior-related neuronal activity

Task-related activity: After chorea-inducing bicuculline microinjection 32 HF GPe and 22 GPi neurons were recorded during periods in which the animals performed the behavioral task. Task related firing rate modulations could be detected in 15/32 HF GPe and 3/22 GPi neurons (Fig. 8). The task-related neuronal responses were diverse with most neurons responding to movement, and some responding to sensory cues or reward. Task-related activity modulations were, in similar proportions, increases or decreases in firing rate. Notably, specific task-related activity modulations could be detected in bicuculline affected neurons classified as BHF (Fig. 8A) or CHF (Fig. 8B).

Neuronal activity associated with bicuculline-induced abnormal behaviors: The intermittent nature of chorea observed in this study enabled us to split the data into periods when the animal expressed choreic movements and time periods when the animal was motionless. This analysis did not reveal any rate or pattern changes in the activity of GPe or GPi neurons between time periods of excessive abnormal behavior and periods without movement. During the expression of chorea, attempts to identify repetitive motion segments to which neuronal activity could be aligned were unsuccessful due to the chaotic nature of choreic movements.

In-vitro effects of bicuculline on rat GPe neurons

A total of 10 GPe neurons were recorded in-vitro from a rat slice preparation. Following bicuculline application all the neurons increased their firing rate, and two types of
firing patterns were observed (Fig. 9). The first type of neurons (6/10 neurons) displayed a bistable firing pattern, spontaneously shifting between periods of HF firing and periods of hyper-polarized membrane potential and no spiking activity (Fig. 9A'). Bursts of activity appeared regularly within the bistable process, manifesting as oscillatory peaks in the autocorrelation function and peaks in the low-frequency range of the PSD. The second type of neurons (4/10 neurons) displayed continuous HF firing, occasionally interspersed with short cessations in firing. The spikes were regularly timed, manifesting as oscillatory peaks in the autocorrelation function and peaks in the PSD. During pauses in spiking activity the membrane potential was depolarized, and they were preceded by a gradual reduction in the size of the action potentials (Fig. 9B'), indicating the pauses were a result of depolarization block.

Discussion

This work examined the electrophysiological correlates of chorea induced by bicuculline microinjection into the GPe. The behavioral effects in the present study are in line with those described earlier (Grabli et al., 2004) showing a direct relationship between injections location in the GPe and the induced abnormal hyper-behavioral symptoms. Bicuculline caused a marked increase in the firing rate of GPe neurons, which was associated with drastic changes in firing pattern. In some bicuculline-affected GPe neurons, pauses became more pronounced (BHF) whereas in others they were almost completely abolished (CHF). These abnormal activity patterns were associated with a reduction in the neurons' information capacity. Bicuculline also induced regularity in both the spiking and bursting activity in the GPe. However, there was very little correlated activity in the GPe either in the temporal or in the spectral domains. There were no gross changes in the activity of GPi neurons after GPe bicuculline microinjection, with overall firing rate, pattern, oscillations and correlations remaining within normal range. Despite the changes in tonic neuronal activity, bicuculline affected GPe neurons were still able to encode movements and sensory events.

The two types of abnormally active GPe neurons (BHF & CHF) were detected following bicuculline administration to both in-vivo (behaving primate) and in-vitro (rat slice). In-vivo, bicuculline had a focal effect on GPe neurons located within a 1.5-2 mm radius around the site of injection, which is in line with estimations of the extent of bicuculline diffusion (Yoshida et al., 1991). Neurons located further away from the injection site were mostly unaffected and displayed normal firing rates and patterns. This suggests that despite the existence of collateral connections between GPe neurons (Kita and Kitai, 1994; Sadek et al., 2007), the abnormal firing patterns of the bicuculline-affected neurons did not propagate to...
other neurons within the nucleus. The localized nature of the bicuculline effect was further evidenced by the spatial organization of the different bicuculline-induced behavioral abnormalities. The nature of abnormal behaviors induced by bicuculline was spatially organized within the nucleus, and followed the known anatomical subdivision of the GPe into motor, limbic and associative territories (Haber et al., 1993; Francois et al., 2004). This suggests that rather than causing a global dysfunction of the GPe, the bicuculline effect is focal in nature and confined to the local area (and functional circuit) surrounding the site of injection.

The changes in firing patterns of GPe neurons brought on by application of bicuculline may be attributed to the block of GABA-A receptors which alters the patterns of input to the neurons. As most of the input to GPe neurons is mediated by GABA-A receptors originating either from the striatum (Parent and Hazrati, 1995) or GPe collaterals (Kita and Kitai, 1994), bicuculline blocks many of these synapses and thus cuts off the neurons from most of their afferents (Chan et al., 2004). This reduction of inhibitory drive could lead to the observed increase in firing rate of the neurons due to the reduced inhibitory shunt. Thus, the two types of firing patterns observed in-vitro might result from the unmasking of cellular properties following the block of GABA inputs. One subgroup of neurons responded to bicuculline with a bistable firing pattern (Fig. 9A`). One mechanism that could account for this response is the activation of SK channels during the bursts inducing long inter-burst afterhyperpolarization. SK channels have been shown to influence GP firing patterns (Deister et al., 2009) and are known to be influenced by bicuculline (Johnson and Seutin, 1997; Druzin et al., 2004). The other group of neurons responded with an increase in firing frequency and a marked decrease in the action potential amplitude (Fig. 9B`). This might be due to a partial depolarization block of the voltage-gated Na$^+$ channels expressed by these neurons. In addition, the same types of abnormal GPe firing patterns, albeit to a significantly smaller extent, were detected following microinjection of saline alone. Saline is not expected to influence GABAergic synapses, but it might have an effect on GPe firing properties via different cellular mechanisms. For example, saline (NaCl) lacks many of the ions normally present in the cerebrospinal fluid; therefore it changes ionic concentrations around the injection site and thus affects the cellular mechanisms modulating neuronal activity. Thus, the activation patterns observed in GPe neurons after bicuculline administration may also be affected by cellular mechanisms unrelated to synaptic inputs, again suggesting a localized effect of bicuculline.

Despite the reduction in information capacity and local disruption of GABAergic inputs, some of the bicuculline-affected GPe neurons and downstream GPi neurons still
demonstrated firing rate modulations directly associated with the behavioral task, comparable to movement-related responses recorded from pallidal neurons of normal animals (Arkadir et al., 2004; Turner and Anderson, 2005; Mushiake and Strick, 1995). This suggests that the neuronal connections and activation patterns associated with the performance of a familiar motor task were largely maintained and undisturbed by the focal disruption of GABA-A inputs. In GPe neurons directly affected by bicuculline such phasic responses might be mediated by glutamatergic inputs from the STN (Wichmann et al., 1994; Nambu et al., 2000; Hanson et al., 2004) or GABAergic inputs activating GABA-B receptors (Charara et al., 2000; Galvan et al., 2005; Kita et al., 2006).

The GPi is a major target for GPe projection neurons (Shink and Smith, 1995; Hazrati et al., 1990); therefore it is surprising that the significant changes in GPe activation after bicuculline microinjection were not reflected in the activity of GPi neurons. A possible explanation for this may lie in the uncorrelated nature of abnormal GPe activity observed in this study. GPe activation was shown to be able to effectively influence the activity of GPi neurons (Kita, 2007), but due to the diffused pattern of GPe-GPi connectivity a synchronized activation of the GPe (or a subgroup of its neurons) is needed to effectively influence the GPi (Kita, 2007). Indeed, in normal animals there is little or no correlated activity between individual GPe-GPi neurons (Elias et al., 2008; Raz et al., 2000). GPe-GPi correlations have been shown to emerge in some pathological conditions in which the GPe neurons throughout the nucleus fire in a synchronized manner (McCairn et al., 2009; Raz et al., 2000).

The GPi is the main output structure of the BG, and it is associated primarily with motor activity (Turner and Anderson, 1997; DeLong et al., 1985). Current theoretical models of the BG predict that hyperkinetic states such as chorea should be associated with a global reduction of GPi activity which will disinhibit the cortex and facilitate the activation of excessive action sequences (Albin, 1995; Albin et al., 1989). In the current study we did not find evidence of significant changes in the firing rate, pattern or synchrony of GPi neurons. Findings from previous studies looking at GPi activity during choreic states are inconsistent and scarce. GPi recordings from Huntington's disease patients have so far yielded mixed results, with some indicating reduced GPi firing rates (Starr et al., 2008) and some showing no change in GPi rate compared to Parkinson's disease patients (Tang et al., 2005). Notably, these studies did not find any modulation in GPi activity that could distinguish choreic and non-choreic periods (Starr et al., 2008). STN lesions induce both chorea (hemiballism) and a reduced GPi firing rate, but no evidence links the GPi rate modulation to the onset or maintenance of chorea (Hamada and DeLong, 1992a; Nambu et al., 2000). A previous study
that examined GPi activity associated with chorea induced by GPe bicuculline microinjections found both increases and decreases in GPi activity associated with chorea, such that there was no overall change in GPi firing rate in any one direction (Matsumura et al., 1995).

If chorea is not associated with global changes in the BG output (such as reduced firing rate or increased synchronization) it might be related to dysfunctions of the flow of information through the BG. A focal GABA dysfunction in the GPe, as was used in this study, might interrupt normal information processing within the GPe (Sadek et al., 2007) or disrupt the balance of information going through the direct and indirect pathways (Mink, 2003). This type of effect might disrupt the timing of GPi neuronal activation, thus making its signal irrelevant or inappropriate, without causing major changes to the overall level or pattern of GPi activation. Such a formation of rogue patterns of GPi activation could lead to unintended releases of cortical motor patterns which are expressed as chorea. The difficulty in identifying such subtle changes in neuronal activity patterns might be related to the nature of chorea itself. In contrast to the repetitive and rhythmic nature of the abnormal movements seen in motor tics (McCairn et al., 2009) or parkinsonian tremor (Findley et al., 1981; Heimer et al., 2006), the abnormal involuntary movements of chorea are characterized by a disorganized, unpredictable and random presentation (Mink et al., 2007) which maintains a chaotic nature. This presentation confounds attempts to uncover the neuronal encoding of such movements. The modulations in activity of individual GPi neurons or sub-groups may encode different segments of the complete behavior, but since these segments are unknown and rarely repeat themselves due to the chaotic manifestation of the complete behavior they are impossible to detect. Thus, individual GPi neurons might still display firing rate modulation in response to specific parts of the abnormal movements, but if these movement-related changes are short and non-repetitive they may be lost in the overall spontaneous neuronal activity. When looking at the downstream effect of GPi activation on the cortex, rather than imposing a general state of cortical excitability, specific motor patterns may be encoded in the activity of specific GPi neurons, and chorea might represent the random activation of these patterns.

Our results suggest that chorea, unlike other disorders of the cortico-BG loop, does not involve global changes in the activation of the GPe itself or its downstream target. Despite the fact that the GABAergic dysfunction was limited to a localized area within the GPe and generated asynchronous activity, it still led to major hyperkinetic symptoms in the animals. Thus, it appears that current rate-based models of the cortico-BG circuitry do not reflect the pathophysiology associated with chorea and new models should take into account phasic
patterns of activation and the possible effects of internal computation processes within the BG system. Attempts to understand the complex relationship between chorea and neuronal activity might be hindered by the chaotic nature of chorea itself and may require novel and detailed techniques of both behavioral pattern quantification and neuronal data analyses.

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Figure legends

Figure 1: **Anatomical reconstruction of injection site locations.** Localization of bicuculline microinjection sites marked according to the evoked abnormal behaviors in monkey M (circles) and monkey I (triangles): chorea (yellow), associative abnormalities (green), limbic abnormalities (red), no effect (white). Injection sites which evoked behavioral abnormalities of more than one type are marked with both colors. The injection sites from both animals are overlaid on the same outlines. Control saline microinjections are marked in black. Outline drawings are reconstructed from the coronal sections (AC0 to AC-5) of the right hemisphere of monkey M. (Abbreviations: AC = Anterior commissure, Cd = Caudate nucleus, Cl = Claustrum, OT = Optic tract, Pu = Putamen, Th = Thalamus).

Figure 2: **Neuronal firing rates in the GPe.** (A) Distribution of mean firing rates of GPe neurons recorded from an untreated normal macaque (white bars), post-saline microinjection (gray bars) and post-bicuculline microinjection (black bars). (B) Comparison of GPe mean firing rates recorded from normal untreated macaque (un – white bar), post-saline microinjection (sal – gray bar) and post-bicuculline microinjection (bic – black bar). Error bars indicate standard error of the mean. (C & D) Rate functions, calculated in 2 s bins (smoothed with a Gaussian window with STD=3.9 s), of GPe neurons recorded continuously before and after bicuculline microinjection and development of chorea. Bicuculline microinjection is marked by the dashed line at time zero, and the appearance of chorea is marked by the horizontal black line. (C) A neuron showing a gradual increase in firing rate. (D) A neuron showing an increase in firing rate and a change in firing pattern.

* p<0.001

Figure 3: **Post-bicuculline GPe firing patterns.** Examples of the different types of GPe neurons observed after bicuculline microinjection: (A) unaffected high-frequency pauser (HFP) (B) affected bistable high-frequency (BHF) neuron (C) affected continuous high-frequency (CHF) neuron (D) affected low frequency burster (LFB). Presented for each neuron are: (i) trace of recorded activity at two time scales (12 s and inset of 1.5 s), (ii) autocorrelation function at two time scales (offset ±5 s and inset of ±0.05 s) and (iii) ISI distribution at two time scales (0-1 s and inset of 0-10 ms). The y-axis of the long-scale ISI histograms is truncated for better examination of long intervals.
Figure 4: **Properties of the different post-bicuculline GPe neuronal groups.**

(A) Scatter plot of 1/median ISI vs. mean length of longest 1% ISI values for post-bicuculline (colored circles) and control untreated GPe neurons (white diamonds). Dashed lines indicate thresholds for the two parameters used for the group classification. Blue = unaffected neurons, red = CHF, orange = BHF, color scheme is the same for all the sub-figures. (B) Fraction of neurons classified as BHF, CHF or unaffected recorded after bicuculline and saline microinjections. (C) Mean 3D distance from the bicuculline injection site of BHF, CHF and unaffected GPe neurons. (D) Mean information capacity of BHF, CHF and unaffected GPe neurons recorded after bicuculline microinjection. Error bars in C & D indicate standard error of the mean. * p<0.05, ** p<0.01, *** p<0.001

Figure 5: **Post bicuculline neural oscillations in the GPe.** Oscillations were observed on two scales (A-D) High-frequency oscillations (100-500 Hz) and (A'-D') Low-frequency oscillations (0.2-10 Hz). (A) Short traces (500 ms) of two simultaneously recorded GPe CHF neurons. Subsequent sub-figures (B-D) show data for these two neurons. (B) The spectrogram and PSD of each neuron showing stable high-frequency oscillations. The frequency on the PSD graph is the frequency with maximal power. (C) The cross-correlation function of the neurons showing zero correlation in the temporal domain. (D) Coherence of the neurons showing no significant coherence in the spectral domain. (A') Long traces (10 s) of simultaneously recorded BHF (i) and LFB (ii). Subsequent sub-figures (B'-D') show data for these two neurons. (B'-D'). Same as B-D, respectively, but calculated and presented only for the low frequency range (0-10 Hz). In all the sub-figures, the dashed red line is the confidence limit.

Figure 6: **Post bicuculline neuronal activity in the GPi.** (A) Distribution of mean firing rates of GPi neurons recorded from untreated normal macaque (white bars), post saline microinjections (gray bars) and post bicuculline microinjections (black bars). (B) Comparison of GPi mean firing rates recorded from normal untreated macaque (unt – white bar), post-saline microinjection (sal - gray bar) and post-bicuculline microinjection (bic - black bar). Error bars indicate standard error of the mean.

Figure 7: **Cross-correlations in the GPe and GPi following bicuculline microinjection.**

Cross-correlations are grouped based on the nuclei of the neuronal pairs: (A) GPe-GPe (B) GPi-GPi and (C) GPi-GPe. Top row: Examples of post-bicuculline cross-correlation functions calculated in 1 ms bins with ±4 s offset. The y-axis represents the conditional firing rate in
spikes/s. Dashed lines represent the 99% confidence intervals. The graphs show significant correlations, but the magnitude of the peaks/troughs is very small. Bottom row: Percentage of correlated pairs in each condition (un = untreated normal macaque, sal = post saline microinjections, bic = post bicuculline microinjections), divided by the type of correlation (white – positive, gray - negative). The numbers in parentheses presented under each bar are the number of significantly correlated pairs / total number of simultaneously recorded pairs.

There were no significant differences in the fraction of correlated pairs between the different conditions (untreated, saline, bicuculline) for any correlation type.

Figure 8: **Post bicuculline movement-related firing rate modulations.** (A-C) Behavioral task related peri-event time histograms and raster plots of post bicuculline (A) BHF (B) CHF & (C) GPi neurons. Black dots on the raster represent spikes of the neurons, and triangle markers indicate timing of touch central button (blue), touch side button (green) and delivery of liquid reward (yellow). Dashed lines are 99% confidence limits.

Figure 9: **In-vitro effects of bicuculline on rat GPe neurons.** Intracellular recording trace, autocorrelation function and PSD of GPe neurons recorded in-vitro (A & B) before and (A' & B') after bicuculline application. Left column: Trace of recorded intracellular activity, middle column: autocorrelation function at two time scales (offset ±2 s and inset of ±0.5 s), right column: PSD, dotted line is 99% confidence limit.
Table 1: **Bicuculline microinjections: anatomical and behavioral details.**

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* Over several injections during the session

(+) marks intensity of the behavioral effect, from mild (+) to severe (+++). When several effects were present together, letters indicate corresponding latencies (L=limbic, A=associative, M=motor)
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