Early-onset cortico-cortical synchronization in the hemiparkinsonian rat model

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Changes in synchronized neuronal oscillatory activity are reported in both cortex and basal ganglia of Parkinson’s disease patients. The origin of these changes, in particular their relationship with the progressive nigrostriatal dopaminergic degeneration, is unknown. Therefore, in the present study we studied interregional neuronal synchronization in motor cortex and basal ganglia during the development of dopaminergic degeneration induced by a unilateral infusion of 6-hydroxydopamine (6-OHDA) into the rat medial forebrain bundle. We performed serial local field potential recordings bilaterally in the motor cortex and the subthalamic nucleus of the lesioned hemisphere prior to, during, and after development of the nigrostriatal dopaminergic cell loss. We obtained signal from freely moving rats in both resting and walking conditions, and we computed local spectral power, interregional synchronization (using phase lag index), and directionality (using Granger causality). After neurotoxin injection the first change in phase lag index was an increment in cortico-cortical synchronization. We observed increased bidirectional Granger causality in the beta frequency band between cortex and subthalamic nucleus within the lesioned hemisphere. In the walking condition, the 6-OHDA lesion-induced changes in synchronization resembled that of the resting state, whereas the changes in Granger causality were less pronounced after the lesion. Considering the relatively preserved connectivity pattern of the cortex contralateral to the lesioned side and the early emergence of increased cortico-cortical synchronization during development of the 6-OHDA lesion, we suggest a putative compensatory role of cortico-cortical coupling.

6-OHDA; Parkinson’s disease; in vivo neurophysiology; subthalamic nucleus; cortex

SYNCHRONIZED NEURONAL OSCILLATORY activity is altered in Parkinson’s disease (PD) patients in both cortical and subcortical brain regions (Fogelson et al. 2006; Hirschmann et al. 2013; Silberstein et al. 2005; Williams et al. 2002). The results of surface measurements (EEG, MEG) and recordings of human basal ganglia (BG) in PD patients suggest a link between the observed electrophysiological changes and the pathophysiological mechanisms of PD (Hirschmann et al. 2013; Klassen et al. 2011; Lalo et al. 2008; Litvak et al. 2012; Olde Dubbelink et al. 2012; Silberstein et al. 2005; Stoffers et al. 2007). Evidently, BG recordings from electrode implants are restricted to advanced-stage PD patients, so there is limited information on the time course of the development of the pathological synchronization patterns.

Recent evidence suggests the presence of stage-specific interregional synchronization patterns in PD patients, with the involvement of lower (alpha) frequencies occurring at early and higher (beta) frequencies at more advanced stages of the disease (Moazami-Goudarzi et al. 2008; Olde Dubbelink et al. 2013; Palmer et al. 2010; Silberstein et al. 2005; Stoffers et al. 2008a, 2008b). Experiments in animal models have revealed changes in synchronization similar to those recorded in PD patients (Brazhnik et al. 2012; Degos et al. 2009; Dejean et al. 2012), showing increased intrahemispheric or cortico-nigral synchronization. Interestingly, none of these studies examined interhemispheric synchronization specifically, although this is among the earliest alterations identified in PD patients (Stoffers et al. 2008a). In addition, directionality measures, which may also characterize interregional relationships, have so far described rather diverse effects (Fogelson et al. 2006; Litvak et al. 2012; Magill et al. 2005; Williams et al. 2002).

Besides resting-state measurements, human and experimental animal studies have investigated changes in association with the characteristic motor disturbances (Brazhnik et al. 2012; Vardy et al. 2011; for review see Brittain and Brown 2014). Although the presence of a direct (causal) relationship between the motor symptoms and the excessive synchronized oscillatory activity in parkinsonism is still under discussion (Brown 2007; Chen et al. 2007; Leblois et al. 2007; Syed et al. 2012; Vardy et al. 2011), motor activity was shown to modulate oscillatory activity and interregional coupling in PD patients in both cortical and subcortical areas (Alegre et al. 2005; Defevre et al. 1996; Joundi et al. 2013; Lalo et al. 2008; Litvak et al. 2012; Pollok et al. 2012). The parkinsonism-induced alterations in movement-associated electrophysiological patterns are at present not clear. We therefore investigated how synchronization patterns between cortical and subthalamic regions alter during development of a rat parkinsonian model. In addition, we examined how this is modulated by behavior. To this aim, we recorded local field potentials (LFPs) in a freely moving 6-hydroxydopamine (6-OHDA) rat model at rest and during locomotion to describe changes in oscillatory activity, synchronization, and directionality induced by dopaminergic cell loss at the cortico-cortical and cortico-subthalamic level, and to explore the evolution of these changes during the development of dopaminergic cell loss.
METHODS

Animals. Male Wistar rats (~300 g; Harlan) were kept under standard housing conditions at constant temperature (22 ± 1°C) and humidity (relative, 56%) and a 12:12-h reverse light-dark cycle (daylight period 1900-0700). Food and water were available ad libitum throughout the experiment. Behavioral sessions were conducted during the dark phase, at the same time of the day each day. The study was approved by the Animal Ethical Committee at the VU University of Amsterdam, and it was conducted in accordance with Dutch (Wet op de Dieropvoed 1996) and European (Guideline 86/609/EEC) regulations.

Head stage. A custom-made electrode holder device was designed to allow multielectrode recordings from motor cortex and the subthalamic nucleus (STN). The electrode holder consisted of two parts: two tetrodes for acquiring cortical signal (2 mm below the dura surface, approximately layer V neurons) and two tetrodes (9.5 and 10.0 mm long) targeting STN in a separately adjustable holder. The tetrode tips were used to increase the success rate; the mobility enabled intraoperative recordings to improve STN placement. The tetrodes used for single-unit and LFP recordings were produced of 4 × 13-μm insulated NI RO-800 wire (Kantan Precision Technology, Palm Coast, FL); impedance was adjusted between 0.9 and 1.2 mΩ.

Surgery. Inhalation anesthesia with isoflurane was used to initiate and maintain anesthesia throughout the implantation procedure [mixed isoflurane 2.5%-1.75% in O2 (0.3 l/min)-N2O (0.6 l/min)]. Placement holes for tetrode bundles and the cannula were marked and drilled in the skull, which was fixed in a stereotactic frame (Kopf). The electrode holder targeting the motor cortex bilaterally (AP +4.0 mm, ML ±2.5 mm from bregma, 2.0 mm ventral from dura surface) and the STN [AP −3.6 mm, ML 2.5 mm, DV 7.1–8.4 mm from dura (Paxinos and Watson 2005)] was implanted in the following steps: The frontal piece of the holder containing the tetrodes for motor cortex were fixed with dental cement to the skull under a 15° angle to the front. After this, the tetrode bundle aiming to measure STN was placed. We performed intraoperative recordings and spike discrimination during the slow advancement of the tetrode bundles. Once the desired STN-type activity was recorded (Benazzouz et al. 2002), the device was fixed and secured with anchor screws and dental cement. A common reference/ground wire for all tetrodes was attached to a stainless steel screw placed above the left cerebellar hemisphere. After tetrode placements, the guide cannula for the subsequent 6-OHDA injections was placed above the medial forebrain bundle (under 18° antero-posterior angle, AP −7, ML 1.9, DV 7 mm from dura surface). The injector extended 1 mm below the implanted cannula. After surgery the animals were allowed to recover for 7 days.

Behavior. During the home cage recordings rats (n = 6) were allowed to explore the environment freely. To acquire movement-related LFP, a subgroup of the same group of animals (n = 4) were trained to execute a simple walking task. The behavioral experiments started when the animals were placed in an elongated behavioral box with a 1-m corridor and two cue lights and pellet dispensers (Fig. 1B). The automated behavioral task [executed with the aid of MED-PC behavioral control software (Sandown Scientific, Hampton, UK)] set the beginning: At both ends of the corridor, the cue lights were illuminated and a sucrose pellet was available. Once the animal collected one of the pellets, another pellet (with simultaneous cue light illumination) was offered to the animal at the opposite side of the elongated box. The animal was encouraged to walk from side to side until it collected 40 pellets or the task duration exceeded 20 min. The animals were trained (in 3–6 sessions) before surgery; after the implantation we obtained neuronal signals during the task.

Electrophysiological recordings. After the recovery period, we performed daily recordings of freely moving and behaving animals. For resting (sitting) measurements animals were placed in a home cage (50 × 30-cm Plexi Faraday cage) every day for 3 wk; after this (at >14 days after 6-OHDA injection, when the effects of the neurotoxin are expected to be completed) recordings ran every other day. The animals were left in the cage for 20–30 min, 30 min if only the resting condition was monitored and 20 min if the walking task was also executed (Fig. 1A). For the group of animals trained in the walking task recordings were performed during the task every second day. LFP signal was recorded from all four electrodes of each tetrode. The tetrodes’ position was fixed over the course of experiments, enabling comparison of the same neuronal population over time. However, this did not allow single-unit data collection at a systematic level. The acquired LFPs were amplified 20 times (HST/16V-G20), followed by a preamplifier (PBX/32sp-r G50/16fp-G50, Plexon, TX) with 50× gain. The signal was band-pass filtered to generate LFPs (0.7–170 Hz). The LFPs generated by the preamplifier were alternatively amplified 2 times and 5 times by the AD converter, for a total amplification of 2,000× and 5,000×. Video recordings (Cineplex, Plexon) and behavioral data (MED-PC), both synchronized with the LFPs, were stored for off-line analysis.

Resting and movement intervals were selected with Cineplex Markup software (Plexon). Two major behavioral patterns were dis-

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**Fig. 1.** Experimental setup and 6-hydroxydopamine (6-OHDA) lesion effects on histology and behavior. A: schematic outline of the time course of the experiments. B: schematic drawing of the walking task behavioral setup. The pellet dispenser areas had a cue light (gray circle) switched on when a reward was presented in the food cup (black rectangle). C: electrode and injection cannula placement on the rat skull. Location of tetrodes: X, frontal motor areas; *, subthalamic nucleus (STN). Gray circle, 6-OHDA cannula location. D: representative coronal section immunostained for tyrosine hydroxylase (TH) at the level of substantia nigra (SN). Note the absence of TH-immunopositive cells in the right SN and ventral tegmental area (VTA). E: walking speed (mean ± SD) of rats during the walking task (n = 4). F: performance during walking task (mean ± SD no. of pellets collected). Time relative to 6-OHDA injection (= day 0). T, training sessions; S, day of surgery (implantation). *P < 0.05, significant difference when day compared with baseline sessions (E) or training days (F).
tiblished, rest (closest possible to the human resting state): awake, sitting animal without any major motor activity (washing-grooming, etc.) involving more than one limb/shoulder girdle (considered an alert state; sleep spindles were never observed in our animals) and walk: animal standing on four limbs, performing step-by-step motion (only clear locomotion-associated signal was included in the analysis).

6-OHDA lesion procedure. For the duration of the 6-OHDA injection animals were anesthetized with isoflurane (2.5%). The injection needle was inserted in the guide cannula (extending 1 mm from end of cannula), 6-OHDA (8 μg; Sigma) in 3 μl of 0.9% NaCl saline stabilized with 0.05% ascorbic acid was injected with a Hamilton injector was left in place for 3 additional minutes to prevent the solution from flowing back up the guide.

Historical. Tetrode placements and neurotoxin effects were validated by postmortem histological analysis of the brains. After the final recording, the rats were anesthetized with isoflurane and the recording sites were marked by passing a direct current through every electrode. Then the animals were injected with medetomidine (0.25 ml/kg ip) and ketamine (ketamine 10%, 0.7 ml/kg ip) and perfused intracardially with buffered 4% paraformaldehyde. After perfusion, the brains were removed and immersion-fixed in the same fixative. Coronal brain sections (40 μm) were cut from substantia nigra (SN) (AP: −6.6 to −4.3 mm), STN (AP: −4.3 to −3 mm), and the motor cortex (AP: 3.5 to 4.5 mm) for tetrode placement and dopaminergic cell loss verification. Slides from all structures were stained with cresyl violet for tracing the tetrode tracts; slides from SN were immunostained for tyrosine hydroxylase (TH) for quantitative assessment of dopaminergic cell loss. Briefly, free-floating brain tissue sections were incubated with mouse anti-rat TH (Incstar, Stillwater, MN) diluted 1:2,000 in Tris-buffered saline. Washings between incubation steps were in the same buffer. After incubation with biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA; 1:100), peroxidase was visualized with an ABC immunoperoxidase kit (Vector Laboratories; 1:200) and 3,3′-diaminobenzidine tetrahydrochloride dihydrate (Sigma, St. Louis, MO; 0.5 mg/ml) as chromogen.

6-OHDA lesion verification. The extent of the 6-OHDA lesions was quantified independently by two observers using an ordinal five-point scale to assess microscopically the number of dopaminergic cell bodies that was present in ventral tegmental area (VTA) and SN at the end of the experiment. The lesioned side was compared to the nonlesioned side. In no case had the neurotoxin injection affected the noninjected side. The scale used was 1, no effect of lesion; 2, >50% of the dopaminergic cell bodies are present; 3, <50% but >10 cells are present; 4, <10 cells are present; 5, not a single cell is present. Six or seven coronal sections through mesencephalon, covering a range of bregma AP −6.20 to −4.30, were rated per animal, and ratings were averaged to obtain a final score. Interrater consistency was calculated with Cohen’s kappa agreement between the evaluations of the two raters.

Data analysis. The analysis of power and coherence spectra was performed as follows. For a given behavioral period (e.g., sitting), we divided all available LFP recordings in segments of 2 s. The 50-Hz line noise artifact was removed from the data by fitting a 50-Hz sinusoid to the data and subtracting this (Fries et al. 2008). For every segment, we then estimated the power spectral density and cross-spectral density by using the discrete Fourier transform in combination with multitapering, using a spectral resolution of 2 Hz in the case of the sitting period and 6 Hz in the case of the walking period (we used a coarser frequency resolution, as fewer observations were available). The power spectral density estimates were then averaged across all available channels in a given area. The relative power spectra were computed by dividing the power with the total power. The phase lag index (PLI; Stam et al. 2007) was computed as follows:

\[ \hat{\omega} = \frac{1}{N} \sum_{j=1}^{N} sgn(\mathbb{E}(X_j)) \]

Instead we computed the unbiased estimator of the PLI (Vinck et al. 2011) as

\[ \hat{\Omega} = \frac{N}{2} \sum_{j=1}^{N} d(X_j, Y_j) \]

where

\[ d(U, V) = sgn(\mathbb{E}(U)) \cdot sgn(\mathbb{E}(V)) \]

The PLI computes the nonequiprobability of phase leads and lags, with a value of 0 indicating that there are no systematic phase leads and lags and a value of 1 indicating that one channel is always phase leading or lagging the other channel.

Granger-causal flow was computed with nonparametric spectral density estimation (using spectral matrix factorization) according to Dhamala et al. (2008). It has been shown that additive noise (e.g., because of a common reference or volume conduction) can distort Granger causality measures, leading to erroneous conclusions about who is the causal driver and recipient (Haupe et al. 2013; Nolte et al. 2008). As a control, we therefore computed Granger causality measures on time-reversed signals (Haupe et al. 2013). To establish that Granger-causal flow from area A to area B is stronger than Granger-causal flow from area B to area A, we required not only a significant Granger-causal asymmetry for the actual data (A → B > B → A) but also a significant Granger-causal asymmetry for time-reversed signals in the opposite direction (B → A > A → B). The Granger causality analysis was based on the conventional assumption that there is no third-party source controlling the interaction between two measured sources.

To assess the temporal evolution of the various physiological parameters, we fitted an exponential model to the time courses of PLI and power as a function of day relative to lesion. We first normalized the PLI (or power) values per rat by dividing, for each day, the PLI (or power) by the maximum PLI (or power value) across days. For each time point, we then averaged the normalized PLI values across rats. This yielded an index between 0 and 1, as power and PLI are positively valued quantities. We then fit an exponential model to the data as

\[ y = [1 - \exp(-Ct)] \cdot A + B \]

Here \( y \) stands for the fitted value of the normalized PLI (or power); \( t \) stands for the day relative to lesion, where \( t = 0 \) is taken as all the prelesion data. The main parameter of interest is the increase parameter \( C \). High values of \( C \) indicate a fast rise, whereas low values of \( C \) indicate a slow rise. The parameter \( B \) was estimated as the minimum normalized PLI (or power) across days. The other parameters were estimated with the fminsearch algorithm in MATLAB, minimizing the least-squares error of the fitted versus observed data. To obtain estimates of the standard error of the parameters, we obtained jackknife estimates, by computing the leave-one-out pseudovalue. Using the jackknife estimates of the standard errors of the means, we performed pairwise t-tests to test for differences between the various parameters.

RESULTS

Verification of electrode placements and TH-immunopositive cell loss. Postmortem histological analysis was performed to validate tetrode placement and neurotoxin effects. The tetrode placements in the STN were typically on the dorsal border of the STN; placement of the cortical electrodes was in most cases in deep cortical layers (layer 5–6a), at AP 3.1–3.9, ML 2.3–2.7 (Paxinos and Watson 2005). The extent of neuro-
toxin effects was quantified: In all but one case the 6-OHDA injection had induced major degeneration, with only a few dopaminergic cells remaining (rating 4) (Fig. 1D). In the VTA, more cell bodies were preserved than in the SN (Fig. 1D). The average rating was 3, meaning that <50% of the dopaminergic cell bodies were still present. Agreement between the two independent observers, as calculated with Cohen’s kappa measure, was 1.0 for SN (P = 0.014) and 0.6 for the VTA (P = 0.031). In summary, we collected LFPs from six rats at rest and five rats at walk, four of which performed the walking task.

Behavior. During the development of dopaminergic cell loss induced by the 6-OHDA injection the rats showed altered behavior and task performance. Task performance did not change after electrode implantation, showing that the implantation per se had no effect. As the cell loss developed, the rats initiated fewer walks and the walking speed decreased. The rats’ motor activity (explorative behavior, walking) decreased initiated fewer walks and the walking speed decreased. The rats’ motor activity (explorative behavior, walking) decreased both in the home cage and during the walking task (Fig. 1, E and F). Despite the changing behavior we were able to collect similar amounts of movement-related LFPs at the last recordings as before the lesion, since there were fewer and slower (thus longer) walking intervals available. [The total walk-related signal availability per recording session was 51.56 ± 25 s (mean ± SD) at baseline, 58 ± 25 s (mean ± SD) at day 21.] Task performance and walking speed deteriorated significantly 5 days after the 6-OHDA injection, from the 14th day after the injection both values remained significantly lower than those of the baseline recordings (Fig. 1, E and F). Data from the single rat not trained in the task did not show any outlier values when compared against the animals trained in the task for any of the measures (power, PLI, Granger causality; data not shown), so walk-related signal from all five animals was grouped together.

Changes in spectral power after 6-OHDA lesion. In the present experiments we recorded LFP from cortical and subcortical brain areas. We consider subthalamic LFPs to be a good indicator of synchronized population activity, as has been shown in human and experimental parkinsonism (Kuhn et al. 2005; Walters et al. 2005). LFPs showed significant spectral changes between baseline (bsl) and postlesioned state (post, defined as recording days before and >14 days after the injection, respectively) in all three measured brain areas: M1 cortical area ipsilateral to the 6-OHDA injection (Mipsi), M1 contralateral to the injection (Mcontra), and STN (raw data shown in Fig. 2). We collected data intervals for “sit” in on average 640 ± 291 s (mean ± SD) and for “walk” 52.6 ± 28.8 s (mean ± SD) per recording session. To evaluate whether the present model shows alterations in the beta frequency band, we plotted the relative power for all areas. On average three bsl and four post measurements were included for both behavioral conditions, in total 18 bsl and 28 post sitting and 16 bsl and 19 post walking recording sessions. In the postlesion sitting condition, we detected significantly increased power between 25 and 35 Hz after the dopaminergic cell loss in all regions (Fig. 3A; t-test over recording sessions, P < 0.05, data not shown). The observed high beta peak at ~26 Hz (~24–28 Hz, due to smoothing of ±2 Hz) was most prominent in the lesioned hemisphere (Mipsi and STN) (Fig. 3A). Relative power during walking showed a wide-band elevation in high beta-gamma frequencies (26–100 Hz) in all regions after the lesion [t-test over bsl (n = 16) and post (n = 19) recording sessions, P < 0.05]. The beta peak observed in the postlesion sitting condition appeared slightly shifted to higher frequencies during walking, but the difference

Fig. 2. Local field potentials in resting state in the 3 measured brain areas in an example rat. A and B: simultaneously recorded raw (top) and filtered (10–50 Hz band-pass filter) (bottom) signal. C and D: time-frequency representations of the corresponding recording sessions (concatenated “sit” intervals throughout the whole recording). A and C: before 6-OHDA injection. B and D: 21 days after 6-OHDA injection. In D, note the beta-band activity in STN and M1 cortical area ipsilateral to the 6-OHDA injection (Mipsi). Mcontra, M1 contralateral to the injection.
between peak values only reached significance in Mipsi [peak frequency corresponding to highest relative power values between 23 and 40 Hz, sit: 28.04 ± 0.55 Hz, walk: 31.32 ± 1.35 Hz (mean ± SE over sessions); compare Fig. 3, A and C]. Since the above-mentioned results are consistent with findings in the hemiparkinsonian model, we investigated additional measures to disentangle interregional coupling and directionality.

Changes in synchronized oscillatory activity after 6-OHDA lesion. In the sitting condition, PLI spectra revealed a strong increase in 30 Hz phase synchronization between STN-Mipsi, Mipsi-Mcontra, and STN-Mcontra after the 6-OHDA lesion (Fig. 4, A and B). A similar observation was made for the walking period, with an increase in 30 Hz phase synchronization between STN-Mipsi, Mipsi-Mcontra, and STN-Mcontra after the 6-OHDA lesion or by behavior (sit, walk), suggesting that it is not dependent on SN dopamine content (Berke et al. 2004). Over the course of the development of dopaminergic cell loss, cortico-cortical synchronization increased at first in lower frequencies (~15–20 Hz) and later shifted toward higher beta frequencies (centered ~30 Hz) over the course of the experiment (Fig. 4B, center). The pattern of changes in the walking condition reflected the resting-state changes, albeit at higher peak values (with significantly higher peak values in walk compared with sit only in Mipsi-Mcontra, 36.5 ± 1.107 Hz and 30.3 ± 0.758 Hz, respectively, peak frequency corresponding to highest PLI values between 20 and 50 Hz, t-test over recording sessions, P < 0.05). The movement-related synchronization increased significantly after dopaminergic cell loss in STN-Mipsi at 32–34.5 Hz and in Mipsi-Mcontra at 31–40.5 Hz (Fig. 4, t-test over bsl and post recording sessions, n = 16
and n = 19, respectively). Interestingly, in contrast to the resting state, the cortico-cortical coherence in the walking period (Mipsi-Mcontra) displayed a characteristic peak at ~73 Hz both in baseline and in the fully lesioned state. To explore the source of the dopamine-dependent pattern, we analyzed the directional relationships between regions.

Cortico-subthalamic directionality. Granger causality analysis revealed that the subthalamo-cortical and cortico-cortical electrode pairs involved different frequencies for interregional communication. The neurotoxin induced changes in the cortico-subthalamic axis (STN-Mipsi, STN-Mcontra). In the resting condition, we detected a significantly increased influence of STN on both cortical areas in the 20–40 Hz (14.5–40 Hz; 18.5–39.5 Hz t-test over sessions bsl n = 18 and post n = 28, P < 0.05) band after the neurotoxin injection (Fig. 5, top left and top right). Both cortical areas showed increased impact on STN: Mipsi in the 26–31 Hz and Mcontra in the 3–5.5 Hz band (Fig. 5, middle left and middle right). The cortico-cortical causality changes involved multiple frequencies; we detected increased directionality from Mcontra to Mipsi in beta frequencies ~20 Hz (which involved lower frequencies than the peak frequency of the characteristic changes of the corresponding regions in power and PLI, around 30 Hz) and loss of directional influence in both directions above 70 Hz, without any frequencies showing bidirectional changes (Fig. 5 top center and middle center). The movement-associated signal (walking condition) showed tendencies similar to those in the resting state when comparing baseline and postlesion states. In general, during walk, the lesion-induced changes showed significant differences in fewer frequencies. Compared with the resting condition, we detected a loss of STN influence on both motor cortices at low (8–13 Hz) and high (~80 Hz) frequencies (Fig. 6, top; significantly different frequencies at STN-Mcontra were too few to present in the figure). Interestingly,
Mipsi had more influence on Mcontra after the lesion during walking in contrast to the resting condition (Fig. 6, bottom). Since Granger causality is a bidirectional measure, we were able to estimate the asymmetry of directional influence in one and the opposite direction (Fig. 5, bottom, and Fig. 6, bottom). Interestingly, when looking at the asymmetry of Granger causality between cortical areas during walk in the postlesion condition (Fig. 6, bottom center), an asymmetric pattern was found: Mcontra drove Mipsi in lower (<20 Hz) frequencies and Mipsi drove Mcontra in higher (35–60 Hz) frequencies. To summarize, we found that interregional synchronization was enhanced in the beta band after a fully developed dopaminergic...
cell lesion in the SN. The directional interactions revealed bidirectional changes in the beta band in the subthalamo-cortical axis and frequency-specific, unidirectional changes between motor cortical areas.

Evolution of changes over the development of dopaminergic cell loss in SN. The analysis above demonstrates increases in 20–40 Hz power, synchronization, and causal flow between signals after a 6-OHDA lesion. Our approach with the regular post-6-OHDA injection recordings allowed us to explore the dynamic changes of these measures throughout the development of dopaminergic cell loss in the SN. Here we focus on power spectral and synchronization changes during the sitting condition as those were the characteristics showing the most robust changes over time. The exponential model used to fit and describe the dynamics is shown in Fig. 7A. The difference between measures is described by the increase parameter $C$.

Fig. 6. Granger causality changes due to dopaminergic cell loss in walking condition. Each plot represents the mean Granger causality values between a region pair (shading indicates SE over recording sessions). Top and middle: differences between baseline and fully lesioned (all data ≥14 days after 6-OHDA injection) conditions (light and dark colors, respectively) per direction. Significant increase (dark colors) and decrease (light colors) after the 6-OHDA lesion are indicated with striped bars (colors respective to “driving” region; STN, red; Mipsi, green; Mcontra, blue). Bottom: comparison between the 2 directions for each pair of regions in the post state. Significant differences are indicated with colored bars (colors respective to “driving” region).
altered in various animal experiments and PD patients (Stein et al. 2012; Mallet et al. 2008; Pollok et al. 2012; Sharott et al. 2013; Fogelson et al. 2006; Lehmkuhle et al. 2009; Litvak et al. 2012; Cassidy et al. 2002; Degos et al. 2009; Florin et al. 2013). Here we refer to the broad BG beta frequencies found to be more, after the lesion, increased cortico-cortical functional connectivity was found in the resting state and during movement. Our findings revealed two aspects of pathological beta synchronization. First, in the course of the degeneration process interhemispheric cortico-cortical synchronization increased prior to increments in STN-cortical synchronization and changes in local power spectrum. Second, after dopaminergic cell loss, a bidirectional information flow was observed between STN and motor cortex of both the lesioned and nonlesioned hemispheres. The increase in beta-band relative power is in agreement with results from other studies (Lehmkuhle et al. 2009; Vorobyov and Sengpiel 2008), this indicates that dopaminergic cell loss in one hemisphere affects the nonlesioned hemisphere, possibly involving contralateral projections originating from SN and interhemispheric cortical connections (Morgan and Huston 1990). The importance of interhemispheric connectivity is strongly suggested by our findings showing increased functional coupling after 6-OHDA infusion between the left and right motor cortices and between motor cortex in the nonlesioned side and STN in the lesioned side. In addition to lesion-induced changes in interhemispheric coupling, enhanced synchronicity of beta oscillations was also seen in STN and cortex in the lesioned hemisphere, which is consistent with the results of previous studies in rats and humans (Brazhnik et al. 2012; Cassidy et al. 2002; Degos et al. 2009; Fogelson et al. 2006; Litvak et al. 2012; Mallet et al. 2008; Sharott et al. 2005b; Williams et al. 2002). The increased coupling in the beta frequency band between STN and cortex in the dopamine-depleted hemisphere was accompanied by directionality changes in the present experiments. Granger causality analysis demonstrated increased drive from motor cortex to STN around 30 Hz in resting as well as walking conditions. This finding is in accordance with.

Fig. 7. Exponential model for evolution of measures over the development of dopaminergic cell loss. A: exponential model fit to normalized values of mean relative power values over 25–35 Hz (top) and mean synchronization values 25–35 Hz (bottom) over time (relative to 6-OHDA injection). Dashed line indicates time where model shows 50% of maximal changes. B: increase parameter C indicating the rise of beta (25–35 Hz) power or PLI of model in sitting condition (*P < 0.05, **P < 0.01).
current concepts on pathophysiological changes in directionality of cortex-BG connectivity after degeneration of dopaminergic neurons (see Oswal et al. 2013 for review). Combining recordings from depth electrodes placed in the STN with MEG and EEG recordings, it could be established in PD patients both on and off levodopa that cortex leads STN oscillatory activity in the beta band (Fogelson et al. 2006; Lalo et al. 2008; Litvak et al. 2012; Williams et al. 2002). It is assumed that cortical activity drives the excessive beta-band oscillations in BG, resulting in increased synchronisation of oscillatory activity in the two brain regions (Oswal et al. 2013).

However, Granger analysis in the present experiments not only showed cortex leading STN but also STN leading cortex in a similar frequency range. For the hemiparkinsonian rat model these increments in directionality appear to confirm the suggestion of bidirectional changes put forward by Sharott et al. (2005a). The bidirectional changes in cortex-STN connectivity are not restricted to the rat model. Litvak et al. (2011) showed that enhanced bidirectional information flow in the beta range might also occur in PD patients. Cortex may influence STN through direct projections, whereas the most likely pathway via which STN may reach cortex is through globus pallidus pars interna and thalamus. It is likely that there is a dynamic interplay between the different components of BG and thalamus. A contribution of, e.g., thalamus to the functional connectivity between the regions investigated in the present study can therefore not be excluded. However, to our knowledge a pacemaker role for thalamus at the frequencies under discussion has so far not been established. Florin et al. (2013), in a study using Granger causality analysis to look at the afferent and efferent functional characteristics of STN in PD, argue that STN updates cortex with “afferent” information and speculate that STN integrates peripheral feedback and afferent information coming from cortex. The present findings, together with those of Litvak et al. (2011) in human patients, suggest that the effective connectivity in this cortico-subthalamic loop might be increased in both directions in the same frequency range. Probably, the process involves different populations of neurons of the same cortical/subthalamic region.

A remarkable finding in the present experiments was a strong increase in functional coupling in the beta frequency range between motor cortex of the lesioned and nonlesioned sides in the resting state, which was also present in the walking condition. After the 6-OHDA lesion, the direction of information flow between the synchronized populations of neurons was from nonlesioned to lesioned hemisphere during rest and walking, although the frequencies at which the asymmetry was observed differed. During rest the nonlesioned cortex drove the lesioned cortex in the higher beta range, whereas the walking condition showed this phenomenon in lower frequencies. Interestingly, during walking, at frequency ranges above 30 Hz, the reverse directionality was seen, with lesioned cortex leading the nonlesioned side.) Beta oscillations in sensorimotor cortex under normal conditions probably reflect nonspecific aspects of sensorimotor integration important for motor preparatory activity (see Cheyne 2013 for review). The present finding that information flow between motor cortices was from nonlesioned to lesioned hemisphere might be indicative of compensatory effects involving a stronger role of the nonlesioned motor cortex in sensorimotor processing. The enhanced interhemispheric coupling is discussed further below.

The cortico-cortical synchronisation was the first to develop over the course of the degeneration of dopaminergic cells. In interpreting these early events, it is important to take into account nonspecific effects of the 6-OHDA lesions, as discussed by Dejean et al. (2012). The increase in interhemispheric synchronisation that was found in the present study first involved lower frequencies and only after some days expanded to include higher beta frequencies (around 30 Hz), which was maintained for the length of the experiment. Enhanced beta power and increased coherence have been reported 1 wk after neurotoxin injection in the hemiparkinsonian rat (Brazhnik et al. 2012; Degos et al. 2009; Dejean et al. 2012; Mallet et al. 2008). The early appearance in the present experiments bears a striking resemblance to patterns reported in PD patients. In early disease stages synchronisation is increased in the alpha band, whereas increased coupling in beta frequencies is not observed until more advanced stages (Olde Dubbelink et al. 2013; Stoffers et al. 2008a). Since a lesion of the dopaminergic system caused the effects described in the present study, the increase in cortico-cortical synchronisation as observed in patients may emerge in response to dopaminergic cell loss. Hence, the cortico-cortical coupling patterns that show changes with disease progression might be a good indicator of neurodegenerative processes involving the BG.

In healthy human subjects, dopamine levels in putamen appear to be higher in the dominant hemisphere (de la Fuente-Fernández et al. 2000). Furthermore, movement with the dominant hand, which obviously implicates dopamine in the putamen, is associated with stronger interhemispheric cortical coupling compared with the use of the nondominant hand (Gross et al. 2005). Although unilateral movements were not investigated in the present study, our findings appear to corroborate the idea that activity in the dopamine-dominant hemisphere is associated with increased cortico-cortical synchronisation and that enhanced interhemispheric coupling may constitute a mechanism compensating for a loss of dopamine. Seemingly in contrast to this contention, excessive interhemispheric synchronisation—be it compensatory or physiological—has been shown to lead to perturbed motor processing in healthy subjects (Houweling et al. 2010). However, the loss of dopaminergic cells may induce compensatory enhanced interhemispheric coupling in the beta band, which beyond a certain point becomes excessive and will result in bradykinesia or akinesia. Such a process might explain why interregional coupling changes have been detected prior to the appearance of motor impairment in the rodent (Dejean et al. 2012). It is also in line with studies showing that stimulation at beta frequencies did not per se elicit behavioral alterations in rodents and had only limited effects in PD patients (Brown 2007; Dejean et al. 2012; Leblois et al. 2007; Syed et al. 2012). The fact that in the present experiments the increase in cortico-cortical beta synchronisation was similar in the walking and resting conditions supports a putative compensatory mechanism contributing to the coupling changes in parkinsonism.

In frequency ranges below 16 Hz, the present findings show moderate changes in relative power. We measured reduced cortico-subthalamic synchronisation in the 11–16 Hz range, which was not observed in other rodent studies (Brazhnik et al. 2012; Dejean et al. 2012), presumably because of the different coupling measures used. According to Stein and Bar-Gad (2013), these frequencies would be part of the lower beta
range, in which case our finding supports the proposition of segregation between lower and higher beta frequencies in PD patients (Fogelson et al. 2006; Litvak et al. 2011; Priori et al. 2004; Stein and Bar-Gad 2013). Antiparkinsonian drugs have been shown to differentially modulate activity in the two frequency ranges (Litvak et al. 2011; Priori et al. 2004).

The cortico-cortical synchronization observed in the present study revealed a behavior-dependent but lesion-independent feature: a characteristic gamma (60–80 Hz) coupling during walking [as seen in healthy rodents (Berke 2009)]. Interestingly, Granger causality in the gamma band during walking was not influenced by the 6-OHDA lesion, either. This suggests that the observed gamma coupling is linked to motor activity (note, however, that other aspects of the task such as movement kinematics, vigor of effort, attention, and presence of reward in the task may be involved; Berke 2009; Jenkins et al. 2013; Muthukumaraswamy 2010; van der Meer and Redish 2009). The fact that in PD patients movement-related gamma activity is modulated by dopaminergic medication would seem to contradict our findings (Alegre et al. 2005; Androulidakis et al. 2007; Lalo et al. 2008; Litvak et al. 2012; Williams et al. 2002). However, since dopaminergic medication restores motor activity in many patients, the effects of dopamine and motor activity cannot be dissociated in most cases.

Taken together, our results present frequency-dependent cortico-subthalamic functional and effective connectivity in the hemiparkinsonian rat. This may support the idea of the involvement of multiple subcircuits in the BG-cortex loop in parkinsonism (Fogelson et al. 2006; Hirschmann et al. 2013; Litvak et al. 2011). On basis of our findings we conclude that interhemispheric cortical synchronization is an early indicator of nigrostriatal dopaminergic neuronal loss. Our results support observations in PD patients that suggest disease stage-dependent changes of synchronization patterns involving distinct frequencies. The observed interregional synchronization changes might reflect a functional compensatory mechanism in response to the neurodegenerative processes, a suggestion that is in line with the view of others, questioning a causal relationship between electrophysiological alterations in the BG and symptoms of parkinsonism (Boraud et al. 2005; Hirschmann et al. 2013). However, the present data did not provide conclusive evidence for such a mechanism. In future experiments, it will be necessary to analyze the quality of locomotor activity in sufficient detail and correlate these findings with electrophysiological parameters to unveil the nature of the relationship between symptoms and neurophysiological changes.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


Gross J, Pollok B, Dirks M, Timmermann L, Butz M, Schnitzler A. Task-dependent oscillations during unimanual and bimanual movements in


van der Meer MA, Redish AD. Low and high gamma oscillations in rats ventral striatum have distinct relationships to behavior, reward, and spiking activity on a learned spatial decision task. *Front Integr Neurosci* 3: 9, 2009.


