Chapter 2

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

Changes in synchronized neuronal oscillatory activity are reported both in cortex and basal ganglia of Parkinson’s disease patients. The origin of these changes, in particular their relationship with the progressive nigrostriatal dopaminergic denervation, 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 the development of the nigrostriatal dopaminergic cell loss. We obtained signal from freely moving rats both in resting and in walking condition, 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 the development of the 6-OHDA lesion, we suggest a putative compensatory role of cortico-cortical coupling.

B. N. Jávor-Duray1, M. Vinck2, M. van der Roest1, A. B. Mulder1, C. J. Stam3, H. W. Berendse4, P. Voorn1

1 Department of Anatomy and Neurosciences, VU University Medical Center, Neuroscience Campus Amsterdam, 1081 BT Amsterdam, The Netherlands;
2 Cognitive and Systems Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 XH Amsterdam;
3 Department of Clinical Neurophysiology, VU University Medical Center, 1081 HV Amsterdam;
4 Department of Neurology, VU University Medical Center, Neuroscience Campus Amsterdam, 1081 HV Amsterdam, the Netherlands

INTRODUCTION

Synchronized neuronal oscillatory activity is altered in Parkinson's disease (PD) patients both in cortical and subcortical brain regions [1–4]. 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 [2, 4–8]. 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 frequencies (alpha) occurring at early, and higher (beta) frequencies at more advanced stages of the disease [2, 9–13]. Experiments in animal models have revealed changes in synchronization similar to those recorded in PD patients [14–16], showing increased intrahemispherical or cortico-nigral synchronization. Interestingly, none of these studies examined interhemispherical synchronization specifically, although this is among the earliest alterations identified in PD patients [10]. In addition, directionality measures, which may also characterize interregional relationships, have so far described rather diverse effects [1, 3, 17, 18].

Besides resting state measurements, human and experimental animal studies have investigated changes in association with the characteristic motor disturbances [15, 19, 20]. Although the presence of a direct (causal) relationship between the motor symptoms and the excessive synchronized oscillatory activity in parkinsonism is still under discussion [19, 21–24], motor activity was shown to modulate oscillatory activity and interregional coupling in PD patients both in cortical and subcortical areas [6, 18, 25–28]. 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 in a freely moving 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 (approx. 300g, Harlan; The Netherlands) were kept under standard housing conditions at constant temperature (22±1 °C), humidity (relative, 56%),
and 12-hour reverse light–dark cycle (daylight period 19:00-7:00). 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 Dierpoeven, 1996) and European regulations (Guideline 86/609/EEC).

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, approx. layer V. neurons) and two tetrodes (9.5, 10.0 mm long) targeting STN in a separately adjustable holder. The two 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 local field potential (LFP) recordings, were produced of 4 x 13 µm insulated NI RO-800 wire (Kanthal Precision Technology, Palm Coast, FL, USA), its impedance was adjusted between 0.9-1.2 mOhm.

Surgery

Inhalation anaesthesia with isoflurane was used to initiate and maintain anaesthesia 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 the 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 [29]) 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 15º 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 [30], 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. Following tetrode placements, the guide cannula for the subsequent 6-OHDA injections was placed above the medial forebrain bundle (under 18º 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 1 m corridor and 2 cue-lights and pellet dispensers (Fig. 1B). The automated behavioral task (executed with the aid of MED PC behavioral control software (Sandown Scientific, Middlesex, 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 minutes. 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 (50x30cm plexi Faraday-cage) every day for 3 weeks, after this (at more than 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 minutes, 30 minutes if only resting condition was monitored, and 20 minutes if also the walking task was executed (Fig1A). 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 LFP-s were amplified 20-times (HST/16V-G20), followed by a pre-amplifier (PBX/32sp-r G50/16fp-G50, Plexon Inc., TX, USA) with 50x gain. The signal was band-pass filtered to generate LFP-s (0.7-170 Hz). The LFP-s generated by the preamplifier were alternatively amplified 2x and 5x by the AD converter, for a total amplification of 2000x and 5000x. Video recordings (Cineplex, Plexon Inc., USA) and behavioral data (MEDPC), both synchronized with the LFP-s were stored for offline analysis.

Resting and movement intervals were selected using Cineplex Markup software (Plexon Inc., USA). Two major behavioral patterns were distinguished: rest (closest possible to the human resting state): awake, sitting animal, without any major motor activity (washing-grooming, etc...) involving more than 1 limb /shoulder girdle (considered as an alert state; sleep spindles were never observed in our animals); and walk: animal standing on...
4 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 anaesthetized with isoflurane (2.5%). The injection needle was inserted in the guide cannula (extending 1mm from end of cannula). 8 µg 6-OHDA (Sigma, Germany) in 3 µl 0.9% saline NaCl stabilized with 0.05% ascorbic acid was injected with a Hamilton needle (rate: 0.250 µl/min). After completing the injection the injector was left in place for 3 additional minutes to prevent the solution from flowing back up the guide.

Histology

Tetrode placements and neurotoxin effects were validated by postmortem histological analysis of the brains. After the final recording, the rats were anaesthetized with isoflurane, the recording sites were marked by passing a direct current through every electrode. Then the animals were injected with medetomidine (0.25 ml/kg, i.p.), ketamine (ketamine 10% 0.7ml/kg, i.p.) and perfused intracardially with buffered 4% paraformaldehyde. After perfusion, the brains were removed and immersion-fixed in the same fixative. Coronal brain sections (40µm) were cut from SN (AP: -6.6 to -4.3mm), STN (AP: -4.3 to -3mm) and the motor cortex (AP: 3.5 to 4.5mm) 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, USA) diluted 1:2000 in TRIS-buffered saline. Washings between incubation steps were in the same buffer. After incubating with biotinylated-horse-anti-mouse IgG, (Vector Laboratories, Burlingame, CA, USA; 1:100), peroxidase was visualised using an ABC immunoperoxidase kit (Vector Laboratories; 1:200) and 3,3 diaminobenzidine tetrahydrochloride dihydrate (Sigma, St. Louis, MO, USA; 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 5-point scale to assess microscopically the number of dopaminergic cell bodies that was present in VTA and substantia nigra at the end of the experiment. The lesioned side was compared to the non-lesioned side. In no case had the neurotoxin injection affected the non-injected side. The scale used was: 1. no effect of lesion, 2. more than 50% of the dopaminergic cell bodies are present, 3. less than 50 % but more than 10 cells are present, 4. less than 10 cells are present, 5. not a single cell 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. Inter-rater consistency was calculated using 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 [31]. 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 case of the sitting period, and 6 Hz in 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, [32]) was computed as follows Denote the estimate of the cross-spectral density for the j-th trial as Xj. The direct PLI estimator is defined

$$\hat{\Psi}_j = \frac{1}{N} \sum_{j=1}^{N} \text{sgn}(\Im\{X_j\})$$

Instead we computed the unbiased estimator of the PLI [33] as

$$\hat{\Omega} = \left(\frac{N}{2}\right)^{-1} \sum_{j=1}^{N-1} \sum_{k=(j+1)}^{N} \text{d}(X_j, X_k),$$

where

$$\text{d}(U, V) \equiv \text{sgn}(\Im\{U\}) \text{sgn}(\Im\{V\}).$$

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

Granger-causal flow was computed using nonparametric spectral density estimation (using spectral matrix factorization) according to Dhamala et al. [34]. 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 [35, 36]. As a control, we therefore computed Granger-causality measures on time-reversed signals [36]. To establish that Granger-causal flow from area A
to B is stronger than Granger-causal flow from area B to 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 > B→A). 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 value) 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

\[ y = [1 - \exp(-C \, t)] \cdot A + B \]

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 pre-lesion 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 using 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 jack-knife estimates, by computing the leave-one-out pseudo-values. Using the jack-knife 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 [29]. The extent of neurotoxin effects was quantified: in all but one case the 6-OHDA injection had induced major degeneration with only few dopaminergic cells remaining (rating 4) (Fig. 1D). In the ventral tegmental area, more cell bodies were preserved than in the substantia nigra (Fig. 1D). The average rating was 3, meaning that less than 50% of the dopaminergic cell bodies was still present. Agreement between the two independent observers as calculated using Cohen’s kappa measure, was 1.0
for substantia nigra (p=0.014) and 0.6 for the ventral tegmental area (p=0.031). In summary, we collected LFPs from 6 rats at rest, 5 rats at walk, 4 of which performed the walking task.

Figure 1. Experimental setup and 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 (filled circle) switched on when a reward was presented in the food cup (black rectangle). C: Electrode, injection cannula placement on the rat skull. Location of tetrodes: frontal motor areas (crosses), subthalamic nucleus (star). 6-OHDA cannula location: filled circle. D: Representative coronal section immunostained for tyrosine hydroxylase (TH) at the level of substantia nigra. Note the absence of TH immunopositive cells in the right substantia nigra (SN) and ventral tegmental area (VTA). E: Walking speed (mean ± SD) of rats during the walking task (n=4). Time relative to 6-OHDA injection (=day 0). F: Performance during walking task (mean ± SD number of pellets collected). Time relative to 6-OHDA injection (=day 0); T: training sessions; S: day of surgery (implantation); * signifies p<0.05 significant difference when day compared to baseline sessions (E) or training days (F).
Behavior

During the development of dopaminergic cell loss induced by the 6-OHDA injection the rats showed altered behavior and task performance. The 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 both in the home cage and during the walking task (Fig 1E, 1F). 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±25s (mean±SD) at baseline, 58±25s (mean±SD) at day 21.) The task performance and walking speed deteriorated significantly 5 days after the 6-OHDA injection, from the 14th after the injection both values remained significantly lower than those of the baseline recordings (Fig1E, 1F). 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.

Figure 2. Local field potentials in resting state in the three measured brain areas in an example rat. A and B show simultaneously recorded raw signal in the top three lines, filtered (10-50 Hz bandpass filter) in the bottom three lines. C, D: time frequency representations of the corresponding recording sessions (concatenated ‘sit’ intervals throughout the whole recording). A, C: before, B,D: 21 days after 6-OHDA injection. In D, note the beta band activity in STN, Mipsi.
Figure 3. Relative power spectral changes after the 6-OHDA lesion.
A,C: Average relative power (shading indicates SEM over recording sessions) at baseline (‘bsl’), fully lesioned (‘post’: all data ≥14 days after 6-OHDA injection) conditions during resting and walking (n=6, n=5 rats, respectively) in Mipsi, Mcontra, STN. Black bars indicate significant difference (where significant differences are present ≥ 3 Hz wide, t test over sessions, p<0.05) B,D: 6-OHDA -induced changes in relative power over time course of lesion development in resting (B) and walking (D) (n=6, n=5 rats, respectively). Time indicated relative to toxin injection (=day 0).
Changes in spectral power after 6-OHDA lesion

In the current experiments we recorded LFP from cortical and subcortical brain areas. We consider subthalamic LFP-s as a good indicator of synchronized population activity, as has been shown in human and experimental parkinsonism [37, 38]. LFP-s showed significant spectral changes between baseline (bsl) and post-lesioned state (defined as recording days before, >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 average 640±291 (mean±SD) seconds, for ‘walk’ 52.6±28.8 (mean±SD) seconds per recording session. To evaluate whether the current model shows alterations in the beta frequency band, we plotted the relative power for all areas. On average 3 ‘bsl’ and 4 ‘post’ measurements were included for both behavioral conditions, in total 18 ‘bsl’, 28 ‘post’ sitting and 16 ‘bsl’, 19 ‘post’ walking recording sessions. In the post-lesion sitting condition, we detected significantly increased power between 25-35 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 (approximately 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 post-lesion sitting condition appeared slightly shifted to higher frequencies during walking, but the difference between peak values only reached significance in Mipsi (sit: 28.04±0.55Hz, walk:31.32±1.35Hz (mean±SEM over sessions)), compare Figs 3A and 3C). 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 following the 6-OHDA lesion (Figs 4A, 4B). A similar observation was made for the walking period, with an increase in ~35 Hz synchronization between Mipsi-Mcontra and STN-Mipsi, but not between Mcontra and STN (Figs 4C, 4D). In the sitting condition, we detected significantly lower PLI values between cortex and STN values between 12-16 Hz after the dopaminergic cell loss. In addition, we observed a peak in the PLI spectrum around 8 Hz (Fig. 4). This synchronization was not modulated by the 6-OHDA lesion, or by behavior (sit, walk), suggesting it is not dependent on SN dopamine content [39]. Over the course of the development of dopaminergic cell loss, cortico-cortical synchronization increased at first in
**Figure 4.** Interregional synchronization (PLI) changes after 6-OHDA lesion. A-C: Synchronization (mean PLI, shading indicates SEM over recording sessions) between STN-Mipsi, Mipsi-Mcontra, STN-Mcontra at baseline (‘bsl’), fully lesioned (‘post’: all data ≥ 14 days after 6-OHDA injection) conditions in resting (A) and walking (C) (n=6, n=5 rats, respectively). Black bars indicate significant difference (where significant differences are present ≥ 3 Hz wide, t test over sessions, p<0.05) B-,D: Changes in average PLI spectrum plotted over the time course of lesion development in resting (B) and walking (D) (n=6, n=5 rats, respectively). Time indicated relative to toxin injection (=day 0). Time points for walking data were averaged over 3 baseline sessions. Post-lesion data points represent pooled data from 2 days (except for days 9, 12, 19, 21).
Figure 5. Granger causality changes due to dopaminergic cell loss in sitting condition. Each plot represents the mean Granger causality values between a region-pair (shading indicates SEM over recording sessions). Top and middle row show differences between baseline (‘bsl’), fully lesioned (‘post’: 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 filled bars, where significant differences are present ≥ 3 Hz wide, colors respective to ‘driving’ region (STN: red, Mipsi: green, Mcontra: blue). The bottom row shows the comparison between the two directions for each pair of regions in the ‘post’ state. Significant differences are indicated with striped bars (colors respective to ‘driving’ region). For example, top left plot shows significant differences of STN drive to Mipsi caused by the 6-OHDA injection: below 10 Hz significantly stronger drive in the ‘bsl’ when compared to the ‘post’ condition; at frequencies ~20-40 Hz STN→Mipsi drive stronger in ‘post’ than ‘bsl’. Bottom left plot shows that Mipsi drives more STN at ~8 Hz than STN→Mipsi and STN drives more Mipsi at ~15 Hz more than Mipsi→STN.
lower frequencies (~15-20 Hz) and later shifted toward higher beta frequencies (centered ~30 Hz) over the course of the experiment (Fig.4B, middle panel). 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 to sit only in Mipsi-Mcontra, 36.5±1.107 Hz, 30.3±0.758 Hz, respectively, 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 31-40.5Hz (Fig 4, t-test over ‘bsl’ and ‘post’ recording sessions n=16, 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-subthalar directionality

The 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 sitting condition, we detected 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 row, left and right plots). Both cortical areas showed increased impact on STN: Mipsi in the 26-31Hz, Mcontra in the 3-5.5 Hz band (Fig 5, middle row, left and right plots). The cortico-cortical causality changes involved multiple frequencies, we detected increased directionality from Mcontra to Mipsi in beta frequencies ~20Hz (which involved lower frequencies than the peak frequency of the characteristic changes of the corresponding regions in power and PLI, around 30 Hz), loss of directional influence in both directions above 70Hz, without any frequencies showing bidirectional changes (Fig 5 top and middle row, middle plots). The movement-associated signal (walking condition) showed similar tendencies as that in resting state when comparing baseline and post-lesion states. In general, during walk, the lesion-induced changes showed significant differences in fewer frequencies. In comparison to the resting condition, we detected a loss of STN influence on both motor cortices at low (8-13 Hz) and high frequencies (~80 Hz) (Fig. 6 top row; 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 row). Since Granger causality is a bidirectional measure, we were able to estimate the asymmetry of directional influence in one and the opposite direction (Fig. 5,6 bottom row). Interestingly, when looking at the asymmetry of Granger causality between cortical areas during walk in the post-lesion condition (Fig. 6, bottom row, middle plot), an asymmetric pattern was found: Mcontra drove Mipsi in lower (<20 Hz), Mipsi drove Mcontra in higher frequencies (35-60 Hz). 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 ones 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. 7B). The increase parameter revealed that the fastest rise in values is present in measures involving Mcontra (power Mcontra and PLI Mipsi-Mcontra), which increase significantly faster when compared to most other measures. In short, the changes over the development of dopaminergic cell loss showed the most rapid changes in synchronization between the motor cortical areas in the two hemispheres.

Figure 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 (first row) and mean synchronization values 25-35 Hz (second row) 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).
DISCUSSION

The effects of unilateral 6-OHDA-induced dopaminergic cell loss in the rat ventral mesencephalon were examined on the relationship between LFPs recorded from motor cortex in the two hemispheres and subthalamic nucleus (STN) in the lesioned hemisphere. Following the lesion, increases were seen in relative power in the beta band in STN and motor cortex, and increased coupling between all region pairs. During movement, a characteristic peak in the beta band was detected after the lesion. Furthermore, 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 interhemispherical 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 non-lesioned hemisphere.

The increase in beta band relative power is in agreement with results from other animal and patient studies [1, 3, 14, 15, 18, 27, 40–45]. Here, we refer to the broad basal ganglia beta frequencies found to be altered in various animal experiments and PD patients [46]. As discussed by the latter authors, there is considerable variance across experimental animals as well as human subjects with respect to the precise frequency ranges that are affected as a consequence of lesions or degeneration. Our results revealed power changes in the same frequencies as reported in numerous freely moving hemiparkinsonian rat experiments [14, 15, 42, 44, 47]. The increase in beta band power observed in the current study was not restricted to the lesioned hemisphere since it could also be detected in motor cortex of the non-lesioned side. In line with results from other studies [43, 44], this indicates that dopaminergic cell loss in one hemisphere affects the non-lesioned hemisphere, possibly involving contralateral projections originating from substantia nigra and interhemispheric cortical connections [48]. The importance of interhemispheric connectivity is strongly suggested by our findings showing increased functional coupling after the 6-OHDA infusion between the left and right motor cortices and between motor cortex in the non-lesioned 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 [1, 3, 14, 15, 18, 40–42].

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 current concepts on pathophysiological changes in directionality of cortex – basal
ganglia connectivity after degeneration of dopaminergic neurons [see 49 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 [1, 3, 6, 18]. It is assumed that cortical activity drives the excessive beta band oscillations in basal ganglia, resulting in increased synchronization of oscillatory activity in the two brain regions [49].

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. [50]. The bidirectional changes in cortex – STN connectivity are not restricted to the rat model. Litvak et al. [51] 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 basal ganglia and thalamus. A contribution of e.g. thalamus to the functional connectivity between the regions investigated in the current 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. [45], 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. [51] 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 non-lesioned side 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 non-lesioned to lesioned hemisphere during rest and walking, although the frequencies differed at which the asymmetry was observed. During rest, the non-lesioned 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 non-lesioned side.) Beta oscillations in sensori-motor cortex under normal conditions probably reflect non-specific aspects of sensori-motor integration important for motor preparatory activity [see 52, for review]. The present finding that information flow between
motor cortices was from non-lesioned to lesioned hemisphere might be indicative of compensatory effects involving a stronger role of the non-lesioned motor cortex in sensorimotor processing. The enhanced interhemispheric coupling will be further discussed below.

The cortico-cortical synchronization 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 non-specific effects of the 6-OHDA lesions, as discussed by Dejean et al. [16]. The increase in interhemispheric synchronization that was found in the present study first involved lower frequencies and only after some days expanded to include higher beta frequencies (around 30Hz), which was maintained for the length of the experiment. Enhanced beta power and increased coherence have been reported one week after the neurotoxin injection in the hemiparkinsonian rat [14–16, 42]. The early appearance in the present experiments bears a striking resemblance to patterns reported in PD patients. In early disease stages, synchronization is increased in the alpha band, whereas increased coupling in beta frequencies is not observed until more advanced stages [10, 13]. Since a lesion of the dopaminergic system caused the effects described in the present study, the increase in cortico-cortical synchronization 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 basal ganglia. In healthy human subjects, dopamine levels in putamen appear to be higher in the dominant hemisphere [53]. Furthermore, movement with the dominant hand, which obviously implicates dopamine in the putamen, is associated with stronger interhemispherical cortical coupling compared to the use of the non-dominant hand [54]. 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 synchronization and that enhanced interhemispheric coupling may constitute a mechanism compensating for a loss of dopamine. Seemingly in contrast to this contention, excessive interhemispherical synchronization –be it compensatory or physiological– has been shown to lead to perturbed motor processing in healthy subjects [55]. However, the loss of dopaminergic cells may induce compensatory enhanced interhemispherical coupling in the beta band, which beyond a certain point becomes excessive and will result in brady- or akinesia. Such a process might explain why interregional coupling changes have been detected prior to the appearance of motor impairment in the rodent [16]. 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 [16, 21, 23, 24]. The fact that in the present experiments the increase in cortico-cortical beta synchronization was similar in the walking and resting condition 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 synchronization in the
11-16 Hz range, which was not observed in other rodent studies [15, 16], presumably due to the different coupling measures used. According to Stein and Bar-Gad [46], 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 [3, 46, 51, 56]. Antiparkinsonian drugs have been shown to differentially modulate activity in the two frequency ranges [51, 56].

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 [57]). 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, presence of reward in the task may be involved, [57–60]. The fact that in PD patients movement related gamma activity is modulated by dopaminergic medication would seem to contradict our findings [1, 6, 26, 51, 61]. 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 basal ganglia-cortex loop in parkinsonism [3, 4, 51]. On basis of our findings we conclude that interhemispherical 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. In addition, we propose that the observed interregional synchronization changes reflect a functional compensatory mechanism in response to the neurodegenerative processes. This suggestion is consistent with the view of others, questioning the causal relationship between electrophysiological alterations in the basal ganglia and symptoms of parkinsonism [4, 62].

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REFERENCES


58. van der Meer MAA, Redish AD: Low and High Gamma Oscillations in Rat Ventral Striatum have Distinct Relationships to Behavior, Reward, and Spiking Activity on a Learned Spatial Decision Task. Front Integr Neurosci 2009, 3:9.


