Untangling cortico-striatal connectivity and cross-frequency coupling in L-DOPA-induced dyskinesia

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Keywords: Cortico-striatal circuits, Levodopa-induced dyskinesia, Parkinson's disease, effective connectivity, Cross-Frequency Coupling.

Abstract

We simultaneously recorded local field potentials in the primary motor cortex and sensorimotor striatum in awake, freely behaving, 6-OHDA lesioned hemi-parkinsonian rats in order to study the features directly related to pathological states such as parkinsonian state and levodopa-induced dyskinesia. We analysed the spectral characteristics of the obtained signals and observed that during dyskinesia the most prominent feature was a relative power increase in the high gamma frequency range at around 80 Hz, while for the parkinsonian state it was in the beta frequency range. Here we show that during both pathological states effective connectivity in terms of Granger causality is bidirectional with an accent on the striatal influence on the cortex. In the case of dyskinesia, we also found a high increase in effective connectivity at 80 Hz. In order to further understand the 80- Hz phenomenon, we performed cross-frequency analysis and observed characteristic patterns in the case of dyskinesia but not in the case of the parkinsonian state or the healthy state. We noted a large decrease in the modulation of the amplitude at 80 Hz by the phase of low frequency oscillations (up to ~10 Hz) across both structures in the case of dyskinesia. This may suggest a lack of coupling between the low frequency activity of the recorded network and the group of neurons active at ~80 Hz.

1.   Introduction

36 The basal ganglia (BG) represent subcortical structures thought to be involved in action selection and
37 decision making (Redgrave et al., 1999; Grillner et al., 2005). Dysfunction of the BG circuitry leads
38 to many motor and cognitive disorders such as Parkinson's disease (PD), Tourette's syndrome,
39 Huntington's disease, and obsessive compulsive disorder (Albin et al., 1989; DeLong, 1990; Singer
40 et al., 1993; Wichmann and DeLong, 1996; Bergman et al., 1998; Blandini et al., 2000; Obeso et al.,
41 2000; Brown, 2002; Leckman, 2002; Albin and Mink, 2006; Hammond et al., 2007; Tippett et al.,
42 2007; Starney and Jankovic, 2008; Evans et al., 2009; André et al., 2011). The striatum, the input
43 stage of the basal ganglia, is an inhibitory network that contains several distinct cell types and
44 receives massive excitatory inputs from the cortex (Webster, 1961; Kincaid et al., 1998; Zheng and
45 Wilson, 2002; Belić et al., 2015). The cortex sends direct projections to the striatum, while the
46 striatum can only indirectly affect the cortex through other BG nuclei and thalamus (Oldenburg and
47 Sabatini, 2015). Understanding the complex nature of cortico-striatal interactions is of crucial
48 importance for clarifying the overall functions and dysfunctions of the BG.
49 PD is the most common movement disorder and is observed in approximately 1% of the
50 population over the age of 60 (Tanner and Ben-Shlomo, 1999; Mayeux, 2003). Although dopamine
51 replacement therapy with L-DOPA is the most effective treatment for PD, within five years of
52 starting the treatment, up to 80% of patients will experience severe side effects and develop L-
53 DOPA-induced dyskinesia characterised by abnormal involuntary movements (Bezard et al., 2001;
54 Fabbrini et al., 2007; Thanvi et al., 2007; Pisani and Shen, 2009). L-DOPA-induced dyskinesia is
55 believed to result from abnormal plasticity in the dopaminoceptive brain regions (Cenci and Konradi,
56 2010), although the neural mechanisms underlying it are unfortunately still far from clear. Thus,
57 animal models are crucial to study L-DOPA-induced dyskinesia and develop potential new therapies
58 (Cenci et al., 2002; Nadjar et al., 2009).
59 Oscillations are present at many levels in the basal ganglia and a wide range of characteristic
60 frequencies have been reported to occur during both health and disease (Boraud et al., 2005).
61 Neuronal oscillations, reflecting the synchronised activity of neuronal assemblies, are proposed to
62 play a major role in the long-range coordination of distinct brain regions (Fries, 2005). Oscillations
63 may interact with each other in the way that the amplitude of high frequency activity occurs at a
64 particular phase of a low frequency band, which has been reported to happen in the basal ganglia, the
65 hippocampus and the neocortex (Canolty et al., 2006; Jensen and Colgin, 2007; Tort et al., 2008;
66 Cohen et al., 2009; Tort et al., 2009; de Hemptinne et al., 2013). Such cross-frequency coupling has
67 been proposed to coordinate neural dynamics across spatial and temporal scales (Aru et al., 2015). It
68 has also been suggested that the activity of local neuronal populations oscillates at lower frequencies
69 and that smaller ensembles are active at higher frequencies. Cross-frequency coupling may, therefore,
70 serve as a mechanism for the transfer of information from large-scale brain networks operating at the
71 behavioural time scale to the smaller group of neurons operating at a faster time scale (Buzsaki,
72 2006; Canolty and Knight, 2010; Aru et al., 2015).
73 We simultaneously recorded local field potentials (LFPs) in the primary motor cortex and
74 dorsolateral striatum in order to study L-DOPA-induced dyskinesia in 6-OHDA lesioned hemi-
75 parkinsonian rats. LFP recordings generally provide a useful measure of the synchronised activities
76 of local neuronal populations. Here, we employ LFP signals to study the directed influence between
77 the cortex and the striatum as well as cross-frequency coupling in the healthy, parkinsonian and
78 dyskinetic states. To investigate directional interactions in cortico-striatal networks in these
79 paradigms, we employed Granger causality as a well-established effective connectivity metric. We
80 found that for pathological states, effective connectivity is bidirectional with an accent on the striatal
81 influence on the cortex. In the case of L-DOPA-induced dyskinesia, we observed a high increase in
82 effective connectivity at ~80 Hz. Interestingly, in the dyskinetic state, our results showed a large
83 relative decrease in the modulation of the LFP amplitude at ~80 Hz by the phase of low frequency
84 oscillations, suggesting a lack of coupling between the low frequency activity of a presumably larger

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85 population and the synchronised activity of a presumably smaller group of neurons active at 80 Hz.
86 This work demonstrates the bidirectional nature of influence between the cortex and the striatum for
87 pathological states, as well as the lack of synchronisation between low frequencies and those at ~80
88 Hz in the dyskinetic state, which to the best of our knowledge have not been reported before.
89   Part of these results was previously presented in the form of an abstract (Belić et al., 2015).

2.   Materials and Methods

91 Seven adult female Sprague Dawley rats (230-250 g) were used in this study. All experiments were
92 approved in advance by the Malmö/Lund ethical committee on animal experiments. A more detailed
93 description of the 6-hydroxydopamine lesions, electrodes, surgery, experiments and signal
94 acquisition can be found in (Halje et al., 2012).

2.1.   6-Hydroxydopamine lesions

97 The animals were anesthetised with Fentanyl/Medetomidine (03/03 mg/kg) and received two
98 injections of 6-hydroxydopamine (6-OHDA) hydrochloride (3.0 µg/µl free base, Sigma-Aldrich;
99 dissolved in 0.02% ascorbate saline) into the medial forebrain bundle of the right hemisphere at the
100 following coordinates from the bregma and cortical surface: Injection site (i), 2.5 µl: tooth bar (TB), -
101 2.3; anteroposterior (AP), -4.4; mediolateral (ML), -1.2; and dorsoventral (DV), -7.8; Injection site
102 (ii), 2.0 µl: TB, +3.4; AP, -4.0; ML, -0.8; DV, -0.8. Moderate motor impairments including
103 asymmetric posture, gait and reduced forelimb dexterity were apparent two weeks after lesioning.

2.2.   Electrodes and implantation surgery

106 Electrodes were manufactured for bilateral implantation in the forelimb area of the left and right
107 primary motor cortex (MI; centre coordinates: AP, +1.5; ML, ±2.8; DV, -1.0 from the bregma and
108 cortical surface) as well as the left and right dorsolateral striatum (DLS; centre coordinates: AP, +0.2;
109 ML, ±3.8; DV, -3.5 from the bregma and cortical surface) (Figure 1A). More specifically, formvar-
110 insulated tungsten wires (33 µm; California Fine Wire Co.) were arranged into four 4x5 arrays with
111 250 µm spacing in each dimension and cut to the length corresponding to the implementation site for
112 each array. Each array consisted of 16 recording channels, two reference channels and one
113 stimulation channel (not used in this study; Figure 1B). Reference wires were positioned in cell
114 sparse regions superficial to the recording sites and 200 µm silver wires were used for the ground
115 connection. The wires were attached to board-to-board-connectors (Kyocera 5602) using conducting
116 epoxy (Epotek EE 129-4). Following implantation, dental acrylic was attached to screws that served
117 as connection points for the electrode ground wire. The animals were allowed to recover for one
118 week after implementation and the extent of the lesions was confirmed by tyrosine hydroxylase
119 immunohistochemistry.

2.3.   Experimental procedure

122 Open-field recordings in a transparent cylinder (250 mm in diameter; Figure 1C) were performed and
123 the rats' behaviour was documented via digital video recordings in parallel with the
124 electrophysiological recordings (synchronised via an external pulse generator; Master-8, AMPI).
125 First, the rat was recorded for 30 min to establish baseline conditions. Second, the rat was

126 intraperitoneally injected with L-DOPA (levodopa methyl ester) and Benserazide (serine 2-(2,3,4-
127 trihydroxybenzyl) hydrazide hydrochloride racemate). Dyskinesia developed 10 to 20 min post L-
128 DOPA injection and affected the contralateral (parkinsonian) side of the body with abnormal
129 involuntary movements involving the orolingual, forelimb, and axial muscles as well as contraversive
130 rotations. The L-DOPA-induced dyskinesia reached peak severity approximately 60 min post L-
131 DOPA injection, and the recordings continued until the dyskinesia diminished spontaneously. The
132 scoring of dyskinesia was conducted off-line according to standard methods for the scoring of
133 abnormal involuntary movements.

134 ## 2.4.   Signal acquisition and preprocessing

135

136 The implant was linked to the acquisition device via a board-to-Omnetics connector adapter. LFPs
137 were recorded using a multichannel recording system (Neuralynx Inc.), filtered between 0.1-300 Hz
138 and digitised at 1017 Hz. Channels with exceptional noise level were excluded upon visual
139 inspection. On average this resulted in 14 ± 0.6 channels in the right MI, 11.3 ± 2.9 in the right DLS,
140 13.26 ± 1.3 in the left MI, and 13.1 ± 2 in the left DLS. Only experiments with high quality LFP
141 recordings and a significant duration were included in further analysis (12 experiments in total).
142 The signals were divided into 2-s epochs (Figure 1D) and analysed separately during baseline
143 (referred to as the healthy state for the intact hemisphere and the parkinsonian state for the lesioned
144 hemisphere) and the peak period of L-DOPA-induced dyskinesia (starting from approximately 60
145 min post L-DOPA injection and referred to as the dyskinetic state for the lesioned hemisphere). The
146 same was done for the lesioned hemisphere after levodopa administration. All epochs were visually
147 inspected for obvious artefacts prior to any analysis, and 50 epochs were extracted from each of the
148 recordings and each state. Furthermore, 50 Hz power-line components were removed and the LFP
149 data were then standardised for each of the electrodes by subtracting the mean and dividing by the
150 standard deviation (z-score).

151 ## 2.5.   Cross-correlation analysis of LFPs

152

153 In order to quantify synchronisation between the cortical and striatal LFPs in the time domains we
154 first calculated the cross-correlation. The cross-correlation R depends on the time lag τ and is given
155 as

156

$$R(\tau) = \begin{cases} \dfrac{1}{N-\tau} \displaystyle\sum_{n=1}^{N-\tau} x_{n+\tau} * y_n, & \tau \geq 0 \\ R(-\tau), & \tau < 0, \end{cases}$$

157

158 where x_n and y_n represent normalised signals of length N at sample n. $R(\tau)$ has the maximum value 1
159 for perfect positive correlations and the minimum value -1 for perfect negative correlations. We
160 calculated the cross-correlation separately for each epoch of each pair of LFP signals (one from the
161 MI and the other from the ipsilateral DLS) for a selected recording and state. The cross-correlation
162 functions were then averaged across each state.

163 ## 2.6.   Spectral analysis and Granger causality

164

165 The power spectra were calculated separately for each epoch of an LFP signal by applying the fast
166 Fourier transform. After subsequent normalisation (integral over selected frequency range normalised
167 to unity), the spectra were averaged across all epochs for each LFP signal.
168   In order to quantify synchronisation between the cortical and striatal LFPs in the frequency domain
169 coherence was estimated using standard Fourier analysis. For each epoch and each pair of LFP
170 signals (one from the MI and the other from the ipsilateral DLS) for a selected recording and state,
171 the magnitude-squared coherence C at frequency f was estimated to
172

$$C_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)},$$

173
174 where $P_{xy}(f)$ is the cross-power spectral density between signals x and y, and $P_{xx}(f)$ and $P_{yy}(f)$
175 correspond to the auto-power spectral densities of x and y, respectively. Pairwise coherence was
176 subsequently averaged across matching epochs.
177   Symmetric measures like the cross-correlation function in the time domain and the coherence
178 function in the spectral domain are not sufficient in studies that also aim to identify directed "causal"
179 interactions from time series data. Wiener-Granger causality (G-causality) (Granger, 1969) is a
180 powerful statistical method that provides a solution to this problem. Prediction in the G-causality is
181 based on Vector Auto Regressive (VAR) modelling and is suitable to be applied to continuous
182 signals as well, unlike to some other measures such as transfer entropy (Kaiser and Schreiber, 2002).
183 Therefore, G-causality has been widely used to detect functional connectivity in neuroscience studies
184 (Ding et al., 2006; Seth, 2010; Barret et al., 2012; Seth et al., 2015).
185   Simply put, a variable x is said to G-cause a variable y if the past of x contains information that
186 helps to predict the future of y over and above information already in the past of y (Barnett and Seth,
187 2014). The following equations show the predictability of both x and y over one another

$$x(t) = \sum_{j=1}^{p} A_{11,j} * x(t-j) + \sum_{j=1}^{p} A_{12} * y(t-j) + E_1(t)$$

$$y(t) = \sum_{j=1}^{p} A_{21,j} * x(t-j) + \sum_{j=1}^{p} A_{22} * y(t-j) + E_2(t),$$

188 where p is the model order (maximum number of lagged observations included in the model), the
189 matrix A contains the coefficients of the model , and E_1 and E_2 are residuals for each time series.
190 Thus, x (y) G-causes y (x) if the coefficients in A_{12} (A_{21}) are significantly different from zero. Spectral
191 G-causality from x to y measures the fraction of the total power at frequency f of x that is contributed
192 by y (Geweke, 1982; Ding et al., 2006; Seth, 2010). If we apply the Fourier transform to these
193 equations we get

$$\begin{pmatrix} A_{11}(f) & A_{12}(f) \\ A_{21}(f) & A_{22}(f) \end{pmatrix} * \begin{pmatrix} x(f) \\ y(f) \end{pmatrix} = \begin{pmatrix} E_1(f) \\ E_2(f) \end{pmatrix},$$

194 where matrix A is given as

$$A_{lm}(f) = \delta_{lm} - \sum_{j=1}^{p} A_{lm}(j) * e^{-i2\pi f j},$$

5

$$\delta_{lm} = \begin{cases} 0, & (l = m) \\ 1, & (l \neq m), \end{cases}$$

195 which can be rewritten in the following form

$$\begin{pmatrix} H_{11}(f) & H_{12}(f) \\ H_{21}(f) & H_{22}(f) \end{pmatrix} * \begin{pmatrix} E_1(f) \\ E_2(f) \end{pmatrix} = \begin{pmatrix} x(f) \\ y(f) \end{pmatrix},$$

196 where H is the transfer matrix that maps the amplitude and phase of the residuals to the spectral
197 representations of x and y. So, the spectral matrix S_p can be given as

$$S_p(f) = H(f) * \sum H'(f),$$

198 in which the apostrophe denotes the matrix complex conjugation and transposition, and Σ is the
199 covariance matrix of the residuals. The spectral G-causality (from j to i) is

200
$$I_{j \to i} = -\ln \left(1 - \frac{\left(\Sigma_{jj} - \frac{\Sigma_{ij}^2}{\Sigma_{ii}} \right) * |H_{ij}(f)|^2}{S_{ii}(f)} \right),$$

201 where $S_{ii}(f)$ is the power spectrum of variable i at frequency f.
202   Here we computed the spectral G-causality by employing the MVGC Multivariate Granger
203 Causality Toolbox (Barnett and Seth, 2014). We pooled data from all epochs for each state and
204 calculated the cortico-striatal interactions in terms of G-causality for each pair of LFP signals. The
205 VAR model order was estimated by using the Akaike Information Criterion (Akaike, 1974).

206 **2.7. Cross-frequency coupling**
207
208 In order to estimate cross-frequency coupling, we calculated the modulation index as described in
209 (Tort et al., 2008). The measure is defined as an adaptation of the Kullback-Leibler distance and
210 calculates how much an empirical amplitude distribution-like function over phase bins deviates from
211 the uniform distribution. Thus, it is able to detect the phase-amplitude coupling between two
212 frequency ranges of interest. The obtained values of 0 correspond to a lack of phase to amplitude
213 modulation, while larger values represent stronger phase to amplitude modulation. For our purposes,
214 time series of the phases were obtained for a lower frequency range with 2- Hz bandwidths and 1- Hz
215 steps (i.e., [1 Hz, 3 Hz], [2 Hz, 4 Hz], [3 Hz, 5 Hz], up to [11 Hz, 13 Hz]), and time series of the
216 amplitude envelope were calculated for a higher frequency range with 4- Hz bandwidths and 2- Hz
217 steps (i.e., [60 Hz, 64 Hz], [62 Hz, 66 Hz], [64 Hz, 68 Hz], up to [86 Hz, 90 Hz]). The modulation
218 index was calculated for each LFP signal and then averaged across each state.

219 **2.8. Statistical analysis**
220
221 The values in different groups were compared using the Mann-Whitney U test and a p-value < 0.05
222 was considered statistically significant.
223
224 **3. Results**
225

226 **3.1.   The dyskinetic state is related to high frequency oscillations and increased coherence**
227 **between the cortex and striatum at ~80 Hz**
228
229 We first characterised and compared the LFPs during the different states by estimating the power
230 spectral density. Overall, we were able to confirm earlier findings (Halje et al., 2012), i.e., we
231 observed an increase in power in the high beta band (20-30 Hz) when comparing the parkinsonian
232 state to the healthy state (Figure 2A and C). This power increase was present in both the MI and DLS
233 of the lesioned hemisphere, although it was more prominent in the DLS. In the time domain, we also
234 observed transient high-voltage oscillations in both the MI and DLS of the lesioned hemisphere. In
235 the dyskinetic state, these oscillations were significantly reduced, as was the power in the high beta
236 band. However, in conjunction with dyskinetic symptoms, another phenomenon in the form of a
237 strong narrowband oscillation at ~80 Hz emerged (Figure 2B). This oscillation was stable and similar
238 for different electrodes and recordings but was never observed in either the MI or DLS in the intact
239 hemisphere, as a good control case concerning confounds of behavioural states, (Figure 2C and
240 Halje et al. 2012) or in the lesioned hemisphere during baseline (i.e. parkinsonian state Figure 2A).
241   Next, we calculated the coherence between the MI and DLS in order to obtain a frequency-domain
242 measure of the relationship between these two structures. In the parkinsonian state (Figure 3A) we
243 observed an increase of coherence values for low frequencies (< 10 Hz) and the high beta band.
244 Those increased coherence values were not present in either the dyskinetic or the healthy state
245 (Figure 3B and 3C, respectively). In the dyskinetic state, a prominent peak coherence value could
246 instead be observed at 80 Hz (Figure 3C). Overall these results demonstrated the existence of strong
247 cortico-striatal synchronicity at 80 Hz during L-DOPA-induced dyskinesia in all recordings (Figure
248 3D).

249 **3.2.   Cross-correlation analysis revealed symmetric values for both pathological states but**
250 **not for the healthy state**

251 Coherence *per se* does not provide information about the direction of coupling (which population
252 leads in time) between the cortical and striatal structures. In order to study the temporal relationship
253 between recorded signals in the cortex and the striatum, we thus performed cross-correlation analysis
254 as a first step. For the lesioned hemisphere cross-correlation analysis revealed symmetric values, both
255 in the parkinsonian and the dyskinetic state (Figure 4A, left and right plot, respectively). In contrast,
256 cross-correlation analysis showed asymmetric values for the healthy state (Figure 4B). In fact we saw
257 increased values when we assumed that the striatal signals were shifted forward in time compared to
258 the opposite scenario. One explanation could be that the cortical population is driving and the striatal
259 population is lagging (however, see also Sharott et al., 2005).

260 **3.3.   The effective cortico-striatal connectivity is bidirectional for the pathological states and**
261 **has a peak at ~80 Hz in the dyskinetic state**

262 In a state where we know cortical activity causes striatal activity an analysis of directionality would
263 only confirm this fact. However, in awake behaving animals and in pathological states the main
264 directionality is generally not known and have been reported to depend on the frequency range
265 investigated and even the amount of neuromodulators present (Williams et al., 2002). It was therefore
266 relevant to conduct this analysis in the present study. We accordingly justify application of Granger
267 causality by highlighting that symmetric measures like the cross-correlation function in the time
268 domain and the coherence function in the spectral domain are not sufficient in studies that also aim to
269 identify directed "causal" interactions from time series data. Granger causality is a powerful
270 statistical method that provides a solution to this problem.

271   We evaluated G-causality in the parkinsonian state (Figure 5A), the dyskinetic state (Figure 5B)
272   and the healthy state (Figure 5C). In the parkinsonian state, we observed that effective connectivity is
273   bidirectional with an accent on striatal influence on the cortex, noticeably in the high beta band. In
274   the dyskinetic state, we also found that connectivity was bidirectional with a specifically high
275   increase at ~80 Hz, which was again more pronounced from striatum to cortex. Finally, in the healthy
276   state, we observed that G-causality was generally lower but still bidirectional, with more coherence
277   being directed from cortex to striatum. The connectivity peaked in the low gamma band, and no
278   pronounced connectivity could be seen in the high beta band or the narrow frequency band at ~80
279   Hz. Overall, it seems that effective connectivity in cortico-striatal circuits is dynamic and depends on
280   the current network state.
281   During L-DOPA-induced dyskinesia, we found that the influence of the striatum over the cortex
282   increased most prominently around the 80- Hz peak between 75-85 Hz (Mann-Whitney U test,
283    $p < 0.001$). In order to study this phenomenon in more detail, we selected all recordings with the same
284   number and position of electrodes (Figure 1B) present in both structures (n=7) and tried to access the
285   network topology in the frequency range 75-85 Hz and low frequencies for comparison (Figure 6A).
286   Whether two nodes (electrodes) interact or not was represented by a weighted graph, which indicated
287   the magnitude of each interaction given by the size of arrows. A pure correlation approach is
288   predisposed to generate false connectivity due to common inputs to the nodes (Gerstein and Aertsen,
289   1985). Granger causality can cope with this problem and we estimated the underlying cortico-striatal
290   network topology in two cases. First, we assumed that the cortex was the source (i.e., the cortex was
291   driving striatal activity) and calculated the average influence on the striatum over selected frequency
292   bands (Figure 6B). Next, we assumed that the striatum was the source and the same calculation was
293   repeated (Figure 6C). Fixed, threshold (mean ± SD of full averaged causal spectra) was used to
294   establish the existence of a link between two particular nodes. We generally observed that for the
295   frequency band between 75 and 85 Hz, the node strength (sum of connection values originating from
296   the particular node) was much higher in the case where the striatum was considered as a source. This
297   observation was also consistent across all nodes (electrodes).

298   **3.4.   The dyskinetic state is characterised by a lack of synchronicity between a small group of**
299   **neurons active at 80 Hz and neurons active at lower frequencies**

300   Information processing has to be integrated and combined across multiple spatial and temporal
301   scales, and mutually-interacting oscillations would be suitable to regulate multi-scale integration
302   (Canolty and Knight, 2010). It has been suggested that the activity of local neural populations is
303   modulated according to the global neuronal dynamics in such a way that populations oscillate and
304   synchronise at lower frequencies while smaller, local ensembles are active at higher frequencies. In
305   one variety of those interactions, the phase of low frequency oscillations modulates the amplitude of
306   high frequency oscillations. In order to further study the 80- Hz phenomenon, we thus calculated the
307   phase-amplitude coupling between low frequencies (1-13 Hz) and high gamma frequencies (60-90
308   Hz).
309   Contrary to the parkinsonian state where mutual interactions show no consistent structure (Figure
310   7A), a characteristic pattern was observed in the dyskinetic state (Figure 7B). In this state, we have
311   seen a large relative decrease in the modulation of the amplitude at 80 Hz by the phase of low
312   frequency oscillations. The findings were very robust and observed in each animal. In the healthy
313   state, a distribution similarly disorganised as that in the parkinsonian state was observed (Figure 7C).
314   Phase-amplitude coupling slightly decreased for low phase frequencies and reached their minimum at
315   around 4-5 Hz for both the MI and DLS, before increasing again towards higher phase frequencies
316   (Figure 8). Therefore, our results unexpectedly suggest a lack of coupling between the low frequency

317 activity of a presumably larger population and the synchronised activity of a presumably smaller
318 group of neurons active at 80 Hz in the case of dyskinesia.

319 **4.   Discussion**

320 The cortico-striatal network is central to the control of motor functions, as is apparent from the broad
321 range of movement disorders that are caused by dysfunctions of the circuitry. The striatum receives
322 massive cortical excitatory input and is densely innervated by dopamine from the substantia nigra
323 pars compacta. It is furthermore segregated into two functionally distinct pathways, where the
324 neurons of the direct pathway predominantly express dopamine D1 receptors and presumably
325 facilitate movements, while the indirect pathway neurons predominantly express dopamine D2
326 receptors and presumably inhibit movements (Smith et al., 2004; Bertran-Gonzalez et al., 2010).
327 Degeneration of dopaminergic neurons has been found to correlate with PD symptoms. While L-
328 DOPA replacement therapy is initially the most effective approach for treating these symptoms, PD
329 patients who receive L-DOPA treatment gradually develop dyskinesia characterised by a variety of
330 abnormal involuntary movements. The classical explanation for the triggering of L-DOPA-induced
331 dyskinesia is the imbalance between the direct and indirect pathways in the striatum. It is suggested
332 that both dopamine D1 and D2 receptors in the striatum are excessively stimulated, leading to an
333 overshoot of activity in the direct pathway and an undershoot of activity in the indirect pathway.
334 According to an alternative view, dyskinetic symptoms are instead induced by alterations in the
335 functional connectivity of neuronal networks in several parts of the cortico-basal ganglia-thalamic
336 loop, leading to pathophysiological activity patterns at a systems level (Richter et al., 2013).
337 Although over the last few years there has been an increased research effort addressing this issue, the
338 neural mechanisms underlying L-DOPA-induced dyskinesia in PD are still far from clear.
339   While the cortex sends direct projections to the striatum, the striatum can in turn affect the cortex
340 only indirectly through other BG nuclei and thalamus. Cortico-striatal interactions have been studied
341 at the single neurone level for many years (Oosschot, 1996; Kincaid et al., 1998; Ramanathan et al.,
342 2002; Zheng and Wilson, 2002). However, the underlying mechanisms by which the activities of
343 large populations of cortical and striatal neurones are coordinated in healthy and pathological states
344 are still unclear (Sharott et al., 2005). Here, we simultaneously recorded LFPs (i.e., population
345 signals) in the cortex and striatum in order to study L-DOPA-induced dyskinesia in hemi-
346 parkinsonian rats. We used G-causality to study the direction of activity in the cortico-striatal
347 network, and provide new insights into the network's functional organisation in terms of directed
348 coherence. So far, only three studies have investigated directed interactions in the cortico-striatal loop
349 of rats: directed measures were used to study interactions in the basal ganglia structures of healthy
350 anesthetised rats (Sharott et al., 2005), healthy freely behaving rats (Nakhnikian et al., 2014) and in a
351 rat model of epilepsy (David et al., 2008). Thus, for the first time, directed measures are here
352 employed to study the pathological states of PD and L-DOPA-induced dyskinesia in rats. We found
353 that effective connectivity is generally bidirectional for both pathological states, with a peak at ~80
354 Hz in both directions in the dyskinetic state. Somewhat unexpectedly, this peak was larger in the
355 direction from the striatum to the cortex than vice versa (notably similar results have however
356 previously been reported for high-frequency oscillations following levodopa treatment between
357 cortex and STN; Williams et al., 2002). This indicates that, in the dyskinetic state, the coupling in the
358 striato-thalamic loop via other BG nuclei is rather strong at ~80 Hz. Further experiments will be
359 necessary to address the role of thalamic inputs to the striatum and generally the cortex-basal ganglia-
360 thalamic loop in this case.
361   In order to further investigate the 80- Hz phenomenon, we analysed phase-amplitude coupling
362 between low and high frequencies before and after L-DOPA administration. How neural activity is
363 coordinated between different spatio-temporal scales is one of the most important questions in

364 neuroscience. It has been suggested that slow oscillations are necessary for network synchronisation
365 over large distances, whereas faster gamma rhythms serve to synchronise assemblies that encompass
366 neighbouring cells (Jensen and Colgin, 2007; Aru et al., 2015). Therefore, gamma oscillations can
367 appear at a particular phase of an integration process of lower frequencies. Phase-amplitude coupling
368 has been investigated across different brain structures and is considered to have profound
369 implications for normal brain functions (Canolty et al., 2006; Jensen and Colgin, 2007; Tort et al.,
370 2008; Cohen et al., 2009; Tort et al., 2009; Hemptinne et al., 2013). Here, we report for the first time
371 characteristic patterns for phase-amplitude coupling in the dyskinetic state in both the cortex and the
372 striatum. We have seen a large relative decrease in the modulation of the amplitude at ~80 Hz by the
373 phase of low frequencies (up to ~10 Hz). Therefore, our results unexpectedly suggest a lack of
374 coupling between the low frequency activity of a presumably larger population and the synchronised
375 activity of a presumably smaller and potentially partially overlapping, group of neurons active at 80
376 Hz. Recently, another study reported decreased coupling between the phase of the beta rhythms and
377 the amplitude of broadband activity in the primary motor cortex upon acute therapeutic deep brain
378 stimulation that correlates with a reduction in parkinsonian motor signs (de Hemptinne et al., 2015).
379 Further experimental and modelling studies could reveal the underlying mechanism of the observed
380 80- Hz decoupling phenomena.

381 Acknowledgments

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535 **Figure legends**
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537 **Figure 1: Data acquisition.** (A) Schematic illustration of the positioning of electrodes relative to the
538 bregma. Coronal plane indicating vertical positions for the cortex (left) and the striatum (right)
539 together with AP positions. Electrodes were implanted bilaterally. (B) The electrodes were arranged
540 over four structures: the left primary motor cortex, the right primary motor cortex, the left
541 sensorimotor striatum and the right sensorimotor striatum. Each array consisted of 16 recording
542 channels, two reference channels and one stimulation channel (not used in this study). (C) An
543 example of the open-field recordings. (D) Extracted LFPs from the intact and lesioned hemisphere
544 that represent one epoch for a duration of 2s.

545 **Figure 2: Power spectral densities for the lesioned and intact hemisphere.** (A) Power in the
546 lesioned hemisphere before levodopa administration has shown an increase in higher beta frequency
547 band. Traces for single electrodes and their average value (bold red line for the cortex and bold blue
548 line in the striatum) are illustrated. (B) Power in lesioned hemisphere after levodopa administration
549 has shown an increase in higher gamma frequency band (around 80 Hz). (C) Power in the intact
550 hemisphere has not shown an increase for any frequency band.

551 **Figure 3: Coherence spectra analysis.** (A) Coherence has increased for low frequencies (< 10 Hz)
552 and for high beta frequencies in the case of the lesioned right hemisphere. (B) Coherence has peak
553 values at high gamma frequencies (around 80 Hz). (C) In the case of the intact left hemisphere, we
554 did not see any prominent increase in the coherence. (D) Average values of coherences for the intact
555 and lesioned hemispheres.

556 **Figure 4: Cross-correlation analysis of the lesioned and intact hemispheres.** (A) Average
557 correlation values before (the left panel) and after (the right panel) levodopa administration in the
558 case of the lesioned hemisphere. (B) Average correlation values in the case of the intact hemisphere.
559 Arrows are pointing to observed asymmetry when the striatal signals were shifted forward in time
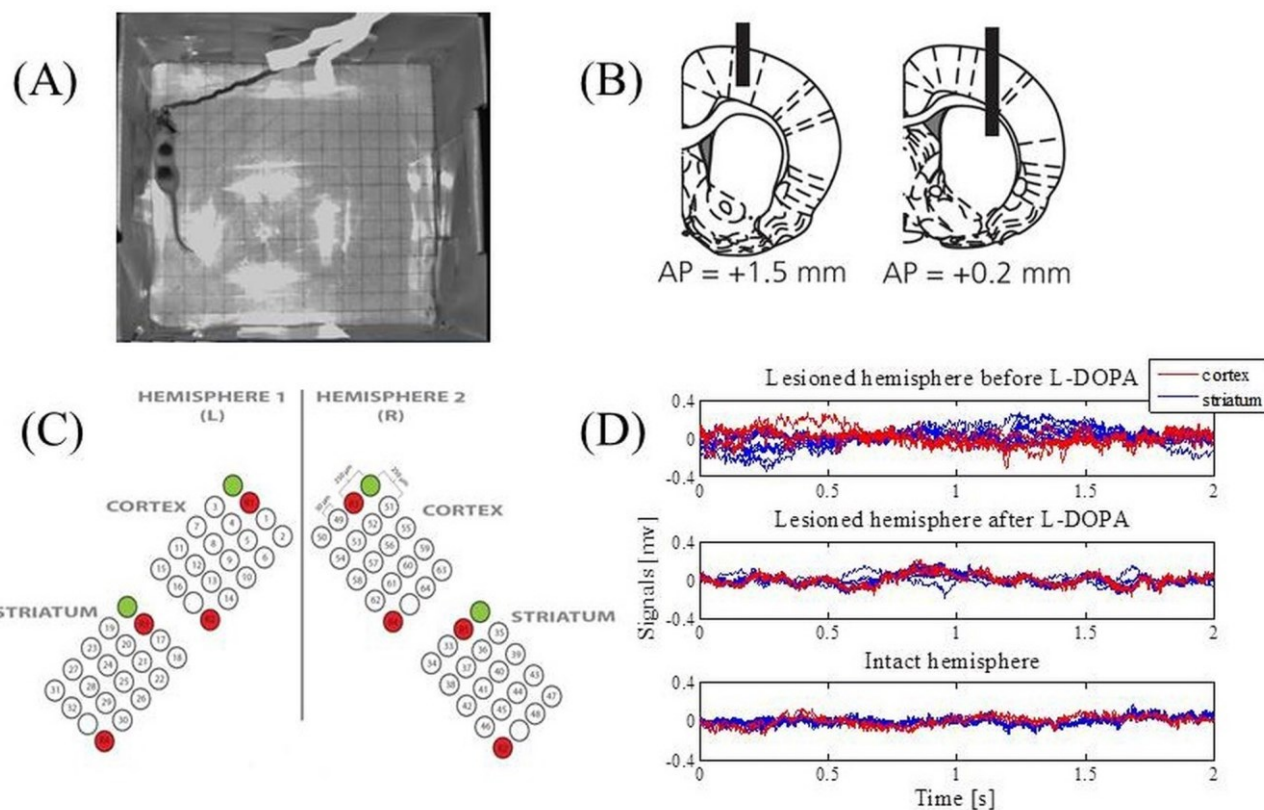560 compared to the opposite scenario.

561 **Figure 5: Causality spectra analysis.** (A) Granger causality for the lesioned hemisphere before
562 levodopa administration. Left panel illustrates GC values when the cortex is assumed to be a source
563 (driving striatal activity) and represents the traces for all pairs of the cortico-striatal electrodes.
564 Middle panel shows GC values in the case where the striatum is assumed to be a source. (B) The
565 same analysis as in (A) for the lesioned hemisphere after levodopa administration. (C) Granger
566 causality for the intact hemisphere before levodopa administration (the same analysis as in (A)).

567 **Figure 6: Reconstructing cortico-striatal network topology for frequency ranges 75-85 Hz and**
568 **low frequencies.** (A) Directed, weighted architectures of the cortico-striatal network. (B) Cumulative
569 strength of directed connections in the case where the cortex is assumed to be the source. The x-axis
570 corresponds to the cortical electrodes and how they influence the striatal electrodes (bars with
571 different colours). (C) The same as in (A) in the case where the striatum is assumed to be the source.
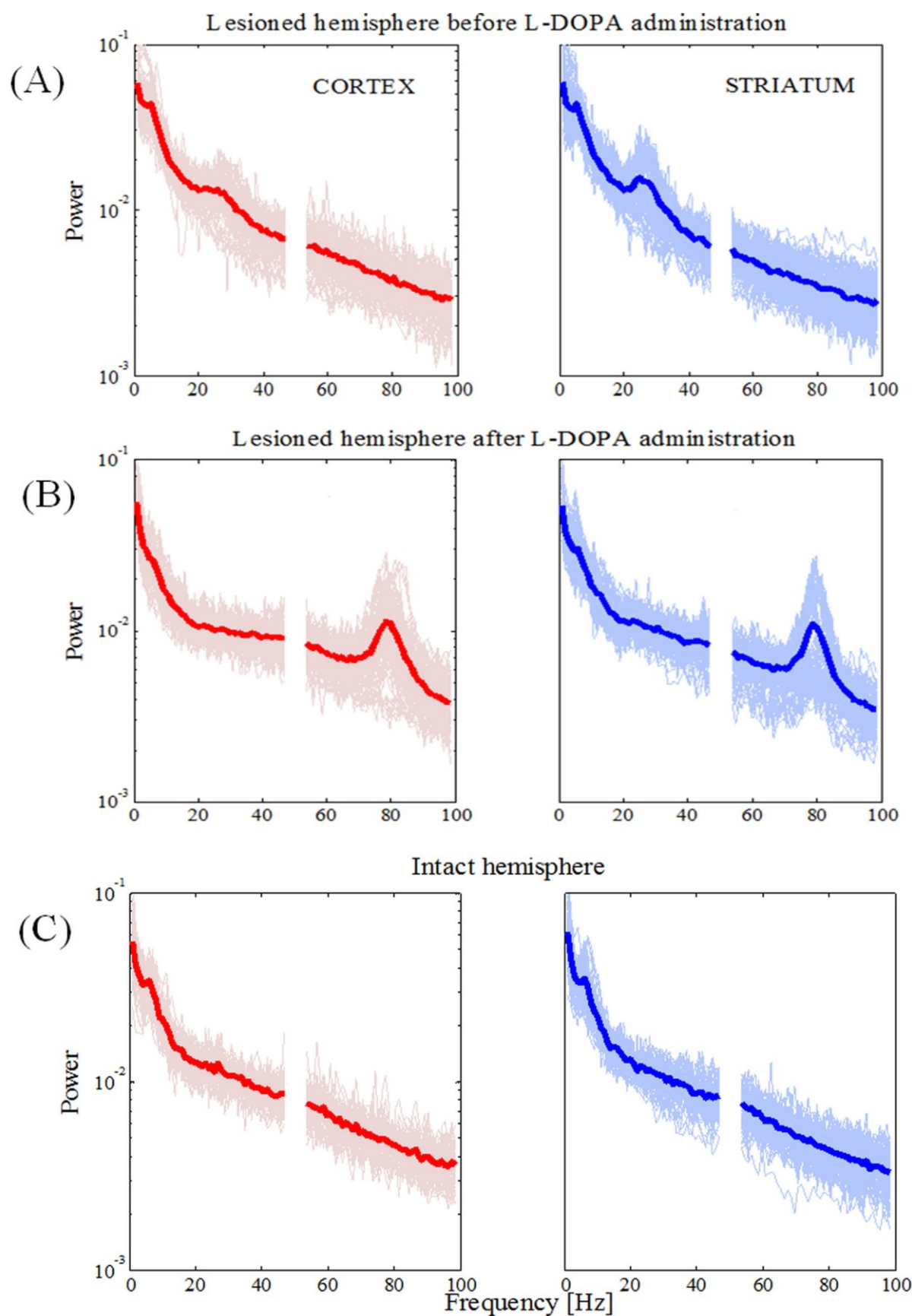
572 **Figure 7: Amplitude modulation of fast rhythms by the theta phase.** (A) Average phase-
573 amplitude coupling values for the lesioned hemisphere before levodopa administration. Values were
574 normalised separately for each column by dividing all values with the maximum value across it. (B)
575 The same analysis as in (B) for the lesioned hemisphere after levodopa administration. (C) Phase-
576 amplitude coupling values for the intact hemisphere before levodopa administration (the same
577 analysis as in (A)).

578 **Figure 8: Statistics of coupling values between phase of low frequencies and amplitude at 80 Hz**
579 **across all electrodes.** Coupling values were normalised separately for each column by dividing all
580 values with the maximum value across it.

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