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1           **Systems level neurophysiological state characteristics for drug**  
2           **evaluation in an animal model of levodopa-induced dyskinesia**

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18  
19          Running Head: Systems level brain states  
20

21          **Abstract**

22          **Disorders affecting the central nervous system have proven particularly hard to treat and**  
23          **disappointingly few novel therapies have reached the clinics in the last decades. A better**  
24          **understanding of the physiological processes in the brain underlying various symptoms could**  
25          **therefore greatly improve the rate of progress in this field. We here show how systems level**  
26          **descriptions of different brain states reliably can be obtained through a newly developed method**  
27          **based on large-scale recordings in distributed neural networks encompassing several different**  
28          **brain structures. Using this technology we characterize the neurophysiological states associated**  
29          **with parkinsonism and levodopa-induced dyskinesia in a rodent model of Parkinson's disease**  
30          **together with pharmacological interventions aimed at reducing dyskinetic symptoms. Our results**  
31          **show that the obtained electrophysiological data add significant information to conventional**  
32          **behavioral evaluations and hereby elucidates the underlying effects of treatments in greater**  
33          **detail. Taken together, these results potentially open up for studies of neurophysiological**  
34          **mechanisms underlying symptoms in a wide range of neurologic and psychiatric conditions that**  
35          **until now have been very hard to investigate in animal models of disease.**

36          Keywords:       Systems neurophysiology, Parkinson's disease, Levodopa

37 Diseases affecting the central nervous system (CNS) are a rapidly growing concern that puts a great  
38 economic burden on society (Olesen et al., 2012) and cause major suffering for afflicted individuals  
39 and their families. Unfortunately, these diseases have also proven particularly hard to treat. In spite  
40 of the last decades' impressive advances in the field of molecular biology few novel therapeutic  
41 options have reached the clinics, notwithstanding recent corporate and regulatory efforts to break  
42 the trend (Graul, 2008). A major challenge in the development of new CNS therapies is the limited  
43 understanding of the basic processes governing normal brain functions, as well as the  
44 pathophysiological changes that ultimately cause the symptoms. For these reasons, the  
45 methodological approaches in drug discovery and development have often been limited to rather  
46 simplistic experimental read-outs. In pre-clinical studies, the evaluation of novel compounds typically  
47 involve characterization of changes in animal behavior in combination with *post mortem* tissue  
48 analyses with little information about the ongoing CNS changes causing the actual symptoms or the  
49 underlying physiological effects of an intervention. To make matters worse, several neurological and  
50 psychiatric conditions are not directly associated with overt changes in behavior, which makes them  
51 even more challenging to model in experimental animals. To gain an insight into such internal CNS  
52 processes, chronic electrophysiological recordings are a particularly promising approach which can  
53 give real-time access to neurophysiological activity patterns associated with physiological processes  
54 during natural conditions (Gervasoni et al., 2004; Lehew and Nicolelis, 2008). Building on this  
55 technology, large-scale sampling of neurophysiological signals from diverse brain regions could allow  
56 for the characterization of brain states that explains the difference between healthy and diseased  
57 states, as well as how these states are altered by drugs aimed at treating the disease. Though clearly  
58 a great experimental challenge, such detailed information on neurophysiological states obtained in  
59 valid animal models of CNS disease could significantly help to increase the rate of progress in  
60 research aimed towards new treatments for CNS disorders.

61 In fact, even recordings performed in single locations of the brain, such as those that have  
62 been obtained in Parkinson's disease (PD) patients implanted with electrodes in the subthalamic  
63 nucleus (STN) and the internal globus pallidus for the purpose of therapeutic deep-brain stimulation,  
64 have provided novel insights into pathological processes potentially underlying symptoms in this  
65 disease (Brown et al., 2001; Lalo et al., 2008; Brücke et al., 2012). In similar experiments on animals  
66 implanted with multiple electrodes, additional neurophysiological features have been identified that  
67 are thought to be associated with motor symptoms on and off medication. In particular, the  
68 parkinsonian hypokinetic state has been linked to an increased cell firing rate in the STN (Albin et al.,  
69 1989; Bergman et al., 1994; Levy et al., 2000), synchronized cell-firing in cortex (Goldberg et al.,  
70 2002), striatum (Goldberg et al., 2004), globus pallidus (GP; Nini et al., 1995) and STN (Bergman et al.,  
71 1994), as well as abnormally strong local field potential (LFP) oscillations in the beta band (~10-35 Hz)  
72 present across the entire cortico-basal ganglia network (Costa et al., 2006; Hammond et al., 2007;  
73 Fuentes et al., 2010; Stein and Bar-Gad, 2013). Dopamine replacement therapy alleviating  
74 parkinsonian symptoms has been shown to concomitantly suppress these aberrant activity patterns  
75 (Kreiss et al., 1997; Costa et al., 2006; Gilmour et al., 2011; Santana et al., 2014). Unfortunately,  
76 following long-term dopamine replacement therapy the therapeutic window frequently narrows to  
77 such an extent that treated subjects rapidly transition from parkinsonism to dyskinesia as the drug  
78 plasma concentration rises. In this situation, oscillatory activity in other parts of the LFP frequency  
79 spectrum has been reported to be markedly altered following levodopa administration in patients  
80 suffering from involuntary dyskinetic movements as a medication side-effect. Low-frequency  
81 oscillations in the theta range (4-10 Hz), for example, have attracted particular attention over the  
82 years (Alonso-Frech et al., 2006; Alegre et al., 2012; Alam et al., 2013), and more recently,  
83 characteristic gamma-oscillations (at ~ 80 Hz) in a rat model of PD were found to be strongly  
84 associated with levodopa-induced dyskinesia (Halje et al., 2012; Dupre et al., 2013). Equivalent high-



85 frequency oscillations have also been reported in STN-recordings in Parkinson patients, sometimes  
86 referred to as finely tuned high-gamma, but have in these studies primarily been thought to reflect  
87 the prokinetic state associated with the therapeutic effect of the medication (Cassidy et al., 2002;  
88 Brown, 2003; Sharott et al., 2005; Cagnan et al., 2014).

89 In order to clarify the association between different aberrant neuronal activity patterns and  
90 the expression of motor symptoms and to obtain a more comprehensive description of the  
91 neurophysiological state on a systems level, we have here made use of a technology developed in  
92 our lab that lets us perform large-scale multi-structure recordings in awake behaving rats (Fig. 1A, B;  
93 Ivica et al., 2014) in the most commonly used model of PD (the 6-OHDA lesioned rat; Nadjjar et al.,  
94 2009). Applying this technology, we have investigated: 1) the neurophysiological state of the cortico-  
95 basal ganglia-thalamic circuit that is associated with parkinsonism, 2) the neurophysiological state  
96 that is associated with levodopa-induced dyskinesia and 3) the behavioral and electrophysiological  
97 effects of experimental and clinically used drug interventions aimed at alleviating dyskinetic  
98 symptoms.

99

## 100 **METHODS**

101

### 102 *Animals*

103 Four adult female Sprague Dawley rats (230–250 g) were used in the study. The animals were  
104 kept on a 12 h light cycle and received food and water *ad libitum*. All experiments were approved in  
105 advance by the Malmö/Lund ethical committee of animal experiments.

106

107 *6-Hydroxydopamine lesions and levodopa priming*

108 Rats were anesthetized with fentanyl/medetomidine (0.3/0.3 mg/kg, intra-peritoneal (i.p.)  
109 injection) and fixed in a stereotaxic frame. The animals received two injections of 6-  
110 hydroxydopamine (6-OHDA) hydrochloride (3.0 µg/µl free base dissolved in 0.02% ascorbate saline)  
111 into the medial forebrain bundle of the right hemisphere at the following coordinates from bregma  
112 and cortical surface (Lundblad et al., 2002): Injection site (I), 2.5 µl: tooth bar (TB): -2.3;  
113 anteroposterior (AP): -4.4; mediolateral (ML): -1.2; and dorsoventral (DV): -7.8; Injection site (II), 2.0  
114 µl: TB: +3.4; AP: -4.0; ML: -0.8; DV: -8.0. Moderate motor impairments including asymmetric posture  
115 and gait and reduced contralateral forelimb dexterity were generally apparent two weeks after  
116 lesioning. One week after lesioning animals were given daily doses of levodopa (6mg/kg) for two  
117 weeks. After two weeks of treatment, the animals that showed moderate to high levels of dyskinetic  
118 symptoms after having been challenged with 12mg/kg levodopa were implanted and included in the  
119 study.

120

121 *Implantation surgery*

122 Implantations were performed under fentanyl/medetomidine anesthesia (0.3/0.3 mg/kg, i.p.) at  
123 least three weeks after 6-OHDA lesions. Microwire electrodes were implanted in both hemispheres.  
124 The eight regions targeted in each hemisphere were: Rostral Forelimb Area (RFA - a rodent  
125 supplementary motor area), primary motor cortex (MI), dorsolateral striatum (DLS), dorsomedial  
126 striatum (DMS), globus pallidus (GP), ventrolateral/ventroanterior nuclei of the thalamus (VL/VA;  
127 projecting to motor cortex), subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr).  
128 Center coordinates in relation to bregma and the cortical surface were in the following structures:

129 RFA, AP: +3.75, ML:  $\pm$ 2.0, DV: -1 (Neafsey and Sievert, 1982); MI, AP: +1.5, ML:  $\pm$ 2.8, DV: -1.0  
130 (Gioanni and Lamarche, 1985); the DLS, AP: +0.2, ML:  $\pm$ 3.8, DV: -4 and DMS, AP: +0.2, ML:  $\pm$ 2.8, DV: -  
131 4, (West *et al*, 1990); GP, AP: -1.0, ML:  $\pm$ 3, DV: -5.5-7.2 (Chen *et al*, 2011); VL/VA, AP: -2.6, ML:  $\pm$ 1.75,  
132 DV: -6.5 (Paxinos and Watson, 2007); STN, AP: -3.5, ML:  $\pm$ 2.3, DV: -7.5-8.2 (Tai *et al*, 2003); SNr, AP: -  
133 5.4, ML:  $\pm$ 2.4, DV: -7.8-8.8 (Wang *et al*, 2010). The implant was fixated with dental acrylic, which was  
134 attached to screws in the skull. After surgery, the anesthesia was reversed by atipamezole  
135 hydrochloride (5 mg/kg, i.p.) and buprenorphine (0.5 mg/kg, subcutaneous (s.c.) injection) was  
136 administered as postoperative analgesic. The animals were allowed to recover for one week after  
137 surgery before testing commenced.

138

### 139 *Experimental procedure*

140 During recording sessions animals were placed in a transparent cylinder (250 mm in diameter),  
141 and their behavior was recorded with digital video in parallel with the electrophysiological recordings  
142 (synchronized via an external pulse generator; Master-8, AMPI). The same paradigm was used in  
143 each experiment: First, the rat was recorded for  $\sim$ 30 min to establish baseline conditions. Second,  
144 the rat received an i.p. injection with 12mg/kg levodopa (levodopa methyl ester hydrochloride) and  
145 12mg/kg benzerazide (serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride). The time point of  
146 this injection marks the beginning of the experimental timeline, i.e.,  $t = 0$  min. Dyskinesia developed  
147 10 to 20 min post-levodopa injection and reached its peak severity  $\sim$ 60 min post-levodopa injection.  
148 In experiments not involving further pharmacological intervention, the recordings continued until the  
149 dyskinesia diminished spontaneously ( $\sim$ 2-3 h after dyskinesia onset). Experiments involving  
150 additional drug treatment are described below.

151

152 *Pharmacology*

153       Following levodopa injection and the manifestation of dyskinesia a number of pharmacological  
154 substances were evaluated with respect to their anti-dyskinetic effects. Injection time points were  
155 chosen such that each drug would exhibit its therapeutic effect during the time of peak dyskinesia.  
156 Once the pharmacological effect of the serotonin 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT (1 mg/kg & 0.4  
157 mg/kg i.p., t = ~60 min) had been established the specificity of the intervention was verified by  
158 injection of the 5-HT<sub>1A</sub> antagonist WAY-100,635 (0.5 mg/kg & 0.4 mg/kg i.p., t = ~100 min), which  
159 effectively reversed the effect of 8-OH-DPAT. The neurophysiological and behavioral effects of the  
160 clinically used anti-dyskinetic drugs amantadine hydrochloride (a NMDA receptor antagonist,  
161 50 mg/kg & 50 mg/kg i.p., t = ~60 min), diazepam (a positive allosteric modulator at GABA<sub>A</sub>  
162 receptors, 5 mg/kg i.p., t = ~60 min) and levetiracetam (a pre-synaptic calcium channel inhibitor,  
163 80 mg/kg & 120 mg/kg i.p., t = ~30 min) were also evaluated. All drugs used in this study were  
164 attained from Sigma Aldrich, Sweden and doses were chosen according to previously published  
165 studies (Kannari et al. 2001; Peixoto et al. 2005; Tronci et al. 2014; Coppola et al. 2010).

166

167 *Assessment of dyskinesia severity*

168       The scoring of dyskinesia was performed offline, using an adapted version of the scoring  
169 methods of abnormal involuntary movements (AIMs) described by Cenci and colleagues (Lundblad et  
170 al., 2002). In summary, three different types of AIMs (orolingual, forelimb, and axial dyskinesia) were  
171 scored with respect to their severity for monitoring periods of 1 min with 5-minute intervals. In  
172 addition, contraversive rotations with respect to the lesioned side were also quantified, as rotational  
173 behavior is correlated with general dyskinetic symptoms in this model (Breger et al., 2013). Forelimb  
174 and axial AIMs and rotations were rated on a scale ranging from zero to three where zero equals no

175 dyskinesia and three equals continuous dyskinesia. Orolingual dyskinesia was less clearly detectable  
176 in the videos and was therefore scored as one when detected and zero otherwise. The measures for  
177 all AIMs and rotations were normalized per category [0, 1] and then added together to produce a  
178 total AIM value [0, 4] for each assessed 1-minute period. This combined value was taken to indicate  
179 the overall severity of the dyskinesia at any given time.

180

### 181 *Recording electrodes*

182 For details on electrode design see (Ivica *et al*, 2014). In brief, formvar-insulated tungsten  
183 wires (33  $\mu\text{m}$ ) were arranged into sixteen groups of arrays (eight per hemisphere; Fig. 1A) with  
184 250  $\mu\text{m}$  wire spacing in each horizontal dimension and fixed to the length corresponding to the  
185 implantation site for each group. Each array consisted of a minimum of five recording channels and  
186 one reference channel. All wires were connected to a custom made printed circuit board and linked  
187 via connectors/adaptors to the pre-amplifier of the acquisition system. A 200  $\mu\text{m}$  thick silver wire  
188 was attached to the skull screws and used as a ground connection from the animal to the recording  
189 system.

190

### 191 *Signal acquisition*

192 Neuronal activity was recorded with the Neuralynx multichannel recording system using a unity gain  
193 pre-amplifier (HS-36, Neuralynx, MT, USA). LFP signals were filtered between 0.1 and 300 Hz, and  
194 were digitized at 1017 Hz. Unit activities were filtered between 600 and 9000 Hz, and were digitized  
195 at 32 kHz. Thresholds for storage of spiking events in each channel was automatically set to three SDs  
196 of the unfiltered signal.

197

198 *Spike sorting*

199           Action potentials were sorted manually into unit clusters using Offline Sorter (Plexon Inc.).  
200   Waveform features used for separating the units were, e.g., valley and peak amplitude or the first  
201   three principal components of all the 32-element vectors defining the sampled waveforms for a given  
202   dataset. A cluster was classified as single unit (SU) when less than 0.1 % of the spikes in a defined  
203   cluster occurred within the refractory period (set to 1.6 ms), and as multiunit otherwise (Harris *et al*,  
204   2000).

205

206 *Frequency analysis of LFPs*

207           To emphasize local sources of the measured electrical potential (and to minimize effects of  
208   the choice of amplifier reference), bipolar LFP time series were computed offline from all unique  
209   pairs of electrodes from the same structure. For each of these time series, time-frequency  
210   spectrograms were calculated over the entire frequency range with a multitaper method (Pesaran,  
211   2008) (50%-overlapping 8-s windows, time-bandwidth product 4, 7 tapers) implemented in Chronux  
212   2.0 (Mitra and Bokil, 2008). Power line noise ( $50 \pm 2$  Hz and 1st harmonic at  $100 \pm 2$  Hz) was removed  
213   from the power spectral densities (PSDs). To better identify oscillations in certain part of the  
214   frequency spectrum, each individual power spectrum was normalized to the pink noise background.  
215   That is, the noise background was estimated once for each 8-s window and for each bipolar channel  
216   separately. Due to the complexity of the data it was not possible to manually pick enough frequency  
217   bands with pure pink noise in all structures and conditions to get unbiased estimates of the noise  
218   background. Instead we divided the whole frequency axis (from 1 to 200 Hz) into 20 logarithmically  
219   spaced bands (1-1.3, 1.3-1.7, ..., 151.3-200) and used the median power of each band for the fitting

220 of the pink noise power curve  $S(f)=b/f^a$ . The pink noise normalization allowed us to describe  
221 deviations from the pink noise floor in terms of the unit  $\text{dB}_{\text{pink}}$ , defined as

$$222 \quad S_{\text{dB}(\text{pink})}(f) = 10 \log_{10} \frac{S(f)}{S_{\text{pink}}(f)}$$

223 where  $S(f)$  and  $S_{\text{pink}}(f)$  have the dimension power per frequency (i.e.  $\text{V}^2/\text{Hz}$ ) and  $S_{\text{dB}(\text{pink})}$  is expressed  
224 in the dimensionless unit  $\text{dB}_{\text{pink}}$ .

225 As a final step, an average spectrogram was calculated for each structure, based on the pink noise  
226 corrected spectrograms for each individual local bipolar LFP time series.

227 In Figs. 2-4 and 7, the obtained spectrograms were further averaged over time for each behaviorally  
228 classified state in order to obtain average spectra for the different states.

229

### 230 *Systems level neurophysiological states*

231 In order to visualize and identify systems level neurophysiological states we relied on the average,  
232 pink-noise normalized spectrograms that were calculated for each structure in each recording  
233 session, as described above. Each such spectrogram consists of a series of individual spectra  
234 reflecting the frequency content between 2 and 120 Hz with 0.5 Hz resolution in that structure  
235 during an 8-s window. The electrophysiological samples (made up of 8-s recording segments)  
236 included from each state were selected from within a time interval during a steady state, as defined  
237 by behavioral criteria (dyskinesia score; see Table 1A for a summary of the number of samples  
238 obtained in each animal and state). Samples were defined, such that one sample contained the  
239 concatenated spectra from all structures for one such 8-s window. Thus, for one recording session  
240 the number of samples  $n$  becomes equal to the number of 8-s windows, and the number of variables  
241  $p$  becomes equal to the number of frequency bins (2 x the frequency range) times the number of  
242 structures. Pooling all recording sessions in one animal results in a number of samples  $n_{\text{pooled}}$  equal to

243 the number of 8-s windows in all these recording sessions, while the number of variables  $p$  stays the  
 244 same.

245 A first aim was to obtain a two-dimensional visualization of the samples describing the spectral  
 246 differences to the control state along the axes <Control vs. PD > and <Control vs. Dyskinesia>. The  
 247 following steps have been taken: The data is normalized such that the mean and standard deviation  
 248 over each variable become zero and one, respectively. The samples normalized in this way are  
 249 denoted by  $\mathbf{s}_i$ ,  $i = 1, \dots, n$ . Next, the origin of the coordinate system is shifted to become equal to the  
 250 cluster center of the control state, i.e., the mean over all samples belonging to the control state,  
 251  $\bar{\mathbf{s}}_{control}$ , is subtracted from each sample:  $\tilde{\mathbf{s}}_i = \mathbf{s}_i - \bar{\mathbf{s}}_{control}$ . By this, each shifted sample  $\tilde{\mathbf{s}}_i$  describe  
 252 the spectral differences to the mean control state. In order to obtain a two-dimensional  
 253 representation of the data, an x- and y-axis are defined to point from the cluster center of the control  
 254 state, i.e., the origin of the shifted coordinate system, to the PD and dyskinesia cluster center,  $\bar{\mathbf{s}}_{PD}$   
 255 and  $\bar{\mathbf{s}}_{dys}$ , respectively. However, for the y-axis its projection on an axis orthogonal to the x-axis will  
 256 be shown. The projection onto the x- and y-axis is furthermore normalized such that the PD and  
 257 dyskinesia cluster centers will have an x- and y-value equal to zero and one, respectively.  
 258 Mathematically, the value on the x-axis for the shifted sample  $\tilde{\mathbf{s}}_i$  can be obtained from

259 
$$x_i = \tilde{\mathbf{s}}_i \cdot \frac{\bar{\mathbf{s}}_{PD}}{\|\bar{\mathbf{s}}_{PD}\|_2^2} ,$$

260 and the value on the orthogonal y-axis can be obtained from

261 
$$y_i^{ortho} = \tilde{\mathbf{s}}_i \cdot \frac{\bar{\mathbf{s}}_{dys}^{ortho}}{\|\bar{\mathbf{s}}_{dys}^{ortho}\|_2^2} \text{ with}$$

262 
$$\bar{\mathbf{s}}_{dys}^{ortho} = \bar{\mathbf{s}}_{dys} - \frac{\bar{\mathbf{s}}_{PD} \cdot \bar{\mathbf{s}}_{dys}}{\|\bar{\mathbf{s}}_{PD}\|_2^2} \bar{\mathbf{s}}_{PD} ,$$



263 where  $\cdot$  denotes the dot-product, and  $\| \cdot \|_2$  denotes the L2-norm. Figures 3A (and 9D) show the  
 264 results of this visualization. Furthermore, in Fig. 3B the vectors  $\bar{\tilde{\mathbf{s}}}_{PD}$  and  $\bar{\tilde{\mathbf{s}}}_{dys}^{ortho}$  are illustrated, while  
 265 Fig. 7D illustrates the eight structure components that makes up the vector  $\bar{\tilde{\mathbf{s}}}_{8-OH-DPAT}$  (i.e., the  
 266 cluster center of the 8-OH-DPAT treatment state in the shifted coordinate system). Finally, for Figs.  
 267 3C,D and 9 the above analysis has been performed for each structure separately (i.e., without  
 268 concatenating the spectra from all structures), and the distributions of  $x_i$  for the control and the PD  
 269 cluster are shown in Fig. 3C, while Fig. 3D shows the distributions of  $y$  for the control and the  
 270 dyskinetic cluster. Note that we chose to not use  $y_i^{ortho}$  in Fig. 3D, but rather the more intuitive  
 271 distribution defined by

$$272 \quad y_i = \tilde{\mathbf{s}}_i \cdot \frac{\bar{\tilde{\mathbf{s}}}_{dys}}{\|\bar{\tilde{\mathbf{s}}}_{dys}\|_2^2},$$

273 which exclusively depends on the difference between the control and the dyskinetic states.

274

### 275 *Quantification of state separability*

276 To quantify the separation between states in terms of classification performance it was  
 277 necessary to first reduce the dimensionality of the data using principal component analysis (PCA). We  
 278 used the singular value decomposition PCA algorithm with variance weighting (Matlab). Generally  
 279 speaking, given a dataset with  $n$  samples and  $p$  variables, all samples can be represented in a  $p$ -  
 280 dimensional coordinate system. PCA can be thought of performing a high-dimensional rotation of  
 281 this coordinate system according to

$$282 \quad \mathbf{T} = \mathbf{S}\mathbf{W},$$

283 where  $\mathbf{S}$  and  $\mathbf{T}$  are  $n \times p$  matrices representing the samples in the original and the rotated coordinate  
284 system, respectively, and  $\mathbf{W}$  is the  $p \times p$  rotation or weight matrix. In PCA, the weight matrix  $\mathbf{W}$  is  
285 constructed such that the  $p$  variables in the new coordinate system are uncorrelated over the  
286 dataset. Furthermore, the first variable in this coordinate system, i.e., the first principal component,  
287 will by definition capture the most variance of the dataset, the second principal component will  
288 capture the second-most variance in a perpendicular dimension to the first, and so forth. Thus,  
289 would one only keep the first two principal components, one would automatically obtain a  
290 representation of the dataset in the two-dimensional plane in which the data is most spread out,  
291 allowing a convenient visualization of the high-dimensional data and, e.g., the identification of  
292 clusters. For example, in Figure 9B the first three principal components, obtained from applying PCA  
293 to the pooled data in one animal, are shown. Such a visualization can complement visualizations  
294 based on the method described in the previous section, where differences between selected states  
295 are emphasized by projection onto the state difference vector.

296 After dimensionality reduction with PCA a Gaussian mixture model was fitted to the data (Matlab  
297 `fitgmdist` function). The number of Gaussian components in the model was set to be equal to the  
298 number of experimental conditions (e.g. control, control+levodopa, PD and Dyskinesia) and the  
299 starting conditions for the optimization (means, covariances and mixing proportions) were calculated  
300 by assigning samples from the same experimental condition to one Gaussian component. The  
301 performance of the model was then estimated by assigning each sample to the Gaussian component  
302 with the largest posterior probability (weighted by the component probability) and calculating the  
303 average number of correct classifications. Generally, the classification performance improved as  
304 more principal components were added, until a plateau was reached (c.f. Fig. 10). Chance level of  
305 correctly assigning a data point to one of  $n$  states corresponds to  $p = 1/n$ . As a compromise

306 between the risk of over-compressing the data and the cost of performing heavy calculations we  
307 settled on consistently using 30 principal components for all quantifications of classification  
308 performance.

309 To complement classification performance as a measure of state separability, classical frequentist  
310 hypothesis tests were performed to test for significant differences between states. For each possible  
311 state pair the data was projected orthogonally onto a line going through the means of the two  
312 distributions, i.e. for example the line defined by the vector  $\bar{\mathbf{s}}_{PD} - \bar{\mathbf{s}}_{dys}$ . The distributions (now 1-  
313 dimensional) were then tested with a standard Wilcoxon rank sum test corrected for multiple  
314 comparisons (Bonferroni).

315

#### 316 *Tissue preparation and immunostaining*

317 Animals were anesthetized with a lethal dose of sodium pentobarbital (100mg/kg) and heads  
318 were fixated in 4% paraformaldehyde. Brains were removed, post fixed in paraformaldehyde  
319 overnight and then transferred to 30% sucrose PBS (phosphate-buffered saline) solution at 4°C  
320 overnight for cryoprotection. Using a cryostat, tissue was sectioned in 50 µm thick coronal slices and  
321 mounted on charged slides. The placement of electrodes was verified in coronal brain sections  
322 stained with cresyl violet in two animals. The extent of the lesions was confirmed by tyrosine  
323 hydroxylase (TH) immunohistochemistry.

324

#### 325 *Cresyl violet staining*

326 Sections were stained with 0.1% cresyl violet (CV) powder in dH<sub>2</sub>O and 0.3% glacial acetic  
327 acid solution for 5 min. Sections were then rinsed for 1 min in dH<sub>2</sub>O and dehydrated with 70%, 95%

328 and 99.5 % EtOH for 1 min each and then immersed in 100% xylene for 5 min (x2) before mounted  
329 with DPX mounting media.

330

### 331 *Tyrosine Hydroxylase (TH) staining and quantification*

332 Brain sections were washed in PB 0.01M (5 min), hydrogen peroxide 0.3% diluted in  
333 methanol (20 min), PBS/Tween 0.05% (5 min) and then were incubated in 10% normal goat serum for  
334 30 min, followed by incubation with primary antibody rabbit anti-TH (1:500, Chemicon) overnight at  
335 room temperature. On the following day, sections were rinsed in PBS/Tween 0.05% (5 min) and  
336 incubated with biotinylated goat anti-rabbit (1:200, Vector) for 2 hrs. After that, all sections were  
337 rinsed in PBS/Tween 0.05%, incubated in avidin-biotin complex (ABC Kit, Vector) for 1 hr and stained  
338 with 3,3-diaminobenzidine and H<sub>2</sub>O<sub>2</sub>.

339 TH striatal optical densitometry was assessed using the ImageJ software (National Institutes of  
340 Health) as described previously (Fuentes *et al*, 2009) in areas adjacent to the striatal recording sites.  
341 The optical density of the ipsilateral corpus callosum was used as staining background and was  
342 subtracted from striatal values prior to comparison.

343

### 344 *Statistical methods*

345 All statistical tests used to assess significant group difference are specified in the Result section  
346 of the main text and in the respective figure legends.

347

## 348 **RESULTS**

### 349 *Experiments performed*

350 To clarify which neurophysiological activity patterns are associated with parkinsonism and  
351 dyskinetic states in PD we performed parallel multi-structure neuronal recordings in eight different  
352 parts of the cortico-basal ganglia-thalamic loop using the described novel methodology. In total, 15  
353 separate recording sessions were performed in four unilaterally 6-OHDA lesioned dyskinetic rats  
354 (repeatability was evaluated by performing nine separate recordings in the same subject in  
355 experiments performed several weeks apart and reproducibility by performing similar recordings in  
356 four different subjects). *Post mortem* TH staining adjacent to the recording electrodes showed a  
357 complete loss (100%) of dopaminergic terminals in posterior parts of the striatum ipsilateral to the  
358 lesion with some remaining terminals in anterior areas (average striatal denervation ~74%). In seven  
359 of these experiments additional pharmacological interventions aimed at reducing dyskinetic  
360 symptoms were also investigated as a proof-of-principle for the use of the developed technology in  
361 characterization of experimental treatment of disease.

362

### 363 *Recordings in STN/M1 confirm previously reported changes in neuronal activity patterns*

364 From the obtained recordings, we could confirm the presence of narrow-band high-frequency  
365 gamma oscillations in M1 (as previously documented in rodents) and theta-oscillations in STN (as  
366 previously documented in humans) after the transition from the parkinsonian to the dyskinetic  
367 condition following levodopa treatment (Fig. 2A, B; Alonso-Frech et al., 2006; Halje et al., 2012). A  
368 notable difference between these two phenomena was however that narrow-band gamma-  
369 oscillations at no instance were observed in neither the intact hemisphere during dyskinesia nor the  
370 lesioned hemisphere of non-levodopa treated animals, as opposed to theta oscillations that were  
371 more abundantly present (in particular during periods of increased motor activity). From the  
372 spectrograms presented in Fig. 2A, it is clear that the spectral contents in the parkinsonian condition

373 varies over time (examinations of the video recordings revealed that these changes were associated  
374 with changes in behavioral state of the animal, in agreement with previous studies (Avila et al., 2010;  
375 Brazhnik et al., 2014; Delaville et al., 2014)). In contrast, following a transient frequency-tuning at the  
376 onset of dyskinesia, the spectral characteristics in the dyskinetic state was relatively stable  
377 throughout the dyskinetic period.

378 Within an individual the theta/gamma power changes in STN/M1 were consistent across recordings  
379 (average power spectra from nine example recordings from the animal shown in Fig. 2A are  
380 presented in Fig. 2B). On the other hand, between rats, peak frequencies within the different bands  
381 were found to vary somewhat. On average over all the recordings, there was an increase in LFP  
382 power for the theta band [3-9 Hz] when comparing the dyskinetic state to the baseline prior to  
383 levodopa administration (Wilcoxon signed rank tests revealed that the 1.9 dB increase in STN was  
384 significant ( $p=0.0004$ ), while the 0.3 dB increase in M1 was weakly significant ( $p=0.05$ ) and did not  
385 survive correction for multiple comparisons). For the gamma band [65-100 Hz] a significant increase  
386 was only found in M1 (Wilcoxon signed rank tests revealed +0.1 dB,  $p=0.4$ , for STN and +3.0 dB,  
387  $p=0.0004$ , for M1). In this context it is also interesting to note that a comparison to the levodopa  
388 treated control side revealed that the theta increase following levodopa administration may partly  
389 be related to the induced increase in motor activity in contrast to the changes in gamma which are  
390 more specific to the dyskinetic state. Wilcoxon signed rank test for differences in the increase of  
391 power in the theta band between the STN in the two hemispheres before and after treatment,  
392 showed that the side difference was not quite significant ( $p=0.054$ , after Bonferroni correction for  
393 multiple comparisons [ $n=4$ ]). For the gamma band, on the other hand, the corresponding power  
394 increase in M1 after treatment was significantly higher in the lesioned hemisphere compared to  
395 control ( $p=0.0032$ ).

396

397 *Multi-structure recordings reveal systems level brain states*

398           Based on these confirmatory findings in M1 and STN, it is expected that abnormal activity  
399 patterns should arise under similar conditions also in other parts of the highly interconnected circuits  
400 making up the cortico-basal ganglia-thalamic loop. Moreover, although these specific frequency-  
401 bands which have been highlighted in earlier studies indeed showed clear changes in relation to the  
402 transition from parkinsonian to dyskinetic state, it is evident that other parts of the frequency  
403 spectrum also displayed changes (which appeared to differ between M1 and STN; Fig. 2A, B). In the  
404 subsequent analyses we therefore included all simultaneously recorded structures from both  
405 hemispheres and did not restrict LFP-analyses to delimited frequency bands. Recordings from four  
406 different conditions were analyzed: 1) from the intact hemisphere OFF-levodopa, representing the  
407 control condition, 2) from the lesioned hemisphere OFF-levodopa, representing the parkinsonian  
408 state, 3) from the lesioned hemisphere ON-levodopa during periods with dyskinesia, representing the  
409 dyskinetic state, and 4) from the intact hemisphere ON-levodopa, representing a second control  
410 condition in the drug-treated state. Recordings from these different conditions were divided into  
411 separate data sets and analyzed individually for each rat. LFPs and the firing rates of individual  
412 neurons were both examined. For LFPs, frequency power spectra (based on the spectral contents  
413 between 2 and 120 Hz with a 0.5 Hz resolution) were calculated during 8-s sample periods for all  
414 brain structures. To describe the neurophysiological state of an animal at different time points during  
415 the experiment the power spectra from the different structures were combined into a single vector,  
416 thereby essentially creating a unique coordinate in this high-dimensional space for each 8-s time bin.  
417 Similarly, for firing rates, the neurophysiological state was also described by a unique coordinate for

418 each 8-s period created from the vector comprising the average firing rate of all recorded neurons  
419 during each sample period.

420 In all recorded experiments, behavioral observations confirmed that animals quickly  
421 transitioned into a stable severely dyskinetic state following levodopa treatment and remained in  
422 this condition with uninterrupted dyskinesia for an average time period of  $160 \pm 22$  min  
423 (corresponding to the reported period of elevated levodopa concentrations Carta et al., 2006) unless  
424 other pharmacological interventions were carried out. This was expected given that medial forebrain  
425 bundle lesions are known to cause a severe model of PD where practically no therapeutic window for  
426 dopamine replacement therapy remains following a brief period of levodopa treatment (Winkler et  
427 al., 2002). When pooling LFP data from parkinsonian and dyskinetic animals from multiple recordings  
428 and plotting the coordinates in a 2D-space chosen to facilitate the comparison of the two  
429 pathological states (i.e. where the x-axis represents the difference vector between the parkinsonian  
430 and control state and the y-axis the difference vector between the dyskinetic and control state in the  
431 direction orthogonal to the x-axis) it became obvious that data sampled from time periods belonging  
432 to each specific state clustered in separate parts of the plane (Fig. 3A). Moreover, this LFP-based  
433 state description proved to be very robust across experiments performed in each animal (denoted by  
434 filled triangles in Fig. 3A; see Table 1B for details on state classification performance).

435 To get a better understanding of the underlying physiological differences separating the  
436 states, the spectral content in each structure was analyzed in further detail. In Fig. 3B the mean of  
437 the spectral differences that chiefly separate the control from the parkinsonian state and the  
438 dyskinetic from the control state in Fig. 3A (i.e. the axes spanning the plane) is plotted for all eight  
439 brain structures in Animal I, which has the largest number of recordings. Note the increase in relative  
440 LFP-power in the beta band in several structures in the parkinsonian state, as well as the theta and



441 gamma-peaks in the dyskinetic state (Fig. 3B top and bottom, respectively). However, certain  
442 variability between subjects in terms of the exact difference spectra that separate the states were  
443 also observed (Fig. 4). These inter-individual differences could be expected given inherent variability  
444 between individuals relating to brain circuit anatomy, the exact locations of the recording electrodes,  
445 signal-to-noise levels etc. On the other hand, the great similarities in the state representations (Fig.  
446 3A) show that comparisons of similar states across subjects can be made even though the absolute  
447 differences between states in terms of LFP spectral contents may vary between individuals.

448         To investigate the relative contribution from the eight different brain structures for state  
449 separation we also analyzed state classifications based on the LFPs recorded in each single structure  
450 (for details on calculations see Methods). In Fig. 3C the state separations [Control vs. PD] and  
451 [Control vs. Dyskinesia] obtained for each structure are shown separately. These analyses show that  
452 the LFP spectral contents in e.g. cortex and STN constitute relatively reliable biomarkers for these  
453 three states (see also Halje et al., 2012). Nonetheless, state separation for any individual structure  
454 was clearly not as robust as the multi-structure data – for example, whereas the average  
455 classification performance for all recordings was 98.6% using data from all structures it was reduced  
456 to 85.6% when using data from M1 and STN only, (cf. Fig. 2B), which corresponds to a >10-fold higher  
457 error rate than when all eight structures are included (histograms for all animals are included in Fig. 5  
458 and classification performance in Table 1B).

459         We next analyzed changes in neuronal activity. Here, the requirement of sampling unit activity  
460 from the same neurons across states limits comparisons to changes observed within each structure  
461 across different experimental conditions. Hence, in the unilateral 6-OHDA PD-model direct  
462 comparisons between the control and the parkinsonian/dyskinetic state cannot be obtained with  
463 single-cell resolution. Even so, when analyzing unit activity of cells located in the lesioned

464 hemisphere we found that several neurons clearly altered their firing rates during dyskinesia  
465 compared to the parkinsonian state (increased: RFA 2/6, DMS 7/10, DLS 6/11, GP 0/9, Thal 9/9, STN  
466 2/9; decreased: RFA 3/6, DMS 3/10, DLS 3/11, GP 9/9, Thal 0/9, STN 5/9;  $p < 0.05$ , Wilcoxon rank sum  
467 test). Consequently, these two states could be reliably separated in a similar manner to the LFP-  
468 based clustering (in the corresponding multi-variate analysis across the two states, i.e. ON/OFF  
469 levodopa). See Fig. 6 for example state plots based on unit data (average classification performance  
470 for PD vs. dyskinesia was in this case 99.3%).

471

#### 472 *Ameliorating dyskinetic symptoms using a serotonin agonist*

473 For the vast majority of Parkinson patients, dopamine replacement therapy effectively  
474 improves a range of symptoms and remains the therapeutic approach of choice (PD MED  
475 Collaborative Group, 2014). The possibility to prolong the levodopa treatment period before  
476 complications arise, by reducing drug-induced dyskinetic symptoms has therefore attracted a lot of  
477 interest in recent years (Olanow et al., 2000; Crosby et al., 2003; Huot et al., 2013). One such  
478 approach is the use of serotonin (5-HT) agonists aiming to control the efflux of dopamine from  
479 serotonergic terminals of dorsal raphe neurons by stimulation of 5-HT auto-receptors (Carta et al.,  
480 2007; Svenningsson et al., 2015). The rationale for this method stems from the notion that dyskinesia  
481 is partly caused by a dysregulation in dopaminergic signaling and that serotonergic terminals  
482 synthesizing dopamine via Aromatic L-amino Acid Decarboxylation (AADC) release it in an  
483 uncontrolled manner (the AADC-enzyme is in serotonergic neurons responsible for the synthesis of 5-  
484 HT but can also convert levodopa into dopamine). Accordingly, a pharmacological intervention  
485 targeting presynaptic 5-HT receptors on these neurons could potentially harness the uncontrolled  
486 synaptic release of dopamine. To evaluate the potential of this approach from a systems level point

487 of view, we first administered the 5-HT<sub>1A</sub> agonist 8-OH-DPAT systemically during peak dyskinesia and  
488 subsequently reversed the effect of the drug by treatment with the 5-HT<sub>1A</sub> antagonist WAY-100,635  
489 ~40 minutes later. Dyskinetic symptoms were quantified during different phases of the experiment  
490 with respect to prevalence of the abnormal involuntary movements observed (Lundblad et al., 2002).

491 The 5-HT<sub>1A</sub> agonist was found to effectively ameliorate dyskinesia and this effect was fully  
492 reversible by the antagonist (Fig. 7A; mean normalized scores [0, 1]: Dyskinesia=0.72, 8-OH-  
493 DPAT=0.01, WAY100635=0.75; Kruskal-Wallis  $p<0.001$ , Dunn's post-test for group differences: Dys vs.  
494 8-OH-DPAT,  $p<0.01$ ; 8-OH-DPAT vs. WAY100635,  $p<0.001$ ; Dys vs. WAY100635, n.s. based on  
495 dyskinesia scores >5 min after each injection [second injection for L-DOPA]). It was noted, however,  
496 that while the dyskinesia was practically eliminated other behavioral abnormalities appeared to be  
497 present in the 8-OH-DPAT treated state (i.e. an abnormally flat body posture and recurring forepaw  
498 movements, resembling previous observations connected to excessive serotonergic stimulation;  
499 Jacobs, 1974). The recorded neurophysiological signals in the eight different brain regions revealed a  
500 clear shift away from the dyskinetic state in both LFPs (Fig. 7B; MANOVA [ANOVA with frequency  
501 bands as dependent variables],  $p<0.001$ , mean distance in first canonical dimension were: Dys vs. 8-  
502 OH-DPAT = 24.3; 8-OH-DPAT vs. WAY100635 = 17.1; Dys vs. WAY100635 = 7.1 and all groups were  
503 significantly different to each other;  $p<0.001$ , Wilcoxon rank sum cf. Dupre et al., 2013) and in unit  
504 activity (Fig. 7C; MANOVA,  $p<0.001$ , mean distance in first canonical dimension were: Dys vs. 8-OH-  
505 DPAT = 10.9; 8-OH-DPAT vs. WAY100635 = 9.5; Dys vs. WAY100635 = 1.4 and all groups were  
506 significantly different to each other;  $p<0.001$ , Wilcoxon rank sum). However, a closer comparison to  
507 the control state revealed that certain differences remained between the 8-OH-DPAT treated state  
508 and the control condition. In particular, low frequency oscillations (delta/theta and beta) showed a  
509 deviant pattern (Fig. 7D; see Fig. 8 for corresponding spectrograms from all structures for the intact

510 hemisphere). These remaining differences between the 8-OH-DPAT treated state and control  
511 conditions, together with the observation that normal behavior was not fully reinstated with this  
512 drug, suggest that aspects of the motor behavior, other than those captured with the dyskinesia  
513 score are relevant for the interpretation of the electrophysiological state in this case.

514

#### 515 *Investigating drug effects in a systems level neurophysiological state space*

516 Because pharmacological manipulations targeting 5HT<sub>1A</sub> receptors clearly have the potential  
517 to reduce dyskinesia but nevertheless induce neurophysiological activity patterns that in some parts  
518 of the brain differ considerably to the control state, it would be of relevance to characterize the drug-  
519 induced state at a systems level. More generally, condensed systems level descriptions could  
520 conceivably offer a more straightforward way to compare complex brain states following  
521 interventions that involve diverse changes in different parts of the CNS and in different  
522 neurotransmitter systems. Thus, to test the potential of the developed technology for the  
523 experimental evaluation of drug candidates and other novel therapeutic interventions we next  
524 characterized brain states, based on LFPs recorded in the eight structures in the same animal,  
525 following treatment with 8-OH-DPAT and three other drugs with putative anti-dyskinetic effects –  
526 amantadine, levetiracetam and diazepam (Pourcher et al., 1989; Pahwa et al., 2006; Stathis et al.,  
527 2011). In parallel, behavioral assessments of the reduction of dyskinesia was quantified for all four  
528 drugs. The drugs were administered systemically to reach maximum effect at the time point of peak  
529 dyskinesia (where the animals displayed severe dyskinesia corresponding to 79±4% of the maximum  
530 compound dyskinesia score). The alleviation of dyskinetic symptoms differed between the drugs  
531 ( $p < 0.001$ , Kruskal Wallis; ranging from no detectable effect for levetiracetam (Wolz et al., 2010) to  
532 clear alleviation of symptoms for e.g. 8-OH-DPAT) and in some cases the effect also varied either

533 during or across experiments. In specific, following diazepam treatment intermittent periods of AIMs  
534 were present even though dyskinesia was otherwise almost completely abolished, and in the case of  
535 amantadine the alleviation of dyskinesia was relatively weaker in one of the two experiments  
536 performed (Fig. 9A).

537 To get an overview of the entire data set, LFP data from six recordings (one experiment was  
538 excluded due to poor recording quality) were first represented in a common principal component  
539 (PC) space spanned by the first three PCs. Remarkably, unique and clearly separable clusters were  
540 found for each of the drugs even in this low-dimensional representation (Fig. 9B; for details on  
541 calculations see Methods). The state separation was quantified with a classifier with eight states that  
542 achieved near-perfect classification performance (>99.9%) using 30 PCs (Fig. 10). This tight clustering  
543 of neurophysiological states induced by the same treatment in separate experiments performed  
544 weeks apart clearly indicates that the drug-induced systems level states were specific and robust.

545 To further clarify to what extent activity patterns in different brain structures contributed to  
546 the combined state description we next analyzed how well the different drug-induced states could be  
547 separated using only a subset of the recorded structures. Hence, the classification performance of  
548 the eight states shown in Fig. 9B was calculated for all 255 possible combinations of structures  
549 (Fig. 9C). As expected, a higher number of brain structures generally improved classification  
550 performance. It was also noted that although motor cortex and STN together turned out to be the  
551 most informative pair, different combinations of structures resulted in the most accurate state  
552 classification depending on the total number included because the fraction of shared (redundant)  
553 information in a given structure will depend on which other structures that are included.

554 To compare the contribution from specific frequency bands we analyzed how well the states  
555 could be separated using only the theta band (3-9 Hz), only the beta band (10-35 Hz), only the

556 gamma band (65-100 Hz), or any combination with two or three of these bands. To make the  
557 comparison fair we used PCA to reduce the dimensionality to 8 in all test cases before classifying. The  
558 classification performances were: theta=24%, beta=20%, gamma=25%, theta+beta=22%,  
559 theta+gamma=35%, beta+gamma=29%, theta+beta+gamma=32%. This should be compared to the  
560 classification performance of 96% obtained when using the full spectrum (when also reduced to 8  
561 PCs).

562 Having confirmed that each drug-induced state could be reliably identified based on multi-  
563 structure LFP data, we wanted to plot the different states using the same 2D space as in Fig. 3A to  
564 facilitate the comparison to the two pathological states that the pharmacological interventions were  
565 aimed to alleviate (i.e. PD and dyskinesia). To enable us to pool data from different subjects despite  
566 potential inter-individual differences the parkinsonian and dyskinetic states were used as reference  
567 states defining the sub-space onto which other states were then projected (the robustness of this  
568 approach was initially verified in a control experiment by training a PD/dyskinesia/amantadine  
569 classifier in one rat and cross-validating it in another rat with or without calibration to the PD and  
570 dyskinesia reference states; see Fig. 11).

571 In this sub-space, spanned by the basis vectors [Control vs. PD] and [Control vs. Dyskinesia]<sub>Ortho</sub>,  
572 the state induced by each drug was plotted separately (Fig. 9D). In agreement with the results from  
573 the 8-OH-DPAT experiments, it is clear that while several of the drugs produced reductions of  
574 dyskinetic symptoms (as shown in Fig. 9A) the neurophysiological state was nevertheless not fully  
575 normalized and in many cases partly reverted towards the PD-state. In this context it deserves  
576 mentioning that although dyskinetic symptoms were clearly reduced by some of the drugs other  
577 aspects of the motor behavior appeared somewhat abnormal (amantadine: poor hindlimb to  
578 forelimb coordination, arching of back, postural deficits; levetiracetam: very minor reduction in

579 dyskinesia, flat body position; diazepam: mostly immobile but dyskinetic in association with  
580 movements).

581

## 582 **DISCUSSION**

583 Experimental treatment of CNS disease is conventionally evaluated in animals by documentation of  
584 changes in behavior. This approach has however a number of drawbacks. First, assessments of motor  
585 behavior only gives indirect information on the underlying brain states that the therapy aim to treat,  
586 making it almost impossible to deduce the specific pharmacological/neurophysiological effects of the  
587 treatment. Second, the sensitivity and robustness in assessments of animal behavioral are usually not  
588 sufficient to allow for differentiation between several related CNS states. Third, unbiased measures  
589 based on automated procedures are still rare making the testing procedures highly dependent on  
590 proper training of skilled observers and reduces reproducibility between labs.

591         Ever since the first electrophysiological measurements were carried out in awake subjects it  
592 has been known that the electrical activity of neurons frequently tend to synchronize into rhythmic  
593 patterns that vary depending on the state of the brain (Berger, 1929). The results presented in the  
594 current study confirm previous findings suggesting an association between LFP oscillations within  
595 certain frequency intervals in specific regions of the cortico-basal ganglia thalamic circuit and various  
596 motor symptoms in PD (Hammond et al., 2007; de Hemptinne et al., 2015). More importantly  
597 however, through the use of the developed techniques previous findings can now be complemented  
598 with significantly more elaborate state characterizations based on large-scale multi-structure  
599 recordings. The added value of these large-scale multi-structure recordings were tested  
600 quantitatively by comparisons against the same recordings where state classifications were based on  
601 information obtained from fewer structures, showing a higher classification performance with higher

602 number of structures (Fig. 9C). Similarly, we show that using the entire spectral contents in LFP-  
603 recordings rather than the power in a few pre-selected frequency bands greatly improves state  
604 classification. It should also be noted that, by aligning the assessed systems level states in each  
605 individual to a number of reference states inter-individual differences in activity patterns associated  
606 with each state is compensated for, which makes it possible to pool data across subjects without *ad*  
607 *hoc* re-alignment of data (this is a well-known problem in, for example, comparisons of spectral LFP-  
608 contents between parkinsonian subjects (Kühn et al., 2009)). In particular, in practical applications  
609 where for example therapeutic effects of a drug are evaluated and disease mechanisms are not of  
610 primary concern this approach can be beneficial.

611         Using the developed method, we have here shown that robust and detailed representations  
612 of the pathophysiological conditions associated with motor symptoms in a rodent model of PD can  
613 be attained. In addition, the complex and diverse effects of a number of different pharmacological  
614 interventions aimed at treating motor symptoms could also be characterized on a systems level. It  
615 may be worth noting in this context, that while a representation based on the systems level  
616 electrophysiological differences between the parkinsonian, dyskinetic and control states is a natural  
617 starting point for the investigation of anti-dyskinetic interventions, adding other reference  
618 states/conditions to the analyses (e.g. information about the behavioral state, the effects of other  
619 drugs etc.) will further help elucidating additional features of each state. Also, for pairwise  
620 comparisons of states such as direct comparison of the effects of two different drugs, difference  
621 spectra is a natural starting point for further analyses. In any case, the very rich data-sets obtained  
622 with the described method potentially open up for a much more exploratory/data-driven approach  
623 which can be very beneficial in this field of research due to the extreme complexity of the systems  
624 studied (Finkbeiner et al., 2015).



625           With regard to the animal model used in these experiments, it should be cautioned that the  
626 unilateral 6-OHDA medial forebrain bundle lesion model of PD has certain limitations. First, and most  
627 importantly, because the non-lesioned hemisphere is used as control some comparisons cannot be  
628 made in a straightforward manner between pathological and non-pathological states (for example,  
629 changes in the firing rate of individual neurons) and a certain degree of variability is inevitably  
630 inherent to the model due to differences of the exact recoding locations in different hemispheres.  
631 Second, it cannot be assumed that the physiology of the intact hemisphere in a hemi-lesioned rat is  
632 entirely comparable to that of a non-lesioned animal due to potential biological adaptations that  
633 have occurred to compensate for the lesion-induced contralateral deficits. A few examples of such  
634 physiological changes have in fact been reported (Kish et al., 1999; González-Hernández et al., 2004;  
635 Breit et al., 2008). In an attempt to estimate how large these differences are we quantitatively  
636 compared differences between intact hemispheres of lesioned and non-lesioned rats using multi-  
637 structure recordings in different animals. While not reaching significance, group differences were  
638 nevertheless confirmed (the average difference in median Euclidian distance to the non-lesioned  
639 references condition were for intact hemispheres in hemilesioned rats 140% higher than that of  
640 contralateral hemispheres in non-lesioned animals; i.e. the median distance to Group 1 for 5 vs.  
641 mean[2&4] in Fig. 12). Third, while the severe lesions used in the model is beneficial for the study of  
642 dyskinesia the limited therapeutic window for levodopa treatment precludes detailed analyses of the  
643 therapeutic effects of this drug. A strength of the unilateral model is, on the other hand, that certain  
644 factors affecting the general neurophysiological state are easier to control for in bilateral recordings  
645 with an internal control condition, such as the degree of drowsiness/alertness, periods of  
646 immobility/locomotion etc.

647 In relation to previous publications using 6-OHDA lesioned rats it is worth pointing out that  
648 certain differences have been observed between recordings in anesthetized preparations as  
649 compared to awake behaving animals. In particular urethane anesthetized 6-OHDA lesioned rats  
650 have been reported to display beta-oscillations with a somewhat lower oscillation frequency than  
651 awake animals (Brazhnik et al., 2014). Instead, awake rats typically display two types of beta  
652 oscillations that are dependent on the behavioral state (Avila et al., 2010; Brazhnik et al., 2014;  
653 Delaville et al., 2014). These oscillations (<15 Hz and 20-35 Hz, respectively) were indeed present also  
654 in the current study (see e.g. Fig. 2A, M1 prior to levodopa).

655 In addition to the presented measures, changes in functional connectivity between different  
656 structures, reflected in increased LFP-coherence and correlated spiking activity of cells in  
657 anatomically connected structures has also been implicated in the pathophysiology of PD (Hammond  
658 et al., 2007; Fuentes et al., 2010; Santana et al., 2014). Such measures have not been included in the  
659 state analyses to this point, but it is probable that the addition of pairwise coherence/correlation  
660 measures of neuronal activity within and between structures would help to further improve the  
661 performance of state classifications and would be a natural complement given the multi-structure  
662 recording design.

663 This methodology could also be combined with several of the recently developed techniques  
664 for genetic manipulations of neuronal sub-populations that are to date primarily performed in mice.  
665 The presented findings indicate that several brain structures should preferably be targeted. Thus, to  
666 adapt the method to a smaller brain it would be recommendable to scale down the number of  
667 electrodes used to target each brain structure in such experiments rather than reducing the number  
668 of structures.

669           Because motor symptoms are cardinal features of PD, neurophysiological states in  
670 parkinsonian and dyskinetic rats could here be directly matched to quantitative behavioral  
671 assessments of the displayed symptoms – essentially providing a validation of the neurophysiological  
672 read-outs for the studied conditions. Following anti-dyskinetic treatment, an apparent mismatch was  
673 sometimes observed between the reduction in dyskinesia score and the corresponding changes in  
674 systems level brain state (although the coordinate values in the dimension [Control vs Dyskinesia]  
675 indeed correlated well with dyskinesia scores, see Fig. 9 Legend). The discrepancy observed can  
676 however largely be explained by the fact that a behavioral characterization solely based on  
677 dyskinesia score does not capture a whole range of other motor symptoms that were here only  
678 described qualitatively. If more detailed behavioral assessments had been carried out with  
679 quantitative assessment scales that were adapted to include a wider range of motor symptoms it is  
680 probable that the behavioral state descriptions would be better correlated to the neurophysiological  
681 activity states recoded in these motor circuits. Notably, however, such behavioral assessments are  
682 technically very challenging to carry out and will likely require more advanced automated procedures  
683 (see e.g. Palmér et al., 2012; Santana et al., 2015). In addition, it is well known that PD also includes  
684 non-motor symptoms and in several other disorders few overt signs, if any, may be associated with a  
685 specific pathological condition. In this situation, CNS state characterizations on a more holistic level  
686 could help opening up a new window into otherwise hidden internal processes in conditions such as  
687 persistent pain states, psychosis, depression etc. We therefore envision that this technology could  
688 have an important use in the development of future treatments for a range of neurologic and  
689 psychiatric conditions. More fundamentally however, the knowledge gained from improved  
690 descriptions of how different brain structures interact to create mental states and complex behaviors  
691 in health and disease using a technology that bridges all the way from the scale of single cell activity

692 to systems level states has potentially wide-reaching implications for neuroscientific research in  
693 general.

694

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697

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900

901

902 **LEGENDS**

903 **Fig. 1 Parallel recordings in eight different structures of the cortico-basal ganglia-thalamic loop in**  
904 **each hemisphere made possible with high-density recording arrays.**

905 A: Microelectrode recording wires (n=128) are distributed to target relevant brain structures (circles  
906 mark positions of single 30  $\mu\text{m}$  tungsten wires; 250  $\mu\text{m}$  center-to-center spacing within groups). B:  
907 The relative arrangement of wire groups is guided by a custom made 2D-array and a 3D-aligner.  
908 Wires are electrically linked to a connector via a printed circuit board (PCB).

909 *RFA*: rostral forelimb area, *M1*: primary motor cortex, *DLS*: dorsolateral striatum, *DMS*: dorsomedial  
910 striatum, *GP*: globus pallidus, *Th*: thalamus, *STN*: subthalamic nucleus, *SNr*: substantia nigra pars  
911 reticulata.

912

913 **Fig. 2 Changes in neurophysiological activity patterns in the subthalamic nucleus and primary**  
914 **motor cortex with the onset of dyskinesia.**

915 A: Top: Examples of LFP spectrograms from recordings in the subthalamic nucleus and primary motor  
916 cortex in the lesioned hemisphere during a 90-min period including a time period prior to, and  
917 following, the onset of dyskinesia (dashed line; t=0 min corresponds to time point of levodopa  
918 injection). Bottom: Close-up of the low-frequency range of the spectrograms shown in the top row  
919 (power is expressed in dB relative to the estimated pink-noise floor). B: Time-averaged spectra from  
920 9 recordings ( $\geq 20$  min per state and recording) for the parkinsonian period (grey: individual  
921 recordings; black: average) and the dyskinetic period (pink: individual recordings; red: average).

922

923 **Fig. 3 Systems level neurophysiological states associated with parkinsonism and dyskinesia.**

924 A: Systems level state descriptions in four rats based on LFP recordings in the cortico-basal ganglia-  
925 thalamic loop (dark blue for control, black for PD, red for dyskinesia and light blue for control with  
926 levodopa). The x-axis denotes the direction in LFP spectral space where the difference between the  
927 control condition and the parkinsonian state is the largest and the y-axis represents the largest  
928 difference between the control and dyskinetic state orthogonal to the x-axis. Note the close  
929 clustering of data points from each state (each small dot represents the state coordinate during an  
930 8s-period and shaded clouds denote dot densities) and the great similarity of the states in separate  
931 recordings (filled triangles indicate cluster centers for the states in each recording; Animal I: n=9;  
932 Animal II: n=4; Animal III: n=1; Animal IV: n=1; classification performance were for the four animals:  
933 0.9910, 0.9782, 1 and 1; all pairwise comparisons of cluster medians were significant,  $p < 0.001$ ,  
934 Wilcoxon rank sum). B: The average spectral differences in the eight structures for [Control vs. PD]  
935 and [Control vs. Dyskinesia]<sub>ortho</sub> over all nine recordings in Animal I. (C-D) Histograms illustrating the  
936 state separability in each structure with data from all nine recordings; (C): [Control vs. PD] and (D):  
937 [Control vs. Dyskinesia]. The distributions were obtained by projecting the data onto the one  
938 dimension represented by the spectral difference vector.

939

940 **Fig. 4 Spectral state differences per structure divided by animal**

941 The average LFP spectral difference vectors in the recorded structures for Top: [Control vs. PD],  
942 Middle: [Control vs. Dyskinesia] and Bottom: [Control + levodopa (LDA) vs. Dyskinesia] over all  
943 recordings averaged per animal. Note that the spectral difference [Control vs. Dyskinesia] is shown  
944 rather than [Control vs. Dyskinesia]<sub>ortho</sub> (to illustrate the true spectral difference without  
945 orthogonality constraints). Colored dots indicate significant differences between the compared



946 states for the corresponding frequency bin and structure (Wilcoxon rank sum,  $p < 0.05$ , Bonferroni  
947 corrected for multiple tests).

948

949 **Fig. 5 Histograms illustrating the state separability of all recordings shown per animal**

950 Top: [Control vs. PD] and Bottom: [Control vs. Dyskinesia]. The distributions were obtained by  
951 projecting the data onto the one dimension represented by the spectral difference vector (for  
952 example, the vector pointing from the center of the control cluster to the PD cluster). Three of the  
953 animals were used for evaluation of electrical microstimulation in a separate set of experiments and  
954 are consequently lacking recording electrodes in that structure, Th [n=2] and GP [n=1]. Notably this  
955 missing information was largely compensated for by the parallel recordings in the other structures as  
956 indicated by the histograms in the rightmost column.

957

958 **Fig. 6 State plots based on changes in neuronal firing rates**

959 Left: Heat plots of all individual unit activities from the lesioned hemisphere during different states.  
960 Each row on the y-axis represents the activity of a unit throughout an experiment, normalized to its  
961 respective maximal firing rate (color codes denoting recording structures as in Fig. 3B). Vertical white  
962 lines indicate times of drug injections during the recording and onset of dyskinesia (based on manual  
963 behavioral scoring). Right: Systems level state descriptions based on unit activity in the cortico-basal  
964 ganglia-thalamic loop in the lesioned hemisphere. The x-axis denotes the direction in unit activity-  
965 space where the difference between the parkinsonian and dyskinetic state is the largest and the y-  
966 axis represents the largest difference between the parkinsonian and drug induced state orthogonal  
967 to the x-axis. The firing rate difference between PD and dyskinesia for units in the respective

968 structures were, expressed in Z-scores (median/iqr): RFA: 0.56/0.63, DMS: 0.63/1.19, DLS: 0.58/1.07,  
969 GP: 0.79/1.93, Th: 1.33/0.93, STN: 0.68/0.78. Classification performance of the three states in this  
970 2D-projection were for the four panels: 0.9708, 0.9645, 0.8556 and 0.7641. All pairwise comparisons  
971 of cluster medians were significant,  $p < 0.001$ , Wilcoxon rank sum.

972

973 **Fig. 7 Systemic treatment with a 5-HT<sub>1A</sub> receptor agonist alleviates dyskinesia and alters the**  
974 **neurophysiological state.**

975 A: Severity of dyskinesia scored during 1-min periods once every 5 min (marked by crosses). Dashed  
976 lines indicate times of drug injections (levodopa was administered twice in this experiment to reach  
977 the dyskinetic state - represented by the first two lines). B: Spectrogram from all recorded structures  
978 in the lesioned hemisphere showing the relative change in LFP spectral contents throughout an  
979 example experiment where a dyskinetic rat was treated with the 5-HT<sub>1A</sub> agonist 8-OH-DPAT  
980 (0.4 mg/kg i.p. at  $t = 123$  min) to reduce dyskinesia. This drug effect was subsequently reversed by  
981 treatment with the 5-HT<sub>1A</sub> antagonist WAY-100,635 (0.4 mg/kg i.p. at  $t = 163$  min). C: Cellular activity  
982 showed clear differences between states (color code represents deviation from the mean firing rate  
983 across all four conditions for each unit; units are ordered in rows according to the mean firing rate  
984 during the non-treated parkinsonian state and the colored boxes to the left of each unit indicates  
985 structure recording, with same color codes as in Figure 3B). D: The mean differences in LFP spectral  
986 contents between the control condition and the non-dyskinetic 8-OH-DPAT treated state shown in  
987 (B), summarized for each structure separately.

988

989 **Fig. 8 LFP spectrograms from the intact hemisphere in recording with 8-OH-DPAT administration**

990 Spectrograms from the non-lesioned hemisphere from two experiments with 8-OH-DPAT  
991 administrated as a dyskinesia reducing agent (bottom panel was recorded in parallel with the data  
992 shown in Fig. 7). Vertical lines indicate times of drug injections, effects and key events during the  
993 recording.

994

995 **Fig. 9 Systems level characterizations of pharmacological interventions alleviating dyskinesia.**

996 A: Reduction in normalized dyskinesia scores following systemic treatment in the same rat with four  
997 different drugs in seven separate recordings. Wilcoxon signed rank tests for significant effects on  
998 individual AIM scores between *pre* and *post*-treatment showed significant reductions ( $p < 0.05$ , after  
999 Bonferroni corrections with  $n = 16$  comparisons) for OL: Amantadine 2, 8-OH-DPAT 2; FL: Amantadine  
1000 2, 8-OH-DPAT 1, Diazepam; Ax: Amantadine 2, 8-OH-DPAT 1 & 2, Diazepam. B: Overview of the  
1001 corresponding systems level neurophysiological states induced by the different pharmacological  
1002 interventions based on the spectral contents of recorded LFPs. Note that each drug clusters in a  
1003 separate region of the illustrated space spanned by the first three PCs (classification performance  
1004 with 3 PCs was 0.82, cf. Fig 10; all pairwise comparisons of cluster medians were significant,  $p < 0.001$ ,  
1005 Wilcoxon rank sum). C: Cluster classification performance shown as a function of number of brain  
1006 structures included in the electrophysiological measurement (red=average value for all possible  
1007 combinations of  $x$  structures; blue=best combination of  $x$  structures [the composition of the best  
1008 combinations are listed for one to six structures]; classification performance when all eight structures  
1009 were used reached 99.94% for the  $n = 5421$  samples with eight states; this performance was  
1010 significantly higher than what was attained using fewer structures except for  $n = 7$  structures,  $p < 0.05$ ,  
1011 Wilcoxon signed-rank test, with Bonferroni correction for multiple comparisons). D: Representation  
1012 of the systems level state induced by each of the drugs in 2D-space with axes defined by the main

1013 spectral differences [Control vs. PD] and [Control vs. Dyskinesia]<sub>Ortho</sub> Pearson correlation ( $R^2$ ) between  
1014 the individual AIM scores shown in 9A and mean coordinate value in the [Control vs Dyskinesia]<sub>Ortho</sub>  
1015 dimension of the states shown in 9D were: OL=0.729 , FL=0.777, Ax=0.621, Rot=0.566, Total=0.724.

1016

1017 **Fig. 10 Classification performance as a function of the number of principal components utilized**

1018 The classification performance for the eight states shown in Fig. 9B, plotted as a function of the  
1019 number of PCs used to represent the full space. The black line shows the performance when all eight  
1020 structures are used together. The colored lines show the performance when only data from a single  
1021 structure is used. The dashed line represents chance level of correctly assigning a data point to one  
1022 of the eight states. In this comparison each structure was represented by the average LFP spectral  
1023 contents of all electrode pairs in the structure. It can be noted that despite that the number of  
1024 electrodes differed (range: 5-9) classification performance was similar using the different individual  
1025 structures. As expected, classification performance was higher when combining the information in all  
1026 structures. It was also confirmed that the number of PCs used ( $n=30$ ) to compress the data prior to  
1027 numerical comparisons of state separability (e.g. in Fig. 9C) was sufficiently high to avoid significant  
1028 information loss (the performance curves appear to have plateaued much earlier).

1029

1030 **Fig. 11 Robustness of state space calibration across subjects shown by cross-validation of the**  
1031 **amantadine treated state**

1032 A classifier with three states (A Gaussian mixture model for parkinsonian, dyskinetic and dyskinesia  
1033 treated with amantadine;) was trained in the subspace spanned by the parkinsonian and dyskinetic  
1034 state in one animal and tested in the analogous subspace in a second animal. The concentric circles

1035 represent the Gaussians corresponding to the three states (black=PD, red=dyskinesia,  
1036 green=amantadine) that were fitted using data from the first animal only. The green crosses show  
1037 the positions of the samples from the amantadine treated state from the second animal after  
1038 calibration using the two reference states. The purple crosses show the positions of the same  
1039 samples but without calibration. With calibration the amantadine samples from the second animal  
1040 was correctly identified 85% of the time (i.e. the true positive rate), which is only a slight decrease  
1041 from the 89% achieved with the samples from the first animal, i.e. the data on which the classifier  
1042 was trained. The corresponding false positive rates were 7% and 3%, respectively. As a comparison,  
1043 the true positive rate without calibration was 26%.

1044

1045 **Fig. 12 Control experiment with LFP spectra of the intact hemisphere in hemi-lesioned rats are**  
1046 **similar to LFP spectra in non-lesioned animals.**

1047 Two experiments each were conducted in four non-lesioned rats, A-D, in the following referred to as  
1048 RecA<sub>1</sub>-D<sub>1</sub> and RecA<sub>2</sub>-D<sub>2</sub>. From each of these recordings, 10 min were chosen for further analysis. The  
1049 power spectral densities (PSDs) in dB<sub>pink</sub> during each 10-min period were then computed for each  
1050 structure as described in methods, i.e., based on 8-s windows with 50% overlap. Samples containing  
1051 the concatenated spectra from all structures in one hemisphere were constructed for each such 8-s  
1052 window, resulting in 149 samples each for the left and right hemisphere during the analyzed 10-min  
1053 period. The same was done for a 10-min period during the off- and on-L-DOPA period, respectively,  
1054 in one recording each of the hemi-lesioned rats I-IV. In summary, this resulted in the following data  
1055 sets, each having a size of 149 samples x n=4; Group: 1 - Left hemispheres from RecA<sub>1</sub>-D<sub>1</sub>, 2 - Right  
1056 hemispheres from RecA<sub>1</sub>-D<sub>1</sub>, 3 – Left hemispheres from RecA<sub>2</sub>-D<sub>2</sub>, 4 – Right hemispheres from RecA<sub>2</sub>-  
1057 D<sub>2</sub>, 5 – Control hemispheres from hemi-lesioned rats I-IV off L-DOPA, 6 – Control hemispheres from

1058 hemi-lesioned rats I-IV on L-DOPA, 7 – Lesioned hemispheres from hemi-lesioned rats I-IV off L-DOPA  
1059 (i.e., PD state), 8 – Lesioned hemispheres from hemi-lesioned rats I-IV on L-DOPA (i.e., dyskinetic  
1060 state). Displayed in this figure is the similarity of the samples in each dataset to the mean over all  
1061 samples in Group 1, with the similarity being measured as the Euclidean distance of each sample to  
1062 this mean. Box represents 25<sup>th</sup> to 75<sup>th</sup> percentile, i.e., the interquartile range (IQR) and red line marks  
1063 median value. Whiskers mark the range for values 1.5 x IQR above or below the 75<sup>th</sup> or 25<sup>th</sup>  
1064 percentile, respectively; data points outside this range are marked as outliers. Blues asterisks denote  
1065 median values for individual hemispheres in each group. Significant differences between these  
1066 median values on a group level were found between group 7, 8 and the control group (1;  $p < 0.05$ , t-  
1067 test with Bonferroni correction for multiple comparisons [ $n=7$ ]).

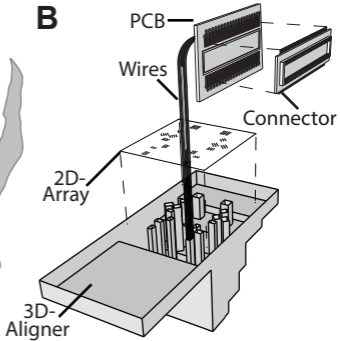
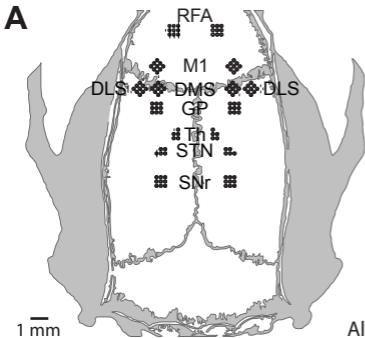
1068

1069 **Table 1**

1070 A: Number of samples used. Summary of the total number of 8s samples simultaneously collected in  
1071 all structures, per state (rows) and animal (columns). B: Comparison of classification performance  
1072 between individual structures and all structures. Classification performance for four states (control,  
1073 control + L-DOPA, PD, Dyskinesia) was evaluated for each and all structures (rows) in all animals  
1074 (columns) using the 30 first PCs, and is presented in the table as the fraction of correctly classified  
1075 states. Note that the best performance was always reached when all structures were utilized.

1076

1077 *Materials, data, Matlab-code and protocols used in this publication are readily available upon*  
1078 *request.*



**A)**

	Animal I	Animal II	Animal III	Animal IV
Control	4281	1661	509	434
Control + LDA	4311	1346	217	194
PD	4281	1661	509	434
Dys	4311	1346	217	194
Levetiracetam	449	-	-	-
Amantadine	1723	-	-	-
8-OH-DPAT	973	-	-	-
Way	298	-	-	-
Diazepam	749	-	-	-

**B)**

	Animal I	Animal II	Animal III	Animal IV
RFA	0.7242	0.5793	0.5970	0.9865
M1	0.6650	0.8387	0.6233	0.9570
DMS	0.5725	0.6875	0.6306	0.8631
DLS	0.5710	0.7220	0.5763	0.9100
GP	0.5743	0.5148	0.8957	-
Th	0.6182	-	-	0.9005
STN	0.5152	0.3755	0.6366	0.8997
SNr	0.4305	0.4487	0.7746	0.9514
All	0.9910	0.9782	1	1



