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Research Paper

Asymmetric dopaminergic degeneration and levodopa alter functional corticostriatal connectivity bilaterally in experimental parkinsonism

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ABSTRACT

Asymmetric dopamine loss is commonly found in early Parkinson's disease (PD), but its effects on functional networks have been difficult to delineate in PD patients because of variations in age, disease duration and therapy. Here we used unilateral 6-hydroxydopamine-lesioned (6-OHDA) rats and controls and treated them with a single intraperitoneal injection of levodopa (L-DOPA) before performing diffusion weighted MRI and resting state functional MRI (rs-fMRI). In accordance with a neurodegeneration of the nigrostriatal dopaminergic pathway, diffusion tensor imaging showed increased radial diffusivity and decreased fractional anisotropy in the lesioned substantia nigra. Likewise a deterministic connectometry approach showed increase of isotropic diffusion values in the medial forebrain bundle. rs-fMRI showed reduced interhemispheric functional connectivity (FC) between the intact and the 6-OHDA lesioned caudate-putamen. Unexpectedly, there was an increased FC between the 6-OHDA lesioned caudate-putamen and sensorimotor cortices of both hemispheres. L-DOPA reversed the FC changes between the dopamine denervated caudate-putamen and the sensorimotor cortices, but not the reduced interhemispheric FC between caudate-putamina. Similarly, L-DOPA induced *c-fos* expression in both sensorimotor cortices, but only in the dopamine-depleted caudate-putamen. Taken together, these data suggest that asymmetric degeneration of the nigrostriatal dopamine pathway results in functional asynchrony between the intact and 6-OHDA-lesioned caudate-putamen and increased interhemispheric synchrony between sensorimotor cortices. The results also indicate that the initial effect of L-DOPA is to restore functional corticostriatal connectivity rather than synchronize caudate-putamina.

1. Introduction

Parkinson's disease (PD) is characterized by a progressive and asymmetric loss of nigrostriatal dopaminergic neurons in substantia nigra pars compacta resulting in bradykinesia, rigidity and rest tremor (Kalia and Lang, 2015). PD is symptomatically treated with dopamine replacement, among which the dopamine precursor levodopa (L-3,4-dihydroxyphenylalanine) (L-DOPA), together with a peripheral dopa decarboxylase inhibitor, is the golden standard (Ibid.).

PD diagnosis is based solely on clinical assessment (Postuma et al., 2015), which often leads to misdiagnosis (Adler et al., 2014). Reproducible and robust objective biomarkers to support diagnosis and quantify disease progression would represent a scientific breakthrough (Mehta and Adler, 2016). A promising method for aiding diagnosis is diffusion weighted magnetic resonance imaging (dw-MRI), particularly diffusion tensor imaging (DTI). Indeed, a consistent finding in PD patients is a reduction of fractional anisotropy (FA) and an increase of mean diffusivity in the substantia nigra (e.g. Vaillancourt et al., 2009; Rolheiser et al., 2011; Skorpil et al., 2012; Cochrane and Ebmeier, 2013; Schwarz et al., 2013). Additional changes have also been observed in other brain regions, particularly in different white matter structures, but without consensus. Resting-state functional magnetic resonance imaging (rs-fMRI) is favorable to apply to PD as it requires nearly no effort from the patient. It has therefore been utilized in PD patients, but with conflicting results (e.g. Palmer et al., 2010; Luo et al., 2014; Yu et al., 2013; Agosta et al., 2014). Accordingly, the variety of disease severity, treatment conditions and analysis methods has prevented a conclusive meta-analysis of the results from rs-fMRI in PD (Tahmasian et al., 2015). In contrast to clinical studies, animal models of parkinsonism offer advantages, including well-controlled age, therapy, environmental settings and genetic background.

Abbreviations: 6-OHDA, 6-hydroxydopamine; CPu, caudate putamen; DAT, dopamine transporter; DTI, diffusion tensor imaging; dw-MRI, diffusion weighted MRI; FA, fractional anisotropy; FC, functional connectivity; FOV, field of view; GQI, generalized Q-ball imaging; iso, isotropic value of the diffusion orientation density function; L-DOPA, levodopa; MFB, medial forebrain bundle; MRI, magnetic resonance imaging; PD, Parkinson's disease; QSDR, Q-space diffeomorphic reconstruction; RD, radial diffusivity; ROI, region of interest; rs-fMRI, resting-state functional MRI; SSC, sodium chloride–sodium citrate buffer..

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Table 1
Statistical tests performed on the datasets obtained with resting-state functional MRI.

Experiment 1			
One sample *t*-tests	Group tested		*p*-Value threshold
	6-OHDA		0,01
	Control		0,01
Two sample *t*-tests	Group 1	Group 2	*p*-Value threshold
	6-OHDA	Control	0,05
Experiment 2			
One sample *t*-tests	Group tested		*p*-Value threshold
	6-OHDA drug naive		0,01
	6-OHDA acute treated		0,01
	Sham drug naive		0,01
Two sample *t*-tests	Group 1	Group 2	*p*-Value threshold
	6-OHDA drug naive	Sham drug naive	0,05
Paired *t*-tests	Group 1	Group 2	*p*-Value threshold
	6-OHDA acute treated	6-OHDA drug naive	0,05

To experimentally study effects of asymmetric dopamine loss in a highly controlled manner, unilateral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) of rodents is commonly used (Herrera-Marschitz et al., 2010). 6-OHDA is retrogradely taken up via the dopamine transporter into dopaminergic neurons where it leads to cell death by oxidative stress within a few days. Using structural MRI it has been reported that 6-OHDA injections in the nigrostriatal pathway results in an edema followed by a hypointensity and an increase in T2 relaxation time interpreted as iron accumulation (Kondoh et al., 2005; Soria et al., 2011; Virel et al., 2014). In contrast to the aforementioned data with DTI in PD patients, it was reported that intrastriatal injection of 6-OHDA increases nigral FA in rats (Van Camp et al., 2009).

To elucidate structural and functional connectivity in experimental PD and in response to L-DOPA under controlled conditions, we performed DTI, connectometry and rs-fMRI in the 6-OHDA experimental model of parkinsonism at baseline and following a single injection of L-DOPA.

2. Materials and methods

2.1. Animals and lesioning protocol

Male Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany), weighing 150 g, were used in this study. Experiments were performed in agreement with the European Communities Council (86/609/EEC) and approved by the Stockholm North Ethical Committee (Ethical permit # N245/11). Animals were arranged in two experimental cohorts. Both cohorts included rats lesioned with 6-OHDA application in the MFB. These 6-OHDA-lesioned rats were compared with naive control rats (experimental cohort 1) or sham-lesioned rats (experimental cohort 2 and ex-vivo experiment). To protect noradrenergic neurons, rats were systemically treated (i.p.) with a mixture of 25 mg/kg desipramine (Sigma–Aldrich, Saint-Louis, Missouri, USA) and 5 mg/kg pargyline (Sigma–Aldrich). They were then anesthetized with 80 mg/kg ketamine (i.p.; Pfizer, New-York, USA) and 5 mg/kg xylazine (i.p.; Bayer, Leverkusen, Germany) and mounted in a stereotactic frame. A hole in the skull was drilled and 12.5 μg of 6-OHDA in 2.5 μL of 0.01% ascorbate (Sigma–Aldrich) (6-OHDA groups) or 2.5 μL of 0.01% ascorbate (sham groups) was injected into the MFB of the right hemisphere (AP, −2.8 mm; ML, −2.0 mm; and DV, −9.0 mm relative to bregma) (Paxinos and Watson, 1998). The skin incision was sutured and post-operative pain relief was given using 0.01 mg/kg injections of Temgesic (s.c.; Indivior UK Limited, Slough, UK) directly after the surgery and twice more within 48 h. Two weeks after unilateral 6-OHDA lesioning, rats were administered 1 mg/kg of the dopamine D1/D2 receptor agonist apomorphine (i.p.; Sigma–Aldrich) and observed for 30 min. Since 6-OHDA-lesioning results in supersensitization of dopamine receptors, a contralateral rotational response can be quantitated in successfully dopamine denervated animals (Ungerstedt, 1971; Jorge et al., 1975). Only rats rotating >100 turns contralateral to the lesioned hemisphere during these 30 min were included in further experiments. MRI scanning was performed four weeks after the 6-OHDA lesioning.

2.2. Ex vivo dw-MRI experiment

2.2.1. Animals and tissue preparation

Eight rats were lesioned (four 6-OHDA-lesioned and four sham-lesioned) using the aforementioned protocol. Four weeks after the surgery, the rats were anesthetized with ketamine/xylazine and perfused

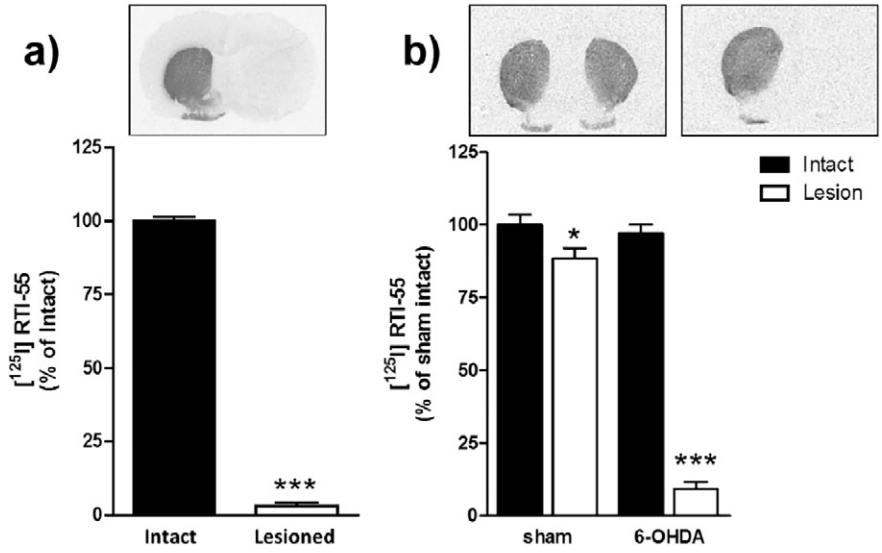

Fig. 1. 6-OHDA injection in the right medial forebrain bundle caused a near-complete dopaminergic denervation of the corresponding caudate putamen. Autoradiograms of DAT as detected by [^{125}I]RTI-55 at the level of the caudate putamen (**a** and **b**) and quantified relative to the intact hemisphere for experiments 1 (**a**) and relative to the intact hemisphere of sham-lesioned animals for experimental cohort 2 (**b**). DAT, Dopamine Transporter. Number of animals per group: a) 6-OHDA lesioned rats ($n = 8$); b) 6-OHDA lesioned rats ($n = 8$) and sham-lesioned rats ($n = 5$). $^{*}p < 0.05$; $^{***}p < 0.0001$. Intact, intact hemisphere; Lesion, lesioned hemisphere.