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Neuronal firing activity and gene expression changes in the subthalamic nucleus after transplantation of dopamine neurons in hemiparkinsonian rats

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ABSTRACT

Dopamine (DA) depletion in the nigrostriatal system leads to basal ganglia dysfunction both in Parkinson's disease (PD) and in 6-hydroxy dopamine (6-OHDA)-lesioned rats with neuronal hyperactivity in the subthalamic nucleus (STN), i.e. increased firing rate and burst activity, together with enhanced beta oscillatory activity. Moreover, intrastriatal transplantation of DA neurons has been shown to functionally re-innervate the host striatum and restore DA input.

However, the effects of those transplanted cells on the STN are not well characterized. Therefore, we transplanted cells, derived from the ventral mesencephalon of E12 rat embryos, intrastriatally in the unilateral 6-OHDA-lesioned rat model of PD. We combined behavioral and histological findings with electrophysiological extracellular recordings in the STN, as well as qRT-PCR analyses of dopaminergic, GABAergic, and glutamatergic transporter and receptor genes in the striatum and the STN. Transplanted animals displayed improved rotational behavior after amphetamine injection by 50% in rats with small grafts (586 ± 109 SEM dopamine cells), or even overcompensation by 116% in rats with large grafts (3486 ± 548 SEM dopamine cells). Electrophysiological measurements revealed, that in rats with large grafts burst activity was not affected, while STN neuronal firing rate, as well as beta oscillatory activity was alleviated, whereas small grafts had less impact. Interestingly, both behavioral and electrophysiological measures were dependent on the number of surviving tyrosine hydroxylase positive cells. Although grafted rats displayed restored expression of the GABA synthesizing enzymes *Gad65* and *Gad67* in the striatum compared to naive rats, the grafts induced a decreased mRNA expression of dopamine receptor *Drd2*, glutamate receptors AMPA3, NMDA2A, and NMDA2B, and glutamate transporter *Eaat3*. Interestingly, the NMDA receptor subunit 2B and glutamate transporter *Eaat3* were also less expressed in the STN of grafted animals compared to naive rats.

In summary, DA grafts restore functional deficits and cause partial improvement of subthalamic neuronal activity. Incomplete recovery, however, may be due to decreased receptor gene expression induced by DA grafts in the striatum and in the STN.

Introduction

The nigrostriatal dopaminergic pathway modulates the cortical glutamatergic input of the striatum, which is then further processed within the basal ganglia (BG). In Parkinson's disease (PD), the progressive degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) leads to decreased striatal DA levels. This causes excessive excitatory glutamatergic activity of the subthalamic nucleus (STN) and increased activity of the BG output nuclei, the substantia nigra pars reticulata (SNpr) and the globus pallidus internus (GPi). Changes in the firing patterns within and between BG regions, e.g. a greater tendency to discharge in bursts and a higher oscillatory beta band activity (13–30 Hz), as recorded in the STN and the GPi of PD patients, are also associated with the pathophysiology of PD (Hashimoto et al., 2003; Litvak et al., 2011; Trottenberg et al., 2007; Wichmann and DeLong, 2006).

Standard PD therapy restores DA deficiency by administration of the DA precursor L-3,4-dihydroxyphenylalanine (L-DOPA) and DA receptor agonists, e.g. apomorphine. The positive effect of those drugs on PD

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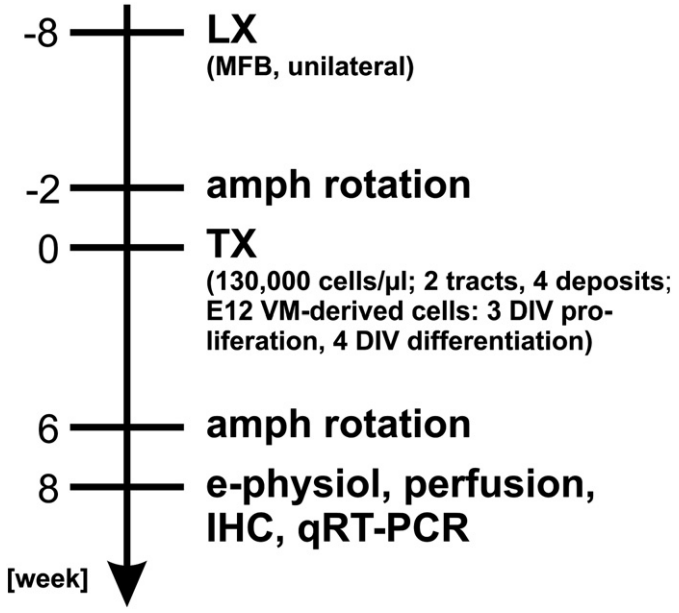

Fig. 1. Experimental design. *HP-Control*, *HP-Small Graft*, and *HP-Large Graft* rats were lesioned eight weeks before intrastriatal transplantation of in vitro expanded and differentiated murine fetal DA cells (for detailed description of experimental groups see Table 1). *Naive Control* animals served as healthy controls. Lesion severity and graft functionality were evaluated by means of amphetamine-induced rotation two weeks before and six weeks after the transplantation respectively. A subset of rats was subjected to electrophysiological experiments recording subthalamic neural activity and immunohistochemical analyses. Another group was subjected to qRT-PCR examination. LX = lesion surgery; amph rotation = amphetamine-induced rotation; TX = transplantation surgery; E12 VM-derived cells = embryonic day 12-derived cells; DIV = days in vitro; e-physiol = electrophysiological recordings; IHC = immunohistochemistry; qRT-PCR = quantitative reverse-transcribed polymerase chain reaction.

motor symptoms is accompanied by both reduced neuronal firing rate and beta oscillatory activity in the STN (Levy et al., 2001; Weinberger et al., 2006). Long-term treatment with L-DOPA, however, causes severe motor complications (Cenci and Lindgren, 2007). Intrastriatal transplantation of DA neurons may be an alternative to restore deficient DA supply allowing a more physiological and less pulsatile DA delivery. Although intrastriatal transplantation trials have been halted due to unwanted side effects (Freed et al., 2001; Olanow et al., 2003), this treatment holds great promise: it might both stop the progression of disease and restore altered physiology (Brundin et al., 2010; Dunnett and Rosser, 2011; Hickey and Stacy, 2011). In this context, Richardson et al. (2011) recently reported on a PD patient with partially restored GPi neuronal activity after intrastriatal transplantation of DA cells.

Injection of 6-hydroxy dopamine (6-OHDA) into the rat medial forebrain bundle (MFB) leads to the striatal loss of DA and abnormal neuronal activity in the BG, which closely parallels the findings in PD (Alam et al., 2012; Deumens et al., 2002; Mallet et al., 2008a). In this model, intrastriatal transplantation of embryonic dopaminergic neurons ameliorates DA agonist-induced rotations (Jungnickel et al., 2011; Klein et al., 2007). Intriguingly and also close to the patient situation, the DA-depleted striatum of 6-OHDA-lesioned rats contains increased levels of GABA-synthesizing enzymes (Gad65, Gad67; Soghomonian and Martin (1998)) and GABA (Coune et al., 2013). This is accompanied by enhanced neuronal activity in the striatum and altered neuronal activity in downstream BG nuclei (Hammond et al., 2007).

One study reported that after striatal implantation of mouse-to-rat xenografts in hemiparkinsonian rats the downstream parkinsonian basal ganglia firing patterns were normalized to some extent (Gilmour et al., 2011). In our study we further characterized intrastriatal rat allografts in the 6-OHDA PD rat model by electrophysiological extracellular recordings and local field potentials in the STN and gene expression analysis in the STN and striatum, and correlated these data with results from the amphetamine-induced rotation paradigm.

Material and methods

Experimental design

Forty-nine adult female Sprague Dawley rats from Charles River (Germany) weighing 200–250 g at the beginning of the experiments were used in this study. Animals were housed in cages of three to four and kept in temperature- and humidity-controlled rooms on a 14 h light/10 h dark schedule with food and water available ad libitum. All experimental protocols followed the German animal protection act and were approved by the local authorities (Bezirksregierung LAVES Hannover, Germany).

Thirty-eight animals received a unilateral 6-OHDA lesion of the right medial forebrain bundle. Eleven rats served as naive controls. Six weeks after lesion surgery amphetamine-induced rotation was performed with all lesioned hemiparkinsonian (HP) animals (Fig. 1), and the animals were matched into experimental groups based on their rotation scores (see Table 1 for details). Seven to eight weeks after 6-OHDA infusion, 19 animals were intrastriatally transplanted with in vitro expanded and differentiated DA progenitor cells derived from ventral mesencephali (VM) of embryonic day 12 (E12) rat embryos. Rotational bias was re-analyzed six weeks post grafting. One to two weeks later either electrophysiological and immunohistochemical or qRT-PCR analyses were performed (Fig. 1).

6-OHDA lesion surgery and amphetamine-induced rotational behavior

Lesion surgery was performed under general anesthesia with chloral hydrate (370 mg/kg; i.p.). Animals received two stereotaxic injections of 6-OHDA hydrobromide (free base 3.6 µg/µl in 0.02% L-ascorbate-saline, Tocris) into the right MFB at the following coordinates (in mm according to bregma and dura (Paxinos and Watson, 2006): first tract anterior–posterior (AP) −4.4, lateral (LAT) −1.2, dorso-ventral (DV) −7.8, tooth bar (TB) −2.4, injection volume 2.5 µl; second tract AP −4.0, LAT −0.8, DV −8.0, TB +3.4, injection volume 3 µl. Injections

Table 1
Experimental groups: HP = hemiparkinsonian; 6-OHDA = 6-hydroxy dopamine; IHC = immunohistochemistry; E-Physiol. = electrophysiology; Amph Rotation = amphetamine-induced rotation; qRT-PCR = quantitative reverse-transcribed polymerase chain reaction; STR = striatum; STN = subthalamic nucleus; TX = transplantation; E12 VM cells = embryonic day 12 ventral mesencephalon-derived cells; n.d. = not determined.

ID	Comments	E-Physiol. Amph rotation IHC (n)	qRT-PCR Amph rotation (n)
Naive Control	No surgery	4	7
HP-Control	6-OHDA injection, no sham surgery (*HP-Only*)	4	7
	6-OHDA injection, capillary injection, vehicle infusion, no cells (*HP-Cannula*)	4	n.d.
	6-OHDA injection, capillary injection, no vehicle, no cells (*HP-Vehicle*)	4	n.d.
HP-Small Graft	6-OHDA injection, TX of E12 VM cells	6	n.d.
HP-Large Graft	6-OHDA injection, TX of E12 VM cells	6	7