Neuroscience 360 (2017) 197–209

NEURONAL FIRING ACTIVITY IN THE BASAL GANGLIA AFTER STRIATAL TRANSPLANTATION OF DOPAMINE NEURONS IN HEMIPARKINSONIAN RATS

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Abstract—The loss of nigral dopaminergic neurons and the resulting dopamine (DA) depletion in the striatum (STR) lead to altered neuronal activity and enhanced beta activity in various regions of the basal ganglia (BG) motor loop in patients with Parkinson's disease and in rodents in the 6-hydroxydopamine (6-OHDA)-lesioned rat model. Intrastriatal DA graft implantation has been shown to re-innervate the host brain and restore DA input. Here, DA cell grafts were implanted into the STR of 6-OHDA-lesioned rats and the effect on neuronal activity under urethane anesthesia (1.4 g/kg, injected intraperitoneally) was tested in the entopeduncular nucleus (EPN, the equivalent to the human globus pallidus internus), the output nucleus of the BG, and the globus pallidus (GP, the equivalent to the human globus pallidus externus), a key region in the indirect pathway. In animals, which were transplanted with cells derived from the ventral mesencephalon of embryonic day 12 rat embryos into the STR, the rotational behavior induced by DA agonists in 6-OHDA-lesioned rats was significantly improved. This was accompanied by alleviated EPN firing rate and reinstated patterns of neuronal activity in the GP and EPN. Analysis of oscillatory activity revealed enhanced beta activity in both regions, which was reduced after grafting. In summary these data indicate restoration of BG motor loop toward normal activity by DA graft integration.

Key words: 6-OHDA, basal ganglia, dopamine, electrophysiology, oscillatory activity, transplantation.

INTRODUCTION

In Parkinson's disease (PD), loss of nigrostriatal dopamine (DA) leads to reduced dopaminergic transmission in the striatum (STR) and disturbed neuronal activity in the direct and indirect pathway of the basal ganglia (BG) motor loop. This includes not only altered firing rates as suggested by the classic BG scheme, but also higher events of burst patterns and irregular firing, as well as disturbed oscillatory activity. Together, these changes functionally compromise related thalamic and cortical areas (DeLong and Wichmann, 2007; Galvan et al., 2015). Motor symptoms of PD are initially treated by administration of DA receptor agonists, which restore neuronal firing rates and beta oscillatory activity in the subthalamic nucleus (STN), although burst activity is not affected or even further enhanced (Levy et al., 2001; Weinberger et al., 2006). Long-term treatment with L-DOPA, however, causes severe motor complications, possibly because of the pulsatile application of treatment (Cenci and Lindgren, 2007). Intrastriatal transplantation of dopaminergic neurons may be an alternative to restore deficient DA supply allowing a more physiological and less pulsatile DA delivery. Recently, there has been renewed interest in this treatment because, in contrast to other therapies, it might both stop progression of disease and restore altered physiology (Barker et al., 2015, 2016). In this context, Richardson et al. (2011) reported on a PD patient with partially restored globus pallidus internus neuronal activity after transplantation of DA cells into the STR.

Using the 6-hydroxydopamine (6-OHDA) rat model of PD, we previously showed that eight weeks after implantation, DA grafts restore functional deficits and cause partial improvement of STN neuronal activity, i.e., neuronal firing rate and beta oscillatory activity were normalized. Burst activity and measures of irregularity,

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Abbreviations: 6-OHDA, 6-hydroxydopamine; AP, anteroposterior; BG, basal ganglia; CV, coefficient of variation; DA, dopamine; DBS, deep brain stimulation; DV, dorsoventral; ECoG, electrocorticogram; EPN, entopeduncular nucleus; GP, globus pallidus; GPe, globus pallidus externus; GPi, globus pallidus internus; IHC, immunohistochemistry; ISI, inter-spike interval; LFPs, local field potentials; MCtx, motor cortex; MFB, medial forebrain bundle; ML, mediolateral; PBS, phosphate-buffered saline; PD, Parkinson's disease; REM, rapid eye movement; SEM, standard error of the mean; STN, subthalamic nucleus; STR, striatum; STWA, spike-triggered waveform average; SU, single unit; TB, tooth bar; TH, tyrosine hydroxylase; TX, transplantation.

http://dx.doi.org/10.1016/j.neuroscience.2017.07.069
0306-4522/

however, were not affected or even enhanced (Rumpel et al., 2013).

To further increase our understanding of neuronal activity in the BG motor loop of a grafted brain, under urethane anesthesia, we examined the effect of striatal DA grafts on neuronal activity of the globus pallidus, which is divided into an internal part (globus pallidus internus, GPi) and an external part (globus pallidus externus, GPe). While in the classical scheme of BG, the GPi is one of the output regions, so far, the GPe has been regarded a relay of the indirect pathway, which sends information to the glutamatergic STN. However, more recent work has redefined the GPe region as central for BG information processing (Deffains et al., 2016; Gurney et al., 2001), since it is reciprocally connected to the STN and to the BG output nuclei (substantia nigra pars reticulata and GPi), but also directly innervates the substantia nigra pars compacta, as well as thalamic and cortical regions. Also, about one third of GPe neurons project back to the STR (Bevan et al., 1998; Kita and Kitai, 1994; Sato et al., 2000).

The aim of our present work was to examine the effect of DA graft implantation into the STR of 6-OHDA-lesioned rats on neuronal activity of the entopeduncular nucleus (EPN, the homologous structure to the GPi in the rat) and globus pallidus (GP, the homologous structure to the human GPe). Notably, in this study, we chose to evaluate the animals 12 weeks after graft implantation to allow long-term and full integration of grafts into the STR.

EXPERIMENTAL PROCEDURES

All experiments were conducted in accordance with the German animal protection act and were approved by the local authorities (Bezirksregierung LAVES Hannover, Germany).

Animals and experimental design

Twenty-one adult female Sprague–Dawley rats from Janvier (St. Berthevin, France) were used in this study. The animals weighing 250 g at the start of the experiments were housed under 14-h light/10-h dark cycle with free access to food and water. Five animals served as naive *Control* group ($n = 5$). Sixteen animals received a unilateral lesion of the right medial forebrain bundle (MFB) using 6-OHDA and were evaluated in the apomorphine- and amphetamine-induced rotation test six weeks after lesion (pre-TX). Three animals died during the surgical procedure. Ten of the lesioned animals exhibited $\geq$four full contralateral body turns/min (apomorphine) and $\geq$six full ipsilateral body turns/min (amphetamine), respectively, and were matched into experimental groups *6-OHDA* and *Transplant* based on their rotation scores. Eight weeks after lesion, the *Transplant* group ($n = 5$) received implantation of DA cell grafts into the right-lesioned STR. The *6-OHDA* group ($n = 5$) was kept as hemiparkinsonian control. Ten weeks after grafting, animals from both groups were re-tested in the drug-induced rotation tests (post-TX). The experiment was terminated 12 weeks after transplantation surgery (20 weeks after the 6-OHDA lesion). Electrophysiological recordings in the EPN and GP were performed with subsequent sacrifice and immunohistochemical (IHC) analysis of the STR (each group $n = 5$; Fig. 1).

Lesion surgery

Stereotaxic lesion surgery was performed by unilateral injection of a total of 19.8 μg 6-OHDA hydrobromide (3.6 μg/μl (calculated as free base) in 0.02% L-ascorbate-saline; Tocris Bioscience, Bristol, UK) under general anesthesia with chloral hydrate (370 mg/kg; injected intraperitoneally, Sigma–Aldrich, Steinheim, Germany) as described previously (Rumpel et al., 2013). Briefly, animals received two injections of 6-OHDA to target the right MFB at the following coordinates (in mm according to bregma and dura according to Paxinos and Watson (2006): 1) anteroposterior (AP) −4.4, mediolateral (ML) −1.2, dorsoventral (DV) −7.8, tooth bar (TB) −2.4, injection volume 2.5 μl; 2) AP −4.0, ML −0.8, DV −8.0, TB + 3.4, injection volume 3 μl. 6-OHDA was delivered using a 10-μl Hamilton syringe with an injection rate of 1 μl/min. The needle was left in the brain for additional 3 min to allow diffusion before being slowly retracted. In addition to chloral hydrate, we applied a few drops of 1% lidocaine (AstraZeneca GmbH, Wedel, Germany) on the skull of the animals prior to removal of periost and drilling. Also, animals received intraoperative analgesia (metamizol, 100 mg/kg; injected subcutaneously, Zentiva Pharma GmbH, Frankfurt, Germany). Analgesic treatment was continued for additional three days post-surgery in the drinking water. As post-operative care, rats were supplied with 5 ml 0.9% saline (injected subcutaneously, Braun, Melsungen, Germany) and maintained under infrared lamps until recovery.

Preparation of fetal ventral mesencephalic tissue and transplantation surgery

Ventral mesencephalic tissue was harvested from 12-day-old (E12) rat embryos (crown-rump length of 6 mm) and a single-cell suspension prepared according to a modified version of the cell suspension technique (Bjorklund et al., 1983; Nikkhah et al., 1994). In our previous study (Rumpel et al., 2013), the differentiation period was four days and the survival of TH-ir cells was analyzed eight weeks after transplantation. Thereafter, we modified our protocol, i.e., we used co-layer instead of monolayer with only two days of cell differentiation together with a survival time of 12 weeks after transplantation. This procedure resulted in a higher TH-ir cell number of surviving grafted neurons, as reported in Rumpel et al. (2015). Therefore, in the present study, we used a similar protocol, i.e., cells differentiated *in vitro* for two days prior to transplantation and analysis of TH-ir cells after survival of 12 weeks after transplantation. In brief, the cells were plated on 6-well plates coated with polyornithine (0.1 mg/ml, Sigma–Aldrich) and laminin (6 μg/ml, Sigma–Aldrich). After one day of attachment, cells were proliferated for three days and differentiated for two additional days. For implantation, cells were washed with