Experimental Neurology 291 (2017) 8–19

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

# Transplantation site influences the phenotypic differentiation of dopamine neurons in ventral mesencephalic grafts in Parkinsonian rats

Marija Fjodorova *[,1], Eduardo M Torres, Stephen B Dunnett

*Brain Repair Group, School of Biosciences, Cardiff University, Museum Avenue, Cardiff, Wales CF10 3AX, UK*

ARTICLE INFO

*Article history:*
Received 27 October 2016
Received in revised form 17 January 2017
Accepted 24 January 2017
Available online 25 January 2017

*Keywords:*
Parkinson's disease
Cell transplantation
Ventral mesencephalon
Striatum
Dopamine
A9 neuron
Girk2

ABSTRACT

Foetal midbrain progenitors have been shown to survive, give rise to different classes of dopamine neurons and integrate into the host brain alleviating Parkinsonian symptoms following transplantation in patients and animal models of the disease. Dopamine neuron subpopulations in the midbrain, namely A9 and A10, can be identified anatomically based on cell morphology and ascending axonal projections. G protein-gated inwardly rectifying potassium channel Girk2 and the calcium binding protein Calbindin are the two best available histochemical markers currently used to label (with some overlap) A9- and A10-like dopamine neuron subtypes, respectively, in tyrosine hydroxylase expressing neurons both in the midbrain and grafts. Both classes of dopamine neurons survive in grafts in the striatum and extend axonal projections to their normal dorsal and ventral striatal targets depending on phenotype. Nevertheless, grafts transplanted into the dorsal striatum, which is an A9 input nucleus, are enriched for dopamine neurons that express Girk2. It remains to be elucidated whether different transplantation sites favour the differential survival and/or development of concordant dopamine neuron subtypes within the grafts. Here we used rat foetal midbrain progenitors at two developmental stages corresponding to a peak in either A9 or A10 neurogenesis and examined their commitment to respective dopaminergic phenotypes by grafting cells into different forebrain regions that contain targets of either nigral A9 dopamine innervation (dorsal striatum), ventral tegmental area A10 dopamine innervation (nucleus accumbens and prefrontal cortex), or only sparse dopamine but rich noradrenaline innervation (hippocampus). We demonstrate that young (embryonic day, E12), but not older (E14), mesencephalic tissue and the transplant environment influence survival and functional integration of specific subtypes of dopamine neurons into the host brain. We also show that irrespective of donor age A9-like, Girk2-expressing neurons are more responsive to environmental cues in adopting a dopaminergic phenotype during differentiation post-grafting. These novel findings suggest that dopamine progenitors use targets of A9/A10 innervation in the transplantation site to complete maturation and the efficacy of foetal cell replacement therapy in patients may be improved by deriving midbrain tissue at earlier developmental stages than in current practice.



## 1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised pathologically by a substantial loss of melanised dopamine neurons in the substantia nigra pars compacta (SNpc) and a subsequent failure to supply dopamine to the putamen, which results in impaired motor, cognitive and neuropsychiatric functions in patients. There is no therapy available to cure PD and current pharmacological treatments, such as levodopa, only address the symptoms by restoring dopamine transmission in the striatum, alleviating some motor deficits but often having little effect, or even impairing, cognitive functions (Hernandez et al., 2014; Obeso et al., 2011; Schneider et al., 2013). In contrast, a series of proof-of-principle clinical trials demonstrated that human foetal ventral mesencephalic (VM) dopamine progenitors, ectopically transplanted into the putamen and in some cases also into either the caudate nucleus or the SNpc, resulted in significant long-term clinical improvement of motor function in patients and in several cases there was no further need for levodopa treatment (Freed et al., 1992; Hagell et al., 1999; Kefalopoulou et al., 2014; Lindvall et al., 1992; Lopez-Lozano et al., 1997; Mendez et al., 2002, 2005, 2008; Wenning et al., 1997). We have recently demonstrated that human VM transplants are also capable of ameliorating non-motor dysfunctions in a rat model of PD (Lelos et al., 2016).

The subtypes of dopamine neurons within embryonic VM grafts have been the focus of many studies. Based on anatomical morphology

* Corresponding author at: Neuroscience and Mental Health Research Institute, School of Biosciences, Cardiff University, Hadyn Ellis Building, Maindy Road, Cardiff, Wales CF24 4HQ, UK.
*E-mail address:* fjodorovam@cf.ac.uk (M. Fjodorova).
[1] Present address: Neuroscience and Mental Health Research Institute, School of Biosciences, Cardiff University, Hadyn Ellis Building, Maindy Road, Cardiff, Wales CF24 4HQ, UK.

http://dx.doi.org/10.1016/j.expneurol.2017.01.010

from histochemical descriptions, out of nine dopamine cell groups in the brain three are present in the developing mammalian midbrain – A9 neurons of the SNpc, A10 neurons of the ventral tegmental area (VTA), and A8 neurons of the retrorubral field (Dahlstrom and Fuxe, 1964). Anatomically the key sites are the SNpc and VTA collectively containing ≈90% of all dopamine neurons in the rat midbrain (German and Manaye, 1993; Nair-Roberts et al., 2008). In a very simplified model, A9 neurons and their ascending axonal projections to the dorsal striatum (dSTR) in rodents (caudate nucleus and putamen in primates) comprise the nigrostriatal pathway while A10 neurons in the mesocorticolimbic pathway extend their axons primarily to the ventral striatum, also known as nucleus accumbens (N.Acc) and cortical areas, but also to the olfactory tubercle, septum and amygdala (reviewed in Björklund and Dunnett, 2007).

Subsequently, A9 and A10 neurons in the midbrain can be identified based on cell morphology and expression profiles. Tyrosine hydroxylase-immunoreactive (TH-ir) A9 neurons located in the ventral tier of the SNpc as well as the ventrolateral region of the VTA are large and angular in shape and most co-express G protein-gated inwardly rectifying potassium channel 2 (Girk2), while TH-ir A10 neurons located in the VTA and dorsal tier of the SNpc are smaller and round in shape and mostly co-express the calcium binding protein Calbindin (Reyes et al., 2012; Thompson et al., 2005). However, authors also reported that strong levels of Girk2 protein can be detected in up to 5–25% of Calbindin-ir/TH-ir neurons in the dorsal tier of the SNpc in mice and humans. Nevertheless, several studies have demonstrated that these distinctive morphological features of the two dopamine neuron populations are retained after transplantation of embryonic VM and Girk2 and Calbindin currently are best available surrogate markers that can be used to define A9- and A10-like neurons once their positional criteria are lost within grafts (Bye et al., 2012; Grealish et al., 2010; Mendez et al., 2005; Thompson et al., 2005).

Increasing lines of evidence indicate that the restoration of function by dopamine grafts is mainly due to the presence of A9-like neurons (Grealish et al., 2010; Kuan et al., 2007). Although both subpopulations of dopamine neurons are present in VM grafts in the dSTR, these grafts are enriched for the A9-like neurons (Bye et al., 2012; Somaa et al., 2015). The use of younger donor tissue and presence of meningeal cells overlying the VM in graft preparation have already been identified among the factors that favour the survival of A9-like neuron population in grafts in dSTR and result in a more effective cell replacement therapy in animal models of PD (Bye et al., 2012; Somaa et al., 2015; Torres et al., 2008, 2007). We set out to determine whether other variables, such as the host environment external to the graft, may have influenced this improved yield of functionally important A9-like neurons in transplants.

In particular, we questioned whether targets of A9 innervation in the dSTR might have contributed to these effects. To this extent, we investigated the commitment of dopamine neuron progenitors to the A9- and A10-like fates by grafting younger, embryonic day 12 (E12), and conventional age (E14) rat VM tissue into cerebral regions containing targets of either A9, A10 or noradrenaline innervation. We examined whether different transplantation sites favour the differential survival and/or development of concordant dopamine neuron subtypes within the grafts. We believe this to be the first systematic comparison of A9-/A10-like cell populations in rat VM grafts transplanted at different developmental stages into various environments in the context of PD.

## 2. Methods

### 2.1. Subjects

Sprague Dawley rats (Charles River, UK) were housed under standard conditions on a 14 h:10 h light/dark cycle with ad libitum access to food and water. All experiments were conducted under UK Home Office personal and project licences in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 and with the approval of the local Cardiff University Ethics Review Committee. Every effort was made to minimise the number of animals used and their suffering.

### 2.2. Experimental design

To test our hypothesis, female rats were unilaterally lesioned with the selective catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) into the nigrostriatal pathway and the lesions confirmed with amphetamine-induced rotations at 4 weeks post-lesion. Prior to transplantation, rats were separated into 8 balanced groups based on rotation performance [see Table 1; Group, $F_{(7,28)} = 0.03$, n.s.]. All transplants were made on the dopamine-depleted side of the brain. To reduce the number of animals used in the experiment each animal received 2 grafts implanted into widely separated sites in the denervated hemisphere, with the first transplant into either dSTR or N.Acc and the second transplant into either prefrontal cortex (PFC) or hippocampus (HPC). Efficacy of the grafts was confirmed with amphetamine-induced rotation tests at 4 and 6 weeks post-graft. Following the final rotation test animals were perfused and brain tissue taken for histological analysis.

### 2.3. Medial forebrain bundle lesions

All surgeries were performed in a Kopf stereotaxic frame where anaesthesia was maintained at 2–3% Isoflurane (AbbVie Ltd., Maidenhead, UK) in a 2:1 mixture of oxygen and nitrous oxide. For the unilateral nigrostriatal lesion, rats received an intra-cerebral injection of 6-OHDA neurotoxin into the right medial forebrain bundle following a recently refined protocol (Torres et al., 2011). Each animal was injected with 12 μg (free-base) of 6-OHDA hydrobromide (Sigma-Aldrich, Gillingham, UK) in 3 μl of 0.025% ascorbate saline at the following stereotaxic coordinates: −4.0 mm caudal from bregma (AP); −1.3 mm lateral from the midline (ML); −7.0 mm deep from dura (DV).

### 2.4. Procurement of embryonic ventral mesencephalic cells

Pregnant dams were bred in-house following a previously established protocol (Weyrauch et al., 2009). Briefly, females in oestrus were established by the vaginal lavage method on the morning of breeding day. Eligible females were paired with a male at 1:1 ratio in the male's home cage for a maximum of 3 h, typically between 10:30 a.m. and 1:30 p.m. Females then returned to their home cages and the day of mating was recorded as E0. At 10 a.m. on the morning of E12, rats were lightly anaesthetised in an induction chamber with 2–3% Isoflurane in oxygen and the pregnancy was confirmed if palpation of the abdomen revealed several swellings in the uterine horns. Embryos were harvested straight away if the desired embryonic age was E12, alternatively pregnant dams returned to their home cages until E14. The short mating-period protocol used here produced embryos with a crown-rump length (CRL) of 6.1 ± 0.3 mm at E12 and 11.2 ± 0.4 mm at E14 (CRL reported as mean ± standard deviation).

**Table 1**
Group allocation for transplantation.
Animals were distributed in 8 balanced groups based on the number of amphetamine-induced net ipsilateral rotations following 6-OHDA lesion. Data are presented as group means ± SEM, (number of animals per group [surviving primary grafts, dSTR or N.Acc/surviving secondary grafts, PFC or HPC]).

| Donor age | Transplantation site | | | |
|---|---|---|---|---|
| | dSTR PFC | N.Acc PFC | dSTR HPC | N.Acc HPC |
| E12 | 1242 ± 273 (n = 5 [5/5]) | 1268 ± 260 (n = 4 [4/3]) | 1307 ± 500 (n = 4 [4/4]) | 1245 ± 264 (n = 5 [5/5]) |
| E14 | 1190 ± 168 (n = 5 [6/5]) | 1388 ± 380 (n = 4 [3/2]) | 1298 ± 428 (n = 4 [4/4]) | 1245 ± 227 (n = 5 [5/4]) |