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Research Paper

Amelioration of non-motor dysfunctions after transplantation of human dopamine neurons in a model of Parkinson's disease

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ABSTRACT

Background: Patients suffering from Parkinson's disease (PD) display cognitive and neuropsychiatric dysfunctions, especially with disease progression. Although these impairments have been reported to impact more heavily upon a patient's quality of life than any motor dysfunctions, there are currently no interventions capable of adequately targeting these non-motor deficits.
Objectives: Utilizing a rodent model of PD, we investigated whether cell replacement therapy, using intrastriatal transplants of human-derived ventral mesencephalic (hVM) grafts, could alleviate cognitive and neuropsychiatric, as well as motor, dysfunctions.
Methods: Rats with unilateral 6-hydroxydopamine lesions to the medial forebrain bundle were tested on a complex operant task that dissociates motivational, visuospatial and motor impairments sensitive to the loss of dopamine. A subset of lesioned rats received intrastriatal hVM grafts of ~9 weeks gestation. Post-graft, rats underwent repeated drug-induced rotation tests and were tested on two versions of the complex operant task, before post-mortem analysis of the hVM tissue grafts.
Results: Post-graft behavioural testing revealed that hVM grafts improved non-motor aspects of task performance, specifically visuospatial function and motivational processing, as well as alleviating motor dysfunctions.
Conclusions: We report the first evidence of human VM cell grafts alleviating both non-motor and motor dysfunctions in an animal model of PD. This intervention, therefore, is the first to improve cognitive and neuropsychiatric symptoms long-term in a model of PD.

1. Introduction

In Parkinson's disease (PD), a common neurodegenerative disorder characterized neuropathologically by Lewy body formation and deterioration of the nigrostriatal pathway, patients present with motor, cognitive and neuropsychiatric symptoms. The non-motor dysfunctions are frequently reported as reducing quality of life and impacting on the well-being and health status of people with PD to a greater degree than any motor complications (Duncan et al., 2014; Hinnell et al., 2012). Non-motor symptoms are increasingly recognized as core dysfunctions of the disease, and indeed are typically observed prior to the onset of overt motor impairments (Hawkes, 2008; Meissner, 2012). Multiple aspects of function can become compromised, including impaired executive function, increased apathy, depression, impaired visuo–spatial abilities, and reduced motivation.

The effective alleviation of non-motor dysfunction is a critical element of any effective therapy, but there are currently no interventions that are capable of adequately targeting both motor and non-motor impairments. While pharmacological therapies, such as L-dopa, alleviate some motor dysfunctions in the majority of patients, they often have no impact upon, or can even impair, cognitive performance (Hernández et al., 2014; Obeso et al., 2011; Schneider et al., 2013; Zibetti et al., 2013). Systemic administration of dopamine-enhancing drugs is unlikely to improve cognitive dysfunctions effectively, given that both too little and too much dopamine are known to impair neural processing, and that optimal levels differ across different sub-regions of the brain (Cools and D'Esposito, 2011).

Cell replacement therapies hold considerable promise as a therapeutic intervention. In open-label clinical studies, intrastriatal grafts of foetal ventral mesencephalon (hVM; the region of the human foetal brain in which the nigrostriatal dopamine cells develop) produced long-term improvements in motor function, removing the need for pharmacological interventions in some patients (Freed et al., 1992; Kefalopoulou et al., 2014; López-Lozano et al., 1997; Wenning et al., 1997; Lindvall, 1997). Transplants of hVM have provided proof-of-principle that implantation of dopamine neurons can produce functional benefit and are pivotal in paving the way for transplantation of stem

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cell-derived dopamine neurons. However, little is known about the ability of hVM grafts to alleviate any non-motor dysfunctions observed in PD patients. Cognitive disability has not been comprehensively assessed in many clinical trials of transplantation in PD, making it difficult to determine the impact of cell therapies on this functional output (Ostrosky-Solís et al., 1988; Sass K et al., 1995).

Results obtained from rodent PD models, utilizing rat-derived VM, have revealed some improvements in visuo–spatial performance (Dowd and Dunnett, 2004; Heuer et al., 2013). While these studies provide encouraging data regarding the ability of cell therapies to target one aspect of non-motor impairment, it has yet to be revealed whether hVM can endow such effects (Lelos et al., 2012) and whether multiple domains of non-motor dysfunctions can be improved. Although several studies report long-term survival of hVM tissue in the rodent brain (Brundin et al., 1986; Strömberg et al., 1989, 2001, 2010), few studies investigated the functional efficacy of these grafts. For example, it has been reported that hVM grafts of 6–9 weeks gestation can reduce drug-induced rotational bias (Brundin et al., 1986, 1988; Clarke et al., 1999; Grealish et al., 2010; Strömberg et al., 1995, 1986). What has not been demonstrated is the capacity of these cells to impact upon the more debilitating non-motor dysfunctions that manifest in PD. Thus, the aim of this study was to investigate the functional impact of hVM grafts on non-motor and motor impairments.

2. Methods

To test our hypothesis, rats were pre-trained on a complex operant choice reaction time task in a 9-hole box apparatus, which measures visuospatial function and incentive motivation. Rats received lesions of the nigrostriatal pathway, by infusion of the selective neurotoxin 6-hydroxydopamine. After the grafting of hVM ectopically into the dorsal striatum, rats were tested for drug-induced rotation, followed by testing on two versions of the choice-reaction time task.

2.1. Subjects

Twenty-six female Lister-hooded rats (Charles River, UK) were maintained on a 14:10-h light/dark cycle. All rats were food restricted one week before commencing operant training/testing and for the duration of any operant experimental work, in order to motivate learning in the operant task. During food restriction, rats were maintained at 90% of their baseline weights. All experiments were conducted in compliance with the UK Animals (Scientific Procedures) Act 1986 under Home Office Licence No. 30/2498 and with the approval of the local Cardiff University Ethics Review Committee.

2.2. Apparatus

2.2.1. Operant chambers

The 9-hole chambers (Paul Fray, U.K.) were constructed of aluminium (25 × 25 cm) with a grid floor, and the back wall housed an array of nine holes, each of which contained a light-emitting diode to provide a visual stimulus, and a vertical infrared beam with a photocell detector, which detected nose poke responses into the hole. A food magazine in the middle of the opposite wall signalled the delivery of 45-mg sucrose reward pellets (TestDiet, IN, USA). On-line data collection was controlled by BNC software (Campden Instruments, UK).

2.2.2. Rotometers

A bank of 16 automated rotometer bowls (Rotorat, Med Associates, VT) (Ungerstedt and Arbuthnott, 1970) recorded the frequency of amphetamine-induced rotation for 90 min after i.p. injection of 2.5 mg/kg methamphetamine hydrochloride (Sigma-Aldrich, UK). Apomorphine-induced rotations were recorded for 60 min after subcutaneous injection of 0.05 mg/kg apomorphine hydrochloride hemihydrate (Sigma-Aldrich, UK). Rotation scores expressed as an average of ipsilateral minus contralateral rotations.

2.3. Experimental design

Rats (n = 26) were pre-trained for 7 weeks in the 9-hole operant chambers on the Bilateral choice reaction time task. A cohort of rats (n = 18) received infusion of 6-OHDA into the MFB. Eight rats remained as unoperated controls. At 2 weeks post-lesion, all rats underwent amphetamine-induced rotations and were tested in the operant box apparatus for 5 days to measure lesion-induced deficits. Rats were separated into equal groups (lesion only versus grafted) based on rotation and operant performance. One cohort of lesion rats (n = 10) then received intrastriatal transplantations of hVM tissue. All rats were immunosuppressed daily with intraperitoneal injections of 10 mg/kg cyclosporine (Sandoz Pharmaceutical, U.K.). Amphetamine-induced rotations were tested at 3 week intervals. At 14 weeks post-graft, rats were re-tested in the operant boxes, using two distinct versions of the task. At 20 weeks, rats underwent an apomorphine-induced rotational challenge and were then perfused and brains tissue taken for histological analysis.

2.4. Behavioural testing

Pre-training was conducted as previously described (Lindgren et al., 2014) (Fig. 1). Briefly, the central location in the array and a lateral "response" location one space away on the left and right of the central hole were utilized. Rats were trained to hold their noses in the central location for a variable delay to initiate brief presentation (200 ms) of a lateralised light. A correct response into the lateralised hole triggered delivery of a sucrose pellet into the magazine (45 mg, TestDiet, IN, USA). Errors were recorded and resulted in a 2 s time out. Failure to maintain hold in the central location for the variable delay duration resulted in a time out and an unusable trial. The session duration was 30 min.

The behavioural outputs were calculated as follows. *Trials usable*: trials in which the rat responded to the illuminated centre hole for the required delay, initiating the presentation of the stimulus light. *Accuracy*: percentage of correct responses, divided by the total number of responses made into the two available locations. *Reaction time*: mean latency to initiate a response by withdrawal of the nose from the centre hole location after the onset of the lateral stimulus light (on correct responses only). *Movement time*: mean latency to execute the lateralised nose poke response after removal of the nose from the centre hole (on correct responses only).

Post-graft, rats were tested in alternating one week blocks on the version of task described above ('Bilateral') and on an alternative version of the task ('Unilateral'). The Unilateral version of the task, in which both response locations are located on one side only, was used to probe response choice within contralateral space (Heuer et al., 2013; Ungerstedt and Arbuthnott, 1970). This more challenging version probes visuo–spatial performance by revealing more precisely the ability of the rat to generate and execute responses on the side of the body contralateral to the dopamine-depleting lesion. The same response location on the left was utilized (now referred to as 'Near') and the space immediately to the left of it was exposed ('Far').

2.5. Surgical procedures

2.5.1. Lesions

Rats were anaesthetised with isoflourane (2–4% with carrier gases oxygen and nitrous oxide) in a stereotaxic frame. The MFB was targeted with an injection of 12 μg (freebase) of 6-OHDA (Sigma; 3 μl of 4 μg/μl solution at 1 μl/min with 2 min diffusion) in 0.01% ascorbate saline at stereotaxic coordinates AP −4.4 and ML ±1.0 (from bregma) and DV −7.8 below dura.