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# Dopamine-rich grafts alleviate deficits in contralateral response space induced by extensive dopamine depletion in rats

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ABSTRACT

Unilateral infusion of 6-hydroxydopamine into the nigro-striatal pathway in the rat is the most common dopamine lesion model of Parkinson's disease. In the present study, we explore the impact of near complete unilateral loss of dopamine along the nigro-striatal pathway and subsequent cell replacement therapy in a choice reaction time task in rats, with assessment of spatial responding towards either side of the body (ipsilateral or contralateral to the lesion) on alternate days. Results indicated a stable contralateral deficit in response accuracy, reaction times and motor function for 50 consecutive days of testing, with no signs of recovery or compensation. All lesioned rats developed a near-hole bias and displayed prolonged movement and reaction times when responses had to be directed towards a distal response location on the side of the body contralateral to the lesion, as well as a smaller ipsilateral impairment in response accuracy and movement times. Grafts of dopamine-rich tissue into the denervated striatum improved some, but not all, of the deficits induced by the lesion. Specifically, grafted rats performed at a similar level to control animals when assessed on the ipsilateral side, they demonstrated a partial restitution of their ability to respond to far contralateral stimuli, and they exhibited a marked reduction in the time to complete all lateralised responses on both sides. The present characterisation of the task and the effects of cell replacement via primary fetal mesencephalic tissue demonstrate restorative properties in alleviating the marked spatial response bias induced by unilateral loss of dopamine.



## Introduction

To model Parkinson's disease (PD) in the laboratory, loss of dopamine cell projections from the SN to the striatum is typically induced in the rat using the unilateral 6-hydroxydopamine (6-OHDA) lesion (Bove et al., 2005; Deumens et al., 2002; Grealish et al., 2008; Kirik et al., 1998; Ungerstedt and Arbuthnott, 1970). This results in a 'hemiparkinsonian' syndrome with degeneration of dopaminergic cells on the ipsilateral side of the toxin injection. Behaviourally these unilateral lesioned rats will display impairments on the side opposite (contralateral) to the side of toxin injection. Specifically, lesioned rats display marked impairments in detecting and responding to visual stimuli (e.g. lights) on the contralateral side of the body (Brown and Robbins, 1989a, 1989b; Carli et al., 1985, 1989; Dowd and Dunnett, 2004, 2005a, 2005b; Ljungberg and Ungerstedt, 1976). A characteristic behavioural response profile of such a lesion can be found when unilateral lesioned rats are tested in a choice reaction time task conducted in the 9-hole operant chamber, in which rats have to report the occurrence of a lateralised stimulus light presented to either side of the animals' head. This paradigm reveals a characteristic response bias, which manifests as impaired accuracy, reaction and movement times when responses are directed to the side contralateral to the lesion, irrespective of the side of stimulus delivery (Carli et al., 1985, 1989; Dowd and Dunnett, 2004, 2005a, 2005b). This bias to respond preferably to the ipsilateral side of the lesion was characterised further by Brown and Robbins (1989a) who have shown that lesioned animals were able to respond accurately in contralateral space, but developed a response bias to a proximal response location when offered a choice between two response options (Brown and Robbins, 1989a, 1989b). The authors argue that dopamine depletion results in a distortion of spatial coding when responses have to be directed into a spatial location on the contralateral side of the lesion while remaining unimpaired when responding towards a spatial location on the ipsilateral side (Brown and Robbins, 1989a, 1989b).

To replace dopamine innervation in denervated target areas, cell replacement therapies have been developed involving transplantation of dopamine rich tissue harvested from the developing fetal ventral mesencephalon (VM) to provide a long-term restitution of synaptic DA release in the denervated striatum (Björklund et al., 1980, 2003; Perlow et al., 1979). Ectopic engraftment into the denervated striatum has shown to be a promising intervention to alleviate certain aspect of the impairment induced by 6-OHDA lesions in rats (Björklund and Stenevi, 1979; Dowd and Dunnett, 2004; Dowd et al., 2005; Dunnett and Bjorklund, 1999; Fray et al., 1983; Torres et

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al., 2008). Detailed analysis of lesion-induced impairments is imperative for the development and assessment of therapeutics for PD. Whereas on the lateralised version of the choice reaction time task the effects of the lesion were assessed long term, on the novel version introduced by Brown and Robbins (1989a, 1989b) the stability of the deficit is unknown and spontaneous recovery might impede the usefulness of the task to assess therapeutic interventions (Dowd and Dunnett, 2004, 2005a, 2005b). With an increase in the development of alternative cell sources to primary fetal tissue (Kriks et al., 2011), it is imperative not only to provide a detailed analysis of behavioural deficits induced in animal models of the disease, but also to fully characterise the parameters that can be improved by a given intervention. Choice reaction time tasks have been valuable in characterising response profiles induced by lesions (Baunez et al., 1995a, 1995b; Brasted et al., 1997; Brown and Robbins, 1989a, 1989b; Carli et al., 1985, 1989; Courtiere et al., 2005; Döbrössy and Dunnett, 1997; Dowd and Dunnett, 2005a, 2005b; Ward and Brown, 1996), to probe pharmacological challenges (Baunez et al., 1995a, 1995b; Blokland, 1998; Blokland et al., 2005; Scholtissen et al., 2006), assess transgenic animals (Fielding et al., 2012; Temel et al., 2006), test cell replacement therapies (Brasted et al., 1999a, 1999b, 2000; Dowd and Dunnett, 2004) and evaluate deep brain stimulation (Darbaky et al., 2003; Temel et al., 2005, 2006).

Although the acute effects of dopamine depletion have been tested after terminal lesions 5 days and 4 weeks post-lesion, no long-term assessment of the stability of the lesion has been reported. Stable lesions are imperative for the assessment of cell replacement therapies as there is a risk of spontaneous recovery with incomplete lesions due to compensatory mechanisms affecting the lesion stability and the risk that re-learning might overshadow the effects of the transplant (Dowd and Dunnett, 2004, 2005a, 2005b).

Here we use a novel variant of the lateralised choice reaction time task to provide a more in-depth analysis of both motor and non-motor deficits and recovery in dopamine-depleted and dopamine cell-rich grafted rats, as well as extending our understanding of the role of dopamine in responding in contralateral space. Specifically, we explore (i) the effects of near complete lesions aimed at the medial forebrain bundle (MFB) on a near/far version of the lateralised choice reaction time task, (ii) whether the lesion-induced deficit is stable over time, and (iii) the effects of restoring striatal dopamine levels by means of grafting dopamine rich neural precursors derived from E14 embryonic VM, on both motor and non-motor parameters.

## Materials and methods

All procedures were performed according to the United Kingdom Animals (Scientific Procedures) Act, 1986 and approved by local ethical review at Cardiff University.

### *Subjects*

A cohort of 32 female Lister Hooded rats (Charles River, UK) started operant training at 12 weeks of age (mean weight: 200–225 g). Rats were housed in standard laboratory cages with 3–4 rats per cage, at a constant temperature of $21 \pm 1$ °C and 50% humidity. The day–night cycle was set to 12:12 h, with the light turned on at 07:00 am. Rats were food restricted to 90% of their free-feeding body weights starting one week prior to operant training, by providing them with weighed amounts of food at the completion of each day's testing. They were allowed ad libitum access to water in the home cages throughout all stages of the experiment.

### *Apparatus*

Operant testing was conducted in standard 9-hole operant chambers (Campden Instruments, Loughborough, UK), which have been described in detail previously (Carli et al., 1983; Dowd and Dunnett, 2004). Briefly, the operant chamber is fitted with a horizontal curved array of 9 response holes which can be illuminated by a green LED and into which a nose poke is detected by break of an infrared beam. A food magazine is fitted in the opposite side of the chamber, to which a pellet dispenser is connected. During operant training and testing, only the centre hole in the array and the two adjacent holes on either the left or the right of the centre hole (depending on the day of testing, see below) were left open, whereas all other holes were covered with well blanks. The 9-hole boxes were controlled by the Cambridge Cognition Control software (Campden Instruments, version 1.23) running on a standard desktop PC using the Windows XP operating system.

### *Training*

Rats were habituated to the operant boxes during a 30 min session in which 30 precision food pellets (45 mg, Sandown Scientific, Hampton, Middlesex, UK) were delivered via the pellet dispenser to the food magazine at the beginning of the session. During the second magazine training session, rats were put into the operant chambers with the house light illuminated. After a short interval the house light was extinguished and the light behind the panel of the magazine was illuminated. Each nose poke into the illuminated magazine resulted in a pellet reward.

Upon conclusion of magazine training, nose poke training commenced. Initially, the centre hole (hole 5) in the array was uncovered and illuminated at the beginning of each trial while all other lights were turned off. A nose poke into the illuminated centre hole resulted in the delivery of a food pellet in the magazine. At the same time, the centre hole light was extinguished and the light in the magazine was illuminated until the food pellet was collected, at which point a new trial commenced.

Once all rats learned to nose poke, the two holes on just one side of the centre hole were uncovered (holes 3 and 4 to the left, or holes 6 and 7 to the right) alternating between the two sides on successive days. Rats were required to respond in the illuminated centre hole (hole 5), which resulted in illumination of one of the lateral holes (holes 3 and 4 on odd days, holes 6 and 7 on even days; see Fig. 1). The lateralised stimulus light remained lit until the rat responded with a nose poke, at which point a food pellet was delivered into the magazine. After achieving >80% correct trials on the basic task, the stimulus duration was gradually reduced from continuous to 200 ms and the required duration of the centre nose poke (hole 5) hold was gradually increased. Once all the rats performed at an asymptotic level on the task, at the longest centre poke duration (400 ms), the rats were trained on the final configuration for a period of three weeks. This consisted of sessions in which the centre hold duration was pseudo-randomly chosen between 4 different hold durations (50 ms, 100 ms, 150 ms, and 200 ms), and the duration of stimulus illumination was always 200 ms. The variable centre holds served to reduce the incidence of anticipated nose withdrawals from the centre hole, thereby enhancing the accuracy of the reaction time measure. The last two weeks of pre-lesion training was recorded as baseline data. A correct response resulted in the delivery of a food pellet into the food magazine, whereas an incorrect response or a premature withdrawal resulted in a "punishment" of a time out period of 5 s where all lights were extinguished (schematic outline in Fig. 1).

The main outcome measures recorded for each session are:

- *Trials usable*, the number of usable trials was defined as those in which the rat responded to the illuminated centre hole for the required delay, initiating the presentation of the stimulus light.
- *Accuracy*, the percentage of correct responses made on each side, for the near and the far hole respectively, divided by the total number of usable trials with the matching stimulus.