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Partial dopaminergic denervation-induced impairment in stimulus discrimination acquisition in parkinsonian rats: A model for early Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) produces progressive nigrostriatal dopamine (DA) denervation resulting in cognitive and motor impairment. However, it is unknown whether cognitive impairments, such as instrumental learning deficits, are associated with the early stage PD-induced mild DA denervation. The current study sought to model early PD-induced instrumental learning impairments by assessing the effects of low dose (5.5 μg), bilateral 6OHDA-induced striatal DA denervation on acquisition of instrumental stimulus discrimination in rats. 6OHDA ($n = 20$) or sham ($n = 10$) lesioned rats were tested for stimulus discrimination acquisition either 1 or 2 weeks post surgical lesion. Stimulus discrimination acquisition across 10 daily sessions was used to assess discriminative accuracy, or a probability measure of the shift toward reinforced responding under one stimulus condition (Sd) away from extinction, when reinforcement was withheld, under another (S^{Δ} phase). Striatal DA denervation was assayed by tyrosine hydroxylase (TH) staining intensity. Results indicated that 6OHDA lesions produced significant loss of dorsal striatal TH staining intensity and marked impairment in discrimination acquisition, without inducing akinetic motor deficits. Rather 6OHDA-induced impairment was associated with perseveration during extinction (S^{Δ} phase). These findings suggest that partial, bilateral striatal DA denervation produces instrumental learning deficits, prior to the onset of gross motor impairment, and suggest that the current model is useful for investigating mild nigrostriatal DA denervation associated with early stage clinical PD.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder associated with the loss of dopaminergic (DA) neurons in the nigrostriatal pathway. The nigrostriatal DA denervation produced by PD leads to compromises in motor function, such as akinesia, resting tremor, and rigidity, as well as cognitive impairment, including learning and memory deficits (Dubois and Pillon, 1997; Grahn et al., 2009; Kulisevsky and Pagonabarraga, 2009; Williams-Gray et al., 2009). While major maladaptive cognitive impairment tends to appear only in the later stages (Apaydin et al., 2002), evidence suggests that mild cognitive impairment is observable during the early stages of PD (Cooper et al., 1991; Costa et al., 2009; Laatu et al., 2004; Pillon et al., 1997). However, the difficulty in diagnosing individuals with PD prior to the onset of motor symptoms leaves uncertainty as to whether the PD-induced motor impairment contributes to these cognitive impairments, or whether the cognitive impairments are independent of motor impairment.

One area in which the relationship between motor and cognitive impairment is especially unclear relates to instrumental learning. Deficits in instrumental learning have been observed as a particular consequence of PD (Foerde and Shohamy, 2011; Frank et al., 2004; Sarazin et al., 2002). Instrumental learning is mediated by the dorsal striatum (dSTR) in a manner that is functionally and anatomically distinct (O'Doherty et al., 2004; Yin et al., 2004, 2005b), where habit learning is mediated by the dorsolateral STR (Yin et al., 2004)

Abbreviations: PD, Parkinson's disease; DA, dopamine; 6OHDA, 6-hydroxydopamine; dSTR, dorsal striatum; TH, tyrosine hydroxylase; RT, room temperature; VR, variable ratio; VI, variable interval; FR, fixed ratio; S-R, stimulus-response; R-O, response-outcome; PBS, phosphate buffered saline; HSD, highly significant differences; SEM, standard error of the mean.

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and goal-directed learning is mediated by the dorsomedial STR (Yin et al., 2005b). Since PD leads to a loss of nigrostriatal DA to both regions, instrumental learning can become impaired after DA denervation (Beeler, 2011; Grahn et al., 2009; Redgrave et al., 2010). The primary objective of the present study was to determine whether impairment in instrumental learning is apparent prior to the onset of gross motor symptoms, especially those that might negatively affect the expression of learning, such as akinesia.

The 6-hydroxydopamine (6OHDA) lesion model in rats is a well-established method for investigating the motor symptoms associated with nigrostriatal DA denervation (Blesa et al., 2012; Deumens, 2002). Previous research has indicated that partial, bilateral lesions to dSTR also produce cognitive impairment, even at low levels of DA denervation (Chen et al., 2011; Courtière et al., 2005; Haik et al., 2008; Lindner et al., 1999; Tadaiesky et al., 2008); however, there are conflicting reports in regards to the effects of low dose 6OHDA on behavior and dSTR DA depletions. For example, partial, bilateral 6OHDA (12 μg) lesions to dSTR produce a decrease in DA and deficits in spatial water T-maze performance. The spatial memory impairment was present in the absence of motor impairment (Haik et al., 2008). Lower concentrations of 6OHDA (e.g. 10.5 μg) in dSTR failed to produce deficits of both motor function and spatial water maze performance (Chen et al., 2011). Conversely, there are reports that a similar lesion using almost the same concentration of 6OHDA (12.5 μg) lead to large decreases in striatal DA intensity and substantia nigra DA cell counts and corresponded to spatial memory deficits as well as motor impairment (Lindner et al., 1999). These conflicting reports show that, within a specific range of 6OHDA concentration, there can be large variations in their effect on motor impairment and that the presence and extent of cognitive symptoms can vary greatly as a function of dose of 6OHDA.

Aside from variations in the dose of 6OHDA, another potential confound is the method of behavioral measurement, which may lead to the conflicting reports of motor impairment at low concentrations of 6OHDA. In particular, some behaviors may be more prone to both deficits in cognition and motor function. For example, reaction time performance in a choice task has been found to be impaired by bilateral (8 μg) lesions to dSTR (Courtière et al., 2005), indicative of cognitive impairment in choice behavior. However, measures of reaction time to model cognition are also associated with motor impairment in animal models (Scholtissen et al., 2006; Spirduso et al., 1985), either resulting from hypokinesia or akinesia, and motor timing is known to be impaired in clinical PD (Franz and Miller, 2002; Low et al., 2002). Additionally, a similar bilateral 6OHDA lesion (8 μg) has also been reported to produce greater depletions in dSTR DA tissue content which corresponded to both motor and cognitive impairment in reaction time performance (Amalric et al., 1995). Reaction time performance is highly sensitive to changes in motor function, especially the initiation of movement, depending on the dose of neurotoxin and resultant striatal dopamine depletion (Smith et al., 2002) These factors raise the question as to whether changes in reaction time can be interpreted purely as a result of cognitive impairment and highlight the need for a precise measure of cognitive performance independent of akinesia-like motor impairment.

The limiting factor of dose and measurement suggest that a less concentrated bilateral 6OHDA lesion to dSTR may provide the most effective model for displaying cognitive impairment without any gross motor deficits. These limitations may be addressed with an instrumental paradigm that can measure cognitive and motor function separately and simultaneously.

The current study sought to examine the effects of partial dopamine depletion on instrumental behavior using a stimulus discrimination acquisition procedure. The stimulus discrimination acquisition paradigm was designed to be sensitive to changes in striatal learning, independent of motor impairment such as akinesia. Rats were first trained to respond on a variable ratio (VR) schedule for reinforcement. After training they were stereotactically injected with low dose 6OHDA (5.5 μg in 2 μl saline/side) bilaterally to dSTR on each hemisphere. Then, either 1 or 2 weeks post-surgery, rats were tested for acquisition of stimulus discrimination across 10 daily testing sessions. Testing sessions were designed to maximize response rate and simultaneously measure instrumental learning, namely stimulus discrimination, dissociated from motor function. The separate post-surgical periods were examined to provide a time-course of these deficits, because findings have shown that 6OHDA may produce alterations in efficacy across time (Courtière et al., 2005; Deumens, 2002; Marinova-Mutafchieva et al., 2009; Na et al., 2010; Tadaiesky et al., 2008). After acquisition testing was completed, striatal tissue sections were assayed for tyrosine hydroxylase (TH) staining density to determine the extent of dSTR DA denervation. Lower dSTR TH is associated with incidental Lewy body disease and presymptomatic PD (Beach et al., 2008; Dickson et al., 2008, 2009), and therefore is a reliable measure of dSTR DA denervation. A separate cohort was tested for low dose 6OHDA-induced akinesia in the rotarod and open field activity test. It was hypothesized that low dose, bilateral 6OHDA would produce marked deficits in stimulus discrimination acquisition, measured by discriminative accuracy changes over time. No changes were expected in overall rates of responding, as an index of akinesia, or in separate tests of motor function after the low dose.

2. Material and methods

All studies were approved by the Institutional Animal Care and Use Committee (IACUC) at Central Michigan University and followed guidelines laid out in the *Guide for the Care and Use of Laboratory Animals, 8th edition* (National Research Council, 2011).

2.1. Subjects

Male CD rats ($N = 30$; Charles River), starting at 8 weeks old, were maintained at 85% of their free-feeding weight throughout the course of the study. Animals were housed 2 rats/cage and kept on a 12:12 h light/dark schedule (lights on 07:00–19:00 h). All training and testing was administered during the light schedule.

2.2. Operant apparatus

The operant training and stimulus discrimination acquisition testing was conducted in a standard rat operant chamber (Med-Associates Inc., St. Albans, Vermont), containing a houselight, sound attenuating fan, two levers to the left and right of the pellet delivery aperture, and an automated pellet dispenser which delivered 45 mg sucrose pellets. Chambers were housed within a sound attenuating box to control for external light and background noise. A computer located in the same room was programmed with Med-PC IV (Med Associates) and recorded behavioral data.

2.3. Training

After rats were successfully reduced to 85% of their free feeding weight, they were manually shaped for one session to press a lever to obtain sucrose pellet reinforcement. After shaping, rats trained on progressively increasing fixed ratio (FR) schedules of reinforcement until reaching a FR30. All training sessions lasted for 1 h or 100 pellets maximum, whichever occurred first. Once rats reached the criterion of 50 reinforcers during the FR30 session, they were placed on a two-phase multiple variable ratio 15 (VR15), VR15 schedule of reinforcement. The signal to turn the houselight on and off was set on a variable time schedule (2 min; ranging from 1 to 3 min) and