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The histone deacetylase inhibitor, sodium butyrate, alleviates cognitive deficits in pre-motor stage PD

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ABSTRACT

Parkinson's disease (PD) patients often times experience impairment in their cognitive abilities early on in the progression of the disease. The reported deficits appear to mainly involve functions that are associated with frontal lobe and frontal-striatal pathways subserving attentional set-shifting, working memory and executive function.

The current study explored executive function deficits in a rat model of PD in the pre-motor deficit stage. The rats were lesioned with 12 μg of 6-hydroxydonpamine (6-OHDA) in the striatum in a two step process (10 μg/μl followed by 2 μg/μl) 48 hours apart. Executive function was tested at 3 weeks post-surgery using a rat analogue of Wisconsin card sorting test called the Extra Dimensional/Intra Dimensional (ED/ID) set-shifting task. The results demonstrated that performance by the pre-motor rat model of PD was equivalent to that of the control groups in the simple and the compound discriminations as well as the intra-dimensional set-shifting. However the PD group exhibited attentional set-shifting deficits similar to those observed in PD patients.

Additionally, sodium butyrate, a short chain fatty acid derivative and inhibitor of class I and II histone deacetylase (HDACi), was tested as a potential therapeutic agent to mitigate the pre-motor cognitive deficits in PD. The results indicated that the sodium butyrate treatment not only effectively alleviated the set-shifting deficits, but also improved the attentional set formation in the treated rats.

1. Introduction

Parkinson's disease (PD) patients can experience diminution of their cognitive abilities early in the disease process (Downes et al., 1989; Sharpe, 1992; Cools et al., 2001; Adler, 2005). The proportion of patients with cognitive deficits increases with disease progression from 40% at stage 1 to almost 86% at stage 4 on Hoehn and Yahr scale (Growdon et al., 1990).

The current study explored executive function deficits in a stepwise 6-hydroxydonpamine (6-OHDA) striatal lesion rat model of PD in the pre-motor deficit stage. The 6-OHDA lesion protocol used in this study has been shown to result in 23% loss of substantia nigra dopamine cells and 27% loss in striatal dopamine levels at 3 weeks post-lesion (Rane and King, 2011). This dopamine depletion was not associated with any apparent motor deficits (Rane and King, 2011), which is in concord with human studies reporting loss of 80% striatal dopamine and 60% depletion in substantia nigra dopamine cells before the onset of motor symptoms (Fearnley and Lees, 1991; Gaig and Tolosa, 2009; Hawkes et al., 2010). The current study also explored the effect of sodium butyrate (NaBu), a short chain fatty acid derivative and inhibitor of class I and II histone deacetylase (HDACi) (Chuang et al., 2009), as a potential therapeutic agent to mitigate the pre-motor cognitive deficits in PD. Importantly, even though dopamine plays a significant role in multiple neuronal networks involved in cognition (Nagano-Saito et al., 2008; Winter et al., 2009), dopaminergic treatments, proven to relieve the motor symptoms of PD, are generally ineffective at alleviating the co-morbid emotional and cognitive symptoms (Gotham et al., 1988; Downes et al., 1989; Jubault et al., 2009). In contrast, NaBu and other HDACi agents known for their tolerability by humans, as well as rodents (Liu et al.,

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2006; Hoshino and Matsubara, 2010), exert strong therapeutic effects in various preclinical models of neurodegenerative disease, and broadly improve cognitive functioning (Fontan-Lozano et al., 2008; Kilgore et al., 2009). However, these drugs have not yet been tested in the context of disabled executive functioning in PD, particularly at a stage when motor deficits have not yet emerged. This was the goal of the present study.

2. Methods

Adult male Long Evans rats (300–350 g) were obtained from Harlan Sprague–Dawley Laboratories (Indianapolis, IN) and were group-housed (2 per cage) in Plexiglas cages at ambient temperature (22–24 °C) on a 12/12 h light/dark cycle. They were provided with food and water *ad libitum*. Study protocols were approved by the Institutional Animal Care and Use Committee at the University of Massachusetts Medical School, Worcester, MA.

Rats were randomly divided into control, sham lesion, 6-OHDA lesion (3WKPD) or 6-OHDA lesion with NaBu treatment (3WKPD + NaBu) groups ($N = 8$/group). The surgery protocol was similar to the one described in our previous publication (Rane and King, 2011). Briefly, the 3WKPD and 3WKPD + NaBu rats received a total of 12 μg of 6-OHDA in the striatum (0.7 mm rostral to bregma, ±3 mm lateral to midline, 5 mm ventral to skull surface) in two steps of 10 μg and 2 μg in 1 μl of vehicle (0.1% Ascorbic acid in 0.9%NaCl) two days apart using precision Hamilton micro-syringes connected to PE50 tubing. The sham lesioned rats received no infusions, but empty PE tubings were used to lesion the same locations on the two surgical days. The control group did not receive any lesions and was not administered any treatment. The 3WKPD + NaBu group received daily intra-peritoneal injections of NaBu treatment (250 mg/kg/ml in 0.9%saline; Sigma Aldrich, St. Louis, MO) starting 2 week post-surgery for 5 days during the dark phase of the light/dark cycle. NaBu dose chosen here was guided by earlier work showing that doses in the range of 200 mg/kg elicit robust behavioural changes after exposure to stimulant drugs and activation of dopaminergic signalling (Schroeder et al., 2008; Febo et al., 2009). NaBu solution was prepared fresh weekly. To minimize stress, no treatment was administered 2 days prior to the test day. Non-treatment groups were handled every day for the five treatment days, to serve as controls.

Executive functioning was tested using the rat analogue of the Wisconsin Card sorting test called the ED/ID test (Extra Dimensional/Intra Dimensional set-shifting task) (Birrell and Brown, 2000). The test apparatus consisted of a custom-built black Plexiglas arena (90 cm × 90 cm). Two adjacent corners, separated from each other using a black partial divider served as test areas, each with a paper plate filled with bedding with a spotlight over it. During the test, stimuli from two dimensions (lights/odours) were used. The light dimension included pairs of lights (orange versus blue, purple versus yellow or green versus red) while the odour dimension consisted of pairs of 100 μl of different aromatic oils mixed with the bedding (vanilla versus lemon, peppermint versus thyme or rosemary versus cinnamon). Testing was performed 3 weeks post-surgical procedure. Four days prior to test day, rats were individually housed, maintained on a restricted diet (15 g of pellets/day) and trained to search for pieces of food as reward underneath the unscented bedding on the paper plates. On the test day each rat randomly started with either the light or the odour dimension as the initially relevant dimension (IRD) that was associated with a food reward, making the other dimension the initially irrelevant dimension (IID). Tests included simple discrimination (SD; Reward is associated with one of the two stimuli from IRD, IID absent), compound discrimination (CD; SD with a second set of stimuli from IID which needs to be ignored), intra-dimensional shifting (ID; Discrimination similar to CD, but with a complete new set of stimuli from the IRD and the IID) and extra-dimensional set-shifting (ED; A complete new set of stimuli from IRD, as well as IID. The reward association is switched from IRD to IID). A trial was marked as successful when the rat retrieved the food from the reward stimulus plate without going towards the non-reward stimulus. Each task was marked as completed when the rat performed six consecutive successful trials. The total number of trials to completion of each task was noted. Repeated measures ANOVA with bonferroni correction for multiple comparisons was used to analyze the group and task effects using SPSS (IBM Corp., Somers, NY). The groups were further compared within individual tasks using univariate ANOVA with bonferroni correction. For each group, the effect of task difficulty was analyzed using repeated measures ANOVA on individual group results. Finally, to analyze the effects of NaBu treatment on attention after 6-OHDA lesions, 3WKPD group was compared with 3WKDP + NaBu group in the SD and ID tasks using repeated measures ANOVA.

3. Results

A significant main effect of task ($F_{(9,81)} = 31.729$, $p < 0.001$), as well as, a significant main effect of group ($F_{(3,27)} = 5.786$, $p < 0.004$) qualified by significant group x task interaction ($F_{(9,81)} = 7.651$, $p < 0.001$) was revealed by repeated measures ANOVA (Fig. 1). Overall, there was no significant difference between the number of trials needed to complete SD and CD tasks ($t_{(27)} = 0.13$, $p = 1.0$). However, there was an increase in the number of trials needed to complete ID compared to SD ($t_{(27)} = 3.51$, $p < 0.01$) and CD ($t_{(27)} = 4.08$, $p < 0.003$) tasks. The ED task required an even higher number of trials to get to completion as compared to SD ($t_{(27)} = 6.79$, $p < 0.001$), CD ($t_{(27)} = 9.71$, $p < 0.001$) and even ID ($t_{(27)} = 4.63$, $p < 0.001$) task, indicating a progressive increase in difficulty level across the tasks (Fig. 1).

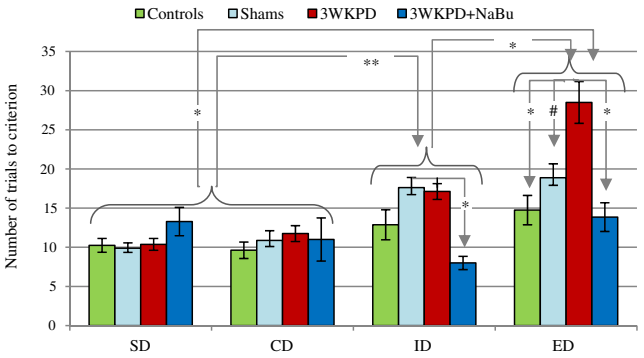

Fig. 1. Results of simple discrimination (SD), compound discrimination (CD), intra-dimensional shift (ID) and extra-dimensional set shifting (ED) tasks. There was a significant main effect of task ($F_{(3,81)} = 31.729$, $p < 0.001$), as well as, a significant main effect of group ($F_{(3,27)} = 5.786$, $p < 0.004$), qualified by significant interaction ($F_{(9,81)} = 7.651$, $p < 0.001$). There was no significant difference between the number of trials needed to complete SD and CD tasks. However, there was an overall increase in the number of trials needed to complete ID and ED tasks. (*$p < 0.001$, **$p < 0.01$, #$p < 0.05$).

The within task univariate analysis revealed that there was a significant main effect of group ($F_{(3,27)} = 10.388$, $p < 0.001$) during the ID task. The post-hoc tests revealed that the NaBu treatment group needed significantly fewer trials as compared to the sham ($t_{(14)} = 4.93$, $p < 0.001$) and the 3WKPD ($t_{(14)} = 4.67$, $p < 0.001$) groups, implying a probable improvement in attentional set formation in the treatment group. There were no significant within task differences between the groups for SD ($F_{(3,27)} = 2.02$, $p = 0.135$) and CD ($F_{(3,27)} = 0.318$, $p = 0.811$).

Significant main effect of group was observed in the ED ($F_{(3,27)} = 10.312$, $p < 0.001$) task. 3WKPD group needed significantly higher number of trials as compared to controls ($t_{(15)} = 4.72$, $p < 0.001$), sham lesioned ($t_{(15)} = 3.31$, $p < 0.02$), as well as, the 3WKPD + NaBu group ($t_{(14)} = 4.85$, $p < 0.05$) as per the post-hoc tests.

The within group repeated measures ANOVA of the control group (Fig. 2A) revealed a significant effect of task on the number of trials needed to complete the four tasks ($F_{(3,21)} = 4.11$, $p < 0.02$) with a significant difference between the CD and ED tasks ($t_{(15)} = 4.11$, $p < 0.03$). Sham lesioned rats (Fig. 2B) exhibited a main effect of task ($F_{(3,21)} = 18.12$, $p < 0.001$) with significantly higher number of trials to complete the ED task as compared to both the SD ($t_{(15)} = 6.3$, $p < 0.003$) and CD ($t_{(15)} = 4.5$, $p < 0.02$). Additionally, sham rats needed significantly higher number of trials to complete the ID task as compared to the SD ($t_{(15)} = 5.42$, $p < 0.007$) and CD ($t_{(15)} = 7.49$, $p < 0.002$) implying a weaker attentional set formation. Similar to the sham lesioned rats, the 3WKPD group exhibited significant main effect of task ($F_{(3,21)} = 34.26$, $p < 0.001$) with significantly higher number of trials for the ID and ED tasks as compared to both the SD ($t_{(15)} = 8.25$, $p < 0.001$ with ID; $t_{(15)} = 6.66$, $p < 0.002$ with ED)and CD ($t_{(15)} = 3.98$, $p < 0.04$ with ID; $t_{(15)} = 8.91$, $p < 0.001$ with ED) tasks (Fig. 2C). In addition, there was a significant difference between the trials needed to complete the ID and the ED tasks ($t_{(15)} = 3.76$, $p < 0.05$), implying a weaker attentional set formation as compared to controls, as well as, a set-shifting deficit.