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Neuroprotective effects of voluntary running on cognitive dysfunction in an α-synuclein rat model of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is no longer primarily classified as a motor disorder due to increasing recognition of the impact on patients of several nonmotor PD symptoms, including cognitive dysfunction. These nonmotor symptoms are highly prevalent and greatly affect the quality of life of patients with PD, and so, therapeutic interventions to alleviate these symptoms are urgently needed. The aim of this study was to investigate the potential neuroprotective effects of voluntary running on cognitive dysfunction in an adeno-associated virus-α-synuclein rat model of PD. Bilateral intranigral administration of adeno-associated virus-α-synuclein was found to induce motor dysfunction and a significant loss of nigral dopaminergic neurons, neither of which were rescued by voluntary running. Overexpression of α-synuclein also resulted in significant impairment on hippocampal neurogenesis-dependent pattern separation, a cognitive task; this was rescued by voluntary running. This was substantiated by an effect of running on neurogenesis levels in the dorsal dentate gyrus, suggesting that the functional effects of running on pattern separation were mediated via increased neurogenesis.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide (reviewed by Tysnes and Storstein, 2017) and is characterized by degeneration of the dopaminergic neurons of the substantia nigra (SN) in the midbrain. The resulting loss of the neurotransmitter dopamine in the striatum, the target region of these neurons, causes the typical motor symptoms of PD, including tremor at rest, akinesia, rigidity, and postural instability. The pathological hallmark of PD is the appearance of insoluble proteinaceous inclusions called Lewy bodies that are primarily composed of α-synuclein (Spillantini et al., 1997). The Braak hypothesis (Braak et al., 2003a) theorized that α-synuclein proliferates throughout the brain in an ordered and predetermined manner and that each stage of this process correlates with increased severity and type of disease symptoms. A growing number of nonmotor symptoms (NMS) are associated with PD, such as cognitive dysfunction and neuropsychiatric problems as well as gastrointestinal and olfactory disturbances (reviewed by Cooney and Stacy, 2016), and it is thought that the variety of NMS may reflect spreading of α-synuclein pathology throughout the nervous system (Braak et al., 2003b).

Cognitive impairment is common in PD patients, with a reported prevalence of up to 36% (Foltynie et al., 2004), and includes deficits in working memory, visuospatial processing, language fluency, and verbal learning (Aarsland, 2015; Goldman and Postuma, 2014). Recent studies employing the Movement Disorder Society diagnostic criteria for mild cognitive impairment in PD (PD-MCI) reported a prevalence of 42.5% of MCI in PD patients (Domellöf et al., 2015; Yarnall et al., 2014). Although the exact cause of this symptom remains unclear, there is growing evidence to support a role for the hippocampus in the pathogenesis of cognitive impairment in PD (Calabresi et al., 2013). PD-MCI is associated with hippocampal atrophy (Bruck et al., 2004; Foo et al., 2016; Schneider et al., 2017; Tanner et al., 2017), and decreased hippocampal volume has been shown to be correlated with deficits in recognition memory and in memory-encoding processes in PD patients (Camicioli et al., 2003; Weintraub et al., 2011). Moreover, Lewy body pathology has been detected postmortem in the hippocampus of individuals with PD (Flores-Cuadrado et al., 2016; Hall et al., 2014) and has been positively correlated with the degree of cognitive impairment (Churchyard and Lees, 1997). Evidence from in vivo studies in mice has shown that overexpression of α-synuclein in the hippocampus can significantly alter the survival and dendritic development of newlyborn neurons in the dentate gyrus

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(Winner et al., 2004, 2012). Furthermore, data from animal studies suggest that this process of adult hippocampal neurogenesis is necessary for certain forms of learning and memory, specifically spatial pattern separation (Clelland et al., 2009). Postmortem analysis of PD patients has also shown decreased neurogenesis in the hippocampus (Hoglinger et al., 2004).

Despite the prevalence of PD-MCI, there is relatively little evidence to inform treatment (reviewed by Yang et al., 2016). Given that it is estimated that approximately 46% of patients with PD-MCI will further decline to Parkinson's disease with dementia within 10 years (Williams-Gray et al., 2013), there is a clear need for preventative strategies to alleviate the disease burden. Thus, therapies that can reverse PD-induced impairments in neurogenesis may prove useful in treating hippocampal-associated cognitive symptoms of the disease.

Aerobic exercise is known to be a potent inducer of adult hippocampal neurogenesis (van Praag et al., 1999). Epidemiological evidence points to the fact that exercise can decrease the risk of developing PD in later life (Yang et al., 2015), as well as decreasing mortality in PD patients (Kuroda et al., 1992). Furthermore, exercise may also play a role in restoring motor function and ameliorating NMS in patients who have already been diagnosed with PD (reviewed by Cusso et al., 2016 and Dashtipour et al., 2015). There are several lines of evidence demonstrating the beneficial effects of exercise on motor symptoms and some of the NMS in acute animal models of PD, including the 6-hydroxydopamine (6-OHDA) model (Cho et al., 2013; Goes et al., 2014; Tillerson et al., 2003) and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model (Gorton et al., 2010; Pothakos et al., 2009; Sung, 2015). However, there is currently no evidence for an impact of exercise on cognitive function in an animal model which recapitulates the long-term progressive nature of PD.

The adeno-associated virus (AAV)-α-synuclein rat model has been shown to replicate many of the clinical features of PD that are seen in other models, while also resulting in α-synuclein accumulation and a progressive loss of dopaminergic neurons that is more consistent with the degeneration reported in the human disease (reviewed by Lindgren et al., 2012). Thus, this is an ideal preclinical model in which to investigate the efficacy of novel therapeutic approaches. Studies to date have used unilateral intracerebral injection of AAV-α-synuclein, resulting in motor impairment on the affected side of the body, which can be easily measured on standard laboratory tests. However, for testing cognitive function, unilateral lesions are not appropriate due to compensation of behavioral function by the unaffected side of the brain. The present study reports a novel adult rat model involving bilateral intranigral injection of AAV-α-synuclein, which can be used to investigate performance on a range of cognitive behavioral tests. This bilateral AAV-α-synuclein model of PD was used to examine the potential neuroprotective effects of voluntary running on motor function and associated dopaminergic neuronal integrity, and on hippocampal-dependent cognitive behaviors and associated alterations in neurogenesis.

2. Methods

2.1. Virus preparation

An α-synuclein plasmid was kindly donated by Dr Eilis Dowd (National University of Ireland, Galway) and Professor Deniz Kirik (Lund University, Sweden), and a viral vector was constructed from this by Vector Biosystems Inc, Philadelphia, USA. In brief, AAV2 inverted terminal repeats coding for human wild-type α-synuclein were packaged using AAV6 capsid proteins to create an AAV2/6 viral vector. Transgene expression was driven by a synapsin-1 promoter and enhanced using a woodchuck hepatitis virus post-transcriptional regulatory factor. Viruses were titered by quantitative polymerase chain reaction using the following primers: Forward 5′ tcc ttg tat aaa tcc tgg ttg ctg 3′, Reverse 5′ agc tga cag gtg gtg gca at '-3'. The final viral titer for AAV2/6-αsyn was 5.2×10^{13} gc/mL.

2.2. Animal husbandry

Adult male Sprague Dawley rats (Envigo, UK) were maintained on a 12 h:12 h light:dark cycle (lights on at 08.00 h) under regulated temperature (21 ± 2 °C) and humidity (30%–50%). Standard rat chow and water were available ad libitum, unless behavioral testing dictated otherwise temporarily. All experiments were conducted in accordance with the European Directive 2010/63/EU, and under an authorization issued by the Health Products Regulatory Authority Ireland (AE19130/P010) and approved by the Animal Ethics Committee of University College Cork (approval number 2013/030).

2.3. Stereotaxic surgery

All surgery was conducted under general anesthesia induced by inhaled isoflurane. Animals were placed in a stereotaxic frame (Kopf Instruments) and an incision was made through to the skull. Animals were administered with 3 μL of either AAV-α-synuclein (3.1×10^9 gc/3 μL) or 0.9% sterile saline solution (sham) bilaterally into the SN at coordinates AP −5.3, ML ±2.0, and DV −7.2 relative to bregma. All solutions were infused at a rate of 1 μL/min, with an additional 2 minutes allowed for diffusion before the needle was withdrawn. Following injection, incisions were sutured, and rats were allowed to recover on a heating mat before returning to their home cages. Animals were administered the analgesic carprofen (Rimadyl 5 mg/kg, subcutaneous (s.c.), Zoetis Ireland Ltd) and 5% glucose solution immediately after the procedure.

2.4. Experimental design

One week after surgery, 5-Bromo-2′-deoxyuridine (BrdU) (Sigma, Ireland) was administered intraperitoneally to all animals at a dose of 75 mg/kg in 4 injections over the course of 6 hours. Animals were randomly divided into 4 groups: "sham sedentary" (n = 7), "α-synuclein sedentary" (n = 8), "sham running" (n = 6), and "α-synuclein running" (n = 8). They were pair housed either in cages with free access to running wheels ("running" groups) (Activity Wheel, Tecniplast, UK) or in standard housing cages ("sedentary" groups). Running wheels were connected to counters which allowed wheel revolutions to be continuously monitored. Motor and cognitive testing was carried out at selected time points after surgery, based on a previous pilot study (see Fig. 1 for experimental timeline).

2.5. Motor performance on the rotarod

The protocol was adapted from Marei et al. (2015) and consisted of 3 training sessions, each containing 3 trials of

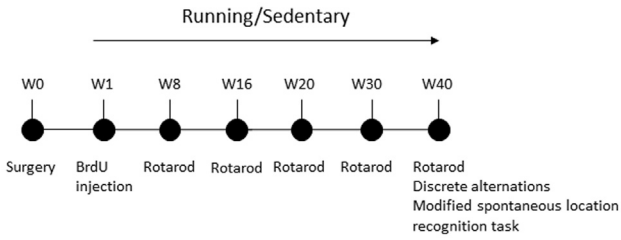

Fig. 1. Experimental design. (W = weeks post-surgery).