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Research report

The novel adaptive rotating beam test unmasks sensorimotor impairments in a transgenic mouse model of Parkinson's disease

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HIGHLIGHTS

- Female Thy1-aSyn mice express less human alpha-synuclein compared to males.
- This could be involved in the lack of motor deficits in female Thy1-aSyn mice.
- A novel beam test reveals sensorimotor deficits in female Thy1-aSyn mice.
- This supports the importance of sensorimotor integration in movement disorders.
- Thy1-aSyn mice can be used to study the mechanisms of sensorimotor impairments.

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ABSTRACT

Development of disease modifying therapeutics for Parkinson's disease (PD), the second most common neurodegenerative disorder, relies on availability of animal models which recapitulate the disease hallmarks. Only few transgenic mouse models, which mimic overexpression of alpha-synuclein, show dopamine loss, behavioral impairments and protein aggregation. Mice overexpressing human wildtype alpha-synuclein under the Thy-1 promotor (Thy1-aSyn) replicate these features. However, female mice do not exhibit a phenotype. This was attributed to a potentially lower transgene expression located on the X chromosome. Here we support that female mice overexpress human wildtype alpha-synuclein only about 1.5 fold in the substantia nigra, compared to about 3 fold in male mice. Since female Thy1-aSyn mice were shown previously to exhibit differences in corticostriatal communication and synaptic plasticity similar to their male counterparts we hypothesized that female mice use compensatory mechanisms and strategies to not show overt motor deficits despite an underlying endophenotype. In order to unmask these deficits we translated recent findings in PD patients that sensory abnormalities can enhance motor dysfunction into a novel behavioral test, the adaptive rotating beam test. We found that under changing sensory input female Thy1-aSyn mice showed an overt phenotype. Our data supports that the integration of sensorimotor information is likely a major contributor to symptoms of movement disorders and that even low levels of overexpression of human wildtype alpha-synuclein has the potential to disrupt processing of these information. The here described adaptive rotating beam test represents a sensitive behavioral test to detect moderate sensorimotor alterations in mouse models.

1. Introduction

There exist a large number of animal models for the second most common neurodegenerative disorder Parkinson's disease (PD) but only few depict some of the progressive hallmarks of the disease, such as degeneration of dopamine neurons of the substantia nigra with loss of striatal dopamine (DA), alpha-synuclein pathology and motor deficits [1]. Most transgenic mouse models were created by modifying the expression of alpha-synuclein,

Abbreviations: Thy1-aSyn, mice overexpressing human wildtype alpha-synuclein under the Thy-1 promoter; ARB, adaptive rotating beam; rpm, rotation per minute; DA, dopamine; SN, substantia nigra.

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which is the major protein implicated in the pathogenesis of PD. Alpha-synuclein aggregates are the primary component of Lewy bodies, a pathological hallmark of this disease [2]. Multiplication of the alpha-synuclein gene causes familial PD while polymorphisms increase the risk of sporadic PD [3,4]. Various transgenic alpha-synuclein mouse models are available and the most robust phenotypes were described in models with several fold overexpression of the human protein [1,5]. This is consistent with the gene dosage effect on disease onset and severity described in PD patients with multiplications of the alpha-synuclein gene [6]. Male mice overexpressing human wildtype alpha-synuclein under the Thy-1 promoter (Thy1-aSyn mice), an animal model of synucleinopathies such as PD, develop robust motor and non-motor deficits, proteinase K resistant alpha-synuclein aggregation at 2–3 months of age and a 40% DA loss at 14 months of age [7]. At 22 months of age tyrosine-hydroxylase positive neurons in the substantia nigra are significantly decreased in diameter and the mice show rapidly declining general health making it difficult to study even older animals for a loss of neurons [7]. Conversely, females of this line do not show these early motor deficits which is thought to be related to the lower expression of human alpha-synuclein due to random inactivation of the X chromosomally expressed transgene [7]. Interestingly, female Thy1-aSyn mice exhibit altered DA modulation of synaptic activity similar to the male counterparts [8–10]. This disconnection between pathophysiological alterations and motor phenotype is a common issue in genetic mouse models of movement disorders [1,11] and may be the result of compensatory mechanisms in rodents. For this reason there is a need for more sensitive tests.

Behavioral tests in rodent models often aim to test a specific feature, for instance motor dysfunction, by reducing any factors with deviating influence such as anxiety, grip strength or fluctuating sensory input. However, recent studies in PD patients show that somatosensory abnormalities contribute to the motor symptoms. Loss of response specificity to sensory information produces incorrect output signals from the basal ganglia for the preparation and execution of voluntary movement [12]. This sensorimotor deficit would unmask if motor performance needs to be adapted according to changing sensory (e.g. tactile, proprioceptive, vestibular) stimuli. We therefore hypothesize that motor deficits in animal models of movement disorders would increase if sensorimotor integration is required. Here we introduce a novel adaptive rotating beam test which requires sensorimotor integration (the use of sensory information to guide movement) that was sensitive to unmask motor deficits in female Thy1-aSyn.

2. Materials and methods

2.1. Animals

Animal care was provided in accordance with the guidelines of the EU Directive 2012/63/EU and the German Animal Welfare Agency and experiments were approved under protocol numbers TVV20/13. Mice used in this study were bred and housed in the institute's facility. The employed well characterized model of PD overexpresses human wildtype alpha-synuclein under the murine Thy-1 promotor (Thy1-aSyn mice) on a mixed hybrid C57Bl/6J × DBA2 (short BDF1) background [7]. Transgenic mice and wildtype littermates were tested on the same day in randomized sequence by an investigator unaware of the genotypes. To examine the impact of the genetic background on performance on the test, we compared wildtype BDF1 mice with mice on a pure C57BL/6J background. N numbers were determined a priori with power analyses in order to limit the number of animals used to the minimum required for statistical analysis (3R principles). Male Thy1-aSyn mice were expected to show strong deficits with low variability (see power analysis in Ref. [7]) and were therefore limited to n = 7. For the other groups larger N sizes were chosen to ensure sufficient power to detect differences (see Table 2 for N). Male and female mice of each line were tested between the age of 3 to 6 months on the pole test and the challenging beam test, and at 6 months on the adaptive rotating beam test. There was no correlation between age and performance on the pole or the beam test (spearman or pearson correlation), therefore mice of ages between 3 and 6 months were grouped together. At 6 months of age male wildtype Thy1-aSyn mice weighed 30 g and transgenics 26 g as described previously [7]. Female wildtype and transgenic Thy1-aSyn mice at 6 months of age weighed 24 g respectively and male C57Bl/6J weighed 29 g and females 25 g. The genotype was verified with polymerase chain reaction (PCR) amplification analysis of DNA [13]. Ear tissue (punch) was used for DNA extraction instead of tail biopsies to reduce stress and pain to the animal. Mice were kept on a reverse 12-h light/12h-dark cycle. All testing was performed between 1 and 5 pm during the dark cycle under low light in an allocated room for behavioral experiments outside the animal holding room. Food (Altromin standard diet) and water were available ad libitum. Mice were housed socially with littermates (1–6 mice) in makrolon cages (Type III) on standard bedding (shredded wood) and material for nest-building (paper rolls, red plastic houses) was provided. Room temperature in the mouse holding room was 22 °C ± 2 °C and relative humidity was about 60%; values were recorded during the daily animal check.

2.2. Quantification of alpha-synuclein mRNA expression

Relative expression of human wildtype alpha-synuclein mRNA (*SNCA*) and mouse alpha-synuclein mRNA (*Snca*) was determined in male and female Thy1-aSyn mice (7–9 weeks of age) with real time qPCR as described previously [14]. Brains were rapidly removed after cervical dislocation of the mice and the substantia nigra dissected on ice. Tissue was flesh frozen in liquid nitrogen and stored at −80 °C. RNA was extracted using the RNeasy Plus Mini Kit (Qiagen). First-strand cDNA was synthesized using the PrimeScriptTM RT Master Mix Perfect Real Time kit (TaKaRa clonetech) and random primers according to the manufacturer's protocol. Specific TaqMan Gene expression assays were used with probes that span exons (Applied Biosystems). High efficacy of the respective assay was evaluated by the manufacturer, but specific sequences are proprietary. One microliter of cDNA was added to the Premix Ex Taq™ kit (TaKaRa clonetech) for a total reaction volume of 5 μl and amplification was performed on a PikoReal 96 Real-Time PCR system (ThermoScientific) following manufacturers protocols. Data was analyzed using the PikoReal Software 2.1. (ThermoScientific). GeNorm was used to analyze candidate reference genes (*Atp5b*, *Eif4a2*, *Gapdh* and *Hprt)* according to average expression stability as described previously [15], and the normalization factor derived from the most stable genes was chosen for normalization.

2.3. Behavioral analyses

For comparisons with the novel type of rotating beam test, we used two established behavioral tests. The pole test is a test to determine motor coordination [16,17]. Mice were put head up on a vertical wooden beam with a diameter of 10 mm that was placed in the home cage. In two days of training mice learned to turn on the pole and to climb downwards. On the third day the time to turn (t(turn)) and the time to climb down after the turn (t(descend)) were recorded in 5 consecutive trials. If the mouse failed to turn or slid downwards, a cutoff time of 30 s (t(turn)) or 30 s (t(descend)) was noted. To further increase the challenge for mice with less phenotype (females), the diameter of the pole was reduced to 6 mm on