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Research Paper

Chronic mild stress accelerates the progression of Parkinson's disease in A53T α-synuclein transgenic mice

Qimei Wu [1], Xiaoyu Yang [1], Yu Zhang, Lei Zhang, Linyin Feng *

CAS Key Laboratory of Receptor Research, Chinese Academy of Sciences, Shanghai Institute of Materia Medica, 555 Zu Chong Zhi Road, Shanghai 201203, PR China

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ABSTRACT

Daily stress is associated with increased risk for various diseases, and numerous studies have provided evidence that environmental stress leads to deleterious effects on the central nervous system. However, it remains unclear whether chronic stress exacerbates the progression of Parkinson's disease (PD). To investigate this hypothesis, we determined the effect of chronic mild stress (CMS) on the pathogenesis of PD in a transgenic mice line that overexpresses the human A53T mutant α-synuclein (A53T Tg mice). We show that when exposed to CMS, male, but not female, A53T Tg mice developed profound motor disabilities and exhibited olfactory sensitivity deficits. Pathological analysis also identified robust dopaminergic neuron degeneration and strong reduction of dopamine levels in A53T Tg male mice who underwent CMS treatment. Systematic examination of the abnormal aggregation of α-synuclein revealed a profound increase of inclusion in A53T Tg male mice subject to CMS resembling key pathological changes of PD. An insight into the mechanism underlying stress leading to the acceleration of neurodegeneration in those with genetic susceptibility, was revealed by evidence of microglia activation and elevated pro-inflammatory factor levels in A53T Tg male mice following CMS. Notably, these effects of CMS on the pathogenesis of PD showed a remarkable sexual dimorphism: only male A53T Tg mice exhibited exacerbation of the progression of PD. However, the molecular and cellular bases for this difference remains to be elucidated. Our results indicate a causative role for chronic mild stress using a PD animal model. Based on these findings, we propose that CMS acts as an environmental risk factor that leads to neuroinflammation and progressive neurodegeneration on a background of PD susceptibility.

1. Introduction

Parkinson's disease (PD) is a late-onset neurodegenerative disease, which is characterized by selective depletion of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the formation of Lewy body (LB) aggregates (Shulman et al., 2011). Impaired motor function is a classic sign used in the clinical diagnosis of PD. Those affected with PD also have non-motor symptoms including olfactory deficits, sleep disturbance, reduced stress tolerance, depression and cognitive decline, which may precede the onset of motor symptoms (Chaudhuri and Schapira, 2009). Although much effort has been devoted to study the cause of PD, the etiology remains unclear.

Environmental challenges (*i.e.* stresses) are part of daily life (de Kloet et al., 2005; Lucassen et al., 2014). Appropriate short-term responses to stress are crucial for adaptation and survival, while chronic stress is predominantly maladaptive, detrimentally impacting both brain and behavior (Carroll et al., 2011; Mo et al., 2014). Stress is one of the earliest proposed causes of PD (Djamshidian and Lees, 2014; Smith et al., 2002), and there is a great deal of evidence indicating that stress contributes to the development of PD. Depression occurs either prior to or concomitant with PD symptoms and indicates a poor prognosis of PD (Hou et al., 2014; Leentjens et al., 2003; Pålhagen et al., 2008; Rod et al., 2013). A considerably higher incidence of PD was reported in the soldiers held as prisoners after their release (Gibberd and Simmonds, 1980). Similarly, a case report described a young woman whose PD was believed to be triggered by stress (Zou et al., 2013). Moreover, dysregulation of stress hormones, such as glucocorticoids also have been linked with PD vulnerability in several clinical studies. Compared to healthy age-matched controls, the cortisol level of PD patients increases (Hartmann et al., 1997), and is positively related to gait abnormalities (Charlett et al., 1998). Of note, the cortisol levels decreases in patients treated with levodopa (Muller et al., 2007). In line with these findings, it has been shown that the DA system is

Abbreviations: PD, Parkinson's disease; CMS, chronic mild stress; LB, Lewy body; 6-OHDA, 6-hydroxy-dopamine; DA, dopamine; CORT, corticosterone; TH, tyrosine hydroxylase; Iba-1, ionized calcium-binding adaptor molecule 1; SNpc, substantia nigra pars compacta; DOPAC, dihydroxy-phenyl aceticacid.

* Corresponding author at: Shanghai Institute of Materia Medica, Chinese Academy of Sciences, PR China.
E-mail address: lyfeng@simm.ac.cn (L. Feng).

[1] Authors Qimei Wu and Xiaoyu Yang contributed equally to this work.

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preferentially vulnerable to the effects of stress. Immobilization-induced stress led to oxidative stress and preferentially impacted the nigrostriatal dopaminergic system (Kim et al., 2005). Chronic stress resulted in the decrease of DA levels in the striatum (Rasheed et al., 2010). Furthermore, in the neurotoxic PD models induced by 6-hydroxy-dopamine (OHDA) (Hemmerle et al., 2014; Smith et al., 2008; Snyder et al., 1985) or lipopolysaccharide (LPS) (de Pablos et al., 2014), chronic stress treatment accelerates DA neurodegeneration. Together, these data suggest that stress is detrimental to the nigrostriatum DA system and exacerbates neuronal damage in condition of other risk factors. However, the effects of stress on a transgenic model of PD have been poorly evaluated to date. α-synuclein is the primary component of Lewy bodies. Missense mutation of α-synuclein (A53T, A30P, E46K) and duplication or triplication of α-synuclein have been linked to familial PD (Chartier-Harlin et al., 2004; Kruger et al., 1998; Polymeropoulos et al., 1997). For sporadic PD α-synuclein (SNCA) is one of several risk factors demonstrated by genome-wide association studies (GWASs) (Satake et al., 2009; Simon-Sanchez et al., 2009). In the present study, a human mutant A53T α-synuclein transgenic mouse model (Giasson et al., 2002) was used to evaluate the effects of chronic mild stress (CMS) on PD progression.

Our results demonstrate that CMS markedly compromises motor ability and olfactory function in A53T Tg male mice, and leads to severe impairment in DA systems. Moreover, CMS promotes aggregation and accumulation of synuclein, which is the hallmark of PD. We further demonstrate that excessive activation of the inflammatory response may participate in the pathology of neurodegeneration of PD induced by CMS in A53T Tg mice. Intriguingly, we observed a sexually dimorphic effect of CMS, with female mice more resilience in the face of stressor-induced neurodegeneration. Together, these results link CMS and microglial activation to depletion of dopaminergic neurons in preclinical stages of PD and ultimately to the development of PD.

2. Materials and methods

2.1. Animals

Transgenic mice expressing A53T mutant human α-synuclein protein driven by the prion promoter were previously generated (Giasson et al., 2002). Homozygous and wild-type age-matched controls were applied in this research (Jackson Laboratories, Bar Harbor, ME, USA). Mice were genotyped using Taqman probe real-time PCR following the procedure on the Jackson Laboratories website. Genotyped mice were housed 4–6 females and 3–5 males per cage at controlled temperature and humidity on 12 h light/dark cycles, until the age of 6 months. Ethical treatments of animals followed the guidelines approved by the IACUC of Shanghai Institute of *Materia Medica*.

2.2. Chronic mild stress treatment

This study involved a total of 45 homogenous transgenic mice (20 males, 25 females) and 45 age-matched WT mice (25 males, 20 females). Mice were single-housed for 7 days of adaption before the CMS procedure began, and then randomized into two groups: an unstressed control group (Control) and CMS treatment group (CMS). Mice were exposed to chronic mild stress (CMS) for a total period of 31 days. Stressors were randomly interspersed throughout the period, and only one stressor was applied within one day (Table 1). The stressors included food and water deprivation (12 h), wet bedding (6 h, 200 ml water in home cage), overnight illumination, cage rotation (20 min), restraint (15 min, 4 °C) and tail pinch (2 min). Age- and genotype-matched control mice were single-housed over an equivalent period of time. Body weight and food consumption were monitored for the duration of experimental testing.

2.3. Quantification of plasma corticosterone

After CMS exposure on day 31, blood was collected into sodium heparin tubes under isoflurane anesthesia. The samples were centrifuged at 2000*g* for 15 min, and plasma was collected and stored at −80 °C until assayed. The level of corticosterone (CORT) was quantified by ELISA according to the manufacturer's instructions and expressed as ng/ml (Enzo Life Sciences, Farmingdale, USA).

2.4. Sucrose preference test

Sucrose preference tests were carried out at the end of CMS procedures, and a two-bottle choice paradigm was performed. Briefly, mice were habituated to sucrose by being given two bottles of 1% sucrose for 24 h, and then one bottle of 1% sucrose was replaced with plain water for 24 h. After 2 days of habituation, mice underwent water deprivation for 16 h and then were supplied with two pre-weighed bottles, one of which contained 200 ml 1% sucrose and the other contained plain water. Twelve hours later (*i.e.* overnight), fluid consumption were determined by weight alteration of the bottles. Sucrose preference was expressed as the ratio of the weight of sucrose *versus* total liquid consumption.

2.5. Olfactory test

This test was conducted in accordance with previous description (Yang and Crawley, 2009). To ensure motivation, mice were placed on a food-restricted diet and maintained body weight above 90% for 2 days. In the buried pellet test, mice acclimated in a test cage (32 × 20 × 17 cm) filled with 3 cm of clean bedding for 3 min, and then returned to their holding cage while a cereal pellet (2 g) was buried 1.5 cm below the bedding surface in one randomly chosen corner. After food placement, each mouse was positioned in the center of the test cage. The time to locate the food pellet was defined as the latency, with a maximum duration of 180 s in each trail. This trial was repeated three times with 15 min intervals, and the test cage were cleaned and filled with fresh bedding between each trial. As control, a visible pellet test was performed similarly except the food pellet was on the top of the bedding. The investigator responsible for timing was blind to genotype and treatment.

Table 1
Chronic mild stress protocol.

Week	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Overnight illumination	Cage rotation	Water and food deprivation	Restraint (4 °C)	Wet bedding	Tail pinch	Water and food deprivation
2	Wet bedding	Restraint (4 °C)	Cage rotation	Tail pinch	Overnight illumination	Cage rotation	Overnight illumination
3	Wet bedding	Water and food deprivation	Restraint (4 °C)	Tail pinch	Restraint (4 °C)	Wet bedding	Overnight illumination
4	Tail pinch	Cage rotation	Water and food deprivation	Overnight illumination	Wet bedding	Restraint (4 °C)	Water and food deprivation
5	Cage rotation	Tail pinch	Wet bedding				