

Early manifestation of depressive-like behavior in transgenic mice that express dementia with Lewy body-linked mutant β -synuclein

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Abstract

Aim: We previously generated transgenic (Tg) mice that expressed P123H β -synuclein (β S), a dementia with Lewy body-linked mutant β S. Notably, these mice recapitulated neurodegenerative features of Lewy body disease, reflected by motor dysfunction, greater protein aggregation, and memory impairment. Since recent studies suggested that non-motor symptoms, such as depression, might be manifested in the prodromal stage of Lewy body disease, the main objective of the present study was to investigate the early expression of behavior in P123H β S Tg mice.

Methods: Nest building, locomotor activity, and depressive-like behavior were assessed using 6- to 10-month-old male and female P123H β S Tg and wildtype mice.

Key Results: P123H β S Tg mice exhibited hyperlocomotor activity in a novel environment, a decrease in mobility time in the tail suspension test, and impairments in nest building.

Conclusions: Importantly, these non-motor behaviors were manifested before the onset of motor dysfunction, suggesting that P123H β S Tg mice could be a valid model for investigating the early phase of Lewy body disease.

KEYWORDS

β -synuclein, behavior analysis, Lewy body diseases, nonmotor symptoms, transgenic mice

MAIN TEXT

β -synuclein (β S) is a presynaptic phosphoprotein that is abundantly expressed in the brain.^{1,2} Although the physiological function of β S has been elusive, β S might be an inhibitor of neurodegeneration that is stimulated by its homolog α -synuclein (α S), a central player in the pathogenesis of Lewy body disease (LBD), such as Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy.³⁻⁵ However, two missense mutations of β S, P123H and V70M, were identified in familial and sporadic cases of dementia with Lewy bodies, respectively.⁶ However, the accumulation of β S was not observed in

a patient who carried the P123H β S mutation,⁶ and the pathological effect of β S was not clearly understood at that time. Therefore, to investigate the pathological effect of the β S mutant, we generated transgenic (Tg) mice that expressed P123H β S. P123H β S Tg mice exhibited several features of LBD.⁷ We observed the accumulation of both α S and β S P123H in the brain in Tg mice, especially in axons and axon terminals.⁷ Moreover, P123H β S Tg mice presented memory impairments and progressive motor dysfunction. These results suggest that P123H β S Tg mice may be a valid animal model of LBD.

Increasing attention has recently been given to nonmotor symptoms in LBD.⁸ These include cognitive dysfunction, neuropsychiatric

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manifestations, depression, autonomic complications, sleep disorders, and other nonmotor manifestations.⁸ The importance of the early pathogenesis of neurodegenerative diseases has been well recognized,⁹ and some nonmotor symptoms, such as depression, might be manifested in the prodromal stage of LBD.¹⁰ Therefore, the main objective of this study was to investigate the early expression of behavior in P123H β S Tg mice.

All of the experiments used 6- to 10-month-old male and female P123H β S Tg and wild-type (WT) mice. Nest building (Data S3) was assessed by leaving a mouse in a cage that was covered with a Palsoft Sheet Type (ie, a sheet-type bedding material; Oriental Yeast, Tokyo, Japan) for one night, followed by visual examination of the formation of a nest. Locomotor activity (Data S1) was assessed in a novel environment as described previously¹¹ using a Supermex apparatus (Muro-machi Kikai, Tokyo, Japan) and a sensor monitor that was mounted above the chamber. Each mouse was exposed to an illuminated chamber (30 cm \times 40 cm \times 25 cm). The intensity of illumination was approximately 120 lx in the center of the chamber. All locomotor activity counts were automatically summed and recorded every 15 minutes for 2 hours. Depressive-like behavior was then assessed in the tail suspension test (Data S2) as described previously,¹² with some modifications. The mice were suspended by the tail for 6 minutes, and motility was recorded from 2 to 6 minutes using an acceleration detector (Neuroscience, Inc., Tokyo, Japan). We conducted these behavioral experiments in the following order: nest building, locomotor activity, and tail suspension test. The data are presented as the mean \pm SEM. For locomotor activity, the analyses were performed using two-way analysis of variance (ANOVA). Individual post hoc comparisons were performed using Scheffe's test. The tail suspension test

data were analyzed using Student's *t* test. Values of $P < .05$ were considered statistically significant. The data were analyzed using Bell-Curve for Excel software (Social Survey Research Information, Tokyo, Japan).

The results showed that locomotor activity in a novel environment increased in P123H β S Tg mice compared with WT mice at 60 min ($F_{1,136} = 5.369$, $P = .022$) and 90 min ($F_{1,136} = 5.1501$, $P = .0248$; Figure 1A), indicating that P123H β S Tg mice became hyperactive in an unfamiliar environment that was distinct from their home cage.⁷ This result also confirmed that locomotor activity was not impaired at this age in P123H β S Tg mice, although motor dysfunction was gradually manifested around 12 months of age in these mice.⁷ Furthermore, although a previous study reported "claspings" in α S/P123H β S bigenic mice but not in P123H β S single Tg mice under tail suspension conditions, based on gross observations,⁷ the present qualitative study unambiguously demonstrated that motility significantly decreased in P123H β S Tg mice compared with WT mice (Figure 1B). Nest building was also impaired in P123H β S Tg mice (Figure 1C,D).

Given that spontaneous locomotor hyperactivity has previously been observed under various neurological conditions, including manic-depressive disorder, viral encephalopathy, attention-deficit/hyperactivity disorder, and several other organic brain diseases,¹³ one may predict that the neurodegenerative status of P123H β S Tg mice might be similar to these clinical pathological conditions. A recent study found that the assessment of nest building might be a valid method for assessing depressive-like symptoms.¹⁴ Therefore, the present results that showed a decrease in motility in the tail suspension test and impairment of nest building suggest that P123H β S Tg mice may be considered to express depressive-like behavior.

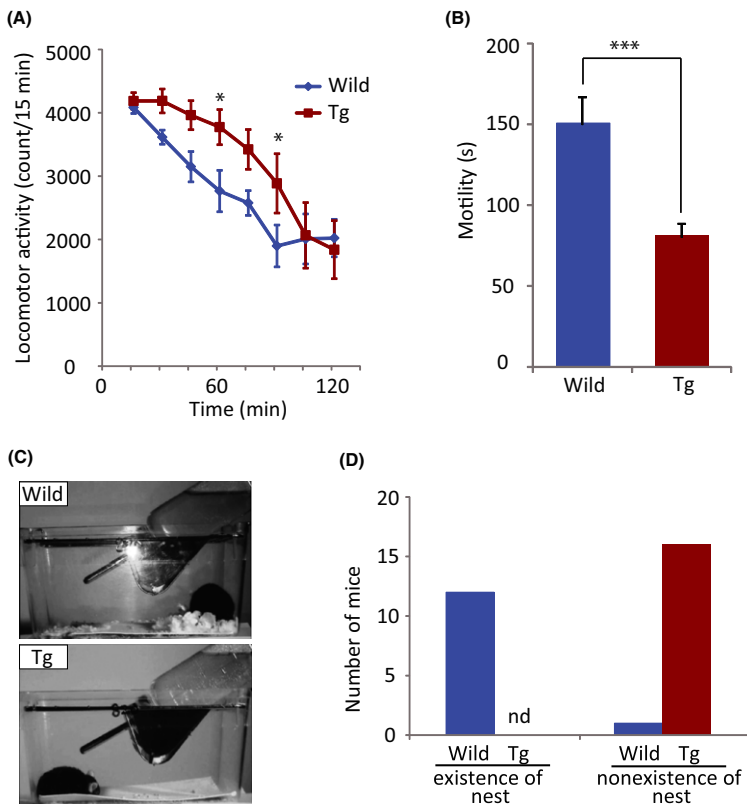


FIGURE 1 Behavioral alterations in P123H β S Tg mice. (A) Locomotor activity in a novel environment in wild-type mice ($n = 10$; blue line) and P123H β S Tg mice ($n = 9$; red line). The data are expressed as mean \pm SEM. * $P < .05$, compared with wild-type mice. (B) Tail suspension test in wild-type mice ($n = 16$; blue bar) and P123H β S Tg mice ($n = 17$; red bar). The data are expressed as mean \pm SEM. *** $P < .001$, compared with wild-type mice. (C) Representative images of nest building in a wild-type mouse (wild) and P123H β S Tg mouse (Tg). (D) Twelve of the 13 wild-type mice built a nest, whereas all of the P123H β S Tg mice ($n = 16$) failed to build a nest



Several mechanisms are assumed to underlie depressive-like behavior in P123H β S Tg mice. Either a toxic gain of function or loss of function that is caused by P123H β S in the cytoplasm may result in alterations of signal transduction and transcription, followed by a decrease in synaptic plasticity, eventually leading to the manifestation of neurological phenotypes, such as depression. Indeed, similar hyperactivity and depressive-like behavior were observed in A53T α S mice¹⁵ and a mouse model of tauopathy.¹⁶ Alternatively, recent findings suggest that neuroinflammation may be involved in depression.¹⁷ Therefore, neuroinflammation might play a key role in depressive-like behavior in P123H β S Tg mice. Consistent with this possibility, P123H β S Tg mice presented mild features of neuroinflammation, including an increase in inflammatory cytokines (eg, interleukin-1 β and tumor necrosis factor- α) in the hippocampus.⁷

In summary, P123H β S Tg mice exhibited depressive-like behavior that was manifested before the onset of motor dysfunction. Thus, P123H β S Tg mice may be a valid model for investigating the early phase of LBD.

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CONFLICT OF INTEREST

Authors declare no conflict of interest for this article.

ANIMAL STUDIES

The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee at Tokyo Metropolitan Institute of Medical Science.

AUTHOR CONTRIBUTIONS

MF, KI, and MH conceived and designed the experiments and wrote the article. MF, YH, YuT, YS, and YoT performed the behavioral experiments and analyzed the data.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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