Olfactory Discrimination Deficits in A53T Alpha-Synuclein Transgenic Mice at 3 Months

Qi Liu[1], Yu Zhou[1]✎, Zegang Ma[1]✎

[1]Department of Physiology, Medical College of Qingdao University, Qingdao, Shandong, China 266071

Abstract: Parkinson's disease (PD) is a kind of typical neurodegenerative diseases. Previously, clinical studies have showed that deficits in olfactory and cognitive functions precede motor-symptoms in PD. However, the mechanisms of olfactory discrimination deficits in early stage of PD is still unknown. In this study, we aimed to clarify the characteristics of olfactory deficits in A53T transgenic mice and test the possible mechanisms involved. By the elevated plus maze and bedding test, we showed that A53T transgenic mice at 3 months exhibit no hyperactivity behavior. Olfactory discrimination test indicated wild-type mice spent more time in old bedding than in new bedding during 5 min test (*P<0.05). However, the A53T transgenic mice could not distinguish between the old bedding and the new bedding at 3 months. In the fine odor discrimination test, we found that as the ratio [+] (Mango juice) to [-] (Almond juice) of odor components declined in task, the correct olfactory discriminations per trial session (%) reduced in A53T transgenic mice and wild type controls. There was no difference between two groups at the ratio of [+] 100:0 to [-] 0:100. However, at the ratio of [+] 60:40 to [-] 40:60, the correct olfactory discriminations per trial session (%) was significantly higher in control group. Rota-rod test indicated that A53T transgenic mice had no motor disorders. These findings reinforce the notion that the olfactory deficits occurred in early phase of PD, prior to motor disorder.

Keywords: Parkinson's Disease; Olfactory Discrimination; Fine Odor Discrimination; Hyperactivity

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized pathologically by the loss of dopamine neurons in the substantia nigra and the formation of intra-neuronal inclusions called Lewy bodies, which are mainly comprised of alpha-synuclein (a-syn) [1]. The typical features of PD including tremor, rigidity, bradykinesia, and postural instability. However, there are some non-motor symptoms, for instance, olfactory disorder, cognitive decline, appearing before or in parallel with motor deficits [2–5]. In both PD patients and mouse models, increasing evidence proves that neuronal dysfunction occurs before the accumulation of protein aggregates (i.e.,a-syn) and neurodegeneration[6]. Presently, neurogenesis was proposed to connected with olfactory discrimination[7]. Adult neurogenesis, the generation of new neurons within two areas in the adult brain, One is the subgranular zone of the hippocampus, the other is the subventricular zone, a structure known to be critical for olfactory discrimination[8, 9, 10, 11, 12], Lateral ventricle ependymal lower (SVZ) of newborn neurons in the rostral migration stream to the olfactory bulb, is very important to maintain normal sense of smell.

A multitude of literatures showed that Parkinson's early olfactory are inhibition[7, 8, 9], moreover, few people do olfactory discrimination with C57BL/6 mice. Therefore we use this PD model to investigate the performance of olfactory discrimination in the bedding test and fine odor discrimination task as well as the correlations of olfactory with other behaviors. We now report that these A53T genetic mutant mice do not produce hyperactivity and anti-anxiety, but olfactory deficits, moreover prior to motor disorders.

Materials and Methods
Animals
In this study, three-month-old and nine-month-old C57BL/6 mice were used. Mice were kept at room temperature under a 12 h light/12 h dark cycle (lights on from6:00 A.M. to 6:00 P.M.) and housed 2-4 animals/cage. Every one week, these mice were provided the chow and the water as an ad libitum. Mice were initially obtained from Jackson Laboratories (Bar Harbor, ME). Mice with the mutant gene were generated on a C57Bl/6 genetic background with wild phenotype [9-10]. The

✎ **Yu Zhou (Correspondence)**

✉ 1040941966@qq.com

☎ +

heterozygous mice were used in this experiment because previous studies have proved that the homozygous mice were stillborn or die. PCR amplifications was used to identify transgenic mice. Genotype was analyzed by gel electrophoresis in a 1% agarose gel for the presence of a 248-base-pair band [11]. All experimental animals were kept with approval of the Institutional Animal Care and Use Committee. Mice used in accordance with the Committee on the Ethics of Animal Experiments of Qingdao University.

Elevated Plus-Maze Test
This test was proceeded before other tests such as rota-rod[7]. The elevated plus maze is a widely used behavioral assay for rodents. Briefly, mice are placed at the junction of the four arms which is named center in the maze, facing an open arm, and entries/duration in each arm are recorded by a video-tracking system and observer simultaneously for 10 min. Other parameters (i.e., average speed, latency and stretched-attend postures) can also be observed at the same time. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior.

Bedding Test
This test was used to assess the olfactory discrimination of mice. At first, mice were kept at home-cage for 3 days. Then we put new bedding in half of the home-cage and the remaining half was placed with the old bedding. Per mouse was placed between the old bedding and the new bedding, a video-tracking system would record the total distance and duration in the new area and old area for 5 min. If the mice spend more time smelling the new bedding, the result showed that the mice occured olfactory disorders.

Fine Odor Discrimination Test
Mice were separated into home-cage individually and were deprived of water for 48 hours. Two sessions involved in this task. Firstly, in the training session, we put 12 μl of double-distilled water into a 50×10 mm tissue culture dish, on the surface of the water, mixed with 1 μl Mango juice, which was designated [+]. We put the dish at the end of the home-cage and the mouse was allowed to find and drink the [+] during 2 min. After 30 sec, a new fresh [+] solution was placed into the home-cage for 2 min. Five trails were conducted using [+] until the amount of Mango juice reached 9 μl. Secondly, in the training session, we put 12 μl of a 1% solution of denatonium benzoate (DB) (Sigma) into the dish and then put 9 μl Almond juice on the surface, the combination of solution was called [-]. Mice did not like bitter DB and learned to associate the bitter taste with the smell of Almond juice. Five trails were conducted to ensure that mice had learned to detest the [-]. In the testing stage, [+] and [-] were placed at the end of home-cage at the same time. If the mouse discriminated or drank the [+] but not [-], we defined this was a successful discrimination. Per testing stage contained 10 trails. [+] and [-] were alternated the positions at random from trail to trail to avoid the influence of the location. This task performed for 4 days continuously, each day for each gradient, mice were allowed to discriminate between solutions containing ratios of Mango juice:Almond juice of 100:0, as well as the discrete odors 60:40, 58:42, and 56:44. We stated the discriminate rates in this task.

Rota-rod Test
Rota-rod apparatus was used for estimating the balance and motor coordination of mice.The animals were placed on the rota-rod for 2 min without rotation. Then the rod was accelerated to a speed of 4-40 rpm for 5 min in total, three trails one day for 3 days continuously. The parameters of the test were recorded by the system.

Statistical analysis
All data were expressed as mean values ± S.E.M. Independent sample t-test was adopted to analyze the fear conditioning test and parameters at the probe session between the control and trans-genetic groups (SPSS 19.0 software, $n \geq 6$ for each group). The escape latency during the training tests was determined by repeated measure ANOVA analysis (SPSS 19.0 software, $n \geq 6$ for each group. $*P < 0.05$ was considered to be statistically significant.

Results
No hyperactivity and reduced anxiety-like exhibited in A53T genetically-modified mice at 3 months.

No anti-anxiety: as is exhibited, there were no obvious differences compared A53T trans-genetic mice with the wild type in the percentage into the open arms and close arms. We notice that 10% increase in the ratio of open and close, although there was no statistical significance(Figure 1 A). The percentage about number of entries into the open arms and close arms had no diversity between two groups, indicating A53T trans-genetic mice had no changes but had the tendency(37% higher than the wild type in the proportion)(Figure 1 B). The results demonstrated that trans-genetic mice and wild type mice displayed similar behaviors on elevated plus-maze test. We do not observe any reduced anxiety-like at 3 months of age.

No hyperactivity: total distance was used to determine the locomotor activity. At 3 months, our results showed traveled less distance compared with wild type mice, which reached statistical significance($*P<0.05$)(Figure 1 C). In our mouse model of PD, we confounded that previous behaviors, trans-genetic mice decreased locomotor activity. Therefore, the trans-genetic mice had no hyperactivity.

In bedding test, two groups behaved similarly in total distance, also observed hyperactivity, coinciding with the results on the elevated plus-maze test(Figure 1 D).

Olfactory deficits emerged in A53T trans-genetic mice

As the study of bedding test, the mice were allowed to smell the new bedding and the old bedding for 5 min in total. We observed wild-type mice spent more time in old bedding than in new bedding during 5 min test (*P<0.05). While the A53T transgenic mice presented a disruption in distinguishing between the old bedding and the new bedding, spending similar time in two compartments.

In the fine odor discrimination test, we found that as the ratio [+] (Mango juice) to [-] (Almond juice) of odor components declined in task, the correct olfactory discriminations per trial session (%) reduced in A53T transgenic mice and wild type controls. There was no difference revealed between two groups at the ratio of [+] 100:0 to [-] 0:100. However, at the ratio of [+] 60:40 to [-] 40:60, the correct olfactory discriminations per trial session (%) was significantly higher in control group, such that 87% of the discriminations succeeded, compared with a performance of 43% in the transgenic mice. At the ratio of [+] 58:42 to [-] 42:58 and [+] 56:44 to [-] 44:56, no statistical significance was revealed in two groups, although the performance of the transgenic mice were inferior to the wild type mice.

Rota-rod test indicated that A53T transgenic mice had no motor disorders(data not show). These findings reinforce the notion that the olfactory deficits occurred in early phase of PD, prior to motor disorder.
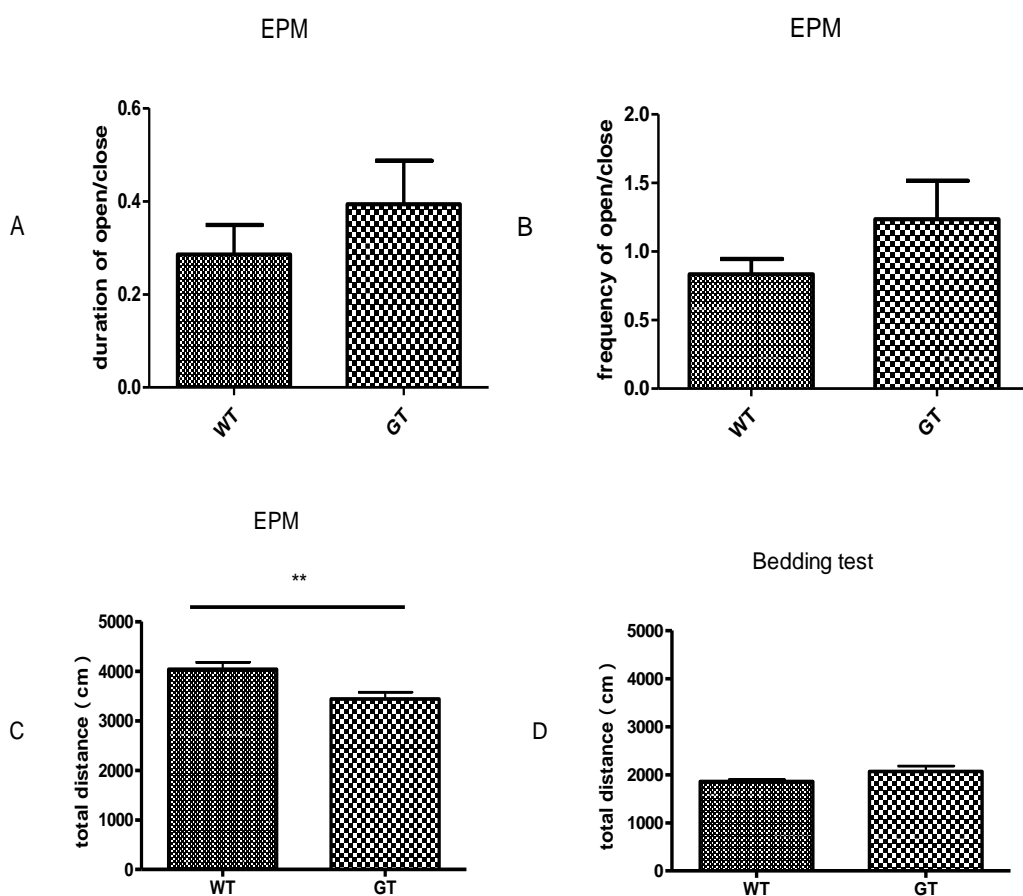

Figure 1. **No hyperactivity and reduced anxiety-like exhibited in A53T genetically-modified mice at 3 months.** **A**, percentage time spent in open arms and close arms. **B**, frequency into the open arms/close arms between wild type mice (WT) and trans-genetic mice (GT). **C**, total distance in all area of elevated plus-maze test. **D**, total distance in the bedding test. *P<0.05, **P<0.01, n ≥7.
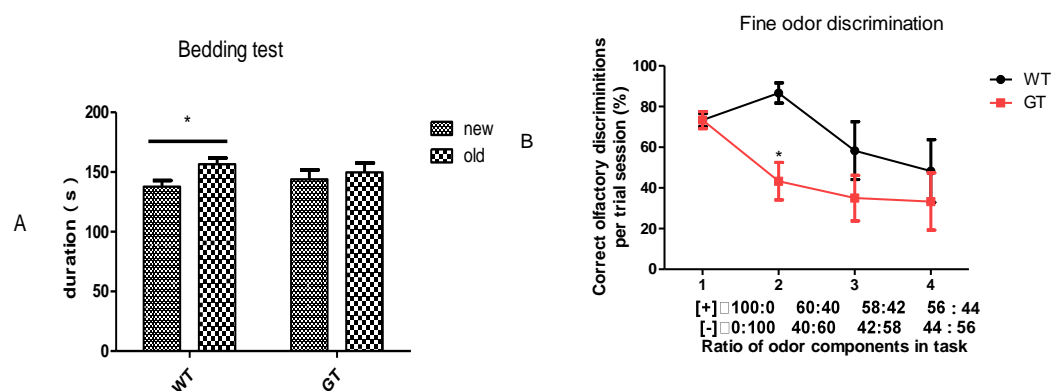
Figure 2. **Olfactory deficits emerged in A53T trans-genetic mice. A**, home-cage was divided into two identical parts, where it could choose between new bedding and old bedding. Then we placed the mice for 5 min in the center of the cage to discrimination the two odors of bedding. Two-way ANOVA. *P<0.05. **B**, Fine odor discrimination generalization gradient. [+] and [-] represented Mango juice mixture with double-distilled water and Almond juice mixture with DB. We recorded the percentage of correct discriminations per gradient. One-way ANOVA. *P<0.05, **P<0.01, n ≥7.

Discussion

Our study confirmed the notion that olfactory deficits occurred in early phase of PD, prior to motor impairments[7, 9]. The evidence suggested that neurogenesis played an important role in olfactory discrimination comparing the mutant mice to their wild type counterparts[15, 16]. Therefore, we suspect the olfactory ability of A53T mice exhibits deficits may be associated with the neurogenesis of the subventricular zone or neurons of differentiation which migrate to the olfactory bulb. Further studies are required to illustrate the underlying mechanisms mediating the olfactory discrimination deficits in PD animal models including A53T transgenic mice. This might draw more attention to olfactory discrimination, expecting to make a contribution to clinical research.

References

1. Savitt JM, Dawson VL, Dawson TM (2006) Diagnosis and treatment of Parkinson disease: molecules to medicine. J Clin Invest 116: 1744–1754.
2. Ferrer I, Lopez-Gonzalez I, Carmona M, Dalfo E, Pujol A, et al. (2012)
   Neurochemistry and the non-motor aspects of PD. Neurobiol Dis 46: 508–526.
3. Lim SY, Lang AE (2010) The nonmotor symptoms of Parkinson's disease—an
   overview. Mov Disord 25 Suppl 1: S123–130.
4. Levy G, Tang MX, Louis ED, Cote LJ, Alfaro B, et al. (2002) The association of
   incident dementia with mortality in PD. Neurology 59: 1708–1713.
5. Shimizu S, Ohno Y (2013) Improving the Treatment of Parkinson's Disease: A
   Novel Approach by Modulating 5-HT(1A) Receptors. Aging Dis 4: 1–13.
6. Paumier KL, Sukoff Rizzo SJ, Berger Z, Chen Y, Gonzales C, et al. (2013) Behavioral Characterization of A53T Mice Reveals Early and Late Stage Deficits Related to Parkinson's Disease. PLoS ONE 8(8): e70274. doi:10.1371/journal.pone.0070274
7. EmekaEnwere,*TetsuroShingo,*ChristopherGregg(2004) Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J.Neurosci.,September22,2004 • 24(38):8354–8365.
8. Oaks AW, Frankfurt M, Finkelstein DI, Sidhu A (2013) Age-Dependent Effects of A53T Alpha-Synuclein on Behavior and Dopaminergic Function. PLoSONE 8(4): e60378. doi:10.1371/journal.pone.0060378
9. Yunhua Zhu, J Clin Invest.Phosphatase WIP1 regulates adult neurogenesis and WNT signaling during aging. 2014;124(7):3263–3273. doi:10.1172/JCI73015.
10. Franz Marxreiter, Martin Regensburger, Jurgen Winkler(2012)Adult neurogenesis in Parkinson's disease. 6 July 2012 @ Springer Basel AG 2012
11. Kawai F, Miyachi E (2001) Enhancement by T-type Ca²⁺ currents of odor sensitivity in olfactory receptor cells. J Neurosci 21:RC144.
12. Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior fore-brain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–457
13. Aungst JL, Heyward PM, Puche AC, Karnup SV, Hayar A, Szabo G, Shipley MT (2003) Centre-surround inhibition among olfactory bulb glomer-uli. Nature 426:623–629.
14. Belluzzi O, Benedusi M, Ackman J, LoTurco JJ (2003) Electrophysiological differentiation of new neurons in the olfactory bulb. J Neurosci 23:10411–10418.
15. CremerH,ChazalG,LledoPM,RougonG,MontaronMF,MayoW ,LeMoalM, Abrous DN (2000) PSA-NCAM: an important regulator of hippocampal plasticity. Int J Dev Neurosci 18:213–220.
16. Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, Geary C, Cross JC,Weiss S (2003) Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science 299:117–120.