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Human LRRK2 G2019S mutation represses post-synaptic protein PSD95 and causes cognitive impairment in transgenic mice

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ABSTRACT

Background: LRRK2 G2019S mutation is associated with increased kinase activity and is the most common mutation associated with late-onset PD. However, the transgenic mouse model has not recapitulated cardinal PD-related motor phenotypes. Non-motor symptoms of PD including cognitive impairments are very common and may appear earlier than the motor symptoms. The objective of this study was to determine whether human LRRK2 with G2019S mutation causes hippocampus-dependent cognitive deficits in mice.

Results: Male (LRRK2-G2019S) LRRK2-Tg mice showed impairments in the early portion of the Two-day radial arm water maze acquisition trial as well as in the reversal learning on the third day. However, their performance was similar to Non-Tg controls in the probe trial. LRRK2-Tg mice also displayed impairments in the novel arm discrimination test but not in the spontaneous alternation test in Y-maze. Interestingly, there was no statistically significant locomotor impairment during any of these cognitive test, nor in the locomotor tests including open field, accelerating rotarod and pole tests. Expression of the postsynaptic protein PSD-95 but not the presynaptic protein synaptophysin was lower in hippocampal homogenates of LRRK2-Tg mice.

Conclusion: Consistent with previous reports in human LRRK2 G2019S carriers, the current data suggests that cognitive dysfunctions are present in LRRK2-Tg mice even in the absence of locomotor impairment. LRRK2 G2019S mutation represses the postsynaptic protein PSD-95 but not the presynaptic protein synaptophysin. This study also suggests that mild cognitive impairment may appear earlier than motor dysfunctions in LRRK2-G2019S mutation carriers.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease characterized by a progressive deterioration of motor functions. Neuropathologic hallmark of PD primarily involves the loss of a specific population of midbrain dopaminergic neurons of the *substantia nigra*. Even though PD is primarily a movement disorder, studies have shown that certain domains of cognitive functions may be affected in PD as demonstrated in animal models and human studies (Chaudhuri & Odin, 2010; Magen et al., 2012; Mochizuki-Kawai, Mochizuki, & Kawamura, 2010; Paisan-Ruiz et al., 2004). The etiology of PD is not clearly known but certain genetic factors may predispose an individual to the disease (Melrose, 2008). Mutations in the Leucine-rich repeat kinase 2 gene (LRRK2) is the most important known genetic risk factors associated with PD (Paisan-Ruiz et al., 2004). The most prevalent LRRK2 mutation associated with late-onset PD is the G2019S mutation characterized by the main pathologic mechanism of increased kinase activity (Melrose, 2008). Although, various LRRK2 mice models have not accurately recapitulated the classic PD pathology and symptoms, some studies, including those employing *in vitro* techniques have demonstrated the role of LRRK2 in cellular and molecular mechanisms that may impact learning and memory. For example, LRRK2 has been shown to play important roles in synaptic functions, apoptosis and perhaps more importantly

Abbreviations: ChaRP, challenge-adjusted rod performance; ITI, inter-trial interval; LRRK2, leucine-rich repeat kinase 2; NAD, novel arm discrimination; ORP, overall rod performance; PD, Parkinson's disease; PSD-95, post synaptic density protein; RAWM, radial arm water maze; SAYM, spontaneous alternation in Y-maze; Tg, transgenic.

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neurogenesis – a hippocampus process that is important for learning and memory (Iaccarino et al., 2007; Jessberger et al., 2009; Matta et al., 2012; Winner et al., 2011). The cognitive features of PD pose a significant challenge for clinical management of the disease because the symptoms are not as responsive to dopamine-based therapy as motor symptoms (Chaudhuri & Odin, 2010). Although, contrary reports exist, several studies have reported cognitive impairments which precede or accompany the appearance of motor symptoms in PD patients and even among healthy LRRK2 G2019S subjects (Mochizuki-Kawai et al., 2010; Saunders-Pullman et al., 2006; Thaler et al., 2012). The objective of this study is to determine whether human LRRK2 with the G2019S mutation causes hippocampus-dependent cognitive deficits in middle aged (9–10 month old) mice.

2. Methods

2.1. Mice

The previously described human LRRK2-Tg mice (with G2019S mutation) in FVB background were crossed with Non-Tg littermate of LRRK2-Tg mice (also with G2019S mutation) in C57BL/6J background. The resulting offsprings were genotyped by tail biopsy with the previously described primers (Melrose et al., 2010). The animals used throughout this study (in behavior and biochemical assays) are twelve (12) male mice consisting of six LRRK2 G2019S-Tg (henceforth referred to as LRRK2-Tg) and six Non-Tg littermates from the cross above, at 8.6–9.7 months of age (9.7 ± 0.9 month average ± standard deviation for both Tg and Non-Tg mice). All procedures were in compliance with University of Mississippi Medical Center Institutional guidelines, approved by the Animal Care and Use Committees (protocol #1242A).

2.2. Materials

Rabbit anti-PSD-95 (Cat# Ab18258), Rabbit anti-synaptophysin (Cat# ab68851) and Mouse anti-β-Actin (Cat# Ab6276) were obtained from Abcam (Cambridge, MA). Pierce™ Fast Western Blot Kit, ECL substrate (Cat# 35050) was obtained from ThermoFisher Scientific, (Waltham, MA).

2.3. Learning and memory tasks

We used a Two-day *Radial-arm water maze* (RAWM) task followed by a one-day reversal learning component to study spatial memory and cognitive flexibility respectively. The tasks were conducted and analyzed as we have previously described (Adeosun et al., 2014; Alamed, Wilcock, Diamond, Gordon, & Morgan, 2006; Hou et al., 2015). Visual cues were included in the RAWM set up both near the tank and on the walls of the room.

Novel arm discrimination (NAD), a hippocampus-dependent spatial recognition memory task was carried out and analyzed as we have previously described (Adeosun et al., 2014; Hou et al., 2015; Wright & Conrad, 2005) with a shorter inter-trial interval (ITI) of 30 min. *Spontaneous alternation in Y-maze* (SAYM), which tests the mice working memory was also carried out and analyzed as previously described (Adeosun et al., 2014; Holcomb et al., 1999). The test lasted for 10-min and the first and last 5 min data were analyzed separately.

2.4. Locomotor tasks

Distance travelled in an *Open field task* was obtained by recording the mice activity in a 40 cm × 40 cm × 40 cm Plexiglas box for 10 min. The total distance travelled every 2 min was obtained by analyzing the captured overhead video offline with Noldus Ethovision XT software (Wageningen, The Netherlands).

An accelerating *Rotarod task* was also conducted using a 4-lane rotarod device as previously described with major modifications described below (Adeosun et al., 2012). Mice were tested in 2 batches of 4 animals per batch. The acceleration settings were 8, 20, 40 and 80 rev/min^2, starting from the lowest acceleration. Mice were settled on the rod facing away from the investigator after which the device, which preset to the right acceleration (starting with 8 rev/min^2), was turned on. The time each mouse was able to ride on the accelerating rod before falling on the padded platform under the rod was recorded. The test was repeated at the same setting after all the mice had fallen off the rod. The second batch of mice were tested at the same setting while the first batch rests. Then the first batch was tested at the next setting (20 rev/min^2) and so on until both batches have been tested at all the acceleration settings. Replicate data for each animal at each acceleration setting was combined as an average before the average for each genotype was calculated.

Although, ours was an accelerating rod, we used the overall rod performance (ORP) method, which is the area under the curve of a fixed speed rotarod, to calculate a performance score for each genotype (Rozas, Guerra, & Labandeira-Garcia, 1997). We also used a novel method that consider performance in each acceleration setting individually and scores the animal such that the relative difficulty (***Co****efficient of* ***C****hallenge*) of each setting is factored into the total score. Thus, a 10 sec ride at 40 revs/min^2 contributes twice as much as a 10 sec ride at 20 revs/min^2 to the total score of the animal. We have termed this scoring method ***Ch****allenge-****a****djusted* ***R****otarod* ***P****erformance* (ChaRP). Thus, for the 8, 20, 40 and 80 revs/min^2 that we used in the current report, the ride time at the four settings were multiplied by coefficients of challenge 1, 2.5, 5 and 10 respectively before being summed to arrive at the ChaRP score.

Pole test was conducted using a 1 cm diameter, 50 cm long wooden pole with rough surface as previously described. The test was repeated four times (10 min inter-trial interval) and the scores were averaged for each mouse before the averages of the genotypes were calculated (Ogawa, Hirose, Ohara, Ono, & Watanabe, 1985).

2.5. Western blot

After all the behavior tests, the mice were sacrificed by isofluorane anaesthesia and cardiac perfusion. The left hemisphere of the brain was fixed in PFA for immunohistochemistry while the right hemisphere was reserved for protein extraction. The hippocampus was micro-dissected out of fresh right hemisphere brain samples and flash frozen in liquid nitrogen. Protein extraction from the hippocampal homogenates were done as previously described (Adeosun et al., 2014; Hou et al., 2015). The proteins were separated in polyacrylamide gel electrophoresis and transferred to PVDF membranes. PSD-95 (1:500) or Synaptophysin (1:1000) were used to probe the membranes. Membranes were also probed with β-Actin antibody (1:5000). Detection of the blots were completed using the Pierce™ Fast Western Blot Kit, ECL substrate. Optical densities were read in a Biorad Chemidoc imager (Hercules, CA).

2.6. Immunohistochemistry

The left hemisphere of the brains were transferred from overnight PFA into 30% sucrose until they sank to the bottom. The brains were sectioned coronally at 40 μm thickness and then subjected to immunohistochemical staining with specific antibodies for PSD-95 or Synaptophysin along with NeuN as previously described (Adeosun et al., 2014; Hou et al., 2015). Appropriate negative controls were done (i.e. excluding either the primary or