Journal of Neuroscience Methods 189 (2010) 180–185

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

A rotarod test for evaluation of motor skill learning

Hiromi Shiotsuki[a], Kenji Yoshimi[b,e], Yasushi Shimo[a], Manabu Funayama[a], Yukio Takamatsu[c], Kazutaka Ikeda[c], Ryosuke Takahashi[d,e], Shigeru Kitazawa[b,e], Nobutaka Hattori[a,e,*]

[a] Department of Neurology, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan
[b] Department of Neurophysiology, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan
[c] Tokyo Institute of Psychiatry, Kamikitazawa 2-1-8, Setagaya-ku, Tokyo 156-8565, Japan
[d] Department of Neurology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawaharacho, Sakyoku, Kyoto 606-8507, Japan
[e] CREST, Japan Science and Technology Agency, Honcho 4-1-8, Kawaguchi-shi, Saitama 305-8575, Japan

ARTICLE INFO

Article history:
Received 9 November 2009
Received in revised form 18 March 2010
Accepted 23 March 2010

Keywords:
Rotarod
Motor skill learning
Dopamine
Phasic neurotransmission
MPTP
Parkinson's disease
Mice

ABSTRACT

The rotarod test is widely used to evaluate the motor coordination of rodents, and is especially sensitive in detecting cerebellar dysfunction. However, mice with striatal dopamine depletion show only mild or no motor deficit on the typical accelerating rotarod. This suggests that dopamine-depleted mice are useful as animal models for non-motor symptoms, because the influence of motor deficit is minimum and easy to discriminate from cognitive aspects of the behavioral change. The typical accelerating rotarod test is designed to evaluate maximal motor performance and is not optimized to detect motor skill learning. In an attempt to make the test more selective to motor skill learning rather than maximal gait performance, we modified the rotarod test by using a slowly rotating large drum to obtain a steep learning curve. Furthermore, administration of nomifensine, a dopamine uptake inhibitor, improved the learning. On the other hand, apomorphine, an agonist of dopamine autoreceptor, a dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) impaired the learning. These pharmacological profiles fit the involvement of the so-called phasic dopamine neurotransmission. Using our modified procedure, we found impaired learning of Parkin-deficit mice, which has not been detected in typical accelerating rotarod. The modified rotarod test would be useful for evaluation of dopamine involvement in the acquisition of motor skill learning.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease characterized by akinesia, rigidity, resting tremor, and postural instabilities. In addition, neuropsychiatric, perceptual and cognitive deficits are increasingly recognized as non-motor manifestations of Parkinson's disease (Carbon and Eidelberg, 2006; Frank et al., 2004; Owen et al., 1992; Taylor et al., 1990). It is generally difficult to discriminate motor and cognitive aspects in behavioral tests of Parkinson's disease patients, because impaired movement can influence all of behavioral performance. If there is an animal model which has Parkinson-like pathological brain degeneration but has no motor deficit, it would be an ideal model for the non-motor symptoms of Parkinson's disease.

The major neurochemical hallmark of Parkinson's disease is the degeneration of dopaminergic neurons in the substantia nigra pars compacta. In animal models, nigral degeneration can be produced by the selective toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP). Although nigral neurons are degenerated in MPTP-treated mice, several studies have failed to detect motor deficit (Jackson-Lewis and Przedborski, 2007; Przedborski et al., 2001). While MPTP-treated primates show Parkinsonism-like motor deficits (Arai et al., 1990; Fisher et al., 2004; Sedelis et al., 2001; Tillerson et al., 2002), the behavior of MPTP-treated mice can be hardly be mentioned as "Parkinsonism".

Among several behavioral tests that measure motor performance, the rotarod is a suitable test for evaluation of cerebellar deficits in rodents (Caston et al., 1995; Lalonde et al., 1995). The motor performance on the rotarod can be influenced by several factors, such as motor coordination, learning and cardiopulmonary endurance. Since several studies have shown that basal ganglia are essential in motor skill learning of serial motor sequence (Hikosaka et al., 1999), we tried to extract the acquisition of motor skill from the original procedure. In the previous study, deficit in the acquisition of rotarod learning was not obvious in MPTP-treated C57 BL/6 mice (Sedelis et al., 2000). Some studies have detected impairment of rotarod performance in dopamine-depleted mice and rats (Monville et al., 2006; Rozas et al., 1998). Monville have shown

* Corresponding author at: Department of Neurology, Juntendo University School of Medicine, Hongo 2-1-1, Bunkyo-ku, Tokyo 113-8421, Japan. Tel.: +81 3 5802 1073; fax: +81 3 5800 0547.
E-mail address: nhattori@juntendo.ac.jp (N. Hattori).

0165-0270/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2010.03.026

that the sensitivity to motor disability improved as rotation speed became higher. But these studies focused on behavior after overtraining whereas the learning curve at the initial acquisition phase was not presented. The learning effect appears as the elongated falling latency along with the trial numbers, but the typical accelerating rotarod does not seem ideal for evaluating acquisition of motor skill learning, because the learning curve is shallow and the performance after training for several days is no more than twice of the first day (Perez and Palmiter, 2005). Various rod sizes and speeds have been tried on a considerable amount of the literature evaluating daily changes in the motor performance on the rotarod (Akita et al., 2006; Caston et al., 1995; Jeljeli et al., 1999; Ogura et al., 2005; Sedelis et al., 2000). But if researchers decide to look for changes in performance over successive testing bouts where "motor learning" can be demonstrated, the rotarod test should have a distinction separating "motor learning" from basal gait/postural ability. Therefore, in the present study, we designed a modified rotarod protocol to evaluate the acquisition of motor skill learning selectively. Our non-accelerating rotarod test employs a wide drum with a hard surface, on which naive mice find it difficult to stay, but its low rotating speed leaves room for improvement after training.

A recent study reported dynamic reorganization of striatal circuits during the acquisition of motor skill on the accelerating rotarod (Yin et al., 2009). Furthermore, Akita et al. (2006) reported that a decrease in synaptic dopamine release induced by blocking the expression of synaptophysin in the nigrostriatal neurons resulted in impairment of acquisition of the rotarod task. Transgenic mice with striatal degeneration could walk on the rotarod but lacked the ability to learn (Kishioka et al., 2009). Based on these findings, one can postulate that the rotarod task reflects the striatum-based motor skill learning. To test this hypothesis, first, we tried our modified rotarod test in MPTP-treated mice. Next, we examined the effects of dopamine uptake inhibitors and a dopamine agonist. Finally, we examined our modified rotarod test in Parkin-deficient mice to compare with past studies using accelerating rotarod. Several studies have failed to detect impairment in gait performance of Parkin-deficient mice (Goldberg et al., 2003; Von Coelln et al., 2004; Perez and Palmiter, 2005; Sato et al., 2006), but impairment of motor skill learning was evident in our modified rotarod test. The results showed that our modified rotarod test is suitable for the selective evaluation of acquisition of motor skill, and cognitive involvement of the nigrostriatal dopamine system.

2. Materials and methods

2.1. Animals

Adult C57 BL/6J male mice (CLEA Japan, Tokyo, Japan, 4-month old, 25–30 g body weight) were used in this study. In addition, *Parkin*−/− male mice (4-months old), which carries a chromosomal replacement of the exon 3 of the *parkin* gene (Kitao et al., 2007) and their littermates were used. The mice were backcrossed into the C57BL/6J mice for 12 generations, then both +/+ (WT) and −/− (PKO) littermates were substrained and maintained in parallel in the same animal facility in Juntendo University. Wild-type and Parkin-deficient mice of close birth date were subjected to the behavioral tests in the same day in a shuffled sequence.

The mice were housed in groups of five to eight per cage and allowed free access to food and water. They were maintained in a temperature-, humidity- and light-controlled environment with a 12 h light–dark cycle. The experimental procedures were in accordance with the Guidelines for Proper Conduct of Animal Experiments by the Science Council of Japan and all experiments were approved by the Ethics Review Committee for animal Experimentation of Juntendo University School of Medicine. All efforts were made to minimize the number of animals and their suffering.

2.2. Motor skill learning test

To assess the acquisition of skilled behavior in mice, we first modified the standard rotarod test to emphasize the learning aspect of the test and minimize the other factors. A rotarod machine with automatic timers and falling sensors (MK-660D, Muromachi-Kikai, Tokyo, Japan) were used. The mouse was placed on a 9 cm diameter drum. The surface of the drum was covered with hard chloroethylene, which does not permit gripping on the surface. Before the training sessions, the mice were habituated to stay on the stationary drum for 3 min. Habituation was repeated every day for 1 min just before the session. Acceleration of the rotation was abandoned and the rotation was set at a relatively slow speed (10 rpm, 2.8 m/min on the surface), to make the task easier for learned animals. The animal was placed back on the drum immediately after falling, up to 5 times in one session. A fall was overlooked when the animal remained on the drum for 180 s. To evaluate long-term memory, the test was repeated one session a day for four consecutive days. The latency to falling was recorded automatically by photo-cells and the total latencies on the rod on each day was analyzed. Next, in order to compare the typical rotarod test with our modified rotarod test, we also tested the accelerating rotarod protocol. The speed of the rod of 3 cm diameter was accelerated from 4 to 40 rpm. The habituation time and daily schedule was the same.

2.3. Drugs and solutions

MPTP-HCl, nomifensine, and (−)-apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in saline. Apomorphine was dissolved just before use. Drugs were administrated subcutaneously in 10 ml/kg. The doses used were 30 mg/kg for MPTP, 1 and 3 mg/kg for nomifensine and 0.1 and 0.3 mg/kg apomorphine. The information on the solution bottle was coded by another individual scientist, and the trainer of the animal was blinded to the drug information.

2.4. Pharmacological treatment

2.4.1. MPTP

Each mouse received one injection of either saline or MPTP solution per day for five consecutive days, and the animals were allowed to recover for three days before the first training session. Several different MPTP dosing regimens were used. We used typical sub-acute intoxication regimen, which involves one injection of 30 mg/kg MPTP daily for five consecutive days (Jackson-Lewis and Przedborski, 2007). We chose this sub-acute regimen in order to minimize the death of the animals during administration. Motor learning training was applied for four consecutive days, and two days after the last training, all animals were deeply anesthetized with an overdose of pentobarbital, decapitated and the striatal tissue was dissected out under a microscope. All needles, syringes and animal housings were cleaned with 1% bleach solution in water according to the safety protocols (Jackson-Lewis and Przedborski, 2007; Przedborski et al., 2001).

2.4.2. Nomifensine and apomorphine

The mice were assigned to three groups for each dose of each compound. Motor learning training was applied for four consecutive days. Each animal received one injection of each drug per day, 10 min before the training.