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Evaluation of cognitive function in adult rhesus monkeys using the finger maze test

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ABSTRACT

In research on cognitive function, the use of experimental animals is essential for the study of human cognitive processes and mechanisms. Furthermore, non-human primates are necessary for understanding higher cognitive functions in humans. However, there are few cognitive function tests available for non-human primates, Thus, we modified a finger maze test for application to non-human primates. In this study, we assessed learning and memory in 12 adult rhesus monkeys using a finger maze test that was developed to assess cognitive functions in captive non-human primates. The monkeys were trained with moving rewards indicating the correct direction, which allowed the monkeys to obtain the reward. Following training, subjects completed a learning trial and a memory trial two months later. Although the time required for training varied among the monkeys, 11 out of 12 monkeys completed the training and achieved a high success rate in the learning trial as well as in the memory trial conducted 2 months later. This is the first study to apply the finger maze test to adult rhesus monkeys. The finger maze test enabled us to assess learning and memory in several adult rhesus monkeys simultaneously.

1. Introduction

Cognitive function refers to the ability to efficiently manipulate knowledge and information through learning, reasoning, problem solving, and memory. It tends to diminish due to aging or neurodegenerative disease (Deary et al., 2009). Therefore, many studies have been conducted to evaluate cognitive function and treat the related disorders. Studying cognitive function using experimental animals is important for the elucidation of the processes and mechanisms involved in human cognitive function (Katz and Witkin, 1993; Parker and Gaffan, 1995; Ridley et al., 1997; Sawaguchi and Goldman-Rakic, 1994). Accordingly, numerous tools for assessing cognitive function in various animals have been developed (Laughlin and Mendl, 2000; Morris, 1984; Shaw and Schmelz, 2017). Despite the importance of research on cognitive function using non-human primates, there is a shortage of assessment tools commonly available to measure cognitive functions (Inoue et al., 2014).

The Wisconsin General Test Apparatus (WGTA) has long been used as a tool for assessing cognitive function in non-human primates (Harlow, 1962). However, WGTA is unsuitable for simultaneous application to a large number of monkeys as only one monkey can be accurately tested at a time and a lengthy preliminary training period is

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required (Yoshida and Fujimoto, 2006). Therefore, a finger maze test was developed to address these drawbacks. The finger maze test is an assessment tool for evaluating cognitive functions such as learning and memory in non-humans (Tsuchida et al., 2003). It is an assessment tool designed as a puzzle feeder-type that attaches to the home cage which is the most familiar environment for monkeys. The finger maze test apparatus is easy to make and affordable regarding material costs. In addition, finger maze tests can be applied to multiple primates simultaneously (Inoue et al., 2014; Tsuchida et al., 2003). Moreover, this puzzle feeder enriches the environment of the monkeys (Evans et al., 1989; Novak et al., 1998).

The finger maze test developed in 1998 has been used in laboratories to assess cognitive function, specifically, the learning abilities and memory function in non-human primates. The most recently developed test is a 4-step non-correction-method-type finger maze that was initially designed for adult cynomolgus monkeys to test their visuospatial orientation, spatial learning, and manual dexterity (Tsuchida et al., 2003). It was used to evaluate the effects of toxic substances on learning in infant cynomolgus and rhesus monkeys and those of amyloid-β targeting immunotherapy on long-term memory in aged African green monkeys and cynomolgus monkeys(Hara et al., 2016; Inoue et al., 2014; Negishi et al., 2006).

In previous studies, the finger maze test was performed on young cynomolgus monkeys, young rhesus monkeys, and adult African green monkeys. However, to date, there have been no studies on adult rhesus monkeys. Non-human primate experiments are important in the preclinical stage (Friedman et al., 2017), and although non-human primates (NHPs) account for 0.5 % of animal studies, they generally have highly significant results in comparison with other animal studies. Among the non-human primates, rhesus monkeys and cynomolgus monkeys are the most widely and frequently used in NHPs studies. Rhesus monkeys are good candidates for establishing an animal model of aging-related diseases (Simmons, 2016). Since aging and cognitive functions are related (Deary et al., 2009; Herndon et al., 1997; Itoh et al., 2001), it is useful to evaluate cognitive functions using adult rhesus monkeys. Therefore, we applied the finger maze test to adult rhesus monkeys to evaluate their learning and memory functions. Moreover, we modified and upgraded the finger maze test using a different reward, that is, monkey chow. In previous studies, a piece of apple (2.5 g) was used as a reward in each trial in the finger maze task. However, the apple crumbled easily if the adult rhesus monkeys tried to pull it into the cage without dropping it. Therefore, we modified the finger maze test proposed by Tsuchida et al. (2003) by replacing the apple with a standard certified commercial monkey chow as the reward. The modified finger maze test may be used to measure the cognitive function of adult rhesus monkeys. It can also be used to measure changes in cognitive function due to drug administration or disease.

2. Materials and methods

2.1. Subjects

Twelve experimentally naïve female rhesus macaques (*Macaca mulatta*) were used as the subjects (Table 1). The monkeys were born at Gaoyao Kangda Laboratory Animals Science & Technology Co., Ltd. (Gaoyao, China) and were transferred to our facility when they were 3 years old. They were housed in individual indoor cages because the finger maze test apparatus was attached to the front of the individual cage and one apparatus had to be used by one monkey. They were fed commercial monkey chow (Teklad 2050™, Envigo, USA), and their diet was supplemented with various fruits daily and water *ad libitum* at the National Primate Research Center of the Korea Research Institute of Bioscience and Biotechnology (KRIBB) for 3 years. As previously reported (Lee et al., 2014; Yeo et al., 2015; Yi et al., 2017), the environmental conditions were controlled to provide a temperature of 24 ± 2 °C, relative humidity of 50 ± 5 %, 100 % fresh air at a rate of ≥ 12 room changes per hour, and a 12:12 h light:dark cycle. All procedures were approved by the KRIBB Institutional Animal Care and Use Committee (Approval No. KRIBB-AEC-18018).

2.2. Hand dexterity test (HDT)

Monkeys performed the finger maze test using their fingers. Therefore, it was vital to make sure that they had no hand motor function deficit. Consequently, we conducted an HDT to confirm no motor dysfunction in the monkeys. We attached an HDT device consisting of a 9-well test plate on the front of the home cage. The 9 wells contained apples (1 × 1 × 1 cm) and the monkeys required to remove apples from each well. Following three training sessions, the HDT was conducted on the monkeys, and their time to collect apples was measured using a tracking software (Tracker 4.9.8, Douglas Brown, www.opensorucephysics.org).

2.3. Finger maze test

The finger maze test apparatus developed at Kyoto University (Tsuchida et al., 2003) was modified to fit each individual cage size (Fig. 1) and was set in front of each individual cage. This maze consists of 4 steps, 4 error boxes, and a feeding box (390 mm length, 320 mm width and 32 mm step height). The front part was made of acrylic plastic and the frame was made of stainless steel. When the monkey selected the correct route, a reward was designed to move to the lower step and finally dropped in the feeding box accessible to the monkey. However, when the monkey selected the incorrect route, a reward dropped into the error box inaccessible to the monkey. In our study, monkey chow was used as a reward instead of an apple.

2.4. Acclimation

Prior to training, monkeys were allowed 2 days to acclimate to the test apparatus. During this period, monkeys adapted to the presence of feeding boxes and the apparatus. Acclimation was considered to be complete when the monkeys were capable of taking the reward from the feeding box.

2.5. Training

A training session was conducted once a week and the session consisted of 8 trials (Fig. 2). A schematic description of our modified finger maze test is depicted in Fig. 1. In each trial, a reward was placed on step 1 and monkeys had to move it into the feeding box to get the reward. The success criterion for each step was achieved when completing 8 trials without a failure within one session. Once the monkeys passed step 1, they progressed to the next level where a reward was placed on step 2. Here, monkeys had to initially move the reward in the reverse direction of step 1 and then move it in the same direction as step 1 in order to place it in the feeding box. In this way, the monkeys progressed to step 3 and step 4 using similar procedures as step 1 and step 2. The training duration, number of training sessions, and success rates of the trials were recorded. Throughout all four steps, the reward was not removed if it was positioned into the error box during training. After the monkeys met the criterion of the final step 4, they underwent a learning test and a memory test.

2.6. Learning test

A learning test was conducted to verify that the monkeys succeeded in the finger maze test (Fig. 2). The monkeys were tested for two days, 10 times a day. A reward was placed on a pseudorandom position outside of the four steps. The monkeys had to identify the step where the reward was placed and moved the reward in the correct direction to drop it into the feeding box. The success rate was measured as follows:

Table 1
Information on experimental animals. Animal ID, gender, birthday, age, body weight. All information was collected prior to the experiment.

No.	ID	Gender	Birth (Day)	Age (years)	Body weight (kg) (before test)
1	R602	Female	2012/03/12	6	5.38
2	R603	Female	2012/10/01	5	4.06
3	R604	Female	2012/06/25	6	4.46
4	R609	Female	2012/05/05	6	3.16
5	R601	Female	2012/04/09	6	4.39
6	R605	Female	2012/06/11	6	4.99
7	R617	Female	2012/07/29	5	4.73
8	R622	Female	2012/04/12	6	4.01
9	R624	Female	2012/04/12	6	3.53
10	R625	Female	2012/09/09	5	4.3
11	R627	Female	2012/08/09	5	4.46
12	R629	Female	2012/05/15	6	4.2

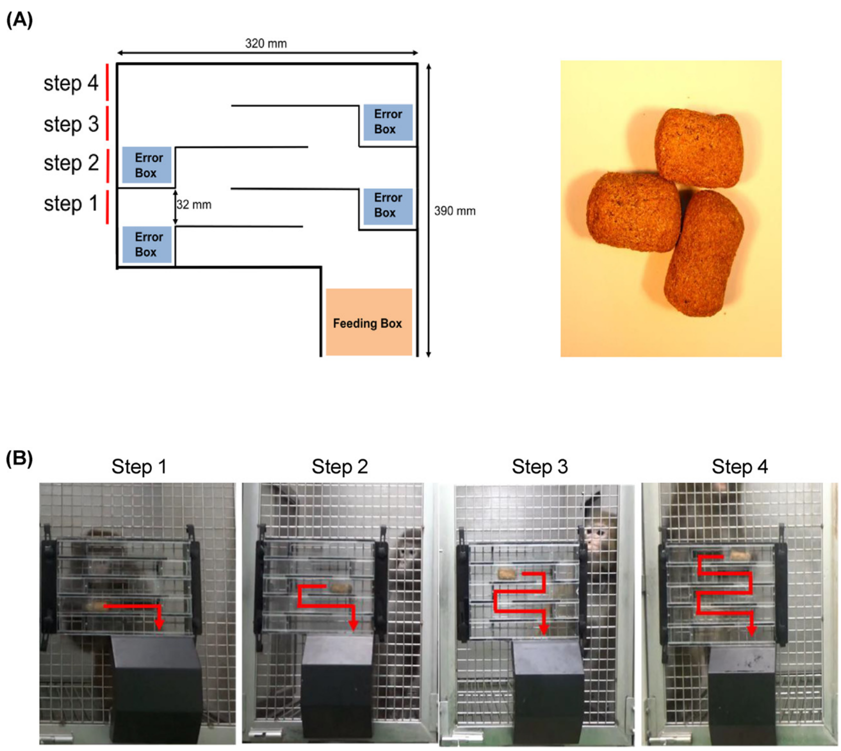

Fig. 1. (A) Schematic diagram of the apparatus and monkey chows used as a reward for the finger maze test. (B) The correct direction (red arrows) of each step and practical application of the finger maze test on the animal's home cage.

success number / trial number.

2.7. Memory test

Two months after the learning test, a memory test was performed in the same way as the learning test, and the success rate was also calculated (Fig. 2).

2.8. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows, Version, 18.0 (SPSS Inc., Chicago,

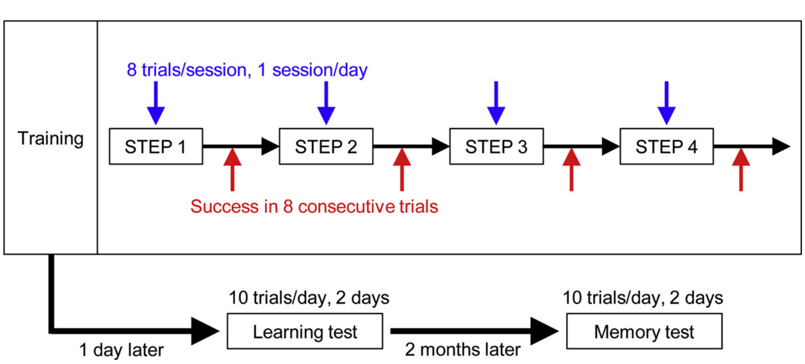

Fig. 2. Schedule and success criteria of finger maze test. The training was conducted in two sessions per day, and one session included four trials. If the monkey succeeded in all the trials in one day, it proceeded to the next step. A learning test was conducted for two days and 10 trials were conducted per day. Two months later, the memory test was performed in the same manner.

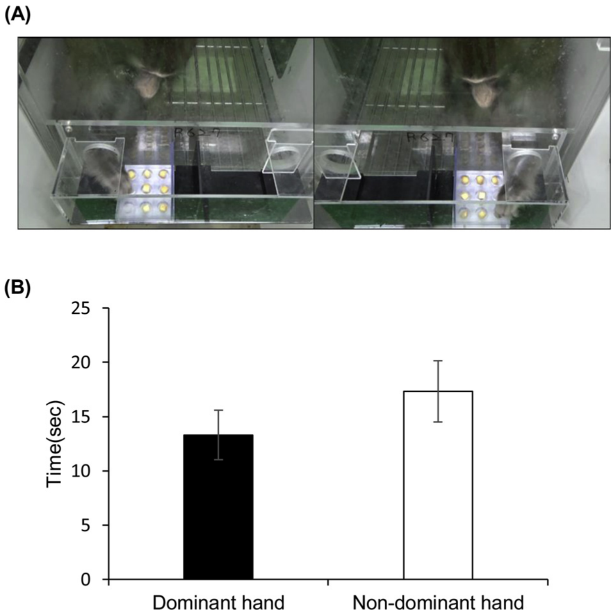

Fig. 3. Retrieval time of each hand measured by the hand dexterity test. Representative images (A), and retrieval time measured by the hand dexterity test (B). Average retrieval time of 11 monkeys. There was no statistical difference in retrieval time for the dominant hand.

IL). Results of the HDT, and the learning and memory tests were analyzed by the Kruskal-Wallis test and paired sample *t*-test, respectively. A difference of $P < 0.05$ was regarded as being statistically significant. All numerical data are expressed as the mean ± standard deviation.

3. Results

3.1. HDT

We defined a hand with a short take-out time as a dominant hand and a hand with a long take-out time as a non-dominant hand. The average time taken by 11 monkeys to take out apples was 13.23 s for the dominant hand and 17.33 s for the non-dominant hand (5 monkeys with a left dominant hand and 6 monkeys with right dominant hand). All of the monkeys were able to retrieve the reward. There was no significant difference in the dominant hand retrieval time among the monkeys (Fig. 3, p = 0.092, Kruskal-Wallis test). Therefore, we confirmed that there were no motoric dysfunctions in finger movement in the monkeys.

3.2. Training

The success rates and the days to complete the training are shown in

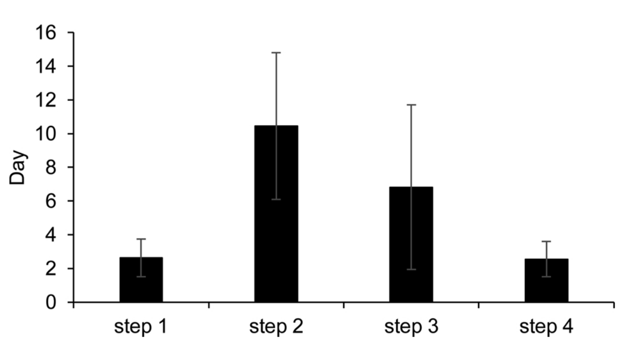

Fig. 4. Training results of the finger maze test. Average number of days to reach criterion for each step.

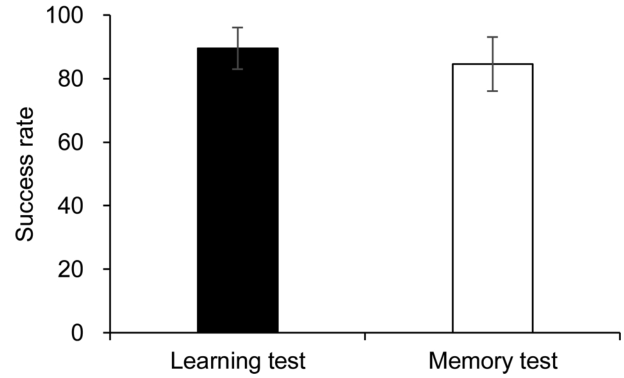

Fig. 5. Results of the learning and memory test. Average of random and memory tests of 11 monkeys. There was no significant difference between the random and memory tests.

Fig. 4. The monkeys spent an average of 2.5 days to achieve the success criteria for step 1. Step 2 took more time than step 1 with an average of 10.45 days. One out of the twelve monkeys did not succeed in the step 2 training. The monkeys took a shorter time in step 3 than in step 2 to learn the correct direction in an average of 5.44 days. Training for step 4 took an average of 2.25 days. Two monkeys completed step 4 at the first attempt without making mistakes. Importantly, the time required to reach the learning criterion decreased as the step progressed from 2 to 4, indicating that the monkeys successfully applied the rule to the next level. Total duration of training took an average of 22.55 days with 180 trials.

3.3. Learning and memory tests

The average success rate of the learning test was 89.6 % (Fig. 5), suggesting that the monkeys successfully performed the finger maze test. The average success rate of the memory test was 84.6 %. There was no statistically significant difference between the learning and memory test results (p = 0.067, paired *t*-test). These results suggest that the monkeys still remembered the rules of the finger maze test correctly even two months later. We have summarized the results of training, and the learning and memory tests (Table 2).

4. Discussion

To the best of our knowledge, we are the first to conduct the finger maze test on adult rhesus monkeys. In general, memory has been classified into three types: short-term memory, medium-term memory, and long-term memory (Teng and Squire, 1999). According to this classification, the learning test and memory test in our experiment evaluated short-term and long-term memory, respectively. Previous studies implemented the finger maze test on young cynomolgus monkeys and rhesus monkeys aged approximately 1 year (Inoue et al., 2014; Tsuchida et al., 2003). In our study, the adult rhesus monkeys completed training much faster than other species of monkeys such as

Table 2
Results of the training, random test, and memory test. Average period required for training. Average success rate of the random and memory test.

Duration of training	Day	Trial
Total day	22.55 ± 8.95	180.10 ± 71.44
Step 1	2.63 ± 1.12	21.09 ± 8.96
Step 2	10.45 ± 4.43	83.64 ± 34.93
Step 3	6.82 ± 4.89	54.55 ± 39.16
Step 4	2.55 ± 1.03	19.63 ± 9.70
Learning test (%)	89.55 ± 6.50	
Memory test (%)	84.55 ± 8.50	

cynomolgus monkeys and African green monkeys (Hara et al., 2016; Inoue et al., 2014). Unlike previous studies, we did not remove the rewards in the error box during the training. This difference might have allowed the monkeys to understand the meaning of the error box clearly, enabling them to learn the rule more quickly.

More interestingly, the time for training decreased as monkey progressed from step 2 to step 4. Surprisingly, even though training in step 3 was more difficult than in step 2, the time for training was shorter in step 3 than in step 2. This trend was also replicated in the comparison between step 3 and step 4. Given these results, we suggest that the monkeys learned the rules and applied them to the next step successfully and that non-human primates have high cognitive functions for learning rule applications. Further, eleven monkeys were successfully trained and demonstrated good performances in both the learning and memory tests with a success rate above 80 %. This indicates that the adult rhesus monkeys were able to learn new rules, apply those rules to more advanced levels, maintain the rules even for an extended period of time, and successfully retrieve the rules even two months later. Therefore, we suggest that our modified finger maze test successfully measured the cognitive function of learning and memory in non-human primates.

The finger maze test has an advantage in that many monkeys can be tested simultaneously even with a short training period. Moreover, it is simple to use and easy to manufacture at a low cost and within a short time. It also provides non-human primates with environmental enrichment using the form of a puzzle feeder. In the present study, we conducted experiments using normal monkeys only and using only young adult monkeys. Additional experiment with younger or older rhesus monkeys may confirm the level of cognitive function at that age and future longitudinal or cross-sectional studies can be conducted to assess changes in cognitive dysfunction.

5. Conclusion

We evaluated cognitive function of learning and memory in captive adult rhesus monkeys. Twelve monkeys were tested simultaneously through our modified finger maze test, of which 11 completed the training and tests successfully, with the tests showing a high success rate. In this study, we suggest that the finger maze test is a useful method for evaluating cognitive function in adult rhesus monkey in captive condition.

Declaration of Competing Interest

None.

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