Neurosci Bull June 1, 2014, 30(3): 394–400. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1426-8

·Original Article·

A modified light-dark box test for the common marmoset

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ABSTRACT

The common marmoset (*Callithrix jacchus*) has attracted extensive attention for use as a non-human primate model in biomedical research, especially in the study of neuropsychiatric disorders. However, behavioral test methods are still limited in the field of marmoset research. The light-dark box is widely used for the evaluation of anxiety in rodents, but little is known about light-dark preference in marmosets. Here, we modified the light-dark test to study this behavior. The modified apparatus consisted of three compartments: one transparent open area and two closed opaque compartments. The closed compartments could be dark or light. We found that both adult and young marmosets liked to explore the open area, but the young animals showed more interest than adults. Furthermore, when one of the closed compartments was light and the other dark, the adult marmosets showed a preference for the dark compartment, but the young animals had no preference. These results suggest that the exploratory behavior and the light-dark preference in marmosets are age-dependent. Our study provides a new method to study exploration, anxiety, and fear in marmosets.

Keywords: marmoset; behavioral test; light-dark box; exploration; anxiety

INTRODUCTION

The common marmoset (*Callithrix jacchus*) is a small New World monkey. Due to its unique biological characteristics[1], the marmoset has attracted increasing attention, and has been used as a non-human primate model in many fields, such as neuroscience, immunology, drug toxicology, and stem-cell research. Compared to the commonly-used Old World monkeys, such as rhesus and cynomolgus monkeys, marmosets show faster sexual maturation (~1 year), a shorter gestation period (~144 days), and many more offspring during a lifetime (40–80). Furthermore, transgenic marmosets with germline transmission have been generated recently[2]. Thus, marmosets show great potential as transgenic non-human primate models of human diseases, especially neuropsychiatric disorders that cannot be faithfully mimicked in rodent models.

Behavioral tests are crucial for the study of almost all animal models, especially in neuroscience research. For example, various behavioral tests are well-established for rodent models to investigate aspects of motor ability, learning and memory, anxiety and depression, fear, and social activity. However, as a relatively new animal model, the marmoset still lacks effective behavioral test methods. Thus, besides the development of genetic manipulation, the establishment of standard behavioral assays is also important for the field of marmoset research. The marmoset is one of the smallest primates, and is easy to handle with an adult body weight between 300 and 500 g, similar to that of the adult rat. Thus, it is feasible to transfer behavioral test methods from rats to marmosets. Indeed, marmosets have been tested on a spatial delayed non-match to sample task in a T-maze, in which they perform better than macaques and at a level similar to rats[3]. Also, marmosets show spontaneous alternation behaviors in the Y-maze[4].

Here, we examined the behavior of marmosets by another method commonly used in rodents, the light-dark box test[5]. This test is based on the conflict between the innate aversion of rodents to bright light and exploratory behavior in response to a novel environment. The light-dark box is widely used to evaluate anxiety and test anxiolytic drugs in rodents[5, 6], but little is known about the light-dark preference of marmosets. In the present study, we modified the rodent light-dark box and assessed the light-dark exploratory behaviors in both adult and young marmosets.

MATERIALS AND METHODS

Animals

Seven adult common marmosets (average age 41 ± 5 months) were used as subjects in the conventional light-dark box test. Eight other adult (average age 38 ± 6 months) and six young (average age 3 ± 1 months) marmosets were used as subjects in the modified light-dark box test. The animals were maintained and tested in the Jiuting Non-human Primate Facility of the Shanghai Institute of Neuroscience. They were housed in families with male/female pairs in cages and a nest-box hung in each cage. Food and water were available *ad libitum*, except during the experimental sessions. Animal care and experimental procedures were approved by the Animal Care Committee of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

Apparatus

The conventional two-box apparatus consisted of two cubes of the same size with a side-length of 40 cm (Fig. 1A). One was an opaque closed area and the other was a transparent open area. The two boxes were connected by a square tube (inner dimensions 12 × 12 cm, length 30 cm), which was half opaque and half transparent to reduce the entry of light into the closed compartment. A lamp was fixed in the opaque box and could be turned on or off by an experimenter outside the test room. Animals were put into the apparatus through a door in the open compartment.

The modified light-dark box for marmosets consisted of three compartments: two closed areas and one open area (Fig. 2). The two closed compartments were the same as the closed box above. The open compartment was a smaller transparent cube with a side-length of 20 cm. The three compartments were connected by square tubes (inner dimensions 12 × 12 cm, length 30 cm) with the open compartment in the middle. The tubes were half opaque and half transparent as above. Animals were put into the apparatus through a door in the open compartment.

Experimental Protocol

The test was performed in a sound-isolated room. Animals were removed from their home cages and handled by experienced breeders, and then put into the open compartment of the conventional or modified light-dark test apparatus. In the conventional two-box test, the lamp in the closed box was turned on first, the animal was allowed to adapt for 10 min, and was observed for another 30 min. Then, the lamp was turned off for 30-min observation. In the modified three-box test, both the lamps in the closed compartments were turned on, and the animals were allowed to adapt for 10 min and freely explore for 30 min in the open compartment and two lighted closed compartments. Then, the experimenter outside the test room randomly turned off one lamp so that one closed compartment became dark while the other was still light. The animals were observed for another 30 min.

Data Recording and Analysis

The behaviors in all experiments were recorded by digital video cameras. All data were scored offline by two independent experimenters in a blinded manner. All the data are shown as the mean ± SEM, with statistical significance assessed by Student's *t* test. All statistical analysis was performed using Origin 7.0 (OriginLab, Northampton, MA).

RESULTS

We first conducted the conventional two-box light-dark test (Fig. 1A) with seven adult common marmosets (average age 41 ± 5 months). In the first 30-min observation with the lamp on in the closed box, adult marmosets showed a preference for the open box (time spent, closed-light, 569 ± 91 s; open, 1231 ± 91 s; $n = 7$, $P < 0.001$, Fig. 1B). Then, the lamp was turned off to make the closed box dark. We found that marmosets showed the same preference for the open box (time spent, closed-dark, 442 ± 78 s; open, 1358 ± 91 s; $n = 7$, $P < 0.001$); the time spent in each box did not change significantly after turning off the lamp (Fig. 1B). This

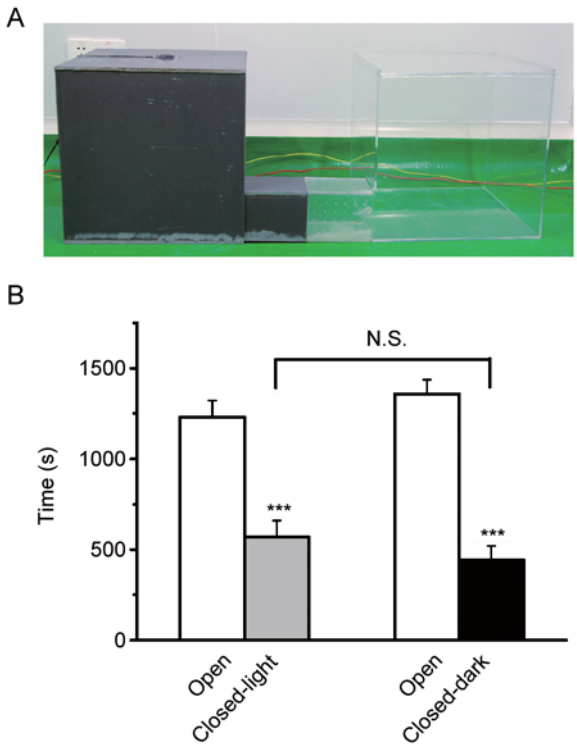

Fig. 1. Conventional light-dark test in marmosets. A: Apparatus for the conventional two-box light-dark test. B: Time spent in the open and closed compartments during the first (closed-light) and second (closed-dark) 30 min-test. ***P <0.001; N.S., no significant difference; Student's t test.

result suggests that adult marmosets prefer the open area no matter whether the closed area is lighted or dark. Thus, this conventional two-box test is not suitable to study light-dark preference in marmosets.

We thus modified the light-dark test to three boxes (Fig. 2) and tested eight other adult marmosets (>1 year old, average age 38 ± 6 months). We found that during the first 30-min period, the animals showed no preference between the two lighted closed compartments, as indicated by the numbers of entries (left, 14 ± 2.4; right, 13.6 ± 2.6; n = 8, P >0.1, Fig. 3A) and the time spent in the right and left boxes (left, 408 ± 51 s; right, 356 ± 52 s; n = 8, P >0.1, Fig. 3B), while they spent more time in the middle open compartment (1032 ± 70 s; n = 8, P <0.001 compared to both closed compartments, Fig. 3B). After the lamp in one closed compartment was randomly turned off, we found that the marmosets still spent most time in the open area (1207 ± 104 s; n = 8, P <0.001 compared to both closed compartments, Fig. 3D) but showed a significant preference for the dark compartment (entries, light, 6.8 ± 2.6; dark, 11 ± 2.6; n = 8, P = 0.02, Fig. 3C; time, light, 153 ± 51 s; dark, 440 ± 65 s; n = 8, P = 0.001, Fig. 3D). Consistent with the results in the conventional two-box test, marmosets in this modified apparatus also showed interest in exploring the open area. Furthermore, this modified test showed

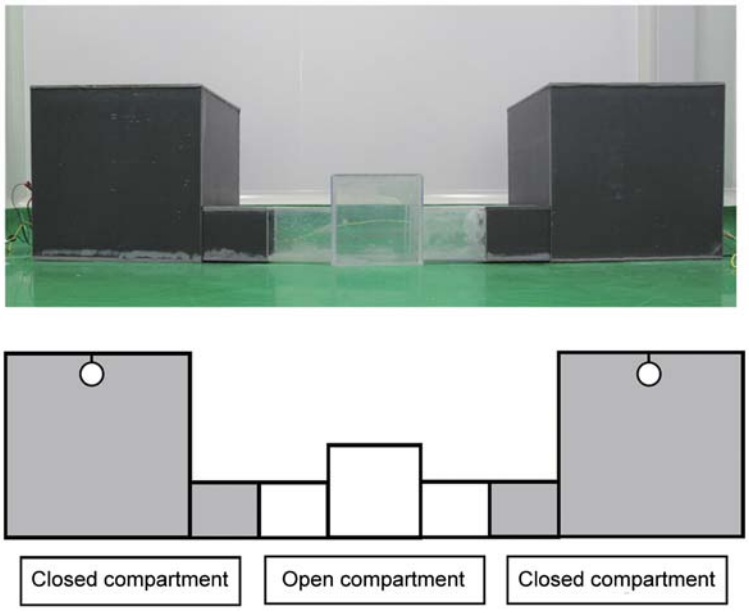

Fig. 2. Apparatus for modified light-dark test in marmosets. The modified apparatus consisted of two closed compartments and one open compartment. The lamp in each closed compartment could be turned on or off by an experimenter outside the test room.

that adult marmosets preferred staying in the dark closed compartment rather than in the lighted one.

Then, we determined whether this preference depends on the age of the marmosets. We chose six young marmosets that were just weaned (average age 3 ± 1 months); their motor ability was similar to that of adults[7]. During the first 30 min, these young marmosets also showed no preference between the two lighted closed compartments, as indicated by the numbers of entries (left, 8.7 ± 2.5; right, 6.8 ± 2.3; n = 6, P >0.1, Fig. 4A) and the time spent in the right and left boxes (left, 157 ± 54 s; right, 125 ± 44 s; n = 6, P >0.1, Fig. 4B), while they spent more time in the middle open compartment (1519 ± 93 s; n = 6, P <0.001 compared to both closed compartments, Fig. 4B). Furthermore, in the following 30 min with one lighted and one dark closed compartment, the young marmosets still spent most time in the open area (1500 ± 93 s; n = 6, P <0.001 compared to both closed compartments, Fig. 4D), but showed no preference between the lighted and dark closed areas (entries, light, 6.3 ± 1.4; dark, 7.8 ± 2.9; n = 6, P >0.1, Fig. 4C; time, light, 130 ± 42 s; dark, 170 ± 97 s; n = 6, P >0. 1, Fig. 4D), which differed from the adults. Taken together, these results suggest that young marmosets also show interest in exploring the open area, but have no preference between the light and dark areas.

To further investigate the differences between adult and young animals, we analyzed their activity in the modified three-box light-dark test during the first 30-min with both lamps turned on. Compared to adult marmosets, young marmosets showed less time in the closed compartments (adult, 768 ± 70 s, n = 8; young, 282 ± 93 s, n = 6; P = 0.001, Fig. 5A) and spent more time in the open compartment (P = 0.001, Fig. 5A), suggesting that young marmosets have more interest in the open area than adults. Consistently, young animals showed fewer entries into the two closed boxes (adult, 27.6 ± 4.7, n = 8; young, 15.5 ± 4.4, n = 6;

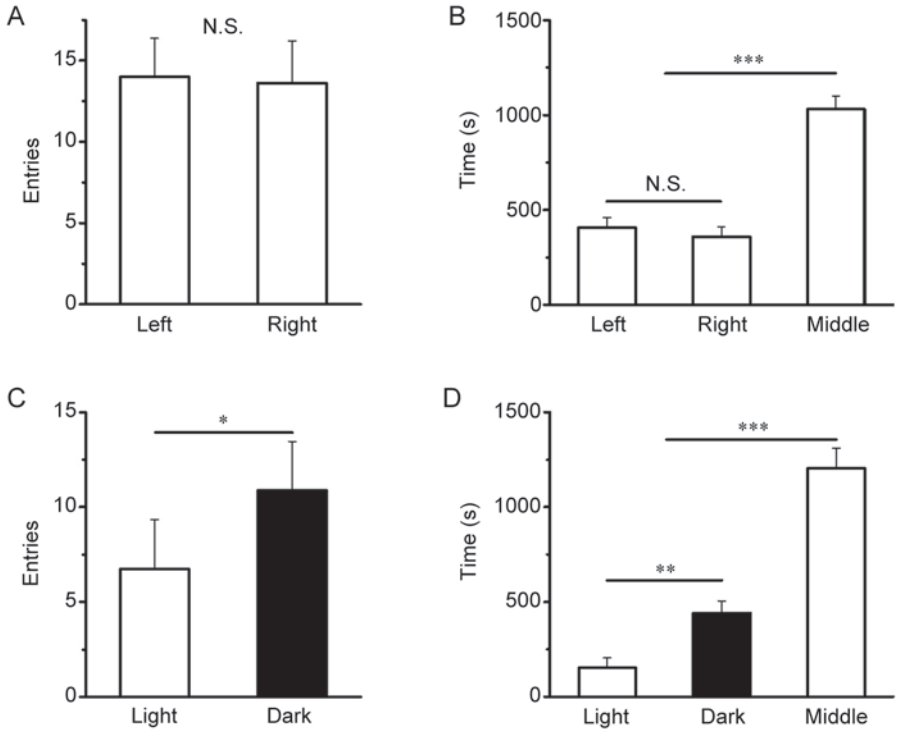

Fig. 3. Exploratory behaviors of adult marmosets in light-dark box test. A: Quantification of the left and right compartment entries during the first 30 min-test when both the closed compartments were lighted. B: Time spent in each of the three compartments during the first 30 min-test. C: Quantification of the light and dark compartment entries during the second 30 min-test when one lamp was turned off randomly. D: Time spent in the light, dark, and middle compartments during the second 30 min-test. *P <0.05; **P <0.01; ***P <0.001; N.S., no significant difference; Student's t test.

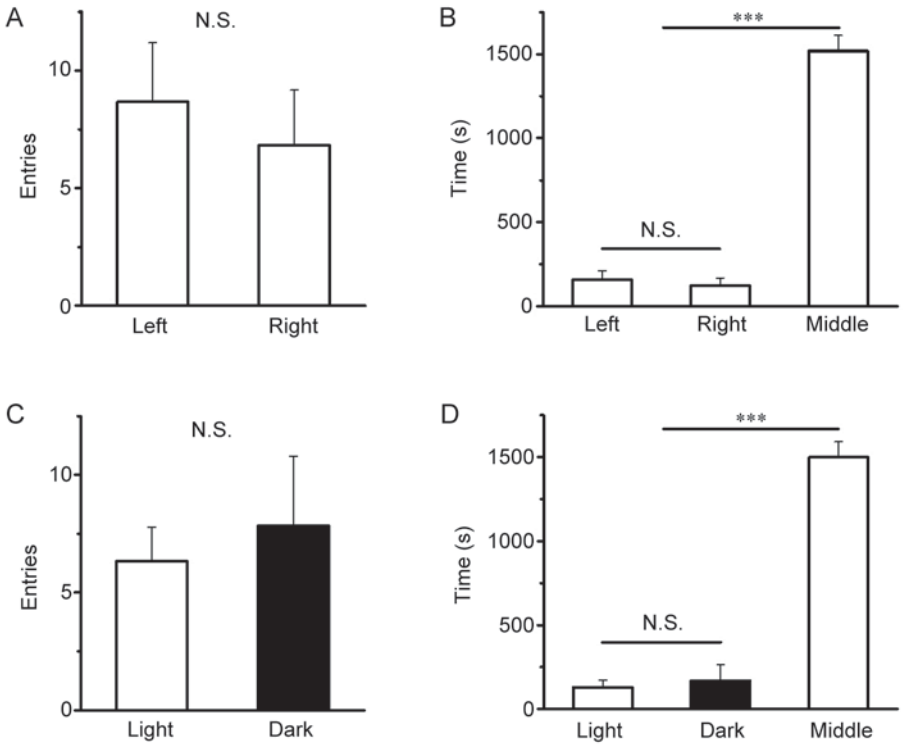

Fig. 4. Exploratory behaviors of young marmosets in light-dark box test. A: Quantification of the left and right compartment entries during the first 30 min-test. B: Time spent in each of the three compartments during the first 30 min-test. C: Quantification of the light and dark compartment entries during the second 30 min-test. D: Time spent in the light, dark, and middle compartments during the second 30 min-test. ******P* **<0.001; N.S., no significant difference; Student's *t* test.

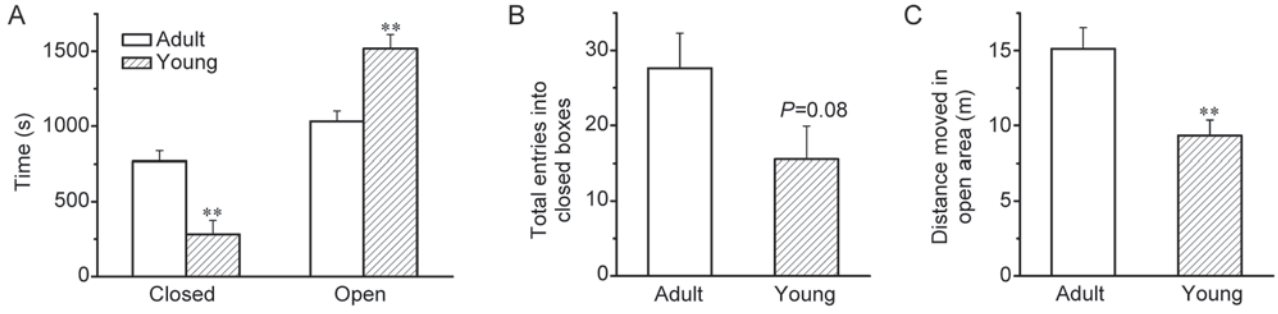

Fig. 5. Young marmosets spent more time but showed less motility in the open area than adults. A: Time spent in the open and closed compartments by adult and young marmosets during the first 30-min observation with both lamps turned on. B: Total entries into the closed compartments. C: Distance moved in the open area. *****P* **<0.01; Student's *t* test.

P = 0.08, Fig. 5B). Interestingly, although spending more time in the open box, young animals exhibited lower motility as shown by the distance moved in the open area (adult, 15.1 ± 1.4 m, *n* = 8; young, 9.3 ± 1.0 m, *n* = 6; *P* = 0.008, Fig. 5C). Taken together, these data further suggest that the exploratory behaviors of marmosets depend on age.

DISCUSSION

In the present study, we developed a novel light-dark box apparatus to test the behavior of marmosets. This method is derived from the classical light-dark box test for rodents, but we made some modifications based on the characteristics of marmosets. In the wild, each marmoset

family has a nest in a tree. For laboratory marmosets, there is also a dark nest-box hanging in each cage. In the normal life of marmosets, the dark nest-box represents a safe area to avoid threats, feed infants, and sleep. This characteristic can be used to examine a marmoset's emotional state. Thus, we designed open and closed areas to mimic the space outside and inside the nest-box, respectively. However, according to our results (Fig. 1), the conventional test with the two-box test only reflected open-closed preference but not light-dark preference. To assess the light-dark preference, we further divided the closed area into two compartments which could be controlled to be light or dark. In this apparatus, it is easy to observe marmoset behaviors in response to dark and light. We found that both adult and young marmosets liked to explore the open area, with the young animals showing even more interest. However, the adult marmosets showed a significant preference for the dark compartment, while the young animals had no such preference.

In rodents, the light-dark box has been widely used to assess the level of anxiety[5, 6], based on the conflict between exploratory interest and an aversion to bright light. However, the use of this light-dark test in measuring anxiety in the marmoset needs further validation. Compared to the simple light-dark preference of rodents, marmosets clearly have more complicated emotional states, including both open-closed and light-dark preferences. Furthermore, it is unknown whether light is as a stressful factor for marmosets as it is for rodents, although adult marmosets showed a dark preference in the closed boxes. If light is stressful for marmosets, it would be predicted that turning off of the light would result in more time spent in the box. However, as shown in Fig. 3, the time spent in the dark box was comparable to that spent before turning off the lamp, while less time in the lighted closed box contributed to the light-dark difference. Among the three boxes, adult marmosets showed much more preference for the open area than the dark closed area, and did not prefer lighted closed areas. Our findings that young marmosets spent more time but were less active in the open area than adults, and showed no light-dark preference in the closed boxes, suggested the developmental changes in marmoset behavior. An abundant literature supports the idea that human adolescents in general are less anxious, more risk-taking, and less sensitive to aversive consequences[8], providing a possible explanation for the differences between adult and young marmosets in the present study. These findings may be useful to the field of marmoset behavioral studies in the future, but the question as to whether these open-closed and light-dark preferences are related to anxiety or fear needs further studies. There are already several ethologically based tests of anxiety/fear in marmosets, such as involuntary isolation, social interaction, human threat, and predator confrontation[9, 10]. In future studies, the effects of classical anxiolytic drugs, such as benzodiazepines[11, 12], need to be tested in marmosets by the light-dark test, to further clarify the anxiety-related behaviors in this test.

ACKNOWLEDGEMENTS

We thank Xuebo Li and Jianhong Li for assistance in the breeding of marmosets. This work was supported by the Youth Innovation Promotion Association of the Chinese Academy of Sciences and an SA-SIBS scholarship, the National Basic Research Development Program (973 Program) of China (2011CBA00400), and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB02020100).

Received date: 2013-04-03; Accepted date: 2013-06-03

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