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Research Article

Establishing a Special Open Field Test Appliance for Tree Shrews Evaluates Their Stressed Locomotor Behavior

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Abstract

It has a great significance to establish a high repetitive and special system to evaluate locomotion for tree shrew animal models, especially neuropsychiatric models. Thus, we designed a special open field test appliance for tree shrews by using a polymer PMMA board (plexiglass). The locomotion of naïve tree shrews was detected twice in it. By using Any-maze Software tracing the motion trajectory, we found that the motion trajectory and the distance were very similar in both tests. It demonstrated that the appliance designed for tree shrews had extremely high reliability and stability. Then we used this system to evaluate the activity of the stressed tree shrews, and observed the reduced activity of the tree shrews after 2-week light-stress. Consistent with this, the ACTH level in the urine of the stressed tree shrews was enhanced. Taken together, the light stress could lead to a significant reduction of locomotion which could be investigated by our open field experiment system. Furthermore, the data from our special open field experiment system is stable and accurate for testing the locomotion behavior of tree shrews.

Keywords: Tree shrews; Open field test; Stress; Motion trajectory

Abbreviations

OFT: Open Field Test; ACTH: Adrenocorticotropic Hormone; ELISA: Enzyme-Linked Immunosorbent Assay; ICC: Intra-Group Correlation Coefficient; HPA: Hypothalamic-Pituitary-Adrenal; CRH: Corticotropin-Releasing Hormone; PVN: Paraventricular Nucleus.

Introduction

In recent years, the role of stress-induced neuroendocrine system changes in the pathogenesis has attracted much attention. Stress caused by abominable and demanding conditions can cause different biological responses according to different stress conditions. It is generally believed that transient and moderate stress can trigger adaptive responses, such as a 'fight or flight response', and promote adaptation and resilience to stress. Conversely, chronic or excessive stress can lead to cognitive and affective dysfunction, and can easily lead to mental disorders [1,2,3]. There are many ways to establish animal stress models depending on the purpose of the study. Among the many methods for creating models, the closest method to human stress is the animal stress modeling method [4]. The tree shrews belong to the sister of primates [5]. Its shape is small, and the whole body is fluffy similar to the squirrel. It was found that the tree shrews had timid and docile characters. They are very sensitive to psychological stress. Stress from bondage can lead to the increased cortisol in tree shrews. The stress depressed tree shrew model showed that there was a continuous increase in cortisol level of subordinate tree shrews, which could lead to atrophy of hippocampal CA3 neurons. This phenomenon is consistent with the atrophy of the hippocampus in patients [6]. Another important index in the evaluation of the stress model inactivity is the open field test. Open Field Test (OFT), which is also called an open box experiment, is widely used in the study of neuropsychiatric diseases represented by stress behavior. However, most of the current OFT boxes are designed for rodents, because of the large differences between tree shrews and rodents in their living habits, which is not suitable for evaluating the activity of the tree shrews. Moreover, the activity characteristics of the tree shrews are mainly jumping, while the OFT box of the existing rodents is selected to evaluate their activity, the data of the vertical plane will be not true enough. Recently, most of the locomotor evaluation has been conducted in the home-cages of tree shrew. Although the problem of authenticity can be solved, the background is too complex, which can cause the problem of poor tracking and data loss. Considering that the equipment of the tree shrews' open field experiment equipment is lacking. In this study, we designed a locomotor detection system. By it we did the OFT twice, and assessed the accuracy and stability of the data of the tree shrews' behavior experiment. Then we further investigated the alteration of the activities in the tree shrews induced by light-stress for 2 weeks through this system.

Materials and Methods

Experimental animals

Since the female tree shrews have menstruation, we only used male tree shrews in the present experiments to avoid the interference of it. Moreover, we wanted to observe the effect of stress on male tree shrews' behavior by self-control (before and after stress) and to investigate the stability of our new OFT instrument. Thus, in the experiments, 15 male adulthood (aged 1-2 years, and weighted 120-160 g) Chinese tree shrews were selected. The tree shrews were provided by the Kunming Institute of Zoology, Chinese Academy of Sciences, and the tree shrews were raised at the Animal Core Facility of Nanjing Medical University.

All animals were given ad libitum access to food and water and

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were housed under a 12-h light/dark cycle (house light on from 8:00 am to 8:00 pm). According to local standards in Yunnan province, the working illumination of tree shrews' rooms are set to 150-300 lux, and that of the area to 50-100 lux. All animal experiments were performed following the recommendations of the Experimental Animal Ethics Committee at Nanjing Medical University.

Methods

Preparation of special OFT box for tree shrews: The open-field experimental process and equipment for rodents have been accurately designed, but those for tree shrews are lacked. At present, most of researchers use tree shrews' breeding cages or open field experiment boxes for rodent rats to do tree shrews' behavior test. The biological behavior between rats and tree shrews is very different, and the obtained data is inconsistent with the living habits of tree shrews to a certain extent. The repeatability and stability of the data of multiple experiments are low. Therefore, to set up a suitable behavioral experiment box for tree shrews has become the focus of this research.

According to the relevant literature [7,8] and combining with the characteristics of tree shrews, we designed the special OFT box for tree shrews (Figure 1A). The special material is a kind of polymer PMMA polymethyl methacrylate sheet (plexiglass). The size of the box is 50cm×50cm×80cm (length×width×height). Considering the tree shrews' habits from the instinctive behavior in the wild environment, the beam is designed at a height of 40cm which is made of the frosted material to prevent slippage and make it easier for tree shrews to move autonomously in the direction of the ground. Moreover, the frosted material is easy to be cleaned and has a high degree of safety. The open-field box is composed of a clear plexiglass top, detachable fencers, a recessed white bottom plate and a frosted clear plexiglass rod running through the box. Moreover, in the rodents, we use a central area to evaluate the anxiety-like behavior in the open field test, while the alteration in jumping was used in tree shrews [9]. In the present experiments, the open-field box is divided into three parts from top to bottom: upper part, middle part, and the lower part (Figure 1B). In the behavior test, both the total distance and the changed activity of the tree shrews in the middle or upper part would be investigated.

Open field test: A camera was set in front of the transparent plexiglass front baffle on the front of the box to monitor the activity of the tree shrews. Before the experiment, the door of the sleeping box was opened. After the tree shrews entered the box through the round hole, the door of the sleeping box was closed quickly and video recording was started. The movement parameters of the tree shrews in the horizontal and vertical directions, the average movement speed and the number of jumps were recorded respectively during the experiment. At the end of the experiment, the door of the sleeping box was opened. After the tree shrews entered the sleeping box through the round hole, the door was closed quickly. After removing the sleeping box, we successively removed the top cover with the air hole, front baffle on the front, frosted glass rod through the box and detachable silicone non-slip pad, and then washed and dried with water. After wiping the residual urine and feces in the tank with flat paper, spray 75% alcohol to eliminate the residual odor of the experimental tree shrews. The whole operation process eliminates the need to grab the tree shrews and eliminates the artificial stress caused by grasping the tree shrews. Therefore, the most basic activity and emotional state of the tree shrews can be objectively evaluated.

Selection of Detection Software for Tree shrews' OFT System: The Any-maze Animal Behavior Video Analysis System (ST-60000, Sterling, USA) is a scientific analysis software that provides an advanced grayscale recognition algorithm that adjusts the threshold when the animal's color contrasts with the background color. It's easy to lock the target and accurately track the trajectory of the animal's movement with powerful analytical statistics function. We used this system to record the animal's original activity video, the activity track, and get the real-time changes in animal activity patterns (diet, sleep, move, chase, social, etc.) and the animal trajectory data.

Stress experimental process: Fifteen naïve tree shrews were tested in the Open Field Test (OFT) at the end of the first week and the end of the second week. The Any-maze software was used to track the locomotion. Then, the house light being on was prolonged by 4 hours. That is the house lights on from 8:00 am to 12:00 pm. After 2 weeks of treatment, the tree shrews' locomotion was detected by OFT again. What's more, the concentration of Adrenocorticotropic Hormone (ACTH) in the urine of tree shrews before and after stress was detected by enzyme-linked immunosorbent assay.

Enzyme-linked immunosorbent assay: Urine was collected in the morning before and after stress. The concentration of corticotrophin in urine before and after stress was measured by Enzyme-Linked Immunosorbent Assay (ELISA). It was measured using an ELISA kit commercially available according to the manufacturer's instructions (ACTH, ml001895, mlbio, China).

Statistical methods

Statistical analysis was performed by using SPSS 19.0 software, and statistical mapping was performed by using Origin Pro 8.5 software. The data were presented as means ± SEM. The differences between the two groups were compared via a two-tailed Student's t-test. $P<0.05$ was considered statistically significant.

Results

Tree shrews' special OFT box can stably detect tree shrews' activity

After putting the tree shrews into the special OFT box, the Any-maze software was used for tracking and identification immediately. By tracking the trajectory map, we observed that the tree shrews moved freely in the special OFT box. Each of them ran and jumped freely in line with the basics of the tree shrews' living habits (Figure 2A). Through the comparison of the initial and re-measurement trajectories, the activity patterns of the same tree shrews are similar in the dedicated squatting box, indicating that the tree shrews' special OFT box is relatively stable in detecting tree shrews' activity (Figure 2A).

The distance and average speed in the open field test experiment have extremely high reliability

The Intra-group Correlation Coefficient (ICC) analysis was carried out for the initial distance and the mean speed of the tree shrews in the open field test experiment. The reliability was evaluated by the ICC parameters. The results showed that the activity distance's ICC is 0.951 ($p<0.001$) and the average speed's ICC is 0.962 ($p<0.05$)

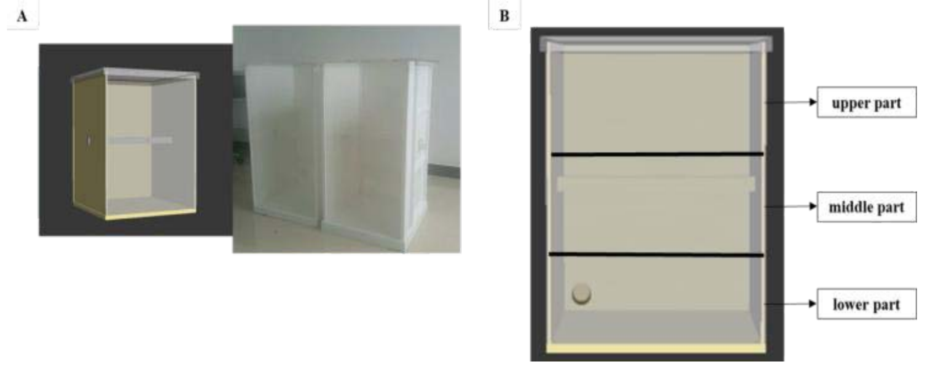

Figure 1: Special OFT box for tree shrews.
A: The side view of an open field box.
B: The front view of the open field box and the distribution pattern of the OFT box.

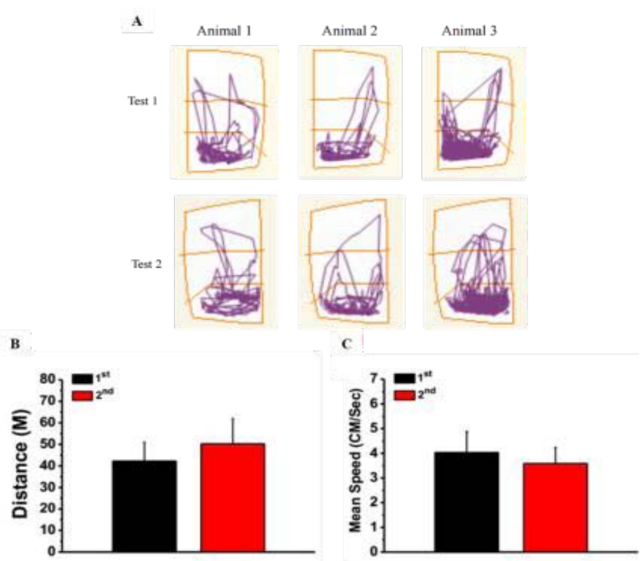

Figure 2: Partial trajectories of initial and remeasured OFT in tree shrews.
A: Representative images of trajectories from 3 tree shrews are illustrated: Test 1: Trajectories of initial OFT; Test 2: Trajectories of re-measured OFT).
B and C: Quantitation of the activities of the tree shrews from 2 tests by our OFT system showed the data were stable. Data are presented as the means ± SEM.

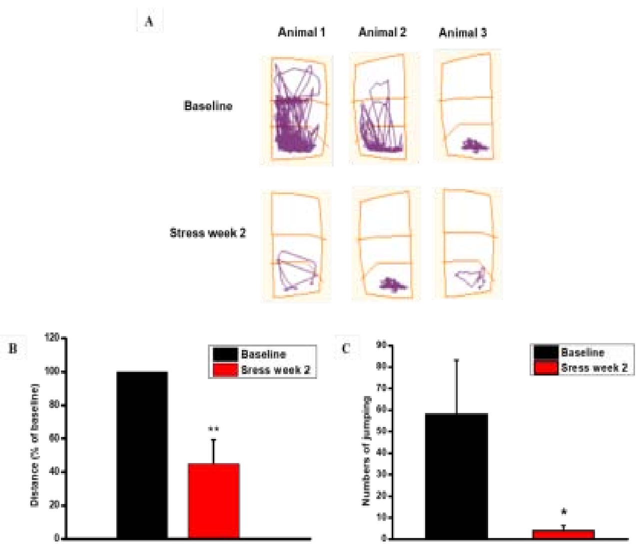

Figure 3: After two weeks of stress, the activities of tree shrews were significantly decreased.
A: The representative trajectories of tree shrews after 2-week stress are illustrated.
B: The distance of tree shrews was reduced after 2-week light stress.
C: The effects of light stress on the numbers of jumping of tree shrews.
Data are presented as the means ± SEM. **P*<0.05 and ***P*<0.01 compared with baseline.

between the first test and the second test. It suggested that the distance and the averaged speed reliability in the open field test experiment were extremely high. Moreover, after paired t-test on the above parameters, it was found that the data of the two measurements were stable and there was no significant difference (Figure 2 B&C, A: p=0.591; B: p=0.685, n=11).

Stress significantly reduced the activity of the tree shrews

To investigate the applicability and generality of our open field test experiment system, the activity of tree shrews in pre-stress (Baseline) and 2 weeks after stress (Stress week 2) in the OFT experiment were observed. As shown in Figure 3, the activity of tree shrews was significantly reduced after light stress for 2 weeks (Figure 3A). Comparing the distance of each tree shrew with its baseline, it was found that the tree shrews' activity distance was significantly decreased after 2 weeks of stress (Figure 3B, p<0.01, n=15). Moreover, we not only observed the alteration of horizontal movement, but also investigated the numbers of jumping in these tree shrews. Among these tree shrews, the numbers of jumping for 12 tree shrews showed the significant decrement (Figure 3C, p<0.05, n=12) while 3 in 15 tree shrews showed the increased jumping (data not shown). All these suggested that 2-week staying up late contributed to the change of the activities in the tree shrews and this has a lot to do with individual differences in tree shrews. Furthermore, to further validate the stress-induced stress behavior tree shrew model, we also collected tree shrew morning urine before and after the stress. We found that the ACTH concentration in the urine of the tree shrews was significantly increased after 2 weeks of stress (Figure 4, p<0.001, n=15). All these data suggesting that our OFT system was stable and sensitive to

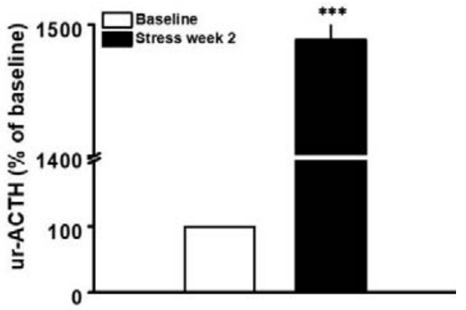

Figure 4: 2-week light stress enhanced the urine ACTH concentration in tree shrews.
After 2-week light stress, the concentration of ACTH in the urine of tree shrews at the morning was significantly increased. (***P<0.001 compared with baseline. Data were normalized to the baseline).

investigate the alteration of the activities of the tree shrews.

Discussion

Physiological and behavioral responses to acute stress can be adaptive, but exposure to chronic stress, especially chronic psychosocial stress, can have negative consequences and increase susceptibility to chronic diseases [10].

Stress refers to the internal reaction of the body to the environment and is caused by the loss and damage of the body caused by the outside world. Classification according to the results of stress may have pathological stress and physiological stress. In recent years, the role of stress-induced neuroendocrine system changes has attracted much attention. The most important pathological change is the excessive drive of the Hypothalamic-Pituitary-Adrenal (HPA) axis [11]. The core driver of the HPA axis activation response is the Corticotropin-Releasing Hormone (CRH) located in the Paraventricular Nucleus (PVN) site [12]. In human patients, there is a significant increase in neuronal apoptosis in the norepinephrine-blue-spot system and the amine-based system, with the norepinephrine-blue-spot system being the most severely damaged [13]. By placing the animals in a mild stress-stimulated environment, after several weeks of action (typically 2-3 weeks), animals exhibit clinically common symptoms in depressed patients [14]. OFT is a classic method for evaluating the motor function and stress behavior in experimental animals and is widely used in the basic research of neuroscience. In the open field test experiment of tree shrews, the horizontal movement in the ground direction reflects the locomotor activity, and the vertical ground movement including climbing and jumping reflects the curiosity of the tree shrews to the fresh environment. The number of fecal particles reflects the degree of tension of the tree shrews; the number of crossings reflects the anxiety state of the tree shrews, and the stress state can lead to a certain decrease in value. Therefore, the reliability of OFT and the stability of its repeated test parameters are very important [15,16]. It is also important to establish a behavioral experimental system with high reproducibility and suitable for tree shrews' habits. Degree refers to the degree of consistency of multiple measurement results, which reflects the stability and reliability of a measurement method or a tool. The test-retest reliability is a kind of reliability, which reflects the measurement method or a tool that consistently measures the consistency of two measurements at different times. The commonly used statistical evaluation parameter for the test-retest reliability is the ICC parameter. The ICC parameter, the intra-group correlation coefficient, was first proposed by Bartko in 1966 for evaluating the reliability of continuous or grade variables [17,18]. If the ICC is greater than 0.75, the credibility is good. If it is between 0.5 and 0.75, the credibility is generally acceptable and when it is less than 0.5, the credibility is poor. The results of ICC for tree shrews tested in the special OFT box suggested that the common parameters of the active distance and the average speed had extremely high reliability, confirming that the tree shrews' specific OFT system has a good test-retest reliability. The system is stable. Meanwhile, there was no signal loss when we used Any-maze software to track tree shrews in the special OFT box. The trajectory of the tree shrews in the OFT box was well captured.

As our data showed, the open field test experiment which is carried out by our special OFT box for tree shrews, restored the basic activities of the tree shrews to a large extent and the data from our OFT system is stable. Moreover, the moving distance and average speed have extremely high reliability in selecting the evaluation parameters. Besides, the results of the tree shrews' urine test showed that ACTH was significantly increased after two weeks of light-stress. Consistent with this, after 2 weeks of stress, the activity of tree shrews by our OFT system changed significantly, that is, the distance of tree shrews was significantly reduced after stress. Taken together, our OFT system can investigate the change of activity of tree shrews after stress.

Conclusion

The tree shrews' specific OFT system that we designed can help us to get the stable and reliable data. Moreover, it is sensitive to test the effect of light-stress on the activities and anxiety of tree shrews, which is consistent with the increased ACTH level after 2-week staying up late.

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