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Tree shrew models: A chronic social defeat model of depression and a one-trial captive conditioning model of learning and memory

WANG Jing[1, 2], ZHOU Qi-Xin[1], TIAN Men[1], YANG Yue-Xiong[1], XU Lin[1,*]

(1. *Key Laboratory of Animal Models and Human Disease Mechanisms*, *the Chinese Academy of Sciences & Yunnan Province*, *Kunming Institute of Zoology*, *Kunming* 650223, *China*; 2. *Graduate School of the Chinese Academy of Sciences*, *Beijing* 100049, *China*)

Abstract: Recent genome studies indicate that tree shrew is in the order or a closest sister of primates, and thus may be one of the best animals to model human diseases. In this paper, we report on a social defeat model of depression in tree shrew (*Tupaia belangeri chinensis*). Two male tree shrews were housed in a pair-cage consisting of two independent cages separated by a wire mesh partition with a door connecting the two cages. After one week adaptation, the connecting door was opened and a brief fighting occurs between the two male tree shrews and this social conflict session consisted of 1 h direct conflict (fighting) and 23 h indirect influence (e.g. smell, visual cues) per day for 21 days. The defeated tree shrew was considered the subordinate. Compared with naïve animals, subordinate tree shrews at the final week of social conflict session showed alterations in body weight, locomotion, avoidance behavior and urinary cortisol levels. Remarkably, these alterations persisted for over two weeks. We also report on a novel captive conditioning model of learning and memory in tree shrew. An automatic trapping cage was placed in a small closed room with a freely-moving tree shrew. For the first four trials, the tree shrew was not trapped when it entered the cage and ate the bait apple, but it was trapped and kept in the cage for 1 h on the fifth trial. Latency was defined as the time between release of the tree shrew and when it entered the captive cage. Latencies during the five trials indicated adaptation. A test trial 24 h later was used to measure whether the one-trial trapping during the fifth trial could form captive memory. Tree shrews showed much longer trapping latencies in the test trial than the adaptation trials. The N-methyl-d-aspartate (NMDA) receptor antagonist MK-801 (0.2 mg/kg, i.p.), known to prevent the formation of memory, did not affect latencies in the adaptation trails, but did block captive memory as it led to much shorter trapping latencies compared to saline treatment in the test trial. These results demonstrate a chronic social defeat model of depression and a novel one-trial captive conditioning model for learning and memory in tree shrews, which are important for mechanism studies of depression, learning, memory, and preclinical evaluation for new antidepressants.

Key words: Social defeat; Depression; Captive conditioning; Learning and memory; Tree shrew

树鼩模型：抑郁症的社会竞争失败与学习和记忆的被捕获条件反射

王 静[1,2], 周启心[1], 田 孟[1], 杨跃雄[1], 徐 林[1,*]

(1. 中国科学院和云南省动物模型与人类疾病机理重点实验室, 中国科学院昆明动物研究所, 云南 昆明 650223;
2. 中国科学院研究生院, 北京 100049)

摘要：最近基因组研究表明树鼩属于灵长类或是与灵长类亲缘关系最密切的姐妹种。因此, 树鼩可能是应用于建立人类疾病动物模型的最佳动物之一。该文报道一种抑郁症的社会竞争失败病因学树鼩 (*Tupaia belangeri chinensis*)模型。一对雄性树鼩被饲养在一个双笼中, 用网格把双笼隔开, 网格上有一小门。适应 1 周后, 把小门打开, 这一对树鼩产生短暂的争斗, 每天一次，连续 21 天。其中争斗失败者被称为服从者。这个过程可导致每天 1 h 的直接社交冲突和 23 h 的间接相互影响 (比如通过气味、视觉等)。与正常对照相比, 失败者在第三周也就是社交冲突的最后一周显示了体重、自主活动、躲避行为、尿液皮质醇水平等的变化, 并且这种改变可持续至少 2 周以上。此外, 还报道全新的记忆模型, 一种被捕获条件反射树鼩模型。在一个封闭的小房间中放置捕获笼, 其中挂有

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* Corresponding author (通信作者), Email: lxu@vip.163.com
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一片苹果, 小房间中有一只自由活动的树鼩。训练的前 4 次树鼩进入捕获笼吃苹果并不触发捕获笼关闭, 但在第 5 次时触发捕获笼关闭, 并持续一小时才释放树鼩。第 1—5 次树鼩进入捕获笼的延迟时间作为适应性指标, 其中第 5 次才是作为被捕获的一次学习训练。24 h 后, 测试树鼩进入捕获笼的延迟时间作为被捕获记忆能力指标。树鼩经过第 5 次被捕获训练, 能形成很好的被捕获记忆, 因为 24 h 后的延迟时间极大地增加。在训练前腹腔注射已知能阻断记忆形成的 NMDA 受体拮抗剂 MK-801 (0.2 mg/kg, 腹腔注射), 对适应指标没有显著影响, 但是极大地缩短了 24 h 后测试的延迟时间, 即阻断了被捕获记忆。这些结果表明了一种抑郁症的慢性社会竞争失败与学习和记忆的一次被捕获条件反射树鼩模型。这两种树鼩模型对抑郁症与学习和记忆的机理研究、抗抑郁症新药的临床前药效学评价具有潜在的重要意义。

关键词：社交竞争; 抑郁; 被捕获条件反射; 学习记忆; 树鼩
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Tree shrews are small diurnal mammals from the tropical forests of Southeast Asia including southwest China, Burma, Indonesia, and the Philippines. There are abundant tree shrew (*Tupaia belangeri chinensis*) populations in Yunnan, Hai nan, Tibet, and Gui zhou provinces in China (Helgen et al, 2005). Previous molecular phylogeny studies suggest that tree shrews are in the same order as primates, which include the closest relatives to human beings (Janecka et al, 2007). It is important, therefore, to establish tree shrew models for human diseases, learning and memory, as well as preclinical evaluation for new drugs.

While psychosocial stress-based models of depression have been examined for tree shrews (Holst, 1977), such studies have only examined short fighting episodes between two males. Increasing evidence indicates that chronic psychosocial stress leads to a variety of alterations in behaviors, endocrinology, physiology, and central nervous systems in the tree shrew (Fuchs & Flügge, 2002; Meyer et al, 2001; Magariños et al, 1996), and these alterations can be reversed by antidepressant treatment (Fuchs et al, 1996; van Kampen et al, 2000; Czéh et al, 2001;van der Hart et al, 2005). It is important, therefore, to establish a chronic fighting model of depression.

Conversely, however, tree shrew models of learning and memory have been rarely studied. Holeboard learning and eight arm maze tasks have been utilized in learning and memory models for tree shrews (Ohl et al, 1998; Bartolomucci et al, 2002; Takahashi et al, 2008). These models are complicated, however, due to the repeated training processes. In the present study, we report on a social defeat model of depression as indicated by depression-like behaviors as well as urine cortisol levels in subordinate (who has experienced chronic social defeat) tree shrews. Furthermore, we established a novel one-trial captive conditioning model of learning and memory in tree shrews.

1 Materials and Methods

1.1 Animals

All experiments were performed on adult male tree shrews (*Tupaia belangeri chinensis*, *n*=15) obtained from the breeding colony at the Animal House Center of the Kunming Institute of Zoology, Kunming, P. R. China, and weighing 120−150 g. They were housed individually in a 12h/12h dark/light cycle (light, 8:00−20:00; dark, 20:00−8:00) and thermoregulated rooms (T: 25−27℃, RH: 55%−70%) with free access to water and food. Animal care and experimental protocols were approved by the Animal Ethic Committee of Kunming Institute of Zoology, Chinese Academy of Sciences, P. R. China.

1.2 Drugs

MK-801 (dizocilpine maleate, Sigma), a non-competitive NMDA receptor antagonist, was dissolved in saline (0.2 mg/mL) and injected intraperitoneally (i.p., 0.2 mg/kg) 30 min before the first trial of training (Vales et al, 2006).

1.3 Experimental design

1.3.1 Depression model

Tree shrews were adapted to a pair-cage (size 67 cm ×50.5 cm×65 cm, w×d×h) (Fig.1) consisting of two independent cages separated by a wire mesh partition with a small door, for one week. Control animals (Ctrl, *n*=2) remained undisturbed until behavioral tests. Four pairs of tree shrews were housed in four pair-cages, and the door was opened for 1 h per day for 21 days, during which time a brief fighting episode occurred between each pair of the tree shrews. The defeated tree shrew was called the subordinate (Sub), while the winner was called the dominate (Dom). The fighting episode consisted of when the Sub was attacked by the Dom for 1 h at an unpredictable time point between 9:00 and 18:00 by opening the door. For the remaining time of each day,

each animal was exposed to visual, auditory, and olfactory cues. Body weight was measured once at the beginning of each week. Urine was collected at the beginning of the third and fourth week twice per day at 7:30−9:00 and 17:30-19:00.

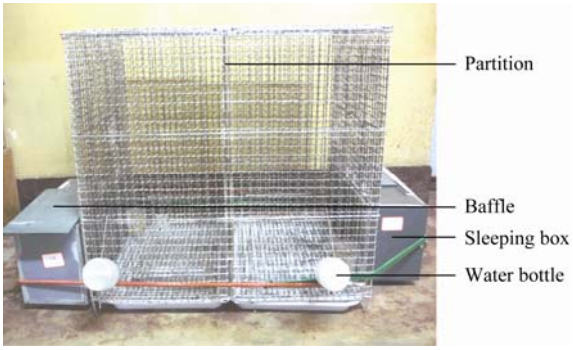

Fig. 1 Frontal view of a pair cage

1.3.2 Learning and memory model

The training/test apparatus consisted of an automatic trapping cage (size 24 cm×12.5 cm×10 cm, w×d×h) with a hook at the end on which was placed a small piece of apple (about 2−3 g). When a tree shrew ate the apple, it triggered the door to close.

Five naive tree shrews were divided into a saline group (n=2) and a MK-801 group (n=3). Tree shrews were fasted with free access to water for 24 h before training. The tree shrews were then weighed and injected with saline or MK-801. Thirty minutes later, they were placed into a small closed room with white noise (60 dB) and 55 lux illumination (Fig. 2A), and trained to find the apple in the trapping cage. During the first four training trials, the tree shrews ate the apple without triggering the door. During the fifth training trial, however, the trapping process was set and the door closed when the apple was eaten. Imprisonment lasted 1 h, after which the animals were returned to their home cage. Latency in the five training trials from release to catching the apple was used to measure adaptation. The latency on day 2 (24 h later) was used to test whether the animals had formed a captive memory (Fig. 2B), and this test trial was terminated if the animal did not catch the bait within 30 min, indicating that the animal had formed a fear memory related to being trapped. Each animal had an individual trapping cage that was placed on a new piece of black plastic so that the contrast of apple and floor was apparent. Additionally, alcohol was sprayed in the room after each trial. As a reward, animals were fed half a banana and some melon seeds after the training and tests.

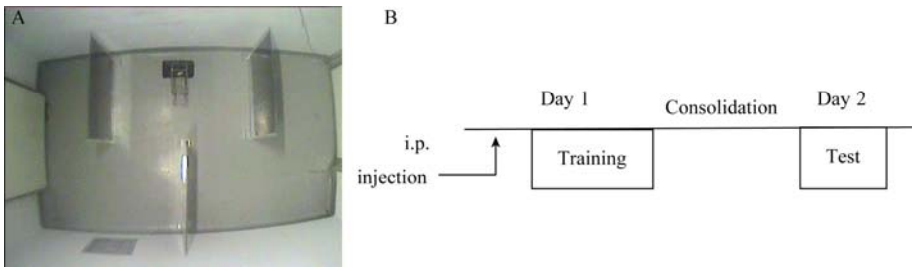

Fig. 2 Experimental design and aerial view of the tree shrews environment

A: Aerial view of environment for tree shrews. There were three black clapboards and a trapping cage in a small closed room. Black plastic was placed under the cage as a visual contrast. In the small cage, a piece of apple about 2~3 g was placed on a hook as bait. B: Experimental design. The training trials included accommodation and trapping processes.

1.4 Behavioral analysis

During the depression experiment, all animals were videotaped twice a day between 8:00−10:00 and 17:00−20:00, when their self-grooming behaviors (licking, cleaning, scratching, and washing), locomotor activities (jumping and moving in their home cage), and avoidance behavior (time spent in the sleeping-box) were measured manually from the videos. In the learning and memory experiment, performance was videotaped from 17:00 to 20:00 to avoid any unwanted confounding factors, such as human activity around the animals.

1.5 Analysis of urine samples

Urine free cortisol was measured with an Iodine [^{125}I] cortisol radioimmunoassay kit (Bei Jing North Institute of Biological Technology, China) and γ radio-immunity counter (GC-2010, Zonkia, Anhui, China). To distinguish radioimmunoassay and immunoradiometric assay, the results were determined with a log-logit process mode.

1.6 Data analysis and statistics

Data was statistically analyzed using SPSS 16.0. Cortisol and behavioral data of each animal for each day was averaged and a statistic method one-way ANOVA was used. The significance level was set at P<0.05. Data is expressed as mean±SEM.

2 Results

2.1 Depression model

Compared with naive animals, subordinate tree shrews (Sub group, n=4) showed a significant decrease in the number of self-grooming [$F_{(1, 4)}$=7.864, P=0.049; $F_{(1, 4)}$=12.180, P=0.025; Fig. 3A] and locomotion [$F_{(1, 4)}$ =15.840, P=0.016, Fig. 3B] activities between their cages and sleeping-boxes [$F_{(1, 4)}$=18.190, P=0.013; $F_{(1, 4)}$ =17.747, P=0.014; Fig.3C,D] in the final week of 21 days of social conflict. Conversely, the time spent in the sleeping-box by the Subs showed a significant increase by the third week [$F_{(1, 4)}$ =14.644, P=0.019; Fig. 3E]. The difference activity between Sub and control group (Ctrl) was significant in the afternoon, especially from 18:30 to 19:30, the activity peak period in tree shrews [$F_{(1, 4)}$ =216.000, P=0.000; Fig. 3F].

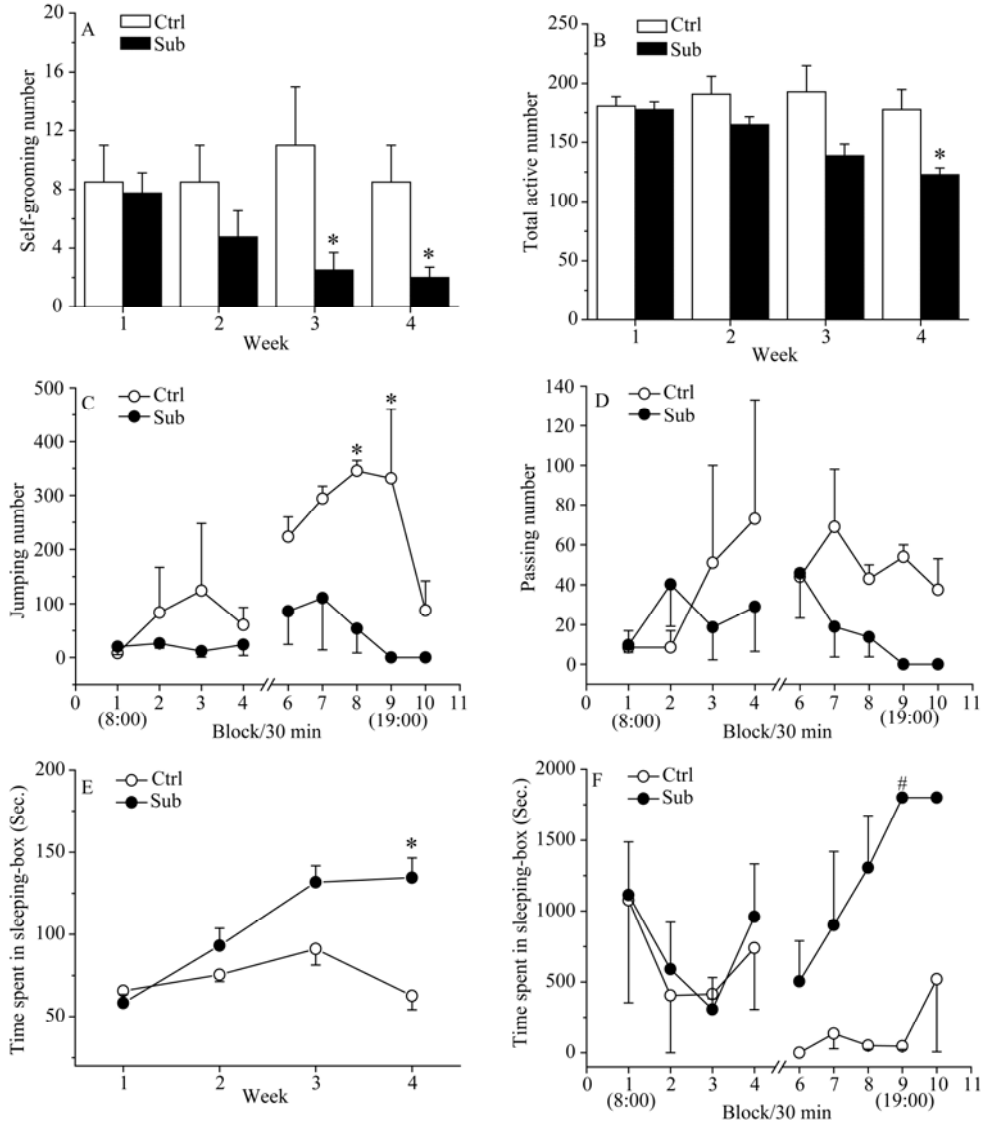

Fig. 3 Effect of chronic social defeat on different behavioral indexes

The subordinate tree shrews (Sub) showed a significance decrease in self-grooming behaviors (A) and locomotor activities (B−D) compared to the controls (Ctrl). The Sub group showed more inactivity by staying in the sleeping-box during their active phase (E, F). A, B, E shows statistical data for a 15-min period between 18:00 and 19:30; C, D, F shows statistical data from 8:00 to 10:00 and 17:00 until 20:00 in the fourth week. The blocks of 1−4 indicate 8:00−10:00; the blocks of 6−10 indicate 17:30−20:00. * P<0.05, # P=0.000.

The Sub tree shrews lost 5%~10% in body weight after chronic social defeat, while the Ctrl group exhibited a small increase over the same time period (Fig. 4A). Animals subjected to chronic social defeat showed an increase in urinary cortisol levels (Fig. 4B,C), especially during their activity peak period [$F_{(1, 4)}$=7.837, P=0.049; $F_{(1, 4)}$ =25.353, P=0.007; $F_{(1, 4)}$ =10.149, P=0.010; Fig. 4C].

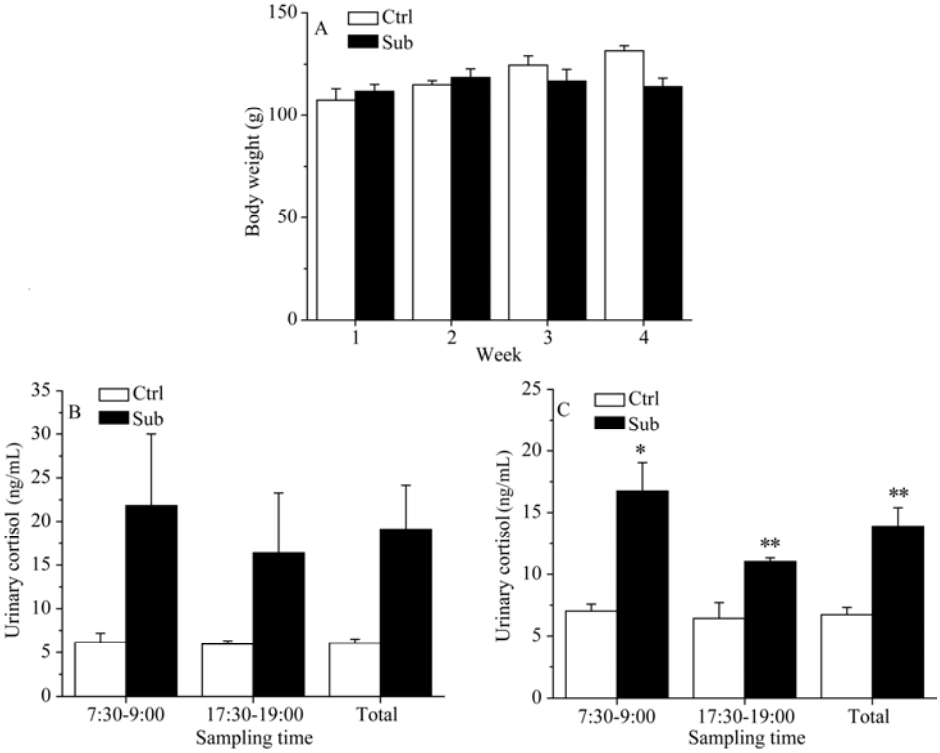

Fig. 4 Effects of chronic social defeat on body weight and urinary cortisol

Chronic social defeat reduced body weight in the subordinate (Sub) group (A). The Sub group showed a significant and sustained elevation of urinary cortisol (B was measured in the third week, and C was measured in the fourth week). * P<0.05, ** P<0.01.

2.2 Learning and memory model

There was no difference between the saline and MK-801 groups in regards to latency to catch the bait and freezing time induced by apple replacement during the training process, indicating that the injected drug did not affect adaptation and learning (Fig. 5). Twenty-four hours after the fifth trapping trial, the latency to catch the bait increased significantly compared with training in saline-injected animals but not in MK801 injected animals [$F_{(1, 4)}$=72.792, P=0.001; Fig. 5A].

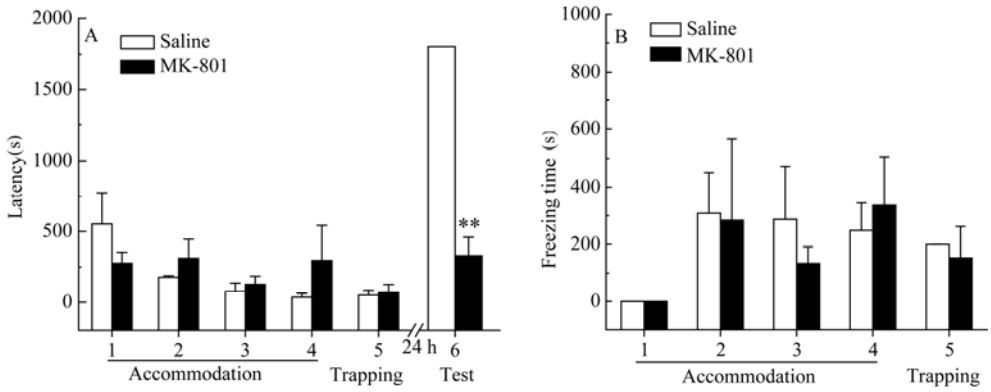

Fig. 5 The NMDA receptor antagonist MK-801(0.2 mg/kg) prevented the formation of captive memory in tree shrews

A) Twenty four hours after the final training trial, the MK-801group had significantly shorter latency than the saline group. B) Tree shrews showed freezing behavior after technician changed apple piece. Freezing time was not significantly different by groups. ** P<0.01.

3 Discussion

We demonstrated a chronic social defeat model of depression and a novel one-trial captive conditioning model of learning and memory in tree shrews. Although these pilot studies require further study, the results already broaden understanding of the behaviors in the tree shrew, the closest relative to primates. The present

study is important for establishing tree shrew models of depression, learning, and memory for mechanism studies and preclinical drug evaluation. One could expect that the data from the tree shrew may be more appropriate than those from other mammals to conditions in human beings.

In the depression model, we investigated the effects of chronic social defeat on the tree shrew (*Tupaia belangeri chinensis*). Subordinates showed alterations in body weight and behaviors, and increases in urinary cortisol levels. Remarkably, these alterations were persistent for at least two weeks. The time spent in the sleeping-box also increased. All these data suggest a set of depressive symptoms similar to those observed in human depression patients with stress symptoms. The present results are consistent with previous reports (Kozicz et al, 2008; Fuchs & Flügge, 2002). Future research should focus on what drug mechanisms can prevent or treat these depression-like symptoms in tree shrews.

In the one-trial captive condition model of learning and memory, latency progressively decreased in trials 1-5, suggesting a process of adaptation. In the final trial, however, tree shrews entered the cage and were trapped for 1 h after they ate bait apple. The one-trial trapping led to a large increase in latency in the test trial conducted on day 2 (24 h later), which suggested that tree shrews formed a captive memory and avoided entering the cage. Furthermore, we demonstrated that a NMDA receptor antagonist given before the adaptation process did not affect adaptation as indicated by the latency in adaptation. It did, however, prevent the formation of captive memory as indicated by much shorter latency in the test trial than the saline treatment. These results are similar to previous rodent studies (Boultadakis & Pitsikas, 2010). NMDA receptor plays a critical role in learning and memory in a variety of species (Bliss & Collingridge, 1993; Kovacic & Somanathan, 2010). Thus, the fact that the NMDA receptor antagonist MK-801 prevented the formation of captive memory strongly suggests that one-trial captive conditioning indeed forms a captive memory. Since adaptation latency was not affected by MK-801 (Fig. 5), other explanations were excluded. Nevertheless, future study would better test whether captive memory depends on the final trapping trial or whether the formation of a captive memory requires 24 h. Most importantly, the underlying mechanism for establishing captive memories needs to be studied.

The present results demonstrate a pilot study on behaviors of the tree shrew and suggest a promising future in animal research fields of depression, learning and memory as tree shrews are closely related to primates. One of the best applications of tree shrew models could be preclinical evaluation of drugs, as previous evidence shows that rodent models are very different to humans, which might be one of the major reasons contributing to high failure in clinical trials for new drugs.

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