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Tail Suspension Test for Evaluating Mice's Depression-Like Behaviors

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Abstract: As depression has become a serious global health issue, animal models are essential for understanding its underlying mechanisms and developing new treatments. The Tail Suspension Test (TST) is a widely used assay that induces a state of behavioral despair in rodents, which serves as a key indicator of depression-like behavior. In this study, mice first undergo a period of light deprivation to induce a depressive state. Subsequently, the TST is used to assess their behavior. The primary metric is immobility time—the duration for which a mouse ceases to struggle when suspended. It is hypothesized that mice subjected to light deprivation will exhibit significantly longer immobility times compared to control mice, reflecting a state of learned helplessness. This model is expected to be valuable for screening new antidepressant compounds and providing a theoretical basis for novel therapeutic strategies.

1. Introduction

Depressive disorder is a serious mental health condition affecting people worldwide. According to the World Health Organization, approximately 3.8% of the global population, or 280 million people, experience depression at some point in their lives. This prevalence includes 5% of all adults (specifically, 4% of men and 6% of women) and 5.7% of adults older than 60 [1]. The duration of light exposure can affect mood, which helps explain why individuals in regions with prolonged periods of darkness have a higher risk of developing depression [2]. Therefore, it is essential to develop animal models that can mimic depressive conditions and allow for the assessment of depression-like behaviors to support research in this field. This study will first induce a depression-like state in mice through a light deprivation protocol. Subsequently, the TST will be used to observe and analyze the severity of their resulting behaviors.

Previous studies have established that the TST is an effective model for assessing depression-like behaviors in mice. It can also be used to evaluate the efficacy of new potential antidepressant compounds. The test operates by inducing a state of behavioral despair; when an animal is suspended by the tail, it will eventually cease struggling and become immobile. This immobility time is the key metric, with a longer duration indicating a more severe depression-like state. Based on these findings, past research concluded that the TST is a reliable tool for both quantifying depression-like symptoms and screening for effective drug treatments. The current study will build upon this by first using a

light deprivation protocol to induce depression-like behaviors in mice. Subsequently, the TST will be used to assess the severity of these behaviors by measuring changes in immobility time.

The TST procedure involves securing a mouse's tail with adhesive tape and suspending it, so the animal hangs clear of any surfaces. Initially, the mouse will struggle, but under these stressful conditions, it will eventually adopt an immobile posture. This period of immobility is precisely measured. The core hypothesis is that mice with more severe depression-like behaviors will spend a significantly longer time immobile, reflecting a state of learned helplessness. The significance of this research model is threefold. First, it provides a standardized and quantifiable method for assessing the severity of depression-like behaviors, offering more objective data than simple observation. Second, it serves as a valuable tool for investigating the neurobiological mechanisms underlying depression. Finally, the TST is crucial for preclinical drug screening, allowing researchers to efficiently test the efficacy of new potential antidepressant compounds.

2. Materials and Methods

2.1 Experiment materials

Control group mice were housed in standard cages (30 cm × 20 cm × 15 cm) under a standard 12:12h light-dark cycle. Environmental conditions were maintained at a temperature of 20-26 ℃ and a humidity of 40-70%. Food and water were available ad libitum. All mice were purchased from the Laboratory Animal Center of the Institute of Zoology, Chinese Academy of Sciences.

Medical-grade, double-sided tape was used to secure the mouse's tail. A suspension bar served as the apparatus from which the mouse was hung.

2.2 Experimental method

2.2.1 The protocol for this experiment

The TST was used as a model of behavioral despair. The aim of this experiment was to observe and record the behavior of the mice during this test, thereby characterizing the specific responses associated with a depression-like state.

2.2.2 Experimental method

The experiment method of this test is the behavioral observation method, which could objectively and systematically observe and record the reaction of the mouse, in order to further conclude the feature of the depression-like behavior.

2.2.3 Equipment for experiment and measurement

The TST utilizes medical-grade, double-sided tape to secure a mouse's tail to a suspension bar or loop. This setup places the mouse in an unsupported, suspended position. Each test session, lasting a total of 6 minutes, was recorded using a high-definition camera. From these recordings, the total immobility time was scored manually and entered into a spreadsheet. Immobility was defined as the period during which the mouse remained completely motionless, without any active struggling. The recorded data were then analyzed using GraphPad Prism software.

2.2.4 Experimental design process

Each mouse was suspended individually inside an observation box (45 cm × 25 cm × 35 cm). The mouse was hung at a height positioned between one-third and one-half of the box's total height,

ensuring it could not touch any surfaces. Adhesive tape was affixed to the tip of the tail for suspension. To prevent the animal from climbing its own tail, a small piece of tubing was placed around the base of the tail. The total test duration was 6 minutes, and the entire session was recorded using a video camera.

Figure 1: The experimental setup

As shown in Figure 1, the experimental setup consisted of an observation box (45 cm × 25 cm × 35 cm). A camera was used to record the entire experimental process.

Figure 2: The mouse turned around and grasp its tail

As illustrated in Figure 2, if a mouse managed to turn and grasp its own tail during the test, its data were excluded from the final analysis, and the trial was repeated with a different mouse.

Figure 3: Mice are hung upside down through tape in the box

When two tests were conducted simultaneously, an opaque divider was placed between the setups to prevent the animals from visually influencing one another, which could affect the results. This setup is illustrated in Figure 3.

Once suspended, a mouse initially struggles. However, it eventually ceases this activity and enters a state of immobility. The duration of this immobility, rather than the struggling time, serves as the primary indicator of the severity of depression-like behavior.

2.2.5 Specific operating steps

First, while wearing gloves, the experimenter gently removed one mouse from its home cage and placed it on the laboratory bench. The cage was then immediately closed. A suspension apparatus with a loop was used to hang the mouse at a consistent, predetermined height. Medical-grade, double-sided tape was used to affix the tip of the mouse's tail to the suspension loop. Care was taken to ensure the tape was applied with moderate tightness to secure the animal without causing tissue damage or restricting its movement. Once the mouse was fully suspended, video recording was initiated. The animal's identification number and the trial start time were recorded simultaneously. Throughout the process, the experimenter remained at a distance from the apparatus to avoid influencing the animal's behavior. After the 6-minute trial concluded, the mouse was taken down, the tape was gently removed, and the animal was placed in a separate holding cage to distinguish it from untested mice. Between each trial, the suspension apparatus and bench surface were cleaned with 75% ethanol to remove any residual odors or feces that could influence subsequent tests. A new piece of tape was used for each mouse.

2.2.6 Method for data analysis

We name the original video using their ID and save it on the computer. We use the analysis software 'Pot Player' and simultaneously record the data in Excel for further video evaluation and statistical analysis. We record the time when the mouse first hangs up and manually record the start and end times of the struggling period. We calculate the differences to determine the duration of each movement period and sum these differences to obtain the total struggling time. We compare the total struggling times of each mouse to identify features indicative of depression-like behavior.

All statistical analyses and data visualization were performed using GraphPad Prism 9 (GraphPad Software, Inc., USA). All data are presented as mean ± SEM. The Shapiro-Wilk test was first used to assess data normality. For normally distributed data, comparisons between the two groups were made using an unpaired Student's t-test. If the data were not normally distributed, the non-parametric Mann-Whitney test was used instead. Significance levels were defined as $P < 0.05$, $*P < 0.01$, and $**P < 0.001$. A result was considered not significant (n.s.) if $P \geq 0.05$. Figure 4 provides a detailed summary of these results, including group means for immobility time, individual data points, and measures of variance.

3. Results

Figure 4 shows a comparison of the immobility times between the control and LD groups. The mean immobility time for the control group was approximately 100 seconds, whereas the mean for the LD group was significantly longer, at approximately 230 seconds. The variability in the data was also greater for the LD group compared to the control group. A statistical comparison revealed a highly significant difference between the two groups ($****P < 0.0001$). These results indicate that mice subjected to light deprivation exhibited more pronounced depression-like behaviors in the Tail Suspension Test.

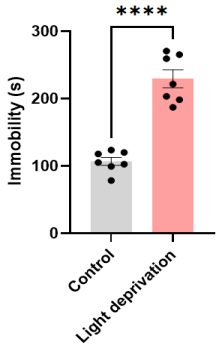

Figure 4: Graph made in SEM

4. Conclusion

This study investigated the depression-like behaviors of mice subjected to light deprivation using the TST. This behavioral assay induces a state of learned helplessness, allowing for the observation of differences between mice with induced depression-like symptoms and a control group. The primary metric recorded was immobility time—the duration for which a mouse ceases struggling when suspended. The results demonstrated that mice with more severe depression-like behaviors exhibited significantly longer immobility times, indicating a reduced desire to struggle in a hopeless state. This finding supports the use of the TST as a method to confirm the presence of a depression-like phenotype in this animal model. However, this study has several limitations. These include a lack of strict control over environmental variables, such as ambient light and noise, which may have varied between trials. Furthermore, manual timing and subjective judgment in scoring struggling behaviors could introduce errors. Finally, the small sample size limits the generalizability and reliability of the results.

Because this animal test successfully characterizes the features of depression-like behavior, it has significant applications. It can be used to assess depressive phenotypes and to screen for the efficacy of potential antidepressant compounds by observing behavioral changes before and after drug administration. As such, it is a valuable method that can contribute to the development of new treatments for depression. However, several limitations and challenges persist. The translatability of findings from animal models to human clinical outcomes remains a significant hurdle, as highlighted by previous research [3]. Furthermore, the influence of subtle environmental variables on mouse behavior is not fully understood [4]. To improve the reliability of this test, future efforts could focus on automating the data collection process using AI-powered tracking systems to enhance objectivity. As the Tail Suspension Test has its own limitations, a more robust assessment could be achieved by synthesizing results from a battery of tests (e.g., the Forced Swim Test and the Sucrose Splash Test). Finally, adopting an interdisciplinary approach to explore the pathogenesis of depression—integrating findings from various scientific fields—could provide novel insights for future drug discovery and clinical treatments.

A comparison of behavior between mice that experienced light deprivation and normal control mice suggests that a lack of light can induce depression-like behaviors. These findings may offer insights into how similar conditions contribute to depressive feelings in humans. Consequently, light therapy has become a useful method for improving patients' mental states and treating depression (especially SAD) in recent clinical practice. Combining light therapy with physical approaches, such as massage, to promote holistic relaxation may represent a novel direction for future depression treatments.

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