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Light Deprivation Induces Anxiety-Like Behaviors in Mice

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Abstract

To gain a more profound understanding of the nature of anxiety, this study utilized two animal behavioral models: the Open Field Test (OFT) and the Light/Dark Box Transition Test (LDT), using two groups of C57BL/6J mice: one with anxiety — like symptoms and the other without. In the LDT, the control group (healthy mice) showed a significantly higher tendency for active movement ($P < 0.05$) and a greater preference for exploring unfamiliar environments compared to the group of mice with anxiety, while the differences revealed by the OFT were less pronounced. Our findings highlight the effectiveness of these two animal models and the correlation between locomotive behavior and anxiety, which can be applied in future research.

Keywords: open field test, Light/Dark Box Transition Test, anxiety, motor performance

1. Introduction

Anxiety disorders are often referred to as panic attacks, and are characterized by recurrent, sudden and intense anxiety and fear. These episodes can escalate severely within minutes, which in turn can cause unbearable disruption to daily life. Moreover, the symptoms are difficult to control and can persist over extended periods, significantly impairing an individual's ability to function in social environments (Mayo Clinic, 2018). These anxieties usually cause patients to exhibit physical symptoms such as increased heart rate, shortness of breath, dizziness or nausea, as well as psychological distress such as persistent worry and apprehension.

Over the past decade, anxiety disorders have increased rapidly among the adolescents. Perhaps several social environmental factors caused this phenomenon, such as the isolation caused by the COVID-19 pandemic. It is estimated that approximately one in seven of adolescents aged 10–19 experience mental health issues, and anxiety disorders are among the most common. Furthermore, roughly one in three adolescents between the ages of 13 and 18 experience anxiety disorders at some point during their growth (World Health Organization: WHO, 2024). Despite these alarming statistics, the National Institutes of

Health (NIH) reports that anxiety disorders in adolescents remain largely unrecognized and undertreated.

Animal models play an important role in the study of mechanisms underlying anxiety disorders and other psychological disorders. Animal models of rodents have been used particularly extensively. For instance, an earlier study found that GAL 3 receptor KO mice exhibit an anxiety-like phenotype using the OFT (Brunner et al., 2014).

Seasonal Affective Disorder, a type of depression, tends to occur more frequently in autumn and winter due to shorter days. Because the daylight hours are relatively short in the northern winter, Seasonal Affective Disorder is also more common in regions with northern climates (such as the Nordic countries and Canada). Based on the theory that light exposure affects emotions, we adopted the method of light deprivation to create an animal model — the light deprivation model. Specifically, we suppressed light exposure to induce anxiety behaviors in the mice participating in the experiment.

Commonly used animal models of anxiety include the Open Field test (OFT), Light Dark Box Transition Test (LDT), the Elevated Zero Maze (EZM) etc.

The OFT provides a well-controlled and standardized setting that can be conveniently used to observe behavioral traits such as locomotive activity, exploration, and anxiety-like behaviors. OFT is particularly useful for studying anxiety in rodents as it simulates a mildly stressful environment where the animals are exposed to a large, open area. Studies based on the OFT have revealed that factors like sex, breed, and genes may cause mice to exhibit different patterns of locomotion and exploratory behavior. These behavioral patterns are often linked to the animals' psychological state (Kraeuter, A., Guest, P. C., & Sarnyai, Z., 2018).

The LDT is based on rodents' natural aversion to bright light and their tendency to explore new environments when mildly stressed. The model consists of two connected chambers, a dark one and a lit one. By observing behaviors such as transitions between the two chambers, researchers can evaluate the anxiety levels of rodents.

In a study published in Behavioral Ecology and Sociobiology by Springer Nature, researchers assessed the behavioral responses of rodents to stress and explored potential correlations between these behaviors and physiological stress markers using the OFT model (Mazza et al., 2019b). The study found that animals exhibiting heightened anxiety-related behaviors in the experiment also exhibited increased levels of corticosterone, suggesting a link between behavioral and physiological markers of anxiety.

While much research has examined the psychological connections between rodent behavior and various characteristics, the current body of knowledge remains limited in the specific relationship between locomotor activity and anxiety. Animal models like the OFT however, can be invaluable in providing insights into the fundamental biological and behavioral underpinnings of anxiety disorders.

Our study aims to address this gap by comparing the behaviors of mice exhibiting anxiety-like symptoms with those that do not based on the OFT and LDT model. By focusing on the contrast between these two groups, we identified specific behavioral markers associated with anxiety in rodents.

By understanding anxiety behavior in animal models, we can provide a theoretical framework for the treatment of adolescent anxiety disorders which helps not only to develop better diagnostic tools, but also to improve our understanding of the neurobiological mechanisms underlying anxiety, thus ultimately creating more effective treatment strategies. This research could serve as a basis for interventions aimed at alleviating the psychological distress experienced by young people, potentially offering a path to more effective treatments for adolescent anxiety disorders and contributing to a broader understanding of how mental health challenges affect youth on a global scale.

Our study, however, still holds its limits. We failed to explore the inner mechanisms that drive rodent behaviors and how the anxiety behaviors of rodents can be related to that of humans. Therefore, these two questions remain an area of ongoing research we plan to address in the future.

2. Materials and Methods

2.1 Open Field Test

The OFT is a commonly used behavioral test designed to assess an animal's exploratory behavior, activity level, and anxiety state.

In this study, mice were placed in an open environment to examine their natural reactions in an unfamiliar environment. Mice with lower levels of anxiety showed a stronger tendency to explore, boldly venturing into the center of the open field for deeper investigation. Conversely, mice with higher levels of anxiety showed lower mobility and exploratory tendencies, preferring to remain along the perimeter of the field for a sense of safety.

In general, mice are averse to bright, open spaces and prefer dim, enclosed spaces, presumably to prevent detection by predators.

In our experiment, we used C57BL/6J mice as experimental model organisms. By observing and analyzing their behavior in the box area, we can determine how they behave in an unfamiliar environment.

Experimental method: We adopted a control-based approach, maintaining the control group under normal light conditions (twelve hours each day) and exposing the experimental group to reduced light (eight hours per day).

The experimental tools included seven three-month-old C57 mice for each group (two groups in total), a 40×40×25 cm open white acrylic box and a camera.

The experimental steps are as follows:

a. Preparation:

There are fourteen three-month-old C57BL/6J mice divided into two groups, seven mice for control group and the other seven mice for experimental group (light depression). The mice in the control group were treated with normal light (twelve hours each day), while the mice in the experimental group were treated with unusual light and had eight hours of light deprivation per day (four hours of light per day). Before the experiment officially began, these fourteen mice had been acclimated in the laboratory for an hour.

b. Process of experiment:

One mouse was randomly selected at a time, placed in the acrylic box for five minutes, and its behavior was recorded with the camera. Before the next mouse was put into the box, we use 75% alcohol to clean the box to eliminate the smell of the last mouse.

2.2 Light/Dark Box Transition Test

LDT is a classic method to evaluate anxiety and depression, and is widely used in the basic research of psycho-neuropharmacology because of its good structural validity. The LDT can be used to study disease models associated with mood disorders. The light-dark chamber can be used to test the unconditional anxiety response in rats and mice. It is based on innate light avoidance and spontaneous exploration behaviors in rodents faced with mild stressors, such as novel environments and light/open Spaces. By comparing the behavioral differences between normal mice and mice that mimic specific diseases (such as anxiety, depression) in the light-dark box, we can obtain correlations between emotional states and behavioral performance. Rodents in the light — dark box tend to explore the novel environment. However, due to their aversion to the bright light in the lit chamber, they are compelled to retreat, creating a state of conflict. Consequently, the frequency of their transitions between the two chambers is reduced.

In our experiment, we used C57BL/6J mice as experimental model organisms, by observing and analyzing their behavior in a certain light and dark box. We can recognize which kind of area they prefer to stay, and then analyze their anxiety level.

The experimental method is the same as that of the OFT.

The experimental tools included seven three-month-old C57 mice for each group (there are two groups in total), a 45×25×25 light dark acrylic box (the light area is 30×25×25, and the dark area is 15×25×25).

PS: The light area is composed of transparent acrylic plates, while the dark area is made up of opaque black acrylic plates. There is also a 15×25 black opaque acrylic plate that is placed on top of the box in the dark area during the experiment.

The experimental steps are as follows:

a. Preparation:

The LDT used the same experimental mice as OFT experiments. Before the experiment officially began, these fourteen mice had been acclimated in the laboratory for three days.

b. Process of experiment:

One mouse was randomly selected at a time, placed in the acrylic box for ten minutes, and its behavior was recorded with the camera. Before the next mouse was put into the box, we needed to use 75% alcohol to clean the box to eliminate

the smell of the last mouse.

2.3 Statistics and Analysis

The results of the experiments were expressed as mean ± standard deviation; Normality analysis with t-test was applied to analyze significant differences between the samples (*P<0.05, significant difference).

GraphPad Prism was used to analyze other figures and draw images.

a. OFT

Use EthoVision to identify the video and extract the data of mice.

b. LDT

Since this experiment was not suitable for video analysis by software, we personally observed and analyzed the number of times the C57 mice entered and exited in the dark area and the duration of their stay.

3. Results

3.1 OFT

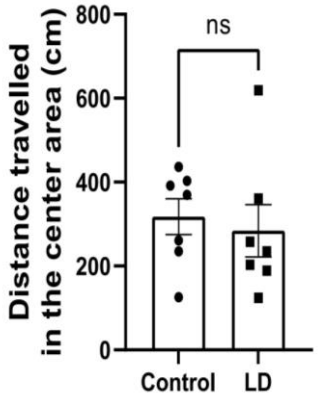

Figure 1. Distance travelled in the center area

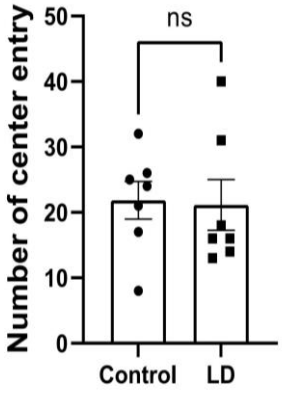

Figure 2. Number of center entry

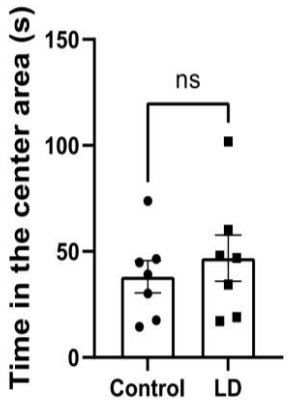

Figure 3. Time in the center area

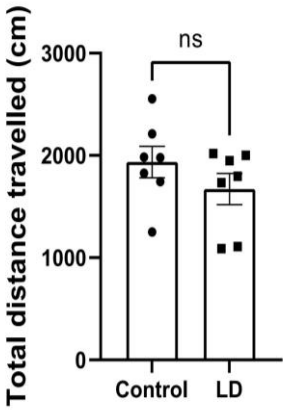

Figure 4. Total distance travelled

Figure 1 shows the distance travelled in the center area. The distances travelled by the control-group and light-deprivation group mice were nearly the same (*p > 0.05), indicating similar anxiety levels.

Figure 2 shows the number of center entry. The travel frequency of mice in the control and light-deprivation groups was almost the same, and the difference between the two was not significant (*p > 0.05). These data indicate that the mice from the light deprivation group have the same anxiety level as the control group.

Figure 3 shows the time in the center area. As we can see, the time the mice stayed in the center area in the light deprivation group was less than that of the control group, and the difference between the two was significant (*p < 0.05). These data indicate that the anxiety level of the light deprivation group was higher than that of the control group.

Figure 4 shows the total distance travelled. As we can see, the distance travelled by the mice in the control group was almost the same as that of the light deprivation group, and the difference between the two was not significant (*p > 0.05). These data indicate that the mice from the light deprivation group have the same anxiety level as the control group.

Based on the above four sets of data, we can see that these experiment mice did not show significant behavioral differences in this experiment. Therefore, the inhibition of light did not cause the light deprivation group of mice to have a significantly higher anxiety level in the open field test.

3.2 LDT

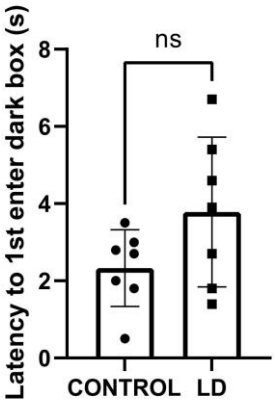

Figure 5. Latency to 1st enter dark box

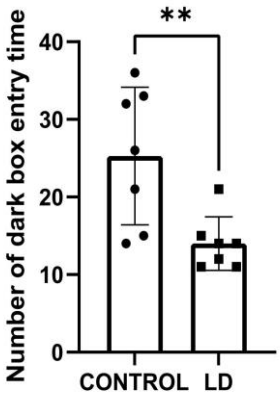

Figure 6. Number of dark box entry time

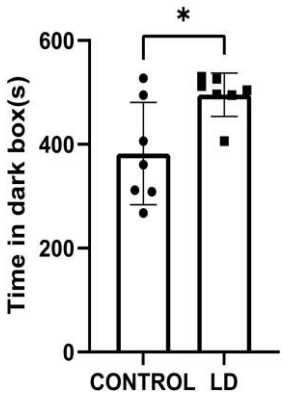

Figure 7. Time in dark box

In the light/dark box experiment, we detected data in three aspects, latency to 1st enter dark box, number of dark box entry time, time in dark box, which correspond to figure 5, 6, and 7 respectively.

Figure 5 shows the latency to 1st enter dark box. We can see that the latency of the mice in the control group was almost the same as that of the light deprivation group, and the difference between the two was not significant (*p > 0.05). These data indicate that the mice from light deprivation group have the same anxiety level as the control group.

Figure 6 shows the number of dark box entry times. From this graph, we can see that the number of dark box entries of the mice in the control group was more than that of the light deprivation group (which also means that the mice of control group entered light box more frequently), and the difference between the two was significant (*p < 0.05). These data indicate that the mice from the light deprivation group have a higher anxiety level than control group.

Figure 7 shows the total time the mice were in the dark box. From this graph, we can see that the control group mice persisted in the dark box

for a shorter period than that of the light deprivation group, indicating that the mice from control group are more willing to stay in open and unfamiliar environments. The difference between the two groups was significant (*$p < 0.05$). These data indicate that the mice from light deprivation group have a higher anxiety level than the control group.

Based on the above four sets of data, we can see that these C57 mice did show significant behavioral differences in this experiment — the mice in deprivation have significantly higher anxiety level than the mice in control group. Therefore, we can infer that depriving light exposure does indeed affect the mood of living organisms and increases their anxiety index.

4. Conclusion and Discussion

In this experiment, our main objective was to construct an anxiety model using mice and conduct research on the behavioral characterization of anxiety onset based on the results. The results of this study provide a foundation for work on the neural mechanisms of anxiety disorders.

First and foremost, we constructed an acute light deprivation model of mice. This method, which is commonly used, simulates human mood disorders by altering the light conditions in the mice's living environment to affect their moods. In this experiment, we established an acute light deprivation model by controlling the light exposure time of mice and investigated the effect of light duration on the emotional behavior of mice.

This study mainly conducted two experiments: the Open Field Test (OFT) and the Light/Dark Box Transition Test (LDT), using a control-based experimental method.

The first experiment we conducted was the OFT. In this experiment, we detected the data about total distance traveled, time in center area, distance traveled in center area and Number of center entry time. By analyzing these four types of data, we determined that in OFT, there was no significant difference in the performance between control group and light deprivation group.

We propose several reasons for the lack of significant differences in the experimental results. First, the adaptation period for the mice was too short, so they had not fully adapted to the experimental environment. As a result, they exhibited excessive tension, which affected their exploratory behavior and led to insignificant differences between groups. Second, there were errors made by experimental operators introduced by irregular or inconsistent operation of the experimenters. For example, placing mice in different positions in the open field would affect their initial direction of exploration and range of activity; inconsistent standards for observing and recording behavior would lead to inaccurate data and make it difficult to detect differences between groups. Third, too small a sample size reduced the statistical validity of the experiment and made it difficult to detect real differences between groups.

The second experiment we conducted was the LDT. In this experiment, we detected the data about the time when the mouse first enters the dark box (referred to as the latency period), the time the mouse stays in the dark box, the number of times the mouse enters the light box.

Based on the above two experiments, we can infer that suppressing light exposure has a significant impact on organisms, such as the anxiety level, which we are concerned about.

The limitations of this study lie in the small number of mice involved in the research and the relatively small sample size, which may affect the generalizability of the results.

The open field test can be widely applied to assess the behavioral effects of various drugs, such as anti-anxiety drugs, antidepressants, and other central nervous system drugs. By comparing the behavioral changes before and after drug treatment, researchers can evaluate the efficacy and potential side effects of the drugs. For instance, central stimulants can increase the spontaneous activity of mice. (Ye et al., 2011)

The light-dark box test is mainly based on the natural aversion of mice to bright areas and their exploratory behavior in new environments. This test is often used to study the efficacy of anti-anxiety drugs. Anti-anxiety drugs can significantly increase the number of times mice enter the light box and the time they spend in it, thereby reflecting their improvement of anxiety behavior. In addition, this experiment can also be used to evaluate the potential anxiogenic effects of drugs. Besides that, by observing the behavior of mice in the light-dark box, researchers can infer their emotional responses

to different lighting conditions, thereby revealing the mechanisms of emotional regulation and stress response in animals. For instance, anxiety model mice exhibit more pronounced light-avoiding behavior in the light-dark box experiment.

Thus, through this experiment, we have not only analyzed and simply summarized the possible causes and factors that may lead to the onset of anxiety, but also laid a certain foundation for the research of drugs for treating related mental disorders.

Regarding future development, the research will place greater emphasis on the integration of multiple disciplines, such as the combination of techniques from neuroscience, immunology, genetics, and behavioral science. For instance, the research of Yu Xiaofei's team has revealed the role of IL-22-mediated "gut-brain axis" in alleviating mental stress, indicating that the interaction between the immune system and the nervous system is of significant importance in anxiety disorders (Xia et al., 2024). Such interdisciplinary research will contribute to a more comprehensive understanding of the pathological mechanisms of anxiety disorders.

Research directions for mouse models of anxiety also include in-depth exploration of neural circuit mechanisms, in-depth study of gene-environment interactions, application of non-invasive techniques, and exploration of brain-gut axis mechanisms, which are expected to provide more effective strategies for the treatment and intervention of anxiety disorders.

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