Exploring Anxiety like Behaviors Through Locomotor Activity Testing

Tianyue Wang
Beijing No.80 High School
Email: wangtianyue2021ss@163.com

Abstract: Anxiety disorders represent a highly prevalent class of psychiatric conditions worldwide, yet their complex pathogenesis remains incompletely understood. In recent years, the potential relationship between light exposure conditions and the mechanisms underlying anxiety disorders has garnered increasing attention from the scientific community. Light serves as a critical environmental factor that influences biological rhythms and neuroendocrine function, potentially modulating emotional regulation through multiple physiological pathways. This study employed the open field test to systematically investigate the specific effects of light deprivation on anxiety like behaviors in mice. During the experimental procedure, the behavioral performance of mice in the light deprivation group and the normal light control group was meticulously observed and recorded. The experimental results revealed that, compared to mice housed under normal light conditions, light deprived mice exhibited significantly reduced time spent in the center zone of the open field apparatus, demonstrated a marked preference for peripheral areas, and displayed more pronounced anxiety like behaviors including reduced exploratory activity and increased vigilance. These findings suggest that insufficient light exposure may represent a significant contributing factor to the development of anxiety related emotional states. This research not only provides important animal experimental evidence for understanding the relationship between light conditions and anxiety disorders but also draws attention to the often overlooked environmental factor of light insufficiency and its potential impact on human mental health, thereby offering new perspectives for the prevention and treatment of anxiety disorders.

Keywords: Light deprivation; open field test; anxiety; animal behavioral experiment

1. Introduction

Anxiety disorders are the most common mental disorders globally. In 2019, 301 million people were living with an anxiety disorder. It is estimated that approximately 4% of the global population currently suffers from an anxiety disorder. Anxiety disorders interfere with daily activities and can impair a person's family, social, school, or work life [1]. As a psychiatric condition characterized primarily by persistent psychological anxiety, somatic anxiety, sleep disturbances, and other major clinical features, the prevalence rate of anxiety disorders ranks first among adult mental disorders in China. Anxiety disorders typically onset during childhood, adolescence, and early adulthood, with over 90% of patients experiencing onset before 35 years of age. Unlike transient anxiety induced by stress, anxiety disorders are persistent and impair daily functioning. Clinical symptoms of anxiety disorders include poor sleep, decreased appetite, reduced energy, and increased irritability, all of which are common anxiety related behaviors [2]. Anxiety related behaviors have been recognized as important in the psychopathology and treatment of generalized anxiety disorder (GAD). Among the various approaches to investigating human pathology, animal models represent one of the most commonly used methods [3]. Therefore, to thoroughly investigate the mechanisms underlying the effects of light conditions on anxiety related behaviors, this study plans to employ a classic mouse model combined with the open field test, a well established behavioral assay, to systematically examine the characteristics of anxiety like behavioral changes under light deprivation conditions. Based on existing research evidence, mice subjected to light deprivation treatment may exhibit reduced exploratory behavior, locomotor inhibition, and other typical anxiety like behavioral phenotypes through disruption of the biological rhythm system or alterations in neurotransmitter balance. The core objective of this experiment is to test whether light deprivation significantly induces anxiety like behaviors in mice and to provide a preliminary assessment of its effect magnitude.

Currently, many European regions and underground workers receive limited daily light exposure and show higher prevalence rates of depression or anxiety disorders. Scientists believe that light plays an important role in regulating mood. Insufficient light is also considered one of the causes of depression or anxiety disorders. Seasonal affective disorder frequently occurs at high rates in high latitude regions where light exposure is limited [4]. To investigate whether reduced light exposure duration induces anxiety like behaviors in mice, this study will employ the open field test. The open field test is a widely used behavioral research method in scientific research. It is a method for evaluating spontaneous behavior, exploratory behavior, and tension in experimental animals within a novel environment. Based on the exploratory and avoidance instincts of rodents, it observes emotional

changes and spontaneous locomotor activity in mice, which can reflect, to some extent, the cognitive function, anxiety, tension, and psychological state of the mice. Rodents have a natural preference for moving along edges and corners and a fear of open spaces, a trait known as thigmotaxis. The open field test uses a large open area as a stimulus to induce anxiety in animals. By examining parameters such as time spent and distance traveled in peripheral versus center zones, the anxiety level of the animal can be assessed. Animals with higher anxiety levels spend more time in the peripheral areas of the open field [5].

2. Methods and Materials

2.1 Experimental Materials

The experimental materials included an open field box, a video camera, a data processing system, mice in the experimental group (subjected to light deprivation), and mice in the control group (not subjected to light deprivation). A total of 14 eight week old specific pathogen free (SPF) male C57BL/6 mice were used in this study. All animals were purchased from the Animal Experiment Center of the Institute of Zoology, Chinese Academy of Sciences. The experimental animals were housed in individually ventilated cages measuring 30 × 20 × 15 centimeters, with 5 mice per cage. The mice had free access to standard rodent chow and drinking water. The ambient temperature was maintained between 20° C and 26° C, and the relative humidity was maintained between 40% and 70%. After one week of acclimatization to the housing conditions, mice with a body weight of 20 ± 2 grams were selected for formal experimentation. All experimental animals were handled in accordance with the guidelines outlined in the Laboratory Animal Ethics Review and Welfare Guidelines [6].

2.2 Experimental Methods

The experimental procedures were carried out in a quiet, dimly lit environment with an illumination intensity of 50 ± 5 lux. Behavioral testing was conducted at a fixed time each day, specifically between 9:00 and 12:00, to control for circadian variations in activity levels.

At the beginning of each trial, a mouse was gently transferred from its home cage to the center of the open field arena. Once the mouse was placed in the center of the arena, the video recording system was started immediately, and behavioral data were collected for a duration of 5 minutes.

After each trial, the floor and inner walls of the open field box were thoroughly cleaned with 75% ethanol solution. This cleaning procedure removed any urine, feces, or other biological residues that could provide olfactory cues to subsequent mice. After cleaning, the apparatus was allowed to air dry completely, and the experimenter waited until the ethanol odor had fully dissipated before proceeding with the next trial. This waiting period prevented residual odors from influencing the behavior of subsequently tested mice [7].

The primary observational parameters recorded in this study were as follows. The time spent in the center zone, measured in seconds, reflected the duration the mouse remained in the central area of the open field. The frequency of entries into the center zone, counted as the number of times the mouse crossed the boundary into the central area, indicated the mouse's willingness to explore the more anxiogenic region of the apparatus. The total distance traveled, measured in centimeters, served as an index of general locomotor activity and allowed the experimenter to determine whether any group differences in center zone behavior were attributable to differences in overall movement capacity rather than anxiety levels [8].

2.3 Data Analysis

Statistical analysis and data visualization were performed using GraphPad Prism 9 (GraphPad Software, Inc., United States). All results are presented as mean ± standard error of the mean (s.e.m.) throughout this paper. Before conducting t tests and descriptive statistics, the Shapiro Wilk normality test was used to confirm that the residuals conformed to a Gaussian distribution [9].

For comparisons between two groups, either paired or unpaired t tests were employed as appropriate. In this study, the light deprivation group and the normal light control group represented independent samples, so unpaired t tests were used to compare their behavioral measures. For analyses involving multiple experimental groups in future studies, one way analysis of variance (ANOVA) followed by Bonferroni post hoc tests would be applied. When the data did not meet the assumptions of normality, nonparametric tests, including the Wilcoxon matched pair signed rank test and the Mann Whitney test, were to be used as alternatives [10].

Data are displayed as individual values or expressed as mean ± standard error of the mean

(SEM). The threshold for statistical significance was set at $P < 0.05$. Significance levels are denoted in the results section as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, with nonsignificant results labeled as n.s. [11] The experimental design and group allocation are summarized in Table 1.

Table 1. Experimental Design and Group Allocation

Parameter	Control Group	Light Deprivation Group
Number of mice (n)	7	7
Sex	Male	Male
Age (weeks)	8	8
Body weight (g)	20 ± 2	20 ± 2
Light condition during housing	Normal light-dark cycle	Complete light deprivation
Open field test illumination (lux)	50 ± 5	50 ± 5
Testing duration (minutes)	5	5

3. Results and Analysis

3.1 Movement Trajectory Analysis

The movement trajectories of representative mice from both groups were recorded and analyzed to provide a visual representation of spatial exploration patterns in the open field apparatus. These trajectories reflect the paths taken by each mouse during the 5 minute testing session and reveal preferences for different zones within the arena. Light serves as a central modulator of circadian rhythms, sleep, and affective states, and disruptions to normal light exposure patterns can profoundly influence behavior [12].

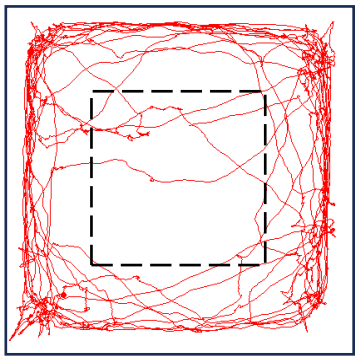

Figure 1(left panel). Control group movement trajectory.

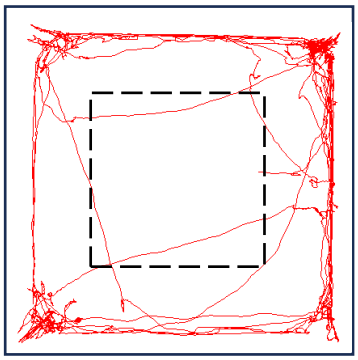

Figure 1(right panel). Light deprivation group movement trajectory.

Figure 1 presents the representative movement trajectories of control and light deprived mice in the open field test, with the two panels placed side by side for direct comparison. The left panel of Figure 1 shows the movement trajectory of a representative control group mouse. This mouse was primarily active in the peripheral area but occasionally ventured into the center zone, demonstrating a balanced pattern of thigmotactic behavior with intermittent center exploration. The trajectory reveals that while the mouse preferred to stay near the walls, it was willing to cross into the center area at multiple time points during the session. The right panel of Figure 1 shows the movement trajectory of a representative experimental group mouse subjected to light deprivation. This mouse spent the vast majority of its time moving along the peripheral walls and rarely entered the center zone. The trajectory appeared more compressed and confined to the outer edges of the arena, with very few crossings into the central area.

Comparing the left and right panels of Figure 1, it is clearly visible that the control group mice produced a denser pattern of center zone movement trajectories than the experimental group mice. This visual evidence strongly supports the conclusion that the light deprived mice were more fearful of remaining in the center zone. Consequently, the experimental group mice exhibited more pronounced anxiety like behavior compared to the control group. The melanopsin containing retinal ganglion cells and their projections to the suprachiasmatic nucleus provide the primary pathway through which light information reaches the circadian system, and disruption of this pathway may contribute to the behavioral changes observed in light deprived animals [13].

Figure 1. Representative movement trajectories of control and light deprived mice in the open field test. (Left panel) Control group movement trajectory. The control group mice were primarily active in the peripheral area but occasionally moved into the center zone. (Right panel) Experimental

group (light deprivation) movement trajectory. The experimental group mice spent most of their time in the peripheral area and rarely entered the center zone. Comparison of the left and right panels clearly shows that the control group mice had a denser pattern of center zone movement trajectories than the experimental group mice.

3.2 Locomotor Activity Assessment: Total Distance Traveled

The total distance traveled by each mouse during the 5 minute open field test session was measured to assess general locomotor activity. This parameter served as an important control measure to ensure that any observed differences in anxiety related behaviors between the two groups were not confounded by differences in basic motor function or spontaneous activity levels. The synchronization of circadian rhythms within the suprachiasmatic nucleus and throughout the brain is essential for normal behavioral regulation, and disruption of this synchronization can lead to affective disturbances [14].

The analysis revealed that the control group mice and the experimental group mice showed comparable total movement distances. Specifically, the mean total distance traveled by the control group was similar to that of the light deprivation group. This finding indicates that the locomotor function of the mice in both groups was equivalent. There was no evidence of motor impairment, lethargy, or hyperactivity in either group that could otherwise explain differences in zone specific behaviors. The preservation of normal locomotor activity in the light deprived mice is critical for interpreting the subsequent results, as it confirms that any reduction in center zone exploration reflects increased anxiety like behavior rather than a generalized decrease in movement capability.

3.3 Anxiety Like Behavior Assessment: Time Spent in the Center Zone

The time spent in the center zone of the open field apparatus is one of the most reliable indicators of anxiety like behavior in rodents. Animals with higher anxiety levels tend to avoid the exposed, brightly lit center area and instead prefer to remain in the peripheral zones near the walls, a behavior known as thigmotaxis. Conversely, animals with lower anxiety levels are more willing to explore the center zone. The inputs and outputs of the mammalian circadian clock involve complex neural pathways connecting the suprachiasmatic nucleus to multiple brain regions that regulate mood and anxiety related behaviors [15].

The results of this study demonstrated that the control group mice spent significantly more time in the center zone compared to the experimental group mice.

3.4 Anxiety Like Behavior Assessment: Frequency of Center Zone Entries

The frequency of entries into the center zone provides an additional measure of anxiety like behavior that complements the total duration measure. While total duration reflects the cumulative time an animal is willing to spend in the anxiogenic center area, entry frequency reflects the animal's willingness to initiate exploration of that area. A low entry frequency indicates that the animal is reluctant to cross the boundary from the safe peripheral zone into the potentially dangerous center zone.

It is worth noting that the pattern of results across the two anxiety related measures was consistent. Both the total time spent in the center zone and the frequency of center zone entries were reduced in the light deprivation group relative to the control group. This convergence of evidence from two complementary measures strengthens the validity of the conclusion that light deprivation increases anxiety like behavior in mice. Furthermore, because total locomotor activity did not differ between groups, the observed differences in center zone behavior cannot be attributed to differences in general movement capability. Instead, they reflect a genuine shift in the emotional state of the animals toward increased anxiety.

3.5 Quantitative Results

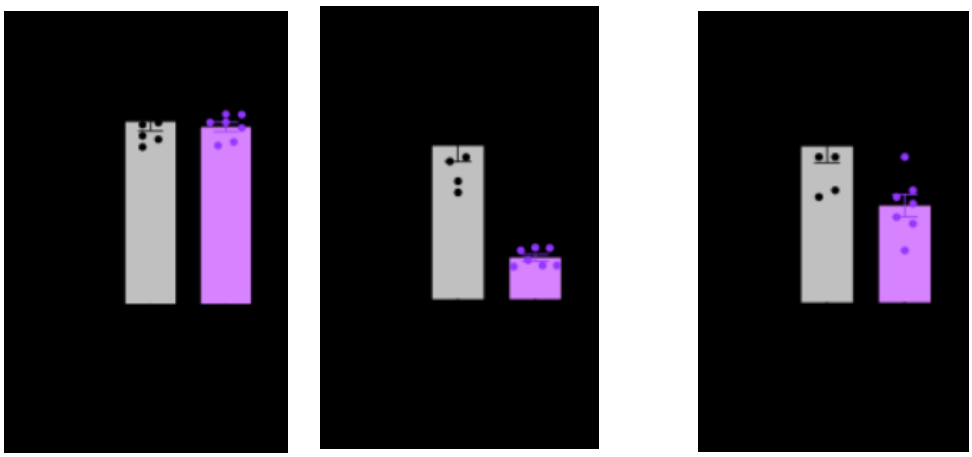

Figure 2(left panel). Total distance traveled. **Figure 2(middle panel).** Total time in center zone. **Figure 2(right panel).** Frequency of center zone entries.

Figure 2 presents the quantitative comparison of behavioral parameters between the control group and the light deprivation group. The left panel of Figure 2 shows the total distance traveled. The bar graph shows that the two groups exhibited nearly identical mean total distance values, with overlapping error bars indicating no significant difference between groups. This observation was further supported by the statistical analysis. An unpaired t test comparing the total distance traveled between the control group and the light deprivation group yielded a P value greater than 0.05, confirming that the difference was not statistically significant. Therefore, the two groups were well matched with respect to baseline locomotor activity. The left panel demonstrates that the control group mice and experimental group mice showed comparable total movement distances, indicating equivalent locomotor function between the two groups.

The middle panel of Figure 2 shows the total time spent in the center zone. The mean center zone duration for the control group was notably higher than that for the light deprivation group. The light deprived mice exhibited a marked reduction in the amount of time they were willing to remain in the center area. This behavioral pattern suggests that the experimental group experienced a higher level of anxiety when exposed to the open and potentially threatening center zone. The middle panel demonstrates that the control group mice spent significantly more time in the center zone compared to the experimental group mice.

The right panel of Figure 2 shows the frequency of center zone entries. The control group mice entered the center zone more frequently than the experimental group mice. Specifically, the mean number of center zone entries was higher for the control group compared to the light deprivation group. This finding indicates that the light deprived mice were not only spending less time in the center zone overall but were also less willing to initiate entries into this area. The reduced entry frequency further supports the interpretation that light deprivation induced a state of heightened anxiety in the experimental animals. The right panel demonstrates that the control group mice entered the center zone more frequently compared to the experimental group mice.

Figure 2. Quantitative comparison of behavioral parameters between control and light deprivation groups. (Left panel) Total distance traveled. The control group and experimental group mice showed comparable total movement distances, indicating equivalent locomotor function between the two groups. (Middle panel) Total time spent in the center zone. The control group mice spent significantly more time in the center zone compared to the experimental group mice. (Right panel) Frequency of center zone entries. The control group mice entered the center zone more frequently compared to the experimental group mice.

3.6 Summary of Experimental Findings

Taken together, the results of this open field experiment can be summarized as follows. First, the total movement distance did not differ significantly between the control group and the light deprivation group, indicating that both groups had equivalent locomotor function. Second, the control group mice spent a longer duration in the center zone compared to the light deprivation group mice. Third, the control group mice entered the center zone more frequently compared to the light deprivation group mice. These three findings collectively demonstrate that mice subjected to light deprivation were more fearful of entering and remaining in the center zone. The experimental group mice exhibited more pronounced anxiety like behavior than the control group mice.

In behavioral terms, the light deprived mice displayed a pattern of increased thigmotaxis, reduced

center zone exploration, and heightened vigilance, all of which are characteristic features of the anxiety like phenotype in rodent models. These results provide clear evidence that light deprivation leads to the development of anxiety like behaviors in mice. A summary of all behavioral parameters is presented in Table 2.

Table 2. Summary of Behavioral Parameters

Behavioral Parameter	Control Group	Light Deprivation Group	Group Difference
Total distance traveled (cm)	Comparable	Comparable	Not significant ($P > 0.05$)
Time spent in center zone (s)	Higher	Lower	Significant
Frequency of center zone entries	Higher	Lower	Significant
Locomotor function	Normal	Normal	Equivalent
Thigmotaxis level	Moderate	High	Increased in light deprivation group
Exploratory behavior	Present	Reduced	Decreased in light deprivation group
Vigilance level	Normal	Heightened	Increased in light deprivation group

4. Conclusion and Future Perspectives

This study employed the open field test to investigate whether light deprivation induces anxiety like behaviors in mice. The experimental results demonstrated that mice subjected to light deprivation exhibited a marked preference for remaining in the peripheral, darker areas of the open field apparatus and spent very little time in the center, brightly lit zone. In contrast, the control group mice, which were not subjected to light deprivation, spent significantly more time in the bright center zone compared to the light deprived mice. These findings indicate that mice subjected to light deprivation display anxiety like behaviors.

Specifically, the light deprived mice showed significantly reduced time spent in the center zone and a lower frequency of center zone entries relative to the control group. The total distance traveled, however, did not differ between the two groups, confirming that the observed differences in center zone behavior were not attributable to differences in general locomotor function. These results provide clear evidence that light deprivation leads to the development of anxiety like behaviors in mice. The light deprived mice exhibited more pronounced anxiety like behavior than the control group mice, as reflected by their increased thigmotaxis, reduced exploratory activity, and heightened vigilance.

The findings of this study have important implications for understanding the relationship between environmental light conditions and mental health. Insufficient light exposure, which is common in high latitude regions and among individuals working in underground or windowless environments, may represent a significant contributing factor to the development of anxiety disorders. Seasonal affective disorder, which occurs more frequently in regions with limited sunlight, shares features with the anxiety like phenotype observed in light deprived mice. This parallel between human and animal studies strengthens the translational relevance of the current findings.

This study has several limitations that should be acknowledged. First, only male mice were used in the experiment. The inclusion of only one sex limits the generalizability of the findings, as previous research has suggested that light deprivation may produce sexually dimorphic effects on neural excitability and behavior. Second, the sample size of 14 mice, while sufficient for detecting group differences in the primary outcome measures, may not have been adequate for detecting more subtle effects or for conducting subgroup analyses. Third, the light deprivation condition was applied as a continuous treatment, and the study did not systematically vary the duration of light deprivation to examine dose response relationships. Fourth, the study focused exclusively on behavioral outcomes and did not include neurobiological measurements such as hormone levels, neurotransmitter concentrations, or neural activity patterns that could help elucidate the mechanisms underlying the observed behavioral changes.

Future research should address these limitations and extend the current findings in several important directions. First, subsequent studies should expand the sample size to increase statistical power and improve the reliability of effect size estimates. Larger sample sizes would also enable more complex experimental designs and subgroup analyses. Second, future experiments should include female mice to investigate potential sex differences in the effects of light deprivation on anxiety like behavior. Understanding sex differences is critical for developing sex specific prevention and treatment strategies for anxiety disorders. Third, researchers should systematically vary the duration of light deprivation to establish a quantitative relationship between the length of light deprivation and the severity of anxiety like behaviors. Such dose response studies would provide valuable information for determining the threshold of light exposure necessary to maintain normal emotional regulation.

Fourth, future investigations should incorporate detailed neurobiological analyses to elucidate the mechanisms by which light deprivation induces anxiety like behaviors. Potential mechanisms include disruptions to circadian rhythms mediated by the suprachiasmatic nucleus, alterations in

neurotransmitter systems such as serotonin, dopamine, and norepinephrine, changes in neuroendocrine function including cortisol and melatonin secretion, and modifications of neural circuit activity in brain regions involved in fear and anxiety, such as the amygdala, hippocampus, and prefrontal cortex. Experimental manipulations targeting specific components of the brain circuits that constitute the neurobiological basis of psychopathology could be performed to establish causal relationships.

Fifth, longitudinal studies could be designed to track the time course of anxiety like behavior development following light deprivation and to assess whether the effects are reversible upon re exposure to normal light conditions. Such studies would have important implications for the development of light based interventions for anxiety disorders, including bright light therapy, which has already shown efficacy in treating seasonal affective disorder. Sixth, future research could screen for novel pharmaceutical compounds with clinical potential by testing their ability to prevent or reverse the behavioral effects of light deprivation in animal models.

In conclusion, this study provides important animal experimental evidence for understanding the relationship between light conditions and anxiety disorders. The findings demonstrate that light deprivation reliably induces anxiety like behaviors in mice as measured by the open field test, including reduced center zone time, decreased center zone entries, and preserved locomotor activity. These results draw attention to the often overlooked environmental factor of insufficient light exposure and its potential impact on human mental health. By highlighting the link between light deprivation and anxiety like behaviors, this research offers new perspectives for the prevention and treatment of anxiety disorders. Public health initiatives should consider promoting adequate light exposure as a low cost, accessible strategy for supporting mental health, particularly in regions and occupations characterized by limited access to natural light. Clinical interventions for anxiety disorders might incorporate light based therapies as adjunctive treatments, and future research should continue to explore the therapeutic potential of optimizing light exposure patterns.

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