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Behavioural Investigation of Light Deprivation on Anxiety-Like Symptoms in Mice: A Preliminary Study

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Abstract: Anxiety disorders affect over 300 million individuals globally, representing a significant public health and economic burden. Understanding environmental risk factors for anxiety is important for the promotion of prevention and treatment. The current exploratory research investigates the impact of light deprivation on anxiety-like behaviour in mice to create conditions endured by humans in chronically low-light environments such as high-latitude regions or underground working conditions. Mice were randomised into a control or light deprivation (LD) group. The LD group was given reduced daily light, while the control group was exposed to a normal 12-hour light/dark cycle. Both groups were subsequently tested on the Elevated O Maze, an established behavioural measure of anxiety, following conditioning. Measures included latency to open arms entry, total time in open arms, and entries. Results revealed significant differences in behaviour: LD mice exhibited greater latency to enter open arms, lower time spent in open arms, and fewer number of entries—all indicative of greater levels of anxiety. Movement patterns also differed by group, with LD mice showing greater hesitation and linear movement, again in support of the presence of increased anxiety-like behaviour. These findings show that protracted deprivation of light may induce strong anxiety-type behaviours in mice, a convenient model for the study of light-related anxiety disorders in humans. The shortcomings of limited sample size and lack of analysis of molecular or neural pathway abbreviations restrict the value of these findings. Future research must expand sample size and employ neurobiological methods (e.g., c-Fos staining, AAV manipulations) to identify underlying mechanisms. With the comorbidity of anxiety and depression, light treatments may be explored more widely across affective disorders. This study contributes to mounting evidence for environmental influences on mental health and the importance of adequate light exposure to emotional modulation.

1. Introduction

Anxiety is defined as "the brain response to danger, stimuli that an organism will actively attempt to avoid"[1]. This brain function serves an evolutionary purpose as it facilitates organisms across species to avoid danger more efficiently. However, when the symptoms become extreme—frequent, severe, and persistent—so that they affect normal operations of life, it would exacerbate into a

disorder. [1]. Globally, it is estimated that anxiety disorders affect approximately 4.05% of the population (equivalent to 301 million people). This number increased by over 55% between 1990 and 2019, highlighting the pressing need for new clinical treatments [2]. Indeed, in the year 1990 alone, the diseases posed an economic burden of $42.3 billion USD in the United States [3]. Compounding with growing medical expenditures and patient population, the cost is only going to be much more substantial in the future. Hence, it is imperative to understand the mechanisms underlying anxiety to provide a solid foundation for clinical research into these disorders.

Pertinent ethical concerns and the rare nature of data from human subjects necessitate a model organism to study anxiety. A foundation for an animal to be a model organism is that it must mimic the physiological processes being studied; meanwhile, the more similar a species is to humans, the more complicated the animal ethics are, the less understanding researchers have, and the harder it is to sustain such an animal. Amongst potential mammal candidates, mice stand out the most, in that they achieve a balance between efficiency and efficacy: they displace anxiety-like behaviours while staying economical to maintain[4]. Therefore, this essay will use mice as a model organism to study anxiety.

It is well-recognised that light deprivation leads to various psychological abnormalities; it causes depression in mice[5] [6]. For humankind, light deprivation is considered to be a major risk factor for SAD, and light therapy is effective in treating SAD[7]. Currently, the majority of the research focus on the correlation between light deprivation and depression. On the other hand, not only were depressive symptoms relieved, but also some of the anxious symptoms by light exposure therapy. In addition, depressive patients are usually accompanied by severe anxiety-like phenotypes or concur as secondary symptoms of other diseases[8][9][10]. Therefore, it is worth investigating how light deprivation affects not only depression but also anxiety disorders. In addition, mainstream predominantly uses either complete darkness from birth treatment or shortly-extended darkness for LD group, which, however, cannot fully reflect real life situations such as that of coal miners or residents of high latitudes, as they receives adequate light-exposure in during development but only very limited light exposure later in life. Hence, this study adopts a light-deprivation model that strives to simulate conditions of the situations described above, seeking to lay a base for future research targeting light-deprived occupations.

Therefore, this study conditions mice with a control group and an LD group that receives a prolonged darkness treatment. Then, the study tested their level of anxiety in the level by Elevated Maze apparatus. The goal of this paper is to investigate whether a prolonged deprivation of like would induce significant anxiety behaviours in comparison with the control group. The experimental group would show a significant increase in anxiety level in comparison with the control group. This paper hopes to add new evidence to the existing debate on the effect of the light-dark cycle on mental health, fostering treatment and alleviation for related clinical conditions.

2. Materials and Methods

All animals used in the experiment were acquired from the Laboratory Animal Centre of the Institute of Zoology, Chinese Academy of Sciences, and treated according to the Guidelines for Ethical Review and Welfare of Laboratory Animals of the Chinese Academy of Science, Institute of Zoology. Mice (n = 14) were housed under a 12-hour light/dark cycle with food and water available ad libitum. Temperature was maintained between 20° and 26°, and humidity was maintained between 40% and 70%. The mice were 8-10 weeks old when the conditioning started. The mice were randomly and independently assigned to the LD group and the control group. In the LD group, the 8 hours of light were removed in the 12 hours of lighted periods; in the control group, 0 hours of light were removed in the 12 hours of the lighted periods.

Elevated O Maze test is used to determine the level of anxiety between the control (n=7) and LD group (n=7). The mice were allocated to the test room 1 hour before the experiment to familiarize them with the experimental environment. An elevated O Maze was used to test the level of anxiety of mice (Fig. 1). Mice were gently placed on the apparatus where the tail half of their body is on the open arm while the head half is on the closed arm. A Canon Camera was placed above to record the movement of the mice for 6 minutes. The mice were placed into a separate cage after the recording was finished. 75% Ethanol solution is sprayed on the Maze, which is then wiped to remove any odour and residues.

The quantifiable indices being measured are the amount of time a mouse stays in the open arm, the frequency of entering the open arm, and the latency of entering the open arm. Vision XT is used for tracking the movements of mice. Prism 9 is used for statistical analysis.

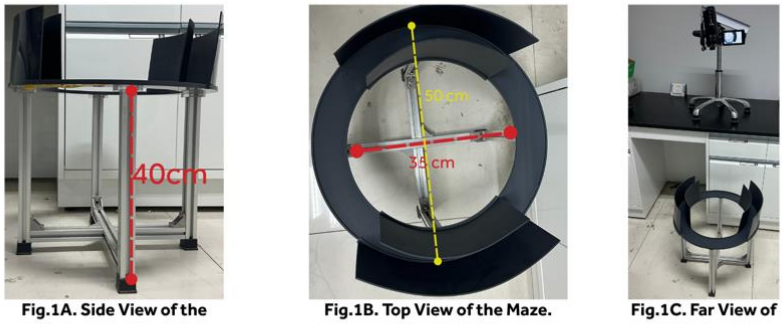

Fig.1A. Side View of the Maze. The elevation abovethe ground is 40cm

Fig.1B. Top View of the Maze. The inner diameter is 35 cm and the outer diameter is 50 cm

Fig.1C. Far View of the Maze.

Fig. 1 The experimental setup for the Elevated O-Maze test.

A: Side View of the Maze. The elevation above the ground is 40cm; B: Top View of the Maze. The inner diameter is 35 cm and the outer diameter is 50 cm; C: Far View of the Maze.

Statistical analyses and data visualization were conducted using GraphPad Prism 9 (GraphPad Software, Inc., USA). Results are expressed as mean ± s.e.m. throughout the manuscript. The Shapiro- Wilk normality test was used for conformity with Gaussian- distributed residuals before t tests and descriptive statistics. We conducted statistical comparisons between two groups using paired or unpaired Student's t tests. One-way ANOVA and Bonferroni post hoc analyses were used for analyses of multiple experimental groups. Nonparametric tests were conducted when data are not normally distributed, including Wilcoxon matched- pairs signed- rank test, Mann-Whitney test, Data are shown as individual values or expressed as the means ± SEMs, and the significance levels are indicated as *P < 0.05, **P < 0.01, ***P < 0.001, and not significant (n.s.). All the statistical tests, significance analyses, number of individual experiments, and other relevant information for data comparison are specified in table S1.

3. Results

The two groups were found to be normal. Unpaired t-tests were conducted between the control group and LD group on latency entering open arms, time in open arms and the Number of times entering open arms. The control and LD groups show significant differences in all three measures (Fig. 2.).

Compared to the control group, the Light Deprivation (LD) group took significantly longer to enter

the open arms (i.e., had a higher latency), spent less time within them, and entered them less frequently. Since mice have a natural aversion to open, elevated spaces, this avoidance is a well-established measure of anxiety. Therefore, these results strongly indicate that the LD group exhibited more significant anxiety-like behaviors than the control group.

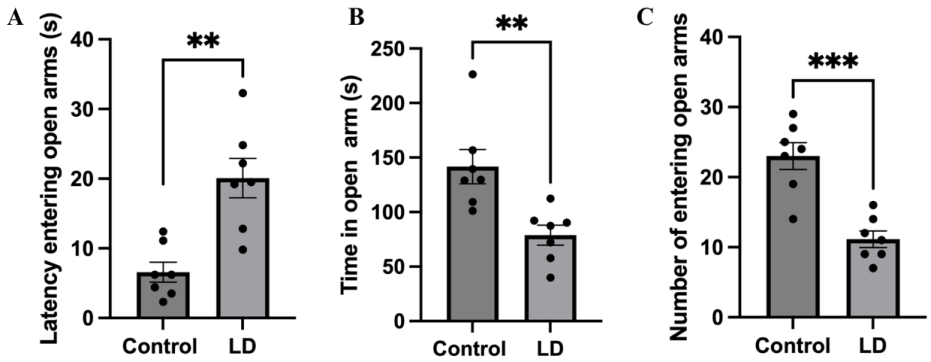

Fig. 2. Graphs showing Measures in the Elevated Maze Test.

A: A Bar Graph Comparing the Latency of Entering Open Arms. LD group takes significantly longer to enter the open arms. B: A Bar Graph Comparing Time in Open Arms. Control groups stay in the open arm significantly longer than the LD group. C: A Bar Graph Comparing the Number of Entering Open Arms. Control groups enter the open arm significantly more frequently than the LD group. Error Bar indicates a one SEM.** represents p <O.01.** represents p<0.001.

The movement patterns of the mice also differed between the control and LD groups. Mice in the control group tended to explore the closed arms more thoroughly and made multiple stops in the open arms (Fig. 3A). In contrast, mice in the LD group tended to move quickly and linearly through the open arms, often hesitating at the junctions between the open and closed sections (Fig. 3B).

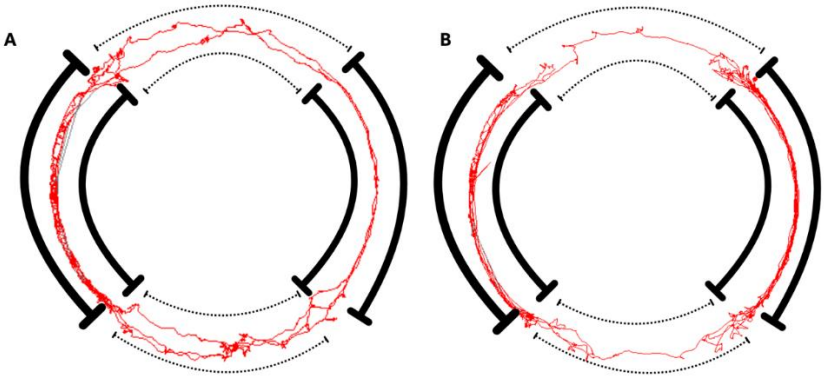

Fig. 3. Graphs Showing Movement of Mice on Open Arms.

A: Graph Showing Movement of Mice in Control Group. B Graph Showing Movement of Mice in LD Group. Red lines represent the movement of Mice. Curved black bars indicate the location of walls in the close arm section Thus, the movement patterns furthermore confirm the LD group's reluctance to enter the open arm, reflecting their anxiety.

4. Conclusions and Perspective

The results confirm the hypothesis: Light Deprivation leads to anxiety behaviour. This showcases the value in doing further research in the following direction. Indeed, anxiety from the LD group is comparable to symptoms in people facing similar light-deprived environments.

There are major limitations to this study. First, the sample size (n=7) is barely satisfactory for the results to be recognised as conclusive. Due to practical reasons such as lack of funding, conflicting schedules and a shortage of comprehensive institutional resources, the study is confined to the behavioural levels. Finally, the study only used the Elevated O Maze to test the level of anxiety, which requires cross-examination from other anxiety-testing methods such as the light-dark box and open field test.

Given the constraints, future studies should expand the scale in the same direction. First, a larger sample size should be adopted to achieve a result with higher credibility and significance. In addition, future studies should also investigate the topic on a cellular and molecular level. More advanced instruments and procedures, such as injection, AAV, and c-fos staining, should be used to look into not only the appearance of anxiety-like behaviour, but also the mechanism behind it. In addition to anxiety behaviour, other related fields are worthy of research. Light Therapy has proven to be useful for both anxiety and depression, which suggests a common pathology behind both disorders. Therefore, future researchers should tackle the disorders holistically by comparing and contrasting the neurocircuitry of both anxiety and depression disorders. Aside from focusing on the cerebrums alone, future studies should also investigate the role of ganglion, bipolar and photoreceptor cells on anxiety, as they not only convert light signals into electro-physiological neuro signals but also start preliminary processing of such signals. Investigating the retinocortical pathway would also foster the development of more effective light therapy by improving the current light-exposure model. For example, selected wavelengths or combinations of multiple wavelengths might alleviate the symptoms better. Finally, mice provide only a rough model for comprehending anxiety in humans, as their brain is far less complicated and convoluted as that of humans. Also, the underdeveloped associative areas hinder researchers from using the mouse model to reflect cognitive processes in human subjects.

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