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Forty-Hertz light oscillations did not change the natural fear behavior of the 5xfAD mice with looming visual stimuli

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Abstract. In recent studiesresearchers have found that gamma oscillations (40Hz) significantly reduced in some neurological diseases. In this article, we will investigate whether the looming stimulation experiment to mice can be used as a way to test the efficacy of 40Hz light stimulation. The results showed that the fearful characteristics displayed by the treated 5xFAD mice, including the response of returning to the nest, freezing, etc. were changed compared with the control group, but did not reach a significant difference. 40Hz light oscillation did not improve the natural fear behavior of 5xFAD mice, and it may be that this physical therapy mode has limited effect on the basic physiological response of AD mice. The effects of intensity and frequency of therapeutic stimulation on the fear cognition of 5xFAD mice will be tested in subsequent experiments.

Keywords: Gamma oscillations, 5xFAD mice, Natural fear behavior, Looming visual stimuli

1. Introduction

gamma oscillations are the synchronization with a frequency of 20-50 Hz of neural oscillations [1-3]. Abnormal gamma oscillations have been observed in cognitive disorders such as Alzheimer's disease [4]. Stimulated by external gamma (40 Hz) frequencies, AD model mice exhibited decreased levels of amyloid -β (Aβ)1–40 and Aβ 1–42 isoforms and the transformation of microglia [5]. In these studies, 5xFAD mice were used to detect the role of 40Hz photic stimulation in the treatment of Alzheimer's disease. In addition, in other studies, researchers have used the Barnes maze to test the effects of this therapy [6].

The defensive behavior shown by mice in response to looming stimuli is largely dependent on visual perception and it is an innate, non-learning, activity. So, in the looming stimulation experiment, we can observe the innate fear of mice [7]. Besides that, some studies have shown that there are some changes in the fear behavior of elderly people with Alzheimer. In this study, we will use visual looming stimulation experiment as a way to detect the behavioral differences between 5xFAD mice (control group) and 40Hz treated 5xFAD mice (experimental group) to explore whether this experiment about innate fear behavior can be used as a detection method to Alzheimer's disease.

2. Animals and Methods

For the consistency with previous studies, 5XFAD mice were also used in this experiment. Mice were divided into experimental group,which had eight mice, and control groups, which had nine mice. For the experimental group, imitating the previous experiment, we placed three-month-old 5XFAD mice in

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a dark box and exposed to light at a frequency of 40 Hz for 1 hours once a day for two weeks [5]. For 5XFAD mice in the control group, we do not do any special treatment.

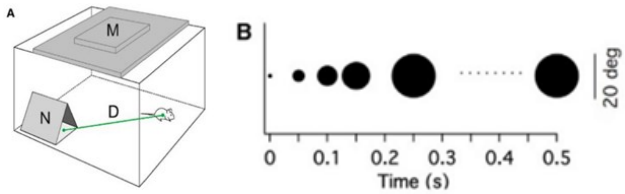

Figure 1. The experimental device and methods are based on Melis Yilmaz's methods about dark expanding disc in the upper visual field triggers flight and freezing [7].

Again, to imitate previous looming experiments (Fig1. A) [7], mice are placed in a box (D) with a display (M) covering the top of the box. A corner of the box provides a place, nest, for mice to hide from stimuli (N). Mice have ten minutes acclimation time in the box before stimulation. Stimulation begins after the acclimation: on a gray background, directly above the mouse, the display begins to appear with a black disk with a 2 degrees diameter visual angle, expanding to a 20 degrees visual angle in 250 milliseconds and maintaining that angle for 250 milliseconds. This stimulus is repeated fifteen times, each 500 milliseconds apart (Fig1. B). Researchers observe the behaviors of mice when stimulated: flight, freeze, etc.

3. Results

Treatment group (as shown in Fig 2.A1 and A2): the number of times of flight but did not enter nest (flight) was 7, accounting for 30.43% in proportion; The number of times of freezing (freezing) is 4, accounting for 17.39% in proportion; The number of flight and enter the nest was 7, accounting for 30.43% in proportion; The number of rearing (rearing) is 1, accounting for 4.34% in proportion; The number of other reactions was 4, accounting for 17.41% in proportion.

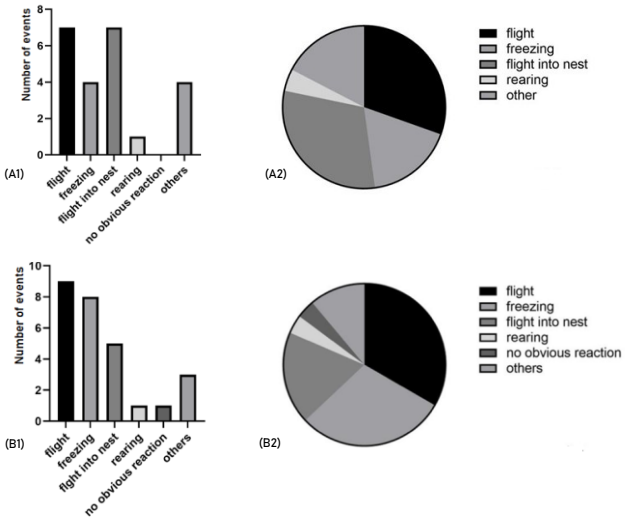

Figure 2. Statistics of Reactions to the Looming Stimulus. A1 and A2 respectively represent the number and proportion of various behavioral responses in the treatment group(N=23). B1and B2 respectively represent the number and proportion of various behavioral responses in the control group(N=27). It is very unusual for mice to appear rearing when they encounter a threat, and it is still recorded here to ensure data integrity. Other reactions (others) are features that mice show their fear (such as wagging its tail) but does not flight or freeze obviously.

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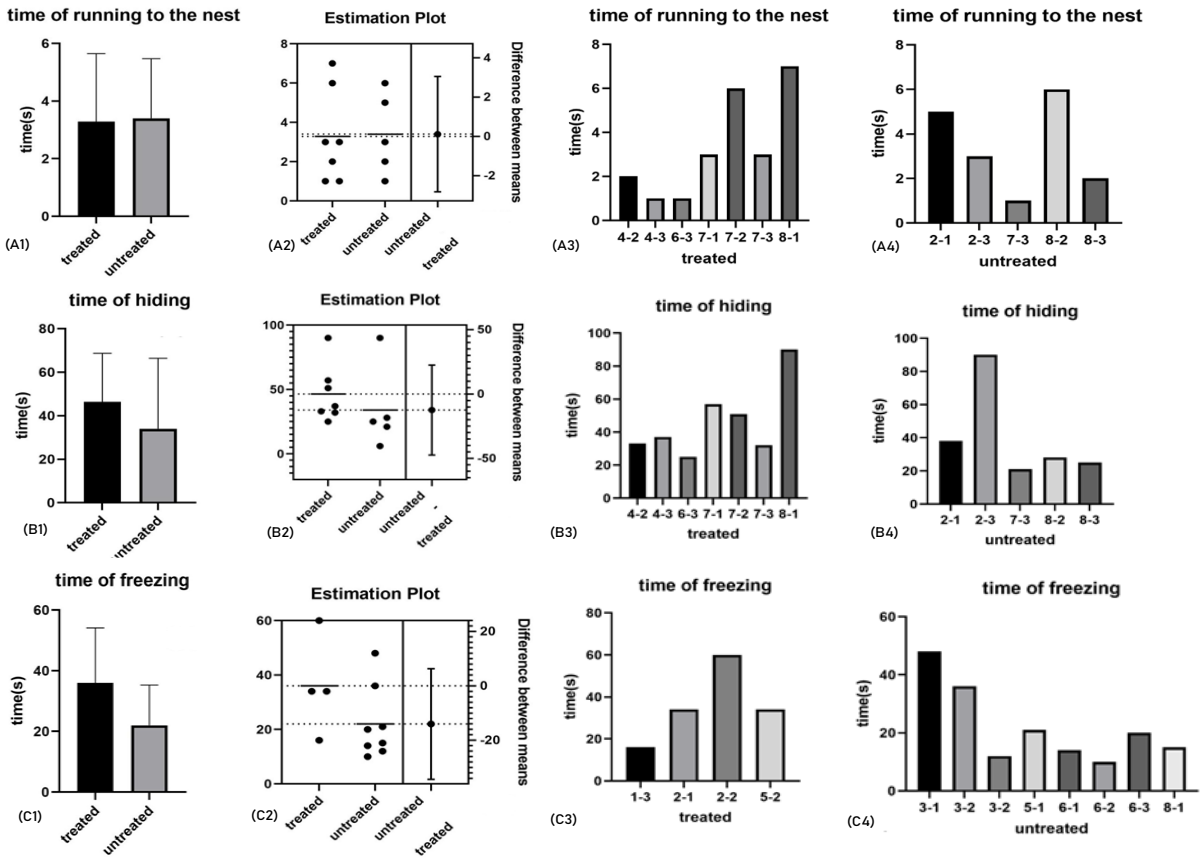

Figure 3. Statistics of the time mice ran into the nest after being stimulated(A1~A4), hiding time (the time the mouse hid in the nest after being stimulated,B1~B4), and time of freezing(C1~C4). A1: Overall comparison of time of running to the nest. A2: Comparison of average time of running to the nest. The average time of the experimental group (treated) was 3.29s, and the average time of the control group (untreated) was 3.40s. A3: The specific running time of the treatment group (treated).A4: The specific running time of the control group (untreated).Note: The coordinates of the horizontal axis of A3 and A4 refer to "mouse number-first stimulus", such as "4-2" refers to the second stimulus of mouse No. 4. According to the obtained data, the independent-samples T test showed the P value of 0.93, that is, there was no significant difference in the time of running to the nest between the treatment group (treated) and the control group (untreated). B1: Overall comparison of hiding duration. B2: Comparison of average hiding time. The average hiding time of the treatment group (treated) was 46.43s, and the average hiding time of the control group (untreated) was 34.00s. B3: The specific length of time the mice in treatment group (treated) hid. B4: The specific length of time the mice in control group (untreated) hid. According to the obtained data, the independent-samples T test showed the P value of 0.45, that is, there was no significant difference in time of hiding between the treatment group (treated) and the control group (untreated). C1: Overall comparison of freezing duration. C2: Comparison of average length of freezing. The average time of freezing was 36.00s in the treatment group and 22.00 s in the control group (untreated). C3: The specific length of freezing in the treatment group (treated). C4: The specific length of freezing in the control group. According to the obtained data, the independent sample T test showed a P value of 0.16, that is, the difference in stiffness between the treatment group (treated) and the control group (untreated) was not significant.

Control group(as shown in Fig 2.B1 and B2): the number of times of flight but did not enter nest (flight) was 9, accounting for 33.33% in proportion; The number of freezing (freezing) is 8, accounting for 29.62% in proportion; The number of times of flight and enter the nest (flight into nest) was 5,

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accounting for 18.51% in proportion; The number of rearing (rearing) is 1, accounting for 3.70% in proportion; The number of no obvious reactions was 1, accounting for 3.70% in proportion; The number of other reactions was 3, accounting for 11.14% in proportion.

In addition to recording the behavior of mice after being stimulated, we also recorded the following indicators(Fig 3): the time mice ran into the nest after being stimulated, hiding time (the time the mouse hid in the nest after being stimulated), and time of freezing. P=0.05 is used as the significance criterion, that is, if $P<0.05$, the data have a significant difference, and vice versa.

4. Discussion

The results of this experiment show that the proportion of 5XFAD mice entering nest increased (18.51% to 30.43%), the average hiding time increased by 12.43s (34.00s to 46.43s), and the average of freezing time by 14s (22.00s to 36.00s). However, we still cannot say that the looming experiment is a suitable method for detecting 40Hz photic stimulation. Whether it was the time of running to the nest ,time of freezing, and time of hiding, there is no significant differences between the treatment group (treated) and the control group (untreated). As for the reason for this result, we cannot be sure whether the reason is that the looming experiment cannot detect Alzheimer's disease, or whether the effect of 40Hz photic stimulation therapy of Alzheimer's disease is not significant, or if there are some other reasons.

In recent years, it has also been pointed out that 40Hz photic stimulation therapy cannot entrain the native gamma oscillations of 5XFAD mice [8]. They observed no reduction of amyloid -β (Aβ)1–40 and Aβ 1–42 and no significant morphological changes in microglia. Therefore, further research on this therapy needs to be carried out.

5. Conclusion

Looming stimulation experiment cannot be used as a valid behavioral test for the efficacy of 40Hz photic stimulation therapy of Alzheimer's disease. We speculate that the possible reason is that the 40Hz photic stimulation treatment cycle is insufficient, thus it still does not achieve a significant therapeutic effect; Or the visual looming stimulation experiment may not able to detect differences in treatment outcomes between the treatment group and the control group. In future follow-up experiments, we will further set more conditions to study whether innate fear behavior can be used as one of the criteria for the detection or diagnosis of Alzheimer's disease. The principle of this test and its pre-feasibility need further study.

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