Title: The 40 Hz White LED Alleviates Psychiatric Symptoms Induced by STZ in Vivo: Evidence from Behavioral and Molecular Studies

Authors: Elham Soleimani[1], Abolhassan Ahmadiani[1,*], , Maryam Bazrgar[1], Fariba Khodagholi[1], Afsaneh Eliassi[2]

1. *Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

***Corresponding Author**: Abolhassan Ahmadiani, Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: aahmadiani@yahoo.com

To appear in: **Basic and Clinical Neuroscience**

Received date: 2024/01/08

Revised date: 2024/01/15

Accepted date: 2024/01/16

Please cite this article as:

Soleimani, E., Ahmadiani, A., Bazrgar, M., Khodagholi, F., Eliassi, A. (In Press). The 40 Hz White LED Alleviates Psychiatric Symptoms Induced by STZ in Vivo: Evidence from Behavioral and Molecular Studies. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2024. Doi: http://dx.doi.org/10.32598/bcn.2024.1856.1

DOI: http://dx.doi.org/10.32598/bcn.2024.1856.1

Abstract

Introduction: The use of a 40 Hz white light emitting diode (WLED) has emerged as an alternative non-pharmacological and non-invasive approach to Alzheimer's disease (AD). Here we used behavioral tasks and measurements of some mitochondrial factors to address the therapeutic effects of 40 Hz WLED on psychiatric symptoms (PS) and the contribution of mitochondrial factors in the early stages of sporadic AD in rats.

Materials and methods: The AD model was induced via intracerebroventricular (ICV) injection of streptozotocin in male wistar rat. After recovering (7 days) from stereotaxic surgery, 40-Hz WLED exposure was performed for 7 consecutive days lasting 15 min/day. Behavioral (elevated plus maze, force swim, and social interaction test), enzymatic, and molecular assays were conducted after 24 h of the last 40-Hz WLED exposure.

Results: Behavioral tasks revealed that 40-Hz WLED exposure in stz-induced toxicity rats, lowered anxiety and depression and increased social interaction. furthermore the 40-Hz WLED therapy in stz-induced toxicity rats, led to increased catalase activity in the amygdala, decreased the activity of monoamine oxidases A and B in the whole brain, and increased mitochondrial DNA in the hippocampus.

Conclusion: The current study explains that 40-Hz WLED therapy in the early stages of sporadic AD improved psychiatric symptoms and biomarkers. also it seems that there is a potential relationship between alterations in mitochondrial markers in certain brain regions and PS.

Keyword: Alzheimer's disease, 40-Hz WLED, Psychiatric symptoms, mtDNA, MAO, Catalase

1 Introduction

Alzheimer's disease (AD), a neurodegenerative disease that progresses with age, accounts for the largest proportion of dementia globally (Chakraborty et al. 2019). Most patients with AD suffer from various behavioral and mood disorders that accelerate the progression of the disease. Studies have recently shown that psychiatric symptoms (PS), such as depression, anxiety, and apathy, occur before the onset of cognitive symptoms in AD patients (Vuic et al. 2022; Zhang et al. 2018). Currently, there are no FDA-approved interventions for PS, and the available pharmacological treatments, such as monoaminoxidase inhibitors (MAOI) prescribed for depression and anxiety, have toxic side effects, including hepatotoxicity, orthostatic hypotension, and hypertensive crisis (Chakraborty et al. 2019; Depression in Older Adults - A Review n.d.; Nandi et al. 2023).

Scientists are investigating nondrug, noninvasive alternatives, such photobiomodulation (PBM) using red light (RL) or near infrared light (NIR), for treating brain diseases because current treatments have limited effectiveness and more side effects (Li et al. 2023). The use of a 40 Hz white light scintillator through the eyes has been shown to attenuate the pathological characteristics of AD (Tian et al. 2023), and the PBM has multiple benefits such as reducing inflammatory factors, improving memory in AD model mice (Yu et al. 2022), colocalizing microglia with Aβ and eliminating senile plaques (Iaccarino et al. 2016; Tao et al. 2021), and reducing superoxide and Aβ-induced inflammation in astrocytes (Lu et al. 2017).

The impact of 40-Hz WLED on PS in AD has not been proven, and the underlying mechanisms are not fully understood despite the hypothesis that it may affect certain structures and biomolecules in mitochondria. As demonstrated by researchers, the use of 40-Hz WLED in the Aβ1-42 toxicity model increased mitochondrial respiratory chain complex I and IV activity, decreased ROS production, mitochondrial membrane potential, and improved the structure-function of potassium channels (Nazari et al. 2022).

Moreover, the mitochondrial dysfunction is acknowledged as an early event in the development of AD (Wang et al. 2020). Excessive production of reactive oxygen species through ICV-STZ in the sporadic AD (sAD) model targets mitochondria, which are the main source of ROS production, resulting in a reduction in mtDNA frequency and contributing to AD pathology and cognitive and non-cognitive behavioral disorders (Ansari Dezfouli et al. 2019; Dhapola et al. 2022; Harerimana et al. 2022; Wan Chik et al. 2023).

Previous studies have acknowledged the significance of Aβ in generating H2O2 as the primary free radical produced in mitochondria. They suggested that it is rapidly catalyzed by CAT and preserve the mitochondrial function in AD (Reddy 2006).

In addition, monoaminoxidases (MAOs), found in the mitochondria's outer membrane, are associated with AD psychiatric symptoms pathophysiology because of their impact on monoamine neurotransmitter levels, as well as their role in oxidative stress through hydrogen peroxide production (Behl et al. 2021; Emilsson et al. 2002). Therefore, it has been proposed that inhibiting MAOs could potentially enhance PS by decreasing oxidative stress and the breakdown of amine neurotransmitters.

Therefore, the objective of the current research is to provide a response to the inquiry posed as follows: Does 40 Hz flashing light improve depression, anxiety, and social interaction at an early stage in STZ-induced AD-like rats? Was the effect of 40 Hz flashing light on

behavioral symptoms due to the regulation of monoaminoxidase A (MAOA), monoaminoxidase B (MAOB), and catalase (CAT) enzyme activity as well as the abundance of mitochondrial DNA in the hippocampus (HIP), amygdala (AMY), and prefrontal cortex (PFC)?

2 Material and Method

2.1 Animal

Male Wistar rats (250±10g) were kept in the laboratory under controlled conditions (12:12-h light/ dark cycle, steady temperature of 23 ±2 ◦C, and free availability to water and food) and all procedures followed ethical guidelines approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.2018.973).

2.2 Induction of STZ Toxicity

Streptozotocin (Sigma-Aldrich) was used to create an Alzheimer-like animal model. according to the paxinos and Watson atlas. Stereotaxic surgery was performed to perform bilateral intraventricular injections (AP = 0.8, ML = 1.5, DV = 3) of STZ (3 mg/kg) or normal saline in a volume of 3 μL and gentle infusion over 5 min, after anesthesia rats with ketamine/xylazine (Alluri et al. 2020; Paxinos G Watson C The Rat Brain- In Stereotaxic Coordinates [Compact 3rd ed].pdf n.d.).

2.3 Experimental Design

The therapy was started one week after the surgery and given once a day for 15 minutes, and continuing for a total of seven days. Following a 24-hour interval since the last session, a group of rats were subjected to behavioral testing, while the other rats had their brain tissue collected for molecular and enzyme studies.

The experimental groups were divided randomly in the following manner.

1. Saline (control): bilateral ICV injection of normal saline .

2. Saline + light (40-Hz WLED).

3. STZ (Alzheimer-like group): bilateral ICV injection of STZ 3 mg/kg.

4. STZ + light (40-Hz WLED).

The rats were exposed to white light with a visible wavelength of 425-550 nm, intensity of 60 W, frequency of 40 Hz, and average irradiance of 12 mW/cm2 in a dark chamber containing LED strip lamps (Iaccarino et al. 2016; Jones et al. 2019; Nazari et al. 2022).

Fig.1. Experimental design**. A)** Experimental groups. **B)** Experimental timeline.

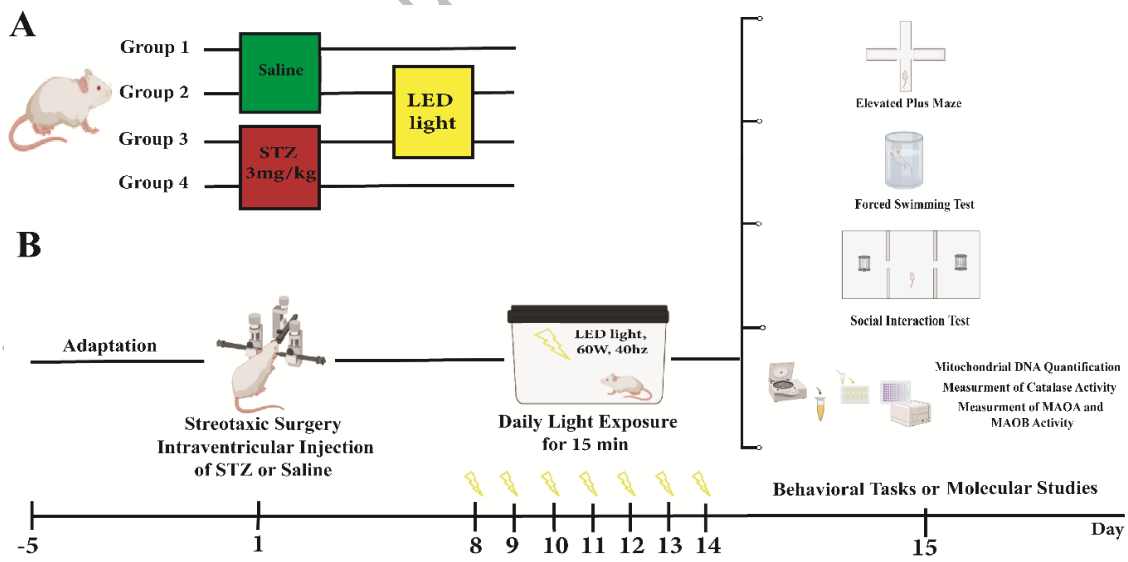
2.4 Elevated Plus Maze Test

The Elevated Plus Maze (EPM) behavioral model, which measures anxiety in rodents based on their searching and avoidance instincts, features four arms in the shape of a positive sign, each measuring 50 × 10 cm, with a high wall surrounding the closed arm and 1 cm high edges around the open arms. The central area, with dimensions of 10 × 10 cm, intersects the arms, and the maze is illuminated by a dim glow (Rodgers and Johnson 1995; Walf and Frye 2007). Animals were individually positioned in the center of the maze, where they faced the open arms and freely moved among the arms for 5 min. A decrease in the percentage entries and/or time spent in open arms is indicative of an anxiolytic index (Beirami et al. 2017).

2.5 Forced Swimming Test

In this test, it is thought that inactivity reflects the state of depression. In this study, the animal was places individually in a plexiglass cylinder containing tab water to a depth of 30 cm, which was kept for 15 min (usually at 25 °C). Twenty-four h later, the subjects were placed in the cylinder and their immobility time was counted in a 5-min test period. A subject is considered motionless when it is passively floating in water in a little bent but vertical status with its head just above the water's surface (Tucci et al. 2022).

2.6 Social interaction test (three chamber paradigm)



In this trial, animals that were unfamiliar with one another were chosen, with two control rats required in each experiment, one for the first stage and the other for the second stage of the trial. A social interaction test was performed for 25 min in an unfamiliar rectangular

space that was divided into three parts by walls. During the habituation process, the rats were allowed to move freely between the rooms for a duration of 5 min. In the first stage (10 min), one of the control rats was placed in one of the chambers to test the animal's social tendencies. The duration of direct contact with stranger rat 1 was recorded. In the second stage (10 min), the level of the animal's desire to create new social relationships was tested, with the parameters stated in the previous step measured and the rat's behavioral differences in interaction with stranger 1 and stranger 2 observed. In this test, sociability, the desire for new connections, and social isolation can be investigated (Davoudi et al. 2023).

2.7 Quantification of mitochondrial DNA

DNA was extracted from the HIP, AMY, and the PFC using the Tissue genomic DNA extraction mini kit (Favorgen, Taiwan) following the provided instructions. The quantification of mtDNA relative amount was performed through the implementation of real-time PCR methodology with SYBR Green detection. the quantification of mtDNA was performed using PCR Master Mix reagents (Ampliqon, Denmark), following specific cycling conditions including activation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, and a total of 40 cycles in the ABi step-one System (USA) (Ansari Dezfouli et al. 2019; Sheng et al. 2012). The primers used for the mitochondrial gene cytochrome c oxidase II and the nuclear gene B actin are documented in Table 1.

Table 1. The primer sequences for COXII and β-actin in real-time PCR for quantifying mtDNA relative to nuclear DNA

Gene name	Abbreviation	Primer direction	Sequence (5´-3´)
Cytochrome c oxidase II	COX II	Forward	ATAGGACACCAATGATACTGAAGC
		Reverse	CATTGGCCATAGAATAGACCTGG
Glyceraldehyde3 phosphate dehydrogenase	β-actin	Forward	GCAGGAGTACGATGAGTCCG
		Reverse	ACGCAGCTCAGTAACAGTCC

2.8 Measurement of the CAT activity

We used TPR's CAT Activity Assay Kit (TEB PAZHOUHAN RAZI (TPR), Iran) to measure CAT activity in tissue.

2.9 Measurement of MAOA and MAOB Activity

To evaluate the activity of MAOA and MAOB enzymes, mitochondria were isolated using the Clark and Nicklas method and then measured by spectrophotometry (Clark and Nicklas 1970). Sodium phosphate buffer (100 mM) and 5-hydroxytryatpamine (4 mM) were

combined in each well to estimate MAOA activity. Subsequently, the enzymatic reaction was initiated by adding solutions of the mitochondrial fraction, and the resulting change in absorbance was monitored at 280 nm using a spectrophotometer (Thermo Scientific/USA) against a blank consisting of sodium phosphate buffer (100 mM) and 5-hydroxytryptamine (4 mM). To estimate MAOB activity, a mixture of sodium phosphate buffer (100 mM) and 0.1 M benzylamine was prepared in each well at a wavelength of 249.5 nm, and a blank containing sodium phosphate buffer and benzylamine was used as a reference. The next step involved adding mitochondrial fraction solutions to start the enzymatic reaction and recording the resulting change in absorbance (Bahaeddin et al. 2022).

2.10 Statistical analysis

The processing and visualization of the data were conducted using Graph Pad Prism 9 software, and the results were presented as the mean ± standard error of mean (SEM). Significant difference was set as p-value <0 .05. The groups were compared using a one-way analysis of variance (ANOVA) and a Tukey post hoc test.

3 Results

3.1 Effect of 40-Hz WLED on STZ-induced anxiety-like responses

One-way ANOVA revealed significant differences in the percentage of time spent on the open arms (OAT%) [$F(3, 28) = 5.403$, $p = 0.0046$] (Fig. 2A) and the percentage of entries into the open arms (OAE %) [$F(3, 28) = 22.33$, $p < 0.001$] (Fig. 2B) during the EPM test. Post-hoc analysis revealed that the levels of OAT% ($p < 0.05$) and OAE% ($P < 0.0001$) in the STZ-injected rats were significantly reduced compared with those in the saline group, suggesting an anxiogenic reaction. In the 40-Hz WLED treatment group (STZ+light), there was a notable rise in OAT% ($P < 0.01$) and OAE% ($P < 0.0001$) compared with the STZ-injected rats, indicating that the 40-Hz WLED reversed the anxiogenic response induced by STZ administration.

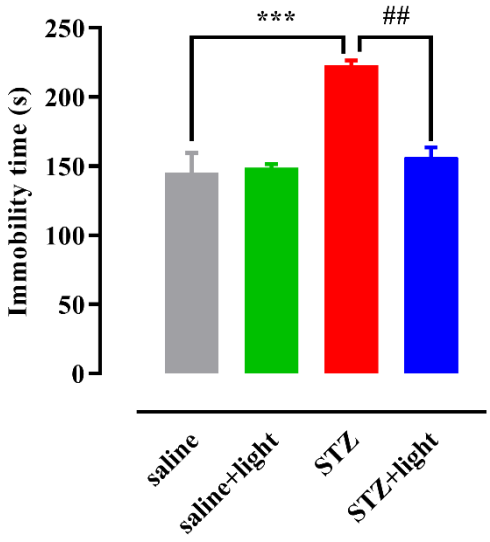


Fig.2. The effects of 40-Hz WLED treatment (15 min/day, for 7 days/ start 8 day after Stereotaxic) on anxiety-like behavior triggered by STZ administration (3 mg/kg) during a 5-min exploration in the EPM. Sample size in each group = 8. *P < 0.05 and ***P < 0.001 vs. saline group, ##P < 0.01 and ###P < 0.001 vs. STZ+light group. (A) The percentage of time spent in open arms (OAT%) and (B) the percentage of entries into open arms (OAE%).

3.2 Effect of 40-Hz WLED on STZ-induced depressive-like responses

Figure 3 indicates the behavior of rats subjected to FST. One-way ANOVA showed that there were significant differences in immobility time between the groups [F (3, 24) = 9.967, p= 0.0002] (Fig. 3). Post-hoc analysis (Tukey test) indicated that STZ induced a depression-like response, as evidenced by a significant increase in immobility time ($p<0.001$) compared with the saline group. The Application of 40-Hz WLED lead to a significant decrease in immobility time ($p<0.01$) and removed the STZ-induced depression in rats.

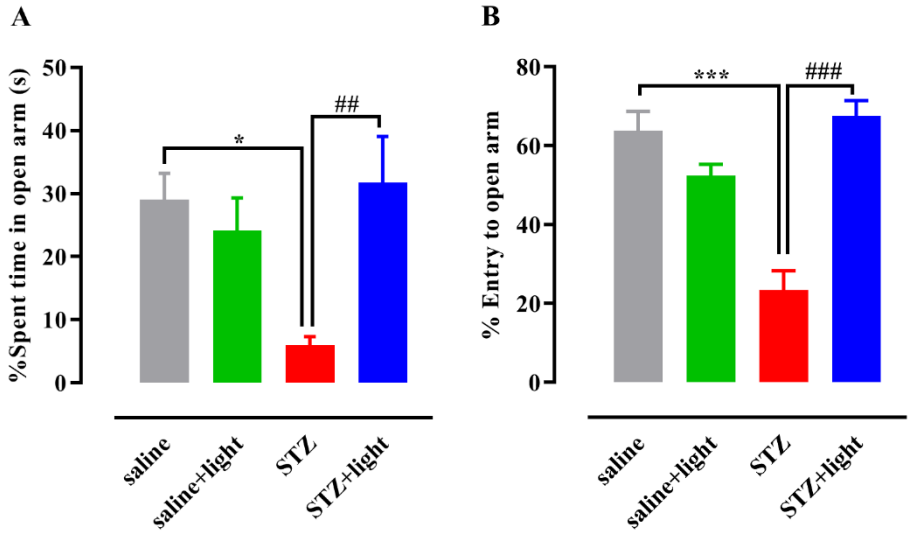


Fig. 3. The Effect of 40-Hz WLED treatment (15 min/day, for 7 days/starting 8 days after stereotaxic) on STZ (3 mg/kg)-induced depressive behavior during a 5-min forced swim test. Sample size = 6-10 in each group. ***P < 0.001 vs. salin group, ##P < 0.01 vs. STZ+light group.

3.3 Effect of 40-Hz WLED on STZ-induced impairment of social interactions

One-way ANOVA analysis in phase 1 of the experiment showed that there was a significant group difference in the time spent with the empty basket and stranger 1 (respectively [F (3, 32) = 25.04, P<0.0001] and [F (3, 32) = 9.261, P=0.0001]). In the control group, the increase in time spent around Stranger 1 and the decrease in time spent around the empty basket, indicate, normal sociability, whereas the opposite behavior in the STZ group suggests a decrease in the desire to make social connections (Fig.4A). On the other hand, we showed that light significantly improved social relationships in the STZ+light group. One-way ANOVA analysis in phase 2 shows a significant group difference in the time spent with stranger 1 [F (3, 32) = 26.68, P<0.0001] and stranger 2 [F (3, 32) = 28.52, P<0.0001]. The STZ group showed less desire to create new relationships and preferred social isolation by spending less time with stranger 2 than the control group. While light therapy was able to have a significant positive effect on social novelty in STZ+light, by increasing the time spent around the stranger 2. It is possible that the results were influenced by the decrease in anxiety that occurred following exposure to light (Fig.4B).

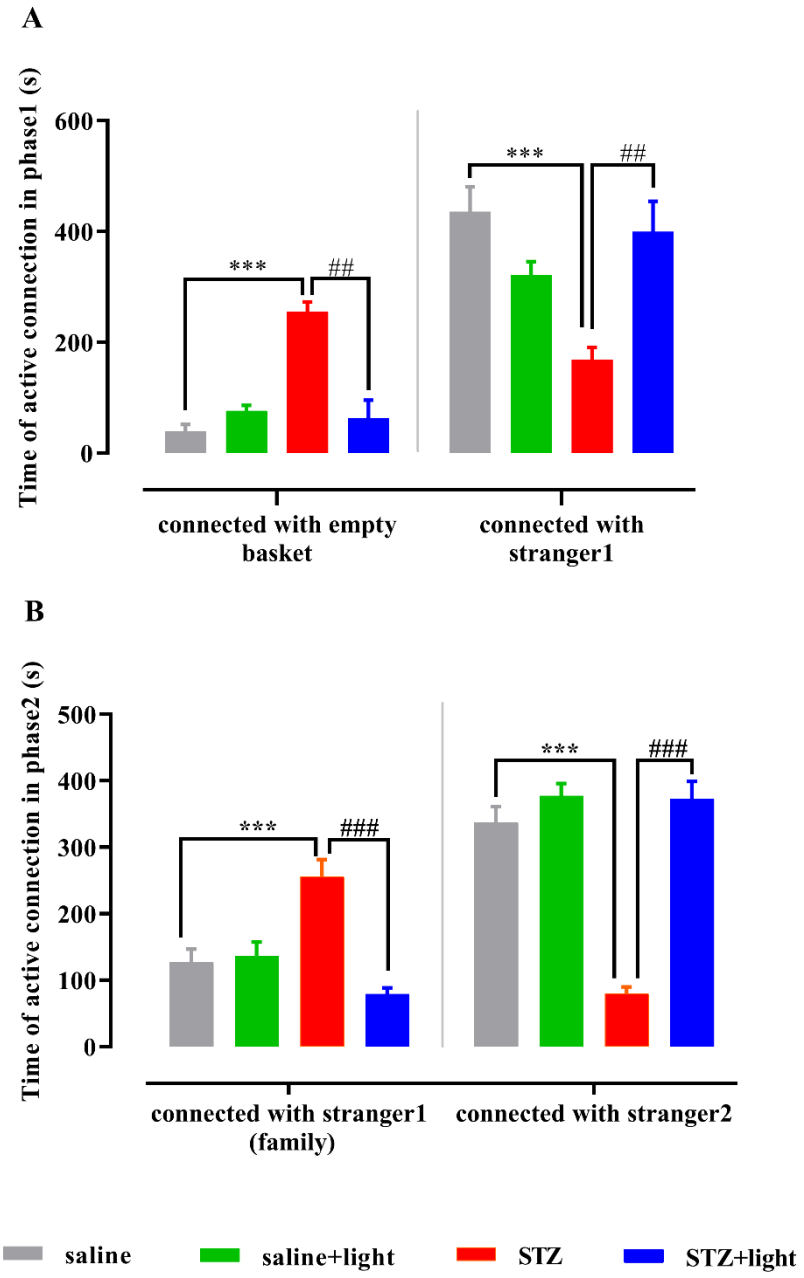


Fig.4. Effect of 40-Hz WLED treatment (15 min/day, for 7 days/starting 8 days after stereotaxic) on STZ (3 mg/kg)-induced social behavior during the three-chamber social interaction test. Sample size = 9. **A)** phase 1: sociability. Determination of difference in time spent around empty basket and stranger 1 between groups using one-way ANOVA. **B)** phase 2: Social novelty. The difference in the time spent around stranger1 and stranger 2 between the groups was determined. (**p < 0.01, ***p < 0.001 vs. saline) & (##p < 0.01, ###p < 0.001 vs. saline+light)

3.4 Effect of 40-Hz WLED on CAT activity in the HIP, AMY, and PFC

A significant difference in CAT activity between different groups was observed in the AMY [F (3, 14) = 60.37, p<0.001] (Fig.5C) and PFC [F (3, 10) = 4.588, P=0.0288] (Fig.5B). The average CAT activity of the STZ group in the AMY and PFC was significantly lower than that of the saline group, (respectively P < 0.0001 and P < 0.05). 40-Hz WLED treatment in the AMY prevented the decrease of CAT activation in STZ+light group compared with the STZ group (p<0.01). However, in the other two brain regions, HIP and PFC, CAT activity did not show any significant difference in light-treated rats respect to STZ group.

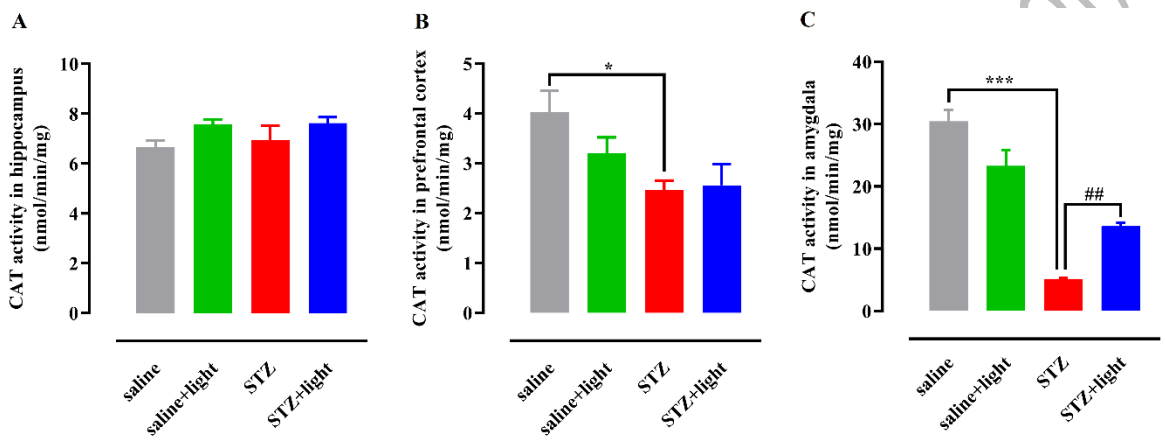


Fig.5. The effect of 40-Hz WLED treatment (15 min per day, for 7 days / starting 8 days after stereotaxic) on the amount of CAT activity in **A)** HIP, **B)** PFC, and **C)** AMY (n =3-5). (***p<0.001 and *p<0.05 vs. saline, ##p<0.01 vs. STZ+light).

3.5 Effect of 40-Hz WLED on the activity of MAOA and MAOB

One-way ANOVA showed that there were significant differences in MAOA and MAOB enzymatic activity between the groups; respectively [F (3, 20) = 8.035, ***P=0.001 and F (3, 8) = 9.857, ** P=0.0046] (Fig. 6). STZ administration resulted in a significant increase in MAOA and MAOB enzymatic activity (p<0.05) compared with the saline group. 40-Hz WLED, significantly reduced MAOA and MAOB (p<0.01) enzymatic activity in STZ-light group compared to STZ-treated rats. The decrease in anxiety and depression in animals through WLED therapy may be due to the decrease in MAOA and MAOB activity, leading to the reduction of monoamine oxidation involved in anxiety and depression.

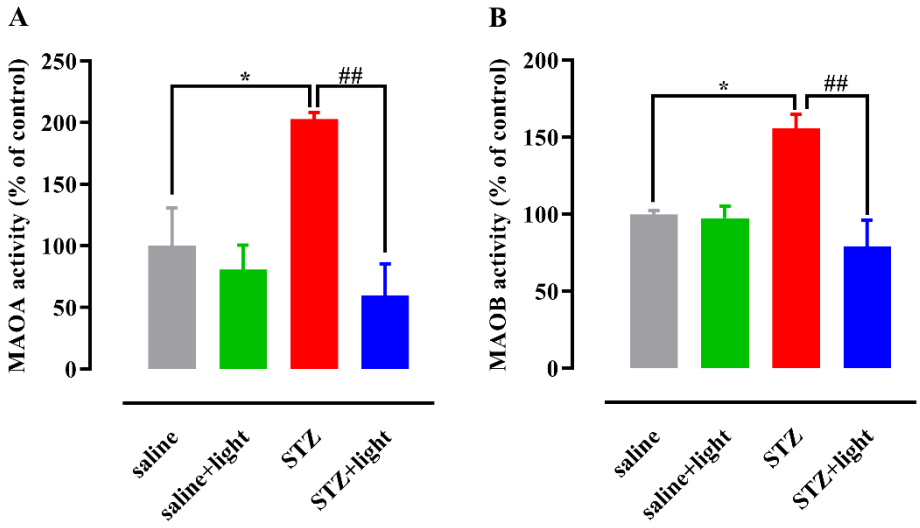


Fig.6. The effect of 40-Hz WLED treatment (15 min per day, for 7 days / starting 8 days after stereotaxic) on MAOA and MAOB activity in the rat brain (n = 3-6). (*p<0.05 vs. saline, ##p<0.01 vs. STZ+light)

3.6 Effect of 40-Hz WLED on mtDNA relative amount in the HIP, AMY, and PFC

To evaluate the mtDNA relative amount in brain tissue, the ratio of mtDNA to total DNA was measured using real-time PCR. A significant difference between the groups was observed in the HIP [F (3, 12) = 6.414, P=0.0077] (Fig.7A), PFC [F (3, 12) = 6.645, P=0.0068], (Fig.7B) and AMY [F (3, 12) = 8.135, P=0.0032] (Fig.7C). STZ injection decreased mtDNA relative amount in all three brain regions compared to saline (control) groups (p<0.01 and p<0.05) (Fig.7A). 40-Hz WLED treatment in HIP prevented the decrease of mtDNA relative amount in STZ+light group compared with STZ group (p<0.01). However, the observed increase in mtDNA relative amount in the PFC and AMY following 40 Hz WLED treatment did not show statistical significance.

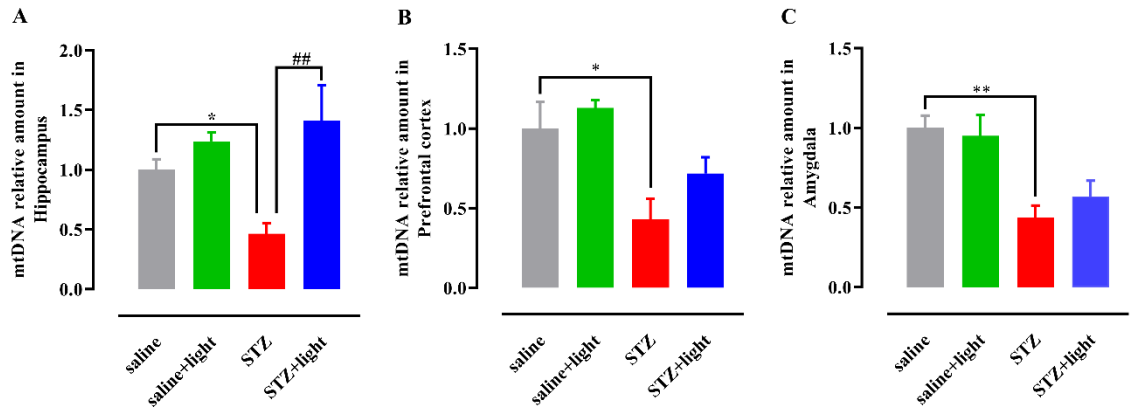


Fig.7. The effect of 40-Hz WLED treatment (15 min per day, for 7 days / starting 8 days after stereotaxic) on the mtDNA relative amount was determined using RT-PCR by the ratio of cytochrome c oxidase II to β-actin (**A**. HIP, **B**. PFC and **C**. AMY tissues from 4 animals in each group were prepared for the real-time PCR. (**p<0.01 and *p<0.05 vs. saline, ##p<0.01 vs STZ+light.

4 Discussion

The increasing prevalence of AD and the adverse effect of PS including anxiety and depression on patients highlights the necessity for more effective and also low-cost treatments, (Degawa et al. 2021; Kenwood, Kalin, and Barbas 2022); Recent research suggests that investigating the involvement of the AMY, HIP, and PFC in anxiety reactions and the potential role of mitochondrial dysfunction in the pathogenesis of AD may lead to improved treatment options for psychiatric disorders in Alzheimer's patients (Ashleigh, Swerdlow, and Beal 2023; Yaojing Chen, Dang, and Zhang 2021; Duval, Javanbakht, and Liberzon 2015).

Our results indicated that: 1) anxiety and depressive-like behaviors and social isolation occur in STZ-induced AD model rats (on day 14), 2) in the STZ toxicity model (on day 14), a decrease in the frequency of mtDNA in the HIP, PFC, and AMY, a decrease in the activity of CAT in the PFC and AMY, and an increase in the activity of MAOA and MAOB enzymes in the whole brain were observed compared to control animals, 3) 40-Hz WLED improves depression and anxiety-like behaviors and social interactions, 4) 40-Hz WLED increases the mtDNA relative amount in the HIP, improves the activity of CAT enzyme in the AMY, and also decreases the activity of MAOA and MAOB.

STZ mimics several pathological and behavioral changes observed in humans with AD, including cognitive dysfunction, depression, anxiety, oxidative stress, and mitochondrial dysfunction. According to independent research studies, the cognitive performance and memory of rats decreased on days 21 (Kumar et al. 2016), 35 (Correia et al. 2012), and 60 (Roy et al. 2022) after receiving a single dose of ICV-STZ (3 mg/kg). Also, Chen et al. (2013) reported an increase in anxiety-like behaviors three weeks after STZ treatment (Yanxing Chen et al. 2013). In this study, behavioral, enzymatic, and molecular tests were

performed in the early stage of AD, 14 days after STZ injection, which distinguishes that from previous research. PS was observed and documented in a 14-day Alzheimer's disease model, indicating a novel finding in the current study. In our study, STZ-injected-rats showed a ~23% and ~40% decrease in the anxiety indices OAT% and OAE% (in EPM), respectively. In addition, the STZ-injected-rats demonstrated a notable rise of ~53% in immobility time, indicating hopelessness and depression (in FST). Moreover, in this study, animals showed reduced sociability in the three-chamber test 14 days after exposure to STZ, which was a novel finding. This study yielded significant results regarding the early onset of PS at 14 days after STZ administration. Hence, manifestations of anxiety and depression and social withdrawal are evident not only in the later phases but also in the initial stages of sAD. Thus, early diagnosis of AD through these symptoms before the onset of proteinopathy can prevent the progression of the disease and minimize its speed.

There is significant evidence of oxidant-antioxidant enzyme imbalance in a sporadic AD model induced by ICV-STZ, which leads to increased impairment in cognitive and non-cognitive behaviors and potentially plays a role in the pathophysiology of depression and anxiety (Akhtar, Dhaliwal, and Sah 2021; Ghaderi et al. 2022; Postu et al. 2022). Neurons are more vulnerable to oxidative damage caused by hydroxyl free radicals because of the low level of CAT (Lee, Cha, and Lee 2020), whereas, mitochondria are more sensitive because of the abundant production of H2O2 and lack of CAT production (Palma et al. 2020). Hence, in the current study, an assessment was conducted on the CAT activity in the AMY, HIP, and PFC, which are regions that contribute to the development of anxiety and depression responses (Duval, Javanbakht, and Liberzon 2015). For example, Hajizadeh et al. demonstrated that a reduction in cerebral cortex antioxidant enzymes, including SOD, CAT, and GRx, and a subsequent increase in anxiety behaviors occurred 21 days after ICV-STZ (3 mg/kg) (Hajizadeh Moghaddam et al. 2020). In addition, a study conducted by Abu-Taweel et al. revealed that depression and anxiety behaviors were observed along with reduced CAT activity in the HIP after 35 days in a sporadic model caused by AlCl3 in mice (Abu-Taweel and Al-Mutary 2021). This study investigated the timing of oxidative disorders to expedite diagnosis of sAD by examining CAT activity 14 days after model induction. It revealed a significant reduction in the AMY and PFC of STZ-injected rats (~83% and ~40% respectively). Further investigation is needed to explore the possible influence of CAT-mediated oxidative stress modulation on the pre-primary phase of AD based on our findings.

Furthermore, It has been suggested that changes in mitochondrial structure and function contribute to the progression of AD and related behavioral changes; For example, MAOA and MAOB potentially play a significant role in the pathophysiology of AD through the oxidation of neurotransmitters associated with mood (Santin et al. 2021). In the present study, 14 days after STZ administration, an elevation of over two times in MAOA activity and a ~56% rise in MAOB was noticed in the brains of STZ-injected rats. Hafez et al. and Abdulghani et al. documented that AD rats had reduced serotonin and increased MAO activity in the HIP and cerebral cortex three months after AlCl3 administration in compared with control rats (Abdelghany et al. 2023; HA et al. 2021). The study's findings reveal that MAOA and MAOB activity changes report earlier than what was observed before, aligning with the emergence of anxiety and depression-like behaviors in the initial phase.

In addition, mtDNA abnormalities and copy number reduction are other mitochondrial factors that may be associated with cognitive and psychological changes in AD. For example, Ansari et al. demonstrated that the injection of 1-42 Aβ into the CA1 region of the HIP results in anxiety-like behavior, impaired working memory, and a decline in mtDNA copy number (21). The present study showed that mtDNA relative amount in the HIP, PFC, and AMY was decreased by ~53%, ~57% and ~56%, respectively, in STZ-injected-rats (on day 14). According to the reviewed literature, due to its close proximity to high concentrations of ROS, inefficient DNA repair mechanisms, and lack of protective histones, mtDNA is more susceptible to oxidative stress (Phillips, Simpkins, and Roby 2014); Damage to mtDNA and loss of mitochondria leads to disturbances in oxidative phosphorylation (OXPHOS), alterations in brain energy metabolism, increased ROS levels, and decreased mtDNA abundance has been directly linked to poor cognitive performance in AD (Ansari Dezfouli et al. 2019; Klein et al. 2021; Shang et al. 2022). Nazari et al. also showed that Aβ1-42 amyloid toxicity reduces the activity of mitochondrial complexes I and IV (OXPHOS members), increases ROS production, and leads to memory deficits (Nazari et al. 2022). In this study, we suggest that decreased mtDNA relative amount, probably through the aforementioned alterations, can result in depressive and anxiety-related behavior, as well as social seclusion in STZ-injected rats after 14 days. Thus, the utilization of mtDNA relative amount as an early biomarker for AD, along with interventions that maintain balanced mtDNA levels, may effectively manage AD progression and associated mood disorders.

In recent years, researchers have introduced PBM as a non-pharmacological and non-invasive approach with less cost and side effects, safer to treat AD (55,56). PBM describes light therapy using different wavelengths of light as a noninvasive neuroprotective strategy (Heinig et al. 2020; Tian et al. 2023). PBM is employed as a novel technique for enhancing brain function by stimulating neural activity through increased activity of complexes in the mitochondrial electron transport chain (Pope and Denton 2020; Salehpour et al. 2018). The use of LEDs has been recommended over lasers in recent research because lasers have a narrow beam width and are not well-suited for treating large areas, in addition to generating excessive heat on the tissue. (M.R. 2016; Nazari et al. 2022; Rojas and Gonzalez-Lima 2013). In 2018, Singer et al introduced 40-Hz flickering white light exposure as a protocol to non-invasively reduce Aβ levels by changing the morphology of microglia to a phagocytic state, leading to improved memory in transgenic Alzheimer's rats (Singer et al. 2018). In a study by Park et al. in 2020, the 40-Hz flickering light treatment group showed a reduction in tau and Aβ phosphorylation in the HIP and improved spatial learning, memory, long-term memory, mitochondrial function, and neural plasticity (Park et al. 2020).

In this study, it was shown for the first time that exposure of the whole body to 40-Hz WLED for 15 min a day for 7 days leads to improvement of anxiety-like and depression-like behaviors and social interactions in STZ-induced AD. Therefore, it can be claimed that treatment with 40-Hz WLED improves not only the cognitive performance of the animal but also PS in the Alzheimer's rat model. nevertheless, the mechanism of 40-Hz WLED effects on mitochondrial activity in PS is still unclear. Recent studies have shown that 630-nm RL can activate CAT by photoelectric coupling to the tyrosine residue, leading to H2O2 degradation (Park et al. 2020). However, more research is needed to clearly justify the increase in CAT activity after the application of 40-Hz WLED. In addition, no convincing

evidence has been discovered to explain the mechanism by which light reduces the activity of monoamine oxidase A and B enzymes.

In this study, the COX II gene, one of the subunits of complex IV, was evaluated as an indicator for measuring mtDNA relative amount by RT-PCR (Ansari Dezfouli et al. 2019). Our findings indicate that the decrease in mtDNA relative amount caused by STZ was more than two-fold by 40-Hz WLED irradiation in the HIP. However, there were no significant increases observed in other experimental regions. The PBM effects are related to the capacity of brain tissue chromophores to absorb photon energy, depending on the wavelength, and the resulting effect on cellular metabolism and brain physiology (Cardoso, Gonzalez-Lima, and Gomes da Silva 2021). Nazari et al. showed that exposure of Aβ-injected rats to 40 Hz WLEDs with a wavelength of ~425-550 nm significantly increased complex I and complex IV activity and reduced ΔΨm and ROS production (Nazari et al. 2022), as well as other Studies have shown that mitochondrial cytochrome c oxidase (COX; complex IV) as a chromophore absorbs light at about 420-450 nm (Covian et al. 2023), suggesting that the beneficial effects of the 40 Hz WLEDs in this study may be attributed to these factors. However, many questions about the effect of 40 Hz WLED on mitochondrial activity remain unanswered, and more experimental researches are needed to make a conclusive statement about the underlying mechanisem behind the beneficial effect of 40 Hz WLED in AD models.

Conclusion

This study provides the first documentation of the effect of light therapy (40-Hz WLED) on PS related to AD, and the possible effect of 40 Hz WLED on some mitochondrial factors involved in it. Light improved anxiety and depression, and social interaction in the early stages of sAD by increasing CAT activity and decreasing MAOA and MAOB activity, increasing mtDNA relative amount. In the future, 40-Hz WLED may serve as a noninvasive therapeutic option for mitigating early-stage Alzheimer's PS.

5 Acknowledgements

This work was a part of the Ph.D. thesis of Elham Soleimani at the Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References

Abdelghany, Asmaa K. et al. 2023. "Evaluating the Neuroprotective Effect of Spirulina Platensis–Loaded Niosomes against Alzheimer's Disease Induced in Rats." *Drug Delivery and Translational Research*.

Abu-Taweel, Gasem Mohammad, and Mohsen Ghaleb Al-Mutary. 2021. "Pomegranate Juice Moderates Anxiety- and Depression-like Behaviors in AlCl3-Treated Male Mice." *Journal of Trace Elements in Medicine and Biology* 68.

Akhtar, Ansab, Jatinder Dhaliwal, and Sangeeta Pilkhwal Sah. 2021. "7,8-Dihydroxyflavone Improves Cognitive Functions in ICV-STZ Rat Model of Sporadic Alzheimer's Disease by Reversing Oxidative Stress, Mitochondrial Dysfunction, and Insulin Resistance." *Psychopharmacology* 238(7): 1991–2009.

Alluri, Ramesh et al. 2020. "Phosphoinositide 3-Kinase Inhibitor AS605240 Ameliorates Streptozotocin-Induced Alzheimer's Disease like Sporadic Dementia in Experimental Rats." *EXCLI Journal* 19: 71–85.

Ansari Dezfouli, Mitra, Maryam Zahmatkesh, Maryam Farahmandfar, and Fariba Khodagholi. 2019. "Melatonin Protective Effect against Amyloid β-Induced Neurotoxicity Mediated by Mitochondrial Biogenesis; Involvement of Hippocampal Sirtuin-1 Signaling Pathway." *Physiology and Behavior* 204: 65–75.

Ashleigh, Theophania, Russell H. Swerdlow, and M. Flint Beal. 2023. "The Role of Mitochondrial Dysfunction in Alzheimer's Disease Pathogenesis." *Alzheimer's and Dementia* 19(1): 333–42.

Bahaeddin, Zahra et al. 2022. "Almond Intake during Pregnancy in Rats Improved the Cognitive Performance of Adult Male Offspring." *Nutritional Neuroscience*.

Behl, Tapan et al. 2021. "Role of Monoamine Oxidase Activity in Alzheimer's Disease: An Insight into the Therapeutic Potential of Inhibitors." *Molecules* 26(12).

Beirami, Elmira et al. 2017. "Intranasal Insulin Treatment Alleviates Methamphetamine Induced Anxiety-like Behavior and Neuroinflammation." *Neuroscience Letters* 660: 122–29.

Blivet, Guillaume, Aroa Relano-Gines, Mélanie Wachtel, and Jacques Touchon. 2022. "A Randomized, Double-Blind, and Sham-Controlled Trial of an Innovative Brain-Gut Photobiomodulation Therapy: Safety and Patient Compliance." *Journal of Alzheimer's Disease* 90(2): 811–22.

Cardoso, Fabrízio dos Santos, F. Gonzalez-Lima, and Sérgio Gomes da Silva. 2021. "Photobiomodulation for the Aging Brain." *Ageing Research Reviews* 70.

Chakraborty, Saikat et al. 2019. "Serotonergic System, Cognition, and BPSD in Alzheimer's Disease." *Neuroscience Letters* 704: 36–44.

Chen, Yanxing et al. 2013. "A Non-Transgenic Mouse Model (Icv-STZ Mouse) of Alzheimer's Disease: Similarities to and Differences from the Transgenic Model (3xTg-AD Mouse)." *Molecular neurobiology* 47(2): 711–25.

Chen, Yaojing, Mingxi Dang, and Zhanjun Zhang. 2021. "Brain Mechanisms Underlying

Neuropsychiatric Symptoms in Alzheimer's Disease: A Systematic Review of Symptom-General and –Specific Lesion Patterns." *Molecular Neurodegeneration* 16(1).

Clark, J. B., and W. J. Nicklas. 1970. "The Metabolism of Rat Brain Mitochondria. Preparation and Characterization." *Journal of Biological Chemistry* 245(18): 4724–31.

Correia, S C et al. 2012. "Mitochondrial Abnormalities in a Streptozotocin-Induced Rat Model of Sporadic Alzheimer's Disease." *Curr Alzheimer Res*. http://www.ncbi.nlm.nih.gov/pubmed/23061885.

Covian, Raul, Lanelle O. Edwards, Bertrand M. Lucotte, and Robert S. Balaban. 2023. "Spectroscopic Identification of the Catalytic Intermediates of Cytochrome c Oxidase in Respiring Heart Mitochondria." *Biochimica et Biophysica Acta - Bioenergetics* 1864(2).

Davoudi, Shima et al. 2023. "Chronic Inhibition of Astrocytic Aquaporin-4 Induces Autistic-like Behavior in Control Rat Offspring Similar to Maternal Exposure to Valproic Acid." *Physiology and Behavior* 269.

Degawa, Tomohide et al. 2021. "T-Type Ca2+ Channel Enhancer SAK3 Administration Improves the BPSD-like Behaviors in AppNL−G-F/NL−G-F Knock-in Mice." *Journal of Pharmacological Sciences* 146(1): 1–9.

"Depression in Older Adults - A Review."

Dhapola, Rishika et al. 2022. "Recent Advances in Molecular Pathways and Therapeutic Implications Targeting Mitochondrial Dysfunction for Alzheimer's Disease." *Molecular Neurobiology* 59(1): 535–55.

Duval, Elizabeth R., Arash Javanbakht, and Israel Liberzon. 2015. "Neural Circuits in Anxiety and Stress Disorders: A Focused Review." *Therapeutics and Clinical Risk Management* 11: 115–26.

Emilsson, Lina et al. 2002. "Increased Monoamine Oxidase Messenger RNA Expression Levels in Frontal Cortex of Alzheimer's Disease Patients." *Neuroscience Letters* 326(1): 56–60.

Ghaderi, Shahab et al. 2022. "P-Coumaric Acid Ameliorates Cognitive and Non-Cognitive Disturbances in a Rat Model of Alzheimer's Disease: The Role of Oxidative Stress and Inflammation." *International Immunopharmacology* 112.

HA, Hafez et al. 2021. "Ameliorative Effects of Astaxanthin on Brain Tissues of Alzheimer's Disease-like Model: Cross Talk between Neuronal-Specific MicroRNA-124 and Related Pathways." *Molecular and cellular biochemistry* 476(5): 2233–49.

Hajizadeh Moghaddam, Akbar, Hananeh Ahmadnia, Sedigheh Khanjani Jelodar, and Mojtaba Ranjbar. 2020. "Hesperetin Nanoparticles Attenuate Anxiogenic-like Behavior and Cerebral Oxidative Stress through the Upregulation of Antioxidant Enzyme Expression in Experimental Dementia of Alzheimer's Type." *Neurological Research* 42(6): 477–86.

Harerimana, Nadia V. et al. 2022. "The Role of Mitochondrial Genome Abundance in Alzheimer's Disease." *Alzheimer's and Dementia*.

Heinig, Nora et al. 2020. "Photobiomodulation Mediates Neuroprotection against Blue Light Induced Retinal Photoreceptor Degeneration." *International Journal of Molecular Sciences* 21(7).

Iaccarino, Hannah F. et al. 2016. "Gamma Frequency Entrainment Attenuates Amyloid Load and Modifies Microglia." *Nature* 540(7632): 230–35.

Jones, Marggie et al. 2019. "Gamma Band Light Stimulation in Human Case Studies: Groundwork for Potential Alzheimer's Disease Treatment." *Journal of Alzheimer's Disease* 70(1): 171–85.

Kenwood, Margaux M., Ned H. Kalin, and Helen Barbas. 2022. "The Prefrontal Cortex, Pathological Anxiety, and Anxiety Disorders." *Neuropsychopharmacology* 47(1): 260–75.

Klein, Hans Ulrich et al. 2021. "Characterization of Mitochondrial DNA Quantity and Quality in the Human Aged and Alzheimer's Disease Brain." *Molecular Neurodegeneration* 16(1).

Kumar, Anil et al. 2016. "Possible Role of P-Glycoprotein in the Neuroprotective Mechanism of Berberine in Intracerebroventricular Streptozotocin-Induced Cognitive Dysfunction." *Psychopharmacology* 233(1): 137–52.

Lee, Kyung Hee, Myeounghoon Cha, and Bae Hwan Lee. 2020. "Neuroprotective Effect of Antioxidants in the Brain." *International Journal of Molecular Sciences* 21(19): 1–29.

Li, Xianqian et al. 2023. "Non-Drug Therapies for Alzheimer's Disease: A Review." *Neurology and Therapy* 12(1): 39–72.

Lu, Yujiao et al. 2017. "Low-Level Laser Therapy for Beta Amyloid Toxicity in Rat Hippocampus." *Neurobiology of Aging* 49: 165–82.

Luo, Guangxin et al. 2023. "Effectiveness of Non-Pharmacological Therapies on Cognitive Function in Patients with Dementia—A Network Meta-Analysis of Randomized Controlled Trials." *Frontiers in Aging Neuroscience* 15.

M.R., Hamblin. 2016. "Shining Light on the Head: Photobiomodulation for Brain Disorders." *BBA Clinical* 6: 113–24. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L612598299%0Ahttp://dx.doi.org/10.1016/j.bbacli.2016.09.002.

Nandi, Nilay Kumar et al. 2023. "Design, Synthesis, Pharmacological and in Silico Screening of Disubstituted-Piperazine Derivatives as Selective and Reversible MAO-A Inhibitors for Treatment of Depression." *Journal of Molecular Structure* 1276.

Nazari, Maryam et al. 2022. "The 40-Hz White Light-Emitting Diode (LED) Improves the Structure–Function of the Brain Mitochondrial KATP Channel and Respiratory Chain Activities in Amyloid Beta Toxicity." *Molecular Neurobiology* 59(4): 2424–40.

Palma, Flavio R. et al. 2020. "Mitochondrial Superoxide Dismutase: What the Established, the Intriguing, and the Novel Reveal about a Key Cellular Redox Switch." *Antioxidants and Redox Signaling* 32(10): 701–14.

Park, Sang Seo et al. 2020. "Physical Exercise during Exposure to 40-Hz Light Flicker Improves Cognitive Functions in the 3xTg Mouse Model of Alzheimer's Disease." *Alzheimer's Research and Therapy* 12(1).

"Paxinos G Watson C The Rat Brain- In Stereotaxic Coordinates [Compact 3rd Ed].Pdf."

Phillips, Nicole R., James W. Simpkins, and Rhonda K. Roby. 2014. "Mitochondrial DNA

Deletions in Alzheimer's Brains: A Review." *Alzheimer's and Dementia* 10(3): 393–400.

Pope, Nathaniel J., and Michael L. Denton. 2020. "Low Irradiance Light Exposure Alters the Activity of Key Enzymes in the Mitochondrial Electron Transport Chain." : 13.

Postu, Paula Alexandra et al. 2022. "Pinus Halepensis Essential Oil Ameliorates Aβ1-42-Induced Brain Injury by Diminishing Anxiety, Oxidative Stress, and Neuroinflammation in Rats." *Biomedicines* 10(9).

Reddy, P. Hemachandra. 2006. "Amyloid Precursor Protein-Mediated Free Radicals and Oxidative Damage: Implications for the Development and Progression of Alzheimer's Disease." *Journal of Neurochemistry* 96(1): 1–13.

Rodgers, R. J., and N. J.T. Johnson. 1995. "Factor Analysis of Spatiotemporal and Ethological Measures in the Murine Elevated Plus-Maze Test of Anxiety." *Pharmacology, Biochemistry and Behavior* 52(2): 297–303.

Rojas, Julio C., and F. Gonzalez-Lima. 2013. "Neurological and Psychological Applications of Transcranial Lasers and LEDs." *Biochemical Pharmacology* 86(4): 447–57.

Roy, Avishek et al. 2022. "Cognitive Dysfunction and Anxiety Resulting from Synaptic Downscaling, Hippocampal Atrophy, and Ventricular Enlargement with Intracerebroventricular Streptozotocin Injection in Male Wistar Rats." *Neurotoxicity Research* 40(6): 2179–2202.

Salehpour, Farzad et al. 2018. "Brain Photobiomodulation Therapy: A Narrative Review." *Molecular Neurobiology* 55(8): 6601–36.

Santin, Yohan, Jessica Resta, Angelo Parini, and Jeanne Mialet-Perez. 2021. "Monoamine Oxidases in Age-Associated Diseases: New Perspectives for Old Enzymes." *Ageing Research Reviews* 66.

Shang, Dehao et al. 2022. "MtDNA Maintenance and Alterations in the Pathogenesis of Neurodegenerative Diseases." *Current Neuropharmacology* 21(3): 578–98.

Sheng, Baiyang et al. 2012. "Impaired Mitochondrial Biogenesis Contributes to Mitochondrial Dysfunction in Alzheimer's Disease." *Journal of Neurochemistry* 120(3): 419–29.

Singer, Annabelle C. et al. 2018. "Noninvasive 40-Hz Light Flicker to Recruit Microglia and Reduce Amyloid Beta Load." *Nature Protocols* 13(8): 1850–68.

Tao, Lechan et al. 2021. "Microglia Modulation with 1070-Nm Light Attenuates Aβ Burden and Cognitive Impairment in Alzheimer's Disease Mouse Model." *Light: Science and Applications* 10(1).

Tian, Zixi et al. 2023. "Photobiomodulation for Alzheimer's Disease: Photoelectric Coupling Effect on Attenuating Aβ Neurotoxicity." *Lasers in Medical Science* 38(1).

Tucci, Paolo et al. 2022. "Glucoraphanin Triggers Rapid Antidepressant Responses in a Rat Model of Beta Amyloid-Induced Depressive-like Behaviour." *Pharmaceuticals* 15(9).

Vuic, Barbara et al. 2022. "Tailoring the Therapeutic Interventions for Behavioral and Psychological Symptoms of Dementia." *Expert Review of Neurotherapeutics* 22(8): 707–20.

Walf, Alicia A., and Cheryl A. Frye. 2007. "The Use of the Elevated plus Maze as an Assay of Anxiety-Related Behavior in Rodents." *Nature Protocols* 2(2): 322–28.

Wan Chik, Mazzura, Nur Adiilah Ramli, Nurul Aqmar Mohamad Nor Hazalin, and Gurmeet Kaur Surindar Singh. 2023. "Streptozotocin Mechanisms and Its Role in Rodent Models for Alzheimer's Disease." *Toxin Reviews* 42(1): 491–502.

Wang, Wenzhang et al. 2020. "Mitochondria Dysfunction in the Pathogenesis of Alzheimer's Disease: Recent Advances." *Molecular Neurodegeneration* 15(1).

Yu, Yutian et al. 2022. "Transauricular Vagal Nerve Stimulation at 40 Hz Inhibits Hippocampal P2X7R/NLRP3/Caspase-1 Signaling and Improves Spatial Learning and Memory in 6-Month-Old APP/PS1 Mice." *Neuromodulation*.

Zhang, Qin et al. 2018. "Citalopram Restores Short-Term Memory Deficit and Non-Cognitive Behaviors in APP/PS1 Mice While Halting the Advance of Alzheimer's Disease-like Pathology." *Neuropharmacology* 131: 475–86.