Enhanced cognitive effects in snails (*Physa acuta*) after exposure to meditational music and low-level near-infrared laser

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Abstract

Light and sound are form of vibrations that are essential elements for life and knowingly and unknowingly support several functions in living forms. Meditation techniques use sound and light for treating several illnesses and disorders but its mechanisms are yet to be explored. The basis of this study was to determine whether sound vibrations at a known meditative frequency and light at a known therapeutic wavelength can influence cognitive abilities of lower invertebrates. *Physa acuta*, an air breathing freshwater snail was considered for this study and their cognitive abilities were established as time taken to complete the T-maze pre- and post-exposure to a recommended meditational Buddhist hymn and low level near-infrared laser for a period of 5 days. A significant change in cognitive abilities was observed in snails exposed to low level near–infrared laser at a wavelength of 650 ± 10 nm and exposed to meditational music at a decibel output range of 75 – 80 db and frequency range of 260 – 280 Hz, in comparison to the control group. The results of this study indicate that vibrations generated by meditative music at 75-80 db and frequency of 260 – 280 Hz have a possibility of enhancing cognitive abilities in snails. Significance of exposure to low level near infrared laser in comparison to control group confirms the enhanced cognitive ability of the brain demonstrated by the elevated maze performances by snails. Based on results of this study, we can confirm that low level near infrared light and sound vibrations at 260 – 280 Hz induce a significant effect on the neural system at a cellular level, which is evident with the enhanced cognitive abilities; the mechanisms for which need to be evaluated further.

Keywords: Low-level near-infrared laser, music, cognition, snail, meditation, vibration

1. Introduction

Cognition is a mental process involved with acquisition, processing retention and use of information, which engages the numerous electrophysiological, neurochemical, neuropsychological and biochemical processes of the neurons in the brain [1]. Meditation through light and sound has been linked to increased brain activity in the left prefrontal cortex, which is associated with concentration; planning, meta-cognition and positive affect [2]. EEG recordings of skilled Buddhist meditators have shown a significant rise in gamma wave activity in the 80 – 120 Hz range during meditation [3]. Vibration based meditative techniques are used by Qigong masters to enhance or reduce the rate of biochemical reactions involved in plant growth [4].
In a study conducted by Aggio and group, a significant increase in growth rate and reduced biomass yields was observed in *Saccharomyces cerevisiae* cells growing in the presence of music, high frequency and low frequency sound waves and in cells grown in silence, and therefore music based vibrations may play a significant role in cognitive enhancement for all living organisms.

Long wavelength light is known to stimulate energy metabolism and energy production through photoacceptor molecules present in tissues [6]. Mitochondrial respiration via photoneuromodulation of cytochrome oxidase activity in the brain is a known therapeutic target for neuroprotection and cognitive enhancement [7]. Low level light therapy is known to show a biphasic dose response which is highly dependent on the depth of tissue infiltration by near-infrared light and is proportional to the absorption and scattering coefficients [8]. Behavioural studies in invertebrates such as molluscs are usually specific to food and mating and therefore there may be a good probability that their cognitive abilities would be dependent on their behaviours [9, 10]. Molluscs have been useful models in basic neuroscience research [11] but studies have been very limited in the field of neurobehavioral science and cognition.

T-maze based studies, has helped understand the reasons for cognitive abilities and cognitive dysfunctioning in animals [12] and therefore was chosen as a model in this study, to assess the cognitive abilities of the snails. T-maze is a variant form of Y-maze that is widely used to evaluate cognitive and motor capabilities in animals and has helped understand numerous cognitive based behaviours that subsist in animals [13]. The present T-maze study was designed to assess the exposure of vibrations of meditative music and low level near-infrared laser on the neural system and confirm whether it can enhance cognitive abilities and behaviours in snails. The results of this study would help determine the importance of vibrations in the form of light and sound in the life of these animals.

### 2. Material and Methods

#### 2.1 Study Species and Maintenance

Adult *Physa acuta* snails, size 1 – 1.3 cm belonging to family Physidae were collected from a pond in the Sanjay Gandhi National Park, Mumbai, India (19.2500° N, 72.9167° E) and were acclimatized for a period of 1 week in dechlorinated water at room temperature. During the acclimatization period, snails were placed in well ventilated and hydrated PVC plastic boxes filled with dechlorinated water and were maintained on a 12:12 light: dark schedule (7.00 am: 7.00 pm) at room temperature (30 - 33°C). The snails were fed daily on lettuce leaves *ad libitum* which were procured from the local market and was thoroughly washed and cleaned before providing as feed. The PVC plastic housing was washed and cleaned and the water was also changed on a daily basis.

After 1 week of acclimatization, 30 snails were divided into 3 groups of 10 each for Study 1. Based on the results from Study 1, 20 snails were randomly divided into 3 groups of 6 snails each and were labelled as control group, music experimental group and light experimental group. The groups were placed in well ventilated and hydrated PVC plastic boxes with dechlorinated water and were fed daily on lettuce leaves *ad libitum*. Cleaning of the PVC plastic boxes and water change was done on a daily basis. Snails were acclimatized in this setup for a period of 1 week prior to Study 2 and were maintained on a 12:12 light: dark schedule (7.00 am: 7.00 pm) at room temperature (30 - 33°C).

The T-maze runs were carried out during the day, from 6.00 – 12.00 pm and during the study the T-maze was kept well hydrated with dechlorinated water and well ventilated. The studies were carried out at room temperature (30 - 33°C). Post run, the snails were placed in their PVC plastic boxes and were fed fresh lettuce *ad libitum*. After completion of all the T-maze based studies, the snails were released at the collection area in the Sanjay Gandhi National Park, Mumbai, India (19.2500° N, 72.9167° E).

#### 2.2 Equipment

##### 2.2.1 T-Maze

A self-designed, enclosed, well ventilated and hydrated PVC plastic T-maze, with start and goal arms measuring 4 cm x 4 cm x 3 cm was used to conduct the experiments. The dimensions of all the arms in the T-maze were kept similar for all three groups (Figure 1). The T-maze was designed based on some of the T-maze designs used to study rat behaviour [14].
2.2.2 Music Source
Snails were subjected to a Tibetan meditational hymn from Tibetan Incantations (Nascent) “Om Mani Padme Hum” in an Mp3 format with a bit rate of 128 kbps. Frequency analysis of this soundtrack was done using the WavePad NCH software Version 6.18 which uses a FFT analytical tool. The highest frequency recorded was 21371 Hz (21096 Hz + 274.8 Hz) with a range varying from 236 Hz – 21371 Hz and a decibel gain range of – 23 db to -130 db. The nearest sound note recorded for this hymn was E (21096.2 Hz). The Mp3 soundtrack was played on an I-ball Tarang 2.1 music system with one sub-woofer (20 watts RMS max) and two satellite speakers (10 watts RMS max each) and a total output of 40 watts RMS max with the frequency ranges for - woofer as 20Hz-200Hz and satellites as 100Hz-20kHz. The decibel output range for this meditational hymn was 75 – 80 db and frequency range was 260 – 280 Hz which was recorded and averaged by means of an Android based Spectral Audio Analyzer Application from RandonSoft. This meditational hymn is known to generate positive energies within the body through mystical vibrations that are generated while chanting and therefore was chosen for this study [15].

2.2.3 Laser Source
The source for the low-level near-infrared laser light was a classic pen-sized laser pointer with a maximum output power of < 5 mW and a wavelength of 630 ± 10 nm. Lower wavelength lasers of approximately 630 nm have shown therapeutic effects and therefore this wavelength was chosen for the study [16]. The light source for this laser as described by the manufacturer was a 5630smd led, with a luminous flux of 270 - 300. The pen-sized laser pointer was manufactured by Guangzhou Nantian Sources Co., Ltd, China and was procured online from eBay, India. This pen-sized laser was classified under Class IIIa category of lasers and therefore sun glasses were worn as a mode of protection for the eyes during the study.

2.3 Procedure
2.3.1 Study 1
The study was conducted on 50 snails that were acclimatized in a group, for a period of 1 week. The snails were starved for a period of 12 hrs before the experiment. 10 snails were randomly selected and placed in 5 groups, and the run was conducted for each of the groups. During each run, the group of 10 snails were placed at the starting arm of the T-maze with fresh lettuce placed in the right-hand side goal arm, as a reward. For each run, the start time was recorded on placing the 10 snails in the starting point and the end time was recorded when the snails would reach their reward. During the run, behaviour for each of the snails were observed and documented. Similar pattern was followed for all the 5 groups and time taken for each snail to reach the food source was documented. For the snails that completed the run in ≤ 30 minutes, the time was noted and they were placed as a group in a PVC plastic box filled with fresh lettuce. 18 snails from this set were randomly chosen and were divided into control group, music experimental group and light experimental groups with 6 snails each, for the next set
of experiments. The three groups of snails were acclimatized for a period of 1 week prior to study 2. Snails that did not complete the test within 30 minutes, were stored as a group in well-ventilated PVC plastic box with lettuce ad libitum and were later released at the collection area.

2.3.2 Study 2
18 snails that were randomly selected, acclimatized and grouped as control, music experimental and light experimental were used in this study. 6 snails from the music experimental group were exposed to a meditative hymn for a period of 5 days with all sound based factors (db and Hz) kept constant. During exposure time, the PVC plastic box housing the snails was placed in an enclosed chamber which housed the two speakers and woofer. The snails were exposed once a day for a period of 15 mins for the next 5 days. 6 snails from the light experimental group were individually exposed to low-level near infrared laser light for a period of 5 days. During exposure time, the laser light was held exactly 1 cm above the brain region of the snail with a continuous exposure time of 2 minutes. The snails were exposed once a day for 5 days and the maze runs were conducted on Day 6 wherein the snails of all three groups were starved for a period of 12 hrs prior to the run.

A minimum of 5 runs was carried out for each group and were done in such a manner that each group would have sufficient amount of rest time before the next run. Exhaustion in the snails was managed by keeping a gap of approximately 30 mins before the next run for each group. The maze runs for control and experimental groups were conducted as per Study 2. Start time and end time for each snail in each group and their behaviour observed was recorded. Food was only provided once the group of snails completed their 5th run. During the study, the behaviour observed for each of the snails and the run time data obtained was recorded.

2.4 Data Analysis
ANOVA Two factor without replication and Student T-test were the statistical tests used to determine the significance and variation of the data obtained during the study. Significance was determined and confirmed using the F, F critical and P values with the significance level maintained at p ≤ 0.05 and F > F critical.

3. Results
3.1. Study 1
3.1.1 Run Time Analysis
Of the 50 snails that participated in the run, 20 snails successfully completed the run within 30 mins which was considered as the threshold time. A total of 18 snails were chosen for the next set of experiments and were divided into three groups of 6 snails each. Based on the results of this study it is evident that the cognitive capacity or intelligence levels of the brain differs between snails within the same species and same population. Since this experiment was based on a single run, it was difficult to confirm memory-retention/formation and learning behaviour of the snails which could have been possible if multiple runs were conducted.

3.1.2 Behaviour Observed
The snails that did not complete the run, demonstrated behavioural patterns, which comprised of circular movements in the maze and isolation in specific corners of the maze. The snails that took > 30 mins to reach the food source spent most of their time exploring the maze and were unsure about their moves. The snail which completed the run within the threshold time of 30 mins travelled in one specific direction, without changing their paths. Some snails which were slower but completed the task within the threshold time, explored the maze before advancing towards the food source. Most of the snails used the lid of the maze to explore and travel towards the food source rather than utilizing the surface. Some snails were also seen interacting with each other during the run and moving in parallel towards the food source.

3.2 Study 2
3.2.1 Run Time Analysis
No significant difference was observed between the run time for the snails in the control (M = 28.85, SEM ± 2.318) and music experimental group (M = 21.489, SEM ± 2.422) (ANOVA; F value = 2.981, F critical = 6.607, P = 0.144) (Figure 2). A significant difference in run time was observed between control (M = 28.85, SEM ± 2.318) and light experimental group (M = 14.562, SEM ± 1.299) (ANOVA; F value = 25.585, F critical = 6.607, P = 0.00391) (T = 5.715, P = 0.000194) and between music (M = 21.489, SEM ± 2.422) and light experimental groups (M = 13.105, SEM ± 0.638) (ANOVA; F = 15.59, F critical = 6.607, P = 0.01087) (T = 2.7082, P = 0.022) (Figure 2). Overall significance was observed between the three groups (ANOVA; F value = 10.616, F critical = 4.102, P = 0.00336). A difference in time taken to complete the run was observed between snails in each group, but was considered as a confirmatory result to the results observed in Study 1 which showed a remarkable difference between the intelligence and cognitive abilities for each of the snails.
Average run time of snails were significantly reduced in light experimental group when compared to run time of control and music experimental groups (p<0.05) confirming the enhanced cognitive abilities in the snails exposed to low level near-infrared laser. Asterisk indicates a significant difference with a value p<0.05.

3.2.2 Behaviour Observed
No behavioural changes were observed within and between the groups. Some snails used the lid to navigate towards the food well rather than using the surface. The time lost during the runs was due to the exploratory behaviour observed in some of the snails. The snails from all groups showed a similar pattern of movement in each of the runs, with some of them moving first into the left arm and then moving towards the food source in the right arm or rapidly moving towards the food source in the right arm. A similar pattern of movement in every run conducted was observed in the snails that travelled directly from the start arm to the food source in the right arm.

4. Discussion
Meditation and meditative music is known to alter the functional and structural plasticity of the neural network in the brain [17]. Light and sound that form the basis of meditation present around us are known to affect the brain-wave states and patterns and therefore can enhance cognitive abilities linked to the neural network system which may be a possibility even in lower organisms such as invertebrates. This study is an attempt to understanding the effect of low level near-infrared laser and sound vibrations of a meditative hymn on the neural system of snails, wherein its direct effects on the cognitive abilities of learning and memory have been evaluated using a T-maze.

Learning and memory are cognitive abilities supported by the neural network system and studies in invertebrates though limited have demonstrated these forms of cognitive response [18]. Learning and formation of long-term memory has been demonstrated in pond snail Lymnaea stagnalis, which showed a significant variability within the natural occurring populations of these snails [19] and the mechanisms that manage these cognitive related differences in snails are highly conserved and rarely differ from their original type [20]. Snails are known food foragers [21] and since food foraging is a cognitive based behaviour [22] it was considered as a form of attraction to study cognitive abilities in snail, Physa acuta. A self-designed T-maze was used to study the cognitive ability of the snails and to understand the various cognitive patterns of learning and memory-formation/ retention demonstrated by these snails.

Intelligence is a mental ability for reasoning, problem solving and learning and is known to highly differ within human populations [23]. Results from Study 1 confirm that there exists a significant differentiation in cognitive abilities in snails within the same population. A clear difference in intelligence levels was also observed between the snails within the same population, suggesting a highly conserved mix of behavioural patterns which the snails exploit to perform their day to day activities, especially while foraging for food. Molluscan brain which manages
learning and memory-formation consists of photosensitive neurons in the central ganglion which utilizes light to trigger several electrophysiological transmissions [24]. Light is known to support several physiological and cognitive functions in snails [25]. Snails do not have ears to hear but can sense vibrations through their tentacles below their eyes and therefore this study is a first time attempt of understanding the effects of vibrations created during music on the neural system.

In Study 2, a significant enhancement in cognitive abilities of the snails exposed to low level near infrared laser light was observed based on the significant reduction in run time (Figure 2). Laser exposure at 600 – 1150 nm shows better tissue penetration because of light scattering at low wavelengths and absorption of higher wavelengths by water present in the tissue [26, 27]. These findings correlate well with the findings of this paper, wherein repeated exposures of low level near-infrared light at 650 ± 10 nm showed a significant improvement in learning and memory-retention/formation of the snails. The improvements in cognitive abilities post exposure to low level near-infrared lasers are solely related to mitochondrial respiration which has been used as a therapeutic target for neuroprotection and cognitive enhancement in humans [26].

A mild significant enhanced effect of meditative music on the cognitive ability of the snails was also observed based on the reduction in run time but further evaluation is required in terms of dose selection, music type, decibel selection, frequency selection, etc. Vibrations created through music are known to have several neural-based therapeutic effects in humans [28]. Levitin and his group have found that music improves the body's immune system function and reduces stress. Several researchers are exploring whether sound vibrations absorbed through the body can help ease the symptoms of Parkinson’s disease, fibromyalgia and depression [29]. In cell cultures, music can alter cellular morpho-functional parameters, such as cell size and granularity in cultured cells and can directly interfere with hormone binding to their targets, suggesting that music or audible sounds could modulate physiological and pathophysiological processes [30]. A significant increase in growth rate and reduced biomass yields was observed in Saccharomyces cerevisiae cells growing in the presence of music, high frequency and low frequency sound waves and cells grown in the silence [31]. Based on these studies, it is evident that vibrations created at 260 - 280 Hz through meditative music can affect organisms without hearing abilities as these therapeutic effects may occur at cellular level.

Enhanced cognitive abilities observed in the snails exposed to the Buddhist meditative hymn may be due to a direct effect of vibrations created at a decibel output range of 75 – 80 db and frequency range of 260 – 280 Hz on the neural cells, which may have led to an enhanced effect in the biochemical processes involved in cognition. Further evaluation is required to understand how such meditative hymns can affect cell metabolism and their pathways which may be involved in cognition. Vibrations in the environment perceived at cellular level may be used by snails in various regulatory processes and may also regulate the cognitive abilities of the brain. Based on the results of this study, light in the near-infrared zone and sound at specific frequencies and decibel output plays an important role in enhancing cognition and behaviour of the snails. This study is a first time report of vibration induced enhanced cognitive effects observed in snail, Physa acuta. All around us vibrations exist in the form of sound and light as a mixture of wavelengths and through a selective sorting mechanism within neural cells, supports and triggers the neurophysiological processes in the brain of these snails. Understanding these photo- and phonic- based mechanisms would definitely open up a new area of research in the field of invertebrate cognition.

5. Ethics statement
Ethical approval is not required for research work with Physa acuta; however every effort was made to restore suffering of animals, ensuring adequate food, clean oxygenated water and sufficient ventilation. The stress treatments used in the study have no long-term effects on the animals beyond the brief exposure periods and therefore the animals were released back into the wild post experiments. No specific permits were required for the described field collections. The Sanjay Gandhi National Park collection site is accessed via a public highway and is not situated on private or protected land. The collection of P. acuta for this study did not involve endangered or protected species.

6. References
3. Chang K, Lo P. Meditation EEG interpretation based on novel fuzzy-merging
27. Lapchak PA. Transcranial near-infrared laser therapy applied to promote clinical recovery.


