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Music enhances cognitive-related behaviour in snails (*Achatina fulica*)

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Abstract
Music is a collection of sound waves which exist at varying frequencies, some of which may appear melodious while some appear unbearable to our ears and then there are frequencies which our ears cannot perceive e.g. Ultrasound. Most living forms bear the sense of hearing; the ability to perceive sound within specific ranges of frequencies, but organisms without non-auditory features perceive these frequencies in the form of vibrations or via the phenomenon of resonance. In humans, music therapy is a proven relaxation technique that helps manage stress and acts as a form of secondary treatment for several neural based disorders. The basis of this study was to understand whether music or rather sound vibrations, can enhance brain related activities mainly cognitive behaviour in snails, as these invertebrates do not possess auditory features, thus hypothesizing the interaction to be at a cellular level. Cognition is an important attribute of every living organism which although varies considerably from species to species, helps facilitate their day to day activities. Cognitive abilities of snails in this study were monitored, as time taken to complete the T-maze run post exposure to two different soundtracks for 15 minutes for a period of 5 days. A highly significant reduction in run time was observed in snails exposed to music signifying enhanced cognitive effects in comparison to control group. The significance was higher in the snails exposed to meditative music with lesser variation in frequencies, as compared to a rock soundtrack with varying frequencies. A linear and gradual short-term memory learning profile was observed in snails exposed to meditative music with lesser variation in frequencies as compared to snails exposed to rock music which showed a large variation in frequencies. The results of the studies confirmed that sound generated vibrations augments cognitive capacity of the brain and at reduced variation in frequencies can enhance learning ability with an increase in short-term memory gain. Resonating vibrations in the form of waves can definitely bring a change in behaviour of organisms without non-auditory features as observed in this study, which may induce biochemical changes at a cellular level, the mechanism for which needs to be evaluated.

Keywords: Resonance, Vibrations, Music, Cognition, Snail, Behaviour

Introduction
Music articulates our life, evoking emotions from joyous to sadness and regulating moods. Music can be stimulating but depends on structural features such as tempo, pitch, frequency patterns, etc. which can be broadly categorized as pleasant or unpleasant by the listener (Brandt *et al* 2012) [3]. Music has been associated with NAc activation and ventral tegmental area (VTA) regions of the brain which regulate autonomic, emotional and cognitive functions. Dopaminergic neurons that originate in the VTA region directed to the NAc and forebrain regions associated with rewarding stimuli have been activated with vibrations generated through music (Chanda and Levitin 2013) [6]. The potential therapeutic effects of music listening reduces stress and modulates arousal and has also shown reduced anxiety in humans (Thoma *et al* 2013) [29]. The emotional effects observed after exposure to music is due to the mechanosensory hair cells present in the ear that transmit the sound generated mechanical vibrations via neural impulses to the brain (Bryant *et al* 2002) [4].
Music has always been linked to meditation or meditative religious or non-religious techniques which may involve singing in a choir or chanting. Brain regions associated with attention and sensory processing were found to be thicker in persons who would meditate daily in comparison to persons who would not, and the thickness of these areas increased with increasing years of mediation practice (Lazar *et al* 2005) [17]. Meditation is a process that self-regulates the body and mind and maybe associated with psychological and neurophysiological alterations. Meditation studies have been linked to an increased activity in the prefrontal cortex of the brain which is associated with several cognitive based functions (Previc 2006) [24].

EEG recordings of skilled Buddhist monks with years of training have shown a significant rise in gamma wave activity in the 80 – 120 Hz range while this effect was lower in new meditators. For these Buddhist monks, the purpose of meditation is to free oneself from suffering and gain spiritual liberation which is the same reason for meditative practice in other religions (Davidson and Lutz 2008) [10].

Resonant vibrations generated through music are known to affect mood and emotions, which has been mainly focussed on the brain and its cells and not on cellular metabolism, which may be the case in organisms with non-auditory apparatus. Cultured human breast cancer cell line MCF7 showed an alteration in cellular morpho-functional parameters such as cell size and cell granularity when exposed to music generated resonant vibrations conforming to the direct interference of these vibrations with hormonal binding processes that could modulate physiological and pathophysiological processes within these cells (Lestard *et al* 2013) [18]. Yeast cells demonstrated a 12% increase in growth rate and 14% reduction in biomass production with a significant difference in the metabolite profiles on exposure to different sound frequencies, confirming the enhancing effect of these vibrations at a cellular level (Aggio *et al* 2012) [2].

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 (Majovski and Jacques 1982) [20]. The brain as we know differs in shape, size and cellular complexity from invertebrates to vertebrates but in relation to behaviour, memory and learning there is always some sort of similarity. 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 (Elliott and Sussswein 2002; Crook and Walters 2011) [12, 9]. Molluscs have been useful models in basic neuroscience research (Grone and Baraban 2015) [13] but studies have been very limited in the field of neurobehavioral science and cognition. Resonance occurs without regard to distance or time separations and without physical communications. Experiments have provided a confirmation that resonant based experiences are not illusionary or imaginary, but occur through a form of interconnectedness created by resonating frequencies inside and outside the living system (Cambray 2009) [5] but need to be evaluated.

The intention of this study was to validate whether music generated vibrations can enhance cognitive abilities and behavioural responses in the molluscan brain, which may be non-specific in terms of species with neuronal tissue but may differ in mechanisms from invertebrates to vertebrates. Molluscs do not have auditory features and therefore the effect of music and its vibrations in this study may be an indirect sensory effect of vibrations at a cellular level and therefore differentiation in music would intern mean differentiation in vibrations or a mixture of vibrations. This is a difficult procedure to demonstrate and therefore was beyond the scope of this study. T-Maze based studies, has helped understand the reasons for cognitive abilities and cognitive dysfunctioning in animals (Levin 2015) [19] and therefore was chosen as a model in this study, to assess 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 (Sherman *et al* 2013) [26].

The present T-maze study was designed to justify whether there exist a difference in cognitive abilities and behaviours within a known population of snails and to assess the exposure of two different types of music generated vibrations on the brain and whether it can enhance cognitive abilities and behaviours in snails.

Collection and Maintenance

Pre-study

Achatina fulica (Bowdich 1822) snails (4 – 6 cm) were collected from St. Peter’s Catholic Cemetery, Worli, Mumbai, India (18°15’29.5’’N, 72.49’19.5’’E). 40 snails were collected and acclimatized for a period of 2 weeks as a group before Study 1. During the acclimatization period, snails were placed in ventilated and hydrated PVC plastic basket measuring 50 cm x 30 cm x 30 cm, 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 was thoroughly washed and cleaned before providing as feed. The PVC plastic housing was washed and cleaned on a daily basis during which the snails were moved into a similar housing apparatus.

Study 1

Study 1 was conducted to isolate snails with a healthier performance from the collection lot to maintain a uniform set of snails for the experiment. Based on the results from Study 1, 21 snails were isolated of which 18 snails were randomly segregated into three groups of 6 snails each - control, experimental 1 and experimental 2. For identification the snails were numbered with a non-toxic water-proof marker on their shells. All three groups were placed in ventilated and hydrated PVC plastic boxes measuring 30 cm x 20 cm x 20 cm. Snails of all three groups were fed daily on lettuce leaves *ad libitum* and the PVC housing for each of the groups were washed and cleaned on a daily basis. Snails were acclimatized in this setup for a period of 2 weeks 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).

Study 2

Study 2 was conducted to evaluate the effect of different types of music on the cognitive abilities of the snails. The T-maze runs were carried out during the day, from 6.00 am – 2.00 pm and during the study the T-maze was kept hydrated and ventilated. The studies were carried out at room temperature (30 – 33 °C) and the maze was thoroughly washed after each snail run, in order to reduce the interference of mucus trail-following behaviour in the snails. Mucus trail-following is a known behaviour observed in snails that is used for activities such as homing, grouping and reproduction in snails (Ng *et al* 2013, Patel *et al* 2014) [21, 23]. Post run the snails were placed in their individual PVC boxes and were fed fresh lettuce *ad libitum*. After completion of all the T-maze based studies, the snails were released at their collection area.

Equipment

T-Maze

A self-designed, enclosed, well ventilated and hydrated PVC plastic T-maze, with start and goal arms measuring 12 cm x 10 cm x 10 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 T-mazes designed to study rat behaviour (Deacon and Rawlins 2006) [11].

Music Source

During Study 2, snails of experimental group 1 were exposed to a rock soundtrack by Aerosmith - "Stop Messin Around" with a bit rate of 128 kbps, while snails of experimental group 2 were exposed to a Tibetan meditational hymn from Tibetan Incantations (Nascente) - "Om Mani Padme Hum" with a similar bit rate of 128 kbps. Frequency analyses of these soundtracks were done using the WavePad NCH software Version 6.18, which uses a FFT analytical tool to determine the actual frequency recordings of the soundtrack. The highest frequency recorded for the Tibetan meditational hymn 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). Similarly, the highest frequency recorded for the Aerosmith soundtrack was 21468 Hz (21096 Hz + 371 Hz) and a decibel gain range of – 6 db to -130 db. The nearest sound note recorded for this soundtrack was E (21096.2 Hz).

The soundtracks were 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 the Tibetan hymn was 75 – 80 db and frequency range was 260 – 280 Hz, while the decibel output range of the Aerosmith soundtrack was 80 – 85 db with a frequency range of 380 – 420 Hz. These output values were recorded by means of an Android based Spectral Audio Analyzer Application from RandonSoft. "Om Mani Padme Hum" is a meditational hymn, and is known to generate positive energies within the body through mystical vibrations that are generated while chanting (Misra and Shastri, 2014) and due to its static pattern of frequency shift it was chosen for this study. On the other hand, the rock soundtrack from Aerosmith - "Stop Messin Around" is a mix of variations which changes at a rapid rate with the progression of the song which would result in exposure to a large variation of frequencies.

Data Analysis

ANOVA Two factor without replication and Student's t-test and Mann-Whitney U value test were some of 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 $p \leq 0.05$ and F > F critical.

Procedure and Analysis: Study 1

Study 1 was conducted to isolate snails with a healthier performance from the collection lot to maintain a uniform set of snails for the experiment. The study was conducted on 40 snails that were acclimatized in a group, for a period of 2 weeks. The snails were starved for a period of 12 hrs before the experiment. Each snail was randomly selected and 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 in the starting arm at 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 snails and time taken for each snail to reach the food source was documented.

For the snails that completed the run in $\leq$ 13 minutes, the time was noted and they were placed in a PVC plastic box filled with fresh lettuce. 18 snails from this set were randomly chosen and were divided into control, experimental group 1 and experimental group 2 comprising of 6 snails each, to be used in the next set of experiments. The three groups were acclimatized for a period of 2 weeks prior to study 2. Snails that did not complete the test within 13 minutes, were stored as a group in a ventilated PVC plastic box with lettuce *ad libitum* and were released at the collection area.

Results: Study 1

Run Time Analysis

Of the 40 snails that participated in the run, 21 snails successfully completed the run with a maximum time of 16.1 mins and a minimum time of 2.8 mins (M = 8.3, SEM $\pm$ 0.8906) (Figure 2). A threshold time of 13 mins was applied to the run time for all snails. A total of 21 snails were chosen for the next set of experiments. Based on these results it is evident that the cognitive capacity or intelligence levels of the brain differs between snails within the same species and same population.

Behaviour Observed

19 snails that did not complete the run, demonstrated behavioural patterns which comprised of restricted movements in the maze and isolation corners of the maze. The snails that took > 13 mins demonstrated restrictive behaviour. The snail which completed the run in the shortest time travelled in one specific direction, without changing its path. Snails that completed the task within the 13 mins threshold time demonstrated focussed movements towards the right arm but spent most of their time exploring the maze before advancing towards the food source.

Procedure and Analysis: Study 2

The purpose of this study was to evaluate the effect of different types of music on the cognitive abilities of the snails. From the group of 21 snails isolated from Study 1, 18 snails were randomly selected, acclimatized and grouped as control, experimental 1 and experimental 2 with 6 snails each and that were used for this study. The experimental group 1 were exposed to the rock soundtrack while experimental group 2 were exposed to the meditational sound track for 15 minutes, once a day in the mornings, for a period of 5 days. During exposure, the snails were placed as a group in a ventilated and hydrated PVC plastic box measuring 10 cm x 10 cm x 10 cm. The box was placed in between the two speakers and sub-woofer which were housed in another PVC box measuring 80 cm x 60 cm x 50 cm. After an exposure period of 5 days, 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. Three identical T-mazes were used for this study so that runs for all three groups could be carried out simultaneously.

Fresh lettuce was placed at the right-hand goal arm of the T-maze as a reward. Start time and end time for each snail were recorded as in Study 1 and the maze was thoroughly washed after each snail run to prevent the mucus trail-following behaviour. A minimum of 10 runs were carried out for each snail in each group and were done in such a manner that each snail 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 of the snails. The runs for all three groups were conducted simultaneously and the start and end time was recorded. Food was only provided once the snail completed its 10^{th} run and was placed in their individual boxes, which were filled with fresh lettuce.

Results: Study 2

Run Time Analysis

A significant difference was observed between the run time for the snails in the control (M = 15.833, SEM $\pm$ 1.146) and between experimental group 1 (M = 7.891, SEM $\pm$ 0.476) and experimental group 2 (M = 4.416, SEM $\pm$ 0.301) (ANOVA; F value = 160.02, F critical = 4.102, P $\leq$ 0.05) (Figure 4). A significant difference was observed between the control and experimental group 1 (T= 10.850, P<0.00001) (Z=2.8022, P=0.00512), control and experimental group 2 (T=18.741, P<0.00001) (Z=2.8022, P =0.00512) and between experimental group 1 and experimental group 2 (T=6.514, P<0.00001) (Z=2.8022, P=0.00512) (Figure 4). A reduction in run time was observed over the 10 runs carried out for each of the groups which were highly significant in both experimental groups in comparison to control group (Figure 3). An erratic or inconsistent reduction in run time was observed in experimental group 1 as compared to a linear, steady reduction in run time observed in experimental group 2 (Figure 3).

Behaviour Observed

Some snails used the lid to navigate towards the food well rather than using the surface. The snails from the control group showed a similar pattern of movement in each of the runs, with some of them moving into the left arm and then moving towards the food source in the right arm. Snails from the experimental group were quick and would directly move towards the food well in the right arm in comparison to the control group. Experimental group 1 showed a perplexed movement pattern which differed within the snails of this group wherein some snails would restrict their movements and were indecisive about their movement. The movement patterns observed in experimental group 2 were smooth and focussed in comparison to experimental group 1 and control group.

Discussion

Learning, short-term and long-term memory formation and retention are cognitive abilities associated with the brain and its neural network system, which assist animals in several cognitive based behaviours like, recollecting predators, differentiating between foods, locating food sources, mate selection, etc. (Shettleworth 2001, Healy *et al* 2009) [27, 15]. Cognitive based studies in invertebrates are limited, but they are known to demonstrate memory-formation/retention and also perform certain learning-based behaviours (Haszprunar and Wanninger 2012) [14]. In a recent study, learning and formation of long-term memory was demonstrated in pond snail *Lymnaea stagnalis,* which showed a significant variability within the natural occurring populations of these snails (Dalesman *et al* 2011). Food foraging is a dietary based behaviour which helps animals to differentiate and trace their food using cognitive based techniques (Overington *et al* 2009) [22]. Snails are known food foragers (Allen 2012) and therefore food was considered as the major source of attraction to study cognitive abilities in snail, *Achatina fulica*. A self-designed T-maze was used to study the cognitive ability of the snails and to understand the various behavioural patterns of learning and short-term memory demonstrated by these snails.

Based on the results of Study 1, a clear differentiation in the cognitive ability was observed between individual snails of the same population (Figure 2). Intelligence is a mental ability for reasoning, problem solving and learning and is known to highly differ within human populations (Colom *et al* 2010) [7]. From the results of this study, 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. Molluscs use their mucus trails for navigational purposes which interim is used by other snails to search and explore (Ng *et al* 2013, Patel *et al* 2014) [21, 23]. This behaviour was evident in Study 1 and therefore in order to prevent any interference from this behaviour, the mazes were thoroughly washed after each and every run for all studies.

Music enhances cognitive functions of the brain which is dependent on dopaminergic neurotransmission and release of opioid peptides within the brain (Sutoo and Akiyama 2004) [28]. Pulsed transcranial ultrasound has been shown to improve memory functioning in Alzheimer's mice by the break-down of amyloid plaques which may help to boost the weakened cemi-field within the neurons or the microtubules within the neurons (Craddock *et al* 2012) [8]. Music has been shown to enhance cognitive abilities in humans and can interfere with complex cognitive processes in the brain (Wang 2013) [30]. In Study 2, a significant enhancement was observed in cognitive abilities of snails exposed to two different types of music shown as a significant reduction in run time as compared to control group (Figure 4). The enhanced cognitive abilities as increased short-term memory was evident in the groups exposed to meditative music which showed a linear pattern in the run profile in comparison to the control group and experimental group 1 (Figure 3). These patterns clearly suggest the enhanced learning and increased short-term memory capabilities which can be correlated to the affirmative effects of exposure to music with lower variations in frequencies.

Meditation is a process that self-regulates the body and mind and maybe associated with psychological and neurophysiological alterations. Meditation studies have been linked to an increased activity in the prefrontal cortex of the brain which is associated several cognitive based functions (Previc 2006) [24]. EEG recordings of skilled Buddhist monks with years of training have shown a significant rise in gamma wave activity in the 80 – 120 Hz range while this effect was lower in new meditators (Davidson and Lutz 2008) [10]. Significance in increased short-term memory observed in snails exposed to meditative music in experimental group 2 in comparison to experimental group 1 suggests that music with reduced alternation in frequencies can have a much more significant impact on the brain cells. Ordered and memorized movement patterns recorded during the runs from experimental group 2 (Figure 3) were exclusive to this group and based on the similarity in movement patterns observed in the runs, it can be confirmed that these snails utilize their cognitive capacity of their brain to memorize and learn which can be enhanced with exposure to music with lesser variation in frequencies. Patterns once memorized by the brain are stored as memory and is exploited by the snail while carrying out its next move, in this case search for food.

Music is not perceived by snails as they do not have auditory features but based on this study music does cause an effect, the mechanism for which needs to be evaluated and is beyond the scope of this paper. The mechanisms behind the enhanced cognitive effects post exposure to music is unknown but is surely related to vibrations created at different frequencies which have direct impact on the biochemical processes at a cellular level within the neural tissues. Qigong masters have enhanced or reduced biochemical rates during plant growth through their resonating meditative practices which involves determining the position and velocity of the trajectory of an object that needs to targeted via techniques that involve vibrations and resonance (Jahnke *et al* 2010) [16]. Resonance occurs when an object is vibrated at its natural frequency or naturally occurring frequencies. Resonance has been used as a medium to transfer power into all kinds of waves ranging from lasers to microwave ovens and musical instruments. The brain works on electrical activity which exists in the form of brainwaves ranging from high amplitude, lower frequency delta waves to low amplitude, higher frequency beta waves (Zhuang *et al* 2009) [32]. In the present study, exposure of music at frequencies could have resulted in resonating effects inducing biochemical changes in the brain of the snails, resulting in enhanced cognitive abilities observed as improved short-term memory gain and learning.

Music as perceived by humans cannot be perceived by snails as they do not possess any auditory apparatus or functions but it can be hypothetical understood as vibrations at a cellular level which may differ based on factors associated with these vibrations, in other words music cannot be perceived as notes or chords by snails nor would its melody play a role. Based on the results of this study, vibrations certainly play an important role in managing cognition and behaviour of snails which directly affects at a cellular level; the mechanisms for which remain unknown. Cognition has always been linked to the neural tissue or the brain which is a collection of neural cells, but there is more in understanding cognition at a cellular level. Understanding and reasoning forms the basis for memory and intelligence in many organisms which survive, based on the ability to perform cognitive functions without the presence of a neural system (Shapiro 2007) [25] e.g. molluscs. The kind of intelligence and memory formation demonstrated by invertebrates cannot be compared to the intelligence observed in higher organisms, but does show some overlap in areas of mental activity, memory and learning (Westerhoff *et al* 2014) [31]. A better understanding of the mechanism that supports this enhanced effect and a correlation to specific frequencies that induce this effect would help understand the behaviours of these snails.

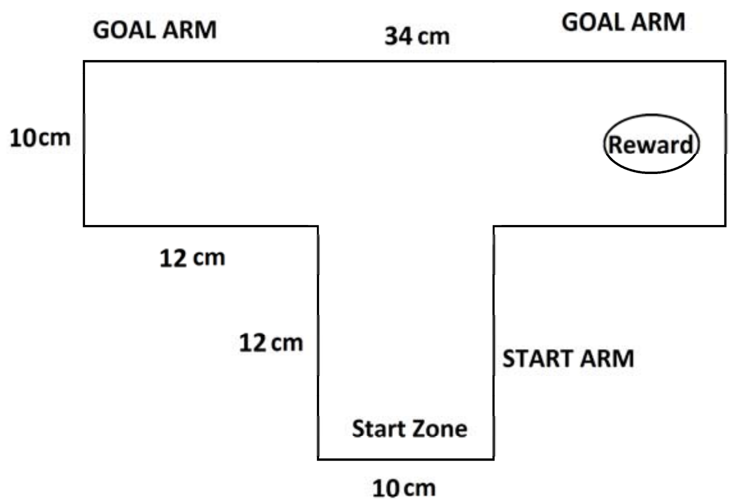

Fig. 1 Self-designed T-Maze sketch.

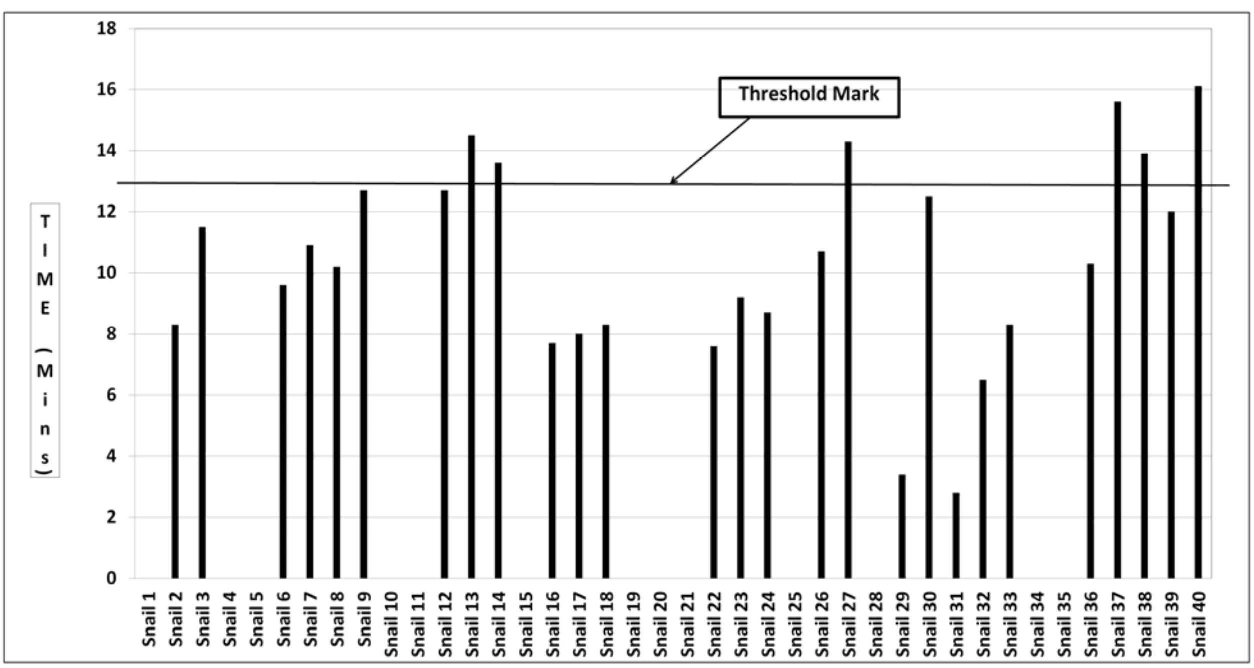

Fig. 2 Actual single run time of 40 snails with a threshold level (*horizontal line*) fixed at 13 minutes.

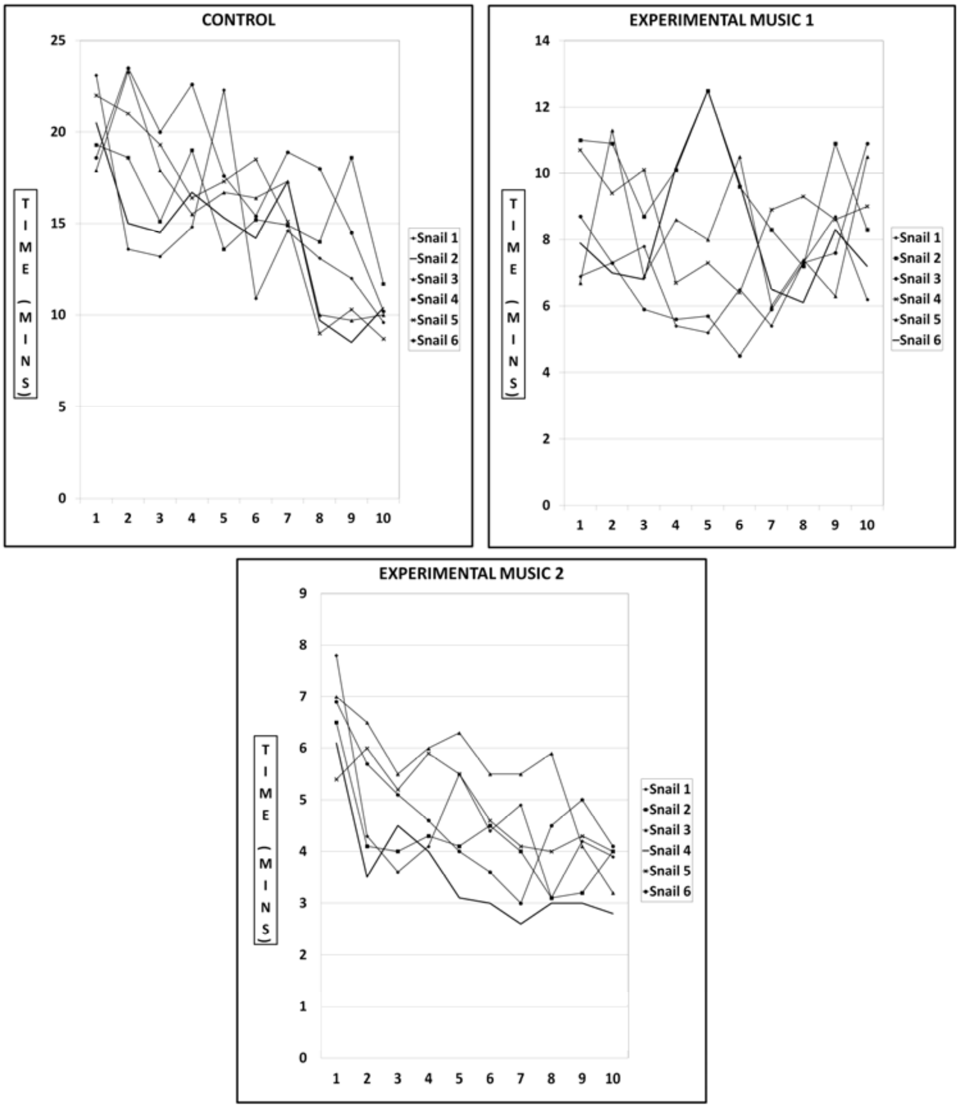

Fig 3 Run time for 10 runs conducted for snails in control, experimental group 1 and experimental group 2 where a significant linear pattern in the run time was observed in experimental group 2 in comparison to experimental group 1 and control group ($p < 0.05$) suggesting an enhanced cognitive ability in short-term memory gain due to exposure to music with lower frequency variations.

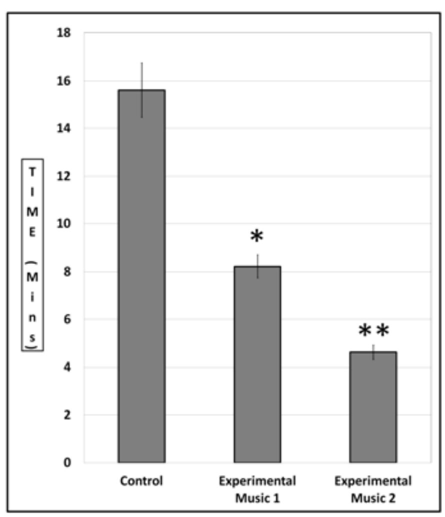

Fig. 4 Average run time comparison of control, experimental group 1 and experimental group where the average run time of snails exposed to music in experimental group 1 and 2 were significantly reduced as compared to control group ($p< 0.05$) confirming the enhanced cognitive abilities in the snails exposed to music. Average run time of snails exposed to music in experimental group 2 was significantly reduced as compared to experimental group 1 ($p<0.05$). *Asterisk* indicates a significant difference between the experimental groups and control group with a value $p<0.05$. Double *Asterisk* indicates a significant difference between the two experimental groups with a value $p<0.05$.

Ethics statement

Ethical approval is not required for research work with *Achatina fulica*; 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 *A. fulica* for this study did not involve endangered or protected species.

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