Effect of tuning fork generated frequencies on cognition in snails (Achatina fulica)

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Effect of tuning fork generated frequencies on cognition in snails (*Achatina fulica*)

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Abstract
The aim of this study was to validate whether individual frequencies generated by tuning forks can enhance and improve cognitive abilities in snails *Achatina fulica*. This paper is a first time report of the effect of individual frequencies generated by tuning forks on the cognition of these snails. The study was conducted in three parts A, B and C, where the snails were exposed to tuning fork generated frequencies of 64, 128, 256, 288, 320, 341, 384, 426, 480 and 512 Hz. From the results of the T-maze tests; it was evident that snails exposed to 320 Hz frequency were extremely active as compared to snails of the control groups and other groups. In the maze these snails were highly focussed and goal oriented, and completed their maze run in a shorter time as compared to the other groups. Based on the results of this study, it was evident that exposure to 320 Hz frequency enhanced the cognitive capacity of the brain in snails *Achatina fulica* which maybe a direct effect of the vibrations created at this frequency at a cellular level. The mechanism involved in the effect of this frequency on the brain to enhance cognitive capacity needs further evaluation.

Keywords: Frequencies, Hertz (Hz), snails, vibrations, tuning fork

Introduction
Meditation and healing practices have always considered vibrations to be the key to resonance, which can induce several conformational changes in the patterns of cognition and consciousness. A living system may have many resonant frequencies due to their degrees of freedom, where each can vibrate as a harmonic oscillator supporting the progression of vibrations as waves that moves as a ripple within the whole system (Pereira 2015c) [1]. Mystics of the past and present have known that the whole universe is vibrating with energy and this energy vibrates at various frequencies (Khan 1996) [2]. According to a renowned sound therapist, sound therapy apparently uses the body's own innate wisdom to bring it back into balance. Sound frequency pulse waves played directed into the body has a profound effect on the nervous system and transmits the vibration to the brain stem, cerebellum, pons, medulla, hippocampus/limbic system and various areas of the of the cerebral cortex (Thompson 2013) [3]. NASA has also been experimenting with sound vibrations and therefore has frequency generators on space shuttles that resemble the frequency of the earth to keep the astronauts healthy during their space missions (Thompson 2005) [4].
Elementary particles are known to vibrate at frequencies interacting with the various growth proteins which demonstrates the mechanism of how plants respond to the stimulation of sound waves, where the effect of sound has shown growth to be twice as fast when exposed to a simple sound frequency for less than one minute (Sternheiner 1984) [5]. Sound has contributed to our quality of life and is coming to the forefront with the advent of Holistic Medicine. Tuning fork is an acoustic resonator that resonates at a particular pitch when vibrated by striking it against a surface that emits a pure musical tone or frequency (Wikipedia) [6]. Tuning fork frequencies play an important role in holistic healing practices and have demonstrated significant healing effects. They provide instantaneous deep state of relaxation, improve mental clarity and brain functioning, increase one's level of physical energy and mental concentration and helps in relieving stress (Beaulieu 2010) [7]. Solfeggio frequencies are those that make up the ancient 6-tone scale thought to have been used in sacred music where their special tones were believed to impart spiritual blessings when sung in harmony. Tuning forks of these frequencies are known to balance the energy and keep the body, mind and spirit in perfect harmony (Paddon 2012) [8].
Vibrational frequency plays an important role in the creation of our physical reality, because it allows energy to express itself into any form, including molecules, atoms, planets, stars,

biological life and even diseases. Frequencies and vibrations that make-up the music of various chants (Pereira 2016a; 2016b) [9,10] are known to augment the cognitive capacity of the brain in snails *Achatina fulica*, which has been demonstrated in earlier studies (Pereira 2016c; 2016d; 2015a; 2015b) [11,12,13,14]. Tuning forks are instruments that can provide a single frequency and therefore has been adopted by healers who use them to increase the amount of energy on parts of the body they are trying to heal (Beaulieu 2010) [7]. The aim of this study was to validate whether individual frequencies generated by tuning forks that have been used by healers can enhance and improve cognitive abilities in the snails *Achatina fulica*. The effect may be non-specific in terms of molluscs that do not possess auditory features and therefore the effect of these frequencies in this study may be considered as an indirect sensory effect of vibrations at a cellular level.

Materials and Methods

Achatina fulica (Bowdich 1822), snails (4–6 cms) were collected from St. Peter’s Catholic Cemetery, Worli, Mumbai, India (18°15’29.5’’N, 72.49’19.5’’E). 90 snails were collected and acclimatized as a group for a period of 3 weeks. The snails were placed in a ventilated and hydrated PVC plastic box measuring 100 cm x 60 cm x 60 cm and maintained on a 12: 12 light: dark schedule (7.00 am: 7.00 pm) at room temperature (30 – 33 °C). The snails were fed on lettuce leaves *ad libitum* which was thoroughly washed and cleaned before providing feed. The PVC plastic housing was washed and cleaned on a daily basis during which the snails were moved into a similar housing apparatus. Post the 3 week acclimatization period, 78 snails were randomly chosen from the batch of 90 snails and were randomly sub-divided into 13 groups of 6 snails each. The snails from each group were numbered on their shells using a water-proof marker for identification purposes. Each group were placed in individual ventilated and hydrated PVC plastic boxes measuring 30 cm x 20 cm x 20 cm and acclimatized for a period of 2 weeks in this setup. The snails were maintained on a 12: 12 light: dark schedule (7.00 am: 7.00 pm) at room temperature (33–35 °C) and were fed on lettuce leaves *ad libitum.* The PVC plastic boxes were washed and cleaned on a daily basis during which the snails were moved into a similar housing apparatus.

Study A, B and C

The study was conducted in three parts – A, B and C on 78 snails that were randomly subdivided into 13 groups of 6 snails each and acclimatized for a period of 2 weeks. The snails in each group were numbered and were placed as a group in individual PVC plastic boxes. Individual control groups were maintained for all three studies. Exposure related studies were carried out during from 9.00 am to 11.00 am. Post exposure the snails were placed in their PVC plastic boxes and their behaviour was observed for a period of 1 hour. All behaviour-based observations were carried out at room temperature (33–35 °C). After the one hour observation period, the housing was filled with fresh lettuce *ad libitum.* Post 5 day exposure the T-maze runs were conducted on the 6th day. The maze runs were carried out during the day, from 9.00 am to 12.00 pm and during the runs the maze was kept hydrated and ventilated. All maze studies and behaviour-based observations were carried out at room temperature (33 – 35 °C). The maze was thoroughly washed after each snail run, inorder 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) [15, 16]. All three studies were completed within a period of 18 days post the 2 week acclimatization period. After completion of the studies, the snails were released back at the 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).

Tuning forks

The 8 piece C type tuning fork set with frequencies specifications of 256 Hz, 288 Hz, 320 Hz, 341 Hz, 384 Hz, 426 Hz, 480 Hz and 512 Hz and a mallet manufactured by Radical Healing Tuning forks was purchased from eBay, India. The C type 68 Hz and 128 Hz tuning forks were purchased individually from different suppliers from eBay, India. The tuning forks were analysed for their respective frequencies on three android based applications – Spectrum Analyzer by Raspberrywood Version 5.0.3, Spectrum Analyzer by Keuwlsoft Version 1.3 and Sound Analyzer by Tinia Soft Version 1.02 using a Lenovo Ideatab A1000-G tablet with a 1.2GHz dual-core Cortex A-9 processor (MediaTek 8317), a 4GB RAM with an Android 4.1 Jelly Bean operating software**.** The frequencies for each tuning fork was analysed by the android applications using a FFT analytical tool of hertz (Hz) against the decibel output value. By comparing the frequencies recorded for each of the tuning forks by all three applications, a final set of frequencies for each tuning fork was confirmed (Table 1). Frequency generation and retention time for each tuning fork was calculated based on the decibel retention levels of 10 mallet hits for 5 mins using the Spectrum Analyzer by Raspberrywood Version 5.0.3.

Tuning fork frequency exposure

Based on the frequency analysis of the purchased tuning forks, the frequencies confirmed upon measurement were 136, 172.6, 255.6, 288.2, 313.6, 336.9, 386, 418.1, 483.8 and 517.9 Hz against the specified factory-set frequency of 64, 128, 256, 288, 320, 341, 384, 426, 480 and 512 Hz (Table 1). Exposure to the respective frequencies was conducted once a day for a period of 5 days for all three studies. During the exposure, the tuning fork was vibrated by striking it manually to a mallet and by placing the end of the tuning fork in the head region of each individual snail, for each individual frequency. Consistency in exposure was maintained by exposing the frequency of the tuning fork in 10 mallet hits within an exposure time span of 5 mins per snail, as the vibration of the tuning forks was approximated to retain for at least 30 seconds pre frequency.

Table 1: Tuning fork frequencies (Hz) analysed by three android based applications – Spectrum Analyzer by Raspberrywood Version 5.0.3, Spectrum Analyzer by Keuwlsoft Version 1.3 and Sound Analyzer by Tinia Soft Version 1.02

	FREQUENCY (Hz)									
Actual	64	128	256	288	320	341	384	426	480	512
Measured - Tinia Soft	139	174	256.8	286.5	310.2	334.4	386.3	417.2	485.1	520.7
Measured - Raspberrywood	132.1	176	254	289.3	312.5	336.4	387.4	417	485	522.5
Measured - Keuwlsoft	137	168	256	288.8	318.3	340	384.3	420.2	481.4	510.6

Data Analysis

ANOVA Two factor without replication and Student t-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 A (Exposure to 64, 128, 256 and 288 Hz), Study B (Exposure to 320, 341 and 384 Hz) and Study C (Exposure to 426, 480 and 512 Hz)

The study was divided into 3 parts. Study A was conducted on 5 groups of 6 snails each, where one group was maintained as a control group while the other 4 groups were exposed to frequencies of 64, 128, 256 and 288 Hz respectively against the actual frequency of 136, 172.6, 255.6 and 288.2 Hz. Study B was conducted on 4 groups of 6 snails each where one group was maintained as a control group while the other 3 groups were exposed to frequencies of 320, 341 and 384 Hz respectively against the actual frequency of 313.6, 336.9 and 386Hz. Study C was conducted on 4 groups of 6 snails each where one group was maintained as a control group while the other 3 groups were exposed to frequencies of 426, 480 and 512 Hz respectively against the actual frequency of 418.1, 483.8 and 517.9 Hz. All snails were placed in their individual PVC plastic boxes as per their groups. Post exposure the snails were placed in their PVC plastic boxes and their behaviour was observed for a period of 1 hour. All behaviour-based observations were carried out at room temperature (33 – 35 °C). After the one hour observation period, the housing was filled with fresh lettuce *ad libitum*. Post the 5 day exposure period the T-maze runs were conducted on the 6th day for all three studies. The runs were carried out during the day, from 9.00 am to 12.00 pm and during the runs the maze was kept hydrated and ventilated. Food was only provided once the run was complete and the snails were placed in their individual boxes. On completion of all the studies, the snails were released at the collection area.

Results

Study A (Exposure to 64, 128, 256 and 288 Hz)

Study A (Maze Run Analysis)

A significant difference in run time was observed between the control group (M = 15.155, SEM $\pm$ 2.339) and Group 1 (M = 5.105, SEM $\pm$ 0.309) (T = 4.243, $P<0.05$), Group 2 (M = 7.38, SEM $\pm$ 0.976) (T = 3.271, $P<0.05$), Group 3 (M = 7.94, SEM $\pm$ 1.053) (T = 2.55, $P<0.05$) and Group 4 (M = 7.78, SEM $\pm$ 0.425) (T = 3.074, $P<0.05$) (ANOVA; F value = 7.549, F critical = 2.866, P = 0.00071) (Figure. 2A).

Study A (Behavioural Observations)

Snails of group 1 and 2 were goal oriented and focussed compared to the snails of the control group. Snails of group 3 were slower and confused in comparison to snails of the control group and other groups. These snails were lethargic and spend a lot of their time in the starting arm. Snails of group 4 were highly sensitive to touch and retracted immediately into their shells when they were picked up, but like the snails of group 3 they were also lethargic and spend a lot of their time in the starting arm.

Study B (Exposure to 320, 341 and 384 Hz)

Study B (Maze Run Analysis)

A significant difference in run time was observed between the control group (M = 14, SEM $\pm$ 2.413) and Group 5 (M = 4.675, SEM $\pm$ 0.560) (T = 4.101, $P<0.05$) and Group 6 (M = 9.07, SEM $\pm$ 0.458) (T = 2.154, $P<0.05$) (ANOVA; F value = 10.120, F critical = 3.287, P = 0.00068) (Figure. 2B). No significant difference was observed in run time between the control group and Group 7 (M = 12.53, SEM $\pm$ 1.49) (T = 0.765, $P>0.05$) (Figure 2B).

Study B (Behavioural Observations)

Snails of group 5 were extremely active as compared to the snails of the control group and other groups. In the maze they were focussed and goal oriented, and completed their run in a much shorter time as compared to the other groups. Snails of group 6 and 7 were slow and confused, and spend a lot of their time in the starting arm. Snails of group 7 were highly sensitive to touch and retracted immediately into their shells when they were picked up, but like the snails of group 6 and 7, they were lethargic and spend a lot of their time in the starting arm.

Study C (Exposure to 426, 480 and 512 Hz)

Study C (Maze Run Analysis)

A significant difference in run time was observed between the control group (M = 17.65, SEM $\pm$ 2.318) and Group 9 (M = 11.37, SEM $\pm$ 2.092) (T = 2.122, $P<0.05$) and Group 10 (M = 13.505, SEM $\pm$ 1.914) (T = 1.879, $P<0.05$) (Figure 2C). No significant difference between run times of control group and group 9 and 10 was confirmed by the ANOVA test (ANOVA; F value = 1.7652, F critical = 3.2873, P = 0.1968). No significant difference was observed in run time between the control group and Group 8 (M = 14.235, SEM $\pm$ 1.660) (T = 1.522, $P>0.05$) (Figure 2C).

Study C (Behavioural Observations)

Snails of group 8, 9 and 10 were very slow and appeared confused. They were lethargic and spend a lot of their time in the starting arm.

Study A, B and C (Overall Observation)

No significant difference was observed between the three control groups of study A, B and C (ANOVA; F value = 1.09164, F critical = 4.1028, P = 0.372). A significant difference between control groups and treated groups was confirmed by an overall ANOVA test (ANOVA; F value = 6.6391, F critical = 2.0621, P = 0.0006) (Figure 2A, B and C).

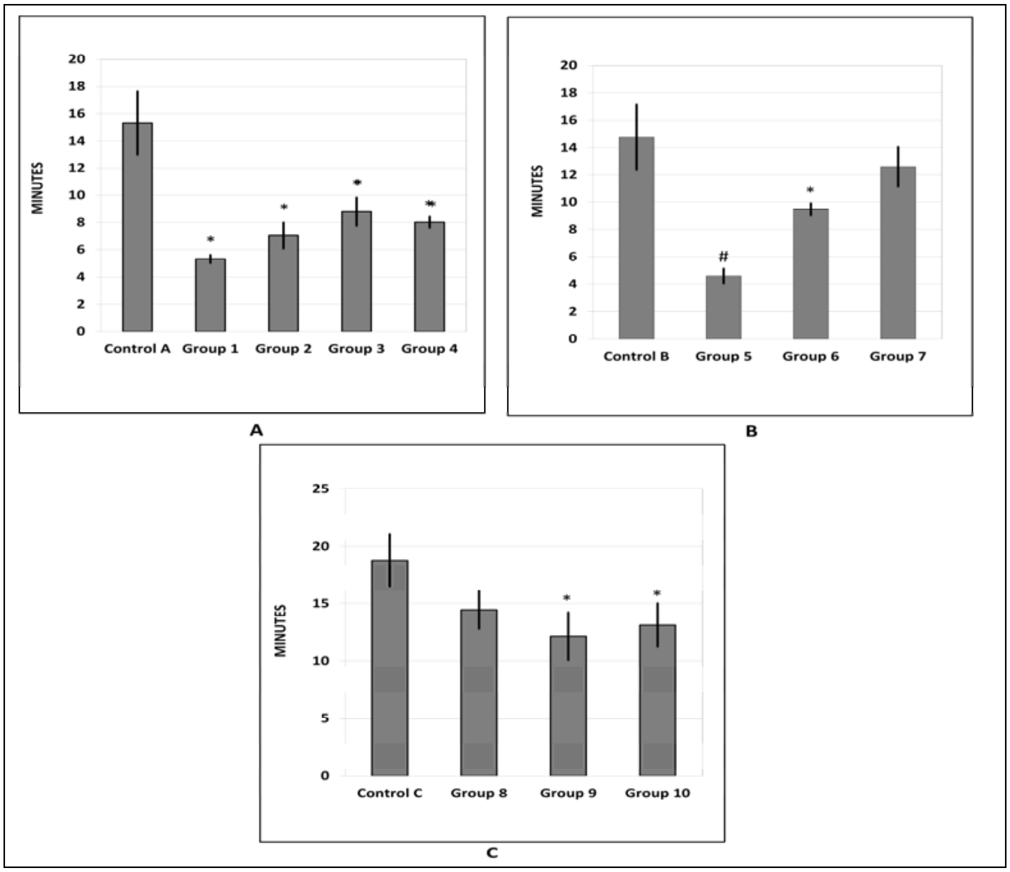

Fig 1: Average run time comparison for studies A, B and C. Study A (Exposure to 64, 128, 256 and 288 Hz frequency) – Significant difference in run time was observed between the control group and group 1, 2, 3 and 4 that were exposed to tuning fork frequencies of 64, 128, 256 and 288 Hz (p<0.05) marked with an *, Study B (Exposure to 320, 341 and 384 Hz frequency) – Significant difference in run time was observed between the control group and group 5 and 6 that were exposed to tuning fork frequencies of 320 and 341 Hz (p<0.05) where the results were highly significant in the group 5 which was exposed to 320 Hz frequency marked with an #, Study C (Exposure to 426, 480 and 512 Hz frequency) - Significant difference in run time was observed between the control group and group 9 and 10 that were exposed to tuning fork frequencies of 480 and 512 Hz (p<0.05) marked with an *.

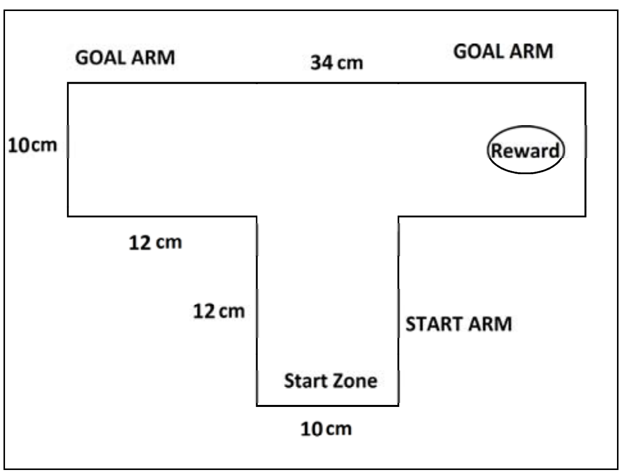

Fig 2: Self-designed T-Maze sketch.

Discussion

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) [17]. 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) [18]. Binaural beats seem to exert some effect on cognitive functioning and mood (Lane *et al* 1998) [19]. They are termed as an auditory illusion that can result in cognitive or neural entrainment (Reedijk *et al* 2015; Hommel *et al* 2016) [20, 21]. In animals, binaural beats are known to produce a stimulus that produce neural patterns of phase locking, or synchronization which begins in the auditory system and propagates to the inferior colliculus (Fritzpatrick *et al* 2009; Cai *et al* 1998) [22, 23]. Tuning forks are precise instruments that have the ability to change our inner tuning by creating a resonance throughout our mind, emotions, and body, and interact with the nervous system is like the string of a musical instrument (Beaulieu 2010) [7]. The use of sound in healing has become a proven tool, as sound travels four times faster through water than it travels through air and he body is 80% water and so a perfect receptor for sound (Heather 2007) [24]. Sound has contributed to our quality of life and is coming to the forefront with the advent of holistic medicine without any life threatening side effects and therefore is different from medical treatment and procedures. (Sylver 2011) [25]. Sound creates a phenomenon called as 'resonance' that occurs when a given system is driven by another vibrating system or external force to oscillate with greater amplitude at a specific preferential frequency (Wikipedia) [26]. Resonance can occur when an object is vibrated at its natural frequency or naturally occurring frequencies, which is possible by frequencies generated by means of a tuning fork.

The effect of meditational music is known to augment the cognitive capacity of the brain in snails *Achatina fulica* and at reduced variation in frequencies e.g. Om Mani Padme Hum enhance the learning ability with an increase in short-term memory gain (Pereira 2016c; 2016d; 2015a; 2015b) [11,12,13,14] but the effect of individual frequencies has never been studied on these snails. This paper is a first time report of the effect of individual frequencies generated by tuning forks on the cognition of snail *Achatina fulica*. Based on the results of studies A, B and C, it was evident that snails exposed to most of the lower frequencies showed significant enhanced cognitive capabilities (Figure 2A, B & C) because of the shorter run-times in the maze. Of all the frequencies, snails exposed to 320 Hz frequency tuning fork (actual measure - 313.6 Hz) were extremely active as compared to the snails of the control group and other groups (Figure 2B). In the maze these animals were focussed and goal oriented, and completed their maze run in a shorter time as compared to the other groups (Figure 2B). Significance was lower in groups exposed to the other frequencies when compared to snails exposed to 320 Hz frequency suggests that, 320 Hz frequency has the potential to enhance the cognitive capacity of the brain in snails *Achatina fulica* with specific effects. 320 Hz frequency tuning forks have been used extensively in holistic healing practices as they are known to activate the Manipura chakra present in the solar plexus, which supports balance and coordination (Gurjar *et al* 2009) [27].

The 320 Hz frequency is termed as the 'OM' frequency by the Hindus, where some call it 'Pranava' while majority call it 'God'; the source; the spirit; known as the default frequency inherent in every form, shape, colour, size and energy within the cosmos, surpassing and transcending time and space (Gurjar and Ladhake 2016) [28]. 320 Hz frequency has also been suggested by healers as a pure healing sound and is used in many healing therapies (Paddon 2012) [8]. The phenomenon of resonance creates vibrations at specific frequencies and has been used as a medium to transfer power/energy into all kinds of waves ranging from lasers to microwave ovens and musical instruments. In this study, 320 Hz frequency as perceived by the snails can be hypothetical understood as a direct effect of the vibrations at a cellular level where the resonating effects of this frequency can induce biochemical changes in the cells of the snails, resulting in an enhanced cognitive effect. The mechanisms involved in the therapeutic effect of these frequencies generated by tuning forks needs to be evaluated in future for which this species of snails could be a recommended model.

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 do have long-term effects on these animals but as observed in the study no significant effects were observed with a complete recovery for animals and therefore the animals were released back into the wild after observing them post the experiments. No specific permits were required for the described field collections. The collection of *A. fulica* for this study did not involve endangered or protected species.

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