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Hypothermia induced reversible state of unconsciousness/ insentience in snails (*Achatina fulica*) and the therapeutic effect of a meditative chant on this state

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Abstract
Unconsciousness in humans or insentience in animals is a state when a living being or organism is unaware or lacks the ability of perceiving its self or its environment. Proving consciousness or sentience in animals is a big problem as they lack the ability to express and communicate like humans and therefore keeping aside the experience of consciousness the presence of its existence can be evaluated. This paper is a series of two studies, one which attempts to prove sentience or consciousness in simple invertebrate organisms by inducing a hypothermic reversible state of unconsciousness or insentience in snails, *Achatina fulica*. The second study suggests a therapeutic effect of vibrations and frequencies generated by a meditative chant "Om Mani Padme Hum" on this hypothermic induced state of unconsciousness or insentience in these snails. Significance in recovery time was observed against the increasing exposure time to hypothermic stress, with a complete recovery in snails induced for 30 and 45 minutes of hypothermic stress. Snails showed a time based recovery in movement and crawling after being knocked out as a loss of sentience or consciousness for all groups, which increased in accordance to the increasing exposure time. Snails exposed to the meditative chant that were exposed to hypothermic stress for 30 and 45 minutes during the period of loss of sentience or consciousness, showed a significant reduction in recovery time in comparison to the group that was not exposed to the chant. Based on the results from Study 1, it can be confirmed that 15 to 20 mins exposure to hypothermic stress is an ideal time range for inducing a state of unconsciousness/insentience for this snail species, as no irreversible short and long-term effects were noted. Results of Study 2, revealed a faster recovery from the hypothermic induced state of unconsciousness/insentience when exposed to the meditational chant "Om Mani Padme Hum", indicating direct therapeutic effects of the vibrations and frequencies generated from this chant.

Keywords: Hypothermia, Unconsciousness, Insentience, Snails, Music.

1. Introduction
Consciousness in humans occurs when one is in a state of awareness of one's self and the external environment (Searle 2005) [22]. Consciousness has always been linked to the nervous system but there are several studies that have recorded conscious behaviours in animals with and without nerve cells (Pereira 2015a) [19]. Sentience in context to consciousness is known as the ability of the organisms to experience pleasure and pain (Breed and Marino 2010) [3]. Animals ranging from micro to macro levels such as cell, worms, ants, bees, mammals, etc., are known to display conscious or sentient behaviours and are known to engage in Hamiltonian descriptions of cooperative interactions such as altruism, where interactions are beneficial to the recipient but costly to the actor and mutualism, that provides a direct fitness benefit to the organism that performs the behaviour, which outweighs the cost of performing the behaviour (Neilands 1995) [17].
Lynn Margulis, in the endosymbiotic theory of organelle evolution, suggested that not only animals but every organized being is conscious (Margulis and Sagan 1995) [12]. Humberto Maturana was the first to propose that living systems though cognitive systems are applicable to all organisms with and without a nervous system (Maturana 1970) [15]. At the first annual Francis Crick memorial conference on consciousness held on 7th July, 2012, a group of scientists formally declared a document entitled "Cambridge Declaration on Consciousness in Non-Human Animals" which stated that the capacity of consciousness emerged very early in evolution and those processes that support consciousness in humans are likely characteristics of many living organisms (Low 2012) [10].

Unconsciousness on the other hand is a state when the ability to maintain awareness of one's self and the environment is lost. Hypothermia is a condition when the body core temperature drops to less than 35 °C and is known to induce unconsciousness in humans when the core body temperature falls approximately to 30 to 32 °C (Luscombe and Andrzejowski 2006) [11]. Hypothermic anaesthesia is a technique which is used to induce coma in humans by lowering the body temperature to 30 to 34 °C (Polderman 2004) [21]. Hypothermia induced unconsciousness is associated with reduction in cerebral blood flow and oxygen requirement with a reduced cardiac function which basically resembles clinical death (Karnatovskaia *et al*. 2014) [7]. Revival from this state is only possible when a patient shows vital signs of brain and cardiac activity. For animals, this technique has been used to incapacitate organisms but its long-term effects have never been evaluated (Diaz and Becker 2010) [6].

Hypothermia is a well know technique used to slow the metabolic rate which may induce a state of unconsciousness with longer exposure time and lower freezing temperatures in animals. In invertebrates, hypothermia has been used as a technique to immobilize and anesthetize them for various invasive procedures (Cooper 2011) [5] Invertebrates such as snails are known to demonstrate freeze tolerance which is usually associated with increase in glycerol and proline reserves in the body which was first observed in an intertidal pulmonate gastropod *Melampus bidentatus* (Loomis 1985) [9]. Alterations in carbohydrate metabolic pathways have been observed during cold acclimatization of freshwater apple snails *Pomacea canaliculata* that confirms the involvement of carbohydrate metabolism in changes to cold hardiness (Matsukura *et al.* 2009) [14]. Land snail *Helix aspersa* demonstrated freezing tolerance based on body size which increased with increase in body size (Ansart and Vernon 2004) [2]. Freeze tolerance or tolerance to hypothermia may help invertebrates survive, but the level of tolerance may differ from species to species, which needs to be evaluated for its short and long term effects.

This study is an attempt to understand the short and long-term recovery post induction of hypothermic stress at varying temperatures in snail *Achatina fulica*. *Achanita fulica* is a robust and sturdy land snail and therefore was chosen for this study. Based on the results of the short and long-term recovery post induction of hypothermic stress, the therapeutic effect of meditative music on this state would also be evaluated. Meditational music is known to generate frequencies and vibrations which augment the cognitive capacity of the brain in snails *Achatina fulica* and at reduced variation in frequencies e.g. Om Mani Padme Hum, can enhance learning ability with an increase in short-term memory gain (Pereira 2015b) [20]. This study would help evaluate whether these vibrations and frequencies can reduce the recovery time post hypothermic stress and whether it has a therapeutic effect on this induced state of unconsciousness/ insentience.

2. Collection and Maintenance

2.1 Pre study

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). 100 snails were collected and acclimatized as a group for a period of 2 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 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.

2.2 Study 1 (Time varied hypothermic induced stress)

Post the 2 week acclimatization period, 18 snails were randomly chosen from the batch of 100 snails and were randomly sub-divided into 3 groups of 6 snails each and were numbered on their shells using a water-proof marker. Each group were placed in individual ventilated and hydrated PVC plastic boxes measuring 30 cm x 20 cm x 20 cm. 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 housings were washed and cleaned on a daily basis during which the snails were moved into a similar housing apparatus. The study was conducted during the day from 9.00 am to 12.00 pm. Post exposure the snails were placed in their PVC plastic boxes and their behaviour was observed for a period of 3 hours and then for the next 20 days. All behaviour-based observations were carried out at room temperature (33 – 35 °C). After the three hour observation period, the housing was filled with fresh lettuce *ad libitum.* After completion of the study, the snails were released back at the collection area.

2.3 Study 2 (Time varied hypothermic induced stress and the effect of a meditative chant on this state)

From the initial set of 100 snails that were acclimatized for 2 weeks, 24 snails were randomly chosen and were further sub-divided into 4 groups of 6 snails each. The snails in each of the groups were numbered on their shells using a water-proof marker. Each group were placed individually in ventilated and hydrated PVC plastic boxes measuring 30 cm x 20 cm x 20 cm. 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 housings were washed and cleaned on a daily basis during which the snails were moved into a similar housing apparatus. The study was conducted during the day from 9.00 am to 12.00 pm. Post exposure to hypothermic stress and the meditational chant, the snails were placed in their PVC plastic boxes and their behaviour was observed for a period of 3 hours on the same day and then for the next 20 days. All behaviour-based observations were carried out at room temperature (33 – 35 °C). After the three hour observation period, the housing was filled with fresh lettuce *ad libitum.* After completion of the study, the snails were released back at the collection area.

2.4 Equipment

2.4.1 Hypothermia Induced Stress

Hypothermia was induced by placing the snails as a group in their individual boxes in a frost-free freezer of a double-door refrigerator at a temperature of -16 °C $\pm$ -2 °C. The LG GL-D292JSFL double-door refrigerator is equipped with a LG Dura Chill option that maintains optimum cooling inside the refrigerator for up to 10 hrs.

2.4.2 Music Source

In Study 2, snails were exposed to a Tibetan meditational chant from Tibetan Incantations (Nascente) - "Om Mani Padme Hum" with a similar 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 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). The 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 100 Hz-20 kHz. The decibel output range for the Tibetan hymn was 75 – 80 db and frequency range was 260 – 280 Hz. These output values were recorded by means of an Android based Spectral Audio Analyzer Application from Randon Soft. "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) [16] and due to its static pattern of frequency shift it was chosen for this study.

2.5 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.

3. Procedure and Analysis: Study 1 (Time varied hypothermic induced stress)
The study was conducted on 18 snails that were subdivided into 3 groups of 6 snails each, 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 that were placed in the freezer for 15, 30 and 45 minutes. Post hypothermic exposures, each of the boxes containing the snails were placed at room temperature (33 – 35 °C) and were observed for a period of 3 hours. Time and behaviour was recorded as: First Movement Observed – This time point was recorded when first movement was observed in the snail post exposure e.g. movement in the foot, tentacles, and head, Revival (Body Movement) – This time point was recorded when complete movement of the body movement in and out of the shell but absence of crawling, Complete Revival (Crawling) – This time point was indicative of complete movement associated with crawling. The reflex actions of the snails were checked by observing withdrawal effects after nudging them with a wooden stick. The three time points were recorded for all groups within the 3 hour observation period on the day of the study and for the next 20 days. The snails were fed with lettuce *ad libitum* after the 3 hour observation period and for the next 20 day observation period. On completion of the study the snails were released at the collection area.

3.1 Results: Study 1 (Time varied hypothermic induced stress)
3.1.1 Time Points Recorded Post Exposure (3 hours Observation Period)
3.1.1.1 First Movement Observed
A significant increase in recovery time for this time point was observed with an increase in exposure time (ANOVA; F value = 15.824, F critical = 2.602, P = 0.00005) (Figure. 1A). Based on these results it is evident that the knockout time for snails when exposed to hypothermia, increases with an increase in exposure to stress. Significant increase in time taken for the first movement to be observed in the 45 min exposure group is indicative of a hypothermic shock which induces a state of unconsciousness or insentience with a longer recovery time.

3.1.1.2 Revival (Complete Body Movement)
Significant increase in recovery time was observed for this time point with an increase in exposure time (ANOVA; F value = 35.696, F critical = 4.102, P = 0.00002) (Figure. 1B). Increase in the knockout time for snails when exposed to hypothermic stress was higher for the snails exposed for 45 mins.

3.1.1.3 Complete Revival (Crawling)
In accordance to the results of the earlier time points recorded, a significant increase in recovery time was observed for this time point with an increase in exposure time (ANOVA; F value = 20.884, F critical = 4.102, P = 0.00027) (Figure. 1C).

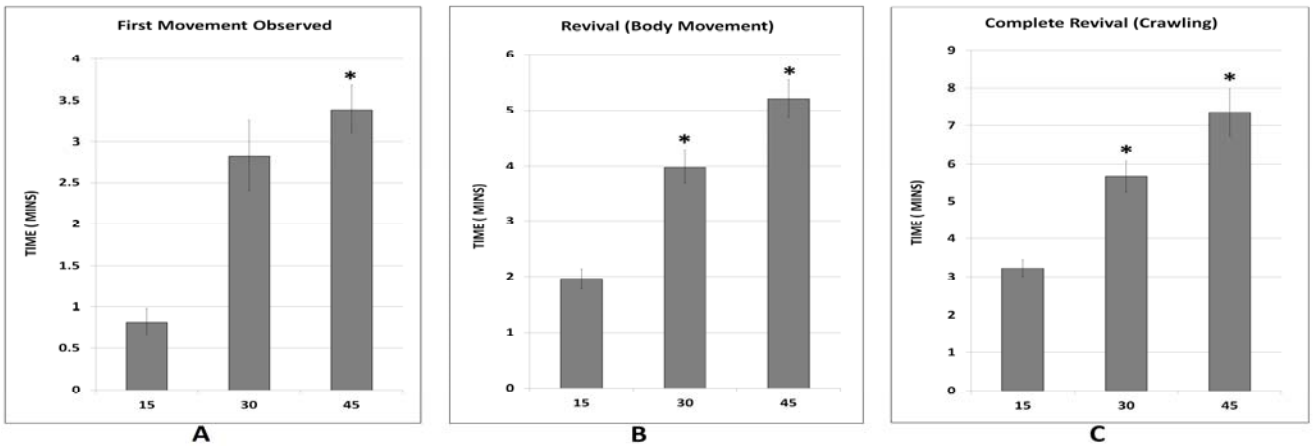

Fig 1: Time varied hypothermic induced stress for 15, 30 and 45 mins. **A** – First movement observed – Significant difference in recovery time was observed between snails exposed for 15 mins in comparison to those exposed for 30 and 45 mins ($p < 0.05$) marked with an *, **B** – Revival (Body Movement) – Significant difference was observed between the snails exposed for 15, 30 and 45 mins ($p < 0.05$) marked with an *, **C** – Complete Revival (Crawling) - Significant difference was observed between the snails exposed for 15, 30 and 45 mins ($p < 0.05$) marked with an *.

3.1.2 Behaviour Recorded Post Exposure (3 hours Observation Period)

3.1.2.1 First Movement Observed

This time point was very critical, where a first movement was recorded after the exposure where the snails were in a complete knocked out or comatose state with no movement. The time point was recorded when the first jerk was observed in the head region of the snail. For all groups, during exposure the snails did not retreat into their shells and therefore the body of the snails were exposed throughout the time point. Groups exposed for 15, 30 and 45 mins showed a jerk in the head region, which followed with a slight swirling of the head, no whole body shell retention was observed.

3.1.2.2 Revival (Complete Body Movement)

This time point was recorded when the snails demonstrated complete body movement, which was evident in the foot region and in the tentacles. The groups exposed for 15, 30 and 45 mins demonstrated partial body retention and therefore this end point could be easily observed as movements in the head and body were visible. Crawling was restricted as the snails were still in a state of unconsciousness/ insentience.

3.1.2.3 Complete Revival (Crawling)

For groups exposed for 15, 30 and 45 mins, the time was noted when complete crawling movement was observed along with apt head and body movements. The reflex actions of the snails were checked by observing withdrawal effects after nudging them with a wooden stick. For all groups, the snails showed complete signs of recovery from the state of unconsciousness/ insentience.

3.1.3 Long-Term Observations Post Exposure (20 day Observation Period)

During the 10 day observation period, snails exposed for 15, 30 and 45 mins showed complete recovery when compared to snails of the higher exposure groups. No significant altered changes in eating habits, crawling patterns, body movements and reflex actions were observed post exposure confirming complete recovery from the state of unconsciousness/ insentience for snails exposed for 15, 30 and 45 mins.

4. Procedure and Analysis: Study 2 (Time varied hypothermic induced stress and the effect of a meditative chant on this state)

The study was conducted on 24 snails that were subdivided into 2 sets, non-exposed and music exposed containing 2 groups each. Each group comprised of 6 snails were acclimatized for a period of 2 weeks. The snails were numbered as per their sets and groups and were placed as a group in individual PVC plastic boxes that were placed in the freezer for 30 and 45 minutes. The study consisted of 2 sets, non-exposed and music exposed, each containing 2 groups with 6 snails each that were exposed for 30 and 45 minutes. Post hypothermic exposure, one set was exposed to a meditational chant for a period of 15 minutes at room temperature (33 – 35 °C) on the day of the study. The animals for both sets were observed for a period of 3 hours on the day of the study and for the next 20 days.

The time and behaviour for each of the snails was recorded as: First Movement Observed – This time point was recorded when first movement was observed in the snail post exposure e.g. movement in the foot, tentacles, and head, Revival (Body Movement) – This time point was recorded when complete movement of the body movement in and out of the shell but absence of crawling, Complete Revival (Crawling) – This time point was indicative of complete movement associated with crawling. The reflex actions of the snails were checked by observing withdrawal effects after nudging them with a wooden stick. These time points were recorded for all groups in both sets during the 3 hour observation period on the day of the study and for the next 20 days. The snails were fed with lettuce *ad libitum* after the 3 hour observation period and for the next 20 days observation period. On completion of the study the snails were released at the collection area.

4.1 Results: Study 2 (Time varied hypothermic induced stress and the effect of a meditative chant on this state)

4.1.1 Time Points Recorded Post Exposure (3 hours Observation Period)

4.1.1.1 First Movement Observed

A significant increase in recovery time for this time point was observed with an increase in exposure time for both music exposed and non-exposed group (Figure 2A). No significant difference was observed between the recovery times of non-exposed (M = 2.795, SEM $\pm$ 0.165) and music exposed (M = 2.575, SEM $\pm$ 0.2268) groups belonging to the 30 mins exposed group (T = 0.7188, P = 0.244) (ANOVA; F value = 0.28103, F critical = 6.607, P = 0.618) and non- exposed (M = 4.035, SEM $\pm$ 0.179) and music exposed (M = 3.105, SEM $\pm$ 0.184) groups belonging to 45 mins exposed group (T = 2.777, P = 0.0097) (ANOVA; F value = 4.800, F critical = 6.607, P = 0.0799) (Figure 2A). The results were similar to Study 1, where knockout time for snails when exposed to hypothermic stress increased with an increase in exposure time (Figure 1A and 2A).

4.1.1.2 Revival (Complete Body Movement)

Significant increase in recovery time was observed for this time point with an increase in exposure time for both music exposed and non-exposed group (Figure 2B). A significant difference in recovery time was observed between the non-exposed (M = 6.075, SEM $\pm$ 0.1543) and music exposed (M = 4.353, SEM $\pm$ 0.251) group belonging to the 45 mins exposure group (T = 5.504, P = 0.00013) (ANOVA; F value = 36.771, F critical = 6.6078, P = 0.0176) (Figure 2A). No significant difference was observed between the recovery times of non-exposed (M = 3.39, SEM $\pm$ 0.186) and music exposed (M = 2.765, SEM $\pm$ 0.203) groups belonging to the 30 mins exposure group (T = 2.505, P = 0.0155) (ANOVA; F value = 4.762, F critical = 6.607, P = 0.080) (Figure 2A). The results were similar to Study 1, where knockout time for snails when exposed to hypothermic stress increased with an increase in exposure time (Figure 1B and 2B). A significant reduction in recovery time for snails exposed for 45 mins when exposed to the meditational chant signifies a therapeutic effect of this chant, resulting in faster recovery from the induced state of unconsciousness/ insentience for this time point.

4.1.1.3 Complete Revival (Crawling)

Based on the results of the earlier time points, a significant increase in recovery time for this time point was observed with an increase in exposure time for both the music exposed and non-exposed groups (Figure 2C). A significant difference in recovery time was observed between the non-exposed (M = 6.145, SEM $\pm$ 0.136) and music exposed (M = 5.11, SEM $\pm$ 0.177) group belonging to the 30 mins exposure group (T = 3.7109, P = 0.00201) (ANOVA; F value = 7.744, F critical =

6.6078, P = 0.0387) (Figure 2C). Significance was lower between the recovery times of non- exposed (M = 8.16, SEM ± 0.435) and music exposed (M = 6.775, SEM ± 0.346) groups belonging to the 45 mins exposure group (T = 2.616, P = 0.0128) (ANOVA; F value = 6.202, F critical = 6.607, P = 0.055) (Figure 2C). A significant reduction in the recovery time for snails exposed for 30 mins when exposed to the meditational chant signifies a therapeutic effect of this chant, resulting in faster recovery from the induced state of unconsciousness/ insentience at this time point (Figure 2C). The lower significance observed between the non-exposed and music exposed group for snails exposed for 45 mins also conforms to the therapeutic effect of this meditative chant (Figure 2C).

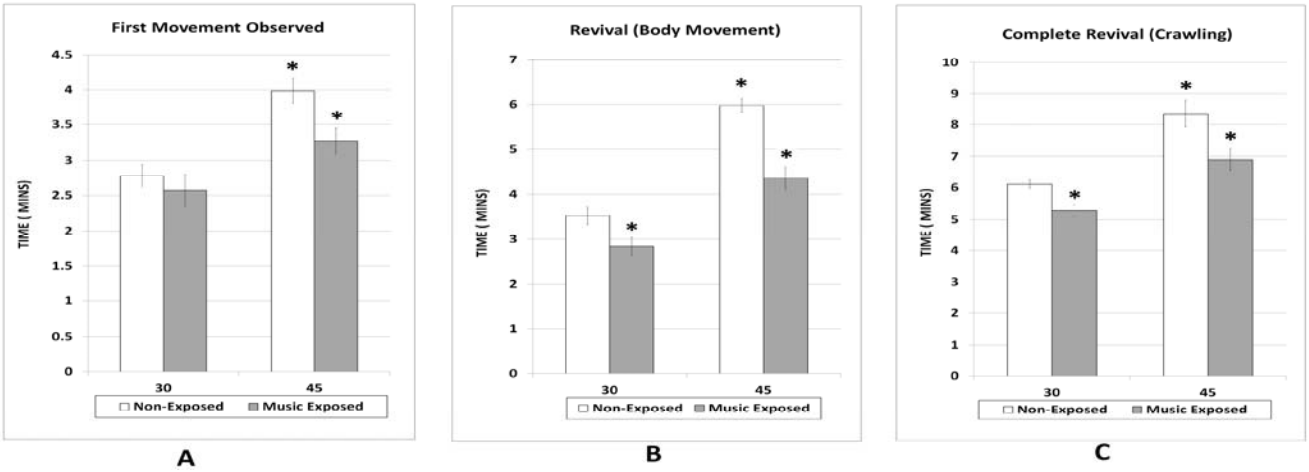

Fig 2: Time varied hypothermic induced stress and the effect of a meditative chant on this state for 30 and 45 mins. **A** – First movement observed – Significant difference in recovery time was observed between non-exposed and music exposed snails belonging to the 45 mins exposed group ($p < 0.05$) marked with an *, **B** – Revival (Body Movement) – Significant difference in recovery time was observed between non-exposed and music exposed snails belonging to the 30 and 45 mins exposed groups ($p < 0.05$) marked with an *, **C** – Complete Revival (Crawling) - Significant difference in recovery time was observed between non-exposed and music exposed snails belonging to the 30 and 45 mins exposed groups ($p < 0.05$) marked with an *.

4.1.2 Behaviour Recorded Post Exposure (3 hours Observation Period)

4.1.2.1 First Movement Observed

For all groups, for this time point, the snails did not retreat into their shells and therefore the body of the snails were exposed right until the first movement was observed. For both non-exposed and music exposed groups belonging to the 30 and 45 mins exposure group, a first jerk was observed in the head region followed with a slight swirling of the head, no whole body shell retention were observed for both these groups.

4.1.2.2 Revival (Complete Body Movement)

This time point was recorded when the snails showed complete body movement especially in the foot and head region for both non-exposed and music exposed groups. Both non-exposed and music exposed groups belonging to the 30 and 45 mins exposure group, demonstrated partial body shell retention and therefore this end point could be easily observed as the snails showed complete head and body movements. The snails belonging to the music exposed group that were exposed for 45 mins to hypothermic stress showed a quicker recovery in terms of head and body movement in comparison to the non-exposed snails of the 45 min exposure group. Crawling was not observed in all groups as the snails were still in a partial state of unconsciousness/ insentience for this time point.

4.1.2.3 Complete Revival (Crawling)

For both non-exposed and music exposed groups belonging to the 30 and 45 mins exposure groups, this time point was recorded when complete crawling movement was observed with appropriate head and body movements. The reflex actions of the snails were monitored by observing the withdrawal effects after nudging them with a wooden stick, which was significantly high in snails exposed to the chant belonging to the 30 and 45 mins exposure groups. The music exposed group showed complete signs of recovery from a state of unconsciousness/ insentience in comparison to the non-exposed groups belonging to 30 and 45 mins exposure groups which correlated well with the reduced recovery time for these groups (Figure 2C).

4.1.3 Long-Term Observations Post Exposure (20 day Observation Period)

During the 20 day observation period, snails exposed for 30 and 45 mins showed complete recovery from the state of unconsciousness/insentience for both non-exposed and music exposed groups. No drastic changes in eating habits, crawling patterns, body movements and reflex actions were observed post hypothermic induced stress, confirming complete recovery for both non-exposed and music exposed groups belonging to the 30 and 45 mins exposure group. Music exposed groups showed a faster recovery as compared to the non-exposed groups. No deaths were observed in the non-exposed and music exposed groups belonging to the 30 and 45 mins exposure group (Table 2 and 3).

Table 1: Snails showed a survival rate of 100% post exposure to 15, 30 and 45 mins of hypothermic stress. Observation period was 3 hours on day of study and 20 days from the day of study.

TIME (Minutes)	15	30	45
Snail 1	Alive	Alive	Alive
Snail 2	Alive	Alive	Alive
Snail 3	Alive	Alive	Alive
Snail 4	Alive	Alive	Alive
Snail 5	Alive	Alive	Alive
Snail 6	Alive	Alive	Alive
Survival Percentage (%)	100	100	100

Table 2: Snails that were non-exposed showed a survival rate of 100% post exposure to 30 and 45 mins of hypothermic stress. Observation period was 3 hours on day of study and 20 days from the day of study.

TIME (Minutes)	30	45
Snail 1	Alive	Alive
Snail 2	Alive	Alive
Snail 3	Alive	Alive
Snail 4	Alive	Alive
Snail 5	Alive	Alive
Snail 6	Alive	Alive
Survival Percentage (%)	100	100

Table 3: Snails exposed to music showed a survival rate of 100% post exposure to 30 and 45 mins of hypothermic stress. Observation period was 3 hours on day of study and 20 days from the day of study.

TIME (Minutes)	30	45
Snail 1	Alive	Alive
Snail 2	Alive	Alive
Snail 3	Alive	Alive
Snail 4	Alive	Alive
Snail 5	Alive	Alive
Snail 6	Alive	Alive
Survival Percentage (%)	100	100

5. Discussion

Consciousness is prevalent in the animal kingdom and in comparison to the neural system, is in lower form, but by division of labour in cells it propagates and attains a higher state, as observed in higher organisms. Whether unicellular or multicellular, we all depend on our past experiences and observation and use this for several actions that need to be performed in our day to day life, which is managed by the conscious decisions that we take, which may be new or retrieved from memory. This helps animals understand complex environments and is therefore an essential tool for all species which allows movements related to feeding, mating and resting. Proving consciousness or sentience in animals is a big problem as they lack the ability to express and communicate like humans and therefore keeping aside the experience of consciousness the presence of its existence can be evaluated. Since consciousness has been associated with the neural system, invertebrates such as snails with a simple cerebral ganglion that demonstrate cognitive abilities such as learning and memory retention (Pereira 2015b) [20] were chosen for this study.

This paper is a first time report of hypothermia induced unconsciousness/ insentience and its recovery from this state, in snail *Achatina fulica*. Based on the results of Study 1, a significant increase in recovery time was observed for the various time points during the 3 hour recovery period which was significant with increasing exposure time (Figure 2). The snails for all groups were completely knocked out and showed no signs of movement which indicated that the snails were in a state of unconsciousness/insentience, where all ability to maintain awareness was lost. The revival or recovery from this state was observed as a first jerk or movement in the head region for snails belonging to all groups but the time of revival increased based on the increase in exposure time. Snails exposed for 15 mins showed a quick recovery as compared to those exposed for 30 and 45 mins (Figure 2). Body movement and crawling also showed a significant recovery time against the increasing exposure time but did take a longer time with the group exposed for 45 mins. During the 20 day observation period these snails did not exhibit any kind of stressful behaviour. No significant altered changes in eating habits, crawling patterns, body movements and reflex actions were observed post exposure confirming complete recovery from the state of unconsciousness/ insentience for snails of all the groups. Based on these results it can be confirmed that 15 to 20 mins hypothermic exposure is an ideal time range for inducing a state of unconsciousness/insentience for this species, as the effects monitored for short and long term were reversible.

Snails are ectothermic and do have the ability to survive in cold and freezing temperatures, but as a form of adaptation they can increase their glycerol and proline reserves in the body and alter their carbohydrate metabolism to adapt to the changing temperature (Nowakowska 2011) [18], but longer exposures leads to hypothermia which when beyond a certain threshold limit can result in knockout or unconsciousness/sentience with reversible and irreversible effects. This paper is a first time report of the recovery of the snail *Achatina fulica* from a hypothermic induced state of unconsciousness/insentience with no long-term effects of hypothermia and therefore can be proposed as a model of studying consciousness in snails which may differ from species to species. Hypothermic anaesthesia has been considered non-reliable as it may sensitize some species to painful stimuli based on the effects observed in higher animals and therefore is minimally used (Martin 1995 [13], Diaz and Becker 2010 [6], Cooper 2011 [5]). This study did not show any signs of distress or irreversible effects in the treated snails when monitored for 20 days which is evident that the hypothermic stresses which lead to unconsciousness/insentience was reversible for this species of snails confirming the rigidity and sturdiness of this species.

Music articulates our life, evoking emotions from joyous to sadness and regulating moods. It 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). The potential therapeutic effects of music listening reduces stress and modulates arousal and has also shown reduced anxiety in humans (Thoma *et al.* 2013) [23]. 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]. 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) [8]. 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) [1].

"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) [16] and has shown significant increase in cognitive capacity of the brain in snails *Achatina fulica* with enhanced learning ability and an increase in short-term memory gain (Pereira 2015b) [20].

This study is a first time report of the effect of the meditational chant "Om Mani Padme Hum" on the recovery time from the state of unconsciousness/insentience induced through hypothermia in snails *Achatina fulica.* Based on the results of Study 2, a significant increase in recovery time was observed for the various time points during the 3 hour recovery period which was significant with increasing exposure time for both music exposed and non-exposed group (Figure 2). A significant difference in recovery time was also observed between the music and non-exposed groups belonging to the 45 min exposure group. Snails that were completely knocked out or that were in a state of unconsciousness/insentience recovered faster from this state when exposed to this meditational chant, indicating a therapeutic effects of the vibrations generated from this chant. 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.

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) [24]. In the present study, exposure of the frequencies generated by the chant could have resulted in resonating effects inducing biochemical changes in the cells of the snails, resulting in an enhanced therapeutic effect on the state of unconsciousness/insentience. The mechanisms involved in the effect of these vibrations and frequencies generated by this chant needs to be evaluated in future for which this species of snails could be a recommended model.

6. 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.

7. References

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