Article

Cell Consciousness Study with Prokaryotic Cell Metabolism Rate Measurement in Response to Acoustical Vibration

Poh Foong Lee*, Sharannaya, A. P. N. Karthiyeyan,
Kok Suen Cheng, William M. Y. Cheung & Bryan Kok

ABSTRACT

Can a single cell response to auditory stimuli and contribute to the cell decision? Here, we aimed to investigate the response of prokaryotic cell to single tone frequency and Pali chant in a time series. Cell metabolism rate was measured to determine the response of the cells after sound treatment. Two groups of specimens in which one batch was fresh for each sound treatment time, whereas another group was a continuous batch of specimens in which the sound treatment was performed to the same cells for the whole series of time duration. We found that the continuous batch specimen responded significantly to Pali chant through the increased in metabolism rate (10 min to 30 min) which indicates the potential of studying cell cognition and memory with prokaryotic cell as a model to gain behavioral information without stereotype feedback. The outcome from this investigation suggests that the unicellular cell consciousness might potentially be used for preliminary study of signaling pathway in consciousness and cognition before study with multi-cell organisms.

Keywords: Prokaryotic cell, cell cognition, single tone frequency, Pali chant. metabolism rate.

Introduction

Consciousness is elucidated as the dualism concept involving the body and mind in material and immaterial objects that is nonphysical in nature (Schweizer 2013). Consciousness is expanded to the awareness of one's existence, sensations, and thoughts, surrounding with an awake and aware mind which spring into the exuberant neural correlate and empirical analysis of cognition and affection on human decision making. This give rises to meta-cognition studies on one's awareness and the capability to regulate their own thinking (Wokke et al. 2020). Consciousness is a polymorphic feature involving different levels of biological organisms that stem from a complex nervous system (Lau and Lau 2020).

However, the complex study seems to derive from a reaction evaluation stemming from consciousness responding to a stimulus by a human being consisting of multi trillion cells. Therefore, a human decision making on an event or a response to stimuli is unidentifiable to

*Correspondence: Poh Foong Lee, Lee Kong Chien Faculty Engineering & Science, University Tunku Abdul Rahman, Malasia.
Email: pfleej@gmail.com, leepf@utar.edu.my

which cells have involved in the action or decision. Furthermore, the duration to response to the stimuli is definite an important parameter on consciousness. Florian Klapproth (Klapproth 2008) had reviewed on the relationship between time and decision making in humans with respect to the evaluation process, which the delay were owing to realizing the options and time available for making that decision.

Besides, prediction ability in decision making is also part of the fundamental for all living system. The decision-making system even extended into animal collection observation(Arganda et al. 2012). Moreover, living microorganism on making decision has been reported which the growth speed of microbiology in different chemicals conditions(Ashino et al. 2019). In addition, surface charge changes with electrophoretic mobility measurement of living and dead microorganism on different chemical conditions and temperatures were also observed its changes which indicated the unicellular decision making experiment(Poh Foong 2009). Microbial behavior has linked to cell cognition where the understanding of the cognitive evolution either unicellular or multicellular organisms is yet to reach a conclusive outcome(Lyon 2015).

The effect of sound on consciousness in neural observation suggested the neural correlates consciousness was dependent on stimulus features, higher cortical levels and different aspects of a single perceptual scene might not be processed simultaneously(Brancucci et al. 2011). Sounds is an essential stimulus for living organism to survive and detect the surrounding necessitates. However, sounds form multiple perceptual bias which dominates the majority studies in psychoacoustic(Moore 2007)'(Li et al. 2010). Neural with auditory paradigm studies is growing rapidly in these recent years(Smith et al. 2013; Hettich et al. 2016; Norman-Haignere and McDermott 2018; Wöstmann et al. 2019). Conscious processing with auditory paradigm response was inferred from neurophysiological measurement suggested that the presence of the global effect might be a signature for conscious processing(Bekinschtein et al. 2009). However, different perception, attention and many minds interpretation(Zeh 1970) to conclude the particular sound effect on multicellular system is remained challenging.

On the other hand, animal consciousness study was reported to be more challenging than human studies although animal are speculated to have a simpler mindset in responding towards stimuli. This is due to the animal has lacking human language to explain their thinking but only can inferred from their reaction to the experimental manipulation(Hoy 2005). A report even highlighted that the animal hearing evaluation depends on few aspects, including anatomical, physiological, economic, spatial and psychosocial factors and evaluation objective(Reis et al. 2017). Therefore, consciousness of multicellular organism in responding to a stimulus required multiple conditions of investigation and measurement but have yet to identify the fundamental study of the exact signaling pathway of a cell on an auditory stimuli response.

A novel method to brim the curiosity in studying a simple cell response to sound stimuli within the human hearing range is inviting. Prokaryotic cell is a classic model for this approach as it recaptures precedent of the pre-Cambrian explosion where the Earth begun with simple cells

organism and latter evolved to vertebrate organism which was estimated to begin 551 million years ago(Condon et al. 2005). Mechanical response to single-tone and two-tone stimuli was inspected on Chinchilla cochlea with Mossbauer spectroscopy measurement(Robles et al. 1986). However, applying the human sound range stimulus on prokaryotic cell has yet to be reported.

In this work, single tone harmonic sound at frequency of 500 Hz and 1000 Hz, added a natural Buddhist Pali from monks hymning sound without music instruments background were employed in this experiment as auditory stimuli onto *Escherichia coli (E.coli)*. The response from the cells was measured with UV spectroscopy on instantaneous metabolism rate. For sing tone frequency auditory stimuli, both 500 Hz and 1000 Hz were clustered as single tone harmonic sound(Persinger 2014). Meanwhile, the single tone frequency at 1000 Hz was proclaimed as high frequency sound or loud(Smyth 2019). A research shows that the auditory frequency range from 500 to 2000 Hz were able to stimulate a profound immediate effects on the lower limb motor function of the healthy people(Yu et al. 2016), this indicated the exist impact of auditory effect on the brain connection with motor muscle. Another interesting work shows that the auditory stimuli within the range of 200 Hz to 1000 Hz on deaf participants reported experienced dizziness, pain and vibration, suggested the sound experiences can occur without functional hearing(Persinger 2014). These are among reports which encourages the work on how the auditory give impacts on the body and mind, in term of cognition and consciousness.

On the other hand, Pali chant from the monk's natural human hymning voice was adopted in the experiment. A review shows that the prayer related to spirituality and religion has increased in healthcare area and being suggested as a non-pharmacological intervention on resources to be included in the nursing holistic care(Simão et al. 2016). A report shows that the neurophysiological correlates of religious chanting are likely different from those of meditation and prayer, suggested that the chanting would possibly induce distinctive psychotherapeutic effects(Gao et al. 2019). Consciousness study on religious chanting or repetitive mantra commonly elicits unequivocal multiple interpretations and perceptions on human response(Zeh 1970), therefore *E.coli k-12* was expected to provide stereotypic feedback to religious chant. Consequently, this study has incorporated the Buddhist Pali chant to measure the response of the unicellular cell together with single tone harmonic frequency sounds in this experiment. For resolving the ambivalent effective durations of the sound stimulus, a series of time was tested for the range of 5 to 30 min. The cells were groups into two different categories for their metabolism rate measurement - continuous batch of specimen (CS) for the whole series of sounds treatment and a new batch of specimen (NS) for each different duration. Both batches of specimen would indicate the accumulation effect for the same bacteria for progressive treatment.

Materials and Methods

Specimen preparation

Lysogeny media (LB media) is a nutritionally rich medium primarily used for the growth of bacteria. First, 100 ml of LB media and 2g of the LB media powder was measured and diluted with 100 ml of distilled water. Once it had been thoroughly mixed without any visible powder clumps, the media solution was then transferred to a conical flask and covered with an aluminium foil and cotton pad. The prepared LB media in the conical flask was autoclaved at 120℃ for 15 minutes. Once the autoclave had been completed the LB media solution was left to completely cool down for the inoculation of bacteria. When the LB media culture medium was fully cooled down, the sterile inoculation loop was used to transfer the *E.coli k-12* into the culture medium. The inoculation loop is a small and lengthy metal instrument made up of a looped wire at one end that is attached to a handle at the other end. The looped wire end is usually used in capturing bacterial samples from a liquid type of reagent as the loop wired end is designed to hold a drop of liquid. The inoculation loop was sterilized on a Bunsen burner before further used in cell culture. After sterilization, the inoculation loop with the wired end was slowly and gently dipped into the bacterial source plate and then dipped back into the conical flask containing LB media. It was followed by a gently mixing of the sample with the prepared LB media culture medium. The next step was to immerse the conical flask containing the bacteria in the culture medium and incubating at 37°C for 12-18 hr in a shaking incubator. After incubation, the growth of the bacteria was characterized by a cloudy haze in the media.

The preparation of the new batch of specimen (NS) was started by taking out the inoculated bacterial culture medium from the shaking incubator and placed the medium in the fume hood. The specimen was taken out from the culture medium by using a sterile pipette tip. 0.4 ml of the bacteria was pipetted out and added into one well on the 96 Greiner well plate. This step was then repeated for another 10 times under the same condition. After the treatment had been performed, the well plate was removed from the Styrofoam box and placed into the microplate reader to measure the absorbance rate.

For the continuous batch of specimen (CS), once the absorbance rate was measured, the well plate was removed from the microplate reader and again being placed back to the Styrofoam box for the next time duration of sound treatment and so forth. The steps were repeated for 10 min, 15 min, 20 min, 25 min and 30 min and also subsequently the same experimental steps for 1000 Hz single tone frequency and Pali chanting. For new batch of specimen on sound treatment, the new fresh group of specimens exposed to the same type of sound stimuli but was discarded after every single time duration metabolism rate measurement.

Microplate reader OD_{600} assay

Quantification on bacterial growth by measuring the optical density at 600 nm (OD600) (Infinite M200 series, Tecan) is a well-established method in giving the value on the turbidity outcome

that results from light scattering by the bacteria in the well plate. It is a fast and cost-effective method in monitoring the instantaneous metabolism rate of the bacteria in LB media(Stevenson et al. 2016).

Auditory stimuli

The sound treatment was performed at six different time durations which included 5 min, 10 min, 15 min, 20 min, 25 min and 30 min using three different types of sounds. Two of them were single frequency sounds at 500 Hz and 1000 Hz, as well as a Pali chanting natural sounds by monks without instrumental music (https://www.peacebeyondsuffering.org/audio-chanting-04.html). The frequency ranges between 200 Hz to 900 Hz for the Pali chanting. (See appendix)

Experiment setup protocol

The complete experimental setup is shown in Fig. 1. These three music frequencies were played through a Bluetooth speaker that was placed directly on the Greinier 96 well plate placed within a Styrofoam box. A Styrofoam box was used because it provided good insulation for the sound without taking up much noise and avoiding the sound escaping out to the surrounding. An empirical study was done on using recycle Styrofoam as porous sound absorption(Rey et al. 2012). The Bluetooth speaker was controlled by a handphone where the sounds were selected and played using a repeat player. Furthermore, the audio length for the 500 Hz and 1000 Hz were only 30 seconds long in a file, hence these two tracks were set to play on loop continuously to fit the time frames set at 5 min, 10 min, 15 min, 20 min, 25 min and 30 min. The length of the Pali chanting is 18 min 11 seconds which was also controlled using the repeat player to the expected prepared time frames of sounds treatment to the bacteria.

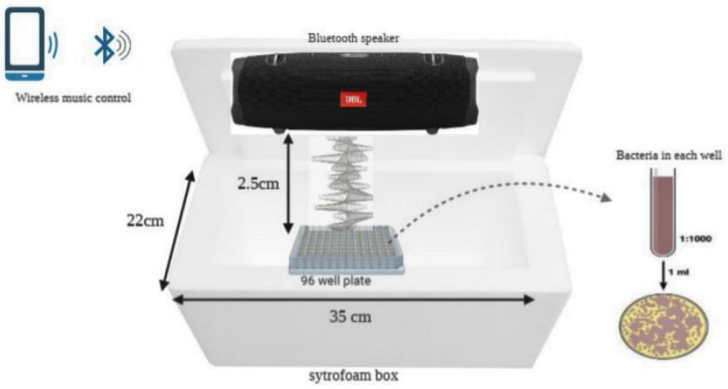

Fig. 1. The experiment setup for the sound treatment on new and continuous batch of specimen in the Styroform box where the speaker with Bluetooth was allocated on top of the 96 well plate which the time series of sound treatment was controlled with smartphone.

Data analysis

For the new batch of specimen, we used 4 x 7 ANOVA with 4 being the between subject Frequency (Control, 500 Hz, 1000 Hz and Pali Chanting) and 7 being the within subject variable Time (0 until 30 min). For the continuous batch of specimen (CS), we used 4 x 6 ANCOVA with the same explanation as above except for the absorbance rate at 0 min being the covariate. For the comparison between the new batch (NS) and continuous batch of specimen (CS), we used 4 x 2 two-way ANOVA with 4 being the Frequency and 2 being the Group (New Specimen and Continuous).

Results

Comparison of the metabolism rate on new batch of specimen (NS) for the sound treatment

There was a significant Frequency main effect ($F(3, 266) = 57.652$, $p < 0.001$, $\eta_p^2 = 0.394$), a significant Time main effect ($F(6, 266) = 15.633$, $p < 0.001$, $\eta_p^2 = 0.261$) and a significant Frequency × Time interaction ($F(18, 266) = 10.888$, $p < 0.001$, $\eta_p^2 = 0.424$). Post-hoc analysis with Bonferroni correction stratifying for the Frequency (Table 1) revealed that for the 500 Hz frequency, the absorbance rate at 0 min was significantly smaller than both 10 min and 25 min ($p < 0.001$ and $p = 0.006$, respectively).

Table 1: Post-hoc analysis for the new specimen group showing the *p* values of the different times for different frequencies (Control, 500 Hz, 1000 Hz and Pali chanting). Significant *p* values are bolded.

Control							
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
0 min	-						
5 min	1.000	-					
10 min	1.000	1.000	-				
15 min	1.000	1.000	1.000	-			
20 min	1.000	1.000	1.000	1.000	-		
25 min	1.000	1.000	1.000	1.000	1.000	-	
30 min	1.000	1.000	1.000	1.000	1.000	1.000	-
500 Hz							
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
0 min	-						
5 min	1.000	-					
10 min	**< 0.001**	**0.024**	-				

15 min	1.000	1.000	**0.002**	-			
20 min	1.000	0.095	< **0.001**	0.702	-		
25 min	**0.006**	1.000	1.000	0.256	< 0.001	-	
30 min	0.231	1.000	0.208	1.000	**0.010**	1.000	-
1000 Hz							
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
0 min	-						
5 min	1.000	-					
10 min	0.060	0.087	-				
15 min	**0.043**	0.063	1.000	-			
20 min	1.000	1.000	**0.004**	**0.003**	-		
25 min	0.337	0.245	< **0.001**	< **0.001**	1.000	-	
30 min	1.000	1.000	1.000	1.000	1.000	0.914	-
Pali chanting							
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
0 min	-						
5 min	1.000	-					
10 min	< **0.001**	< **0.001**	-				
15 min	< **0.001**	< **0.001**	0.069	-			
20 min	< **0.001**	< **0.001**	1.000	1.000	-		
25 min	< **0.001**	< **0.001**	1.000	0.634	1.000	-	
30 min	0.126	**0.001**	0.056	< **0.001**	< **0.001**	< **0.001**	-

The absorbance rate at 5 min was significantly smaller than 10 min (p = 0.024) whereas the result at 10 min was significantly larger than 15 min and 20 min (p = 0.002 and p < 0.001, respectively). For the result at 20 min, it was significantly larger than that of at 25 min and 30 min with p values of 0.001 and 0.010, respectively. For the 1000 Hz frequency, the absorbance rate at baseline was significantly smaller than 15 min (p = 0.043), however, results at 10 and 15 min was both significantly larger than 20 min and 25 min (p = 0.004, p < 0.001, p = 0.003 and p < 0.001, respectively). Similarly, the result at 30 min was significantly larger than that of 25 min with p = 0.006. Meanwhile, the absorbance rate at 0 and 5 min for the Pali chant was all significantly smaller than 10 to 30 min (all p < 0.001) whereas the results at 15 to 25 min was all significantly larger than 30 min (p < 0.001, p < 0.001 and p = 0.004, respectively).

The post-hoc comparisons for each Time are shown in Fig.2A. At baseline, the absorbance rate for the 500 Hz frequency was significantly smaller than Control and 1000 Hz frequency (p < 0.001 and p = 0.005, respectively). At 5 min, the Control frequency result was significantly larger than both 500 Hz and Pali chanting (p = 0.048 and p = 0.004, respectively). For 15 to 30 min, the same trend of Pali chant resulting in significantly larger absorbance rate as compared to all other 3 frequencies was observed (all p < 0.001), with the exception of 30 min being only significantly larger than 500 Hz (p = 0.034).

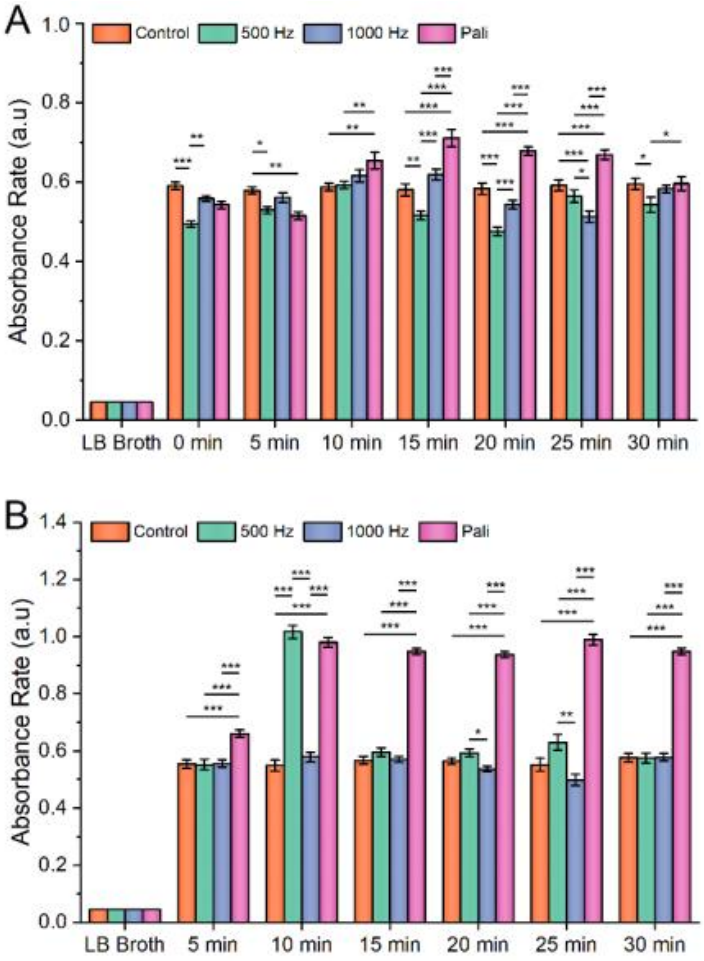

Fig. 2. Comparisons of the absorbance rate when the bacteria E. Coli was subjected to different frequency vibrations at different times for the (A) new specimen group and (B) continuous group. * - $p < 0.050$; ** - $p < 0.010$; *** - $p < 0.001$.

Comparison of the metabolism rate on continuous batch of specimen (CS) for the sound treatment

There was a significant Frequency main effect ($F(3, 37) = 309.452$, $p < 0.001$, $\eta_p^2 = 0.962$), a significant Time main effect ($F(3.558, 37) = 130.876$, $p < 0.001$, $\eta_p^2 = 0.775$) and a significant Frequency × Time interaction ($F(10.659, 37) = 49.515$, $p < 0.001$, $\eta_p^2 = 0.801$). In terms of the Time difference (Table 2), post-hoc analysis revealed that the absorbance rate at 10 min for the 500 Hz frequency was significantly larger than all other 5 times (all $p < 0.001$). For the 1000 Hz frequency, the result at 10 min was significantly larger than that of 25 min ($p = 0.009$) whereas at 15 min the absorbance rate was also significantly larger than both 20 min and 25 min ($p = 0.007$ and $p = 0.003$, respectively). Besides that, both 20 min and 25 min had an absorbance rate that is significantly smaller than that of 30 min, respectively, with $p = 0.002$ and $p = 0.001$. As for the Pali chant frequency, the result for 5 min was significantly smaller than all other 5 times (all $p <$

0.001). Further, the absorbance rate at 25 min was also significantly larger than that of 20 min (p = 0.049).

Table 2: Post-hoc analysis for the continuous group showing the p values of the different times for different frequencies (Control, 500 Hz, 1000 Hz and Pali chanting). Significant p values are bolded.

Control						
	5 min	10 min	15 min	20 min	25 min	30 min
5 min	-					
10 min	1.000	-				
15 min	1.000	1.000	-			
20 min	1.000	1.000	1.000	-		
25 min	1.000	1.000	1.000	1.000	-	
30 min	1.000	1.000	1.000	1.000	1.000	-
500 Hz						
	5 min	10 min	15 min	20 min	25 min	30 min
5 min	-					
10 min	< **0.001**	-				
15 min	0.743	< **0.001**	-			
20 min	1.000	< **0.001**	1.000	-		
25 min	0.270	< **0.001**	1.000	1.000	-	
30 min	1.000	< **0.001**	1.000	1.000	0.484	-
1000 Hz						
	5 min	10 min	15 min	20 min	25 min	30 min
5 min	-					
10 min	1.000	-				
15 min	1.000	1.000	-			
20 min	1.000	0.119	**0.007**	-		
25 min	0.246	**0.009**	**0.003**	0.449	-	
30 min	1.000	1.000	1.000	**0.002**	**0.001**	-
Pali chanting						
	5 min	10 min	15 min	20 min	25 min	30 min
5 min	-					
10 min	< **0.001**	-				
15 min	< **0.001**	0.568	-			
20 min	< **0.001**	0.106	1.000	-		
25 min	< **0.001**	1.000	0.348	**0.049**	-	
30 min	< **0.001**	0.966	1.000	1.000	0.381	-

Fig. 2B shows the post-hoc analysis for each Time for the continuous treatment (CS) and generally two trends can be observed. For 10, 20 and 25 min time, the absorbance rate for the 500 Hz frequency was significantly larger than 1000 Hz ($p < 0.001$, $p = 0.019$ and $p = 0.005$, respectively). The second trend involved all time series from 5 min to 30 min such that the result for Pali chant was significantly larger than all other 3 groups (all $p < 0.001$). The only exception is at 10 min with the absorbance rate of Pali chant being only significantly larger than Control and 1000 Hz (both $p < 0.001$).

The impact of different time duration on the new and continuous batch of specimen

To compare the difference between the new specimen (NS) and continuous specimen (CS), the measurements for each Time were analyzed separately for the Group and Frequency factors (Fig. 3 and Table 3).

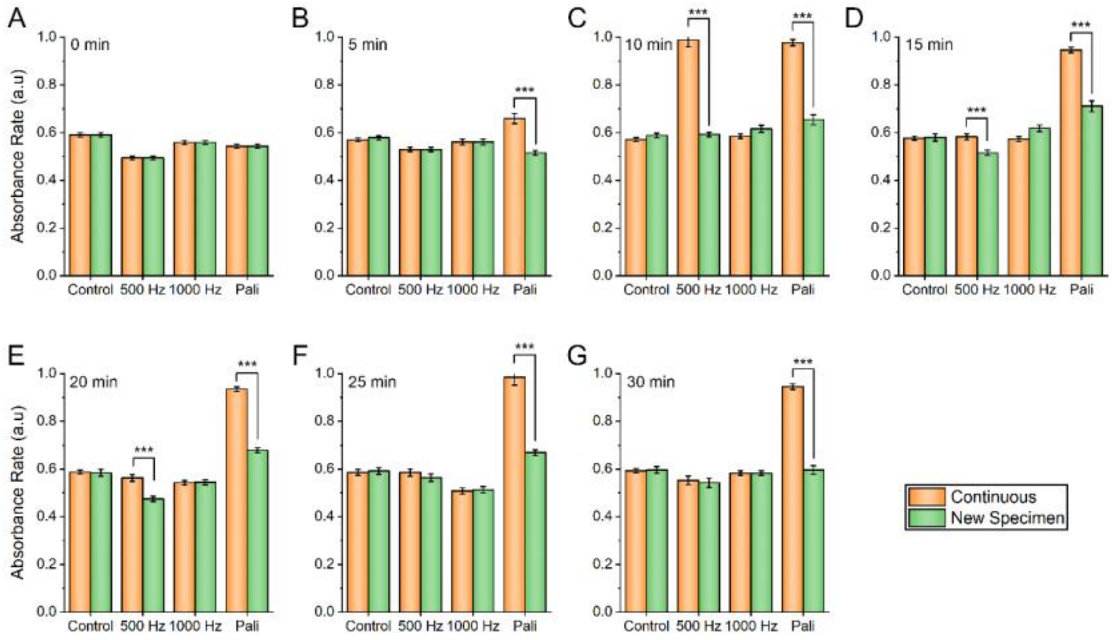

Fig. 3 Comparison between the new specimen and continuous group at different times at (A) 0 min, (B) 5 min, (C) 10 min, (D) 15 min, (E) 20 min, (F) 25 min and (G) 30 min. *** - $p < 0.001$.

Table 3: Post-hoc comparisons of the frequencies at different times for the new specimen and continuous group. The values are the *p* values and significant *p* values are bolded.

New Specimen					Continuous			
5 min					5 min			
	Control	500 Hz	1000 Hz	Pali	Control	500 Hz	1000 Hz	Pali
Control	-				-			
500 Hz	**0.017**	-			0.075	-		
1000 Hz	1.000	0.374	-		1.000	0.374	-	
Pali	**< 0.001**	1.000	**0.044**	-	**< 0.001**	**< 0.001**	**< 0.001**	-
10 min					10 min			
	Control	500 Hz	1000 Hz	Pali	Control	500 Hz	1000 Hz	Pali
Control	-				-			
500 Hz	1.000	-			**< 0.001**	-		
1000 Hz	1.000	1.000	-		1.000	**< 0.001**	-	
Pali	**0.016**	**0.044**	0.566	-	**< 0.001**	1.000	**< 0.001**	-
15 min					15 min			
	Control	500 Hz	1000 Hz	Pali	Control	500 Hz	1000 Hz	Pali
Control	-				-			
500 Hz	**0.006**	-			1.000	-		
1000 Hz	0.296	**< 0.001**	-		1.000	1.000	-	
Pali	**< 0.001**	**< 0.001**	**< 0.001**	-	**< 0.001**	**< 0.001**	**< 0.001**	-
20 min					20 min			
	Control	500 Hz	1000 Hz	Pali	Control	500 Hz	1000 Hz	Pali
Control	-				-			
500 Hz	**< 0.001**	-			0.682	-		
1000 Hz	0.074	**< 0.001**	-		**0.032**	1.000	-	
Pali	**< 0.001**	**< 0.001**	**< 0.001**	-	**< 0.001**	**< 0.001**	**< 0.001**	-
25 min					25 min			
	Control	500 Hz	1000 Hz	Pali	Control	500 Hz	1000 Hz	Pali
Control	-				-			
500 Hz	1.000	-			1.000	-		
1000 Hz	**0.008**	0.251	-		**0.010**	**0.016**	-	
Pali	**0.011**	**< 0.001**	**< 0.001**	-	**< 0.001**	**< 0.001**	**< 0.001**	-
30 min					30 min			
	Control	500 Hz	1000 Hz	Pali	Control	500 Hz	1000 Hz	Pali
Control	-				-			

500 Hz	**0.047**	-			0.257	-		
1000 Hz	1.000	0.317	-		1.000	0.847	-	
Pali	1.000	0.062	1.000	-	**< 0.001**	**< 0.001**	**< 0.001**	-

0 min

There was a significant Frequency main effect ($F(3, 76) = 43.242$, $p < 0.001$, $\eta_p^2 = 0.631$). Post-hoc analysis revealed that the absorbance rate of the Control was significantly larger than all other 3 frequencies ($p < 0.001$, $p = 0.002$ and $p < 0.001$, respectively). Besides that, the result for the 500 Hz frequency was found to be significantly smaller than 1000 Hz and Pali chant as well (both $p < 0.001$) (Table 3).

5 min

There was a significant Group main effect ($F(1, 76) = 17.509$, $p < 0.001$, $\eta_p^2 = 0.187$), a significant Frequency main effect ($F(3, 76) = 9.036$, $p < 0.001$, $\eta_p^2 = 0.263$) and a significant Group × Frequency interaction ($F(3, 76) = 20.107$, $p < 0.001$, $\eta_p^2 = 0.442$). In terms of the frequencies, the results showed that the New Specimen's (NS) absorbance rate when no frequency was applied, i.e. Control, was significantly larger than both 500 Hz and Pali chanting ($p = 0.017$ and $p = 0.001$, respectively). Further, the absorbance rate for the 1000 Hz frequency was also significantly larger than that of Pali chant with $p = 0.044$. For the Continuous group (CS), it was found that the result for Pali chanting was significantly larger than all other 3 frequencies (all $p < 0.001$). In terms of the groups, the continuous treatment (CS) produced an absorbance rate significantly larger than that of the new specimen (NS) ($p < 0.001$; Fig. 3B).

10 min

There was a significant Group main effect ($F(1, 76) = 235.119$, $p < 0.001$, $\eta_p^2 = 0.756$), a significant Frequency main effect ($F(3, 76) = 129.616$, $p < 0.001$, $\eta_p^2 = 0.837$) and a significant Group × Frequency interaction ($F(3, 76) = 104.281$, $p < 0.001$, $\eta_p^2 = 0.805$). As shown in Table 3, the Pali chanting produced an absorbance rate significantly larger than both Control and 500 Hz ($p = 0.016$ and $p = 0.044$, respectively) for the New Specimen group (NS) . As for the Continuous group CS), the result for the Control was significantly smaller than 500 Hz and Pali (both $p < 0.001$). Other than that, the result for the 1000 Hz frequency was significantly larger and smaller, respectively, than 500 Hz and Pali chanting (both $p < 0.001$). In terms of the group difference at fixed frequencies, at both 500 Hz and Pali frequencies, the continuous treatment produced an absorbance rate that was significantly larger (both $p < 0.001$) (Fig. 3C).

15 min

There was a significant Group main effect ($F(1, 76) = 42.907$, $p < 0.001$, $\eta_p^2 = 0.361$), a significant Frequency main effect ($F(3, 76) = 171.067$, $p < 0.001$, $\eta_p^2 = 0.871$) and a significant Group × Frequency interaction ($F(3, 76) = 39.883$, $p < 0.001$, $\eta_p^2 = 0.612$). The New Specimen

group (NS) , the absorbance rate of the 500 Hz was significantly smaller than all other 3 auditory stimuli (p = 0.006, p < 0.001 and p < 0.001, respectively) whereas the Pali chant produced an absorbance rate that was significantly larger than others (all p < 0.001) (Table 3). As for the Continuous specimen group (CS), the result for the Pali chant was also significantly larger than all stimuli (all p < 0.001). Like the results at 10 min, the absorbance rate for the continuous treatment group (CS) was significantly larger than the New specimen group (NS) for both 500 Hz and Pali frequency (both p < 0.001). However, the new specimen (NS) result was significantly larger than the continuous treatment (CS) instead at 1000 Hz frequency (p = 0.027) as shown in Fig. 3D.

20 min

There was a significant Group main effect (F(1, 76) = 117.765, p < 0.001, η_p^2 = 0.608), a significant Frequency main effect (F(3, 76) = 262.233, p < 0.001, η_p^2 = 0.912) and a significant Group × Frequency interaction (F(3, 76) = 56.011, p < 0.001, η_p^2 = 0.689). Similar to 15 min, the new specimen group at 500 Hz frequency was observed to have a significantly smaller absorbance rate than the other 3 frequencies (all p < 0.001) and the result for Pali chanting being significantly larger than all other 3 frequencies (all p < 0.001) (Table 3). On the other hand, for the continuous group (CS), the Pali chanting resulted in a significantly larger absorbance rate than all other frequencies (all p < 0.001) with the addition of the Control group also being significantly larger that of 1000 Hz as well (p = 0.032). As shown in Fig.3E, at 500 Hz and Pali frequencies, the Continuous group (CS) had a significantly larger absorbance rate than the New Specimen group (NS) (both p < 0.001).

25 min

There was a significant Group main effect (F(1, 76) = 44.669, p < 0.001, η_p^2 = 0.370), a significant Frequency main effect (F(3, 76) = 124.428, p < 0.001, η_p^2 = 0.831) and a significant Group × Frequency interaction (F(3, 76) = 40.202, p < 0.001, η_p^2 = 0.613). Post-hoc analysis comparing the frequencies revealed that for the new specimen group, the Pali chanting resulted in an absorbance rate significantly larger than all other 3 frequencies (p = 0.011, p < 0.001 and p < 0.001, respectively). Further, it was also observed that the result for the Control was significantly larger than that of the 1000 Hz frequency (p = 0.008). As for the Continuous group (CS), the absorbance rate for the 1000 Hz was significantly smaller than all other 3 frequencies (p = 0.010, p = 0.016 and p < 0.001, respectively) whereas the result for the Pali chant was significantly larger than all other 3 frequencies (all p < 0.001) (Table 3). In terms of post-hoc comparisons across the groups, the Pali frequency was the only frequency that produced a significantly larger absorbance rate (p < 0.001) when comparing the continuous group (CS) to the new specimen group (NS) (Fig.3F).

30 min

There was a significant Group main effect ($F(1, 76) = 80.423$, $p < 0.001$, $\eta_p^2 = 0.514$), a significant Frequency main effect ($F(3, 76) = 97.902$, $p < 0.001$, $\eta_p^2 = 0.794$) and a significant Group × Frequency interaction ($F(3, 76) = 74.815$, $p < 0.001$, $\eta_p^2 = 0.747$). Post-hoc analysis stratifying according to Group revealed that for the New Specimen group (NS), the Control frequency had a significantly larger absorbance rate than 500 Hz ($p = 0.047$). For the continuous group, the result for the Pali chant was significantly larger than all 3 other frequencies (all $p < 0.001$) (Table 3). Similar to 10 - 25 min, post-hoc analysis stratified for Frequency revealed that the Continuous treatment (CS) produced an absorbance rate that was significantly larger than the New Specimen group (NS) ($p < 0.001$) as shown in Fig.3G.

Discussion

The study was aimed to provide a quantitative study on cell consciousness investigation by providing a new insight into the effect of sound using single tone frequencies at 500 Hz, 1000 Hz and Pali chant on the cell metabolism rate for different treatment time durations ranging from 5 min to 30 min (5 min interval). Further, the effect of the same settings on the new batch of specimen (NS) and continuous batch of specimen (CS) were observed as well. The profound finding shows that the absorbance rate which indicated the metabolism rate for continuous batch of specimen was significantly increased as compared to the new batch of specimen (NS) for Pali chant for all the time durations for sound treatment. Besides that, 10 min of sound treatment at 500 Hz displayed an equally significant modulation on the metabolism rate of the specimen for the continuous batch (CS).

It is worth to take a tour on the speculation for a deeper study on the mechanism of the cell to propagate in a profound manner when stimuli are given. Of note, prior study suggested that the cell membranes on cytoskeletal models with G-protein dynamics is a promising starting point to link psychiatry to quantum models of mind, brain and consciousness (Tonello and Cocchi 2010). A review has reported that lipid rafts, a type of G proteins, exist in the nervous system and these proteins were mostly studied for the regulatory and trafficking of signal transduction(Brady et al. 2012)(Chini and Parenti 2004) and membrane interactions between G proteins and other related proteins was reported(Vögler et al. 2008). The fundamental study on this cytoskeletal element in animals are believed to function in orchestrating the neurotransmitter signalling and the characterization of the element is associated to the area of neurological or psychiatric diseases in human being(Allen et al. 2007). For instance, to clarify neural factors that contribute to depression and to allow a deeper understanding of the neural trafficking causality which leads to depression at the molecular level is highly demanded as this can increase the efficacy of antidepressants(Senese et al. 2018).

Furthermore, the biomolecular pathway related to cell membrane viscosity through Gsα protein and tubulin was hypothesized to enable the measurement of conscious state using electroencephalography on the brain's γ wave synchrony(Cocchi et al. 2010). When sample subject on lipid raft is down-sized to microorganism, eukaryotic cells were studied as a fundamental step to understand the evolution of cellular complexity(Bramkamp and Lopez 2015). In contrast, membrane of single cell without nucleus, i.e. prokaryotic cells have been used to elucidate the effect of FtsZ in regulating cell division(Margolin 2000). The close approach of prior study in association with this is found from a study investigating the lipid raft-mediated transcytotic pathways of *E.coli k-12* to cross the intestinal epithelium indicated that the poor invasive enteric of bacteria to gut epithelial cell during inflammatory stress(Clark et al. 2005). Lipid rafts enriches in cholesterol and sphingolipids that are involved in the lateral compartmentalization of molecules at the cell surface is getting great interest as part of a cellular element that enable cell signalling study. However, association between the metabolism rate of prokaryotic cell relating to lipid raft has yet to be found. In this study, *E.coli k-12* in the prokaryotic cell group has been studied for its response based on the metabolism rate affected by different sound stimuli.

Prior study showed the glucose effect on catabolite repression to induce a positive control of transcription whereby glucose repressed the inducible enzymes(Clark et al. 2005) as well as temperature effect on the metabolism rate of bacteria with optical density measurement (OD)(Membré et al. 2005). Another report was found to obtain an increase in linear vibrational effect on the metabolism rate of bacteria included *E.coli k-12* whom had employed the optical density measurement as metabolism rate monitoring system(College et al. 2001). On the other hand, vibration through ultrasound was adopted to monitor the metabolism rate of the bacterial cells that were adhered to polymer rods revealed that the bacteria growth increased after 30 min of low frequency, low acoustical intensity ultrasound treatment as compared to without ultrasound and this lead to the hypothesis of ultrasound elevating the rate of oxygen and nutrients to and out from the cells which propagated the metabolism rate(Pitt and Ross 2003). *E.coli k-12* was selected as the specimen for this study as a similar strain of specimen was used on different acoustical treatments. These studies had reported an agreeable result of increased *E.coli k-12* metabolism rate with audible sound stimuli compared to control group and this indicated that *E.coli k-12* responded rapidly to sound stress through promoting the synthesis of intracellular RNA(Gu et al. 2016).

One of the prior study which possess a similar aim with our work here had reported that the significant effect on growth promotion at 100 dB and 5000 Hz at multiple variation of the audible sound frequencies for *E.coli k-12* indicated that their activity of antioxidant enzymes increased in which they speculated the audible sound may trigger a secondary oxidative stress(Gu et al. 2013). Similarly, another research had experimented on mono frequency at 300 Hz with different loudness and had found the most significant effect on Chromobacterium violaceum was at the sound level of 13 dB(Kothari et al.). Most of the studies had aimed on understand better the environment audible effect on the growth of the *E.coli k-12* and other

prokaryotic bacteria where variable range of frequencies had been set as parameter. Nevertheless, the use of single tone frequency at 500 Hz and 1000 Hz including different time duration treatments has limited reporting in literature. In our study, the metabolism rate of *E.coli k-12* decreased profoundly at 500 Hz for 15 min and 20 min of sound treatment as compared to the single tone of 1000 Hz whereas after 15 min the result showed a decrease in growth for specimen for the new batch of specimen group. At 25 min, the sound treatment at 1000 Hz showed a decrease in the growth as compared to the control group.

Besides single frequency sound to stimulate the growth of bacteria, Indian classical music consists a range of frequencies from 41 – 645 Hz had been reported bacteria exhibiting antibiotic susceptibility under the influence of music suggesting that the production of cell was linked with the quorum sensing(Sarvaiya and Kothari 2017). There is no similar research studying bacteria response in human hymning sound and moreover for a series of treatment time. In this study, the *E.coli k-12* obtained profound metabolism rate from 5 min to 30 min under the stimulation of Buddhist Pali chant as the natural human hymning sound for both new and continuous batch of specimen in the whole range of treatment time series. For new batch of specimen on each time treatment, the metabolism rate shows significant results at 15 min, 20 min and 25 min in Pali chant sound treatment. However, the appalling finding from this research is the metabolism rate of the continuous batch specimen experienced a great increased surpassing other sound treatment and this trend was the same from 5 min to 30 min. The dominant finding from the Pali chant effects on the metabolism rate in the continuous batch of specimen lead to a new insight on cell memory.

Does cell or bacteria have memory? Chih et at published a very recent work reporting on the memory encoding for bacteria which mimics the neurons whereby the stimuli were based on light and measured on the response of potassium channels in a biofilm and the modelling results predicted from Hodgkin-Huxley model explained that the memory is athletic to ionic perturbations(Yang et al. 2020). Another robust finding on cell memory was studied with single bacterial cells through repeated exposure to salt stress and had discovered that the resiliency of the past exposure cells displayed a memory-like behaviour at the population level(Mathis and Ackermann 2016).

Repetitive religious chanting has been reported to be able to pacify the negative mind of first timer involved in Buddhist chanting and this chanting was able to modulate the brain responses during the late-stage cognitive processing as revealed by event related potential study (Gao et al. 2017), consistent to another report that highlighted the benefit of the repetitive “OM” sound which worked as a brain stabilizer through frequency spectrum analysis(Gurjar et al.). On the other hand, repetitive chant brought significant effect to hypothermia induced stress on cognitive abilities(Pereira 2016). The decision making of human being or animal is defined as the process of selecting an action in which the action stems from memory which is defined as the physical change that carries information about the historical past happening between neurons and wrap into cognition(Del Missier et al. 2013).

The accumulation effect is speculated in this study given that Pali chanting cause the metabolism rate of both the new batch and continuous batch of specimen to have a profound increase in the metabolism rate from 5 min till 30 min with the most significant results happening at 5 min. The metabolism rate has surpassed the duplication rate of the normal metabolism rate of *E.coli k-12* which is about ~20 min. This has enabled the analogy of the cognition of a human participating in this experiment who has received three different stimuli and one's physical response is measured. Similarly, the *E.coli k-12* in this experiment had decided to grow more under Pali chant sound treatment from 5 min till 25 min of treatment durations as compared to single tone frequency.

The limitation of this work is the variety of measurement tools to further observe the changes on the cells. Further investigation is great to include compartments of the unicellular cells, for example, lipid rafts on the cell membranes, RNA, quorum sensing, ion channels and more protein composition changes measurement. These measurements can provide a more in-depth information on the cell propagation mechanism in responding to the auditory stimuli. More religion or healing music can be used as stimuli to measure the metabolism rate of similar specimens. Thus far, the clue on why Pali chant has triggered a great response from this strain of bacteria has gone unknown but enigmatical.

In this study, both new batch specimen and continuous batch specimen of the prokaryotic *E.coli k-12* cells responded with significant metabolism rate in Pali chant as compared to single tone frequency sounds in a short durations of sound treatment, moreover the profound increased in metabolism rate in the continuous batch of specimen. The outcome has indicated its potential in using prokaryotic cells as a simple complex unicellular model on behavioural study which lessen the bias in interpreting the stimuli can contribute to the area of mind and body studies, neuropsychiatry area, experimental quantum mind and neuropharmacology.

Received April 1, 2021; Accepted May 22, 2021

References

Allen JA, Halverson-Tamboli RA, Rasenick MM (2007) Lipid raft microdomains and neurotransmitter signalling. Nat. Rev. Neurosci. 8:128–140

Arganda S, Pérez-Escudero A, De Polavieja GG (2012) A common rule for decision making in animal collectives across species. Proc Natl Acad Sci U S A 109:20508–20513. https://doi.org/10.1073/pnas.1210664109

Ashino K, Sugano K, Amagasa T, Ying BW (2019) Predicting the decision making chemicals used for bacterial growth. Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-43587-8

Bekinschtein TA, Dehaene S, Rohaut B, et al (2009) Neural signature of the conscious processing of auditory regularities. Proc Natl Acad Sci U S A 106:1672–1677.

https://doi.org/10.1073/pnas.0809667106

Brady ST, Siegel GJ, Albers RW, Price DL (2012) Basic Neurochemistry. Elsevier

Bramkamp M, Lopez D (2015) Exploring the Existence of Lipid Rafts in Bacteria. Microbiol Mol Biol Rev 79:81–100. https://doi.org/10.1128/mmbr.00036-14

Brancucci A, Franciotti R, D'Anselmo A, et al (2011) The sound of consciousness: Neural underpinnings of auditory perception. J Neurosci 31:16611–16618. https://doi.org/10.1523/JNEUROSCI.3949-11.2011

Chini B, Parenti M (2004) G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there?

Clark E, Hoare C, Tanianis-Hughes J, et al (2005) Interferon γ induces translocation of commensal Escherichia coli across gut epithelial cells via a lipid raft-mediated process. Gastroenterology 128:1258–1267. https://doi.org/10.1053/j.gastro.2005.01.046

Cocchi M, Tonello L, Rasenick MM (2010) Human depression: A new approach in quantitative psychiatry. Ann. Gen. Psychiatry 9:1–6

College J, Juergensmeyer EA, Elizabeth MAJ, Juergensmeyer A (2001) EFFECT OF VIBRATION ON BACTERIAL GROWTH AND ANTIBIOTIC RESISTANCE

Condon D, Zhu M, Bowring S, et al (2005) U-Pb ages from the neoproterozoic Doushantuo Formation, China. Science (80-) 308:95–98. https://doi.org/10.1126/science.1107765

Del Missier F, Mäntylä T, Hansson P, et al (2013) The multifold relationship between memory and decision. J Exp Psychol Learn Mem 39:1344–1364. https://doi.org/10.1037/a0032379

Gao J, Fan J, Wu BW, et al (2017) Repetitive Religious Chanting Modulates the Late-Stage Brain Response to Fear- and Stress-Provoking Pictures. Front Psychol 7:2055. https://doi.org/10.3389/fpsyg.2016.02055

Gao J, Leung HK, Wu BWY, et al (2019) The neurophysiological correlates of religious chanting. Sci Rep 9:1–9. https://doi.org/10.1038/s41598-019-40200-w

Gu S Bin, Yang B, Wu Y, et al (2013) Growth and physiological characteristics of E. coli in response to the exposure of sound field. Pakistan J Biol Sci 16:969–975. https://doi.org/10.3923/pjbs.2013.969.975

Gu S, Zhang Y, Wu Y (2016) Effects of sound exposure on the growth and intracellular macromolecular synthesis of E. coli k-12. PeerJ 2016:. https://doi.org/10.7717/peerj.1920

Gurjar AA, Ladhake SA, Thakare AP Analysis Of Acoustic of "OM" Chant To Study It's Effect on Nervous System

Hettich DT, Bolinger E, Matuz T, et al (2016) EEG Responses to Auditory Stimuli for Automatic Affect Recognition. Front Neurosci 10:244. https://doi.org/10.3389/fnins.2016.00244

Hoy R (2005) Animal awareness: The (un)binding of multisensory cues in decision making by animals. Proc. Natl. Acad. Sci. U. S. A. 102:2267–2268

Klapproth F (2008) Time and decision making in humans. Cogn. Affect. Behav. Neurosci. 8:509–524

Kothari V, Joshi C, Patel P, et al Influence of a mono-frequency sound on bacteria can be a function of the sound-level. https://doi.org/10.1101/071746

Lau LL, Lau W (2020) All Life Vital phenomena: life, information, and consciousness. https://doi.org/10.1080/26895293.2020.1734670

Li F, Menon A, Allen JB (2010) A psychoacoustic method to find the perceptual cues of stop consonants in natural speech. J Acoust Soc Am 127:2599–2610. https://doi.org/10.1121/1.3295689

Lyon P (2015) The cognitive cell: Bacterial behavior reconsidered. Front. Microbiol. 6

Margolin W (2000) Themes and variations in prokaryotic cell division. FEMS Microbiol Rev 24:531–548. https://doi.org/10.1111/j.1574-6976.2000.tb00554.x

Mathis R, Ackermann M (2016) Response of single bacterial cells to stress gives rise to complex history dependence at the population level. Proc Natl Acad Sci U S A 113:4224–4229. https://doi.org/10.1073/pnas.1511509113

Membré J-M, Leporq BB, Le Vialette M, et al (2005) Temperature effect on bacterial growth rate: quantitative microbiology approach including cardinal values and variability estimates to perform growth simulations on/in food. Int J Food Microbiol 100:179–186. https://doi.org/10.1016/j.ijfoodmicro.2004.10.015

Moore B (2007) Psychoacoustics. In: Springer Handbook of Acoustics. Springer New York, New York, NY, pp 459–501

Norman-Haignere S V., McDermott JH (2018) Neural responses to natural and model-matched stimuli reveal distinct computations in primary and nonprimary auditory cortex. PLoS Biol 16:e2005127. https://doi.org/10.1371/journal.pbio.2005127

Pereira C (2016) Effect of hypothermia on cognitive capabilities in snail (Achatina fulica) and their recovery post exposure to the Buddhist meditative chant " Om Mani Padme Hum ." J Entomol Zool Stud 4:1–6

Persinger MA (2014) Infrasound, human health, and adaptation: An integrative overview of recondite hazards in a complex environment. Nat Hazards 70:501–525. https://doi.org/10.1007/s11069-013-0827-3

Pitt WG, Ross SA (2003) Ultrasound increases the rate of bacterial cell growth. Biotechnol Prog 19:1038–1044. https://doi.org/10.1021/bp0340685

Poh Foong L (2009) ELECTROPHORETIC STUDIES OF SURFACE CHARGE ON UNICELLULAR BACTERIA ELECTROPHORETIC STUDIES OF SURFACE CHARGE ON UNICELLULAR BACTERIA LEE POH FOONG DISSERTATION PRESENTED FOR THE DOCTORATE OF PHILOSOPHY

Reis A dos, Dalmolin SP, Dallegrave E (2017) Modelos animais para avaliação auditiva: revisão de literatura. Rev CEFAC 19:417–428. https://doi.org/10.1590/1982-021620171932117

Rey R Del, Alba J, Arenas JP, Sanchis VJ (2012) An empirical modelling of porous sound absorbing materials made of recycled foam. Appl Acoust 73:604–609. https://doi.org/10.1016/j.apacoust.2011.12.009

Robles L, Ruggero MA, Rich NC (1986) Mossbauer Measurements of the Mechanical Response to Single-Tone and Two-Tone Stimuli at the Base of the Chinchilla Cochlea. Springer, Berlin, Heidelberg, pp 121–128

Sarvaiya N, Kothari V (2017) Audible sound in form of music can influence microbial growth,

metabolism and antibiotic susceptibility. https://doi.org/10.15406/jabb.2017.02.00048

Schweizer P (2013) Mind/consciousness dualism in Sankhya-Yoga philosophy. In: Metaphysics: Indian Philosophy. Taylor and Francis, pp 327–342

Senese NB, Rasenick MM, Traynor JR (2018) The role of G-proteins and G-protein regulating proteins in depressive disorders. Front. Pharmacol. 9:1289

Simão T, Caldeira S, de Carvalho E (2016) The Effect of Prayer on Patients' Health: Systematic Literature Review. Religions 7:11. https://doi.org/10.3390/rel7010011

Smith E, Duede S, Hanrahan S, et al (2013) Seeing Is Believing: Neural Representations of Visual Stimuli in Human Auditory Cortex Correlate with Illusory Auditory Perceptions. PLoS One 8:e73148. https://doi.org/10.1371/journal.pone.0073148

Smyth T (2019) Music 175: Loudness

Stevenson K, Mcvey AF, Clark IBN, et al (2016) General calibration of microbial growth in microplate readers. https://doi.org/10.1038/srep38828

Tonello L, Cocchi M (2010) The Cell Membrane: Is It A Bridge From Psychiatry To Quantum Consciousness? Mood Disorders View project Problem Behavior and Crisis in Autism: a mutlidisciplinary approach View project. https://doi.org/10.14704/nq.2010.8.1.268

Vögler O, Barceló JM, Ribas C, Escribá P V. (2008) Membrane interactions of G proteins and other related proteins. Biochim. Biophys. Acta - Biomembr. 1778:1640–1652

Wokke ME, Achoui D, Cleeremans A (2020) Action information contributes to metacognitive decision-making. Sci Rep 10:1–15. https://doi.org/10.1038/s41598-020-60382-y

Wöstmann M, Waschke L, Obleser J (2019) Prestimulus neural alpha power predicts confidence in discriminating identical auditory stimuli. Eur J Neurosci 49:94–105. https://doi.org/10.1111/ejn.14226

Yang CY, Bialecka-Fornal M, Weatherwax C, et al (2020) Encoding Membrane-Potential-Based Memory within a Microbial Community. Cell Syst 10:417-423.e3. https://doi.org/10.1016/j.cels.2020.04.002

Yu L, Huang Q, Hu C, Ye M (2016) Immediate effects of different frequencies of auditory stimulation on lower limb motor function of healthy people. J Phys Ther Sci 28:2178–2180. https://doi.org/10.1589/jpts.28.2178

Zeh HD (1970) On the interpretation of measurement in quantum theory. Found Phys 1:69–76. https://doi.org/10.1007/BF00708656