Article

Chanting of Nam-Myoho-Renge-Kyo in the Context of the Buddhist Liturgy of Nichiren Shoshu: Study of Sound Frequencies, Brain Activity, and Microbial Metabolism

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CHANTING OF *NAM-MYOHO-RENGE-KYO* IN THE CONTEXT OF THE BUDDHIST LITURGY OF NICHIREN SHOSHU: STUDY OF SOUND FREQUENCIES, BRAIN ACTIVITY, AND MICROBIAL METABOLISM

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Abstract: This study investigates the effects of chanting *Nam-Myoho-Renge-Kyo* by a long-term Nichiren Shoshu Buddhist practitioner on sound frequencies (A), brain activity (B), and microbial metabolism (C). A. Analysis of sound frequencies: Spectral analysis of chanting revealed peaks at 8 Hz and 116 Hz, corresponding to the first Schumann resonance and a frequency linked to chloride ion movements, respectively. Additionally, five peaks (417, 528, 639, 741, 852 Hz) corresponding to solfeggio frequencies were identified. These frequencies are known to exert a positive impact on the human endocrine and autonomous nervous systems; improve survival of human brain cells; decrease anxiety in rats; reverse cognitive and endocrine deficits in zebra fish; reduce total concentration of reactive oxidative species in brain tissue. B. Study of Brain activity: Brain activity changes were measured using functional near-infrared spectroscopy. Prefrontal cortex activity increased modestly during chanting compared to a pre-chanting baseline, but significantly increased afterwards. This suggests a shift towards focused attention during chanting and enhanced activity afterward. C. Effects on microbial metabolism: Probiotic cultures exposed to chanting, either directly or indirectly, showed increased metabolic activity. This suggests local and non-local effects, similar to previously reported phenomena. Importantly, this study clarifies that the spiritual practice of Nichiren Shoshu Buddhism transcends mere neurophysiological explanations. While brain activity and other bodily processes may be correlated with the practice, the religious and spiritual experience of chanting *Nam-Myoho-Renge-Kyo* encompasses a mystical dimension that, though not contradicting science, cannot be fully explained by scientific methods alone.

Keywords: Buddhism; prefrontal cortex; brain activity; sound; solfeggio frequencies; changing frequencies; microbial metabolism

1. Introduction

Nichiren Shoshu is a school of Buddhism established in Japan over 750 years ago. According to Nichiren Shoshu tradition, Shakyamuni, the historical Buddha who lived and taught in India in the sixth century BCE, prophesied in the Lotus Sutra, his final teaching, that the original Buddha from the time without beginning would appear approximately 2,000 years after his passing to guide humanity during a dark age. Nichiren Daishonin is identified by Nichiren Shoshu as this prophesied Buddha. He revealed the Lotus Sutra as the ultimate law and essence of life, teaching that all people have the inherent potential to achieve enlightenment. His practice is universally accessible, allowing anyone, regardless of background, to unlock their inherent potential for happiness and enlightenment in their current lifetime. Central to Nichiren Shoshu practice is the chanting of *Nam-Myoho-Renge-Kyo*, or Daimoku (Japanese for "Sacred Title"), while focusing on the Gohonzon, the True Object of Worship. As per Nichiren Shoshu liturgy, believers chant *Nam-Myoho-Renge-Kyo* vocally every day for a variable number of times, keeping their eyes open and focused on the Gohonzon.

The literal translation and meaning of *Nam-Myoho- Renge-Kyo* are complex. The first word, Nam derives from the word namas in Sanskrit and can be translated as "devotion". Myoho-Renge-Kyo is the title of the Lotus Sutra in Japanese. It's important to note that while the Daimoku is Japanese, its origin is more complex:

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Myoho (妙法) comes from Chinese characters meaning "mystic" or "wonderful law." It refers to the profound and difficult-to-grasp nature of the law or principle enshrined within the Lotus Sutra. It also refers to the dual nature of existence, unfathomable (mystic) and manifest (law).

Renge (蓮華) comes from Chinese characters meaning "lotus flower." The lotus flower is a symbol of purity and enlightenment, as it grows and blooms beautifully despite arising from muddy water. It represents the potential for enlightenment to emerge even amidst life's challenges. It also represents the simultaneity of cause and effect.

Kyo (経) comes from a Chinese character meaning "sutra" or "teaching." It can also be loosely interpreted as "sound".

These characters were adopted and pronounced according to Japanese phonetics, forming the Japanese term Myoho-Renge-Kyo.

According to Nichiren Shoshu, *Nam-Myoho-Renge-Kyo* is the designation, core, characteristic, purpose, and teaching for the fundamental reality that underlies all existence. Because of this complexity, expressing its meaning in a simple way is nearly impossible. However, a basic interpretation could be offered as "uniting with the mysterious essence of all things, embodying both cause and resulting effect."

The School of Nichiren Shoshu teaches that there are four essential powers required to accomplish the attainment of enlightenment. They are called the “Four Powers of the Mystic Law.” These four kinds of powers and functions are present in the Gohonzon, the Object of Worship. They are: the power of the Buddha, the power of the Law, the power of faith, and the power of practice. The power of the Buddha and the power of the Law are the two powers that believers solely rely upon for their enlightenment; the powers of faith and practice refer to the believers own power or effort. When the powers of the Buddha and the Law inherent in the Gohonzon fuse with the powers of faith and practice exerted by the believer, one can enjoy the great benefit of enlightenment. (For review on Nichiren Shoshu practice, please see "The Doctrines and Practice of Nichiren Shoshu").

A recent study (Ruggiero 2023) describes how chanting *Nam-Myoho-Renge-Kyo* within the liturgical practices of Nichiren Shoshu Buddhism produces a unique electromagnetic/vibrational signature. This signature, according to the Orch OR theory of consciousness (Hameroff and Penrose, 2014), enhances quantum computations within brain microtubules. In the article published in 2023, it was described how the brain provides a shared medium for both sound and electromagnetic waves since this medium possesses electrical properties that fluctuate in response to mechanical strain. Microtubules, essential components of the cellular cytoskeleton, act as resonant cavities and these cavities are filled with piezoelectric materials, such as proteins, allowing them to resonate with specific frequencies of either sound waves or electromagnetic waves. The resonant standing waves, either acoustic or electromagnetic in nature, create fixed patterns of acoustic and electromagnetic properties within the microtubules. These patterns are then interpreted by the microtubules' information processing machinery. The concept of microtubules functioning as Fabry-Pérot interferometers explains how they play a role in detecting and interpreting the interaction between sound and electromagnetic waves. The electromagnetic and sound waves produced during chanting interact within the context of microtubules and this interaction influences the information processing capabilities of the microtubules, thus contributing to the phenomenon of enhanced level of consciousness reported by Nichiren Shoshu Buddhism practitioners. The following sequence outlines a recursive chain of events: Brain activity and conscious intention manifest as sound waves through chanting. These sound waves influence the spatial arrangement of tubulin within neurons and potentially other cell types. As proposed in the Orch OR model (Hameroff and Penrose 2014), changes in the spatial arrangement of tubulin impact the information processing ability of microtubules. The outcomes of this modified microtubule-based quantum computation then regulates the firing of axons, ultimately leading to changes of electrochemical activity in the brain. Electromagnetic waves generated by this modified brain activity then interacts with the sound waves within the brain tissue. Microtubules detect this interaction of sound and electromagnetic waves, with their information processing capabilities further modified as a result. These changes in processing ability then influences the results of the microtubule-based computations, leading to enhanced brain activity and consciousness. In other words, voluntary repetitive sound production and exposure lead to a recursive process. On one

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hand, tubulin's sensitivity to sound waves influences the process. On the other hand, microtubules' ability to function as interferometers allows them to interpret the interactions between electromagnetic and sound waves. These interpretations then modulate axonal firing, ultimately leading to enhanced brain activity and consciousness.

Another recent article described how these changes may not be limited to the brain and how chanting *Nam-Myoho-Renge-Kyo* may act directly also on cells of the immune system, a system that has the attributes of consciousness; a system that can gain access to an amount of information greater than what is accessible to the brain since it is not constrained by brain's reducing valve that is responsible for cognitive bottlenecks (Ruggiero and Hiratsuka 2024).

Given the relevance of sound in generating the unique electromagnetic/vibrational signature that characterizes the chanting of *Nam-Myoho-Renge-Kyo*, the present study investigates the spectral frequency analysis of the chant *Nam-Myoho-Renge-Kyo* as well as the effects of chanting on brain activity and microbial metabolism.

2. Methodology

The subject of this study is the author, a long-term Buddhist practitioner introduced to Nichiren Shoshu Buddhism in 1979. The author is currently a member of the Hokkekō, the mainstream lay organization affiliated with Nichiren Shoshu. The informed consent to publish the results of the observations here reported is implicit in the authorship. The three types of observation described in this study, *i.e.* analysis of sound frequencies, study of brain activity, effects on microbial metabolism, were performed independently of each other. The study was conducted between January and June 2024. In all types of observation, the author chanted *Nam-Myoho-Renge-Kyo* in the same manner, *i.e.* at the same pace and voice volume, maintaining the traditional kneeling position called *seiza*. Breath frequency while chanting was approximately 3 breaths per minute (0.05 Hz), characterized by rapid inhalation through the nose and prolonged exhalation without pause. To control for environmental variables, chanting was always performed in the afternoon, in the author's usual practice room, under consistent lighting, temperature, and humidity conditions.

For the analysis of sound frequencies, the author recorded approximately one minute of chanting *Nam-Myoho-Renge-Kyo* on a portable computer (MacBook Air, Apple Inc., Cupertino, CA, USA) using the proprietary application Voice Memos. The audio file was then uploaded on the Free Online Audio Plot Spectrum program. This program provides spectral analysis, that, unlike real-time frequency analyzers, gives a snapshot of how frequently specific frequencies appear within an entire audio file. Instead of showing moment-to-moment fluctuations, it reveals the overall distribution of frequencies. The height of each peak on the graph corresponds to the prevalence of that particular frequency in the audio file. This analysis technique relies on an algorithm called the Fast Fourier Transform (FFT). The FFT breaks down the audio into narrow frequency bands and calculates the magnitude (or strength) of the signal present within each band. These values are then combined to create a visual representation, the spectral plot. The program analyzes the audio in segments of a specific size (samples); FFT uses a sampling size of 8192 for the analysis. It then applies the FFT to each segment and finally averages the results from all segments to create the final plot spectrum. It's important to note that this free program currently analyzes only the first minute of the uploaded audio file; this is the reason why only one minute of chanting was recorded and analyzed.

For the study of brain activity, the author chanted Nam-Myoho-Renge-Kyo approximately 2,000 times over a period of 40 minutes at a steady pace. Brain activity was studied using functional near-infrared spectroscopy (fNIRS) performed with a commercially available, wireless mobile wearable device (Mendi Innovations AB, Stockholm, Sweden). FNIRS has developed rapidly over the past few years and miniaturized, wearable and wireless devices are now widely available (Pinti *et al*. 2018). The device that gave the results described in the present study has the advantage of affordable cost and high usage flexibility; it evaluates in real-time the activity in anterior parts of the prefrontal cortex (Brodmann area 10) by measuring cerebral oxygenation and blood volume changes. Near-infrared light is used to evaluate cerebral hemodynamic responses since it has been demonstrated that synaptic brain activity is directly correlated, in a linear manner, with increase of changes in cerebral

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oxygen consumption (Sheth *et al.* 2004). This fNIRS device delivers continuous real-time measurements of oxygenated hemoglobin (HbO), deoxygenated hemoglobin (HbR). In particular, the device measures changes in: HbO value; HbR value; and dHbO+dHbR value. All changes are considered with respect to initial state and are expressed as 10^-6 mol/L. The optode setup is the following:

Left PD	30mm	2x LED	15mm	Pulse PD	15mm	Right PD

There are 3 photodiodes (PD), Left, Pulse, Right. There is a dual LED at Red 660 nm and Infrared 805 nm. The Left and Right channels are spaced 30 mm from the LED, the Pulse channel is 15 mm away. The sampling rate is at 31.23 Hz, or each row is measured every 32 ms. With respect to filtering, there is a hardware low-pass filter before sampling, at 2.5 kHz. The ambient channel measures the PD value in ambient condition => both LED are off. This value represents the contribution of ambient light and other noise sources in the diode circuitry. Since the device consists of a headband that is placed across the forehead, there is an accelerometer that allows elimination of artifacts due to excessive movement of the head. The raw data obtained with the device are expressed in lines and columns. The units in the columns are based on the digitized value from the analog to digital converter. Given the complexity of analyzing raw data, the manufacturer of the device utilizes a proprietary algorithm that converts the raw data in easily understandable measures of brain activity. Accuracy of the measurements performed by the Mendi device was previously validated by the manufacturer by comparing the results with those obtained with a stationary laboratory equipment (fNIR100, Biopac®) while running a standard cognitive test, that is a stroop color word test (Yennu *et al*. 2016; Högman and Dravniknes 2020). Because of its flexibility the device can be used to evaluate brain activity in a wide range of settings and for a number of purposes; among these, the manufacturer proposes a neurofeedback training program that is based on a proprietary application that can be freely downloaded on a common smartphone. In this program, the subject wearing the device looks at the screen of the smartphone where there is the image of a ball moving horizontally from left to right. The subject has to focus the attention on the task of making the ball move upward and, in so doing, earns points. The ball's movement upwards alongside streaks in collecting points reflects an increase in brain activity. Since brain activity increases during the sessions, the results are expressed as percent changes of brain activity in comparison with basal. Basal is calculated before beginning the neurofeedback training program with the ball. Duration of training sessions can be varied; in this study, the author performed neurofeedback sessions of 5 min. The author performed three 5 min sessions in three different moments; immediately before chanting; during chanting, that is after 20 min of chanting; and immediately after having chanted for 40 min. In the sessions performed before and after chanting, the author focused his attention on the moving ball on the screen of the smartphone as instructed by the manufacturer; however, in the session performed during chanting, the author did not look at the smartphone and kept his eyes focused on the Object of Worship with the intent of maintaining the same type of concentration he normally keeps during chanting. It is worth noting that the author had no prior familiarity with the Mendi device nor had he used the device for training purposes.

For the study on the effects of chanting *Nam-Myoho-Renge-Kyo* on microbial metabolism, commercially available probiotic cultures (Silver Spring Sagl, Arzo-Mendrisio, Switzerland) were used for fermentation experiments. The microbial composition of these cultures has been thoroughly described (Pacini and Ruggiero 2019; Pacini and Ruggiero 2020; Ruggiero 2024). In one series of experiments, the cultures were dissolved according to the manufacturer's instructions in commercial whole bovine milk; in another series of experiments, the cultures were dissolved in commercial apple juice, not from concentrate. The solutions with the dissolved cultures were then divided in six samples. Three of these samples were placed in front of the author, at about 1 meter of distance in the room where he performs his liturgical practice; the other three samples were kept in a distant room. The author chanted *Nam-Myoho-Renge-Kyo* for 30 min having the cultures placed in front of him. During recitation, the author maintained his focus on the Object of Worship and did not concentrate on "sending" any intention toward the cultures. After chanting, the cultures were brought back in the location where the control cultures had been kept and all of them were left sitting undisturbed at room temperature for 48 hours in the case of milk, 24 hours in the case of apple juice. After that, the cultures in milk were examined for changes in pH using a digital pH meter for food (Jinan Runjie Electronic Technology co., Ltd., PRC); the cultures in apple juice were examined for specific gravity

 doi:10.20944/preprints202407.0236.v2

using a hydrometer (HBO Home Brew Ohio, USA). Room temperature and humidity were identical in both locations, that is where the author chanted, and in the distant location where the controls were kept. In another series of experiments designed to assess potential non-local effects of chanting, all six samples were left in the same location, distant from the room where the author chants. Before chanting, however, in the presence of the author three of them were marked with an X; also in this case, however, the author did not attempt to "send" any conscious intention toward those samples. To this point, it is worth noticing that the author has no known psychic ability neither has he ever received any training or has any experience in so-called para-psychological practices. The rational for measuring pH or specific gravity lays in the fact that successful fermentation of milk leads to decrease of pH, whereas fermentation of apple juice leads to production of ethanol with decrease of specific gravity. Therefore changes in pH or specific gravity in comparison to controls (*i.e.* the samples left in a distant location or unmarked) are indicative of changes of microbial metabolism.

Since the chanting of *Nam-Myoho-Renge-Kyo* is characterized by a peculiar pattern of respiration with rapid inspiration and prolonged exhalation, the effects of breathing on brain movements are here reported. Morphology of brain structures was previously studied by the author and his colleagues using transcranial ultrasonography as described in Ruggiero *et al*. (2013); Bradstreet *et al*. (2014); and Ruggiero (2022). Briefly, transcranial ultrasonography of the temporal lobe of the brain was performed using an Esaote MyLabFive (Esaote, SpA, Firenze, Italy) ultrasound system with a linear probe for muscle-skeletal examination (LA 523 by Esaote). The settings were adjusted for adult transcranial imaging as described in Ruggiero *et al.* (2013). The probe was positioned onto the temporal region of the head corresponding to the squama of the temporal bone, gently tilting it until the hyper-echoic line corresponding to the squama of the temporal bone was horizontal. The results depicted in Figure 1 were obtained at the Department of Human Anatomy, Histology and Forensic Medicine of the University of Firenze, Italy. These images are here reported, and their significance discussed, in the context of the object of this article. Figure 1 below is reproduced from Ruggiero *et al*. (2013) under the terms allowed by Firenze University Press. The rationale for investigating brain movements stems from the observation that breathing-induced alterations in brain water content influence sound wave propagation. Since the speed of sound propagation is directly related to the medium's water content, these transient changes in brain hydration modulate the transmission of sound waves within the brain.

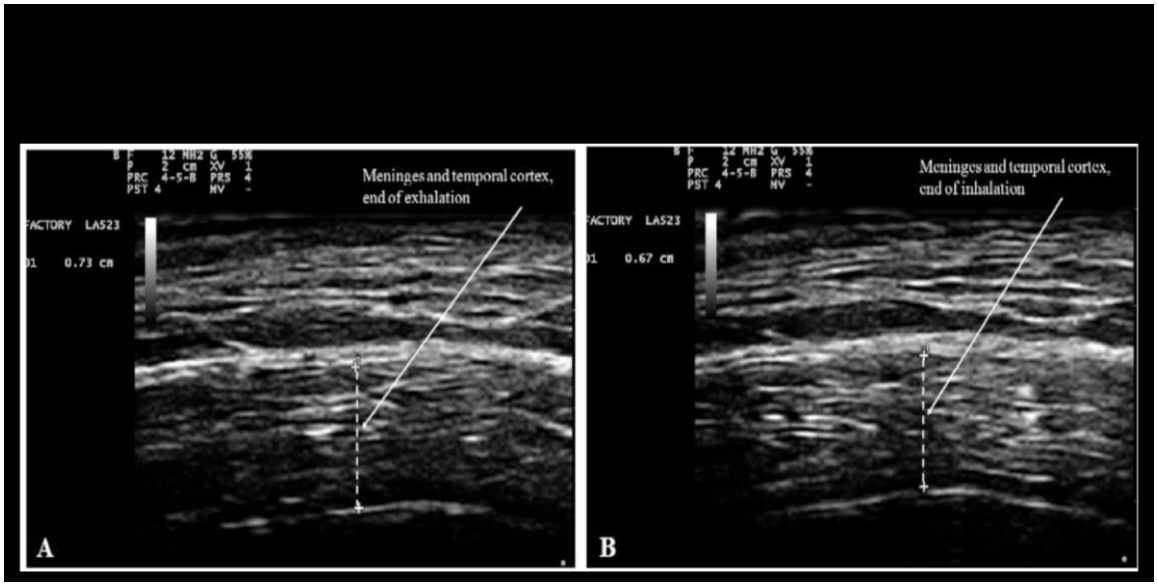

Figure 1. Measurement of combined thickness of the meninges and the cortex of the temporal lobe at the end of forced voluntary exhalation (A: 7.3 mm), and inhalation (B: 6.7 mm).

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3. Results

3.1. Analysis of Sound Frequencies

Figure 2, shows the frequencies generated by chanting *Nam-Myoho-Renge-Kyo.*

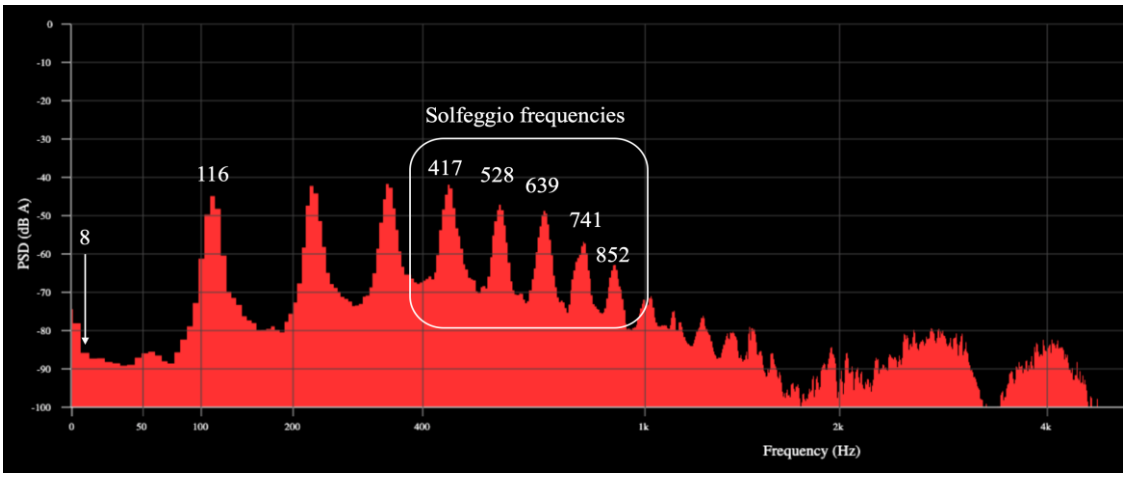

Figure 2. Spectral frequency analysis of the chant *Nam-Myoho-Renge-Kyo* performed by a long-term practitioner of Nichiren Shoshu Buddhism. This graph is representative of 10 other observations that gave qualitatively identical results.

A small, but reproducible peak can be observed around 8 Hz. In the electromagnetic spectrum, this frequency corresponds to the first Schumann resonance, that is approximately 7.83 Hz, with a diurnal/nocturnal variation of about ±0.5 Hz. A recent study suggests that daily autonomic nervous system activity may not only respond to, but also synchronize with, the dynamic magnetic fields generated by Schumann resonances (McCraty *et al*. 2017). A possible mechanism for the influence of Schumann resonance on human biology involves resonant coupling. This coupling could occur between the nervous systems and Schumann resonances that are ultra-low-frequency standing waves within the Earth-ionosphere cavity that share frequencies with physiological rhythms. As mentioned above, sound and electromagnetic waves can interact with each other in the context of microtubules; it is therefore of interest to notice that chanting *Nam-Myoho-Renge-Kyo* generates a frequency that is superimposable to the first Schumann resonance, a resonance of planet Earth that is hypothesized to be responsible for intelligence (Cherry 2003), and is associated with human health (Fdez-Arroyabe et al. 2020).

A much higher peak that corresponds to high prevalence of that particular frequency, can be observed at 116 Hz. This frequency was previously studied in the context of chanting *Nam-Myoho-Renge-Kyo* and was defined as the fundamental sound of life (Hiratsuka and Wakae 2019). It is worth noticing that exposure of seawater to a sound at 116 Hz for 30 min significantly reduced chloride ion concentration (Hiratsuka and Imamura 2020). Within the body, chloride ion reigns as the dominant physiological anion. It plays a crucial role by acting as the primary counterbalancing ion for the movement of key cations like sodium, potassium, and calcium. Focusing specifically on the role of chloride ion within the central nervous system, chloride channels are involved in several critical functions. These functions include maintaining the electrical potential across cell membranes, regulating cell volume, and influencing both cell proliferation and programmed cell death (Elorza-Vidal *et al*. 2019). Therefore, it can be hypothesized that modulation of chloride ion as result of chanting *Nam-Myoho-Renge-Kyo* may influence brain function as well as the function of other systems or the response to cancer (Kunzelmann *et al*. 2005).

Of particular interest are the five high peaks corresponding to the so-called solfeggio frequencies. Although the adjective "solfeggio" for these frequencies is inaccurate from the point of view of musicology, nevertheless, under this definition they have been the object of scientific studies published in peer-reviewed journals. A study published in 2018 (Akimoto *et al*. 2018) explored the potential stress-reducing effects of 528 Hz, one of the solfeggio frequencies, on the human endocrine

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and autonomic nervous systems. This particular frequency has gained recent attention for its purported "healing" properties. The study involved nine healthy participants (one male and eight females, aged 26-37 years) who listened to both 528 Hz and standard 440 Hz sound on separate days. Salivary biomarkers of stress (cortisol, chromogranin A, and oxytocin) were measured before and after music exposure. Additionally, the autonomic nervous system activity was continuously monitored. The Profile of Mood State, Second Edition, was administered to assess subjective stress levels. Results revealed a significant decrease in mean cortisol levels and a significant increase in oxytocin following exposure to 528 Hz. Chromogranin A levels also showed a trend towards decrease. In contrast, no significant changes in these salivary biomarkers were observed after listening to 440 Hz sound. Interestingly, the ratio of low-frequency to high-frequency autonomic nervous system activity decreased after exposure to both sound types. However, only exposure to 528 Hz resulted in a significant decrease in the coefficient of variation of R-R intervals. Furthermore, scores on the Tension-Anxiety and Total Mood Disturbance subscales of the mood state questionnaire showed a significant reduction following 528 Hz exposure, with no significant change observed after 440 Hz. The study provides evidence that 528 Hz frequency, compared to standard 440 Hz, may have a more pronounced stress-reducing effect, even with a brief exposure of only five minutes. Prior research (Babayi and Riazi 2017) suggests that exposure to a frequency of 528 Hz mitigates the toxic effects of ethanol on human brain cells. The study also reported an increase of human brain cell viability by approximately 20% following exposure to this frequency. In another study (Babayi Daylari *et al*. 2019), rats were exposed to 528 Hz and it was observed that this specific frequency increased brain testosterone production through mechanisms involving StAR and SF-1 gene expression, while reducing P450 aromatase activity. Additionally, exposure to 528 Hz led to a decrease of total reactive oxygen species (ROS) within brain tissue. Furthermore, prolonged exposure correlated with reduced anxiety-like behaviors in the rats. These results are consistent with what observed in humans (Akimoto *et al*. 2018) and suggest that the effects of solfeggio frequencies are not limited to humans but appear to be widespread across the animal kingdom. A recent study (Dos Santos *et al*. 2023) supports this suggestion since solfeggio frequencies have a positive impact on zebrafish, a specie quite distant from humans from the point of view of evolution. The study by Dos Santos *et al*. (2023) investigated the effects of solfeggio-frequencies on the cognitive and endocrine responses of adult zebrafish (*Danio rerio*). Zebrafish are increasingly recognized as a valuable model organism for translational research in neuroscience and biomedical fields. The authors, examined how intermittent (2 or 6 hours, twice daily) and continuous (24-hour) exposure to solfeggio-frequencies influenced behavior, cognition, and hormonal parameters in adult zebrafish previously subjected to a disruptive 24-hour light exposure. Their findings revealed that prolonged light exposure (24 hours) induces cognitive decline in zebrafish, evident through performance in the inhibitory avoidance test. Additionally, whole-body cortisol levels, a stress marker, were elevated in these fish. Consistent with previous observations in humans and rodents, exposure to solfeggio-frequencies, regardless of the duration (2 or 6 hours twice daily, or continuous 24 hours), effectively reversed these negative effects. Collectively, these results suggest that solfeggio frequencies can positively influence both cognitive and hormonal responses in adult zebrafish. While the musicological accuracy of the term "solfeggio frequencies" remains a topic of debate, research in humans, rodents, and zebrafish provides intriguing evidence. These studies suggest that specific frequencies may offer a novel, non-invasive approach for promoting stress reduction, cellular health, and cognitive function. Since solfeggio frequencies are generated every time *Nam-Myoho-Renge-Kyo* is chanted, the Daimoku represents a unique method for harnessing the potential life improving effects associated with these specific frequencies.

3.2. Study of Brain Activity

Figure 3, shows the changes of brain activity occurring during neurofeedback training sessions performed immediately before, during, and immediately after chanting *Nam-Myoho-Renge-Kyo.*

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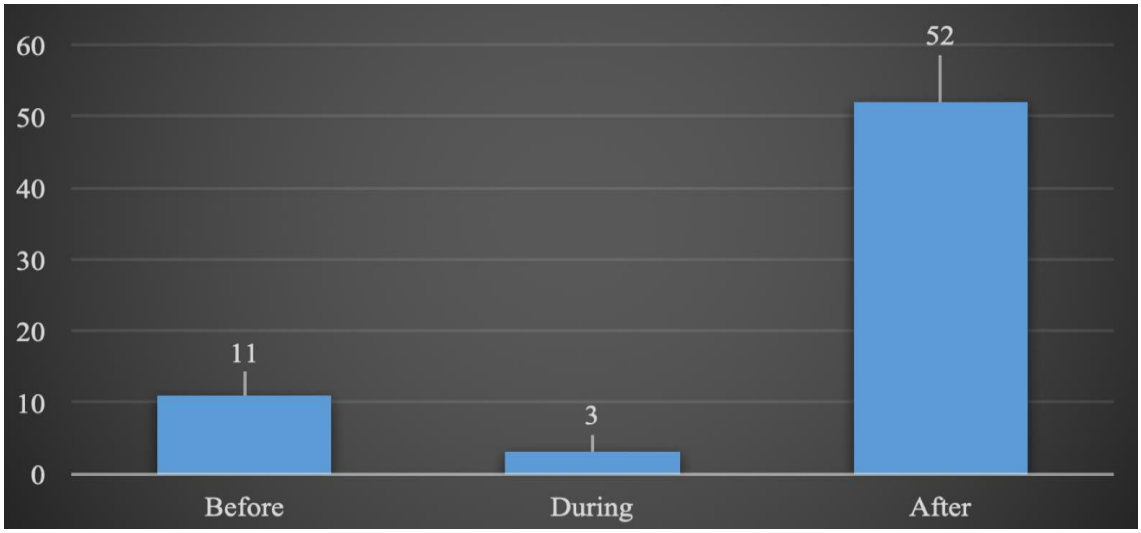

Figure 3. Changes of brain activity in the prefrontal cortex observed before, during, and after chanting *Nam-Myoho-Renge-Kyo* as performed by a long-term practitioner of Nichiren Shoshu Buddhism. These results are representative of 3 other observations that gave qualitatively identical results.

The author's brain activity in the Brodmann area 10 of the prefrontal cortex increased by 11% during the 5 min neurofeedback training session performed before chanting. Such an increase is typical for non-trained subjects. In the session performed during chanting, however, the increase of brain activity in the prefrontal cortex was very modest (3%). Conversely, a large increase (52%) of brain activity in the prefrontal cortex was recorded in the session performed immediately after chanting. The results concerning the increase following chanting are consistent with previous studies demonstrating that meditation enhances blood flow in the prefrontal cortex (Tang *et al*. 2015; Moss *et al*. 2022). The results concerning the relative decrease observed during chanting are consistent with the fact that chanting induces a state of focused attention that leads to a decrease of blood flow to brain regions associated with mind wandering or external stimuli processing. Chanting might induce a shift towards slower brainwaves, such as theta waves that could explain the decrease of blood flow in the prefrontal cortex. For a thorough understanding of the processes associated with chanting, changes of brain activity have to be integrated with changes of brain water content occurring while breathing. As mentioned above, chanting of *Nam-Myoho-Renge-Kyo* is characterized by rapid inspiration and prolonged exhalation and it was demonstrated that the combined thickness of the meninges and cerebral cortex is greater during exhalation (Figure 1). Careful analysis of echogenicity shows that also the water content of the cortex is greater during exhalation. The amount of water in the brain during chanting is further augmented by the increase in blood flow that is associated with brain activity as it is detected by the Mendi device. Therefore, during chanting sound waves travel through the brain for a longer distance, with a faster speed, and greater energy. The increase in speed propagation is due to the fact that myelin, that is responsible for echogenicity, is denser than water. The increase in energy is due to the fact that the energy of a sound wave is related to its intensity, which can be understood as the amount of sound energy flowing through a unit area per unit time. Intensity is directly proportional to the square of the wave's amplitude. The amplitude refers to the maximum displacement of the particles in the medium from their rest position as the wave travels through. For a sound wave to travel faster, the particles in the medium need to be displaced more from their rest positions (larger amplitude). This larger displacement translates to a higher intensity, which signifies more energy carried by the wave. Since sound waves are known to modulate the expression of a number of genes in eukaryotic cells (Kumeta *et al*. 2018), it may be hypothesized that the greater content of water enhances the known beneficial effects of sound waves (Bartel and Mosabbir 2021) on the brain. It may be worth noticing that the effects of sound waves on the brain are independent of the sense of hearing since bone conduction transmits sound waves to the brain (Henry and Letowski 2007). In particular, sound waves generated in the larynx resonate in the oral

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and nasal cavities and the sinuses. They reach the brain through the thin bones of the floor of the skull that have sinuses and foramina *i.e.* the sphenoid and the ethmoid.

3.3. Study of the Effects of Chanting Nam-Myoho-Renge-Kyo on Microbial Metabolism

Figure 4, shows the changes of microbial metabolism as assessed by fermentation of apple juice (left panel) and bovine milk (right panel).

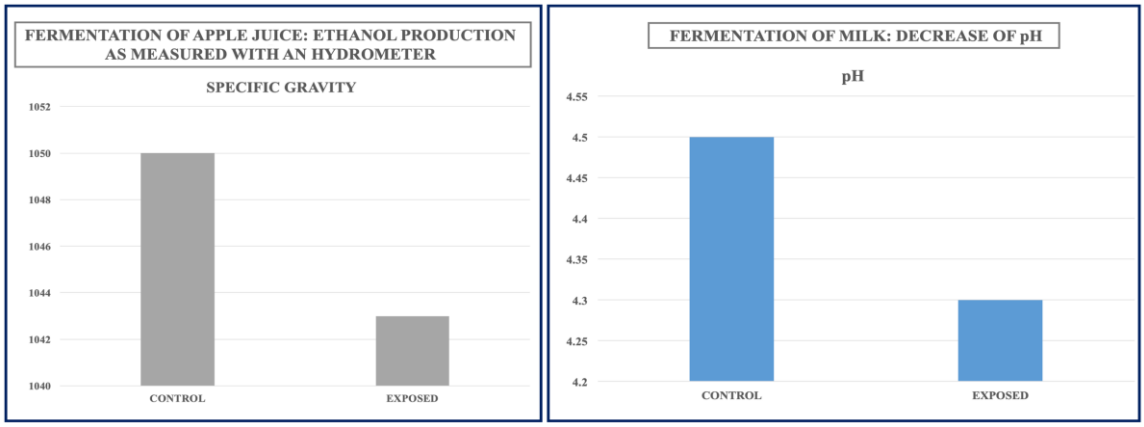

Figure 4. Cultures of probiotic fermenting microbes were exposed to the chanting of *Nam-Myoho-Renge-Kyo* for 30 min either directly in front of the practitioner, or at a distance. Exposure occurred after having dissolved the cultures in the media. Evaluation of the degree of fermentation was assessed after 24 hours (apple juice; left panel) or 48 hours (bovine milk; right panel). These results are representative of 3 other observations that gave qualitatively identical results.

Cultures directly exposed to chanting showed increased metabolism as evidenced by increased production of ethanol, in the case of apple juice, or decrease of pH, in the case of bovine milk. Interestingly identical results were observed when the cultures were not directly exposed to chanting, thus suggesting non-local effects analogous to those described by Shiah *et al*. (2017; 2021; 2022). In this context, it should be considered that microbes exhibit the attributes of consciousness and the molecular mechanisms responsible for microbial consciousness are the same that are at the basis of human consciousness as they are based on phenomena of quantum computations (Reddy and Pereira 2017). It is interesting to notice that such a concept is not in contradiction with Nichiren Daishonin's doctrine. In a writing of Nichiren Daishonin of 1255 is written *Life at each moment encompasses both body and spirit and both self and environment of all sentient beings in every condition of life as well as all insentient beings in every condition of life, as well as insentient beings - plants, sky, and earth, on down to the most minute particles of dust. Life at each moment permeates the universe and is revealed in all phenomena* (The Major Writings 1979). These words are reminiscent of the words written more than seven hundred years later by Nobel Laureate Sir Roger Penrose and Professor Stuart Hameroff *... there is a connection between the brain's biomolecular processes and the basic structure of the universe. ... We conclude that consciousness plays an intrinsic role in the universe* (Hameroff and Penrose 2014).

Even though the effects of chanting on microbial metabolism show some analogy with the results described by Shiah *et al*. (2017; 2021; 2022), there is a significant difference because the latter were observed when three Buddhist monks consciously directed their intention toward Arabidopsis seeds or water with the goal of favoring the growth of the plant (Shiah *et al*. 2017; 2021), or that of mesenchymal stem cells cultured in the intention-treated water (Shiah *et al*. 2022). In this study, however, the observed results were obtained by a single lay believer with no particular training nor psychic abilities, thus making the observations easily reproducible in the context of the so-called citizen science (Parson *et al*. 2011).

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4. Discussion

This study explored the effects of chanting *Nam-Myoho-Renge-Kyo* on sound frequencies, brain activity, and microbial metabolism. The findings provide preliminary evidence for interesting connections. The spectral analysis revealed several noteworthy frequencies generated during chanting. The presence of a peak around 8 Hz aligns with the first Schumann resonance, a natural electromagnetic field phenomenon. While the precise biological implications of this resonance on humans remain under debate, some studies suggest a potential link to physiological rhythms (Martel *et al*. 2023). Further investigation is needed to explore if chanting can synchronize with or influence these rhythms. The observed peak at 116 Hz aligns with a frequency previously linked to chloride ion movements (Hiratsuka and Imamura 2020). Additionally, the identification of five solfeggio frequencies is intriguing. While the historical and musicological basis of solfeggio frequencies is debated, recent studies suggest potential benefits associated with these frequencies, including stress reduction and cognitive improvement (Babayi and Riazi 2017; Akimoto *et al*. 2018; Babayi Daylari *et al*. 2019; Dos Santos *et al*. 2023). These findings, if replicated with larger sample sizes, could contribute to the understanding of sound frequencies and their potential health impacts with particular reference to chanting *Nam-Myoho-Renge-Kyo* in the context of Nichiren Shoshu Liturgy.

The changes of brain activity observed during chanting suggest a shift in cognitive state. The modest increase in prefrontal cortex activity during chanting compared to baseline could indicate a state of focused attention. The significant increase afterward reflects phenomena of enhanced consciousness as it is quite often reported by practicing members of Hokkeko. The observed decrease in blood flow in the prefrontal cortex during chanting, as measured by the Mendi device, merits further exploration. While it suggests a shift of activity from the prefrontal cortex to other areas of the brain, it is important to consider limitations of the Mendi device. Future studies could integrate neuroimaging techniques like fMRI or EEG to gain a more comprehensive picture of brain activity during chanting.

The increased fermentation activity observed in cultures exposed to chanting, both directly and indirectly, is a surprising finding. While the mechanisms underlying this phenomenon remain unclear, it suggests potential non-local effects, similar to other reported observations (Shiah *et al*. (2017; 2021; 2022). Further investigation with controlled experiments is necessary to confirm and understand these non-local effects. Future research should explore the potential mechanisms behind the observed effects. Additionally, exploring the subjective experiences of participants during chanting could provide valuable insights into the cognitive and emotional state changes associated with the practice.

5. Limitations and Conclusive Remarks

This study has significant limitations that deserve consideration. First, the observations were observed only on one subject, the author. Although this is a limitation, the value of the observations remains since they may be considered a series of N-of-1 trials *as per* the definition by Nunn (2011); it has been argued that N-of-1 trials in the field of medical sciences are at the top evidence hierarchy because they *demand serious attention among the health research and clinical care communities given the contemporary focus on individualized medicine* (Lillie *et al*. 2011). In addition, since these observations were performed in the context of citizen science (Parson *et al*. 2011), they give the opportunity to academic as well as non-affiliated researchers to further investigate this fascinating field.

Second, the study does not indicate for how long the enhancement of brain activity and consciousness persists after chanting. From the perspective of a Nichiren Shoshu Buddhist practitioner, however, this is of little importance since the liturgical practice of chanting *Nam-Myoho-Renge-Kyo* is repeated twice a day, every day and, therefore, such an enhancement is continuously regenerated.

As conclusive remark, the author wishes to clarify that this study barely scratches the surface of one of the "Four Powers of the Mystic Law", the power of practice. The spiritual practice of Nichiren Shoshu Buddhism can't be reduced to mere neurophysiological mechanisms in the brain or any other part of the body. The religious and spiritual experience of practicing Buddhism and chanting *Nam-*

Myoho-Renge-Kyo goes well beyond brain activity, neurotransmitters or quantum phenomena of consciousness. It encompasses a mystic dimension that, although not in contradiction with science, can't be explained solely by science. Nevertheless, Nichiren Daishonin's Buddhism states that *Buddhism is reason* (The Major Writings 1985) and *Nothing is more certain than actual proof* (The Major Writings 1986); the author believes that, despite its limitations, this study could be considered a contribution to "actual proof".

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References

1. Akimoto, K. , Hu, A. , Yamaguchi, T. and Kobayashi, H. (2018) Effect of 528 Hz Music on the Endocrine System and Autonomic Nervous System. Health, 10, 1159-1170. doi: 10.4236/health.2018.109088. https://www.scirp.org/journal/paperinformation?paperid=87146
2. Babayi T, Riazi GH (2017) The Effects of 528 Hz Sound Wave to Reduce Cell Death in Human Astrocyte Primary Cell Culture Treated with Ethanol. J Addict Res Ther 8:335. doi:10.4172/2155-6105.1000335
3. Babayi Daylari T, Riazi GH, Pooyan S, Fathi E, Hedayati Katouli F. Influence of various intensities of 528 Hz sound-wave in production of testosterone in rat's brain and analysis of behavioral changes. Genes Genomics. 2019 Feb;41(2):201-211. doi: 10.1007/s13258-018-0753-6. https://pubmed.ncbi.nlm.nih.gov/30414050/
4. Bartel L, Mosabbir A. Possible Mechanisms for the Effects of Sound Vibration on Human Health. Healthcare (Basel). 2021 May 18;9(5):597. doi: 10.3390/healthcare9050597. https://pubmed.ncbi.nlm.nih.gov/34069792/
5. Bradstreet JJ, Pacini S, Ruggiero M. A New Methodology of Viewing Extra-Axial Fluid and Cortical Abnormalities in Children with Autism via Transcranial Ultrasonography. Front Hum Neurosci. 2014 Jan 15;7:934. doi: 10.3389/fnhum.2013.00934. https://pubmed.ncbi.nlm.nih.gov/24459462/
6. Cherry NJ. Human intelligence: the brain, an electromagnetic system synchronised by the Schumann Resonance signal. Med Hypotheses. 2003 Jun;60(6):843-4. doi: 10.1016/s0306-9877(03)00027-6. https://pubmed.ncbi.nlm.nih.gov/12699709/
7. Dos Santos AC, de Abreu MS, de Mello GP, Costella V, do Amaral NR, Zanella A, Poletto J, Petersen EV, Kalueff AV, Giacomini ACVV. Solfeggio-frequency music exposure reverses cognitive and endocrine deficits evoked by a 24-h light exposure in adult zebrafish. Behav Brain Res. 2023 Jul 26;450:114461. doi: 10.1016/j.bbr.2023.114461. https://pubmed.ncbi.nlm.nih.gov/37119977/
8. Elorza-Vidal X, Gaitán-Peñas H, Estévez R. Chloride Channels in Astrocytes: Structure, Roles in Brain Homeostasis and Implications in Disease. Int J Mol Sci. 2019 Feb 27;20(5):1034. doi: 10.3390/ijms20051034. https://pubmed.ncbi.nlm.nih.gov/30818802/
9. Fdez-Arroyabe P, Fornieles-Callejón J, Santurtún A, Szangolies L, Donner RV. Schumann resonance and cardiovascular hospital admission in the area of Granada, Spain: An event coincidence analysis approach. Sci Total Environ. 2020 Feb 25;705:135813. doi: 10.1016/j.scitotenv.2019.135813. https://pubmed.ncbi.nlm.nih.gov/31826805/
10. Hameroff S, Penrose R. Consciousness in the universe: a review of the 'Orch OR' theory. Phys Life Rev. 2014 Mar;11(1):39-78. doi: 10.1016/j.plrev.2013.08.002. https://pubmed.ncbi.nlm.nih.gov/24070914/
11. Henry P and Letowski TR. Bone Conduction: Anatomy, Physiology,
12. and Communication. Army Res Lab. ARL-TR-4138 May 2007 https://apps.dtic.mil/sti/tr/pdf/ADA468459.pdf
13. Hiratsuka, A. and Wakae, K. (2019) Study on Sustainable Rainwater Resource Utilization—Towards Deepening of Homo Environmentics. Jour- nal of Water Resource and Protection, 11, 491-528. https://doi.org/10.4236/jwarp.2019.115029. https://www.scirp.org/journal/paperinformation?paperid=92370

14. Hiratsuka, A. and Imamura, A. (2020) Study on Reduction of Mixed Water Salinity by Sound Wave. Journal of Water Resource and Protection, 12, 155-170. https://doi.org/10.4236/jwarp.2020.122010. https://www.scirp.org/journal/paperinformation?paperid=98429
15. Högman, L. & Dravniknes, H. (2020). Validation of a consumer-grade functional Near-Infrared Spectroscopy Device for Measurement of frontal pole Brain Oxygenation – an Interim Report. Stockholm: Department of Psychology, Stockholm University. Paper retrieved from Mendi innovation AB's website on June 1, 2023. https://mendi-webpage.s3.eu-north-1.amazonaws.com/Mendi_signal_validation_interim_report_final.pdf
16. Kumeta M, Takahashi D, Takeyasu K, Yoshimura SH. Cell type-specific suppression of mechanosensitive genes by audible sound stimulation. PLoS One. 2018 Jan 31;13(1):e0188764. doi: 10.1371/journal.pone. https://pubmed.ncbi.nlm.nih.gov/29385174/
17. Kunzelmann K. Ion channels and cancer. J Membr Biol. 2005 Jun;205(3):159-73. doi: 10.1007/s00232-005-0781-4. https://pubmed.ncbi.nlm.nih.gov/16362504/
18. Lillie EO, Patay B, Diamant J, Issell B, Topol EJ, Schork NJ. The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? Per Med. 2011 Mar;8(2):161-173. doi: 10.2217/pme.11.7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118090/
19. Martel J, Chang SH, Chevalier G, Ojcius DM, Young JD. Influence of electromagnetic fields on the circadian rhythm: Implications for human health and disease. Biomed J. 2023 Feb;46(1):48-59. doi: 10.1016/j.bj.2023.01.003. https://pubmed.ncbi.nlm.nih.gov/36681118/
20. McCraty R, Atkinson M, Stolc V, Alabdulgader AA, Vainoras A, Ragulskis M. Synchronization of Human Autonomic Nervous System Rhythms with Geomagnetic Activity in Human Subjects. Int J Environ Res Public Health. 2017 Jul 13;14(7):770. doi: 10.3390/ijerph14070770. https://pubmed.ncbi.nlm.nih.gov/28703754/
21. Moss AS, Reibel DK, Wintering N, Vedaei F, Porter H, Khosravi M, Heholt J, Alizadeh M, Mohamed FB, Newberg AB. Cerebral Blood Flow and Brain Functional Connectivity Changes in Older Adults Participating in a Mindfulness-Based Stress Reduction Program. Behav Sci (Basel). 2022 Feb 14;12(2):48. doi: 10.3390/bs12020048. https://pubmed.ncbi.nlm.nih.gov/35200299/
22. Nunn R. Mere anecdote: evidence and stories in medicine. J Eval Clin Pract. 2011 Oct;17(5):920-6. doi: 10.1111/j.1365-2753.2011.01727.x. https://pubmed.ncbi.nlm.nih.gov/21815971/
23. Pacini S, Ruggiero M. Natural Plasmids in a Swiss Fermented Milk and Colostrum Product assessed by Microbiome Array. Madridge J Immunol. 2019; 3(2): 100-108. doi: 10.18689/mjim-1000123. https://madridge.org/journal-of-immunology/mjim-1000123.pdf
24. Pacini S, Ruggiero M. Phage composition of a fermented milk and colostrum product assessed by microbiome array; putative role of open reading frames in reference to cell signaling and neurological development. J Neurol Stroke. 2020;10(2):80–90. DOI: 10.15406/jnsk.2020.10.00416. https://medcraveonline.com/JNSK/JNSK-10-00416.pdf
25. Parsons J, Lukyanenko R, Wiersma Y. Easier citizen science is better. Nature. 2011 Mar 3;471(7336):37. doi: 10.1038/471037a. https://pubmed.ncbi.nlm.nih.gov/21368811/
26. Pinti P, Aichelburg C, Gilbert S, Hamilton A, Hirsch J, Burgess P, Tachtsidis I. A Review on the Use of Wearable Functional Near-Infrared Spectroscopy in Naturalistic Environments. Jpn Psychol Res. 2018 Oct;60(4):347-373. doi: 10.1111/jpr.12206. https://pubmed.ncbi.nlm.nih.gov/30643322/
27. Reddy JSK, Pereira C. Understanding the emergence of microbial consciousness: From a perspective of the Subject-Object Model (SOM). J Integr Neurosci. 2017;16(s1):S27-S36. doi: 10.3233/JIN-170064. https://pubmed.ncbi.nlm.nih.gov/29254105/
28. Ruggiero M. Application of ultrasonography to Neuro-COVID-19. Int J Radiol Radiat Ther. 2022;9(3):99–103. doi: 10.15406/ijrrt.2022.09.00331. https://medcraveonline.com/IJRRT/IJRRT-09-00331.pdf
29. Ruggiero M. Study of structural similarities between tubulin, TMC1, AND FTSZ proteins as they relate to mechanosensory transduction in the context of the orch or theory of consciousness. Int J Radiol Radiat Ther. 2023;10(3):65–72. DOI: 10.15406/ijrrt.2023.10.00358. https://medcraveonline.com/IJRRT/IJRRT-10-00358.pdf
30. Ruggiero, M. Genetic Analysis of Bifidobacteriaceae in a Fermented Milk and Colostrum Product in Relation to Immune Function. Preprints 2024, 2024051503. https://doi.org/10.20944/preprints202405.1503.v1. https://www.preprints.org/manuscript/202405.1503/v1

 doi:10.20944/preprints202407.0236.v2

31. Ruggiero, M. & Hiratsuka, A. (2024). On the Natural Intelligence and Consciousness of the Immune System and its Relationship with the Brain. American Journal of Immunology, 19(1), 11-23. https://doi.org/10.3844/ajisp.2024.11.23. https://thescipub.com/abstract/ajisp.2024.11.23
32. Ruggiero M, Magherini S, Fiore MG, Chiarelli B, Morucci G, Branca JJ, Gulisano M, Pacini S. Transcranial sonography: a technique for the study of the temporal lobes of the human and non-human primate brain. Ital J Anat Embryol. 2013;118(3):241-55. https://pubmed.ncbi.nlm.nih.gov/24640587/
33. Sheth SA, Nemoto M, Guiou M, Walker M, Pouratian N, Toga AW. Linear and nonlinear relationships between neuronal activity, oxygen metabolism, and hemodynamic responses. Neuron. 2004 Apr 22;42(2):347-55. doi: 10.1016/s0896-6273(04)00221-1. https://pubmed.ncbi.nlm.nih.gov/15091348/
34. Shiah YJ, Hsieh HL, Chen HJ, Radin DI. Effects of Intentionally Treated Water on Growth of Arabidopsis thaliana Seeds with Cryptochrome Mutations. Explore (NY). 2017 Nov-Dec;13(6):371-378. doi: 10.1016/j.explore.2017.05.001. https://pubmed.ncbi.nlm.nih.gov/28986016/
35. Shiah YJ, Hsieh HL, Chen HJ, Radin DI. Effects of intentionally treated water and seeds on the growth of Arabidopsis thaliana. Explore (NY). 2021 Jan-Feb;17(1):55-59. doi: 10.1016/j.explore.2020.04.006. https://pubmed.ncbi.nlm.nih.gov/32507494/
36. Shiah YJ, Shan L, Radin DI, Huang GT. Effects of intentionally treated water on the growth of mesenchymal stem cells: An exploratory study. Explore (NY). 2022 Nov-Dec;18(6):663-669. doi: 10.1016/j.explore.2021.11.007. https://pubmed.ncbi.nlm.nih.gov/34887234/
37. Tang YY, Lu Q, Feng H, Tang R, Posner MI. Short-term meditation increases blood flow in anterior cingulate cortex and insula. Front Psychol. 2015 Feb 26;6:212. doi: 10.3389/fpsyg.2015.00212. https://pubmed.ncbi.nlm.nih.gov/25767459/
38. The Doctrines and Practice of Nichiren Shoshu. Nichiren Shoshu Overseas Bureau publisher. ASIN: B001I1LD5Q
39. The Major Writings of Nichiren Daishonin. Nichiren Shoshu International Center; Vol. 1; First edition (January 1, 1979). pp. 3-5. ISBN-10 : 4888720088. ISBN-13 : 978-4888720083
40. The Major Writings of Nichiren Daishonin. Nichiren Shoshu International Center; Vol. 3. (January 1, 1985). pp. 238. ISBN-10 : 4888720185. ISBN-13 : 978-4888720182
41. The Major Writings of Nichiren Daishonin. Nichiren Shoshu International Center; Vol. 4. (January 1, 1986). pp. 121. SBN-10 : 4888720223. ISBN-13 : 978-4888720229
42. Yennu A, Tian F, Smith-Osborne A, J Gatchel R, Woon FL, Liu H. Prefrontal responses to Stroop tasks in subjects with post-traumatic stress disorder assessed by functional near infrared spectroscopy. Sci Rep. 2016 Jul 25;6:30157. doi: 10.1038/srep30157. https://pubmed.ncbi.nlm.nih.gov/27452397/