

Neural Tissues Filter Electromagnetic Fields: Investigating Regional Processing of Induced Current in *Ex vivo* Brain Specimens

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Abstract

As has been demonstrated experimentally, the living brain responds to pulsatile electromagnetic fields. Our aim was to investigate the capacities of *ex vivo* neural tissue to process and filter induced current generated by naturally occurring and laboratory-controlled electromagnetic fields. Microvolt potentials within the chemically fixed postmortem brains were collected throughout the field exposures. During strong geomagnetic storms there was a significant increase in power spectra within the 7.5 Hz to 14 Hz range within the right but not the left parahippocampal gyrus compared to days with relatively quiet geomagnetic activity. This finding indicated that ambient electromagnetic fluctuations from natural sources were processed differentially as a function of subsections of the postmortem tissue. Exposing a whole, fixed human brain to two physiologically patterned magnetic fields that have been associated with powerful subjective experiences reported by hundreds of human volunteers in the laboratory setting elicited increased power within the 7.5 Hz to 20 Hz range. The effects required 10 to 20 s to emerge and were primarily represented within tissue subsections of the right and orbitofrontal gyri. Other fields such as simple sine-wave (20 Hz) patterns of comparable intensity (2 to 10 μ T) did not elicit the same configuration of changes. The results indicate that neural tissues filter electromagnetic fields non-randomly.

Keywords: Human brain tissue; *Ex vivo*; Amygdala; Orbitofrontal region; Electromagnetic stimulation

Introduction

The development of living systems from single cell to multicellular organisms occurred within the geomagnetic field. As Dubrov et al. [1] succinctly summarized, there are large numbers of correlational studies that indicate all organisms respond to some component of geomagnetic activity. As reviewed by Persinger [2], the correlations between increased geomagnetic activity and changes in various measures of behavior for human and animal populations usually accommodate 15% to 30% of the variability in these measures. The strongest correlations between changes in geomagnetic activity and behavioral or physiological variables occur within the same day or with changes one to three days previously. The median threshold of intensity for the activity is about 15 to 20 nT, although biological systems can exhibit thresholds below or above this value. One experiment involving more than 100 human subjects individually tested within a quiet, darkened chamber over several years demonstrated a conspicuous global geomagnetic activity threshold of about 20 nT above which the report of vestibular experiences were enhanced during a specific experimental manipulation. This magnitude was similar to that which disrupted homing behavior in pigeons.

That the geomagnetic effect is likely responsible for these correlations rather than the multiple temporally contiguous stimuli (e.g. cosmic rays, Alfven waves) or secondary effects have been shown experimentally. Inspired by the moderate strength correlations between global geomagnetic activity and convulsive seizures in human patients reported by Rajaram et al. [3] and Michon et al. [4]

demonstrated that the magnitude of the correlation between spontaneous seizures in epileptic rats and geomagnetic activity during the same intervals was experimentally reproducible when the rats were exposed to a simulated "magnetic storm" pattern that was generated within the laboratory. Mulligan et al. [5] partially replicated the correlations reported by Babayev and Allahverdiyeva [6] between global geomagnetic activity and specific frequency bands within the quantitative electroencephalographic (QEEG) measures of human volunteers. This included alterations in theta activity (4 Hz to 8 Hz) over the right parietal region. Mulligan and Persinger [7] simulated the geomagnetic activity experimentally and found that after 10 to 15 min of whole body exposure to 20 nT variations there was enhancement of spectral power for theta activity over the right parietal region.

Saroka et al. [8] measured the electroencephalographic coherence between the left and right temporal lobes of 184 volunteers over a three year period while they sat within a quiet, darkened chamber. They found significantly increased coherence between the caudal temporal lobes for the 11 Hz band when the global geomagnetic activity increased above 8 to 10 nT. Source localization as inferred by sLORETA (Low Resolution Electromagnetic Tomography) revealed that the (right) parahippocampal region contributed significantly to the interhemispheric temporal lobe coherence. Within 1 Hz increments between 1 and 40 Hz, the most significant peaks in spectral power occurred at 7.5 Hz and 19.5 Hz. Although intuitions might dictate that direct effects of geomagnetic variations upon the cerebral volume may be too weak to be effective, the energy available according to the classic equation (Equation 1):

$$J = B^2 \cdot (2\mu)^{-1} m^3$$
 (1),

where B is the strength of the field, μ is magnetic permeability (1.26·10⁻⁶ N·A⁻²) and m³ is the volume might be significant.

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Assuming a cerebral volume of $1.3 \cdot 10^{-3}$ m³, a shift of 20 nT would be associated with ~ 10^{-13} J. Considering that each action potential ($1.2 \cdot 10^{-1}$ V) exerts upon a unit charge ($1.6 \cdot 10^{-19}$ As) an energy of 10^{-20} J [9] this means that approximately 10^7 neurons could be affected. Rouleau and Dotta [10] have argued that this aggregate of cells is within the magnitude of the numbers of neurons involved with experiences of a percept. When the work of Cheng-yu et al. [11] is considered, who showed that only one neuron is required to change the electrophysiological state of a (rat) brain, then energies induced within the brain volume from geomagnetic activity may not be trivial. Even single neurons in the prefrontal cortex encode abstract rules [12] and in rats, stimulation of a single neuron in the somatosensory cortex affects response detection [13].

The most intuitive explanation for the correlations between geomagnetic activity (including threshold effects) and specific frequency bands within the human electroencephalogram presumes the role of dynamic processes associated with the living brain. However if structure dictates function and the rudimentary microstructures of human brains remain after clinical death, assuming the tissue is appropriately fixed, then the energies within the volume of the brain during geomagnetic activity should be sufficient to alter the spectral power densities in a subtle but quantifiable manner. As reported by Tsang et al. [14] the inductance and capacitance of fresh brain tissue when stimulated with action potential related durations (1 kHz) is in the order of 7 Hz. The quantitative estimates and imaginative models by Nunez [15] indicates that the intrinsic resonance of bulk velocity of action potentials along the rostral-caudal course of the living cerebrum [16] every approximately 20 to 25 ms ("40 Hz") divided by the typical cerebral circumference solves for a value in the theta range.

Direct application of physiologically patterned magnetic fields with intensities between 1 and 5 μT across the temporal lobes of volunteers has been reported to be associated with a continuum of subjective experiences including the "sensed presence" [17]. One of the most effective protocols to induce the latter experience has been the application of a decelerating, complex pattern with slightly higher strength over the right hemisphere for 30 min followed by more bilateral stimulation of an accelerating, complex patterned magnetic field once every 3 to 4 s across both hemispheres at the level of the temporal lobes [18] for 30 min. The latter pattern was shown to produce analgesia in rats [19]. Saroka and Persinger [20], employing LORETA methodology for source localization supported the hypothesis that enhanced stimulation of the right hemisphere followed by bilateral stimulation across the temporal lobes was associated with the most frequent reports of a sensed presence. Here we report for the first time that fixed human tissue during specific stimulation displayed significant enhancement with geomagnetic activity and the effect was prominent within the right hemisphere. In addition we demonstrate that the specific patterns of physiologically patterned magnetic fields that are most effective for producing powerful subjective experiences also elicited the greatest response from the fixed brain tissue.

Materials and Methods

The present study was based upon the observation that geomagnetic storm conditions influenced microvolt fluctuations within coronal sections of fixed human brain tissue. An experiment was designed to test the hypothesis that electromagnetic fields were processed differentially as a function of spatial loci within the brain. The experimental procedure involved exposing a full (non-sectioned) chemically fixed human brain to different physiologically-patterned electromagnetic fields. Microvolt fluctuations induced by electromagnetic fields were measured within neuroanatomical regions of interest. Analyses were performed to determine whether different electromagnetic field patterns were amplified or attenuated by tissue subsections.

Investigative analysis

Baseline spectral power densities (SPD) derived from electrical potential difference measurements which were obtained from coronal sections (n=3) of fixed human brain tissue from four (n=4) independent experiments completed between May 6, 2015 and April 8, 2016 were aggregated into a database. The brain specimens were acquired over 20 years ago, donated anonymously to Dr. Michael A. Persinger and the Neuroscience Research Group at Laurentian University. Consequently, personal information relating to individual tissue sources was not available. The only structures from which SPD data were abundantly available were the parahippocampal and hippocampal regions bilaterally. Consequently, the responses from these structures were utilized. These data were coded by date and K_pindex values indicative of geomagnetic storm conditions. They had been retrieved from the National Oceanic and Atmospheric Administration (NOAA) Space Weather online database (www.ngdc.noaa.gov). Kp 24 hour maxima were retrieved, which ranged between 1 and 5 on days of testing. A "storm" binary variable was coded where K_p values less than three (K_p <3) were classified as "no storm" and K_p values greater than three ($K_p>3$) were classified as "storm". Data analyses were performed to discern any potential relationship between geomagnetic field disturbances and spectral power fluctuations derived from microvolt measurements within coronal sections of fixed human brain tissue.

Human brain tissue

A full human brain, unsectioned and preserved for over 20 years in ethanol-formalin-acetic acid, served as the tissue specimen which was exposed to different patterned electromagnetic fields. The brain was ethically obtained from an anonymous donor and its use was approved by the institution's research ethics board. As such, the brains could not be identified and all personal information relating to the specimens were unavailable. The brain was neuroanatomically unremarkable, featuring all major gyri and sulci outlined by Crosby et al. [21]. The anterior, middle, and posterior cerebral arteries were intact and partially affixed to the cerebrum and brainstem. This was important as previous electrophysiological measurements involving post-mortem fixed human brain tissue has involved the use of vasculature as the electrical reference point [22,23].

The measurement procedure, similar to previous studies, involved a Mitsar 201 amplifier typically used for human quantitative electroencephalography (QEEG). Instead of the typical multi-sensor cap used in living human studies, five needle electrodes were coupled to the amplifier box. The first four sensors served as references and were directly inserted into the basilar artery of the brain. The average of the sensors constituted the electrical reference point. The fifth sensor was inserted into regions of interest where microvolt fluctuations relative to the reference point were obtained during experimental manipulations.

Six neuroanatomical loci were selected as points of measurement wherein the needle electrode was inserted directly: the amygdala (by way of the uncus), middle frontal gyrus, orbital frontal gyrus, occipital pole, superior parietal lobule, and the parahippocampal gyrus. Both the right and left hemispheric structural equivalents were probed. All measurements were performed with the brain resting on its dorsal surface which rendered both the reference and probe sites accessible. Raw microvolt (μ V) potentials were collected using WinEEG (2.93.59) and subsequently converted to SPD (μ V²·Hz⁻¹) within delta (1.5 Hz – 4Hz), theta (4 Hz – 7.5 Hz), alpha (7.5 Hz – 14 Hz), beta1 (14 Hz – 20 Hz), beta2 (20 Hz – 30 Hz), and gamma (30 Hz – 40 Hz) bands. Filters were applied to exclude 60 Hz ambient noise, 120 Hz harmonics, as well as periodicities below 1.6 Hz and above 50 Hz. All data collection and analyses were performed within SPSS v20 on an HP ENVY laptop computer with a Windows 8 operating system.

Exposure device

Electromagnetic field patterns were delivered to a custom coil by a Zenith-386 (Model ZF-148-41, Zenith Data Systems, Benton Harbor, MI, USA) computer. The patterns were created by converting strings of programmable numbers (0 to 256, with 127=0) into voltage values between -5 and +5 V which were delivered by the complex software© (Professor Stanley A. Koren) to the coils through a digital-to-analogue converter (DAC). The current was then delivered to the coil from which the magnetic fields were generated [19]. The "point durations" or discrete application pulses within each pattern were selected based upon previous studies which identified specific field effects. Six patterns were selected: Burst X (3000, 3), Burst X (3, 3), Thomas (3000, 3), Thomas (3, 3), LTP (4500, 1), and a 20 Hz Sine (3000, 3). The first number within parentheses refers time (in ms) between repetitions of the pattern. The second number refers to the point duration - that is, the duration each voltage equivalent of the numbers between 0 and 256 were generated in ms. The LTP pattern disrupts memory consolidation in rats by potentially competing with Long Term Potentiation [24]. The sine-wave pattern is self-explanatory and was employed to discern the potential differential effectiveness of a physiologically-patterned field that contains a 20 Hz component from the redundant presentation of a symmetrical 20 Hz input. The total numbers of points in Burst, Thomas, LTP and sine patterns were 239, 859, 225 and 200, respectively. Figure 1 includes representations of the complex field patterns.

A coil, built by wrapping 305 m of a single layer of 30 AWG (Belden 9978) wire around a 38 cm \times 33 cm \times 27 cm milk crate (Figure 2), served as the electromagnetic field exposure device. This was the same coil employed by Murugan et al. [25]. The crate was placed face-down over the tissue such that the wire was wrapped along the horizontal plane perpendicular to the surface of the testing table. The strength of the fields ranged between 2 – 10 μ T. The tissue was exposed to one of the time-varying, complex electromagnetic fields for 60 seconds per trial. Each trial involved measurement of a single neuroanatomical locus. Experimental manipulations were counterbalanced to avoid order-dependent confounding factors. There were a total of three replicates per pattern. Each trial was 60 s in duration.

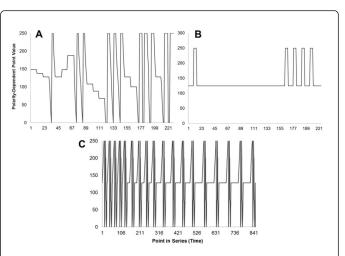


Figure 1: The Burst X (A), LTP (B), and Thomas (C) electromagnetic field patterns. The Y-axes represent polarity-dependent point values ranging between 0 and 250 whereas the X-axes represent the points within each series or, implicitly, time.

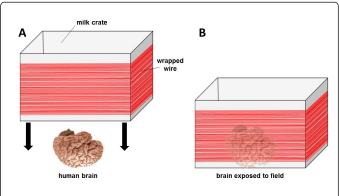


Figure 2: The coil was placed over the human brain (A), exposing the brain to a complex, time-varying electromagnetic field (B).

Analysis

SPD (1.5 Hz – 40 Hz) were extracted from WinEEG as 60 second segments with 4 second epochs. Data were imported to SPSS v.20 and coded by condition. In addition to the classical EEG bands, averages of periodically adjacent bands were computed including theta-alpha (theta+alpha/2) and alpha-beta1 (alpha+beta1/2). Simple tests of mean differences were computed.

Results

Investigative analysis

The geomagnetic values were coded for storm (K_p =4 or 5) and nonstorm (K_p =1 or 2). Statistically significant increases of alpha power (7.5 Hz – 14 Hz) were noted within the right but not the left parahippocampal gyrus during "storm" conditions, t(12)=2.56, p<0.05, r²=0.35 (Figure 3). Other frequencies were unaffected by daily K_p index 24 hour maxima (p>0.05). Hippocampal SPD differences were not statistically significant (p>0.05) These data indicate that right parahippocampal but not hippocampal or left hemispheric background microvolt fluctuation periodicities were affected by geomagnetic storm conditions within fixed human brain tissue. Based upon these observations, experimental applications of electromagnetic fields to brain tissue were performed.

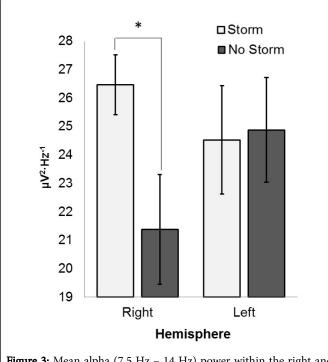


Figure 3: Mean alpha (7.5 Hz – 14 Hz) power within the right and left parahippocampal gyri of fixed human brain sections (coronal) obtained within a 24 hour period within which the daily maximum geomagnetic perturbation (K_p index) value was indicative of storm conditions (K_p >3) or not (K_p <3).

Experimental electromagnetic field exposures

Regardless of the pattern of the magnetic field, when the fixed, unsectioned human brain was exposed (to any field), right hemispheric structures (M=15.83, SEM=0.79) displayed increased alpha-beta1 (7.5 Hz - 20 Hz) power relative to left hemispheric structures (M=13.97, SEM=0.48), t(214)=2.02, p<0.05. The effect size was very weak ($r^2=0.02$). The effects were relatively immediate but not instantaneous. The contribution of each field to the overall effect was then investigated systematically. Burst X (3000, 3) and Thomas (3, 3) patterns elicited increased alpha-beta1 power within aggregates of right hemispheric structures relative to the equivalent aggregates from the left hemisphere, explaining 14% and 12% of the variance respectively (Figure 4). Burst X and Thomas fields generated with the other point duration parameters did not produce equivalent hemispheric asymmetries (p>0.05). Similarly, LTP and Sine (20 Hz) patterns did not produce any statistically significant changes in power as a function of hemisphere (p>0.05). The 20 Hz Sine pattern produced, non-specific statistically significant increases of alpha-beta1 power within all structures relative to all other conditions.

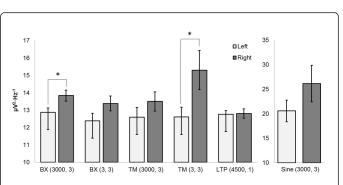


Figure 4: Alpha-beta1 (7.5 Hz – 20 Hz) power within the left and right hemisphere of a full, unsectioned human brain as a function of the electromagnetic field exposure pattern. Burst X (BX), Thomas (TM), long-term potentiation (LTP), and sine (Sine) patterns are shown where significant differences between hemispheres are indicated (p<0.05).

Further analyses revealed that the right hemispheric increases of alpha-beta1 power observed in the fixed, unsectioned human brain during exposures to Burst X (3000, 3) were due to one neuroanatomical locus. The right amygdala, accessed by way of the uncus, generated increased alpha-beta1 power within the right hemisphere (M=15.32, SEM=0.54) relative to the left hemisphere $(M=12.52, SEM=0.66), t(4)=3.30, p<0.05, r^2=0.73$ (Figure 5). This right-left difference emerged within 10 to 20 s after the onset of the field exposures (p<0.05). ANOVAs revealed statistically significant alpha-beta1 power differences across structures within the right hemisphere, F(5,17)=3.56, p<0.05, η^2 =0.60. Homogeneous subsets were revealed. Where the major source of the variance was due to a difference between the right amygdala and right orbital frontal gyrus (p<0.05). There were no statistically significant changes in the left hemispheric structural differences after accommodating for multiple comparisons. Power differences within other frequency bands were not significant during exposures to the Burst X (3000, 3) pattern. Together, these results suggested that the right amygdala was most responsive to the Burst X (3000, 3) electromagnetic field pattern relative to its contralateral paired structure in a fixed, unsectioned human brain.

When exposed to the Thomas (3, 3) pattern, two neuroanatomical loci that were not differential responsive to Burst-X responses demonstrated statistically significant hemispheric differences. During exposure, the right orbital frontal gyrus displayed increased theta-alpha (4 Hz – 14Hz) power relative to the contralateral locus, t(4)= =3.02, p<0.05, r²=0.70 (Figure 6). Although the effects were evident within the 60 sec trial periods, subsequent analyses revealed the onset of the left-right difference emerged between 10 and 20 s after the onset of the field. Similarly, the right superior parietal lobule displayed increased theta-alpha power relative to the left superior parietal lobule, t(4)= 3.34, p<0.05, r²=0.74.

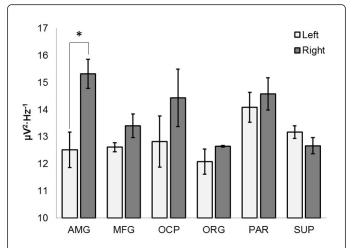


Figure 5: Alpha-beta1 (7.5 Hz – 20 Hz) power within the left and right amygdala (AMG), middle frontal gyrus (MFG), occipital pole (OCP), orbital gyrus (ORG), parahippocampal gyurs (PAR), or superior parietal lobule (SUP) while exposed to Burst X (3000, 3). Significant differences are indicated (p<0.05).

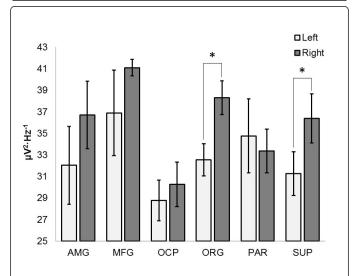


Figure 6: Theta-alpha (4 Hz – 14 Hz) power within the left and right amygdala (AMG), middle frontal gyrus (MFG), occipital pole (OCP), orbital gyrus (ORG), parahippocampal gyurs (PAR), or superior parietal lobule (SUP) while exposed to Thomas (3, 3). Significant differences are indicated (p<0.05).

Whereas orbital frontal effects were discernable between 10 and 20 s after the onset of the field changes within the superior parietal lobule were manifested within 10 s. Exposure to the Thomas (3, 3) pattern was also associated with theta-alpha differences between neuroanatomical loci within the right hemisphere, F(5, 17)=3.26, p<0.05, $\eta^2=0.58$. Homogeneous subsets isolated the power differences between the right middle frontal gyrus and the right occipital polar region as the major sources of variance (p<0.05). Structure-dependent SPD differences within the left hemisphere were not evident F(5, 17)=0.95, p>0.05. These results suggested that the orbital frontal and superior parietal structures of the right hemisphere as well as the

middle frontal gyrus were particularly responsive to the Thomas (3, 3) field pattern.

Discussion and Conclusion

The moderate correlations between brain activity, such as epileptic seizures, or behaviors related to cerebral sensitivity and geomagnetic activity have been reported for decades [1]. The statistically significant and conspicuous effects of experimentally generated weak magnetic fields within the 0.02 (20 nT) to 10 microTesla range, particularly when applied as physiologically-simulated patterns, upon human behaviour, subjective experiences, and current densities within the human cerebrum suggest that the brain volume may be more responsive to these stimuli than classically assumed. The energies associated with this level of magnetic field strength within the human cerebrum would be equivalent to at least the energy from action potentials from about a million neurons each discharging around 10 Hz.

The results of the present experiments indicate that fixed human brain tissue has the capacity to differentially respond to both increases in geomagnetic activity as well as the application of different physiologically patterned magnetic fields. Two major findings were common across the investigative and experimental results. First, the geomagnetic effects as well as the experimentally generated physiologically-patterned electromagnetic field effects were most pronounced within specific right hemispheric structures. Differential hemispheric effects have been shown for living brain. Applications of physiologically-patterned magnetic fields over the left or the right hemisphere produced differential electrophysiological effects within about 15 min [26]. The enhanced sensitivity of the right hemisphere, particularly within the temporal and parahippocampal regions, for geomagnetic activity has been reported by several researchers [6,27,28]. These conclusions have been derived from direct measurements or inferred by right-lateralized experiences reported by participants including visual flashes or phenomena within the upper left peripheral visual fields.

Second, where there were spectral power differences, the alpha band (7.5 Hz – 14 Hz) was always involved. Though SPD differences were observed within the 4 Hz – 20 Hz band, the overlapping alpha signature was conspicuous. The enhancement of the alpha band during increased geomagnetic activity compared to less intense periods suggests that the effects of the applied electromagnetic fields were not artefacts. In addition, some patterns did not evoke significant changes in SPD and each pattern differentially induced changes within different structures. The anisotropy of the response profile would be consistent with differential structural properties rather than non-specific artefacts. Left hemispheric structures reliably demonstrated uniform spectral power across all frequency bands when exposed to both different perturbation levels of the background geomagnetic field or applied electromagnetic fields within the laboratory setting.

Not all regions that were measured within the human brain responded in the same manner. Those areas that differentially responded to very specific patterns of the applied fields displayed the changes relatively quickly, typically within about 10 to 20 s. This latency is much longer than effects produced by spurious induction artefacts. Over the last few decades we have observed and measured multiple electroencephalographic phenomena that were artefacts. All of them occurred within 1 s of the field application. In addition, we found that some areas such as the superior parietal lobule displayed even shorter latencies. The most parsimonious explanation for the requirement for 10 to 20 s exposure to the fields to elicit the changes in a specific band of electrophysiological activity within only some brain regions would involve a property like reluctance or inductance (latency) that is different within those regions particularly within the right hemisphere.

This 10 to 20 s latency is much shorter than the usual latency of 15 min to 30 min that is required for the living brain to respond to these same fields. The latter is not likely to be an impedance effect from the skull because direct measurements of simulated skull thickness and salinity did not attenuate the penetration of these fields [29]. This discrepancy might be employed to estimate the "inertia" of the neuronal fields within the living brain that must be accommodated by the applied, physiologically patterned magnetic fields. We have suggested that one mechanism by which weak, applied, physiologically-patterned magnetic fields influence the dynamic brain is through the hydronium ion. Murugan et al. [30] showed that experimental application of the same field patterns that were effective in this study produced quantitative shifts in the pH of spring water that reflected the intrinsic energies from the applied fields.

We have assumed that the fundamental dynamics associated with cell membrane dynamics that contributes to cognition and to consciousness occurs within the 10 nm space extending from the cell membrane. This is the functional width from which the resting membrane potential emerges [9]. The volume associated with this thin shell is about $0.8 \cdot 10^{-12}$ cc. Assuming there are about 10^{12} neurons and glial cells within the volume that would be equivalent to ~ 1 cc. This volume is equivalent to 5.5.10⁻² M and considering there are 6.02.10²³ molecules per mole and at pH 7.4 the concentration of available protons would be 4.8.10-8 M per L and the number of protons would be $159 \cdot 10^{13}$. If the duration of each hydronium ion is 10^{-12} s [31], then approximately 1600 s would elapse before potentially the vast majority of protons would be affected. This is equivalent to about 30 min which is the same order of magnitude of exposure required to produce demonstrable changes in electroencephalographic activity in live human exposures. Because the response from the fixed tissue was about one thousandths of that value, this would suggest either a modified functional volume around the cells or a slightly more acidic pH associated with the fixative. Our measurements of the fixative solution have yielded values approximating a pH of 3. These calculations do not prove this mechanism is the origin of the differential latency for fixed and live human brains to respond to physiologically-patterned magnetic fields. However the physicalchemical perspective may encourage alternative interpretations and approaches to intrinsic residual properties within significant human tissue in response to weak, time-varying magnetic fields.

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