Article

Spectral Power Densities of the Fundamental Schumann Resonance Are Enhanced in Microtubule Preparations Exposed to Temporally Patterned Weak Magnetic Fields: Implications for Entanglement

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ABSTRACT

Preparations of microtubules (MT) from mouse melanoma cells emitted predictable photon counts when sampled 50 times per s (every 20 ms) that depended upon the numbers of these preparations. Counterbalanced serial 4 min exposures of the same MT to different temporally patterned magnetic fields with intensities between 3 and 10 μT did not alter the absolute photon emissions but shifted their spectral power densities (SPD). Compared to baseline (no field) 4 min periods there were conspicuous increases of power within the 7.7 to 7.8 Hz band during the 4 min exposures to patterned magnetic fields that facilitate long-term potentiation in neurons but not during exposures to a pattern associated with analgesia. *A priori* predictions of the shift in frequency (Δf) based upon the median mass of tubulin dimers, known numbers of unit charges per dimer, and the strength of the applied fields predicted a range between 0.11 and 0.14 Hz. SPD demonstrated two peaks at 7.74 Hz and 7.87 Hz or a Δf=0.13 Hz. The results indicate only 4 min exposures of microtubule preparations to specifically physiologically patterned magnetic fields associated with memory consolidation enhance the power of the numbers of photon emissions in a frequency band that is very similar to the fundamental Schumann Resonance.

Keywords: Tubulin, microtubules, photon emissions, weak magnetic field effects; spectral power density shifts.

1. Introduction

Consciousness in the universe and how it could be manifested in the brain has been the major thrust of Hameroff and Penrose's "Orch OR" theory [1]. They assume that neuronal microtubules are directly necessary for cognition and consciousness. Microtubules are important components of the cytoskeleton [2] that affect the location of organelles within the cell as well as intracellular transport [3]. If microtubules are critical for the emergence of consciousness and the implicit principle of superposition or potential for "excess correlation" between other microtubules independent of distance, they should exhibit photon emissions that respond to natural electromagnetic patterns. These patterns should reflect the frequency context in which living tissue exists upon this planet. We predicted that the spectral densities of photons emitted

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from preparations of microtubules in response to physiologically-patterned magnetic fields should exhibit coherence with the Schumann frequencies that permeate the earth-ionospheric cavity.

From a more integrated perspective, microtubules have been considered to be the most likely substrate for the “collapse of the wave function” to describe “consciousness” [1] and other complex phenomena such as “memory” and “excess correlations” [4]. The physical dimensions of microtubules exhibit interesting geometries that have been described by several researchers. For example Bras et al [5] indicated that the assembled microtubule exhibits approximately 13 protofilaments which compose a hollow tube whose maximum diameter is 24.6 ± 0.6 nm. The length extends in the micron range with a typical value between 1 and 2 μm. The subunits of the polymers are sequestered by weak non-covalent bonds which facilitate rapid assembly and disassembly [2,6,7]. These conditions of dynamic instability [6], in our experience, increase the probability that copious numbers of photons are emitted and could be detected by photomultiplier units. Rahnama et al [8] presented theoretical analyses that interactions between biophotons initiate fluctuations of microtubules between coherent and incoherent states. Dotta and his colleagues [9, 10] provided support for their hypothesis by measuring photon emissions in hyper-dark settings from both intact cells and microtubules preparations.

Alignment of macromolecular microtubules and related semi-rigid protein assemblies to applied static magnetic fields has been reported by several authors [11, 12]. The diamagnetic anisotropy of the dimeric subunits of tubulin in conjunction with the relative rigidity of the microtubules has been considered a major contribution to the phenomenon. As reiterated by Bras et al [5] diamagnetic anisotropy, that is the differential orientation of repelling effects from applied fields, originates from intrinsic properties of chemical bonds and particularly those that exhibit resonance structures such as peptide bonds. The effects are so small when static fields are applied that concentrated aggregates of microtubules must be exposed. Very strong magnetic fields that range from 8 T [13] to 17 T [14] were required to evoke discernable changes in diamagnetic anisotropy. We queried if there were other measurements by which much weaker magnetic fields could affect microtubule activities. One would expect the indicator of this activity to involve very small quantities of energy, such as that which might be associated with photons [15] within aqueous solutions [16]. Water, particularly Pollack [17] interfacial configurations adjacent to hydrophilic surfaces (exclusion zone water), has the capacity to transform disordered energy into coherent photons [18]. Appropriate frequency weak magnetic fields trapped within these coherent domains (~100 nm diameters) may contribute to this energy [19].

Ultraweak biophotons within the visible range are easily and reliably measured from non-malignant and cancer cells when they are removed from incubation [10, 20] and remain at room temperature. The irradiant flux densities are usually in the 10^{-12} to 10^{-11} $W \cdot m^{-2}$ or about 10 to 100 times greater than the background values for cosmic rays on the earth’s surface. Emissions of photons have been measured from bacteria, cells, organs, and entire organisms for decades [21]. Spectral analyses indicate that one major interval for the spectral power densities (SPD) of the numbers of photon emissions from aggregate of cells occurs within the extremely low frequency range (1 to 100 Hz) and is correlated with their functional associations and conditions [9]. Cosic et al [22, 23] applied the Resonant Recognition Model to tubulin by substituting each amino acid in the sequence with pseudopotential values and obtaining a spectral profile. She found peaks between 2.05 and 2.14 μm, 600 to 615 nm, and 440 to 470 nm. The latter is near the maximum sensitivity of our digital PMTs. That these frequencies relate to actual chemical sequences and pathways has been shown by Dotta et al [24].

Although changes in weak (nanoTesla) geomagnetic fields have been correlated with photon emissions [25], experimental elicitation of photons from cells by specific patterns of weak magnetic fields has only recently been reported. Dotta et al [26] tested a biophysical model of the cell plasma membrane by assuming the lateral diffusion of constituents within this "shell" produced a "membrane magnetic moment" which when interacting with a specific intensity, applied magnetic field would result in increased release of energies whose quantities were within the visible wavelength. The measurements strongly supported this hypothesis and indicated that photons are released from aggregates of cells when the appropriately temporally patterned and (weak) intensity magnetic fields are applied.

We designed experiments to test if the numbers of photons were quantitatively related to the numbers of microtubule preparations (MT) as inferred by measured medium only, a single dish, and a double dish of MT. Secondly we exposed the same single dish of MTs for intervals of 4 min to different physiologically patterned weak magnetic fields or baseline periods to discern if there were alterations in the SPD of the photon emissions. By employing a single dish of MTs at any given time as "its own control" and counterbalancing the presentation of the different patterned fields potential artifacts from handling would be minimal. Several of our unpublished studies had shown a specific enhancement of power of the flux density variations within the peaks that coincide with the Schumann Resonances whose fundamental frequency is about 7.8 Hz. Here we report that photons are related to the numbers of microtubule preparations and that the SPDs are enhanced at this frequency during sequential 4 min periods of specific (but not all) weak magnetic fields.

2. Materials and Methods

2.1 Microtubule Preparations and Exposure Parameters

Plates of $\sim 10^6$ B16-BL6 mouse melanoma cells were washed so that only microtubules (MTs) and the cell nuclei primarily remained in the dish [4]. Each standard plastic covered plate of microtubule preparations was then placed at room temperature on a table within 13 m^3 industrial acoustic chamber (also a Faraday Room) which was maintained as a hyperdark environment ($<10^{-11}$ $W \cdot m^{-2}$). The plate was placed on top of the aperture of a digital PMT (Model DM0090C from SENS-TECH, LTD). The plate and the sensor for the PMT were positioned between two containers within which the solenoids were contained. A diagram of the experimental arrangement is shown in Figure 1. This is the same device that was employed to elicit the enhanced release of photons from intact melanoma cells when the appropriate intensity, predicted by modified magnetic moment equations for the entire cell, in previous experiments [26].

In the first series of experiments, to verify the sources of the photons were related to the numbers of MTs, a total of 6 plates containing medium only, 6 single plates of MTs and, 6 double plates (i.e., 12 plates) were place over the PMT shown in Figure 1 and measured for 4 min. We selected this time based upon the requirement for protracted presentations for appropriate temporal sensing by chemical reactions [27, 28]. All plates had been maintained in standard incubators at 37°C until they were placed in the chamber. There was no more than one experiment per week.

In the second series of experiments a single plate of MTs was exposed to a series of 4-min exposures to specific magnetic field patterns or no-field conditions. The order of presentation was counterbalanced. A total of 10 different dishes of MTs were measured. The magnetic fields were generated by transforming a series of numbers from 1 through 256 to -5 to +5 V where 127=0 V by computer software (©Complex; Professor Stanley Koren). This operation was completed by a custom-constructed digital-to-analogue converter that was connected to four pairs of solenoids arranged in a quasi-circle within the containers. The circuit was constructed so that at any given time only one pair (one in the left and one in the right container) was activated for 0.5 s. This value had been selected based upon previous optimal experimental results. Consequently one rotation around the four pairs required 2 s.

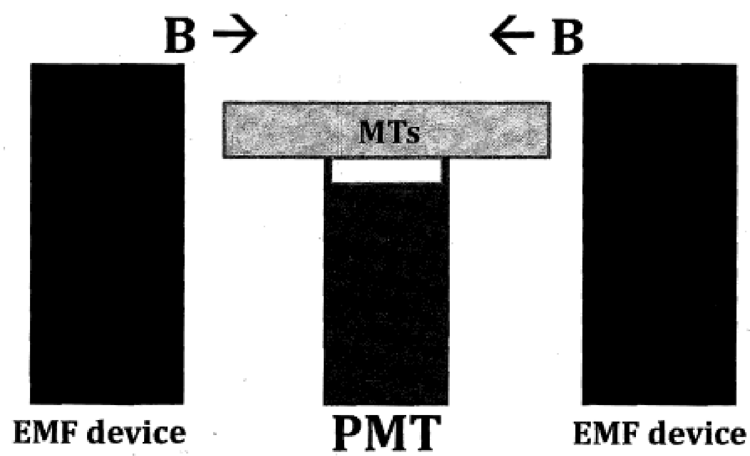

Figure 1. Diagram of the experimental arrangement indicating the placement of the plates of microtubule (MT) preparations over the aperture of the PMT and the placement of the EMF devices.

Three programmable patterns of magnetic fields were generated across the MTs. They are shown in Figure 2 and have been labeled: "Burstx", "Thomas", and "LTP". The Burstx, Thomas and LTP patterns were composed of 230, 849, and 225 points or columns of numbers, respectively, from which the voltages were determined. The point durations, which were programmable, for each of the three patterns was either 1 ms or 3 ms. Point duration is the time (in ms) each number (and hence voltage) is presented before moving to the next value. These values are similar to the binding constants of about 1.5 ms reported by Pilla et al [29] for Ca^{++}/CaM-dependent myosin phosphorylation during nonequilibrium phases. The intensities of the magnetic fields within the area of the dish as measured by power meters were between 3 and 10 μT. In addition to the experimental demonstrations that such weak fields supply relevant energies to molecular targets within cells, the traditional kT "constraint" argument against the effects of such weak fields has been refuted on the bases of its applicability to systems near thermal equilibrium, which biological systems are not [30].

The Burstx pattern was a crude digital representation of an amygdaloid neuron during an "epileptic discharge" from a human patient while the "Thomas" pattern was a more symmetrical decelerating frequency-modulated magnetic field derived from communication-like signals [31, 32]. The third pattern was the magnetic equivalent of the pattern that when applied as an electric current to hippocampal slices [33] results in long-term potentiation which is considered to the primarily biophysical-chemical substrate for representation of information (memory) within brain space. Each of these patterns has been associated with robust and significant changes in behaviors at the level of the organism that reflect the predicted function which have included analgesia and altered learning and memory [34,35].

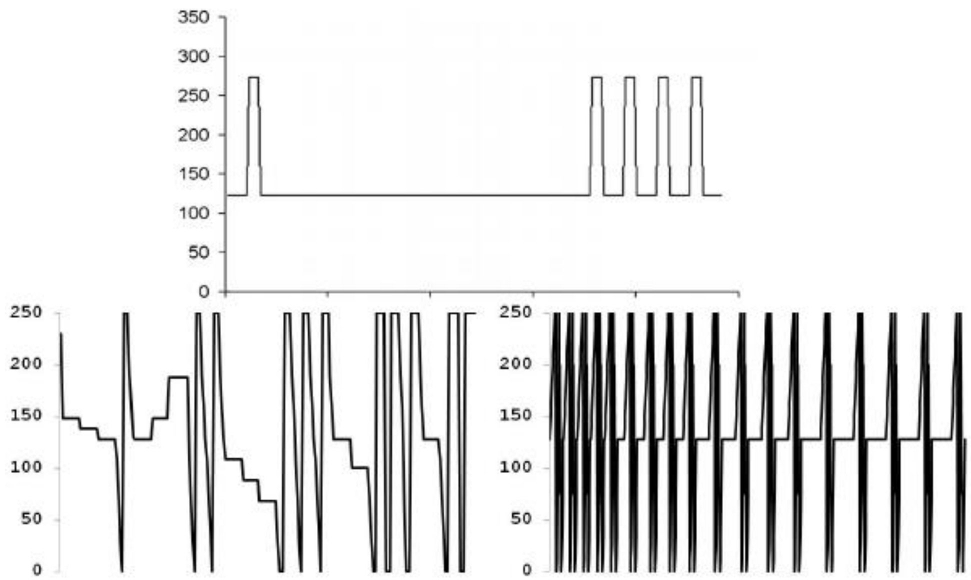

Figure 2. The temporal shape of the three patterns employed in the present study that affected the Spectral Power Density of the photon emissions from microtubule preparations. Top is "LTP", bottom left is the "BurstX" pattern and the bottom right is the "Thomas" pulse. The vertical axis refers to the numbers associated with voltage (-5 to +5 V) while the horizontal axis reflects the order of the points whose durations were either 1 ms or 3 ms.

2.3. Measurement and Analysis Procedures

During the 4 min increments successive exposures to various patterns of magnetic fields or no field intervals, the numbers of photons emitted from the MTs were measured once every 20 ms (50 Hz) which was the upper limit for the collection software of the equipment. The monitoring equipment for the PMT as well as the computer that initiated the different magnetic field patterns were outside of the exposure chamber whose double doors were closed. Consequently once the experiment with a given dish of MTs began there was no human presence or disturbance within the exposure area containing the MTs. The data were then loaded into SPSS 16 programs and analyzed for total photon counts per second for each 4 min interval. In addition, spectral analyses were completed for each 4 min (240 s) segment in order to discern

Spectral Power Densities (SPD). In previous experiments we had found that although the absolute numbers of photons may not increase with some types of patterned magnetic fields there was a robust shift in the amplitude distributions of the photon emissions during the exposures.

3. Results and Discussion

In order to ensure that the photon counts were directly related to the presence of the MTs, the raw measures per 20 ms were obtained for plates containing only medium but no MTs, one plate of MTs or two (stacked) plates of MTs. The results for one experiment are shown in Figure 3. It is important to emphasize that these values are means and standard deviations. This indicates that effectively there was no overlap within 3 standard deviations between the photon values from the no MT (medium only) plates and the two plate photon emissions. There were about twice the numbers of photons emitted from one plate of MTs compared to the medium only and four times this number for two stacked plates of MTs. This specific ratio strongly suggests the photon counts were a function of the numbers of MTs. The increase of 2 counts per 20 ms is equivalent to about 100 counts per s from the preparation. Given the aperture of the PMT is 1 cm^2 and assuming the maximum sensitivity of the PMT (400 nm), the energy equivalent would be about 10^{-12} W m^{-2} per plate. Figure 4 shows the results of all experiments (24 plates of MT) transformed to z-scores because different batches of MTs exhibited different baseline photon counts. The general MT number-dependent relationship is clearly obvious.

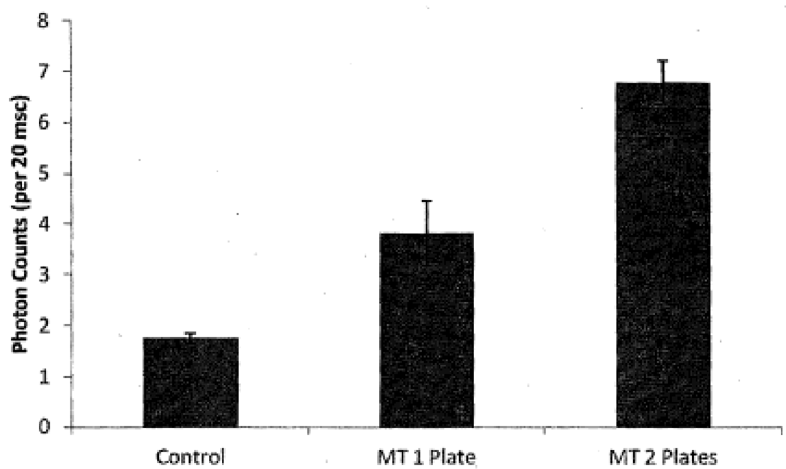

Figure 3. Example of the numbers of photon counts per 20 ms (limits of sampling rate) from plates containing medium only, a single plate containing microtubule preparations and two stacked plates of MTs. Vertical bars indicate SDs.

However there were no significant differences in the numbers of photons per second from single plates of MTs when they were exposed for 4 min each to either the sham condition (no field), Thomas configuration, LTP, or Burst patterns (grand mean=3.2 photons per 20 ms; SD=1.6). The results of the SPD analyses were very clear. The clearest difference over the 0.1 Hz Δf

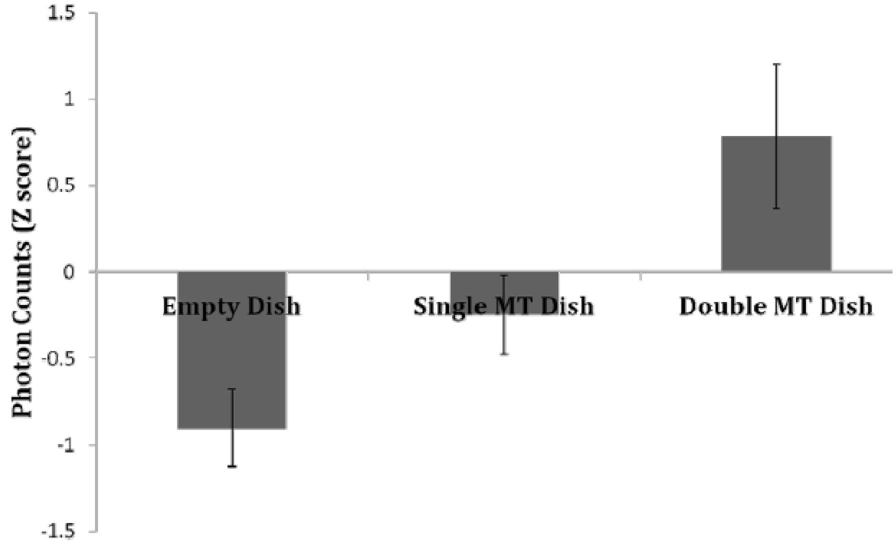

Figure 4. Standardized (z) scores for the numbers of photon counts per second from dishes containing only medium (no MT), a single dish of MTs, and two (superimposed) dishes of MTs for all experiments.

between the minimal and 25 Hz (the Nyquist limit is ½ of 50 Hz) value occurred within the 7.7 to 7.8 Hz interval. The results are shown in Figure 5. During the LTP and Thomas pattern exposures the MTs displayed greater SPD within this frequency interval than during either the Burstx or no field intervals. In other words there was a shift of the amplitude variations of the temporal distributions in the photon emissions during these 4 min of field exposures rather than alterations in the numbers of photons.

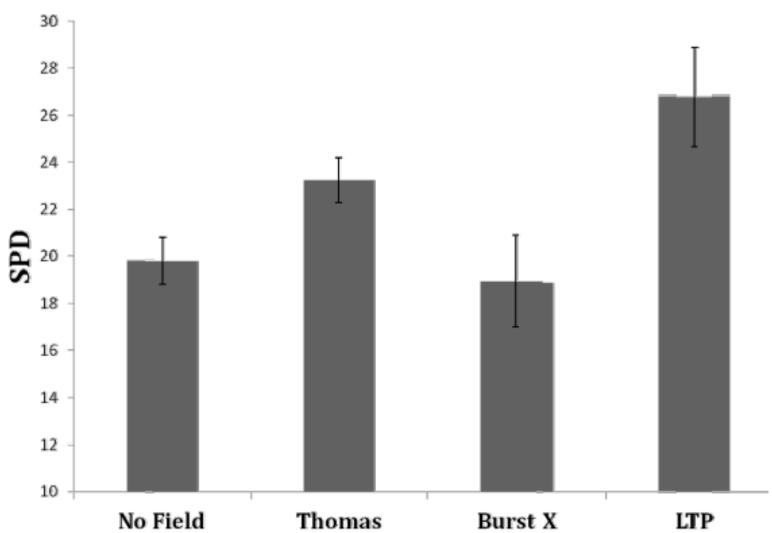

Figure 5. Spectral Power Densities (SPD) of photon emissions within the 7.7. to 7.8 Hz interval from microtubule preparations as a function of the no field and three field conditions. Vertical bars indicate standard deviations.

The 7.7 to 7.8 Hz band is the same as the fundamental for the Schumann Resonance which is generated within the spherical waveguide between the earth's surface and the ionosphere. Our local Schumann Resonance measurement station (compliments of Professor Kevin S. Saroka) as well as a major station in Italy (the data from which our measurements are highly correlated) indicates a comparable peak frequency [36]. We reasoned that if the application of the magnetic field shifted the SPD towards the 7.7 to 7.8 Hz band there should be a bimodal distribution or "split spectra" that would be directly and quantitatively calculable from the intensity of the applied field. This "double peak" might be considered analogous to the Zeeman split found in many spectra. That the Schumann fundamental displays intermittent split spectra of about 0.4 Hz in response to various geomagnetic events has been measured several times [37].

The general relationship between charge, mass and strength of the magnetic field according to dimensional analysis is $q \cdot kg^{-1} \cdot B$, resulting in Hz, where q is unit charge, kg is the mass, and B is the applied field. Assuming the median mass of a tubulin dimer is 55 kD with each monomer carrying 10 unit charges [38], the resulting frequency would be $32 \cdot 10^{-19}$ A·s divided by $9.13 \cdot 10^{-23}$ kg multiplied by the median root mean square for the intensity range (3 to 10 μT) of ~between 3 and $4 \cdot 10^{-6}$ T, or, between 0.11 and and 0.14 Hz. To test this prediction with the data the SPD were analyzed by SPSS according to the maximum Δt associated with the sample number (12,000, i.e., 50 per s, 60 s per min for 4 min) was 25 ms or 0.04 Hz.

The enhanced SPDs within the 7.7 to 7.8 Hz band when the Thomas and LPT were presented reflected this potential. Consequently we combined the data for these two conditions and compared them to periods where there no field conditions. As can be seen in Figure 6, the MTs exposed to either the LTP or Thomas pattern displayed the strongest difference in SPD compared to the sham field exposed MTs in two peaks, 7.74 Hz and 7.87 Hz, which was separated by 0.13 Hz. This difference is within the range of the predicted shift in frequency. The results are shown in Figure 6. The difference was statistically significant ($p < 0.05$). When the two field conditions were analyzed separately they both displayed this double peak but the split did not reach statistical significance. The Burstx pattern did not display this effect. In fact at 7.74 Hz the SPD did not differ from that of the sham field conditions.

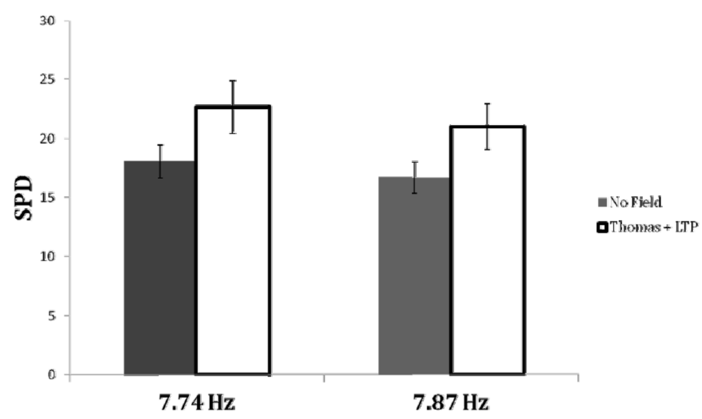

Figure 6. Peaks in the SPD of photon emission from MTs exposed to the two classes of fields demonstrating the potential "split spectrum" around the Schumann fundamental.

Additional analyses indicated that the MTs during the intervals when the Burstx pattern was presented displayed a statistically significant *diminishment* of SPD compared to no field intervals at around 20.5 Hz. This is congruent with the third harmonic of the Schumann Resonance. Exposures to the other two magnetic field patterns did not show this effect.

Considering the significance of photon emissions for intra- and intercellular communication, the reliable productions of these packets of energy from microtubules would be expected. As indicated by Alberts et al [2] microtubules can form noncovalent polymers and some tubulin subunits are enzymes that catalyze the hydrolysis of triphosphates which are often GTP (T-form) filament structures which can expand and GDP (D-form) structures that more rigorously restrict expansion. This rapid interconversion between a shrinking and growing state when there is a relatively homogenous concentration of free subunits produces a dynamic instability. We suggest that this condition would allow weak, appropriately patterned magnetic fields to shift the distribution of the energy from photon emissions within a specific ELF range.

That similarity in the maximum increase in SPD that occurred within a narrow band of 7.7 to 7.8 Hz and the fundamental Schumann resonance that is generated between the earth's surface and ionosphere [37] may be coincidence. The intensity of this magnetic field is about 2 pT which appears to be remarkably small. However the Schumann Resonance has been present since abiogenesis [39] and may have influenced the subtle structural evolution and interactions within proteins in general. The Schumann Resonance and its harmonics are due to global lightning discharges which occur between 40 and 100 times per s. Miller and his colleagues [40] had shown the electrical discharges within preCambrian atmospheric gases produced more than a dozen different amino acids.

The novel observation of a "split" of the SPD around the Schumann fundamental in microtubule preparations that had been exposed to the patterned fields that are known to affect "memory" (the representation of experience) may not be anomalous. Spectral analysis of the quantitative electroencephalogram of human brain activity reveals the recondite presence of Schumann harmonics as well as a bimodal peak [37]. Tanashi [41] while pursuing previous research of a 0.2 to 0.4 Hz spacing in the first mode of Schumann peaks also noted three peaks between 7.1, 7.85 and 8.5 Hz. These occurred within the earth's magnetic field which is about a factor of 10 stronger than the experimental fields employed in this study. The presence of a reliable resonance split in the preparations of microtubules that was enhanced by the appropriately patterned magnetic field might suggest an occluded property in the amplitude variation of photon emissions that reflects the evolutionary history of these fundamental structural proteins.

The shift in spectral power frequencies rather than the elevation of energy or power has significant thermodynamic implications for higher functions such as consciousness. A useful metaphor might be that a defective radio (that delivers static) and a functional radio (that delivers salient information) use the same power. The precision of the frequency might be critical. Harmony [42] who assessed quantitative electroencephalographic activity during different tasks found increased 7.8 Hz power in children that diminished during adulthood. Lisi et al [43] found that human keratinocytes exposed to 7 Hz, ~9 μT magnetic fields for one hour twice daily displayed altered shapes and actin distributions. Altered microtubule organizations, which can be disrupted by psychotropic drugs such as lysergic acid diethylamide (LSD), may be involved with some forms of psychosis [44]. It may be relevant that the isotype of beta-tubulin III, found almost exclusively in neurons, increases tumor aggressiveness.

4. Conclusion

The dynamics of the microtubule and its constituent tubulin dimers during periods of adaptation to a disrupted environment are associated with increased photon emissions. Spectral analyses of the photon emissions from plates of MT preparations within standard media in a Faraday room exhibited weak but significant, reliable peaks of SPD around 7.7 to 7.8 Hz. Exposures for only 4 min to 3 to 10 μT temporally patterned magnetic fields that are associated with the physiological substrates of "learning and memory" enhanced the magnitude of the SPD of photon emissions from MT. There was also a split in the spectra, not observed during the no field conditions, that was predicted by the gross quantitative relationship between the strength of the applied magnetic field, the estimated numbers of unit charges per dimer, and the aggregate's mass. As noted in previous experiments applications of the appropriate, temporally patterned magnetic fields to MTs do not affect the total photon emissions but shift the distributions of the amplitudes power spectra during the brief interval of exposure. If intracellular information is contained within shifting temporal patterns of energy but not the absolute shift in energy within dynamic systems then weak magnetic fields might affect the function of cells through microtubules.

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