PHYSICAL AND CHEMICAL CHANGES IN PLANARIAN AND NON-LIVING AQUEOUS SYSTEMS FROM EXPOSURE TO TEMPORALLY PATTERNED MAGNETIC FIELDS

By

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Abstract

Planarian maintained in spring water and exposed for two hours to temporally patterned, weak (1 to 5 μT) magnetic field in the dark displayed diminished mobility that simulated the effects of morphine and enhanced this effect at concentrations associated with receptor subtypes. A single (5 hr) exposure to this same pattern following several days of exposure to a very complex patterned field in darkness dissolved the planarian and was associated with an expansion of their volume. Spectral power density analyses of direct measurements of the spring water only following exposure to this field in darkness showed emission spectra that were displayed from control conditions by ~10 nm and associated with an energy increment of $\sim 10^{-20}$ J. This value is an intrinsic solution for the physical properties of the water molecule. "Shielding" the exposed water with plastic, aluminum foil or copper foil indicated that only the latter eliminated a powerful spike in photon emission around 280 nm. Continuous measurement of pH indicated that the slow shift towards alkalinity over 12 hours of exposure was associated with enhanced transient pH shifts of .02 units with typical durations between 20 and 40 ms. These results indicate that the appropriately patterned and amplitude of magnetic field that affects water directly could mediate some of the powerful effects displayed by biological aquatic systems.

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Chapter 1 - Introduction

The Nature of Water

Water is often observed to be ordinary as it is transparent, odourless and tasteless at standard conditions. It is the simplest compound of the two most common reactive elements, hydrogen and oxygen. Despite its chemical simplicity, it is highly versatile as it regulates a large variety of processes, including transport of material within plants and animals, formation of geophysical structures such as glaciers atmospheric phenomena, and is ubiquitous as ice in the interstellar space (Campbell et al 2006). In biological processes, water is a fundamental matrix necessary for chemical reactions associated with proliferation and sustaining of life. In all these examples, understanding the properties of water is crucial for elucidating the phenomena.

The unique properties of water that allow it to regulate the aforementioned processes are well known (Tait and Franks 1971; Cooke and Kunts 1974; Halle 2004; Eisenmesser et al., 2005) and are governed predominately by its ability to form hydrogen bonds (Maksyutenko et al. 2006). The distinct geometry that arises from such bonding gives water the ability to rearrange in response to changing conditions and establish dipole and induced-dipole interactions with neighbouring water molecules (Soper and Phillips, 1986). The ability of water to form clusters of 10-100 molecules (Keutsh et al. 2000) gives it a heterogeneous character allowing it to display spatial and temporal variation. Experiments by Narten and Levy (1969) showed a non-random arrangement for two to three water molecules adjacent to a central molecule. Wernet et al. (2004) revealed through X-ray absorption spectroscopy that a single water molecule makes one strong hydrogen bond, and by symmetry accepts one hydrogen bond, resulting in chains or rings rather than a previously hypothesized tetrahedral network.

The existence of any given water cluster is brief, as the equivalent ions that make up the molecule jump to a neighbouring molecule, with a lifetime of 1 picosecond (Geissler et al 2001) and a mobility of 3.6 x 10-3 cm^2/V s (Eigen and De Maeyer 1958). This short-lived exchange in water is similar to macromolecules and water interactions, with a timescale of 1-100 picoseconds (Bernten et al. 2005). These brief but strong interactions allow water to remain as the universal solvent and control crucial biochemical processes such as, protein folding, structure and activity, twisting of the double helix and recognition of the DNA sequence; all necessary for the existence of life.

Being that water is an integral component of biological macromolecules, it is particularly necessary for the activity of all proteins. Recent work has indicated that networks of hydrogen-bonded water molecules cover most of the surface of an enzyme and is mandatory for enzyme activity (Dunn and Daniel 2004). Moreover, enzymes that are in the presented with substrates in the gas-phase show no activity unless some water molecules are present (Smolin et al. 2005). From the point of view of the cell, it must be able to absorb and release water considering 80% of their mass is water (Cooke and Kunts 1974).

Until recently, a widely held misconception was that simple diffusion accounted for all water movement through the selective barrier, the lipidbilayer, and that water channels were not necessary (Agre 2006). Multiple studies (Agre and Kozono 2003; Gonen and Walz 2006; Kuchel 2006; Mitsuoka et al. 1999) have indicated that specialized membrane water transport macromolecules must exist in tissues to allow for high water permeability. In 1992, CHIP28 (channel-like integral proteins of 28kDa), now known as "aquaporin-1" was found. It was the first in a family of sought-after water channels that are vital in regulating intercellular water (Agre 1993).

When a positive charge is added to a water molecule, the resulting ion becomes the fundamental aqueous cation, called "hydronium" (H_3O^+) or "proton". The flow of these protons is as vital to life as the flow of water, as it is coupled to the energetics that fuel cellular metabolism and signal transduction (Bibikov et al. 1991). Hence, it seems advantageous for a cell to have a separate transport mechanism for water and protons so that cell volume and metabolism can be controlled independently.

Decoursey (2003) extensively reviewed the features of proton channels and other proton transfer pathways. He explains that the exchange of protons in intercellular water is of significance as there are much less free protons in physiological solutions in a concentration of 40nM compared to 110M in bulk water. These exchanges or proton conductions occur through Grotthuss mechanism, where each oxygen atom simultaneously passes and receives a single hydrogen atom, forming a water wire through the proton channels (Nichols and Deamer 1980; Nagle 1987). This mechanism, along with the relative small size of the proton: 1.6-1.7 fm in diameter (Cottingham and Greenwood, 1986), explains the high diffusion of protons (3.62 x 10^{-3} cm^2 V^{-1} s^{-1}) relative to other ions such

as sodium (0.519 x 10^{-3} cm^2 V^{-1} s^{-1}) or potassium (0.762 x 10^{-3} cm^2 V^{-1} s^{-1}). The flow of these protons, roughly 6.25 x 10^8 molecules, to the outside of the cell creates a current of 100pA. This current is strongly influenced by local pH, in that; an increase in pH can modulate the current from femtoamperes (10^{-15}A) to picoampere (10^{-7} A) ranges.

Further exploring the nature of water, Preparata (1995) indicated that bulk water consists of two components such that the molecules either synchronously oscillate or oscillate randomly. Coherence domains, which occur where water molecules are found at hydrophilic-hydrophobic interfaces, such as cellular membranes and proteins, exhibit atypical properties compared to their bulk counterparts (Zheng et al. 2003). Such properties include negative electric potentials up to 150mV and a ten-fold increase in viscosity that produces a region where solutes are strongly excluded (Zheng et al. 2003).

These solute-free "exclusion zones" extended several tens of micrometres from the hydrophilic-hydrophobic interface and is equivalent to many molecular layers of water. Water found within this zone displays interesting charge characteristics through proton accumulation, in that, the zone itself is negatively charged while the region beyond is positive. This zone also displays a characteristic absorption peak of approximately 270nm and releases fluorescence when stimulated with this wavelength (Zheng et al. 2003). This unique emission spectrum is attributed to extensive structuring of the water molecules in this zone compared to the bulk equivalents.

Though rarely seen in nature, pure water, which is water completely rid of organic and inorganic compounds displays its own distinctive properties. Zheng et al. (2008) elegantly showed through UV-Vis spectroflourmetry that systematically adding inorganic salt to water. Changes in emission spectra with peaks ranging from 480-490nm were evident. They argue that bulk water in the presence of certain types and concentration of salts exists in a specific arrangement that differs from ultra-pure water or water found within the exclusion zones.

The theoretical pH value of this ultra-pure water as defined by the equation, $pH = -\log_{10} [H^+]$ is 7.0, where $[H^+]$ is the molar concentration of hydrogen ions. Since ultra-high purity water is rapidly contaminated by CO_2 and other gases from the atmosphere more so than solutes, the accurate estimation of pH is through resistivity which is 18 M cm at 25°C and the pH is inherently 6.998.

Integrating these biophysical properties along with water's ability to display diamagnetism (Ikezoe et al. 1998), it is able to interact with all levels of discourse, such as the atom through to the universe, by means of physical forces such as electric and magnetic fields. Since all life on Earth are immersed in a ubiquitous magnetic field and comprised of water, understanding the relationship between the two phenomena is crucial.

Magnetic Fields

Magnetism is a property that is displayed by all matter as a result of the motion of their electrons. One relationship between the size of the force produced by this motion of charge (q in coulombs) and its velocity, (v in meters per second), is mathematically expressed as $F = qv \times B$ and results in the production of a Lorentz force (F), the quintessential description of magnetic fields (B). Thisforce relationship is in the form of a vector product and is perpendicular to both the velocity of the charge and the magnetic field.

The magnetic field is assumed to be composed of flux lines, which are theoretical lines of force that affect objects at a distance. The number of these lines per unit volume of space determines the intensity of the field, such that the more flux lines that exist in space, the more intense the magnetic field. The strength of the magnetic fields is measured in amperes per meter. However, in electromagnetic research the measure of flux density, calculated in units of Tesla (T), is employed.

When magnetic fields are externally applied to materials with partially filled electron shells, they display paramagnetic properties where the resulting dipole aligns along the direction of the flux lines creating an attractive force and enhances the applied field. Oxygen (with two unpaired electrons), a prime component of water and in atmospheric gas, is an example of a paramagnetic material. When it enters into a chemical reaction and interacts with other atoms to form molecules, it becomes diamagnetic. In these compounds, the completed pairs of electrons in the outer most orbital shell align perpendicularly to the direction of the externally applied field (Yamauchi 2008).

The mechanisms by which magnetic fields interact with the environment are dependent on its presentation with respect to time, and are classified as static or time-varying. Static magnetic fields remain largely fixed, except for a slow decay over time, which is due to the normal forces of entropy or disorder. They are a result of the interaction between the magnetic flux lines and induced electric fields. The resultant electric current's lifetime in any system is contingenton the electrical properties (i.e. resistance, capacitance, and inductance) of the medium from which the magnetic field is produced. Static magnetic fields exert their effects on biological and aqueous systems by altering the orientation of asymmetrically distributed charges on cells (Rosen 2003).

Time-varying magnetic fields are defined by the change in the field's intensity over time. These fields are often referred to as electromagnetic fields (EMF), as the resultant change in the magnetic field is associated with changing electric field and vice versa. Applying these fields across or through matter has the potential to continually change the direction and orientation of atoms and molecules found within this matter. This phenomenon was identified independently by Michael Faraday and Joseph Henry in 1831.

Their experiments demonstrated that an electric current could be induced in a circuit by time-varying magnetic fields. This finding led to the discovery of the fundamental law of electromagnetism, Faraday's Law of Induction which states that magnitude of the electromagnetic field induced in a circuit equals the time rate of change of the magnetic flux through the circuit. Biological systems that are submerged in charged aqueous solutions effectively resemble Faraday's circuit, and the application of a time-varying magnetic field may potentially induce a current capable of disrupting the intrinsic electrical signals and consequently alter the structure and behaviour of the system.

Any system that exhibits periodicity or oscillations, such as the coherent domains in water within biological systems can be influenced by any external stimulus exhibiting the same periodicities or oscillations. Several studies (Bramwell 1999; Schevkunov et al. 2002; Wei et al. 2008) have used electricfields as the source of external stimulus and found that they are not as effective or easy to manipulate in experimental conditions compared to that of electromagnetic fields. This is because electric fields are usually distorted at boundary conditions and do not penetrate the biological system with the same efficacy of electromagnetic fields.

Despite the large number of experimental studies showing the in vitro and in vivo effects of electromagnetic fields, these effects are controversial as the energy produced by these fields is several orders of a magnitude lower than the thermal energy or "noise" created by molecules in random motion. This is also known as the k_BT problem, where k_B is Boltzmann constant at T room temperature. The k_BT problem arises from a physical model and is only applicable to systems near thermal equilibrium. Biological systems are among those that do not exist at such equilibrium, which is has been a naturally selected feature for proper physiological functioning (Hartwell et al 1999).

<u>Magnetic Field Effects on Aqueous and Biological Systems</u>

The overwhelming majority of investigations into the effects of magnetic fields on water have been carried out using fields much stronger that theEarth`s natural geomagnetic field which produces intensities of 25 to 65 µT (Shaar et al. 2011). Chang and Weng investigated the effects of static magnetic fields on the hydrogen-bonded structure of water and found that the number of hydrogen bonds increased when the strength of the field was increased from 1 to 10 T. Hosoda et al (2003) suggested that this effect was caused by the increased electron delocalization in the hydrogen-bonded molecules.

Lower, but still strong magnetic fields (0.2T) have been shown to increase the number of monomer water molecules, but increase its tetrahedrality at the same time. Intensity dependant changes in physical properties of water, such as surface tension and viscosity when exposed to 0.16 T or 1 T fields were shown by Gang and Persinger (2011) and Cai et al (2009) respectively. EMFs with very small intensities, comparable to that of the natural geomagnetic fields, have shown to affect the solubility of gases in water, increase rate of evaporation, and the dissolution rate of oxygen. These changes together with changes in other physical properties of water (thermal conductivity, supercooling extent, refractive index, electrical conductivity, and adsorption) as a result of in EMF exposure are due to the weakening of the Van der Waals forces that bind together adjacent water molecules (Krems, 2004). An open, more hydrogen-bonded network structure slows reactions due to its increased viscosity, reduced diffusivities and the less active participation of other water molecules. Thus, the weakening of this structure by EMF exposure should encourage reactivity with other compounds including biomolecules.

These changes in water's structure by EMF exposure affect the optical properties of water and are commonly observed using photoluminescence tools such as spectrophotometry. Pang and Deng (2010) showed that a difference in infrared absorption spectra between 'magnetized' and pure water. This alteration in the spectra does not immediately dissipate after removal of the externally applied EMF but remained for very long periods of time, suggesting water is able to retain the information presented by physical fields. This memory effect was first put forth by Benveniste's controversial experiments in 1988. He not only demonstrated water's ability to retain imprints of EMFs but when he extracted and applied magnetic signatures of chemical reactants to unmagnetized water, the water produced changes as if the compound was physically present in the medium.

As electromagnetic fields influence the polarized properties and structuring of water, they in turn must have an effect on the biological organism that is comprised of these aqueous solutions. The biological effects of electromagnetic fields have been reviewed quiet extensively over the century and all indicate that the direction of effect EMF may have on biological system depends on its intensity, frequency or an interaction between the two. Though it may seem counter-intuitive, most of the therapeutic effects produced EMFs are in the low to extremely low frequency (3 -300Hz) range involve relatively weak intensities. This indicates that 'bigger is not necessarily better'. Fleming, Persinger and Koren (1994) elegantly demonstrated this idea when they reported that whole body exposure of rats to a magnetic field modelled after the burst firing pattern of neurons with intensities of 1 μT, produced analgesia-type effects. These analgesic effects were still apparent 20 minutes after removal of the field and comparable to the analgesia produced by 4mg/kg of morphine.

Along with frequency and intensity, the temporal structure of the applied EMF is critical for eliciting specific biological effects. Since EMFs are able to penetrate all biological tissues and oscillate synchronously with their components, strategically designing the waveforms to imitate natural processes can duplicate or enhance the process, allowing for the complex patterned fields tobe more efficacious and have more biological relevance than a simple structured pattern.

McKay et al (2000) exposed rats to waveforms that were modelled after electrophysiological patterns found within neural structures and simple sinusoidal waves. Rats that had been exposed to the modelled complex field displayed marked attenuation of contextual freezing behaviour than rats that had been exposed to the sinusoidal wave, which did not differ from sham conditions. The importance of waveform shape was also seen when low frequency EMFs at ~20uT in waveforms of continuous sinusoidal in nature produced hyperalgesia in pigeons (Del Seppia et al. 1987) and humans (Papi et al 1995), compared to the morphine-comparative analgesia produced by the aforementioned burst-firing modelled EMFs.

As EMFs are capable of eliciting a plethora of biological effects, a plethora of models for which we can observe and manipulate the effects must also exist. However, to observe the effect of EMF exposure on aqueous solution, its subsequent effect on biological tissue and vice versa, it is evident that an aquatic organism model would be an appropriate model. Such model is the free-living non-parasitic flat-worm, planaria.

Planaria

Classified into the Phylum Platyhelminthes, planaria possess key anatomical features that might have been a platform for the evolution of complex and highly organized tissues and organs found within higher order life. They are the simplest organisms that have bilateral symmetry, distinct excretory and central nervous systems. Planarians also have several diverse sub-epithelialgland cells that are involved in producing the mucous secretions used by the animal for locomotion, protection, adhesion to substrates and capturing prey. These flatworms lack respiratory and circulatory systems and rely on diffusion of materials including oxygen, from their aqueous environments.

The planarian nervous system consists of bi-lobed cerebral ganglia at the anterior end and two longitudinal nerve cords that lie on the ventral surface of the body (Cebria 2007). A sub-muscular nervous plexus runs beneath the body-wall musculature and connects to the main nerve cords. Sensory structures (photoreceptors and chemoreceptors) that are located at the anterior of the animal send projections to the cephalic ganglia, which then process these signals and direct the appropriate behavioural responses.

Classical morphological and physiological studies reveal that planarian neurons more closely resemble the neurons of vertebrates, than those of higher invertebrates. They possess the same neurotransmitters systems (e.g. serotonergic, opioid, dopaminergic) found in mammals (Nicolas et al 2008) making them a good model for neuropharmacological and toxicological studies. Their highly permeable exterior allows Planaria to absorb low molecular weight chemicals from their environments. Because of this high permeability and the presence of fewer receptor sites for competing pharmacokinetic drug-drug interactions, any subtle influences made to a planarian's aqueous environment displays a profound effect on its behaviour.

Planaria possess an extraordinary capacity to regenerate due to the presence of a large adult stem cell population (Reddien and Sanches-Alvarado 2004). Individual planarians are practically immortal, meaning that they are able to regenerate, preventing them from entering senescence, as well as severelyrepair and restore damaged or lost, tissues (Aboobaker 2011). A trunk fragment cut from the middle of an adult planarian has the capability of regenerating into a whole worm, always obeying polarity so that the new head and new tailgrow in the same orientation as the original worm. As little as 1/279th segment of a whole of a planarian (Morgan 1898), or a fragment with as few as 10,000 cells (Montgomery and Coward 1974) can restore into a new worm within 1–2 weeks (Gentile et al, 2001). Planaria have been used for more than a century in research settings, and are a popular model for molecular-genetic and biophysical investigation of the signalling pathways that underlie regenerative patterning (Aboobaker 2001), since they possess more genes in common with humans than does the fruit fly, Drosophila (Lobo et al, 2012).

The Present Study

In the present study the effects of a patterned magnetic field that has been employed to reduce the division of cancer cells but not normal cells was investigated to discern its effects upon: 1) mobility of planarian, 2) dissolution of planarian, and, 3) shifts in energy and photon emissions with spring water, and, 4) shifts in pH that could be quantitatively coupled to relevant changes in membrane activity.

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(submitted to BiochemicaBiophysicaActa)

Chapter 2: Comparisons of Responses by Planarian to microMole to attoMole dosages of Morphine or Naloxone and/or Weak Pulsed Magnetic Fields: Revealing Receptor Subtype Affinities and Nonspecific Effects

Abstract

The behavioral responses of planaria to the exposures of a range of concentrations of morphine (μM to attoM) or the μ-opiate antagonist naloxone or to either of these compounds and a burst-firing magnetic field (5 μT) were studied. Compared to spring water controls, the two-hour exposure to the patterned magnetic field before measurement reduced activity by about 50% which was comparable to the non-specific effects of morphine and naloxone across all dosages except 1 attoMole that did not differ from spring water. The specific dosage of 100 nM produced additional marked reduction in activity for planaria exposed to either morphine or naloxone while only 1 pM of morphine produced this effect. The results support the presence of at least two receptor subtypes that mediate the diminished activity effects elicited by morphine specifically and suggests that exposure to the specifically patterned magnetic field produces a behavioral suppression whose magnitude is similar to the "dose independent" effects from this opiate.

Key Words: planaria · weak analgesic magnetic fields · morphine · naloxone · receptor subtypes

Introduction

Nociception and its avoidance are fundamental to the survival of organisms. Several opiate receptor subtypes each with a different affinity (effective concentration) mediate the effects of morphine within several species. Although there are multiple *in vitro* methods for discerning among these receptor subtypes, we have been pursuing behavioural indicators in aquatic animals, such as planarian, to discern the presence of these receptors by measuring their quantitative responses to a broad range (micro-to attoMole) of concentrations of ligand. Easily measured altered behaviours within narrow ranges of concentration allow relatively inexpensive and quick inferences of receptor subtypes. The possibility that the efficacy of agonists and antagonists, as well as exposure to "physiologically" patterned electromagnetic fields, might involve similar receptors can also be discerned. Here we present evidence of at least two receptor subtypes for morphine displayed by planarian and the likelihood that particularly patterned weak magnetic field that is known to produce analgesia in vertebrates (Baker-Price and Persinger, 2003; Martin et al, 2004; Martin and Persinger, 2004) and invertebrates (Thomas et al, 1997) may involve one of these receptors as well as non-specific effects.

The development by Raffa et al (2003) of a sensitive and convenient metric for planarian locomotor velocity (pLMV) to assess the responses of these organisms has facilitated discernment of subtle changes to gradations of pharmacological substances. This measure was sensitive to dose-related exposures to cocaine (10^{-9} M to 10^{-5} M) and differential responses to κ-opioid receptor antagonists (Raffa et al, 2003). Umeda et al (2004) showed that the decrease in LMV associated with withdrawal from cocaine and κ-opioid compounds could be blocked by D-glucose but not by L-glucose. There are other methods of assessing planarian responses, such as the qualitative changes in motor behavior in response to opioid-dopamine interactions (Passarelli et al, 1999). We selected LMV because of the precision and marked reproducibility. The quantitative values for LMV for our untreated planarian, employing our samples sizes, were not significantly different than those reported by Raffa et al (2003).

The isolation of the convergent operations that would allow mutual equivalence of the spatial organizations of matter as displayed by chemicals and the temporal organizations of information as displayed by temporally patterned electromagnetic fields could be as significant as the discovery of the Rosetta Stone for the equivalence codes for archaeological languages. Several authors have pursued this potential relationship for the domain of nociception because of its importance in adaptation. In a seminal series of studies, Thomas et al (1997) examined the effects of a pulsed magnetic field upon analgesic responses of the land snail as inferred by the pre-injection of either agonists or antagonists for μ,δ, or κ opioid receptors. They found that the specific pulsed magnetic fields elicited significant analgesic effects through mechanisms that involved δ and to less extent μ receptor subtypes. The results were anticipated by Kavaliers and Ossenkopp (1991) andPrato et al (1995) who suggested a resonance-like dependence of the magnetic field effect.

Planarian's responses to different types of magnetic fields have been well documented. The classical work of Brown (1962) and Brown and Chow (1975) established that the orientation and movement directions of planarian could be modified experimentally by applying weak static magnetic fields (less than 10 times the intensity of the earth's magnetic field) in the horizontal plane. Recently Mulligan et al (2012), employing an "energy" resonance approach, showed that the movement of planarian was specific to weak, amplitude-modulated 7 Hz magnetic fields presented for only 6 minutes once per hour during the dark phase. The effect was enhanced with a specific concentration, based upon the predictions from the resonant model, of melatonin in the water medium. There are several researchers who have shown fission rates and regeneration of planarian respond in a non-linear manner to static fields between 10 and 3000 nT (Novikov et al, 2007; 2008) and to specific resonance-related frequencies within the 10 to 40 μT range. Such features may be analogous to differential sensitivities of receptor subtypes. The results also strongly support the role of calcium resonance (Titras et al, 1996; Jenrow, et al 1995). Congruent changes in biomolecular pathways such as ERK (extracellular regulated signal kinases) and heat shock proteins (hsp70) levels have been replicated (Goodman et al, 2009; Madkan et al, 2009).

Planarian contain all of the major classical neurotransmitters, such as serotonin, acetylcholine, the catecholamines, gamma-amino butyric acid, and excitatory amino acids, that are present in mammals (Buttarelli et al, 2008). Synthesis of anandamide and expression of cannabinoid receptors as well as the localization of Met-enkephalin in the neuropil of flatworms has been reported (Passarelli et al, 1999). The general consensus has been that the receptor for this enkephalin is the planarian equivalent of the mammalian κ-opioid receptor (Raffa et al, 2006). Because planaria lack a blood brain barrier and their responses are so stereotyped, discernment of complex interactions should be less affected by the intrinsic impedances encountered with mammalian systems yet reveal potentially applicable information.

Materials and Methods

Brown planaria (*Dugesia tigrina*) were obtained from Carolina Biological Supply (Bulington, NC). The planaria were acclimated to local laboratory conditions (21° C), housed in darkness, and utilized in experiments within 72 hrs of arrival. A given planarian was used as a subject only once. Morphine sulphate solution and naloxone hydrochloride were obtained from Sigma-Aldrich and diluted in spring water to the desired molarity. Spring water was obtained from Feversham, Grey County, Ontario. Ion contents of the water in ppm were HCO_3 270, Ca 71, Mg 25, SO_4 5.9, Cl 2.7, NO_3 2.6 and Na 1.

Worms were removed from group containers and placed in 1.5 mL plastic conical centrifuge tubes (Fisherbrand with flat-top snap cap with dimensions of 10.8 x 40.6 mm) composed of polypropylene. There was one worm per tube containing 1 mL of spring water. Each tube was held upright by placing it in a plastic rack that held 27 tubes per experiment. Consequently there were 27 planarian (27 tubes) per exposure or experiment. Within 1 min of being removed from group containers and placed in the plastic tubes, the worms were exposed for 2 hrs to a magneticfield or sham condition (no field) within a Helmholtz coil. The coil was 32.5 cm by 32.5 cm by 41 cm long and was wrapped with 305 m of 30 (AWG) gauge wire (30 ohm). The tube container was placed in the middle of the coil.

For the first part of the study, two patterns of magnetic fields whose analgesic effectiveness has been demonstrated in several studies for rats (Martin et al, 2004), human patients (Baker-Price and Persinger, 2003; Richards et al, 1993), and invertebrates (Thomas et al, 1997), were tested. The two patterns (Figure 1) are frequency modulated fields generated from converting numbers between 0 and 256 to between – 5 V and + 5 V with 127 as 0 V. The total numbers of points (values between 0 and 256) for the two patterned fields were 849 for the "Thomas pulse" and 248 for the "burst-x" pattern. The former was developed by Thomas and Persinger (1997) based upon theoretical and chirp-features of communication systems while the later was extracted simulation of burst firing from averaged potentials from a human amygdala during the display of complex partial epileptic seizures (Richards et al, 1993).

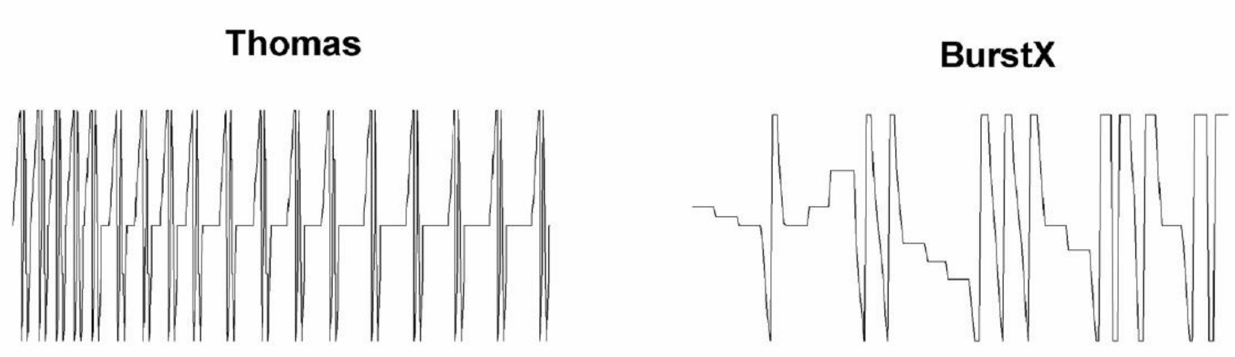

Figure 1. The two patterns of magnetic fields initially examined to compare the efficacy of their effects upon planaria activity when exposed to μM to attoM concentrations of morphine or naloxone.

The point duration, the time each incremental voltage determined by the number between 0 and 256, for both fields was 3 ms. This has been shown to be more effective to produce analgesic effects in rats than point durations that are more or less than that value (Martin et al, 2004). A similar effect was noted for related temporal patterns of magnetic fields for more protracted exposures upon mortality of planarian (Murugan et al 2013). The time between the patterns was 3 ms for the Thomas pulse and 3000 ms for the burst-firing pattern in order to simulate the optimal conditions described for hotplate analgesia for rodents (Martin et al, 2004). The average (root mean square) of the field strength within the center of the coil where the 27 tubes were located was 5 μT (± 0.5 μT). A total of 70 worms were measured.

After this period each worm was placed into a clear glass dish containing spring water in order to measure velocity of movement. This procedure was similar to that first described by Raffa et al (2003). The dish was placed over graph paper with gridlines separated by 5 mm. Velocity was defined as the numbers of gridlines cross during a 5 min interval and expressed as the numbersof gridlines crossed per minute. The cumulative number was calculated as the measure of LMV.

In the second part of the study, the two test compounds (morphine, n=171 planarian, and naloxone, n=156 planarian) were diluted into aliquots of spring water with between 25 x10^{-6} M to 10^{-18} M for morphine and 10^{-6} M to 10^{-15} M for naloxone. We selected this maximum concentration for morphine because 1 and 10 mM produced planarian mortality. Spring water was the "control" or reference condition. The serial dilutions for the two drugs were completed by extracting 0.01 μL of solution with a digital micropipette and adding it to 15 mL of spring water. There were 3 worms per dilution for the 9 dilutions during a given exposure and at least three replicates (different days) were completed. Each worm was placed individually into a conical centrifuge tube containing 1 mL of test solution and allowed to habituate for 2 hrs prior to tests for locomotor activity.

In the third part of the study, based upon the results of part 1, groups of planaria were exposed to different concentrations of the test compounds (morphine, n=91; naloxone, n=91) while they were being exposed to the burst-x firing magnetic fields for 2 hr. The primary statistical procedures involved multiple level analyses of variance, one-way analyses of variance with Scheffee's post hoc tests to differentiate the groups. All analyses involved SPSS 16 PC software.

Results

As shown in Figure 2, the planarian exposed for 2 hrs to either of the two temporally-patterned magnetic fields displayed significantly less movementduring the five minutes after being removed from those conditions than did sham field-exposed planarian. The planarian exposed to the "burstX" pattern displayed significantly less movement than those exposed to the other field pattern. In fact the activity was about one-half of the planarian exposed to the sham treatment. One way analysis of variance demonstrated a significant difference [$F(2,67)=325.63$, $p < .001$; $\Omega^2=.91$]. *Post hoc* analyses indicated that the worms exposed to the burstx pattern field displayed lower LMV then those exposed to the Thomas pulse; however the LMVs of groups exposed to either of these fields were significantly lower than the sham-field exposed groups.

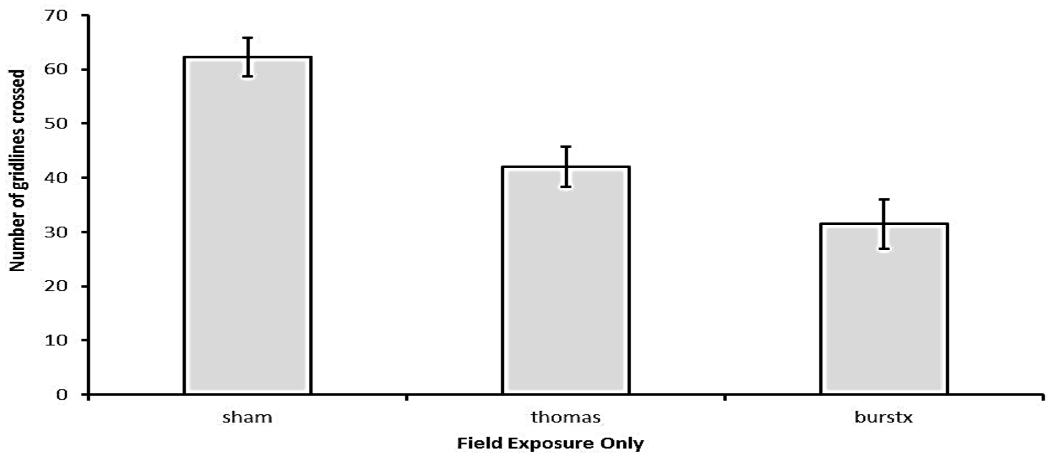

Figure 2. Mean total numbers of grid lines crossed (locomotor velocity or LMV) over 5 min for planarian after exposure for 2 hrs to sham fields or to either the "Thomas" pulse frequency-modulated or "burstx" magnetic fields with strengths around 5 microTesla. Vertical bars indicate standard errors of the mean (SEM).

The mean values for the LMV, as inferred by numbers of grids crossed, of planarian that were exposed within their aqueous environment to various concentrations of morphine between 25 μM and 10 attoM are shown in Figure 3. All dosages of morphine, except 10 attoM, produced suppressions of activitycompared to the water only condition. *Post hoc* analyses of this very significant [$F(12, 158)=93.90$, $p <.001$; $\Omega^2=0.88$] difference in LMV as a function of dosage revealed that the 100 nM and 1 pM concentrations produced the greatest reduction in activity that was significantly lower than 10 fM, 1 uM, or 100 pM, which were in turn significantly lower than all of the other concentrations. There was no significant difference in LMV for the worms exposed to spring water or to 10 attoM of morphine.

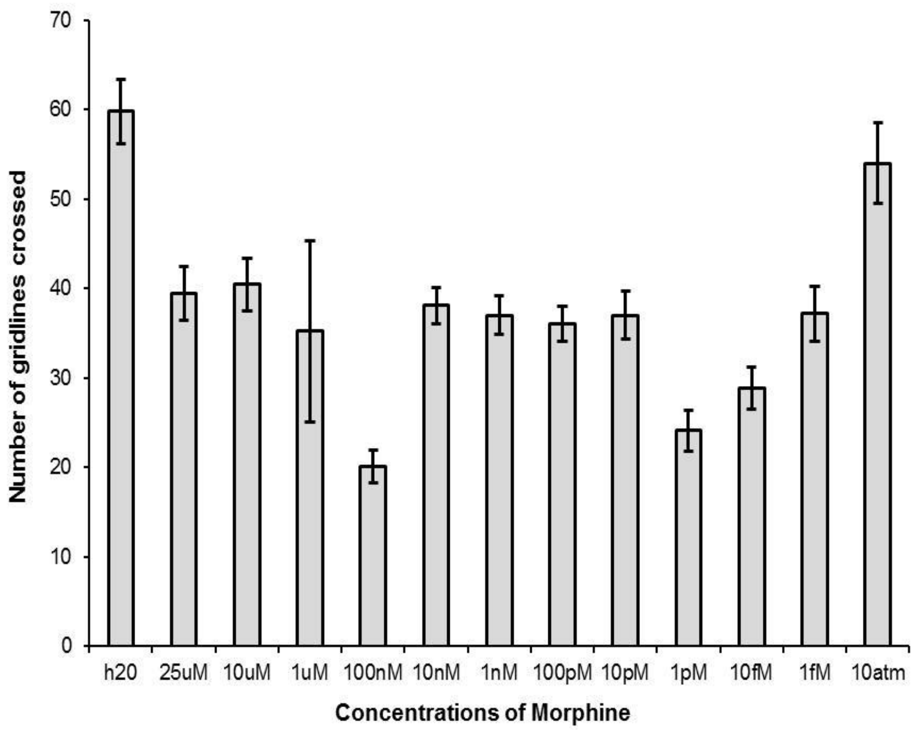

Figure 3. Mean total numbers of lines (LMV) crossed in 5 min for planarian exposed for 2 hrs to different concentrations of morphine. Vertical lines indicated SEMs. There was no statistically significant difference in activity between spring water only and morphine at 10 attoMolar concentrations.

Figure 4 shows the responses of the planarian to different concentrations of naloxone, the classic μ-opioid receptor antagonist. The planarian responded by diminished activity at the same concentrations 100 nM and 1 pM that produced this effect for morphine. *Post hoc* analyses of the significant [$F(11,144)=33.43$, p

<.001; Ω^2=0.71] dosages differences indicated that the 100 nM and 1 pM dosages produced the lowest activity that was significantly different from all other dosages (except 1 fM and spring water) that did not differ significantly from each other. The spring water and 1 fM concentrations did not differ significantly from each other.

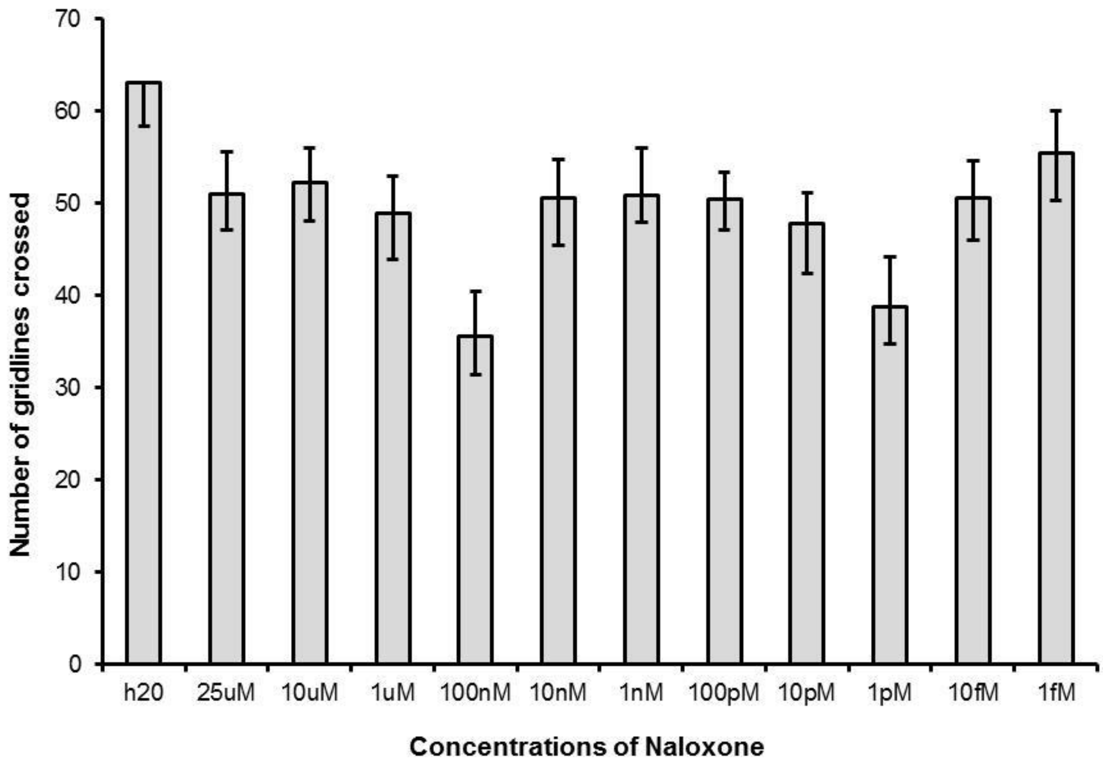

Figure 4. Mean total numbers of lines crossed in 5 min by planarian after being exposed for 2 hrs to different concentrations of the opioid (μ) antagonist naloxone. Vertical bars are SEMs.

Figure 5 shows the activity of the planarian when exposed to variousconcentrations of morphine **and** at the same time to the burstx magnetic fieldpattern. *Post hoc* analyses indicated that the significant dosage differences [F(8,72)=15.40, p <.001; Ω^2=63%] was due in large part to the lower LMV values for planarian exposed to the field+100 nM and the field+1 pM compared to all other concentrations. The 1fM+field and spring water conditions did not differ significantly from each other.

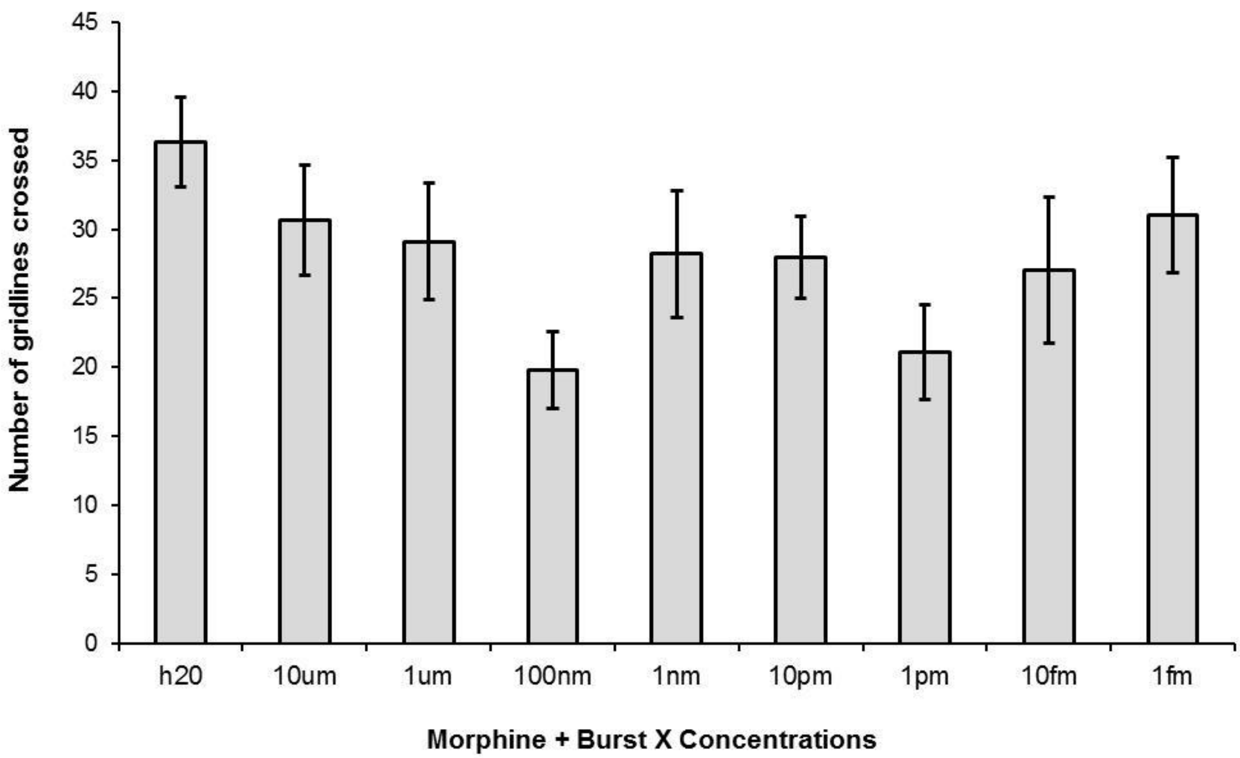

Figure 5. Planarian movements over 5 min following exposure for 2 hr to the burstx magnetic field pattern and various dosages of morphine or water (h20). Vertical bars are SEMs.

For comparison, the effects of simultaneous exposure to the burstx magnetic field and various concentrations of naloxone are shown in Figure 6. The significant dose-differences [$F(8,72)=19.49$, $p <.001$; $\Omega^2=0.68$] were due in large part to the lower activity for the field+100 nm naloxone group which was significantlydifferent from the field +1 pM, 1 nM, 10 pM, and 10 μM groups who displayed less activity than all of the other groups that did not differ significantly (including water) from each other.

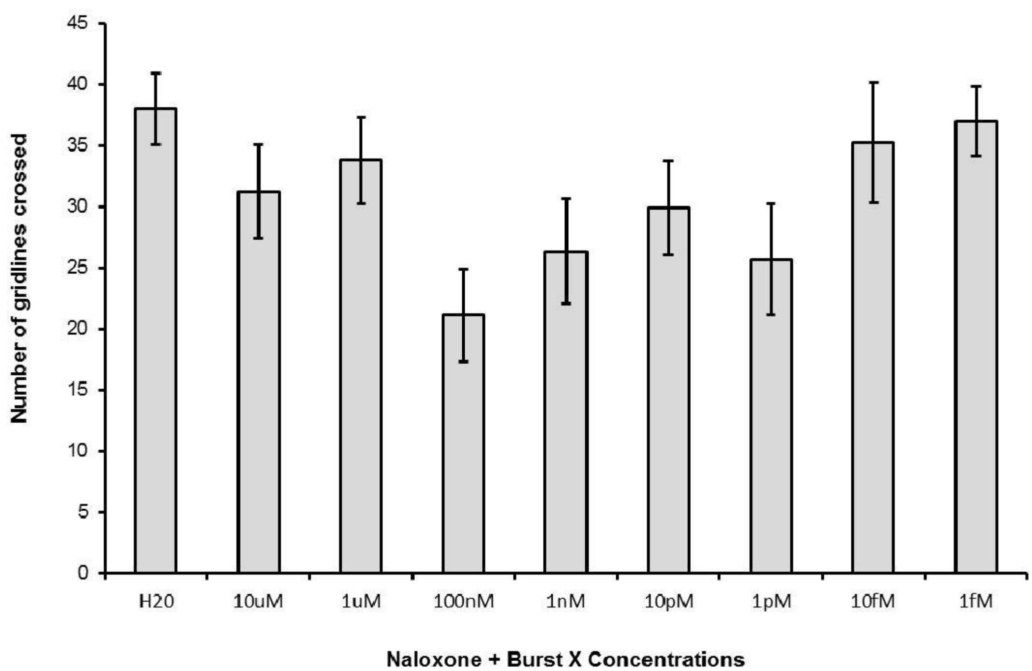

Figure 6. Mean total numbers of gridlines crossed in 5 min by planarian exposed for 2 hrs to the burstx magnetic field and to various concentrations of naloxone. Compared to other concentrations and to spring water, the primary statistically significant diminishment was evident for the 100 nM concentrations. Vertical bars are SEMs.

Clearly, the most conspicuous general effect of the exposure to this particular magnetic field configuration was the diminished activity, compared to sham-field controls, for all concentrations of drugs as well as for spring water. The planarian placed only in spring water averaged about 61 crossing while those exposed in spring water and the magnetic field traversed about 36 crossings (Figures 5 and 6), that is about 60% of control activity which was similar to the effects of all morphine concentrations except for 100 nM and 1 pM levels. On the other hand those exposed in control conditions without any drugs displayed an average of 60 crossings compared to the 20 crossings produced by exposure to 100 nM of morphine, that is, about 33% of no-drug activity. Stated alternatively the diminished activity from the magnetic field was comparable to the "non-specific" effect of the presence of morphine in the aqueous environment. The two exceptions to this effect was the greater diminished activity associated with the 100 nM and 1 pM concentrations.

To discern the specific effects, if any this magnetic field configuration exhibited upon the two putative "receptors" dosages around the two enhanced concentrations, two-way analyses of variance were completed for the morphine and morphine+field conditions, and, for the naloxone and naloxone+field conditions for the concentrations that showed the greatest non-linear effect (diminishment) of activity: 1 μM, 100 nM and 10 nM and 10 pM, 1 pM, 10fM and 1 fM. The values below are reiterations, for convenience, of the means (with SEMs) noted in the figures. The significant interaction [$F(2,65)=4.67$, $p=.01$]

between field+morphine and morphine was due to the significantly lower activity

for the field+morphine (M=28.7, 28.2) compared to the morphine only groups (M=35.6, 37.5) for the 1 μM and 10 nM concentrations, respectively, while there was no difference for the 100 nM concentrations (19.5, 19.8). In other words the field did not increase the dimished activity at the most efficacious dosage but widened the concentration range at which general reduction occurred. A similar two way interaction [F(3,76)=4.71, p <.01] was noted for the lower concentrations. The LMV means for 10 pM, 1 pM, 10 fM and 1 fM for the morphine only and morphine+field treatments were 37.0, 24.4, 28.9, and 37.9, and, 28.0, 21.1, 27.0, and 31.0, respectively.

The two-way interaction for the naloxone and nalxone+field treatments for these concentrations was strongly significant statistically [F(2,60)=10.63, p <.001] and was due to even more diminished activity when both conditions were present for the 100 nM and 10 nM concentrations. The mean LMVs for the 1 μM, 100 nM and 10 nM concentration groups exposed to the nalaxone or naloxone+field conditions were 48.8, 35.5, and 50.8, and, 45.1, 21.1, and 26, respectively. On the otherhand there was no statistically significant interaction [F(3,80)=1.56, p >.05] between field or sham conditions exposed to naloxone for these four concentrations within the pM range. Instead the activity of the planaria exposed to the field+naloxone were reduced across the concentrations compared to naxlone only [F(1,80)=268.27, p <.001] .

Discussion

The results of this study verify the sensitivity of Raffa's locomotor velocity (LMV) measures to discern potent changes in planarian behavior when exposed to remarkably low concentrations of opioid-related compounds in their aqueous environments. The mean value for the control planaria, those exposed to spring water only, in our study was 63 (SD=4.6) while the value reported by Raffa et al (2003) was 60 (SD=3.5). It is clear that diminished activity can be induced in planaria at concentrations of morphine as low as 1 fM. At concentrations of 10^{-18} M the planaria respond as if they were moving in spring water.

These measurements also suggest there are at least two conspicuous receptors. This assumes their presence can be inferred by the enhancement of an agent's effect when it is administered at a specific concentration but not at lower or higher concentrations. This is consistent with other observations. The first putative receptor would have been most responsive to concentrations of morphine in the order of 100 nM while the second was most sensitive to concentrations of about 1 pM.

However by far the most important observation was the non-specific decrease in activity for planarian exposed to any concentration of morphine (that did not kill them) from 25 μM of morphine to 1 fM. A similar but less intense effect was noted for naloxone. This non-specific effect, which diminished the activity by 50% compared to control conditions, was the same magnitude as that produced by the patterned magnetic field only. These results strongly suggest that the multiple weak and strong responses of invertebrates to different opiate receptor subtype agonists and antagonists may reflect a major contribution from these non-specific effects. We have reasoned that appropriately patterned magnetic field effects are not mediated through receptor interaction orsimulation. Instead, they are influencing a more fundamental process shared by all receptors within the plasma membrane.

One possible physical correlate of this process can be inferred by estimating the numbers of molecules required to produce the non-specific effect. The activity of planaria exposed to 10 attoM of morphine did not differ from those exposed to only spring water while those exposed to the next dosage of morphine (1 fM, 100 times greater concentration) showed the same magnitude of non-specific effects as μM dosages (25 billion times more concentrated). The estimated number of molecules within the 1 cc of 10^{-17} M (10 attoM) of solution removed from 15 cc of stock is ~4×10^5 whereas the numbers of molecules within the first effect dosage, 1 fM (4×10^7).

Assuming a planaria length of 6 mm, width of 0.5 mm and thickness of 0.05 mm, the volume would be 1.5×10^{17} nm^3. Consequently the volume of the planarian would be 1.5×10^{-4} of the volume of 10^{21} nm^3 (1 cc of fluid). Assuming homogeneous distribution of the morphine molecules from Brownian movement, this would mean that ~60 molecules of morphine inside the volume of a planarian would not be effective (no different than ambient spring water) to alter activity while ~6,000 molecules would diminish LMV as effectively as billions of molecules. If only brain volume was involved (~10^{16} nm^3, Okamoto et al, 2005) the number of effective molecules would be less by a factor of about 10. Simplistically, this suggests a mechanism involving relatively small numbers of channels or sequestering-points that once inactivated by physical blockage or chemical linkage reduces the activity of planarian by about 50%. Responsespecificity, that is preferential binding to proteins, would be superimposed upon this non-specific effect.

There are several theoretical and empirical reasons to suggest that the ion most associated with the non-specific effect involves H^+. Its influence could occur through the proton (water) channel (Decoursey, 2003) within the cell membrane. Outward H^+ flux is important for reducing intracellular shifts from metabolic activity towards acidic conditions. Unlike typical ion channels proton channels remain open but diminish their activity depending upon internal pH. If large molecules such as morphine or naloxone blocked these channels, then the diminished efflux of H^+ would result in enhanced internal pH that would, by the time the planarian were tested after 2 hrs of exposure, resulted in diminished activity to minimize the accumulation.

We cannot identify with any assurance which receptor subtypes of morphine (or the μ receptor antagonist, naloxone) were associated with the two concentrations, 100 nM and 1 pM. The widening of the effectiveness of morphine around the 100 nM trough would suggest a μ receptor. The largest net change associated with co-exposure to the magnetic field and naloxone compared to naloxone only was also within the 100 nM range. However in this instance the combination enhanced, not attenuated, the decreased LMV values. If we assume that the presence of the magnetic field altered some aspect of receptor dynamics that widened the effective range of the dosage that produced the enhanced decrease of LMV values, then it possible that the naloxone was no longer acompetitive antagonist but stimulated the receptor If this argument is valid, then the 100 nM range effect could be related to the μ receptor.

The marked decrease in activity noted for the 100 nM and 1 pM ranges might also reflect another biochemical entity that is known to affect the behaviour of planaria: melatonin. According to the review by Dubocovich et al (2010) the two melatonin receptors, MT1 and MT2, are regulated by concentrations of melatonin between 30 to 400pM and 1 to 1000 nM, respectively. The net difference in these two concentrations is within the same order of magnitude as the differences between the 100 nM and 1 pM separation measured in our study for the two peaks in minimum LMV values. Inward-rectified potassium channels are activated by melatonin and the best known signalling pathway for melatonin receptors is the inhibition of cAMP. Both processes could result in diminished activity. Itoh et al (1999) measured melatonin levels of 0.4 pM per planarian during the dark phase and 0.1 pM during the light. As expected melatonin binding inhibits regeneration (Yoshizawa et al, 1991).

There are at least two possible mechanisms by which morphine effects could have been facilitated at 100 nM and particularly at 1 pM dosages. First, the disulfide bond between Cys113 and Cys190 residuals maintains the proper receptor confirmation for ligand binding (Dubocovich et al, 2010). A similar property is displayed by δ-opioid receptors. Morphine may have affected disulfide bounds in general and hence affected a receptor subtypes, including those specific to this opioid receptor subtype. Second, some melatonin agonistsdisplay a high affinity for MT1 and MT2 receptors but are antagonists for biogenic amine receptors such as 5-HTc serotonin. Additional research should reveal an underlying commonality between morphine receptor subtypes, melatonin receptor subtypes and the pivotal role of proton movements.

Conclusion

Quantitative measures of movements of flatworms can be sensitive indicators of concentrations of opioid-related compounds ranging from concentrations in the microMolar to picoMolar range in the aqueous environment. General diminishment of movements occurred across all concentrations of morphine from microMolar to picoMolar concentrations with particular enhancement in a narrow range of concentrations that are consistent with the two major receptor subtypes for opiates known to exist for planarian. Below picoMolar concentrations the movement behaviour did not differ from the vehicle (spring water), indicate there is a discrete threshold for the numbers of molecules required for this effect. Exposure to temporally-patterned magnetic fields known to produce analgesia in rodents also produced a generalized suppression of movement and appeared to widen the effective concentration rather than enhance the magnitude of the effect at the concentrations attributed to receptor subtypes.

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Chapter 3: Temporally-Patterned Magnetic Fields Induce Complete Fragmentation in Planaria

Abstract

A tandem sequence composed of weak temporally-patterned magnetic fields was discovered that produced 100% dissolution of planarian in their home environment. After five consecutive days of 6.5 hr exposure to a frequency-modulated magnetic field (0.1 to 2 μT), immediately followed by an additional 6.5 hr exposure on the fifth day, to another complex field (0.5 to 5 μT) with exponentially increasing spectral power 100% of planarian dissolved within 24 hr. Reversal of the sequence of the fields or presentation of only one pattern for the same duration did not produce this effect. Direct video evidence showed expansion (by visual estimation ~twice normal volume) of the planarian following the first field pattern followed by size reduction (estimated ~1/2 of normal volume) and death upon activation of the second pattern. The contortions displayed by the planarian during the last field exposure suggest effects on contractile proteins and alterations in the cell membrane's permeability to water.

Introduction

Whereas chemical effects upon life systems are determined by the complexity of spatial (molecular) structure, alterations by applied electromagnetic fields appear to be determined by the complexity and shape of the specific temporal pattern [1]. The traditional argument that powerful biological effects from weak magnetic fields would be minimal because of obscuration by intrinsic thermal variations (the "kT boundary problem") may not be applicable to systems in non-equilibrium such as life forms [2]. While exploring the effects of exposure of planarian to a digitized complex, amplitude-modulated field that slows the growth rate of melanoma cells *in vitro*[3], we combined two other complex-patterned, electromagnetic fields within the μT (microTesla) range that resulted in planarian being dissolved within a few hours of the exposure to the second pattern. We had never observed any phenomenon of such magnitude with these fields.

Planarians are optimal animals to assess the effects of weak, physiologically patterned magnetic fields in aqueous environments. Their neurons more closely resemble the neurons of vertebrates than even higher invertebrates [4]. Planarian are known for their large proportion of neoblasts, a stem-cell population with the potential to generate every cell type in the adult animal [5]. Their sensitivity to weak (earth magnitude) static magnetic fields has been known for decades [6-8]. Planarian capacity to regenerate and multiply asexually [9] is influenced by weak (~10 μT) power frequency magnetic fields

[10] and frequencies tuned to calcium resonance [11] which is a likely mechanism formembrane voltage-mediated changes in anterior gene expression [12]. Intensities as low as 40 nT and a variety of frequencies such as 1 Hz, 3 Hz, 7 Hz, 32 Hz and 60 Hz can stimulate fission [13].

Goodman et al [14] found that transected planarian exposed twice a day for one hour to weak 60 Hz magnetic fields showed increased regeneration associated with marked activation of the extracellular signal regulated kinases (ERK) and heat shock protein (hsp70). Recently we [15] found that immediately after planarian were sectioned only a single, 30 to 45 min of exposure to asymmetrically patterned, extremely low frequency magnetic fields, about the same duration shown for similar field shapes to increase activity of messenger RNA [16], produced comparable effects. Weak magnetic fields less than 1 μT accelerate oxidation of cytochrome C *in vitro*, an electron transport enzyme, and affect the functions of Na and K-ATPases [17]. None of these effects have been as reliable, conspicuous and qualitatively distinctive as the phenomenon reported here.

Methods

Planaria

A total of 1, 753 planarian *Dugesia tigrina* were employed as subjects; they had been obtained from different sources (North Carolina Biological Supply and Boreal Biological Supplies) and maintained according to standardized procedures [11]. During the experiments there were 15 to 30 worms per jar and 4 to 6 jars. These numbers were the same for any given block. There was the equivalent of 1 cc of spring water from Feversham, Grey County, Ontario per planarian. The length of the planarian varied between batches from 1 cm to 3 cm. However there was no difference in lengths between planarians within a given block that were exposed to the experimental and sham conditions. Ion content (ppm) was $HCO_3$270, Ca 71, Mg 25, SO4 5.9, Cl 2.7, NO3 2.6 and Na 1. In Experiment 1 the different blocks (5) were completed in jars composed of different materials (glass, clear plastic, opaque specimen); this did not influence the effect. For all subsequent blocks the jars were glass.

Exposure system

Two coils were used; one was a classic Helmholtz coil while the other were two rectangular coils (1.15 x 1.15 m). Jars were separated by 10 cm along the outer edge of the Helmholtz coil and by 40 cm (jars placed on a wooden platform) between the two rectangular coils that were separated from each other by 65 cm. The peak intensity of the FM field within the exposure area for the former was 2 μT for the FM and 5.5 μT (55 mG) for the GM field. Within the exposure area generated by the two rectangular coils the peak values for the FM field was 200 nT and 490 to 600 nT for the GM field. The equipment was the same as that employed in previous studies [17, 18].

Magnetic field generation

Pictorial representations of the wave pattern of the applied fields and their spectral analyses are shown in Figure 1. The FM pattern (Thomas.dac) was composed of 849 values (each between 0 and 256). It is known for its capacity toinduce analgesia in several vertebrate and invertebrate species [19-22]. The duration each point was activated, the point duration, was 3 ms.This means that the duration of each FM sequence was 2.5 s before repeating continually for 6.5 hr and each GM sequence was 15.3 s ("65 mHz") before repeating continually for 6.5 hr.

The GM pattern (geomagn5071.dac) was composed of 5,071 points and had been initially designed to imitate sudden geomagnetic storm commencements [23-25] but the point durations were reduced to 1 ms. The duration of each of first 14 wider peaks and troughs was 600 ms (200 points) while the duration of the second 14 narrow peaks and troughs was 300 ms (100 points) with an interface of 1.5 s (500 points). During this time the voltage equivalent (-5 to +5 V) of the number between 0 and 256 was converted by custom constructed digital to analogue converters (DACs) to current that wasdelivered to the coils. The different coils were operated by different computers each loaded with the Complex software required to produce the fields. For the wave file component of the experiment, the sound card generated a voltage from a laptop that was connected directly to the rectangular coils. Theintensity within the exposure area was comparable for both the FM and GM patterns.

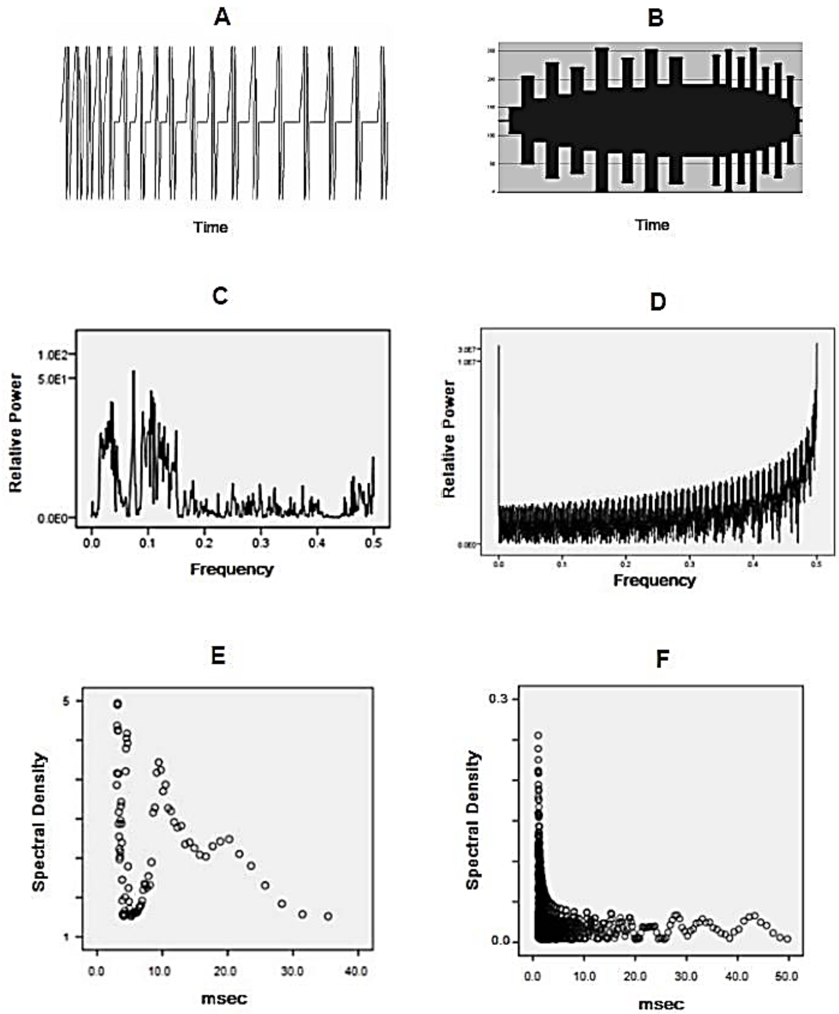

Figure 1.Wave form and spectral characteristics of FM and GM fields.A)the FM ("Thomas") pulse pattern (duration 2.58 s) that was repeated continuously for 6.5 hr. for 5 consecutive days. B) an overall shape of the GM (duration=15.3 s) pattern that was repeated continuously on the 5th day for 6.5 hr. C) raw spectral analyses of FM pattern; D) raw spectral analysis of GM pattern. E) transformation of spectral power (vertical axis) to real time (accommodating the 3 ms points) of duration (inverse of frequency) of the FM pattern. F) transformation of spectral power (vertical axis) to real time for the GM pattern.

Results

The summary for all of our results are listed in Table 1. For the first series of exposures intensities averaged 2.5 μT during the FM field and 5 μT during the GM field and would be similar to those encountered near some electronic equipment. The dissolutions of the planarian were obvious within 6 hours (earliest measurement) after the initiation of the GM, conspicuous after 12 hrs (6 hrs after termination of GM) and maximum by 24 hr (12 hr after GM field termination). Over the course of their termination, planarian exposed to the FM field would expand to about (visually inspection) twice their volume. Once the GM had been applied the planaria would shrink to about half their normal size, display spasmodic contractility, become immobile and then dissolve completely with no fragments (Figure 2).

The effect was *not* apparent if the jars containing the planarian were moved from or disturbed in exposure area or ambient (600 lux) light was present. The phenomena were apparent when the experiments were performed in the dark or in ambient light less than ~10 lux. This "dark" dependence was not considered unusual considering the light-attenuating effects of extremely low frequency magnetic fields upon attenuation of opioid analgesia in mice [26] and nitric oxide activation in the land snail [27].

Figure 2.**Dissolution effect on planaria exposed to FM for 5 days and successive GM exposure on 5th day.** Typical results following exposure to the FM and then GM weak magnetic fields after the fifth day of exposure. C is a sham field or control group of planarian within which there were never mortalities even up to two weeks later in the same environment. E refers to the dissolved debris of the same number of planarian that had been exposed to the FM-GM field combination.

To discern the reliability of this robust phenomenon we exposed jars of planarian (10 to 15 planarian per jar) either to the magnetic field configuration (FM-GM) generated within a traditional Helmholtz coil or sham-field conditions. For 5 blocks (one block per week) all 332 planarian that had been exposed to the configuration were dissolved within 24 hr while none of the 236 planarian in the control conditions died. Within six hours after the onset of the GM field the percentage of worms that had dissolved in the 5 blocks, as discerned by visual inspection (without moving the jars) were 100%, 87%, 80%, 80% and 80% respectively. The results of this component of the experiment as well as the blocks of all the experiments are presented in Table 1 to facilitate clarity.

To insure that the effect could be produced by other equipment, the procedure was repeated with a different computer and a much larger coil system [11] where the intensity of the FM field was 0.2 μT and the GM field was about 0.5 μT. These three blocks of experiments were completed in the basement (completely dark) in another building. Again, within 24 hr after the initiation of the GM field after 5 days of exposure to the FM field 100% of the planarian exposed to the configuration was dissolved. For comparison with the first experiment, after 6 hrs between 35% and 46% of the configuration field-exposed planarian had dissolved while none of the planarian in the control group dissolved.

In all subsequent blocks of experiments there were 15 worms per jar and 3 jars in the field condition and one jar in the control condition per block. We then altered the presentation of different components of the configuration. In three separate blocks, *only* the GM field was presented (instead of the FM field) for 6.5hr per day for 5 days and then for an extra 6.5 hr on the 5th day. Three other blocks were conducted where only the FM field was presented for 6.5 hr per day and then for an additional 6.5 hr on the 5th day in order to discern if the terminal GM component was essential. There were no mortalities for either condition. The reversed presentation of the effective sequence, that is the GM field for 5 days for 6.5 hr per day followed by the addition of 6.5 hr on the fifth day, also produced no mortality. These results strongly suggested that the precise sequence of the FM field first followed by the GM field was the necessary condition to produce the 100% mortality of planarian within 24 hr. None of the control jars containing 230planarian, involving nine blocks, died and were still viable 10 days after the end of the experiment before they were discarded.

Table 1.Percentages of dissolved planarian (in each block of experiments) in reference groups and experimental groups as a function of time 6 hrs.and 24 hrs. after exposure to the final magnetic field. The effective parameters were the FM field for 6.5 hr per day for 5 days and on the fifth day 6.5 hr exposure to the GM field.

Exposure Parameters	**+ 6 hr**		**+ 24 hr**	
	Control	Field	Control	Field
5-d FM (2.5 µT), 1-d GM (5 µT) (3 ms point durations)	0	100	0	100
	0	87	0	100
	0	80	0	100
	0	80	0	100
	0	80	0	100
5-d FM (0.2 µT), 1-d GM(0.5 µT)	0	35	0	100
	0	46	0	100
	0	39	0	100
5-d GM, 1-d GM (GM only)	0	0	0	0
	0	0	0	0
	0	0	0	0
5-d FM, 1-d FM (FM only)	0	0	0	0
	0	0	0	0
	0	0	0	0
5-d GM, 1-d FM (reverse)	0	0	0	0
	0	0	0	0
	0	0	0	0
1-d FM, 1-d GM	0	0	0	0
	0	0	0	0
3-d FM, 1-d GM	0	64	0	100
	0	68	0	100
5-d FM, 1-d GM (5 ms point durations)	0	0	0	0
	0	0	0	0
	0	0	0	0
5-d FM, 1-d GM (wave files)	0	0	0	0
	0	0	0	0
	0	0	0	0

To establish the threshold for the temporal duration required to produce the mortality, planarian were exposed for one day (6.5 hr) to the FM field and then immediately to the GM field for 6.5 hr; there was no mortality. Following three days of daily exposure to the FM field and then to the GM field (two blocks) the average mortality was 66%. This suggested that more than one daily exposure and at least 3 days of exposure were required to start the effect and that 5 days was sufficient to produce 100% mortality.

Both the FM and GM patterns of magnetic fields were generated by computer software that converted columns of numbers (849 for the FM and 5,071 for the GM) to appropriate voltages to generate the magnetic fields in the same space within which the planarian were placed. The point duration of each number between 0 and 256 was programmable in ms. We had selected 3 ms as the point duration (the time each voltage is presented through the circuit to the coil) because of its demonstrated efficacy for analgesia [4] for the FM field when presented to rodents and for its retarding effect upon the growth of several types of cancer cells but not normal (mouse and human) cells for one-hour daily presentations for five days [29]. In the latter *in vitro* setting the same FM pattern employed in this study but presented for one hour per day was not effective when the point durations were either 1, 2, 4, or 5 msec.

o test this application in the present context, planarians were exposed to the same configuration but the point duration was changed from 3 ms to 5 ms for 3 blocks. There were no mortalities. We then copied the magnetic pattern generated from the large coil to a wave file. The fidelity of the pattern was established by listening to the auditory output produced from the magnetic field by a magnetic sensor (solenoid) coupled to an acoustic amplifier. We employ several of these devices in the laboratory routinely to insure the presence and temporal structure of magnetic fields employed in a variety of studies. Planarian exposed to the wave file version of the configuration rather than the one generated by the digital-to-analogue transformation from the complex software exhibited no mortality.

We decided to visualize the phenomena over time by recording the exposed planarian's movements during the 24 hr following the onset of the GM field. An infrared camera recording 1,000 frames per sec of 3 jars (each containing 15 planarians) recorded activity of the planarian for 6.5 hr after the onset of the GM. Within 15 min following the activation of the GM field, the planarian moved more frequently. After 60 min the planarian displayed twisting and contortion movements and no longer adhered to the side of the jars. After 6 hr the planarian no longer ascended to the surface. Within 24 hr all of the planarian were dead and dissolved. A time-lapsed video of this progression is available.

Discussion

We have completed several studies involving planarian in various types and intensities of magnetic fields [29]. The present observation of death and complete dissolution of planarian exposed to this combination of fields isunprecedented in our observations as well as the general literature. Qualitatively, it appears that the exposure to the FM field may weaken the structural protein that maintains the organism resulting in a visually obvious increase in body volume of the planarian followed by rapid contraction and dissolution of the boundary between the ambient water and the intraorganismic fluid after GM field onset. During a subsequent series of unpublished experiments involving mouse B16 melanoma cells, the same exposure paradigm employed in the present study that produced dissolution of the flatworms resulted in fragmentation of the melanoma cells. Within 5 hr of the exposure to the GM field there were no discernable intact cells with the cultures that had been exposed to the procedure. Visually obvious enlargement followed by shrinkage of these cells within a similar time frame was also observed.

Although the role of calcium influx [30,31] membrane resonance[32], opening of calcium and potassium channels [11] coupled to melatonin receptors [33] (the light-sensitive ligand for which is known to be responsive to multiple forms of weak time-varying magnetic fields [34] and direct effects on DNA [35,36] are the most parsimonious sources of mechanism, a yet to be identified process involving coherent domains of water [37,38] during exposure to the fields may be critical. If the temporal pattern is critical to the phenomenon then the “dissolution effect” might occur during exposures to field intensities even less (< 100 nT) than the ones employed in this study. It is relevant that the magnetic fields that produced this effect are not sine-wave or symmetrical patterns and were generated by computer software rather than function generators.

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Chapter 4: Maintained Exposure to Weak (~1 μT) Temporally Patterned Magnetic Fields Shift Photon Spectroscopy in Spring but not Double Distilled Water: Effects of Different Shielding Materials

Abstract

Spring water but not double-distilled water was exposed, in darkness, to a temporally patterned weak magnetic field that has been shown to affect planarian behavior and slow the rate of cancer cell proliferation. Exposure to the magnetic field caused a reliable shift in the peak wavelength for fluorescence emission of ~10 nm to the longer wavelength and a ~20% increase (~100) counts in fluorescence intensity. Spectral analyses verified a shift of 5 and 10 nm, equivalent to ~$1.5 \cdot 10^{-20}$ J "periodicity" across the measured wavelengths, which could reflect a change in the an intrinsic energy corresponding to two lengths of O-H bonds. Wrapping the water sample containers during exposure with copper foil, aluminum foil, or plastic altered these fluorescent profiles. The most conspicuous effect was the elimination of a ~280 nm peak in the UV-VIS emission spectra only for samples wrapped with copper foil but not aluminum or plastic. These results suggest that weak magnetic fields produce alterations in the water-ionic complexes sufficient to be reliably measured by spectrophotometry.

Introduction

There has been a long and colourful history regarding the potential residual effects of exposing water, particularly when it contains concentrations of ions that approach the characteristics of living systems, to weak magnetic fields [1] because of the unique features of water [2]. Toledo and colleagues [3] reviewed the effects from exposure of water to mT to T-intensity magnetic fields, as inferred by measurement of viscosity, enthalpies and surface tension, and suggested intracluster hydrogen bonds were disrupted. Gang et al [4] found that only one hour of exposure to 0.16 T static magnetic fields produced alterations in diffusion velocity of a solute for an additional ~6 to ~9 hrs that was a function of the water volume during exposure. The effect was consistent with the intensity-dependent diminished viscosity reported by Fahidy [5].

Weaker intensity (μT range), time-varying magnetic fields have been shown to affect ion/ligand binding kinetics when bioeffective waveform parameters are employed [6-8]. These alterations in chemical dynamics and biochemical processes are often associated with altered patterns of photon emissions [9-11]. Recent theoretical perspectives have extended the concept that water molecules exists as "flickering clusters" [12] with half-lives of $\sim10^{-11}$ s, several hundred times the period of molecular vibrations. Water containing ions can display coherent domains within which magnetic fields can be "trapped" if the oscillations are optimal [13]. Large polyhedral forms organized from tetrahedral bonded units are considered candidates [14].

We have been investigating the effects of microTesla range magnetic fields presented in temporal patterns that simulate physiological or neuronal patterns. One particular ("frequency-modulated", decelerating) pattern was shown to produce analgesia in rodents (equivalent to 4 mg/kg of morphine) [15], as well as in snails [16], and to inhibit the growth of several different strains of cancer cells without affecting normal cells [17]. Recently Murugan et al [18] showed that exposure to this pattern for several (3-5) days followed by exposure to a naturally-patterned field produced complete dissolution of populations of planarian. However these robust phenomena only occurred when the specimens were exposed to the fields in the dark. The interaction between darkness and magnetic field exposure upon complex systems has been reported by others [19].

In the pursuit of mechanisms we exposed spring water in the dark to this patterned field and found a conspicuous shift in transmittance counts towards longer wavelengths. Pilot studies indicated the effect was not evident when the water samples were exposed in ambient lighting and that 18 days of magnetic field exposure were required to produce reliable effects. In the present experiments we systematically investigated these phenomena with precise instrumentation. To discern if different materials could affect the manner in which exposure to the weak magnetic fields could affect the emitted fluorescence of the treated water, we employed spring water (containing a fixed number of cations and anions) and double distilled water and incorporated four different "shielding" conditions: open, copper foil, aluminum foil and plastic.

Methods

A total of 176 volumes, each of 50 cc, of either spring water or double distilled water contained within 105 cc flint glass jars (9 cm high x 6 cm diameter) were exposed between two coils as shown in Figure 1. Each type of exposure was performed in triplicate; each component of the triplicate was completed on separate days. The two coils were separated by 1 m. Each coil was created by wrapping 305 m of 30 AWG (Belden 9978) wire in a single layer (18 cm wide) around plastic milk crates which were 38 cm x 33 cm x 27 cm. The circuit was organized so that one coil would be activated (A) while the other was not activated (NA). Power meter measurements indicated that the minimum-maximum range in the strength of the field (RMS) were 4.4 to 11.5 μT for the activated area, 0.3 to 0.6 μT for the middle area (MA), and 0.11 to 0.15 μT for the NA area. For comparison the background intensity for ambient 60 Hz was within the 0.1-0.15 μT range. The reference area which also showed the 60 Hz ambient intensity was located 5 m from the two coils.

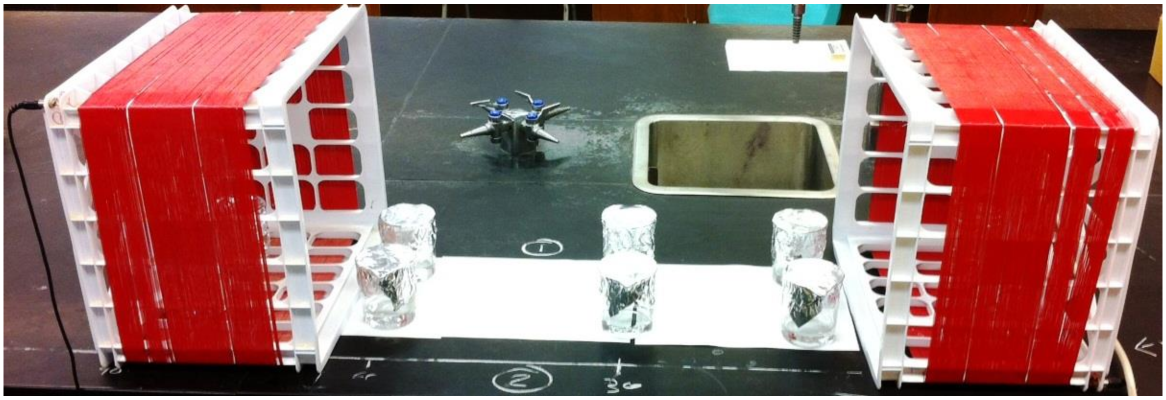

Figure 1.The experimental equipment. When one of the two coils was activated, the proximal containers were location A, the middle were middle MA, and the distal is inactive (NA).

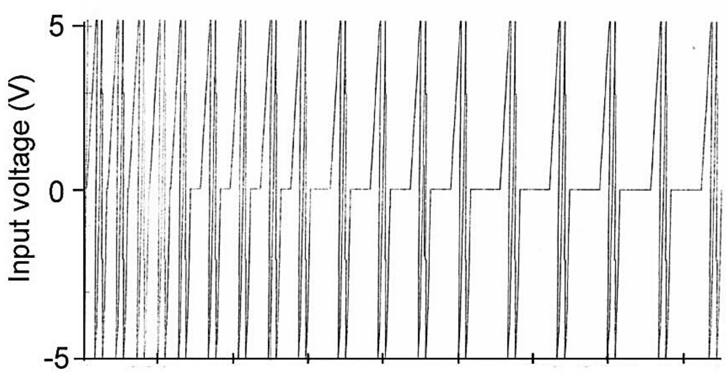

Figure 2. The physiologically-patterned magnetic field that has been associated with significant cellular and biological effects and the one employed in this study.

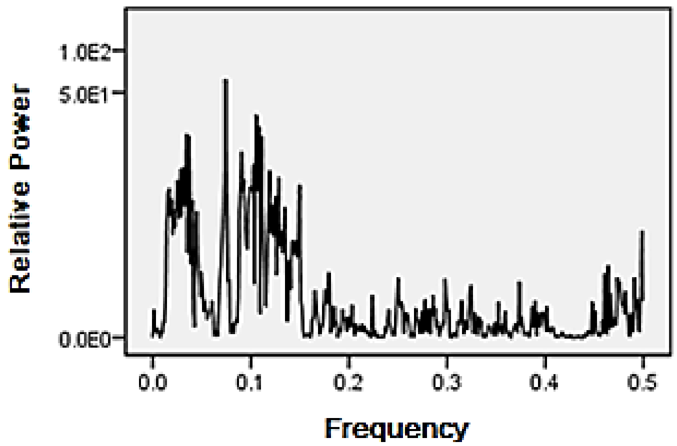

Figure 3. Relative spectral power density of the pattern in Figure 2 as a function of analysis frequency. Actual frequency is the inverse 1 divided by the value multiplied by 3 msec.

The magnetic field pattern was generated by a Zenith-386 compute (Model ZF-148-41, Zenith Data Systems, Benton Harbor, MI, USA) computer employing the complex software ©. A frequency-modulated pattern that has been shown to reduce division rates of cancer cells (by about ½) but not normal cells and to affect nociceptive thresholds in rodents was selected. The pattern was produced by converting a series of 849 numbers between 0 and 255 (equivalent to -5 to +5 V, where 127=0 V) through a custom constructed digital-to-analogue (DAC) converter (DAC). Each point was generated for 3 ms. The pattern and the spectral power are shown in Figures 2 and 3. This duration was selected because it demonstrates the greatest effect on cell proliferation rates and rodent behavior. Point duration values set less (1, 2 ms) or greater (5, 10 ms) than 3 ms do not produce comparable significant effects.

To be comparable with the conditions previously used, in conjunction with the second geomagnetic field pattern, to promote the complete dissolution of planaria [18] after 5 days of exposure, all of the present experiments involved exposing the spring or distilled water in the dark (background level as inferred by photomultiplier tube measurements $\sim 10^{-11}$ $W \cdot m^{-2}$). Exposure to even dim ambient lighting reduced the reliability of the effects. Based upon pilot studies where the samples were exposed continuously for 1, 4, 5, 10 and 18 days, we selected 18 days of exposure because it produced the minimal interexperiment variability. The effect was still apparent after 4 days of exposure.

In an equal numbers of experiments, the containers were exposed to different shielding conditions: they were not wrapped or wrapped with copper foil, aluminum foil, or plastic and then exposed to the three field or reference conditions. Two types of water were employed. Half of the experiments tested spring water (270 ppm HCO_3, 71 ppm Ca, 25 ppm Mg, 5.9 ppm SO_4, 2.7 ppm Cl, 2.6 ppm NO_3 and 1 ppm Na) while the other half of the experiments tested double-distilled (DD; ultra-pure, 18 MΩ at 25°C) water.

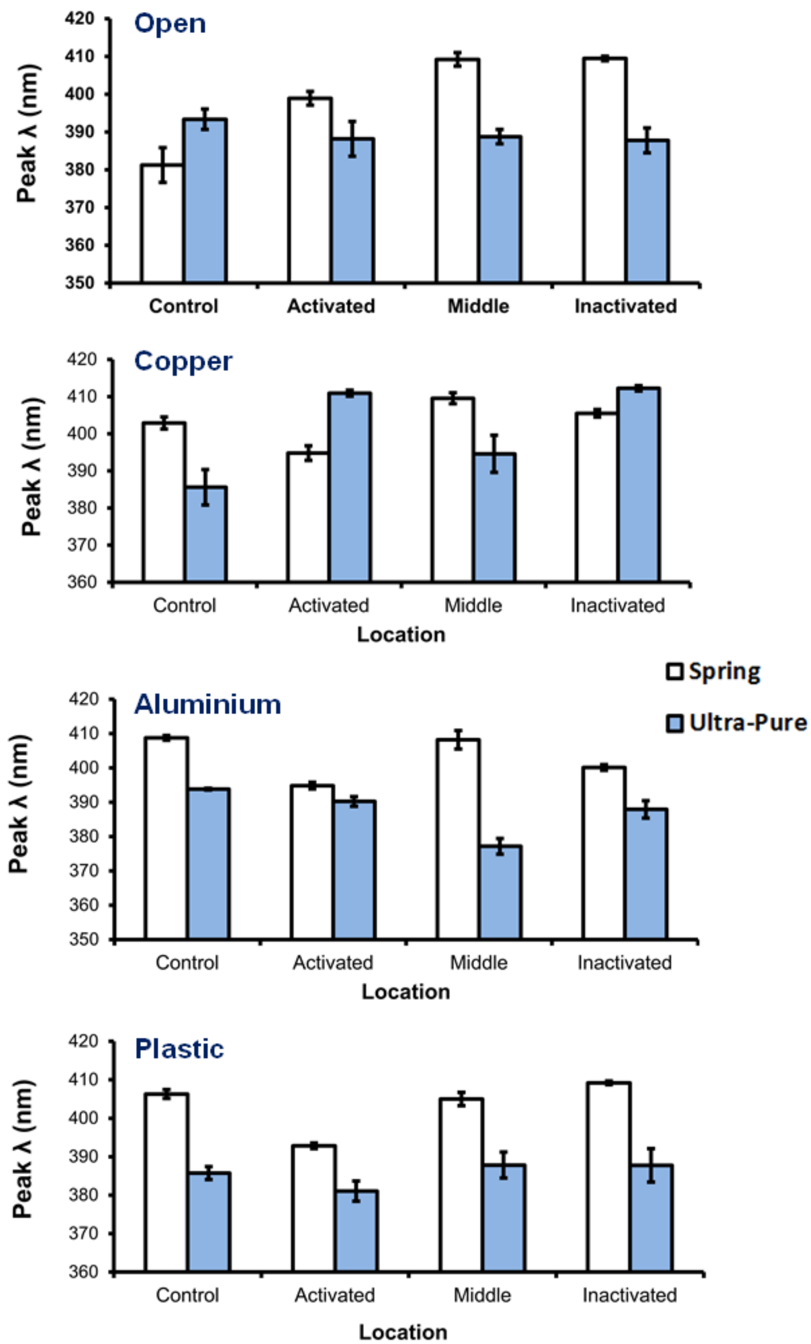

Figure 4. Peak wavelength for maximum numbers of fluorescence counts for spring and ultra-pure (double distilled) water that had been exposed to the different magnetic field conditions and “shielded” (wrapped) with different materials.

After the exposure period, the water samples were assessed by fluorescence spectrophotometry. During the entire procedure the samples were not exposed to ambient light until the actual measurement. The samples were also carried in a black box with minimum perturbation from the exposure room to the measurement room. From the time of removal from the field conditions to the initiation of the measurement the total time was about 5 minutes.

For measurements in the Olis RSM 1000 F1 fluorescence spectrophotometer, 1 cc of the water from each condition was placed in a 1 cc quartz cuvette and exposed to a 120 W Xenon arc lamp (excitation source). Measurements were recorded by a DeSamonochromator/photon counter. Ten nanometer scanning intervals between 320 and 470 nm for emission were recorded using a dual grating monochromator. On the basis of our results, we also exposed additional samples of 1 cc of water from the various conditions in plastic cuvettes to an Ultrospec 2100 pro uv visible spectrophotometer with a stimulation wavelength of 250 nm. The 1 nm steps ranged from 250 to 500 nm. A monochromator with 1200 lines per mm of aberration corrected for concave grating.

For data analyses, the wavelength that displayed the peak measure of photon transmission was obtained by inspection of the curves. Analyses of variance were completed between the four positions (reference, active, middle, inactive coil), type of shield (open, copper foil, aluminum foil, and plastic) and type of water (spring vs DD). Spectral analyses (PC SPSS 16) were completed for the mean values for triplicates measures of the number of counts per 1 nm between 320 and 470 nm for each of the 32 conditions (4 field conditions, two types of water, and 4 "shielding" conditions). To allow greater comparisons, the z-score values determined from the mean of the triplicates was employed. All analyses were performed using SPSS software for PC. Eta^2 refer to the amounts of variance in the dependent variable explained by the treatments.

Results and discussion

The results of these showed that prolonged exposure to a temporally patterned electromagnetic field could alter the fluorescent spectrum of water. The intensity of fluorescence emission and the maximum emission peak of the fluorescence spectrum depended on the position of the water in the magnetic field (intensity), the purity of the water, and the presence of different shielding materials during exposure.

Fluorescence intensity

The most obvious effect involved the differences in fluorescence emission spectra between spring water and DD water. There were approximately three times the number of photons (counts) emitted from the spring water compared to the DD water. Secondly, the shape of the distribution of counts across the measured wavelengths differed conspicuously. While the fluorescence spectra of the DD water exhibited a linear decrease in counts from the stimulation wavelength, the spring water spectrum exhibited a clear non-linear effect with a maximum peak in intensity between 380 and 440 nm. Systematic magnetic field exposure effects were evident for the spring water but not for the DD water.

The triplicate averages of the total numbers of counts for the spring water exposed for 18 days in darkness to the four field conditions are shown in Figure 4A. There was a conspicuous shift towards longer emission wavelengths for the spring water that had been exposed to the middle field (03-0.6 uT) and not activated field (0.11-0.15 uT) conditions (peak around 418 nm) compared to the active field (4.4-11.5 uT) and reference conditions (peak around 400 nm). Between 420 and 475 nm, the intensity of emitted fluorescence for the middle field and not activated field was about 150 counts per unit wavelength higher than for the reference and active field conditions. Neither the non-linear effect nor the differential field condition effects were noted in the DD water (Figure 4B). These results clearly showed that the weaker intensity, patterned field (below 0.6 μT, 6 mG) that were presented in addition to the background 60 Hz ambient intensities produced the greatest shift in wavelength. The spring water exposed to the not activated field produced discernibly higher fluorescence intensity counts between 460 and 470 nm.

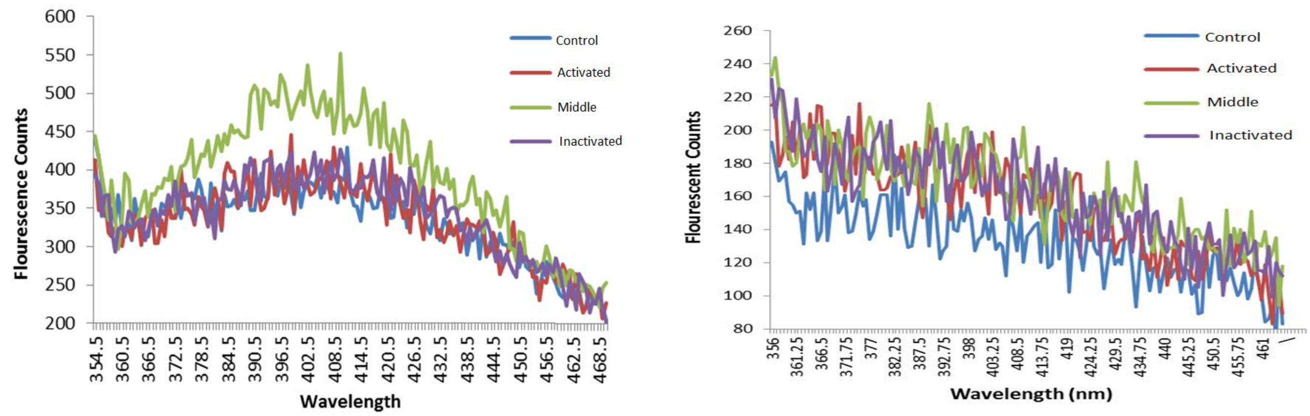

Figure 4. Number of counts by fluorescence spectrophotometry for spring (A) or double distilled (B) water that had been exposed to the reference (blue), active area (red), middle area (green) and inactive area (purple). Note the shift by approximately 10 nm for the latter two conditions for the spring water.

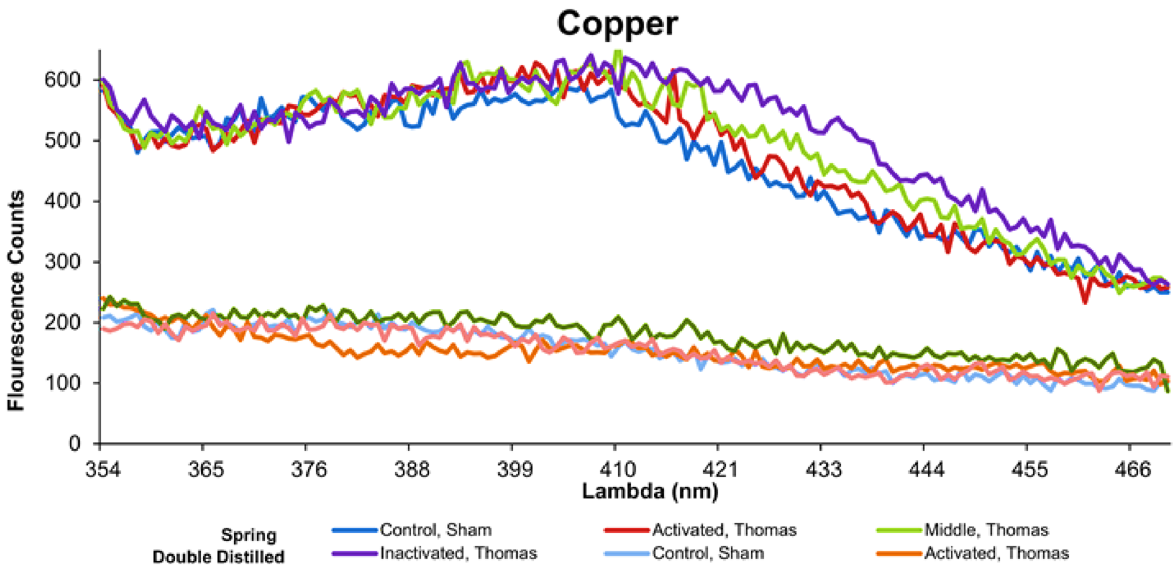

Figure 5. Photon counts from spring water (upper curves) or double distilled (lower section of graph) water that had been exposed to the four conditions of magnetic field but wrapped with copper foil.

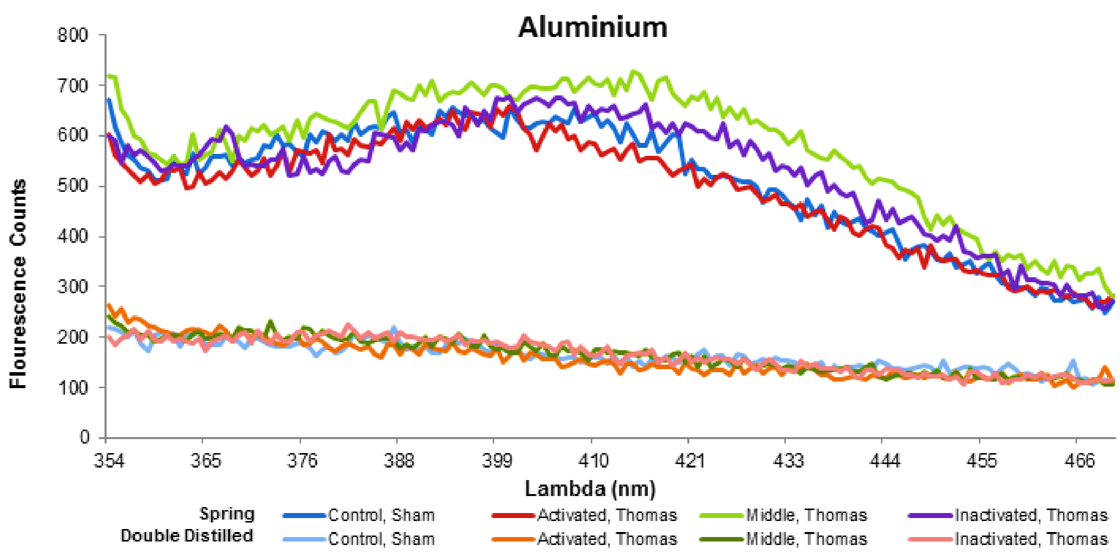

Figure 6. Photon counts from spring (upper curves) or double distilled (lower lines) water that had been exposed to the various magnetic field treatments but wrapped with aluminum foil.

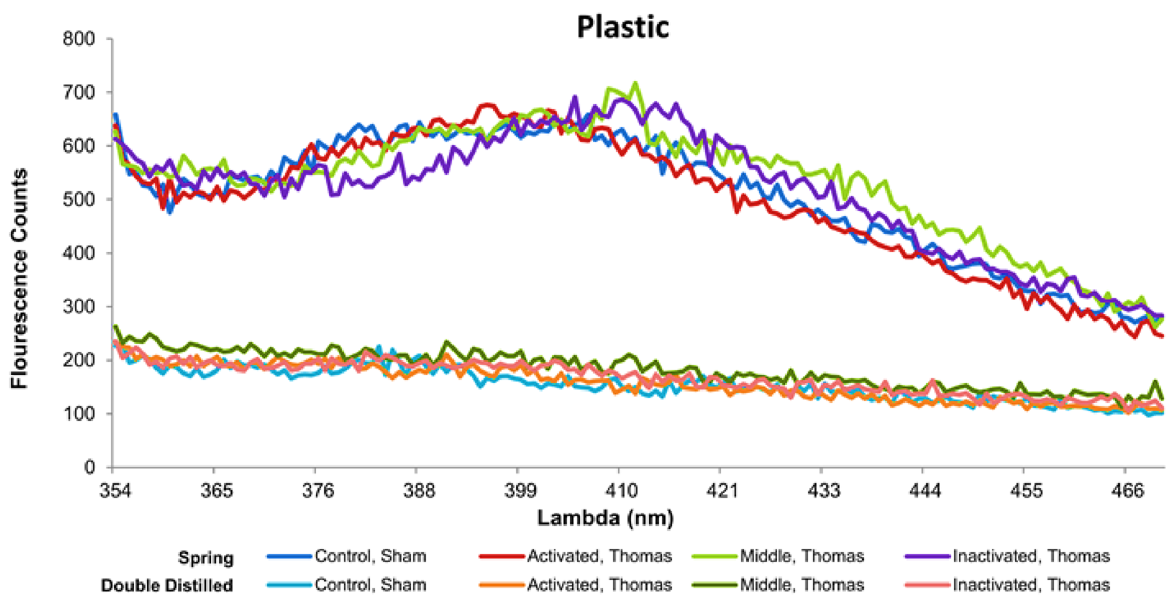

Figure 7. Photon counts from spring (upper curves) or double distilled (lower lines) water that had been exposed to the various magnetic field treatments but wrapped with plastic.

The effects of adding the three different shielding materials during the 18 days of dark magnetic field exposures to the produced fluorescence counts produced by the spring and DD water are shown in Figures 5, 6, and 7. The results for each type of water for each type of shielding are presented in the same graph to facilitate comparison. For the copper foil shielding (Figure 5), the most conspicuous effect (420 to 440 nm) involved samples exposed in the area adjacent to the inactive coil and for spring water only. For the aluminum foil shielding the middle region showed more fluorescence emission counts than the inactive coil region between 420 to 440 nm although both were elevated above the active and reference regions. The plastic shielding produced a markedly attenuated effect within a narrow band of 420 to 430 nm.

Peak Wavelength Effects

The peak wavelength, the value where fluorescence intensity of the spectrum was maximal, was determined for each experimental condition as shown in Figures 4-7. The general results were clear for all experiments combined (Table 1). Two way analysis of variance indicated a statistically significant difference in fluorescence emission for the two types of water [(F1,168)=63.03, $p < .001$; eta^2=24%]. There was a significant interaction between the location of the beakers (field strength) from the activated coil and type of water [$F(3,168)=6.65$, $p < .001$; eta^2=8%]. When analyzed according to each of the types of "shielding" during exposure, the effects of the magnetic field exposure upon the peak fluorescence emission wavelength was very clear.

As shown in Table 1, when field exposures in non-shielded containers produced a strong shift in emission wavelength [$F(3,16)=24.81$] that explained 82% of the variance (equivalent to an r=0.91*). Post hoc* analysis indicated that the emission spectra of the spring water exposed to the middle and non-activated loci displayed significantly longer peak wavelengths (~409 nm) than either the control (~381 nm) are or the activated (~399 nm) locations. The diminished energy difference for the wavelength between the activated and two other loci exposed to the field was equivalent to $1.4 \cdot 10^{-20}$ J. On the other hand, the DD water exposed in the same areas did not differ significantly for the peak emission wavelength [$F(3,20)=0.55$, $p > .05$].

The energy difference of $1.4 \cdot 10^{-20}$ J as reflected by the shift in wavelength may be useful for discerning mechanism. The ratio of the magnetic moment of a proton ($1.41 \cdot 10^{-26}$ $A \cdot m^2$) and unit charge ($1.6 \cdot 10^{-19}$ $A \cdot s$) is a diffusivity aggregate of units ($0.88 \cdot 10^{-7}$ $m^2 \cdot s^{-1}$). When multiplied by the viscosity of water ($8.94 \cdot 10^{-4}$ $kg \cdot m^{-1} s^{-1}$ at biological temperatures) the force is in the order of $7.87 \cdot 10^{-11}$ $kg \cdot m^{-1} s^{-2}$. When applied across the typical length of two O-H bonds spanning between water molecules ($1.92 \cdot 10^{-10}$ m), the functional energy would be ~$1.5 \cdot 10^{-20}$ J.

On the other hand, the emission spectra of both the spring water [$F(3,16)=16.19$, $p <.001$; $eta^2=.75$] and DD water [$F(3,20)=13.67$, $p <.001$; $eta^2=0.67$] wrapped in copper foil displayed a difference in fluorescence intensity depending on the location of the containers (field strength). For the spring water that had been exposed to the active coil, the emission spectra showed a marked elevation in wavelength at 395 nm compared to the control, middle, or NA areas where the maximal value was seen at 403 to 410 nm. *Post hoc* analysis indicated that these peak wavelengths for the control and middle area did not differ significantly from each other but both differed from the peak wavelength for activated and NA loci that did not differ from each other.

In the presence of aluminum shielding, both the spring water [$F(3,16)=19.02$, $p <.001$; $eta^2=14\%$] and the DD water displayed differences in peak wavelengths determined from the emission spectra. *Post hoc* analyses indicated that for the spring water the peak wavelengths for the active and NA locations did not differ from each other (395-400 nm) but were significantly lower than those exposed to the control or middle regions (408 nm) that did not differ significantly from each other. Only the DD water within the middle region displayed a shorter peak wavelength (377 nm) than the other three locations (388 to 393 nm) that did not differ significantly from each other.

The fluorescence emission spectra of the spring water shielded by plastic exhibited significant differences in peak wavelength depending on the different locations [$F(3,16)=41.06$, $p < .01$ $eta^2=50\%$]. *Post hoc* analysis indicated that the water exposed to the active region displayed shorter peak wavelengths (393 nm) than the other three locations (405 to 409 nm) that did not differ significantly from each other. However there was no significant differences in peak wavelengths for the DD shielded by plastic as a function of the four locations [$F(3,20)=1.00$, $p > .05$].

Spectral Power Density Analyses

The results of the spectral analyses for the fluorescence emission spectra for water (spring and DD) exposed to the different positions in the magnetic field and different shielding conditions (open, copper, aluminum, or plastic) versus the z-scores of the total counts revealed intrinsic periodicities within the wavelengths. The phenomena were most evident in the spring water exposed to the four field conditions without any shielding. As shown in Figure 8, the spring water exposed to the active field exhibited a relative power increase around 10 nm and 5 nm. Figure 9, which shows the results of the same analysis for the water from the reference area reflects the expected exponential decay. The conspicuous profile for the active field exposure was not evident for spring water exposed to the MA or IA, although an increase in relative spectral power is discernable visibly for 9 to 10 nm.

At face value, the results suggest that exposure to the patterned magnetic field resulted an in the detection of an intrinsic molecular structure within the spring water, and its constituents. This pattern resembled a standing wave across the 320 to 470 nm, 1 nm increments which is detected within the width of a cell membrane. In addition, the ~10 nm, 5 nm, and 2.5 nm peaks are similar to the quantum steps seen in cell systems when examining the spacing of actin monomers within activated myofibrils [20]. Pollock found these peaks were integer multiples of actin-monomer spacing.

UV-VIS Spectroscopy

Over the last decade of research with biological systems we have noted that the efficacy of the pattern of the applied magnetic field is more related to its spectral characteristics (Figure 3) than to intensity, *per se.*, at least within biologically-effective ranges. The mean of the first peak in power density for the magnetic field presented at 3 ms which was used in this study as shown in

Figure 3 is around 8 Hz. Although not frequently employed for analyses of weak fields, we think it is a potentially relevant difference between the magnetic moments for the electrons spin and orbit ($1.07 \cdot 10^{-26}$ $J \cdot T^{-1}$) multiplied by $0.5 \cdot 10^{-7}$ T (the maximum intensity in MA) is $5.35 \cdot 10^{-33}$ J. When divided by Planck's constant the emergent frequency is ~8 Hz.

We selected the spin-orbit magnetic moment difference because of its theoretical value in the relationship between photon emission and the ephemeral features of the fourth quantum number (spin) that defines location. However this net difference moment is very similar to the proton magnetic moment ($1.41 \cdot 10^{-26}$ $A \cdot m^2$). As mentioned previously the ratio between this value and the unit charge is $0.88 \cdot 10^{-7}$ $m^2 \cdot s^{-1}$ and when multiplied by the mass of the proton is $1.47 \cdot 10^{-34}$ $kg \cdot m^2 s^{-1}$. When 8 Hz is applied the energy is within the range of the 10^{-33} J associated with spin-orbit levels associated with this frequency magnetic field. Although this convergence does not prove a functional connection it does suggest shared frequency and energy levels by which the magnetic fields could directly interact with water structure.

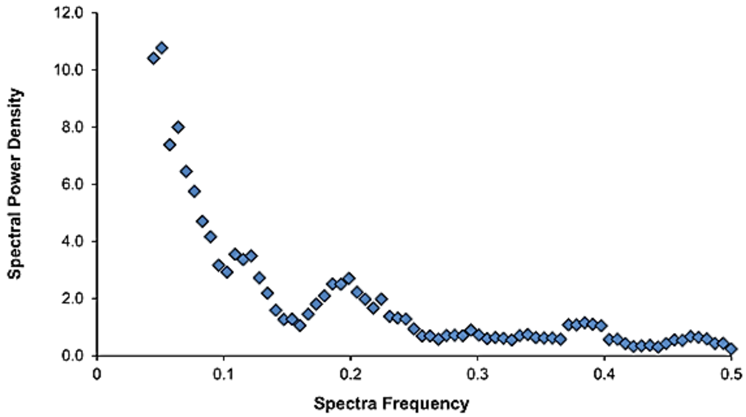

Figure 8. Spectral power density of numbers of photon counts as a function of wavelength for water exposed to the active magnetic field area. In this instance 1 divided by the value in the x-axis reflects the wavelength. Note the peaks around 10, 5, and 2.5 nm.

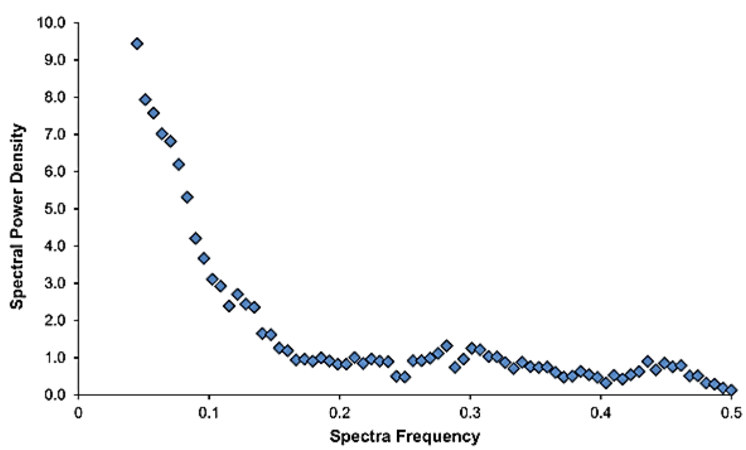

Figure 9. Spectral power density of numbers of photon counts as a function of wavelength for water exposed to the control conditions. Not absence of the enhanced power spectra density around 10 and 5 nm.

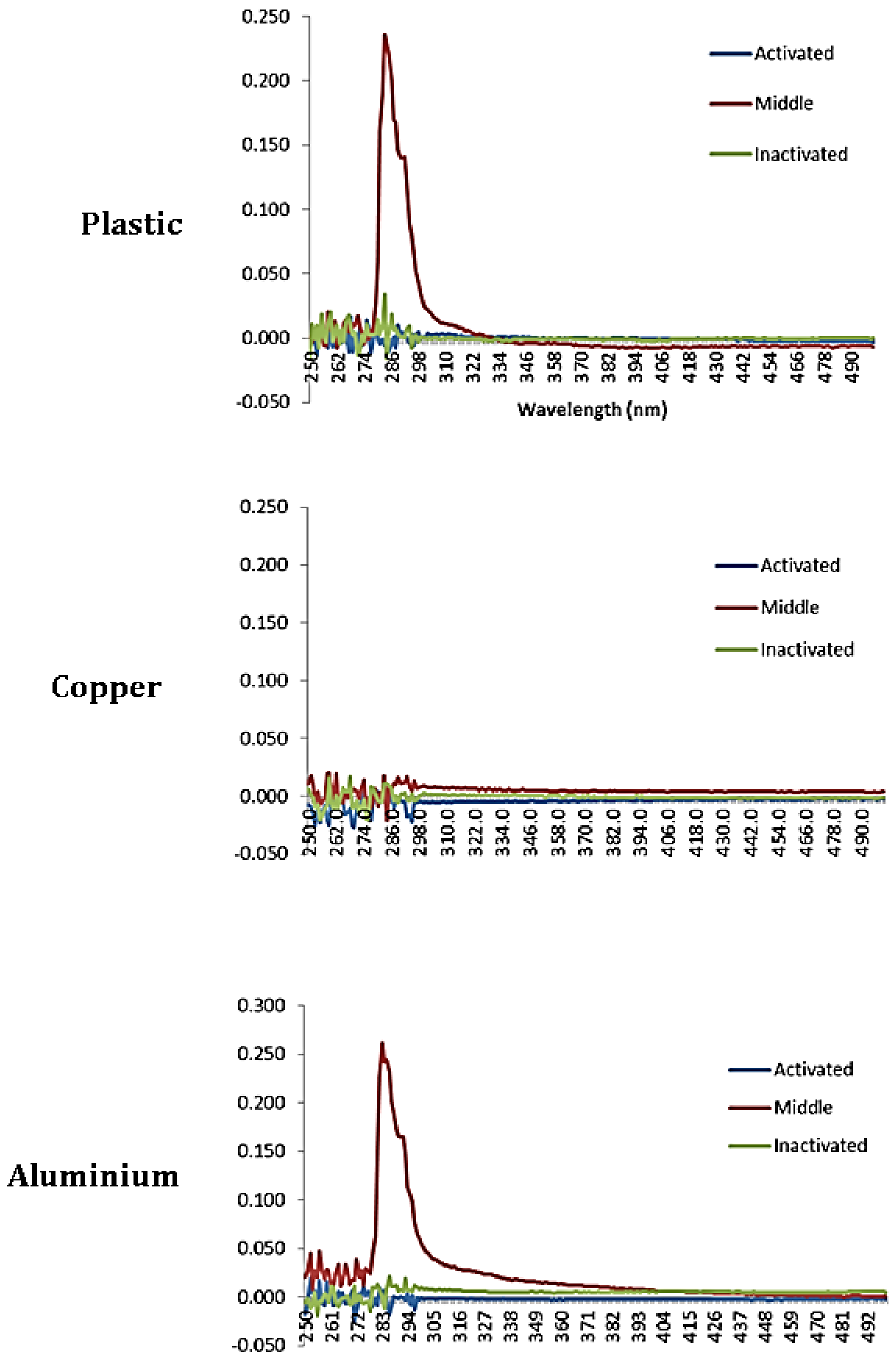

Figure 10. Relative measures for emission (uv) for spring water that had been exposed to the active, middle or inactive areas while being wrapped ("shielded") with either plastic, copper, or aluminum foil. Note the marked

enhancement of emission around 280 nm for the plastic and aluminum foil wrapping that was eliminated by copper foil.

Consequently we decided to discern if a very narrow and enhanced peak in transmittance would be evident if a UV pulse was generated through the spring water covered with different shielding material that had been exposed to this intensity (the MA). The results are shown in Figure 10. For the plastic and aluminum foil conditions the marked enhancement occurred between 275 and 305 nm only for the spring water exposed to the 0.3 to 0.6 uT fields and neither the strongest (active) or weakest (inactive) coil. However this was not observed for the spring water that had been shielded with the copper foil. Copper ions in solution rather than wrapped around water through which magnetic fields are penetrating, have been shown to dramatically decrease fluorescence intensity [22].

Conclusion

In conclusion spring water displayed a non-linear scatter of spectrophotometric transmittance as a function of wavelength while double-distilled water exhibited the expected linear decay as a function of distance from the stimulation wavelength. Exposure of spring water but not double distilled water to a temporally patterned weak magnetic field in the dark for about two weeks produced several classes of reliable effects revealed by photon transmission. The weakest magnetic fields in the order of μT produced approximately 10 nm shift in peak transmittance towards longer wavelengths. Spectral analysis confirmed the presence of 5 and 10 nm periodicities in spectral density across the measured range but this was evident only with the strongest (4-11 μT) magnetic intensity. Concomitant exposure to different types of wrapping around the water samples indicated that copper foil eliminated the ~280 nm spike in transmission compared to aluminum foil or plastic as inferred by UV-VIS spectrometry. These results indicate there is a rich complexity of subtle molecular changes within water, particularly spring water, exposed to this temporally patterned magnetic field.

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Submitted to *Water* (July 2013)

Chapter 5: Serial pH Temporal Increments (~20 to 40 Milliseconds) in Spring Water during Exposures to Weak, Physiologically Patterned Magnetic Fields: Implications for Consciousness

Abstract

The pH of volumes (50 cc) of spring water were measured for 12 hrs while being exposed to a weak (8±4 μT) decelerating frequency-modulated magnetic field that has been shown to diminish the growth of cancer cells and inhibit the movement of planarian. Compared to sham field-exposed water, the magnetic field exposed water displayed greater increased pH (more alkaline) that involved between 0.5 and 1 pH units after about 7 to 8 hr. This shift occurred slowly as successive 0.02 pH of transient peaks (about 7 per s) that were between 20 to 40 ms.The pattern was not measured in the sham field-exposed water. These results are consistent with the hypothesis that properties of water exposed to specific patterns of magnetic fields generated by 3 ms voltage values generated from computer software can produce temporal properties in water that convergence with those associated with the cerebral cortical activity coupled to consciousness.Severalquantitative solutionssupportthispossibility.

Introduction

The chemical and electromagnetic properties of water (House, 1974) have been considered the fundamental substrate that defines the boundaries of living systems (Pollack, 2003; Pollack et al, 2009). The continuous flux of H^+ ions mediated through transient hydronium ions (H_30^+) within a maintained structure (a matrix of H_20) indicates that the spatial pattern of the aggregate is maintained indefinitely while specific microstructure is ephemeral (Decoursey, 2003). This physical combination of statics and dynamics sets the conditions for a substance that can interact with its environment and represent these interactions yet maintain a structural momentum that defines the individuality of the set (DeMeo, 2011).

The universality of the molecule and its connection to fundamental forces is reflected by its diffusivity ($0.88 \cdot 10^{-7}\ m^2 \cdot s^{-1}$) obtained by dividing the magnetic moment of a proton ($1.41 \cdot 10^{-26}\ A \cdot m^2$) by the unit charge ($1.6 \cdot 10^{-19}\ A \cdot s$). When the diffusivity is multiplied by the typical viscosity of water ($1.0 \cdot 10^{-3}\ kg \cdot m^{-1} s^{-1}$) at 20°C the resulting force of $8.80 \cdot 10^{-11}\ kg \cdot m \cdot s^{-2}$ across the width of two O-H bonds ($1.92 \cdot 10^{-10}$ m) is $1.7 \cdot 10^{-20}$ J. This value is within the range of measurement error for the energy of the second shell hydrogen bonds which is $\sim 1.8 \cdot 10^{-20}$ J and reflects proton mobility (Decoursey, 2003). The quantum of 10^{-20} J (Persinger,

2010) may be a universal value that reflects the intrinsic average forces within the fine structure of space distributed across the neutral hydrogen wavelength (Persinger et al, 2008).

The differentiation and measurement of water as bulk and interfacial phases indicated that when this compound was near any surface electrical properties arose that simulated the conditions hereto attributed to plasma membranes and the separation of ions (Del Giudice and Preparata, 1994). Several authors (Pollack, 2003; Del Giudice et al, 2010; 2011) have shown that water adjacent to a surface displays ten times the viscosity than bulk water and exhibits a narrow shell of enhanced proton charges at the interface between the bulk-interfacial zones. The range of potential differences is comparable to the resting plasma membrane potential (~100 mV). The role of proton flux, *per se*, as the source for all membrane phenomena (traditionally attributed to disparity of charge of ion across the membrane) is suggested by the copious nature of proton channels within membranes and the fact that an incredible range of currents within these channels is pH dependent (Decoursey, 2003). In addition clusters of molecules within a coherent phase have the capacity to maintain or represent electromagnetic fields applied exogenously or originating within the interactions between water molecules (Del Giudice and Preparata, 1994).

The spatial segregation of H^+, which defines pH, indicates that this phenomenon could be employed to assess the effects of appropriately-patterned magnetic fields upon water. Minute changes in pH are associated with brain activity (Kaila and Ransom, 1968). Alkaline pH shifts exhibt a rapid onset and can be maintained as long as the stimulus is applied. Increasing extracellular pH and lowering intracellular pH often precedes neuronal activity within the cerebral cortices and hippocampus and is associated with opening of H+ channels (Elderand Decoursey, 2001). Shifts of pH from 7.9 to 7.2 produce currents in outside membrane patches which occur within temporal intervals of 10 and 40 ms (Bevan, 1998). For comparison the three major enzyme complexes of the respiratory chain donate and receive an electron once every 5 to 20 ms (Alberts et al, 2002).

The water molecule has been hypothesized to be the central thread through which even more complex processes, such as consciousness, are mediated if not created (Amoroso, 1999). The fundamental dynamic unit of the neuron, the single ion channel, is associated with a discrete change of 0.2 μV (Kandel et al, 2000). The energy exerted on a charge ($1.6 \cdot 10^{-19}$ A·s) is $0.5 \cdot 10^{-25}$ J (Persinger and Lavallee, 2012). When divided by the mass of a proton ($1.67 \cdot 10^{-27}$ kg) the average velocity is ~4.5 $m \cdot s^{-1}$. This is the bulk velocity of cortical fields moving through the cerebral cortices (Nunez, 1995) and would require ~20 to 30 ms to traverse the linear distance of its length. The time required for the coherent electromagnetic fields (McFadden, 2002; 2007) associated with different cognitive states (particularly waking and dreaming) to move along the rostral-caudal axis of the cerebral cortices is about 20 to 25 ms with phase shifts in the order of 10 to

12 ms (Llinas and Pare, 1991; Llinas and Ribary, 1993). Emoto (2011) has suggested that properties of consciousness may be an intrinsic feature of water or that its kinetics can be influenced by cerebral activity. Radin et al (2006) recently provided experimental evidence to support the latter possibility

The most conspicuous physical feature of consciousness is its electromagnetic features (Persinger and Lavallee, 2012; 2010). Our recent research indicates that application of weak (μT range) physiologically-patterned magnetic fields with the appropriate point voltage durations produced marked and predictable changes in activity within the cerebrum (Saroka and Persinger, 2013) as measured by quantitative electroencephalography and sLORETA (low resolution electromagnetic tomography). Specific changes are associated with subjective alterations in consciousness and phenomena consistent with altered states. The mechanism is more related to representation of the energy from the applied field within the cerebral volume rather than a Faradic-related mechanism (Persinger et al, 2010) although the latter could be significant at quantum energy levels as secondarily induced magnetic fields from the electric fields associated with the applied magnetic fields. It has been known for some time that global shifts in brain pH towards alkalinity are proconvulsant while shifts towards acidity are anticonvulsant (Kaila and Chesler, 1989).

Given these considerations we examined the effects of the same pattern of magnetic fields that produced altered states of consciousness to spring water. It was selected because of its similarity to cellular and extracellular fluids. When 50 cc of spring water were placed in an open beakers and not disturbed for 12 hr there was a gradual drift towards alkalinity (e.g, pH 7.75 to 8.21). Such slow dynamics have been considered optimal to allow interaction with applied magnetic fields. When this volume was exposed to the frequency-modulated magnetic field that is known to induce the conditions to produce altered states in human subjects (Saroka and Persinger, 2013) and electrical lability in epileptic rats (Persinger and Belanger-Chellew, 1999), we observed multiple discrete burst or shifts, with durations primarily between 20 and 40 ms, of pH. The effect was so conspicuous we designed a series of experiments to investigate this phenomenon.

Methods and Materials

"Beakers" (105 cc flint glass jars) containing 50 cc of spring water (4 mM of HCO_3; 1.77 mM Ca; 76 μM of Cl; 1.3 mM; of Mg, 41.9; μM of NO_3; 61 μM SO_4 ; 17.9 μM K; 43.5 μM Na) were exposed continuously for 12 hrs to either a physiologically patterned magnetic field (4.4 to 11.5 μT, RMS) or to sham-field conditions. There were a total of 8 replicates for the field and 8 replicates for the sham conditions. The shape of the applied magnetic field was frequency modulated (Figure 1), that is, it displayed an irregular decrease in phase modulation. The pattern was generated by converting 859 numbers between 0 and 256 to fixed values to -5 to + 5 V (127=0 V) that were transformed by a digital-to-analogue converter to the appropriate current within a coil. It was created by wrapping 305 m of 30 AWG (Belden 9978) wire in a single layer (18 cm wide) around plastic milk crates which were 38 cm x 33 cm x 27 cm.

The point duration is the time each point with a specific number or voltage was generated; it was 3 ms. This duration was selected because it diminishes the division of cancer cells but not normal cells in culture (Buckner, 2011), reduces the growth of melanoma tumors in mice (Hu et al, 2010) and enhances analgesia (equivalent to 4 mg/kg of morphine) to thermal nociceptive stimuli for rats(Martin et al, 2004). Point durations less than 3 ms or greater than 3 ms are less effective or not effective. This was predicted by the neurophysics model of Perisnger and Koren (2007). Volume pH was continuously measured at either 1 s, 100 ms, 10 ms or 1 ms depending on the experiment. The pH values for both beakers in each experiment were recorded separately once per second by Dr. Daq systems (Pico Technology, United Kingdom) which are sensitive to the .01 pH unit. The data were recorded continuously on a laptop operating the appropriate software.

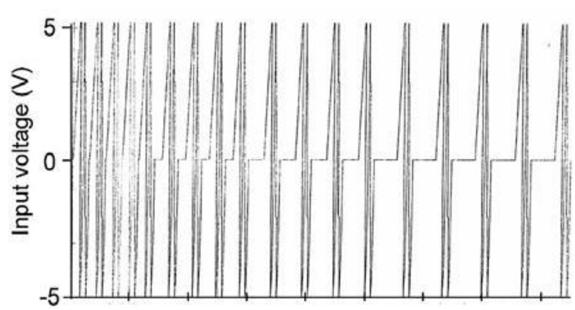

Figure 1.The shape of the magnetic field pattern to which the water samples were exposed.

The pH measurements collected every minute for 12 hrs for the magnetic field-exposed and control water were partitioned into hourly averages for statistical analyses. For the discernment of the optimal increment of time to measure the water (sampling rates of 1 ms, 10 ms, 100 ms or 1000 ms) the numbers of positive displacements (increased pH), which were almost always about .02 to .03 pH were calculated for triplicates of records in each condition. To more precisely isolate the typical duration of the increased pH shift 1000 successive sequences of 10 ms sampling rates were obtained from three different records for the magnetic and for the control conditions. The numbers of positive shifts towards increased pH that displayed 0 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, and 51 to 60 ms increments were counted. All statistical analyses involved SPSS 16 for PC.

Results

The typical shift towards higher pH (0.5 to 1 unit) of spring water over time (12 hr) contained within open beakers is shown in Figure 2. Although the water volumes exposed to both the magnetic field and no field conditions display an increased pH over the 12 hr periods of measurement, the volumes exposed to the magnetic field pattern displayed a greater pH shift. The numbers of discrete pH shifts during hour 1, 3, 4, 6, 7, 9, 10 and 12 for the volumes of water exposed to the no field or magnetic field condition are shown in Figure 3.

Two way analysis of variance as a function of condition (no field, field) and time (increments between 1 and 12 hrs) showed statistically significant differences between field conditions [$F(1,32)=497.70$, $p < .001$] and time [$F(7,32)=55.69$, $p < .001$]. The two-way interaction was statistically significant as well [$F(7,32)=14.86$, $p < .001$].

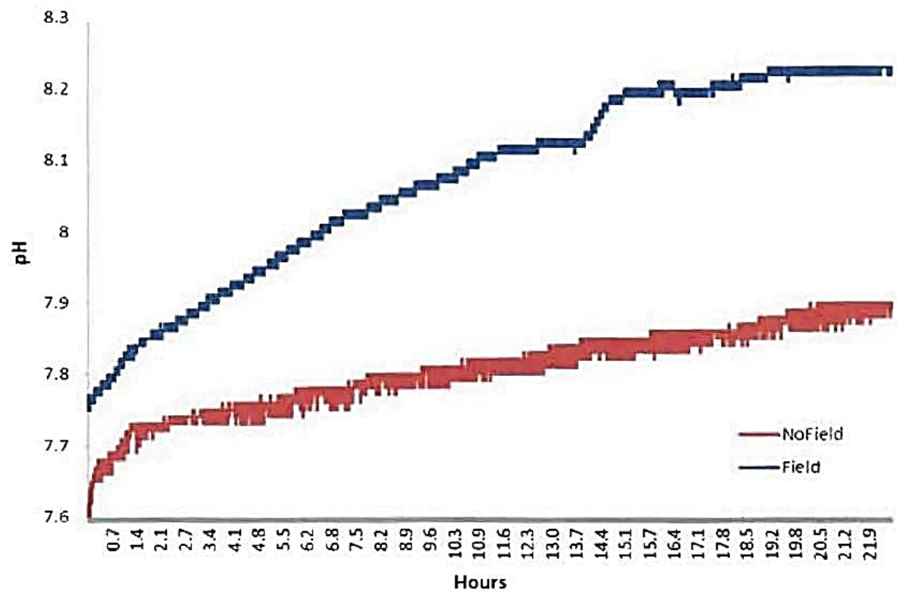

Figure 2. Typical record of successive, slow shift in pH as a function of time (12 hr) for spring water exposed to either the sham field condition or to the temporally patterned magnetic field.

Polynomial analysis for the control (non-exposed) water indicated a significant linear trend for the increase in pH [$F(1,16)=80.83$, $p < .001$] over time. For the water exposed to the magnetic fields there were both a significant linear trend [$F(1,16)=300.00$, $p < .001$] and a deviation from the linear term [$F=5.18$, $p < .001$]. Although there was a weak but significant quadratic term [$F=6.64$], there was also significant deviations from this as well as the cubic, 4^{th}-order and 5^{th} order terms (all $Fs>4.89$, $p < .01$].

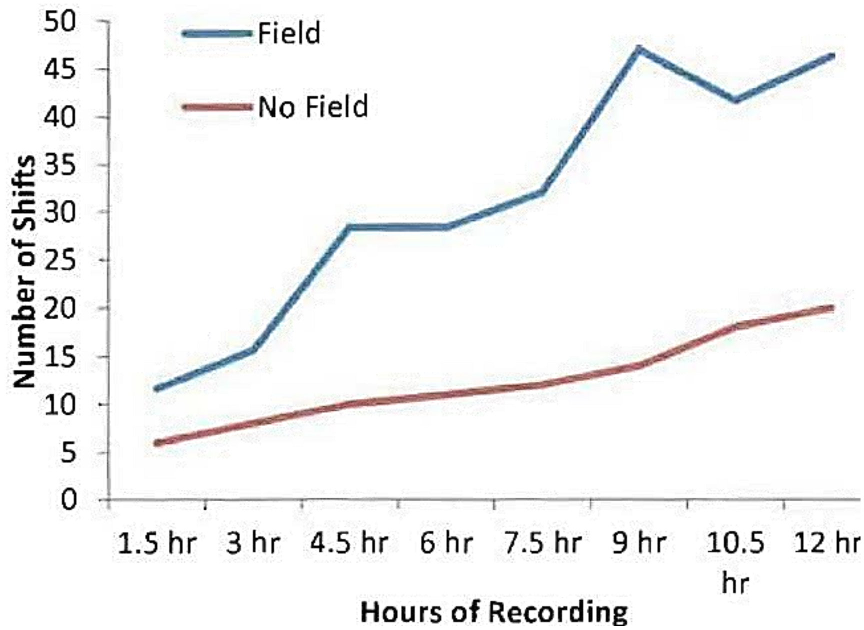

Figure 3. Means and standard errors of the mean for the numbers discrete shifts during hourly intervals for the field and sham field conditions.

To discern the optimal t or increment of analysis by which to discern the pH shifts, the numbers of shifts obtained when the data were collected ever 1 ms, 10 ms, 100 ms, or 1000 ms (1 s) were analyzed as a function of field or no field conditions. As seen in Figure 4, the optimal duration for revealing the numbers of the pH shifts involved 10 ms increments of sampling. Two way ANOVA as a function of duration of sampling and field vs no field condition was dominated by a strong interaction that explained 67% of the variance. *Post hoc* analyses indicated that the major source of the interaction was due to the more frequent pH shifts for the water exposed to the magnetic field when the durations of observation were 10 ms compared to the no field condition and for field and nofield conditions for the other three sampling durations that did notdiffer significantly from each other.

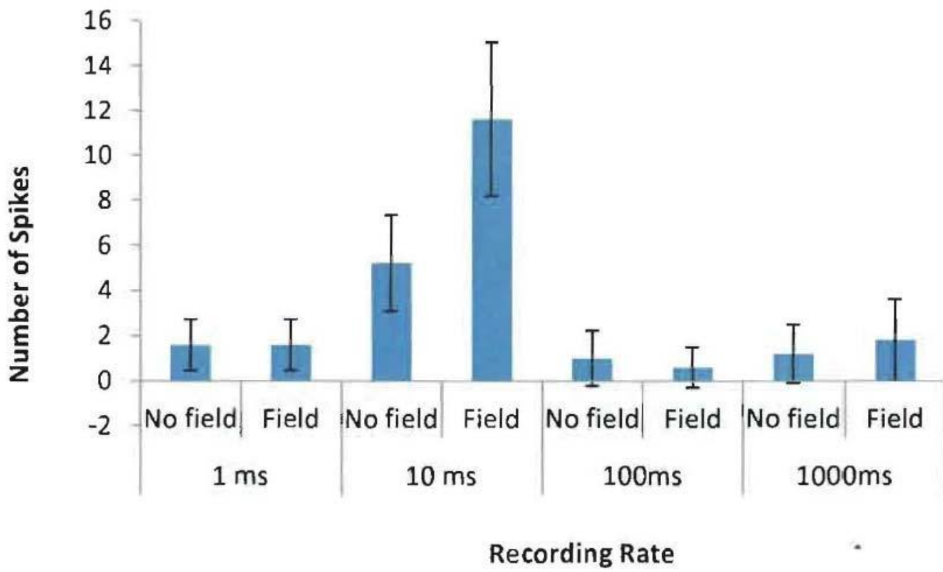

Figure 4. The numbers of pH shifts (>0.02 pH) recorded during increments of sampling of either 1 ms, 10 ms, 100 ms or 1000 ms.

The more precise measurement of samples of the 1 ms measurements is shown in Figure 5 for the mean numbers of occurrences of pH shifts for 10 different random samples for 1000 points each with increments of 10 ms (i.e., 10 s). The peak in the numbers of occurrences of pH shifts occurred for spike durations between 20 to 30 ms and 30 to 40 ms for the samples exposed to the patterned magnetic field. Figure 5 also shows the peaks in the numbers of occurrences in pH units for comparable periods within the water exposed to sham field conditions. There was no obvious non-linear effect with a peak between 20 and 40 ms.

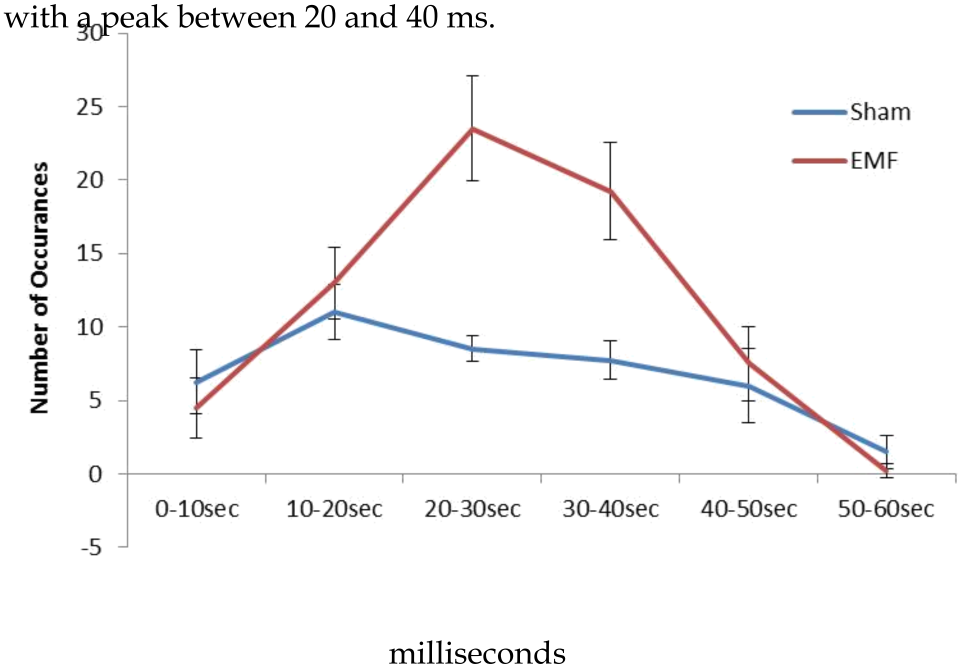

Figure 5. Means and standard errors of the mean (SEM) for the numbers of pH occurrences (increased pH transients) with intervals between 0-10 ms to 50 to 60 ms that were exposed to the sham (blue) or temporally patterned magnetic field (red).

A simple summation of the means of the numbers of positive shifts towards basic for 10 ms increments between 1 and 60 ms indicated an average occurrence of 68 per 10 s or ~6.8 shifts per s. However these shifts were not maintained but returned to antecedent levels when the transient was completed. This would indicate that in an 8 hour period, when the effect began to inflect towards asymptote, there would have been $\sim 2.9 \cdot 10^4$ of these shifts even though the total shift was between 0.5 and 1 pH unit. The shape of the pH shift indicated that over time they did not return to the antecedent value but drifted upward. Consequently, on average, the discrepancy between the antecedent pH and the return pH after the shift would have been about $1.7 \cdot 10^{-5}$ of a pH unit which was below the range of the sensitivity of our sensors. However the integrative effect over the ~8 hr resulted in a cumulative increase of between 0.5 to 1 pH units.

Discussion

The results of these experiments indicate that when spring water was exposed to a physiologically-patterned magnetic field that has been shown to induce conspicuous diminishment of movement in planarian (Murugan et al, 2013), inhibition of division of different lines of cancer cells but not normal cells (Buckner, 2011), marked analgesia in rodents (Martin et al, 2004) and invertebrates (Thomas et al, 1997), and altered states of consciousness in human participants (Saroka and Persinger, 2013), reliable changes in pH occurred. The changes were primarily toward alkalinity and involve between 0.5 to 1 pH unit compared to the sham field-exposed water. Although the latter also displayed a drift towards higher pH the change was slower, more linear, and of less magnitude.

The increase in pH of the water exposed to the magnetic field pattern displayed an inflection around 7 to 8 hrs. The mechanisms for this latency must still be examined. However it is interesting that Gang et al (2012) found that the change in viscosity of water as inferred by diffusion of a solute for 50 cc volumes of water exposed to 0.16 T static magnetic fields was also within this range. Because the water was contained within open beakers the potential interaction of gas exchanges from the surrounding air and the volume of water must still be considered. Even small additions of O_2, N_2 or CO_2 from the air could have been significant. In the mammalian brain interstitial tissue oxygen levels are non-uniform between 1 to 5% relative to the atmosphere concentration of 21% (Richerson, 2004).

The measurement of the incremental shifts in pH of ~0.02 units as a possible "quantum" might be considered analogous to the 0.5 mV increments that were initially measured in postsynaptic space as incremental units. Only later were they associated with the energy from the "spontaneous" release of fixed quantities of neurotransmitter from a presynaptic vesicle. Although the shifts were evident in the sham field-exposed water there were quantitatively more in the water exposed to the magnetic field pattern. That they were not artifacts of simple measurement or a variant of Faradic current by induced electric fields was shown by their maximum detection when the sampling was 10 ms rather than 1, 100, or 1000 ms increments.

In addition, careful measurement of the positive spikes (increased pH) of these discrete shifts indicated there was a non-linear effect as a function of duration for only the water exposed to the patterned magnetic field. There was a conspicuous increase in numbers of increased pH shifts with durations between 20 and 40 ms. This range of duration is within time required for the bulk velocity of the transcerebral electromagnetic fields associated with consciousness (the waking and dream states) to move along the rostral-caudal axis of the cerebrum.

Implications for the Role of Water, Electromagnetic Fields and Consciousness

Although the phenomenon of consciousness may ultimately be relegated to the domain of *phlogiston* once a more detailed understanding of the physical parameters are isolated (Persinger and Lavallee, 2012), the processes and phenomenology associated with this ubiquitous experience will remain. The results of the present experiment as well as the hypotheses and measurements by

others strongly indicate there are properties within water that might mediate if not create the cerebral cortical conditions that are experienced as states of consciousness. That the same physiologically-patterned magnetic field that produced the pH shifts in the study with durations of 20 to 40 ms (the same duration for the "refresh rate" of consciousness) also alters states of consciousness when experimentally applied across the cerebrum indicates that extracellular and intracellular fluids may be the major source of this phenomenon. There is now empirical evidence that these externally applied, physiologically-patterned "weak" magnetic fields are not attenuated significantly through cerebral space (Persinger and Saroka, 2013a). The classical arguments against the influence of weak magnetic fields because of the "kT boundary" apply on in certain conditions Cifra et al (2011).

A shift of .02 units of pH from 7.75 to 7.77, for example, would be equivalent to a shift from $1.7 \cdot 10^{-8}$ M to $1.6 \cdot 10^{-8}$ M of H^+ or a net change of 10^{-9} M. From Avagadro's Number ($6.023 \cdot 10^{23}$ molecules per Mole), which would be 18 cc of water volume), a nanoMole, would be change of $6.0 \cdot 10^{14}$ H^+. When multiplied by the unit charge ($1.6 \cdot 10^{-}$

^{19}A·s) the change would be associated with $9.6 \cdot 10^{-5}$ A·s. Because each increment had a mean of 30 ms ($3 \cdot 10^{-2}$ s) the current equivalent would be ~$3.2 \cdot 10^{-3}$ A. This is the range (mA) required to elicit powerful subjective experiences and images when brain tissue was directly stimulated by intracerebral electrodes (see Persinger and Saroka, 2013b) for a review. With a typical shift of voltage associated with the rostral-caudal domain in the order of 50 to 100 μV (5 to $10 \cdot 10^{-5}$ V), the average steady-state resistance ($V \cdot A^{-1}$) would bebetween .15 to 0.3 Ω-12 cm (length of cerebrum) or about 1 to 2.5 Ω ·m. This range of values includes the resistivity of cellular fluid, 2 Ω m, according to Barnes (1986).

The mean rate of occurrence of shifts in 0.02 pH units was equivalent to about 6.8 per second. Considering the larger number of events during the first few hours, the value may have been proximal to 7 to 8 Hz. With a bulk velocity of about 4.5 $m \cdot s^{-1}$ as measured by Nunez (1995) and the recurrent phase-shifting transcerebral electromagnetic fields measured by Llinas and his colleagues (1991, 1993) , this would indicate that the standing wave of the cerebrum with a circumference of about 60 cm would be between 7 and 8 Hz. These solutions are consistent with hypothesis that discrete transient shifts in pH in the order of the duration involved with the recurrent rostral-caudal fields associated with consciousness (particularly dreams and the waking state) could mediate or even be a major contributor to these processes. A shift towards alkalization in the extracellular fluid adjacent to neuronal membranes precedes their activation. As measured by Bevan (1998) shifts in pH comparable to the ones noted over a longer interval in our study occurred as 20 to 40 ms bursts.

According to Martinez-Banaclocha (2007) the potential decrease from resting to activation (action potential) corresponds to approximately 0.2 μT ($2 \cdot 10^{-7}$ T) at 0.5 mm around a neuronal surface. Alfven (magnetohydrodynamic) waves propagate in the medium with a velocity of $v = B \cdot (\mu_o d)^{-1/2}$ where B is the magnetic field strength, μ_o is magnetic permeability, and *d* is the mass density of the charge carriers (Alvager and Moga, 1997). A change of 0.02 pH units (from 7.77 to 7.75for example) would be associated with a mass density of $5.6 \cdot 10^{-8}$ kg (10^{-9} M x $6.023 \cdot 10^{23}$ H^+ per M x $5.55 \cdot 10^4$ L per m^3 x $1.67 \cdot 10^{-27}$ kg per H^+). The square root of the product of this value with u_o ($1.26 \cdot 10^{-6}$ $N \cdot A^{-2}$) is $2.6 \cdot 10^{-7}$. The quotient is in the order of 1 $m \cdot s^{-1}$. This suggests that small changes in pH within the range would be associated with functional charge densities of H^+ that could mediate the bulk velocity that defines the ~4.5 $m \cdot s^{-1}$ of the recursive waves associated with consciousness.

Diffusivity $(\mu_o \sigma)^{-1}$, where σ~ 2.1 $S \cdot m^{-1}$ is for physiological saline, can reveal the temporal component of the dispersion within water. The value is $0.38 \cdot 10^6$ $m^2 \cdot s^{-1}$. The diffusion time scale, employing Ryskin's (2009) logic, would be estimated by the radius and the thickness of the shell of the volume. Assuming a radius of 6 cm for the cerebrum and an average thickness or 4 mm, the magnetic diffusivity of water within the human cerebrum would be in the order of 10^{-9} s. For an action potential with durations of 10^{-3} s this is the same order of magnitude as the time required for each ion of the ~10^6 ions (Kandel et al, 2000) to traverse a channel that is temporally integrated into the total change in voltage. Thus even at the dynamic level the characteristics of water appear to define the boundary of the likely bases of consciousness.

Global Implications

The conspicuous number of pH shifts per sec are within the range of the fundamental standing resonance of 7 to 8 Hz for the human cerebrum, as inferred by the frequency generated from the bulk velocity (4.5 m s) divided by the typical circumference of the skull (60 cm), and the Schumann resonances(Cherry, 2002) generated by the earth between the surface and the ionosphere. Although the amplitudes are small, in the order of a pT for the magnetic field component and 10s of $mV \cdot m^{-1}$ for the electric field component they are persistent and were present during the origins of life (Cole and Graf, 1974). These electric and magnetic intensities are within the same order of magnitude as those generated during global cerebral activity in the human being. Cosic et al (2006) have shown, as has others, that experimental application of the fundamental frequency and its harmonics affects cerebral systems.

Although water containing various solutes constitutes approximately 70% of the earth's surface, its significance has been relegated primarily to contribution to climate. The intrinsic biological significance may be even greater. If we assume the (median value) volume of water on the surface of the planet is ~$3 \cdot 10^{18}$ m^3, then the energy contained within it from the geomagnetic steady state field would be derived from magnetic field strength would be: $T^2 \cdot (2 \cdot 4\pi 10^{-7}\ NA^{-2})^{-1}\ m^3$ or $(25 \cdot 10^{-10}\ T^2)$ divided by $25 \cdot 10^{-6}\ N \cdot A^{-2}$ multiplied by the volume. Quantitatively this value is $3 \cdot 10^{15}$ J. Because 1 m^3 of water contains $5.5 \cdot 10^4$ M and there are $6 \cdot 10^{23}$ molecules per M, then there would be $\sim 10^{47}$ molecules of water. This means there would be an average of $\sim 10^{-33}$ J of energy available from the average geomagnetic intensity per molecule. When this value is divided by the quantum indicator (Planck's constant, $6.626 \cdot 10^{-34}$ J·s) the intrinsic frequency is within the range of a few Hz. Considering the range of error for estimating the global mass of water, it would not be unreasonable if the actual intrinsic frequency was in the range of 6 to8 Hz.

Within the human brain volume a more direct potential connection to consciousness is suggested. Assuming an average volume of 1350 cc for a cerebrum which is ~945 cc (70%) water, there would be 55.5 M of water (18 cc per Mole). This would involve $3.16 \cdot 10^{25}$ water molecules. The magnetic energy from the static magnetic field from the earth potentially stored within this volume of water would be $0.945 \cdot 10^{-6}$ J or $2.9 \cdot 10^{-32}$ J per water molecule. The equivalent frequency associated with the quantum of energy, obtained by dividing by Planck's constant ($6.626 \cdot 10^{-34}$ J·s), is ~40 Hz. This is within the classic band associated with consciousness and the direct inverse of the 20 to 25 ms intervals associated with both the recursive rostral-caudal waves of electromagnetic coherence produced over the cerebral cortices and the peak duration of pH shifts recorded in this study. We think it is not spurious, in this context, that calcium transients can cause internal pH transients (Schwiening and Thomas, 1998) and the Liboff resonance solution of Ca^{++} in the earth's static magnetic field is ~40 Hz.

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Chapter 6: General Discussion and Conclusion

Water is the major constituent of living systems and occupies a comparable percentage of the earth's surface. The availability of free protons from hydronium ions and electrons from oxygen facilitate an endless dynamic that contributes to an integrated matrix of molecular activity. Physical forces (such as magnetic fields) and energies that can be applied through a volume of water have the capacity to influence its activities as well as its relationship with the solutes contained within its boundaries.

As developed in the introduction within the last chapter the ratio of the magnetic moment of a proton to the unit charge multiplied by water's viscosity provides a force that when applied across the distance of 0-H bonds involves a quantity of energy that is intrinsic to water (10^{-20} J) and clearly with the range of the second shell of electron interactions. The involvement of this quantum which is also the energy associated with the separation between charges that comprise the resting plasma membrane potential, the action potential and even the sequestering of ligands to cell surfaces, suggest that water itself may contain the template for all chemical reactions.

This intriguing possibility has been articulated by researchers who have pursed the concept that there is bulk water and interfacial water. Interfacial water displays specific properties that emerge when water is apposed to a surface. There is increased viscosity, a layer of protons, and the ability to generate voltages that simulate the conditions of the resting membrane potential. When combined with the theoretical solutions that coherent domains of water can "represent" or "trap" appropriately temporally patterned and intensity magnetic fields, it is possible that water itself may be the primary mediator of biomagnetic field effects.

In the second chapter the responsiveness of planarian to a range of concentrations of morphine, from lethal (mM) to femtoMole (where there was no effect) reiterated the importance of knowing the quantity of molecules that are involved with aqueous invertebrates. The magnetic field pattern that has been successful in limiting cell division for cancer cells slowed the movement of these invertebrates after two hours of exposure. The effect simulated the effects of morphine and actually widened its effectiveness at concentrations surmised to be associated with the two subtypes of opiate receptors.

Chapter 3 revealed the powerful effects of exposing groups of planarian for more protracted periods to a geomagnetic-patterned field for a few hours for several days and then to the frequency-modulated magnetic field. The effect was devastating to the organism and resulted in complete dissolution within an hour after the onset of the final field. In both experiments, the effect only occurred if the planarian had been maintained in the dark. If such dissolution occurs in volumes of diminished light, such as within the closed system of a mammalian skull, then magnetic field effects for non-living aqueous would be an important component for successful demonstrations.

Chapter 4 indicated that spring water maintained in the dark while being exposed to the magnetic field showed discrete shifts in fluorescent scatter that simulated the equivalence of 10^{-20} J and approximately 10 nm wavelengths. The effects were evident with very weak magnetic fields in the order of magnitude found in the everyday environment. The wrapping ("shielding") of the water exposed to the magnetic fields by plastic or two different metals generated one of the largest effects, in addition to the dissolution of planarian, that we have seen in this research. The massive peaks around 280 nm found in the magnetized water were completely eliminated by copper shielding. The physical chemical significance of this powerful effect remains to be elucidated.

The importance of the solutes within water may be essential to the effect. The non-linear curves for fluorescent counts across the visible wavelength for the spring water but not for ultrapure water, an observation reported by other researchers, contained the most powerful manifestations of the magnetic field exposures. One might consider that these "impurities" within water might be essential for its optimal interaction with the applied magnetic fields. The situation could be analogous to the transistor where in order to allow the electron avalanche across the boundary a small impurity like germanium is dispersed within the quartz lattices.

Chapter 5 showed that even a measure as crude as pH, H^+ concentrations, responded to the applied fields if the system was in a process of dynamics. In this instance the dynamics was associated with the slow shift toward alkaline when the spring water was exposed to air. The application of the magnetic fields enhanced this effect significantly and did not require darkness.

The revealing component was the emergence of 20 to 40 ms brief transients towards alkalinity that slowly drifted upward during the 12 hours ofexposure. Quantification of the numbers of hydrogen ions that would be involved with this change was consistent with the shift in pH. The generation of these shifts with these time intervals are relevant for biological systems and well as some of the complex functions, such as consciousness.

Even the most simplistic application, such as the energy available from the earth's magnetic field to the water within the oceans, indicates that the energy per unit molecule would be associated with an intrinsic quantum whose frequency is within the range of biological systems as well as the fundamental frequencies of the earth-ionospheric cavity. Consequently it should not be surprising that dissolution of some planarian in some of the experiments occurred during the occasional intense (K6) geomagnetic storms that occurred during this research.