Evidence of “Trapped” Voltage Spectrum Residuals within Mouse Melanoma Tumors for about 30 Minutes following brief Exposures to Treatment-Related, Physiologically-Patterned Magnetic Fields

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Abstract
Theoretical calculations by Del Giudice and Preparata and direct measurements in spring water by us have shown that the effects of applied specifically-patterned weak magnetic fields to volumes of spring water might be “trapped” or maintained within coherent domains. The tumor masses (1-2 gm) within mice that had been exposed for 30 min to 1 μT, patterned magnetic fields known to inhibit malignant cell growth and induce analgesia were measured electrophysiologically. The integrated voltages were spectral analyzed. The predominant 12.5 to 14.1 Hz amplitude in spectral power measured directly from the tumors displayed a conspicuous shift that required about 30 min in real time to asymptote in mice after they had been removed from the magnetic fields. This shift over post-exposure time did not occur in the tumors of sham-field exposed mice. Power spectra within the tumors of mice that had been exposed to the pattern (Thomas pulse) shown to inhibit the growth of a dozen different human and animal malignant cell lines but not normal cells showed elevations of power or discrete spikes within the band of ~8 to ~28 Hz which represents the approximate spectral band of this magnetic field’s frequency-modulation. These results support the concept that representations or “residuals” of appropriately patterned magnetic fields with potential treatment efficacy are present for protracted periods within the tumor mass after the field applications have been stopped.

Keywords: Melanoma tumors, Electrophysiological measurements, Magnetic field residuals, Spectral power density

Introduction
Reductions in size or mass of malignant tumors following whole body exposures to appropriately patterned magnetic fields have been reported by several groups of researchers [1-3]. Hu et al., [4] have found that 3 hr, nightly exposures of C57 mice in which B16 melanoma cells have been injected subcutaneously to a weak, frequency-modulated, extremely low frequency magnetic field reduced the mass of the tumors at the endpoint by more than 50%. Unlike cell cultures tumors are aggregates of cells, blood vessels and other cytological elements that may have infiltrated into the tumor mass. The manner in which applied, physiologically patterned magnetic fields permeate the tumor mass or volume and exert inhibitory effects may involve mechanisms that are different from exposure to cell cultures.

The tumor as a mass with a distinct boundary would be expected to exhibit the capacity to “represent” the information or pattern of the applied field. Del Guiduice and Preparata [5] calculated that applied weak magnetic fields could be trapped within coherent domains of water molecules along boundary conditions. Interfacial
water rather than bulk water has been shown by Pollack and his colleagues [6,7] to exhibit unusual physical chemical properties that could facilitate interaction and maintenance of applied magnetic fields. Murugan et al. [8] demonstrated this capacity for physiological (spring) water to “hold” the energy from applied magnetic fields before the intrinsic structure dissipated and the pH shifted quickly. The “holding” interval, despite the regular addition of small aliquots of proton donors, ranged from 3 min to about 15 min and was intensity dependent within the range of 0.1 to 1.5 μT.

If this assumption is valid, tumors following exposure to these fields would be expected to display electrophysiological profiles that reflect the history of exposure. Detailed examination of these profiles could reveal the discrepancies and hence the potentially intrinsic transformations between the temporal structure of the applied fields and the pattern emitted by the tumor. This difference could be relevant for inferring the physical properties of the tumor as well as for designing potential temporal patterns of magnetic fields that might be effective for terminating the growth of malignant masses. We have imagined a condition where simultaneous application of slight phase- or opposite-polarity modulations of the pattern emerging from the tumor mass could diminish its growth. This type of methodology was reported to be effective to cancel the polarized foci in brains of epileptic patients and to reduce their incidence of seizures [9].

That tumors display electrophysiological profiles reflecting differential impedance was examined by Michalak and Nawrocka-Bogusz [10]. They found that different type of tumors in human patients exhibited a wide range of electrophysiological frequencies.

We designed an experiment to discern if only 30 min of exposure to computer-generated, temporally patterned magnetic fields that: 1) have been shown to diminish the growth of malignant cells from about one dozen human and mouse cell lines but not to affect non-malignant cell growth, and, 2) elicits morphine-equivalent analgesia in planarian [11], molluscs [12] and rats [13], elicit residual “signatures” that can be discerned by spectral power density (SPD) analyses after the fields have been removed. Here we present evidence that residual effects are clearly demonstrated within the SPD profiles of tumor masses in the host mice after their terminations.

**Materials and Methods**

A total of 11 male C57 mice approximately 140 days of age had been maintained in standard plastic cages (2 to 4 mice per cage) within our colony room whose temperature was maintained at 21°C with a L:D (light:dark) cycle of 12:12 beginning at 0800 hr. Each mouse had been injected subcutaneously into the right flank with ~0.5 million B6-B16 mouse melanoma cells (100% confluence) within 50 μL. About 15 to 20 days later when the tumor presence was conspicuous and the mice were approaching the endpoint of the Animal Care Committee-approved protocol the measurements were begun. Over several days mice (1 or 2 per day) were exposed for 30 min to either the Thomas pulse (n=4) which has been shown to reduce malignant cell proliferation in cell culture and mice, the burst-x (n=3) which has been demonstrated to produce the equivalence analgesia of 4 mg/kg of morphine or to control (sham field, n=4) conditions.

The exposure equipment has been described elsewhere [14]. It was a box constructed from plastic. A solenoid in which a metal rod had been inserted (to enhance the strength of the field) was permanently attached to each face of the cube. The circuit from the digital to analogue (DAC) converter was structured such that opposing pairs of solenoids were connected in each of the three planes. The circuitry was programmed to rotate the onset and offset of the pairs of solenoids once every 0.5 s. As a result the fields were generated cross the X plane, Y plane, and Z plane, and then all three planes simultaneously. One cycle required 2 s. The median maximum strength of the magnetic field was 1 to 1.5 μT. The box was filled with ¼ inch corncob bedding such that the mouse ambulated within the center of the focus of the fields from the 3 pairs of solenoids. The control or sham box was constructed identically except no field was activated.

The two patterned magnetic fields were composed of 849 points (Thomas) and 230 points (burst-x). Pictures of the patterns have been published multiple times [14]. Each point was a number (1 through 256) that was transformed to voltages (-5 to +5 V) such that below 127 the polarity was negative, above 127 the polarity was positive, and 127=0 V. The point duration (which the real time plus port latency of the 286 computer that generated the changes from the programmable software) was 3 ms. This meant that each number between 0 and 257 that generated the voltages that produced the patterned was activated for 3 ms. This point duration has been shown to be critical for the effective inhibition of malignant cell growth for the Thomas pattern and the elicitation of analgesia for the burst-x pattern. Each pattern was repeated with an interpattern delay of 3 ms for the 30 min period while the mouse moved freely.

After the 30 min of exposure to the magnetic field or control conditions the mice were euthanized by CO₂. Ten 12 mm long subdermal sensors were placed in each mouse within the tumor (n=3), the left leg (n=2), the chest (n=3) and the left ear (n=2). Each tumor, as measured after the experiment, was about 1.5 to 2 gm. The array of placement provided sufficient channels to allow robust analyses and to correct for artifacts. Twenty-five (25) min of recordings from the 3 tumor leads were imported into MATLAB for each mouse. An average of the tumor-related signal was obtained by computing the temporal root-mean-square of the three sensors inserted in the tumor. Spectral analyses for each of the 5-min intervals in the 25 min recording period were completed for each of the 11 mice. Due to the presence of ~160 frequency bins between 1 and 40 Hz, cursory one-way analyses of variance were completed for the first 5 min of each frequency bin in order to screen for any changes indicative of residual effects from the 30 min of magnetic field exposure. MANOVA (Multivariate Analysis of Variance) was completed to explore the possible temporal changes within a given frequency band.

**Results**

The most conspicuous and robust effect involved the frequency band between 12.45 and 14.1 Hz. The analysis of variance indicated that the tumors within the mice that had been exposed to the burst-x field displayed significantly higher voltages relative to those exposed to either the Thomas pattern or control conditions.
that had grown subsequent to injection of about a 0.5 million of these cells about 20 days earlier. A single 30 min exposure to the patterned magnetic field was followed by a marked shift in this power density over a 30 min period compared to that displayed by the tumors from control (non-exposed) mice. The broader band of between about 8 Hz and 30 Hz where the elevated potentials were noted is congruent with the spectral power density of the Thomas pulse itself when presented in 3 ms point durations [15]. These results are consistent with our prediction that tumor masses could maintain the representation of applied magnetic fields within their volume and that it would dissipate in a systematic and exponential manner following the removal from the magnetic field.

If there is symmetry in the asymptote of the response following the initial exposure to these temporally patterned magnetic fields and the asymptote following their removal, we could surmise that approximately 30 min was required to produce the peak. This is similar to the minimal duration of daily exposures required to produce diminished cell growth in cell culture as well as the latency observed from the increases in calcium influx into melanoma cells as revealed by confocal microscopy [16]. Almost 40 years ago W. Ross Adey et al., [17] found that 6 Hz and 16 Hz but not 32 Hz or 75 Hz electric fields around 10 V·m⁻¹ produced maximum effects upon chick and cat cerebral tissues. The physical-chemical correlate or substrate for this latency must be pursued. However our studies suggest that this duration might be required for the energy associated with the applied field within water molecules to achieve a critical threshold [18].

The complex contribution of water to the multiple processes involved with development of tumors has been discussed extensively by Davidson and his colleagues [18]. Although the role of the proton associated with the hydronium ion is considered paramount [19,20] the role of transient uncoupling of critical
signalling pathways should also be considered. For example Ishido et al. [21] demonstrated that 1.2 μT magnetic fields with specific pulses in the extremely low frequency range could cause uncoupling of signal transduction between adenylyl cyclase and melatonin receptors. In a manner analogous to the strong field of a 1 T magnetic field and accompanying resonance radiofrequency source producing alterations in spin-spin or spin-lattice relations of protons in water, uncoupled signal transductions might exhibit the “relaxation” and rebound recovery to the previous state once the field was removed.

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