Inductively Coupled Electrical Stimulation - Part 3: PEMF Systems for use in Basic Research with Laboratory Animals and In Vitro

Robert Dennis Ph.D. Micro-Pulse LLC

A system is described whereby calibrated pulsed electro-magnetic fields, generally known as PEMF (ICES®-PEMF) can be tested on laboratory rodents (rats and mice) by application of the electro-magnetic pulses through the exterior of the bottom of the cage. These systems may also be reconfigured for various other experimental conditions, and are based on the elucidation of well-defined electro-magnetic parameters, determined by earlier experimental work to result in consistent, reliable, beneficial biological effects while requiring very low levels of RMS electro-magnetic power, and at low frequencies (< 1 KHz primary). The systems described in this report, or their derivatives, are currently in use and are planned for use in numerous basic experiments, and have been used in private testing laboratories as well as academic research. These systems may, for example, involve an array of 4 x 8 (32 total) 40 mm diameter coils powered by 8 synchronized PEMF pulse generators, or a similar system modified for use with cell and tissue samples in a 35 mm diameter Petri dish or other small sample chamber, which may also be used in a cell culture incubator or with a microscope. For testing with laboratory animals, the coil array is built into a magnetically-transparent plate which rests below the bottom of standard plastic caging systems. The pulse generators produce tuned trapezoidal bipolar pulses with 100 micro-second rise times, programmable pulse patterns, and magnetic flux (Gauss) slew rates up to 1600 kG/s. These systems can be used to test the effects of short- or long-term PEMF exposure on untethered, individually or group-housed mice and rats in standard micro-isolation cages. The coil configuration is modified to suit the needs of small sample chambers for cell and tissue culture or microscopic analysis, and can be used for imaging cell growth dynamics, calcium signaling, etc. The system operates on ultra-low power (5.0 Volt USB charging port at 2.1 Amps rated maximum), and has been uniformly approved by institutional animal care and use committees each time its use was proposed, primarily because it has the features that it requires no restriction to the movement of animals within the cage, does not interfere with grooming or feeding or social behavior, is entirely non-invasive, and requires no changes to the standard, approved caging systems in use at the time of their review and approval. The ICES®-PEMF driver circuit remains essentially unchanged, but the coil geometry and configuration can be readily adapted to different coil array configurations, for different sized cages, different cage mounting systems, and for in vitro PEMF testing of cultured cells both in an cell culture incubator, as well as in the form of a solenoid fixture for optical microscopic examination of living cells and tissue samples.

Citation


Introduction
Although inductively-coupled PEMF was a vast improvement over the use of direct stimulation by means of implanted electrodes early in the latter half of the last century [1-3], the application of PEMF to animals in a research setting has remained somewhat problematic due to the cost of typical commercial PEMF systems that are known to be reliably biologically effective, and the difficulty of affixing PEMF coils to ambulatory animals for the application of known and consistent electromagnetic fields [4]. Also, although the veterinary clinical benefits of PEMF are clear [5,6], most PEMF systems are too costly and are not designed for ease-of-use with animals in a research setting, small biological samples, plants, or with a microscope. Therefore, many types of PEMF research, especially animal research with PEMF, which could be done largely in a humane and harmless way, is often not considered because of the high cost of most PEMF systems and the difficulty of use of PEMF with research animals. In many cases, the use of PEMF on animals requires direct and continuous intervention from people [5] manually holding the animal, coils, or both in place during PEMF treatment. This tends to result in the practical experimental limitation to infrequent PEMF treatments of short duration. Therefore, the scientific study of chronic (daily and/or multiple hours, or continuous) treatment of animals with PEMF has largely been avoided by researchers because until recently it has been impractical.

Nonetheless, chronic exposure of laboratory animals to therapeutic PEMF has been attempted, often unsuccessfully (unpublished communications), usually involving daily anesthetization and/or restraint of rats or mice. The reasons that a large number of these pilot experiments have failed are many and varied, but often it is the case that they have been inadequately supported by private PEMF marketing groups that abort the studies before completion due to financial or business pressures, or the studies were poorly conceived, poorly designed, or poorly carried out, and, as is often the case with studies contracted from industry to academic laboratories, the results were shaping up to be equivocal at best. Compounding these difficulties is the fact that the vast majority of commercial PEMF systems are based on pirated technology. Typically, the marketing company promoting the scientific study has no technical skill, usually with no scientists on staff, and therefore is unable to provide systems modified specifically for use in the laboratory setting.

For the occasional well-designed study, more often, institutional animal use committees have rejected and/or restricted the scope of studies requiring the level of animal restraint and frequent anaesthetization that has been proposed to obtain consistent effects. As a result, the scientific literature contains an inadequate number of studies of this kind, and as a result, many people mistakenly take PEMF in general to be “not scientifically supported”, when the reality is that such studies have been unusually difficult both technically and administratively.

This has also been the case with any of a number of promising but non-mainstream medical technologies. However, just because something is difficult to study does not mean that it should be discarded as “scientifically unproven”. To directly address this deficiency in PEMF research, what is required is a PEMF system that is specifically designed for use with laboratory animals or tissue specimens, is easily applied, inexpensive, has reliable biological effects, and will pass muster with institutional animal care and use committees. Until recently, such a system has not generally been available.

Even with the challenges to PEMF research, positive reports on the clinical benefits of PEMF for both humans and animals are numerous, even though typical veterinary PEMF studies have usually involved only a single or just a few PEMF treatments, and usually are limited to 20 minute or shorter treatment duration. Nonetheless, positive benefits of PEMF have been reported widely, by dozens of laboratories over a wide spectrum of injuries and disease conditions, over a period spanning more than 5 decades [5-10].

A number of studies have involved the use of specially designed and fitted PEMF systems [4,11], and while these have also demonstrated significant enhancement of healing, their wider scientific veterinary use has been extremely limited due to the electronic, animal handling, surgical skills and the cost of the PEMF systems required to carry out such studies.
As a result, using current PEMF technology which is often adapted from clinical PEMF systems intended as commercial products for human use, typically all that can be accomplished scientifically is to make individual, incremental demonstrations that PEMF may (or may not) be helpful when applied only for a single or just a small number of sessions to laboratory animals, for short durations, and for specific conditions. These current technical limitations preclude many of the types of studies that need to be done to establish PEMF safety and efficacy, and to elucidate the basic underlying biophysical mechanisms of PEMF. This has been the case for many decades, and the resulting scientific advancement in our understanding of PEMF has been commensurately deficient.

While most clinically important findings related to PEMF can and should involve human subjects research, the basic science of PEMF will nonetheless require the humane use of laboratory animals so that PEMF conditions can be standardized over longer periods of time, using genetically similar or identical individuals (rats or mice typically), in large numbers, and in survival experiments often for extended periods of time, to clearly demonstrate the long-term benefits and safety of PEMF.

In summary, what is required is a low-cost, easily-used, humane, readily-available system for applying PEMF to laboratory animals under standard conditions, for both short or long periods of time, allowing both intermittent or continuous PEMF exposure. The system must allow free and unrestricted movement of the animals, at least within a typical laboratory cage, while also subjecting the tissues to a quantifiable and consistent level of PEMF exposure. Harnesses, lanyards, cables, and tight-fitting jackets to hold coils precisely in place on such small animals is both impractical, and as it turns out, unnecessary. These systems should be amenable to reconfiguration, for use with small samples (cells or tissues), plants, biofluids, and with optical microscopes.

The following report describes several such generic PEMF systems, beginning with two coil array plate systems, both of which have been and are being used for research on the effects of PEMF in both rats and mice ([4,12], several publications in preparation). Later we describe simple variations, basically changes in coil configuration, that have been made for use with small tissue samples, plants and seeds, and other applications. Of the coil array plate systems, a larger system (RCP-48) allows exposure of rats housed either singly or multiply in standard-sized micro-isolation cages, while a smaller system (MCP-48) allows either a singly-housed rat or single or multiply-housed mice in standard-sized micro-isolation cages to be exposed to a programmable repeating sequence of PEMF pulses. In all cases the systems can use the same driver electronics, whereas coils were adapted to fit specific uses in the laboratory; animal housing cages, in cell culture or on a microscope.

**Methods**

A generic driver circuit had been developed [13], and had been used successfully in prior work, both for direct electrical, as well as indirect (non-contact) PEMF tissue stimulation by electromagnetic induction [4,12,14-22]. This basic circuit was progressively adapted and tuned to drive 1 x 4 arrays of coils (28 AWG, 40-turns, 40 to 50 mm diameter), using waveform parameters previously found to reliably elicit beneficial biological effects [4,12,23]. Those specific circuit designs will not be duplicated here. For each design, electronic and coil components were selected to achieve magnetic pulses with the following parameters:

1. Pulse shape: Trapezoidal
2. Rise time: 100 micro-seconds (μs)
3. Fall time: less critical, but typically 60 to 200 micro-seconds (μs). This can be tuned.
4. Magnetic slew rate: up to 1600 kilo-Gauss per second (kG/s)
5. Pulse polarity: bipolar (or selectable as monopolar of either polarity)
6. Pulse rate: programmable from 0.1 to 150 pulses per second (pps), often reported as “Hertz” (Hz)
To reliably achieve this performance, it is necessary to use high-efficiency field-effect transistors (FETs) in an “H-bridge” (https://en.wikipedia.org/wiki/H-bridge) configuration for each coil. Thus, PEMF coils are not ground-referenced but can easily be driven in a bipolar mode, allowing the magnetic polarity to be reversed with each pulse. Bipolar pulses of rapid balanced polarity reversal allows stimulation without net charge flux in the tissue, which is very beneficial for preventing galvanic degradation of implanted metals or electrodes [13], and may also prevent charge-related collateral tissue damage from inductively-coupled tissue stimulation systems, such as PEMF.

As a general design guideline, it is best to select low equivalent series resistance (Low-ESR) aluminum-electrolytic capacitors for the output stage, which are rapidly discharged through the coils. The LCR circuit involving the output-stage FETs, the PEMF coils, and the low-ESR capacitors are selected to result in a tuned response which can be verified by measurement [4] to achieve the above-stated performance parameters.

In the configuration primarily reported here, each driver circuit drives a single 1 x 4 array of coils, which are in turn organized into a planar 4 x 8 coil array using 8 synchronized PEMF drivers operating in parallel.

Figure 1. Adjacent coils (orange) in a PEMF coil array, when placed anti-parallel, will generate oppositely polarized magnetic fields, resulting in the formation of magnetic arcs, spanning from coil to coil with each pulse. Typically, pulse polarity, and therefore, magnetic polarity, is reversed with every 100 ms pulse. The figure shows the magnetic flux lines between two adjacent coils. Not to scale.
Figure 2. Different coil polarity patterns for PEMF arrays. When the polarities of each 1 x 4 coil array are lined up the same (plate above), the resulting magnetic flux lines are created above and below the coil plate, with the magnetic flux arcs following the green arrows as shown in the top figure. When the polarities of the 1 x 4 coil arrays are alternated (plate below), the instantaneous magnetic flux lines form the pattern shown by the green arrows in the bottom figure. The pulsed magnetic flux lines appear and then fade rapidly with each PEMF pulse (in this case, 100 micro-second rise and fall times). A third alternative (not shown) is to wind all coils so that each coil generates the same magnetic polarity (either N or S) as all other coils in the array with each pulse. In that case, magnetic flux lines would not cycle between adjacent coils, but rather around each coil in the array.

The eight output channels from the PEMF pulse generator each drive four coils wired in parallel. The coils in each 1 x 4 array are alternately wound, either clockwise or counter-clockwise. This results in each coil array having opposite polarity to its adjacent coil in each 1 x 4 array. Thus, when energized, the magnetic polarity of each 1 x 4 coil array will be either N-S-N-S or S-N-S-N, depending on the direction of electrical current through the coil array. Placing eight of these 1 x 4
coil arrays into a larger 4 x 8 array, the pattern of magnetic polarities can be either of the two patterns shown in Figure 2. Another alternative is uniform polarity for each coil, where all coils, when activated, have the same polarity (N or S), not shown.

The 4 x 8 coil array is sized to fit the dimensions of the bottom of a standard mouse (or rat) cage. As animals move about the cage, they are subjected to differing magnetic field line orientations of various intensity. With normal activity over time, all animals in the cage are subjected to approximately the same pattern of magnetic field intensities.

Figure 3. Pairs of synchronized PEMF coils may be arranged as Helmholtz coils, as shown. In this configuration, where the separation distance between the coils $h$ is equal to (or less than) the radius $r$ of the coils, a nearly uniform magnetic field is formed between the coils, illustrated by the parallel and equally-spaced flux lines (green) in the region between the coils. Outside of the coils, the magnetic field drops off rapidly in all directions, in general much more quickly that the inverse square law ($1/r^2$) in any direction. For the purposes of mechanistic experiments at the cellular or molecular level, such an arrangement allows an experimentally practical volume of space for test specimens subjected to spatially quasi-uniform fields, while also allowing ample physical clearance and space for optics (such as a microscope objective lens) other sensors/detectors, and other instruments such as electrodes, temperature controlled baths and surfaces, optical components, and laser beams.

Results
A modular, generic design was developed that allows modification to build coil pads suitable for standard plastic rat or mouse micro-isolation cages, as shown in figures 4 and 5. This design is readily modified for different cages or cage rack configurations.

A typical magnetic pulse is shown in figure 6. The total pulse period (rise and fall) is typically less than 200 microseconds, whereas the interval between pulses is typically 100 ms (10 Hz), resulting in a duty cycle of about 0.2%. Thus, the pulses must be measured with a high-speed magnetic flux detector, and a simple “magnetic field meter” is simply not useful or effective because the magnetic fields are in the OFF condition approximately 99.8% of the time.

![Image of MCP-48 coil pad](image)

**Figure 4.** MCP-48, a platform sized for standard laboratory micro-isolation mouse cages, featuring 40 mm nominal diameter PEMF coils in a 4 x 8 array, sized specifically for use with mice housed singly or multiply in standard micro-isolation cages. Plastic cages are simply placed directly on the acrylic plate containing the coil array. No modifications to the cage are necessary. The device is pictured next to a 12-inch ruler for scale.
Figure 5. RCP-48, a platform sized appropriately for standard laboratory micro-isolation rat cages, featuring more power overall, larger coils in a 4 x 8 array, sized specifically for use with rats housed singly or multiply in standard micro-isolation cages. The device is pictured next to a 12-inch ruler for scale.

Other Configurations:

The basic driver circuit was reduced to a 4-channel device that has recently been commercially produced for use in experiments of all kinds, including the application of ICES®-PEMF to seeds prior to planting (Figure 6a), the use of ICES®-PEMF to modulate the rheology of crude oil and other biofluids (not shown), the application of ICES®-PEMF to cells while in a Petri dish in cell culture or when placed on an optical microscope (figure 6b), and for experiments involving drinking water (figure 6c), among others. In each case, the pulse generator has been a Micro-Pulse model B5 (shown in figure 6a stimulating mung beans prior to germination), with the coils variously arranged into a planar array, solenoid, or Helmholtz configuration, as required for each experiment. The model B5 allows the use of up to four synchronized outputs, which can drive single, paired, or arrayed coils. The model B5 also has a simple user interface that allows the intensity and pulse pattern/frequency to be selected, and the entire system operates safely from a single 2.1 Amp capacity USB charger port (5V).
The exact shape of each pulse as shown in figure 7 will depend on the components and the coil geometry. The rising edge of the pulse will show a first-order asymptotic approach because it is limited by the discharge capacitor capacity and bandwidth. Better linearity of the rising edge is attained by truncating the rising edge long before it asymptotically plateaus. Based on earlier
findings ([4,18], unpublished data), the rising edge of the pulse should be set to 80 - 100 μs as a minimum. Because pulse rising edges should be no shorter than 80 - 100 μs, it is necessary to design higher peak pulse levels achievable by the system. Shorter, or excessively longer pulse slew rates may not yield reliable and consistent biological effects.

The electrical components in the output stage (inductor “L”, capacitor “C”, equivalent resistance “R”) must be chosen and adjusted to achieve the desired magnetic slew rates (rising and falling). In the case illustrated in this figure, the desired slew rate of about 900 kG/s (average) was to be retained for 100 μs, whereas the effect of the falling edge was to be set so that the induced electrical field would be short enough to have only minimal physiologic effect, in this case 30μs. It is also possible (and necessary) to adjust the LCR tuning to achieve symmetric, or different asymmetric shapes, depending on the desired induced electrical fields to be studied.

Figure 8. Peak Gauss for any given pulse will diminish rapidly as a function of the distance above the coil array plate. To make sure that animals will be exposed to pulses of quasi-uniform intensity, it is helpful to make sure that the animals do not have toys or platforms in the cage that allow them to spend significant time far above the bottom of the cage. It is also helpful to fill cages with minimal bedding thickness, and also that it also does not vary a great deal from cleaning to cleaning.
Figure 9. The magnetic slew rate of each pulse, measured from the top of the acrylic coil plate.

As shown in figure 9, the magnetic slew rate above an array of coils in a flat acrylic plate is maintained within the biologically-effective range for magnetic pulse slew rate [4], which is in the approximate range of 100 kG/s to 2 MG/s [4,18], but the peak gauss for these 100 microsecond pulses is limited to approximately 90 to 100 Gauss, as shown in figure 8.

Much higher peak Gauss levels can be obtained when coils are stacked into a Helmholtz configuration, as shown in figure 3. Such configurations are not practical for unrestrained animals freely moving in a cage, but they are perfectly suited to use on microscopes or in cell culture incubators. Using the same coil geometry and driver circuitry, much higher peak magnetic fields are achievable within the Helmholtz coil assembly, with correspondingly higher magnetic slew rates as well. Of course, lower peak Gauss and lower slew rates are readily obtained by driving the PEMF coils at lower peak current.
Figure 10. Pulse waveform for stacked coils. Within Helmholtz coils, peak magnetic fields of 200 to 250 Gauss are easily achieved.

Discussion

Presented is a generic architecture for a laboratory-grade experimental PEMF system that is low-cost and applies a regular array pattern of magnetic flux in the form of PEMF pulses, which can be used below the base of standard micro-isolation cages for small laboratory animals. The system can be readily modified to provide smaller volumes of spatially-uniform magnetic fields for studies of fundamental biophysical mechanisms of PEMF for use in cell culture incubators or on microscopes. These systems have been used, and at the time of this writing are in current use, and have proven to reliably and repeatably elicit consistent, beneficial biological effects.

As an unavoidable physical reality, the intensity of the magnetic field will drop as the distance from the coil array plate increases (fig 9). This is because the application of uniform magnetic fields over entire volumes approximately the size of the cages used in this study is not physically practical for many reasons. Most PEMF manufacturers that make claims to the contrary, that a thin plate of approximately the size of these pads can create uniform magnetic fields the size of a human adult, are scientifically incorrect and baseless fraudulent claims, which are easily demonstrated by direct measurement of the magnetic fields surrounding any such devices, which is always much smaller in...
total volume than advertised, and typically is also 10 to 100 times lower in peak Gauss that specified by the manufacturer. We therefore strongly suggest that the *actual* magnetic waveforms and peak magnetic pulse intensities are measured directly and verified when any PEMF system, especially commercial systems, are used in any study. This has been a perennial shortcoming in the published PEMF literature. In a thorough review of the PEMF literature, it was found by the author (unpublished) that only ~ 3% of the papers actually presented data showing that the magnetic pulses and field strengths were measured and verified. In all other cases, the papers presented only theoretical values or manufacturer specifications, which are known to be in error in most cases, and which often proves to be less than specified by the manufacturer by a factor of 10 to 100, or more.

The expectation for the absolute uniformity of magnetic fields is more one of marketing fraud in commercial PEMF systems, rather than a matter of significant scientific concern. In fact, it is a weakness of the PEMF literature in general that the magnetic characteristics of the devices used for their experiments most often are not well characterized, or may not be characterized at all. So, the matter of uniformity of the magnetic fields has not risen to the forefront as a scientific concern. And in no case has non-uniformity been shown to degrade biological effectiveness, provided that the system was well characterized enough to keep the volume of interest within the range of reliable biological effectiveness. Specifically, it is only essential to achieve magnetic slew rates of sufficient duration within a wide biologically relevant range (Dennis 2019b). For laboratory animal studies, absolutely uniform magnetic fields are impractical and unnecessary. What is required is simply a practical, robust PEMF system that provides well-characterized magnetic fields in the broad range that is thought to be biologically effective, as described in the first part of this report. For mechanistic, biophysical studies, a variation of the coil configurations is offered that achieves a high level of magnetic field uniformity for the much smaller volumes required for cell and molecular studies. The same PEMF pulse generators can be used in either system. The difference is in the coil configuration, and the volume of space that is being subjected to PEMF for study.

When studying organismal and clinical effects of PEMF, the configurations described above are practical, simple, cost and space-effective and yield reliable and significant biological effects. As is so often the case, however, many people (including many scientists) confuse the needs of clinical versus mechanistic biophysical studies when striving for ultra-uniform experimental conditions. The value, reliability, and generalizability of clinical findings is almost always increased when diversity in the sample population or wider ranges of interventions are intelligently incorporated into the study design. The improper and ill-advised use of genetically identical test subjects under absolutely uniform conditions to test for clinical effects has increasingly resulted in serious deficiencies in clinical experiments. The basic question always lingers: what if some small change were made? ... which inevitably leads to the larger question: are these findings relevant to a real population of animals or humans at all?!! Indeed, ultra-narrow clinical or animal studies are coming under increasing scrutiny, and they are not bearing it well, as the fundamental relevance of this unnecessarily narrow scientific approach comes under question [24]. But lower uniformity of conditions should not be confused with uncharacterized or poorly characterized experimental conditions. The irony is that modern medical research often suffers from both deficiencies: ultra-narrow conditions that are at the same time poorly characterized. Upon detailed scrutiny, many such studies can only be said to report experimental conditions that are “precisely unknown”.

Complex organisms live in a universe of fluctuations and gradients, and too high a level of monotonic uniformity can (and often does) lead swiftly to physiologic habituation. And magnetic fields are characteristically non-uniform, usually dropping off sharply at a rate inversely proportional to $r^3$ or $r^4$, whereas point sources of light such as a candle, with which people have a generally better intuition, fall off only at the inverse of $r^2$. But this is not how magnetic fields behave. Therefore, it is also especially impractical to expect uniform magnetic fields over any significant volume of space.

Nonetheless, there are cases where uniform or nearly-uniform magnetic fields are of practical and
scientific use. For the unique case of studies of the basic underlying mechanisms on cells in vitro, the elucidation of the underlying biophysical mechanisms of PEMF pulses, specifically the biophysical transduction of these pulses, should be conducted under conditions where the precise magnetic field of interest is known and controlled over a very small volume, where cells or molecules are being subjected to these fields and their biophysical responses are being studied and quantified, and the cells themselves have limited biological variability. Under these unique conditions, the use of uniform magnetic fields is scientifically advantageous. And for this purpose, the variation of this generic system design is offered in the latter half of this report, to describe a means by which cell chambers or Petri dishes could easily have the entire volume of fluid and cells subjected to short- or long-term PEMF, with a highly uniform and well-characterized field throughout the sample chamber. The use of the basic system architecture described above, but with coils arranged in a Helmholtz coil configuration, is easy to configure and directly results in such uniform conditions.

Also, in the case where cell or tissue samples are to be maintained in planar arrays, such as a flat array of Petri dishes, the simpler configuration described above can be used (and has been used, as in Dennis 2019a in the experiments carried out at U-Michigan). For example, an array of 35 mm diameter plastic Petri dishes fits perfectly, one dish over each coil, in the arrays illustrated in Figures 4 and 5, where a simple acrylic plate can be cut with large holes to orient each petri dish over each individual coil. The arrays described above are very low power (2.1 Amps or less from a USB charging port) and moisture tolerant and have worked well inside a cell culture incubator environment, for many months at a time, often working for several years continuously without failure.

Summary

We present the basic architecture of a PEMF system which can be adapted to laboratory animals (rats and mice) housed in standard laboratory micro-isolation cages, as well as variations of the system for use with biofluids, small tissue and cell samples, plants and seeds, and on a microscope. The basic system parameters have been specified, allowing the L-C-R circuit of the PEMF driver to be tuned appropriately. The system described above is readily adapted a wide range of experimental conditions.

Statement of Potential Conflict of Interest

The author of this report declares both a scientific and a commercial interest in ICES®-PEMF technology: He is owner of Micro-Pulse LLC (manufacturer of the technology), holds several patents for ICES®-PEMF technology [25-27] and receives royalty payments from NASA-Johnson Space Center for the commercial licensing of this technology, which he developed in its initial form (TVEMF) as a consultant for NASA in the mid-1990’s [18,19].

References


