

BLOCKADE OF SENSORY NEURON ACTION POTENTIALS BY A STATIC MAGNETIC FIELD in the 10 mT RANGE

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ABSTRACT

To characterize the inhibitory effect of a static magnetic field, action potentials (AP) were elicited by intracellular application of 1 ms depolarizing current pulses of constant amplitude to the somata of adult mouse dorsal root ganglion neurons in monolayer dissociated cell culture. During the control period, <5% of stimuli failed to elicit AP. During exposure to a ~11 mT static magnetic field at the cell position produced by an array of four permanent center-charged neodymium magnets of alternating polarity (MAG-4A), 66% of stimuli failed to elicit AP. The number of failures was maximal after about 200-250 s in the field and returned gradually to baseline over 400-600 s. A direct or indirect effect on the conformation of AP generating sodium channels could account for these results because (a.) failure was preceded often by reduction of maximal rate of rise, an indirect measure of sodium current; (b.) recovery was significantly prolonged in more than half of neurons which were not stimulated during exposure to the MAG-4A field; and, (c.) resting membrane potential, input resistance and chronaxie were unaffected by the field.

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The effect was diminished or prevented by moving the MAG-4A array along the X or Z axis away from the neuron under study and by increasing the distance between magnets in the X Y plane. Reduction of AP firing during exposure to the ~0.1 mT field produced by a MAG-4A array of micromagnets was about the same as that produced by a MAG-4A array of the large magnets above. The ~28 mT field produced at cell position by two magnets of alternating polarity and the ~88 mT field produced by a single magnet had no significant effect on AP firing. These findings suggest that field strength alone cannot account for AP blockade.

Key words: static magnetic fields, magnetic field gradient, adult mammalian neurons in vitro, action potentials, electrophysiology.

INTRODUCTION

Variable effects of static magnetic fields on electrophysiological properties of neural tissue have been reported. A 660 mT field reduced spontaneous firing of action potentials of cockroach subesophageal ganglion neurons (Sittler, 1966). The amplitude of the compound action potential of frog sciatic nerve increased in a perpendicular, but not a parallel, field of 100-712 mT (Edelman et al., 1979). Also, amplitude of the compound action potential of rat tail nerve increased in a field >0.5 T for longer than 30 s (Hong et al., 1986). In a study employing intracellular microelectrode recording techniques, input resistance of inactive identified snail neurons was reduced significantly by static fields of 23-200 mT field; input resistance increased in spontaneously firing neurons exposed to similar fields (Balaban et al., 1990). On the other hand, action potentials and voltage-clamped transmembrane currents of lobster nerve were unaffected by a perpendicular or parallel 1.2 T field (Schwartz, 1979). Technical aspects, such as warming of preparations by heat radiating from electromagnets, could have resulted in falsely positive results, as pointed out by Gaffey and Tenforde (1983).

Calcium flux has been altered at the neuromuscular junction of the mouse by a 120 mT field (Rosen, 1992). In addition, the decrease in amplitude and variability of cat visual evoked responses in a 120 mT field began after a latency of about a minute and persisted for several minutes after the electromagnet was turned off (Rosen and Lubowsky, 1987). Consistent with these results showing an inhibitory effect of static fields on excitable tissues, we have shown reduction of action potential firing by cultured adult mouse dorsal root ganglion neurons positioned near the center of 10-30 mT static fields produced by arrays of four permanent center-charged magnets of alternating polarity (abbreviated here MAG-4A; McLean et al., 1991). This array had previously been shown to have analgesic effects in patients (Holcomb et al., 1991a).

Using cultured sensory neurons, we have determined several features of the biological effect of the magnetic field (McLean et al., 1991). Maximal reduction of firing in the MAG-4A field required several minutes to evolve and recovery of action potentials also occurred over minutes after removal of the array. Although significant, the effect was variable and depended on cell position, reflecting the geometry of the field. No effect was seen on some neurons. Also, the effect was temperature-dependent (McLean et al., 1991; Holcomb et al., 1991 b). Other arrays had different or no effects. Action potentials failed to the same extent in the fields produced by arrays of four magnets of like polarity, but reappeared in seconds after removal of the magnets. A single magnet or two magnets of alternating polarity had no significant effect. We found no prior reports of effects of fields produced by arrays of permanent magnets on mammalian neurons in vitro with which to compare our findings. The present work was undertaken to increase understanding of biologically active magnetic field characteristics and optimize the inhibitory effect seen clinically. We set out to determine (a.) ways to maximize and destroy the effect on action potential firing, (b.) the effect of varying the field around the neuron under study; and, (c.) characteristics of the field responsible for the cellular effect.

METHODS

A. Cell culture methods. The culture and recording methods were published previously (McLean et al., 1988). Briefly, dorsal root ganglia were removed under sterile conditions from adult mice, boarded and sacrificed by methods approved by the Vanderbilt University Animal Care Committee in accordance with provisions of the DHEW Guide for the Care and Use of Laboratory Animals. The ganglia were minced finely and incubated in Eagle's Minimal Essential Medium (MEM) containing 0.1 mg of crude collagenase and 1 mg trypsin per ml for 45-60 minutes at 37 °C. After centrifugation, the pellet was resuspended in culture medium (consisting of 50% (v/v) Eagle's Minimum Essential Medium + 50% Hank's balanced salt solution, supplemented with 1.5 g of dextrose and 0.75 g of NaHCO₃ per 500 ml, 5 ml% heat-inactivated horse serum, 5 ml% fetal calf serum, 1 ml% Nu-Serum and 10 ng/ml of nerve growth factor) and triturated to single cells and small clumps. Aliquots of cell suspension were placed in collagen-coated dishes and maintained in an incubator gassed with 90% room air and 10% CO₂ at 35°C for up to 6 months prior to experimentation. After 1 week, fluorodeoxyuridine (0.5 µg/ml) was added to the medium for 1-2 days to suppress growth of rapidly-dividing, non-neuronal cells. Thereafter, culture medium was changed twice weekly.

B. Intracellular recording. Intracellular electrophysiological recordings were obtained from neurons in a static culture bath or during superfusion (1 ml/min) with modified Dulbecco's phosphate-buffered saline (composition in millimolar: NaCl 143.4; KCl 4.2; CaCl₂ 0.8; MgCl₂ 3.0; and glucose 11 in 9.5 mM sodium phosphate buffer at pH 7.40; pO₂ 190 Tor) or Tyrode's bicarbonate buffered salt solution (composition in mM: 137.0 NaCl; 0.5-2.7 KCl; 0.8 CaCl₂; 3.0 MgCl₂; 0.03 NaHPO₄; 26.0 NaHCO₃; 5.6 glucose; pH maintained by bubbling with 5% CO₂ plus 95% oxygen; pO₂ 340 Tor) at 37°C. The temperature-controlled bath was mounted on the stage of an inverted phase-contrast microscope. The microscope and manipulators were fixed to a stainless steel top of a Micro-g vibration-isolation table (Technical Manufacturing Corporation, Peabody, MA). The table top was isolated from room vibration by air pistons. No vibration of a microelectrode tip was seen at 400x magnification. Use of a bridge amplifier allowed simultaneous measurement of transmembrane voltage and passage of polarizing current through the microelectrodes (>45 MΩ, filled with 3 M potassium acetate). Data was stored on video tape after digitization by a modified audio processor for later analysis and photography.

The rise time of action potentials of the different subtypes of dorsal root ganglion neurons studied here depended on external sodium concentration (see McLean et al., 1988). Membrane potential was differentiated electronically with respect to time (-dV/dt displayed in figures). The maximal rate of rise (V_{max}) of action potentials was proportional to the peak of the differentiated trace and was used as a qualitative assay of sodium currents generating the upstroke of the action potentials.

Lack of effects of magnetic fields on the recording apparatus was indicated by absence of changes in bridge balance and tip potential during 1 Hz stimulation with

the electrode positioned extracellularly near the bottom of the experimental chamber for ten minutes in the magnetic field.

C. Experimental Procedure. The resting potential of an impaled neuron was observed until more negative than -50 mV and stable, i.e. not fluctuating visibly over several minutes. Then, stimulus intensity was increased until less than 10% of stimuli failed to elicit action potentials. If more failures occurred, the intensity was increased or another neuron was impaled. After control recordings, the intensity was kept constant for the rest of the experiment. Then, the neuron was exposed to a magnetic field produced by one or more arrays of magnets mounted on a post on the ocular nosepiece beneath the stage of the inverted phase contrast microscope immediately before rotation into position 5 mm from the bottom of the culture dish.

Positioning required 15-60 sec, thereby averting rapid induction of current in the preparation. The neuron under study was located near the edge of the central hole in the MAG-4A and MAG-2A arrays. The cell position was 5.0 ± 1.5 mm off center (measurement made after positioning array; N=20 trials) in the recess between two magnets of opposite polarity. Cell location was near the pole of a single magnet. The fields varied as much as 15% in the course of removing and replacing the arrays between measurements. For the recovery periods, the nosepiece was rotated and the array of magnets was removed. During baseline recordings and recovery periods, magnets were kept at least 50 cm from the microscope.

D. Magnets and arrays: Arrays were assembled using permanent, cylindrical, center-charged, neodymium magnets (total of 4 magnets and/or non-magnetic blanks, 14 mm in diameter and 5 mm in height) in a 1 mm thick molded plastic case. At least 16 MAG-4A arrays were used for these experiments. Fields measured at the 48 magnetic poles in twelve arrays ranged from 40-90 mT (average 71 mT) at the surface of the case. Variability within an array was substantial. In twelve of the sixteen arrays, the field at the surface of the case (1 mm thick) over one pole differed from the average field of all four poles in a single array by < 10% in 2, <20% in 1, <30% in 5, <40% in 2 and <50% in 2. No reduction of action potential firing was observed if the neuron was positioned between two magnets of an array, with one less than half the strength of the other. Magnetic poles were frequently off center. The effect of this variable on action potential firing was not tested systematically. For some experiments, four magnets 0.4 mm in diameter and 1 mm in height were assembled into arrays with alternating polarity (MAG-4A MICRO, or MAG-4A microarray). A single magnet of this type had a maximum field at cell height (1.5 mm over the array) of about 0.14 mT (measurement limited by spatial averaging of the gaussmeter probe). The MAG-4A microarray was housed in plastic tubing and mounted on an objective of the microscope for positioning.

Different cases allowed mounting the magnets at different interpole distance (the distance between two magnets defining the side of a square connecting the centers of the four magnets in the array). Except in experiments in which interpole distance was varied, the magnets were in contact at an interpole distance of 14 mm around a central hole. The field in the center of the hole did not differ from background (accuracy of gaussmeter probe 0.01 mT).

E. Protocol for magnet experiments. Neurons were stimulated at 1 Hz throughout the experiment, unless noted otherwise, with 1 ms depolarizing current pulses of constant amplitude applied through the recording microelectrode using a bridge circuit in the amplifier. Each stimulus elicited or failed to elicit an action potential. The number of failures per 50 stimuli, or 50 s, was counted. If more than 5 failures occurred per 50 stimuli, pulse amplitude was increased and the control period was restarted. A group of 50 stimuli defined a bin, or study period. Two study periods (C1 and C2; 100 stimuli or 100 s) were recorded to determine baseline (control, pretreatment) failure rate and stability of the recording. Next, the neuron was exposed for a designated number of periods, usually four (M1 -M4), to the magnetic field produced by the selected array of magnets. The number of stimuli which failed to elicit action potentials was counted per period. Then, the magnetic array was removed and four or more recovery periods (e.g., R1 -R4) were counted to determine reversibility.

F. Criteria for stability of recordings and acceptability of data. Stability of resting potential, reversibility of magnetic field effects and similarity of results obtained in both phosphate and bicarbonate buffer were taken as signs of neuronal viability under the represent experimental conditions.

Three criteria were required for data from a given neuron to be accepted for analysis. (1.) Resting potential (E_m) stability was monitored throughout experiments. Initial E_m ranged between -50 to -60 mV (see results for values during different groups of experiments). Different experiments lasted from 15 min to 4 hrs. Data were excluded if E_m of a given neuron changed more than 5 mV, or if the neuron died before completion of four recovery periods. Death was recognized by loss of action potential amplitude and reduction of membrane potential to zero. That is, for data to be acceptable, the resting potential was allowed to vary less than 10% before, during and after exposure of a neuron to the magnetic field(s). Even though E_m was more likely to change during longer experiments, only data obtained at a potential changing less than 5 mV during an experiment were analyzed. (2.) If undershooting action potentials appeared suddenly, with or without immediate change in E_m , the neuron was deemed unstable and a new penetration was obtained. (3.) If the number of stimuli which failed to fire action potentials increased during exposure to a magnetic field (e.g. to 85 of 100 stimuli), return to the baseline number of failures (e.g., 2 per 100 stimuli) was required. That is, increased failures were deemed to result from exposure to the field only if the experiment was reversible. Data analyzed here were from 941 neurons in which penetrations were deemed to be stable. Experiments were begun in about 3,000 others, but data were rejected because of instability of E_m , irreversibility, or death.

This approach avoided confusing voltage-sensitive changes in neuronal properties with response to the magnetic field. Also, each neuron served as its own control and statistical significance of data from relatively small groups of neurons could be tested.

G. Field measurements and experimental error: Magnetic fields were measured with a Model 4048 gaussmeter (F.W. Bell; Orlando, FL). Fields at the cell position in the experimental apparatus varied less than 10% from values obtained outside the rig. For characterization of fields of the arrays, the unidirectional probe (sensing window of 0.5 mm diameter) was mounted on an XYZ micromanipulator and the tip simulated the position of the neuron under study.

To characterize the field produced by the MAG-4A array, the field was measured with respect to the "cell position" (allowing 1 mm for the plastic case and 5 mm for the usual distance from the neuron under study, $x=5$ mm, $y=0$ mm, $z=6$ mm) at which action potential blockade was observed at 1 mm increments to ± 27 mm in the X and Y planes and +27 mm in the Z axis. Three field components (B_x , B_y and B_z) were measured, each with respect to all three axes, e.g., b_{xx} , b_{xy} and b_{xz} . In this notation, the first subscript labels the particular field component and the second labels the direction of scanning. Accuracy of the manipulator micrometer was about 0.1 mm. Alignment of the coordinates of the array and of the manipulator system deviated as much as 1 degree. This could account for errors of 0.3 mm/scanned cm and could result in errors of up to 15 mT/scanned mm, not surpassing 30% of the total field at a particular location, depending on the gradient of the fields. Due to alignment error, our measurements are most accurate near the position of the neuron under study and error increases with distance at the outer edges of the field. Errors shown in table 5 include 10% error for reading the micrometers on the manipulator and error due to angular deviation. The window of the Hall effect probe (0.5 mm diameter) was large relative to the size of the neuronal soma (20-50 μ m).

G. Statistics. Data are plotted as means \pm 1 SEM. The numbers of failures during periods of magnetic field exposure were compared with baseline failures before magnetic field exposure and after recovery and between treatments (e.g., different magnetic arrays or distances) during the exposure periods. Significance was determined with the Wilcoxon signed rank test (with and without Bonferroni correction for baseline failures). Student's T-test was used to compare values of R_{in} , E_m and .

RESULTS

A. Characterization of the MAG-4A field: Measurements of the field of the MAG-4A array in three dimensions (B_x , B_y and B_z ; each analyzed from three perspectives) with respect to the cell position (5 mm off center along the X axis and 6 mm from the magnets along the Z axis) are presented graphically in Figures 1-3 (top rows). The change of field strength per mm is indicated in Figures 1-3 (bottom rows). At $Z = 6$ mm above the MAG-4A array, the maximum 1 mm horizontal variation resulted in a decline in field strength of ~20%. The decline was ~6% for the MAG-2A array and ~1 % for the single magnet.

B. Action potentials of sensory neurons: Two populations of neurons were identifiable on the basis of action potential characteristics (described previously in McLean et al., 1988) as shown in Figures 4: A-like neurons fired action potentials of less than 2 ms duration at the takeoff potential; C-like neurons fired action potentials of greater than 2 ms duration with a hump on the repolarizing phase. Neurons were quiescent until stimulated (see Methods). During control recordings, there were few or no failures of action potential firing in response to stimulation at 1 Hz (Figure 1, CONTROL). During superfusion with phosphate or bicarbonate buffer, more than 50% of stimuli of constant amplitude failed to elicit action potentials in each of 493 neurons (A-like: 63 in Tyrode's solution and 121 in mDPBS; C-like 83 in Tyrode's solution and 226 in mDPBS; Figure 1, MAGNET) positioned off-center in the magnetic field produced by MAG-4A at a distance of 5 mm from the neuron under study (Z field = 10.0 ± 0.8 mT, N = 20). The effect was reversible (Figure 1, RECOVERY).

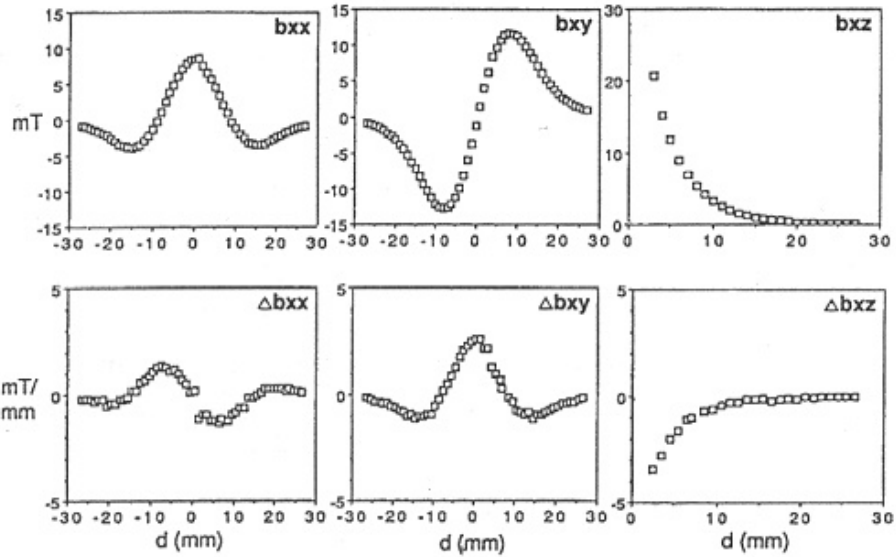


Figure 1: Characterization of the MAG-4A field in the X plane. Graphs prepared from gaussmeter measurements show field strength plotted against distance in three directions (bx with respect to x,y, and z; top row) from the cell position ($x=y=z=0$) and the change of field strength per millimeter (Δbx with respect to x, y and z; bottom row) in each direction. Axes of the Z component are different because it decreased monotonically with distance from the surface facing the neuron under study.

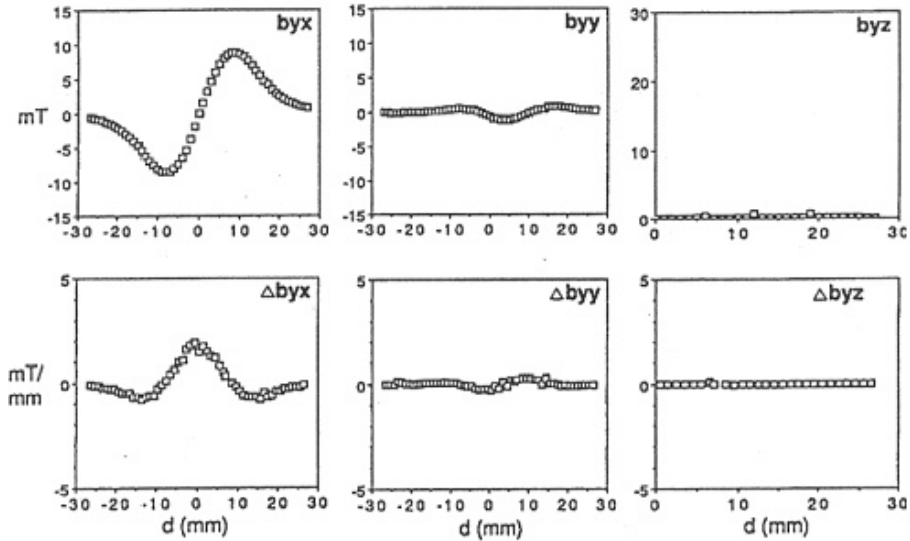


Figure 2: Characterization of the MAG-4A field in the Y plane. As in fig. 1.

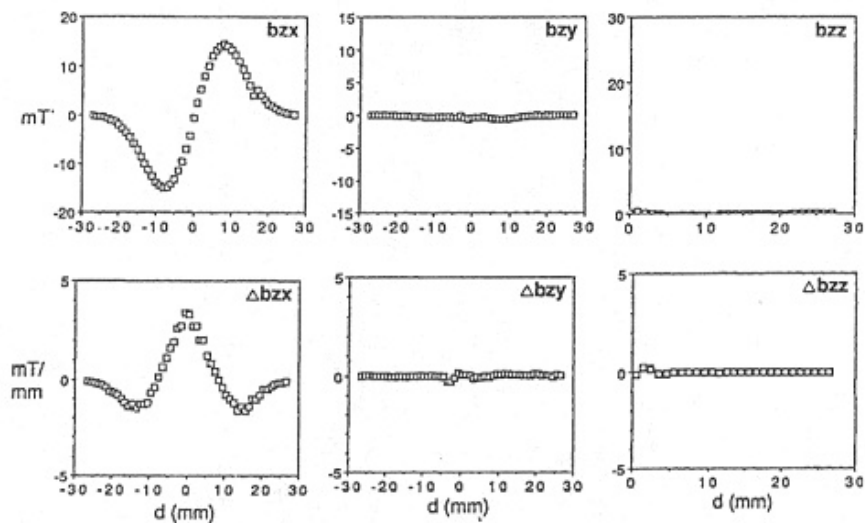


Figure 3: Characterization of the MAG-4A field in the Z plane. As in fig. 1.

Figure 5 shows graphically the results from 8 A-like and 12 C-like neurons exposed to both MAG-4A and MAG-2A arrays sequentially using the protocol described in Methods section E. Order of exposure was varied and 15-20 min was allowed between exposures. For both types of neurons, the maximal number of failures per study period (50 stimuli at 1 Hz, or 50 s) during exposure to the field produced by MAG-4A was significantly more than baseline failures before and after. Exposure

to the MAG-2A field did not increase failures significantly. Maximal reduction of action potentials fired by both A-like and C-like neurons was not significantly different ($p > 0.05$). Also, maximal reduction of firing by both types of neurons in the MAG-4A field did not differ significantly in the two buffers ($p > 0.05$). After removal of the array of magnets, gradual recovery to baseline failure rate occurred over 400 s. Reversibility of the magnetic field effect demonstrates the viability of neurons under study.

In a static bath, there were significantly more failures to fire than during baseline recordings before and after exposure to the magnetic field (1.2 ± 0.5 , 31.1 ± 3.3 and 2.2 ± 0.6 failures during periods C2, M4 and R4, respectively; $N=8$; failures during M4 significantly greater than C2 and R4, $p < 0.02$). The maximal number of failures in the static bath during M4-R1 did not differ significantly from the number observed during superfusion ($p > 0.05$). This suggests that there was no significant interaction between a weak field produced by the flowing ionic solution and that produced by the MAG-4A array.

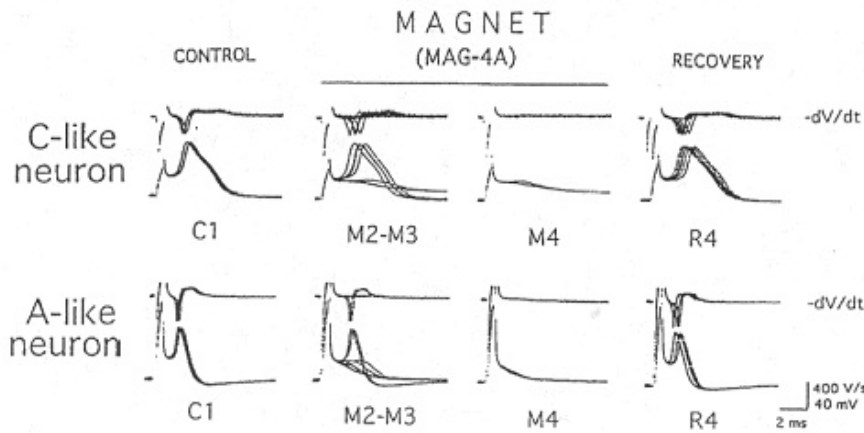


Figure 4: Reduction of action potential firing by A- and C-like sensory neurons exposed in vitro to magnetic fields produced by an array of four permanent magnets of alternating polarity (MAG-4A). Each row shows several traces superimposed from the same neuron at different times. Action potentials were elicited by 1 ms depolarizing current pulses applied through the recording electrode at 1 Hz; current amplitude was not changed during an experiment. In the control study periods (C1-C2; 50 stimuli or 50 s per study period), there were no failures to fire. The number of stimuli which failed to elicit action potentials increased during the four periods of exposure to the MAG-4A array (M1-M4). After removal of the array, action potentials reappeared gradually over 4 recovery periods (R1-R4). Phosphate buffer at 37°C. Top trace gives -dV/dt. Calibrations apply throughout.

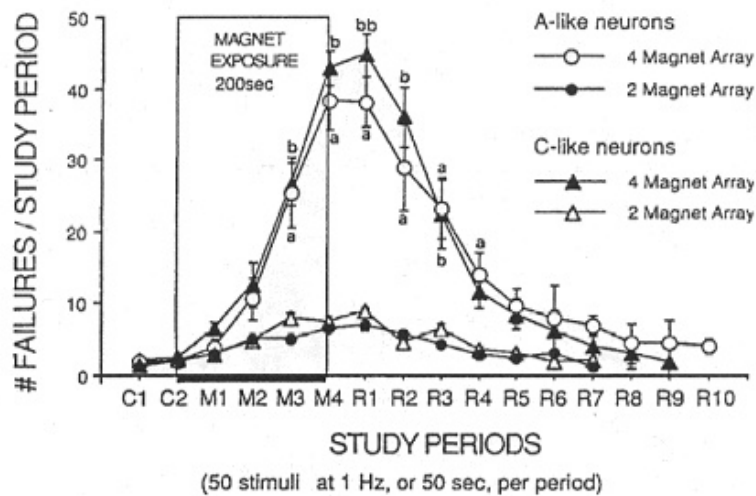


Figure 5: Effects on action potential firing of magnetic fields produced by arrays of two and four magnets of alternating polarity. Data are from 8 A- and 12 C-like neurons exposed sequentially (variable order of presentation; 15-20 min between arrays) to both arrays (symbols labeled on graph). **Ordinate:** number of stimuli failing to elicit action potentials per study period. **Abscissa:** Control (C1-2), magnetic exposure (M1-4) and recovery (R1-10) periods (50 stimuli at 1 Hz, or 50 s, per period). **Significance:** a, b = $p < 0.05$; bb = $p < 0.01$ (a for A-like neurons; b, bb for C-like neurons). Data from A-like neurons did not differ significantly from data from C-like neurons. Bicarbonate buffer at 37°C.

Table 1 shows results from a groups of neurons exposed to magnetic fields produced by different arrays of permanent magnets under the same conditions: (a.) 219 neurons exposed to the MAG-4A macroarray; (b.) 28 exposed to the MAG-4A microarray; (c.) 138 exposed to the MAG-2A array; and, (d.) 27 exposed to the field produced by a single centercharged magnet.

C. No stimulation during magnetic exposure: Fourteen neurons (6 A-like and 8 C-like) were exposed to the magnetic array for 200 s without stimulation, then they were stimulated for 50 s at 1 Hz to determine the number of action potentials fired before removal of the array. Recovery (all neurons were stimulated during the recovery periods) was complete by R4 in 6 neurons and was markedly prolonged (complete by R75) in 8 neurons (Fig. 6A). Recovery time for the eight neurons was significantly prolonged (1900 ± 23.3 s) compared to the six which recovered quickly (450 ± 32.6 s; $p < 0.001$). Figure 6B graphically compares through period R1 4 data pooled from the 14 unstimulated neurons with data from neurons shown in Figure 5 which were stimulated during exposure to the field. Peak reduction of firing of action potentials did not differ significantly under the two conditions ($p > 0.05$). In both cases, maximal failures differed significantly from baseline ($p < 0.05$). After period R11, the number of failures was significantly higher in the neurons not stimulated during exposure to the magnetic field ($p < 0.01$). These findings suggest that the maximal blockade could be elicited whether or not stimulation occurred during the magnetic exposure. Stimulation during exposure to the field markedly abbreviated the second phase of recovery following removal of the array.

D. Effect on excitability: In eleven neurons (6 C-like and 5 A-like), trains of 5 stimuli at control intensity elicited action potentials with constant maximal rate of rise (V_{max}) without failure (not shown). After 5 min of exposure to the MAG-4A field without stimulation, trains were delivered intermittently (1-2 min intervals between

trains). Action potential V_{max} decreased progressively and failures occurred by the fourth or fifth stimulus (Fig. 7 A1). After doubling (Fig. 7 A2) and quadrupling (Fig. 7 A3) the stimulus intensity, action potentials returned initially, but V_{max} decreased and failures occurred. Ten minutes after removal of the array, trains elicited action potentials without failure or reduction of V_{max} in all 11 neurons (not shown). Strength-duration curves were constructed from data obtained before, after 5 min exposure to the MAG-4A field and after removal of the array and maximal recovery. The curves superimposed (Fig. 7B).

Increased threshold, e.g. by changes in chloride conductance, could account for the decrease in action potential firing. Therefore, groups of neurons were exposed to solution containing 10^{-5} M IAA-94, a chloride channel blocker (Landry et al., 1987) or 100 mM sodium gluconate (equimolar substitution for NaCl) to reduce chloride concentration. Resting potential, input resistance and chronaxie of groups of A- and C-like neurons in control and IAA-94 containing buffer and of C-like neurons in low chloride buffer are shown in Table 2. Action potential waveform of A- and C-like neurons (N = 5 and 6, respectively) were prolonged by blockade of chloride conductance and gluconate substitution (N = 6 C-like neurons). Action potentials failed reversibly in both types of neurons by the fourth period of exposure to the MAG-4A ($p < 0.05$ vs. C2 and R5) field with or without increased stimulus intensity.

Table 1. Number of stimuli failing to elicit action potentials before, during and after exposure to steady magnetic fields produced by permanent magnets in different arrays. Data expressed in terms of number of failures per study period (50 stimuli at 1 Hz, or 50 sec, per study period). Neuron positioned 5mm off center and 5 mm from face of macroarrays; positioned near center and 1.8 mm from MAG-4A microarray (MAG-4A MICRO)

Array	mT at cell position	no. of neurons exposed	resting potential (mV)	No. of failures/study period				
				C2	M4	R1	R5	R10
1 MAG-4A	10.8 ± 0.8 (20)	219a	-54.4 ± 2.0	21. ± 0.2 (219)	33.1 ± 0.7 (219) ^{c-e}	31.7 ± 0.8 (219) ^{c-e}	10.1 ± 0.5 (218)	3.3 ± 0.2 (124)
2 MAG-4A MICRO	0.14 ± 0.03(6)	28	-60.8 ± 1.9	1.8 ± 0.3	31.6 ± 3.7 a,b,c	32.4 ± 4.1 a,b,c	8.4 ± 1.8	2.8 ± 0.6
3 MAG-2A	28.5 ± 3.0 (10)	138**	-53.8 ± 1.8	2.4 ± 0.2	8.1 ± 0.6	9.6 ± 0.8	2.3 ± 1.0	2.1 ± 0.3
4 MAG-1	87.6 ± 4.3 (10)	27	-57.4 ± 1.8	1.4 ± 0.2	4.7 ± 0.6	3.0 ± 0.4	2.6 ± 1.4	2.6 ± 0.7

*Data area expressed in terms of number of failures per study period (50 stimuli at 1 Hz, or 50 s, per study period). Neuron positioned 5 mm off center and 5 mm from face of macroarrays; positioned near center and 1.8 mm from MAG-4A microarray (MAG-4A MICRO).

aThe total included 1124 C-like (49 in bicarbonate and 75 in phosphate buffer) and 95 A-like (25 in bicarbonate and 70 in phosphate buffer) neurons.

bThe total included 99 C-like (28 in bicarbonate and 78 in phosphate buffer) and 39 A-like neurons (15 in bicarbonate and 24 in phosphate buffer).

c $P > 0.05$ for M4 (1) vs M4 (2), M4 (1 or 2) and R1 (1) vs. R1 (2)

d $P < 0.05$ for M4 vs. C1-2 and R8-10 (1 and 2, respectively); C1, R8-9 not shown.

e $P < 0.05$ for M4 of (1) and (2) vs. M4 of (3) and (4).

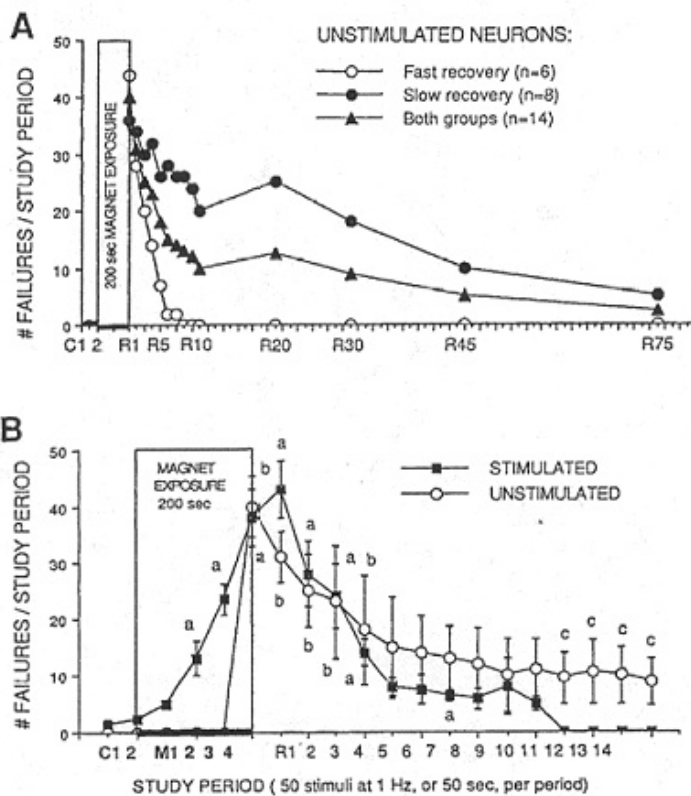


Figure 6: Recovery of action potentials depended on whether or not the neuron was stimulated during exposure to the magnetic field. **A:** Neurons (N=14) which were not stimulated during 200 s exposure to the MAG-4A field fired occasional action potentials when stimulated for 50 s at 1 Hz after removal of the array. Thereafter, recovery was biphasic in 8/14 neurons (closed circles) and for the group as a whole (closed triangles) with an early phase of 50% recovery by ~500 s and prolonged recovery of the group over 50 minutes. Recovery was more rapid in six others with nearly full recovery by 5 min. **Significance:** Data at the end of period M4 differed significantly from data at the end of C2 and R5 ($p < 0.01$). Data at the end of R4-25 differed significantly from C2 ($p < 0.05$). **B:** Comparison of data from neurons which were stimulated (closed squares; N=14) and unstimulated (open circles; N=6) during exposure to the magnetic field. Only the first 15 recovery

periods (750 s) are shown. **Significance:** a = $p < 0.05$ vs. C2 for stimulated neurons; b = $p < 0.05$ vs. C2 for unstimulated neurons; c = $p < 0.05$ vs. C2 for unstimulated neurons and vs. stimulated neurons at R11-14.

E. Effect of axial distance: The above experiments were performed with the MAG-4A array at 5 mm distance along the Z-axis from the cell under study. As the array was moved further away, the effect on action potentials diminished. Table 3 shows the number of failures in groups of 6 neurons exposed to the MAG-4A field for five (Table 3. 1) or ten (Table 3.2) periods at each of three axial distances. Order of exposures varied. These findings indicate that the biological effect depended on the distance of the magnetic array from the neuron under study.

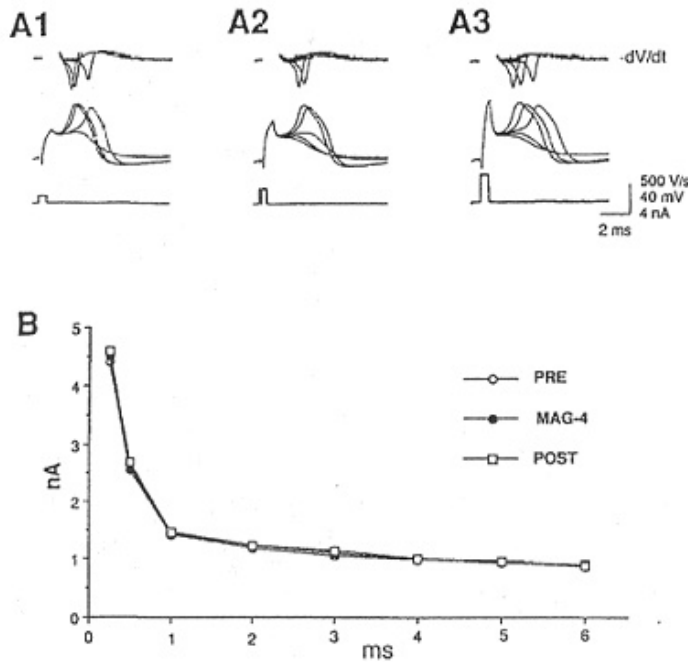


Figure 7: Effect of the MAG-4A field on excitability. **A1:** After 5 min exposure to MAG-4A, action potentials were elicited by 1 nA, 1 Hz stimulation. The fourth stimulus failed to elicit an action potential. **A-2:** Stimulus intensity increased to 2 nA; action potential failure occurred after third stimulus. **A3:** Stimulus intensity increased to 4 nA; failure occurred after fourth stimulus. Note decline in V_{max} before failure in A1 -3. Same neuron throughout. **B:** Strength-duration curves in same neuron before (open circles), after 5 min exposure to MAG-4A field (closed circles) and 5 min after removal of the array (open squares).

F. Effect of increasing interpole distance between magnet: In these experiments, the magnet array was placed 5 mm below the neuron under study in all cases. As the interpole distance (see Methods section D) was increased, the number of action potential failures during exposure to the MAG-4A array decreased (Table 4. 1). The number of failures did not differ from baseline at an interpole distance of 75 mm. These findings indicate that the magnetic field effect depends on the interaction between the magnets in the array and that interaction can be destroyed by separating the magnets in the XY plane.

G. Effect of shifting the array along the X-axis: In these experiments, the MAG-4A array was moved laterally along the X-axis at a Z-distance of 5 mm (Table 4.2). Initially, the array was placed with the neuron 5 mm off center and the neuron was not moved subsequently. The maximum number of failures during exposure to the MAG-4A field decreased at distances ≥ 13 mm and did not differ from baseline failures with lateral displacement ≥ 20 mm. These findings indicate that the biological effect is greatest toward the center of the array and does not occur beyond a critical lateral distance.

TABLE 2. Lack of Effect of the Magnetic Field Generated by MAG-4A on Resting Membrane Potential (E_m), Input Resistance (R_{in}), and Chronaxie (τ) of Neurons in Buffer Containing 10-5 M IAA-94, a Chloride Channel Antagonist, or Low Chloride Concentration (Sodium Gluconate Replacement)

	Before	MAG-4A	After
1. A-like neurons in control buffer (N = 21 for E _m and R _{in} ; 6 for σ)			
E _m (mV)	-58.9 ± 1.3	-59.5 ± 1.3	-58.9 ± 1.2
R _{in} (MΩ _i)	55.2 ± 3.1	55.2 ± 3.1	55.2 ± 3.1
σ (ms)	2.9 ± 0.3	3.0 ± 0.4	3.0 ± 0.4
2. C-like neurons in control buffer (N = 65 for E _m and R _{in} ; 6 for σ)			
E _m (mV)	-56.1 ± 1.0	-56.3 ± 1.0	-56.4 ± 1.0
R _{in} (MΩ _i)	61.4 ± 1.9	61.4 ± 1.9	61.4 ± 1.9
σ (ms)	1.5 ± 0.2	1.5 ± 0.1	1.4 ± 0.1
3. A-like neurons in buffer containing 10 ⁻⁵ M IAA-94 (N = 5)			
E _m (mV)	-60.7 ± 7.0	-58.4 ± 6.1	-57.2 ± 6.1
R _{in} (MΩ _i)	48.1 ± 10.1	48.1 ± 10.1	48.1 ± 10.1
σ (ms)	3.7 ± 0.6	3.9 ± 0.6	3.7 ± 0.6
4. C-like neurons in buffer containing 10 ⁻⁵ M IAA-94 (N = 6)			
E _m (mV)	-60.5 ± 2.5	-60.7 ± 2.2	-60.3 ± 2.1
R _{in} (MΩ _i)	46.4 ± 6.2	36.4 ± 6.2	46.4 ± 6.2
σ (ms)	1.24 ± 0.3	1.26 ± 0.3	1.20 ± 0.4
5. C-like neurons in low-chloride buffer (N = 6)			
E _m (mV)	-59.2 ± 5.2	-55.0 ± 4.1	-56.2 ± 3.7
R _{in} (MΩ _i)	40.5 ± 3.2	40.8 ± 3.3	41.0 ± 3.1
s (ms)	1.05 ± 0.3	1.06 ± 0.3	1.02 ± 0.3

DISCUSSION

The present experiments confirm our previous finding (McLean et al., 1991) that exposure to a static magnetic field of ~11 mT produced by the MAG-4A array reduced the ability of sensory neurons in cell culture to fire action potentials. The effect was time-dependent. Maximum blockade required 200-250 s and was gradual over 400-600 ms following removal of the magnetic array in both types of neurons studied. The timecourse of this effect parallels slow reduction of neurotransmitter release (Rosen, 1992) and prolongation of latency of cat visual evoked responses (Rosen and Lubowsky, 1987) in a 120 mT static field produced by an electromagnet and slow recovery of both after removal of the field. Effects shown here also occurred with slow onset and slow recovery. The maximum number of action potential failures was the same in fields produced by the MAG-4A macroarray (magnets of 7 mm radius; total field at the cell position, B_c, ~11 mT) and the MAG-4A microarray (magnets of 0.2 mm radius; B_c ~0.1 mT). Exposure to fields produced by two magnets of alternating polarity (B_c ~28 mT) and a single magnet (B_c ~88 mT), did not significantly reduce action potential firing. Thus, field strength in the range of 28-88 mT is insufficient alone to cause the observed biological effect.

TABLE 3. Number of Stimuli (1 Hz, 50 Stimuli or 50 s per Study Period) That Failed To Elicit Action Potentials During Exposure to the Steady Magnetic Field Produced by the MAG-4A Array Decreased With Increasing Axial Distance*

Axial distance (mm)	B* (mT)	Study periods					
		C2	M5	M10	R1	R5	R10
1. Exposure to magnetic field for five periods (250 s; E _m -53.3 ± 1.7 mV)							
5	10	1.2 ± 0.6	36.3 ± 7.6 ^b	—	27.5 ± 7.1 ^b	4.2 ± 1.9	—
30	0.2	1.6 ± 0.4	5.2 ± 0.4	—	4.0 ± 1.1	1.6 ± 0.4	—
59	0.02	1.2 ± 0.4	3.8 ± 1.0	—	0.8 ± 0.9	1.0 ± 0.6	—
2. Exposure to magnetic field for ten periods (550 s; E _m -55.6 ± 2.1 mV)							
5	10	2.0 ± 0.7	28.5 ± 4.7 ^b	39.7 ± 6.3 ^b	40.2 ± 5.6 ^c	25.7 ± 4.7 ^b	6.2 ± 1.5
30	0.2	1.7 ± 0.8	8.6 ± 1.9 ^b	14.0 ± 2.9 ^b	16.2 ± 2.4 ^b	5.6 ± 1.8	1.7 ± 0.8
59	0.02	1.7 ± 0.5	3.5 ± 1.2	4.7 ± 0.5	4.7 ± 1.2	3.0 ± 1.7	1.6 ± 1.1

*A group of six neurons was exposed to the MAG-4A magnetic array for five or ten periods at each of the three axial distances (random order), with 10 min between the last recovery period (R5 or R10) and recording of control periods for exposure to the next array. Resting potential did not change more than 3 mV throughout recording from a single neuron.

^bB is the total value of the field obtained by vectorial summation of the field components B_x, B_y, and B_z values plotted in Figures 1-3. Measurements subject to <10% error due to positioning of gaussmeter probe relative to the cell position.

^c^bP < 0.05 and P < 0.01, respectively, for M5, M10, or R1 vs. C2 and R5 (1) or R10 (2) at same distance and vs. M5, M10, or R1 at other two axial distances.

TABLE 4. Number of Stimuli (1 Hz, 50 stimuli, or 50 s per Study Period) That Failed To Elicit Action Potentials During Exposure to the Steady Magnetic Field Produced by the MAG-4A Array Varied With (1) Interpole Distance and (2) Lateral Displacement of the Array Beneath the Neuron Under Study

Distance	B (mT) ^a	E _m (mV)	Study periods				
			C2	M4	R1	R5	R10
1. Interpole distance (six neurons exposed to arrays with all four distances)							
14 mm	10	-59.8 ± 3.1	1.8 ± 0.9	36.6 ± 5.1	40.0 ± 4.9	10.4 ± 3.6	3.2 ± 1.4
		(25)	(25)	(25) ^b	(25) ^a	(25)	(12)
35 mm	4.7	-55.4 ± 2.5	0.7 ± 0.7	34.2 ± 7.4	36.7 ± 8.4	15.3 ± 7.9	1.6 ± 0.8
		(12)	(12)	(12) ^b	(12) ^b	(12) ^b	(4)
53 mm	1.4	-55.9 ± 1.7	1.5 ± 1.2	20.5 ± 6.6	14.8 ± 6.2	0.0 ± 0.0	—
		(6)	(6)	(6) ^b	(6) ^b	(6)	
75 mm	0.3	-57.4 ± 2.2	1.3 ± 0.8	4.9 ± 1.0	3.4 ± 1.4	0.2 ± 0.2	—
		(6)	(6)	(6)	(6)	(6)	
2. Lateral displacement from center (groups of eight neurons per distance)							
5 mm	10	-58.3 ± 2.8	2.4 ± 0.2	40.6 ± 2.2 ^a	42.3 ± 3.1 ^a	7.3 ± 1.6	5.2 ± 0.5
13 mm	7.6	-57.7 ± 3.3	1.5 ± 0.3	30.2 ± 6.6 ^b	28.7 ± 9.6 ^b	2.3 ± 1.0	0.6 ± 0.3
20 mm	2.5	-60.5 ± 3.8	1.9 ± 0.5	14.1 ± 4.3	6.8 ± 0.7	1.5 ± 0.3	1.3 ± 0.3
35 mm	0.2	-59.0 ± 3.1	1.8 ± 0.4	1.9 ± 0.2	1.4 ± 0.2	1.6 ± 0.4	1.6 ± 0.2

^aB is the total value of the field obtained by vectorial summation of the field components B_x, B_y, and B_z. Measurements subject to <10% error due to positioning of gaussmeter probe relative to the cell position.

^b^aP < 0.05 and P < 0.01, respectively, vs. C2 and R10 at same distance and vs. M4 and R1 at 75 mm (1) and M4 and R1 (2).

Plausible explanations for the effectiveness of the MAG-4A array include distortion and concentration of field lines at the edge of the central hole due to interactions between the magnets and a steep gradient between the center of the array and the magnetic poles. The field in the center of the large MAG-4A array is effectively zero (not different from background) and the distance to the poles with maximum field strength is short, indicative of a steep field gradient. To test whether gradient of the field or field strength was the principal determinant of the reduction of action potential firing, experiments were performed with the MAG-4A microarray which had ~1% of the field of the macroarray. The gradient of the field produced by the microarray was steep despite the low field strength because of the close proximity of the magnetic poles. The microarray reduced action potential firing as much as the macroarray. In contrast, the MAG-2A array and the single magnet had greater field strength, but had no significant effect on action potential firing. Fields produced by these two arrays had less variation at the cell position in the XY plane than with the MAG-4A array (see Results section G). On the other hand, the vertical variation of the fields was about the same for all three arrays. These findings suggest that the gradient of the field is a necessary determinant of the cellular effect of fields in the range of 10-90 mT. The relative importance of field characteristics is investigated in greater detail in a manuscript under review, in which fields produced with a current loop model closely fit those produced with gaussmeter measurements plotted in this paper. Computer-assisted three dimensional analysis supports the essential role of the field gradient.

The biological effect of the MAG-4A array was dependent on field variation. Results of the present experiments with the neuron visually positioned ~5 mm off-center in the recess between two magnets were more consistent than those of previous experiments in which the cell was placed as close to the center of the array as possible. Nevertheless, considerable variability in the number of action potential failures from neuron to neuron remained. This was most likely due to positioning of the magnets relative to the neuron under study and, perhaps to some extent, to variability in the strength of magnets in a given array. Even so, the effect was significant. Action potential failures in the MAG-4A field were decreased or abolished by moving the array to greater distances along the Z or X axis and by separating the magnets in the XY plane. The field gradient decreased as field strength decreased at the cell position with these maneuvers. Due to limits of instrumentation, we have modeled the MAG-4A field on the basis of the gaussmeter measurements and have applied the model to fields of several arrays (Cavopoli et al., unpublished). Steep gradients are not unique to the MAG-4A array we used here. We have seen significant action potential failures in several other arrays with steep gradients, including ring magnets (McLean et al., unpublished). However, large regions of the MAG-4A field have steep three-dimensional variation of the field components. The field of the MAG-2A array has domains of steep gradient, but not at the cell position, which was chosen to match that in the MAG-4A field. The Mag-1 field has even less gradient at the cell position in comparison with the other two arrays.

Some of the present findings afford insight into the mechanism of the cellular effect of the MAG-4A field. There was no significant effect of the field on resting membrane potential, input resistance or chronaxie. Action potentials failed despite increased stimulus strength (Fig. 4). This suggests that the effect was on action potential generating mechanisms, rather than other aspects of excitability, e.g. action potential threshold, as might have occurred due to altered chloride conductance. Both could contribute to the slow evolution of the block. Since both types of neurons studied here fired sodium-dependent action potentials (McLean et al., 1988) and V_{max} (an indirect reflection of inward sodium current) declined in the evolution of action potential blockade, the MAG-4A field could have produced conformational changes which limited availability of voltage-sensitive sodium channels involved in generation of the action potential upstrokes (Fig. 6A 1-3; see McLean et al., 1988). This is supported by the markedly slowed recovery of action potentials in more than half of neurons which were not stimulated during exposure to the field. This explanation remains speculative and we have no direct evidence to support it (see McLean et al., 1991). Conformational change has been suggested by others to explain time-dependent reduction of neurotransmitter release (Rosen 1992; Rosen and Lubowsky, 1990) and altered locomotion of Paramecia (Rosen and Rosen, 1990; Male, 1992) in magnetic fields. Alternatively, the field might alter lipoprotein interactions within neuronal membranes, or establish interfering flow patterns in the neuronal cytoplasm and near the outer membrane surface which reduce permeation of ions through activated channels (Cavopoli et al., 1993). Further experiments are needed to clarify cellular mechanisms of the field effect.

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REFERENCES

- Balaban PM, Bravarenko NI, Kuznetsov AN (1990). Influence of a stationary magnetic field on bioelectric properties of snail neurons. *Bioelectromagnetics* 11: 13-25.
- Cavopoli AV, McLean MJ, Holcomb RR (1993). An explanatory mechanism for blockade of action potentials in neural cells by external magnetic fields. Abstract A-1-6, Fifteenth Annual Meeting of the Bioelectromagnetics Society.
- Edelman A, Teulon J, Puchalska IB (1979). Influence of the magnetic fields on frog sciatic nerve. *Biochem Biophys Res Comm* 91:118-122.
- Florig KH (1992). Containing the costs of the EMF problem. *Science* 257:468-492.
- Gaffey CT, Tenforde TS (1983). Bioelectric properties of frog sciatic nerves during exposure to stationary magnetic fields. *Radiat Environ Biophys* 22:61-73.
- Holcomb RR, Parker RA and Harrison MS (1991 a). Biomagnetics in the treatment of human pain - past, present, future. *Environmental Med* 8: 24-30.
- Holcomb RR, Wamil AW, Pickett JD and McLean MJ (1991 b): Temperature dependence of magnetic field effects on subtypes of sensory neurons in vitro. *Soc Neurosci Abs* 17: 1523.
- Hong C-Z, Harmon D, Yu J (1986). Static magnetic field influence on rat tail nerve function. *Arch Phys Med Rehab* 67:746-749.
- Landau L and Lifschitz E (1969). "Electromagnetics of Continuous Media," Moscow, CIS: Mir, p. 168.
- Landry DW, Reitman E, Cragoe EJ Jr., Al-Awqati Q (1987). Epithelial chloride channel. Development of inhibitory ligands. *J Gen Physiol* 90:779-798.

Male J (1992). Biological effects of magnetic fields: a possible mechanism? *Biologist* 39:87-89.

McLean MJ, Bennett PB, Thomas RM (1988). Subtypes of dorsal root ganglion neurons based on different inward currents as measured by whole-cell voltage clamp. *Molec Cell Biochem* 80:9 5- 107.

McLean MJ, Holcolmb RR, Wamil AW and Pickett JD (1991): Effects of steady magnetic fields on action potentials of sensory neurons in vitro. *Environmental Med* 8: 36-45.

Rosen AD (1992). Magnetic field influence on acetylcholine release at the neuromuscular junction. *Am J Physiol* 262(Cell Physiol. 31): C1418-C1422.

Rosen AD and Lubowsky J (1987). Magnetic field influence on central nervous system function. *Exp Neurol* 95: 679-687.

Rosen AD and Lubowsky J (1990). Modification of spontaneous unit discharge in the lateral geniculate body by a magnetic field. *Exp Neurol* 108:261-265. 1

Rosen MS and Rosen AD (1990). Magnetic field influence on paramecium motility. *Life Sci* 46: 1509-1515.

Schwartz J-L (1979). Influence of a constant magnetic field on nervous tissues: 11. Voltage-clamp studies. *IEEE Trans Biomed Engin* BME-26(4):238-243.

Sittler OD (1966). 111 International Biomagnetics Symposium, Chicago, 34-36.

