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Research Article

Resting potential of excitable neuroblastoma cells in weak magnetic fields

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Abstract. The mechanism by which static and low-frequency magnetic fields are transduced into biological signals responsible for reported effects on brain electrical activity is not yet ascertained. To test the hypothesis that fields can cause a subthreshold change in the resting membrane potential of excitable cells, we measured changes in transmembrane current under voltage clamp produced in SH-SY5Y neuroblastoma cells, using the patch-clamp method in the whole-cell configuration. In separate experiments, cells were exposed to static fields of 1, 5, and 75 G, to time-varying fields of 1 and 5 G, and to combined static and time-varying fields tuned for resonance of Na^+, K^+, Ca^{2+}, or H^+. To increase sensitivity, measurements were made on cells connected by gap junctions. For each cell, the effect of the field was evaluated on the basis of 100 trials consisting of a 5-s exposure immediately followed by a 5-s control period. In each experiment, the field had no discernible effect on the transmembrane current in the vicinity of zero current (-50 mV voltage clamp). The sensitivity of the measuring system was such that we would have detected a current corresponding to a change in membrane potential as small as 38 μV. Consequently, if sensitivity of mammalian cells to magnetic fields is mediated by subthreshold changes in membrane potential, as in sensory transduction of sound, light, and other stimuli, then the ion channels responsible for the putative changes are probably present only in specialized sensory neurons or neuroepithelial cells. A change in transmembrane potential in response to magnetic fields is not a general property of excitable cells in culture.

Key words. Neuron; magnetic field; transduction; membrane potential; membrane current; ion resonance.

Exposure to static and low-frequency magnetic fields of the order of 1 G causes changes in brain electrical activity in animals and human subjects [1–6]. The anatomical location and biochemical nature of the receptor system pertinent to these changes are unknown. Several lines of evidence suggested to us that the effects of fields on brain activity were evoked responses similar to those produced as a result of sensory transduction. First, activity changes occurred within 2 s of presentation of the field [1–3]. The rapidity of the response is more consistent with the idea that electromagnetic field (EMF) transduction originated within the nervous system rather than in, for example, connective tissue or the immune system. Second, the fields produced changes in spectral power at specific frequencies within the electroencephalogram, similar to changes produced by light [3, 7]. The occurrence of a common effect due to diverse stimuli indicates that the pathways merged, possibly at the level of the afferent signal. Third, fields as weak as the geomagnetic field are capable of altering brain activ-

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ity [3, 5]. High sensitivity to external stimuli is a property of the sensory system. Fourth, several species of fish possess a sensory transduction system capable of detecting weak EMFs [8]. The cells that mediate EMF detection in fishes have no known specialized histological or biochemical characteristics [9]. Therefore, one or more kinds of mammalian cells could contain the presently undetermined receptors that are responsible for the functional characteristics of fish electrosensory cells.

If magnetic fields are transduced by mammals in a manner similar to the transduction of other sensory stimuli, then fields must trigger one or more of the processes that mediate sensory transduction. The biophysical basis of the initial stages of sensory transduction depend greatly on the nature of the stimulus, but a subthreshold change in membrane potential resulting from a change in mean channel conductance mediates all forms of sensory transduction [10]. The effects of EMFs on membrane potential have been studied [11–14], but EMF-induced changes of less than about 1000 μV could not be detected because the methods of measurement were relatively insensitive. We developed a sensitive measurement method and studied the effect of magnetic fields on the cell resting membrane potential as a means of testing the sensory-transduction hypothesis.

Materials and methods

Cells. The human post-ganglionic neuroblastoma cell line SH-SY5Y (5Y) was used [15]. Cells were seeded into 35-mm petri dishes (10^4 cells) and grown at 37 °C, 5% CO_2 in F12 medium with 10% fetal calf serum (Gibco). Dibutyryl cAMP (50 μM, Sigma) was added to the growth medium to produce the electrically excitable phenotype [16].

Electrical measurements. The cells were studied using the patch-clamp technique in the whole-cell configuration [17]. Microelectrodes (7–9 M in bath solution) were made from borosilicate glass capillaries (1 mm in diameter) pulled in two steps (PB-7, Narishige) and fire-polished in a microforge (MF-9, Narishige). The composition of the pipette solution was (in mM) 155 K-aspartate (monopotassium salt), 4 NaCl, 1 EGTA, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-KOH, pH 7.2. The composition of the bath solution was (in mM) 145 NaCl, 5.4 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-NaOH, 5 glucose, pH 7.3. All electrical measurements were made in bath solution at 25 °C. In some experiments, acetylcholine (Sigma), the K^+ channel blocker tetraethyl-ammonium chloride (TEA) (Sigma), or the Na^+ channel blocker tetrodotoxin (TTX) (Calbiochem) was added to the bath solution. Gigaseals (~ 10 G) were formed under negative pressure (5–10 cm H_2O), typically within 0.5–5 min; the success rate was greater than 50%. After gigaseal formation, a zap voltage pulse was applied to the electrode to obtain the whole-cell configuration. Then, the negative pressure was removed, and the gigaseals usually remained stable for more than an hour. Series resistance compensation was not employed because the changes in total resistance during each measurement were too small to affect the precision of our current measurements.

Current–voltage (I–V) curves, transmembrane current under voltage clamp, and membrane potential (measured as the potential at zero-current clamp) were recorded using a patch-clamp amplifier (Axopatch 200B) and interface (Digidata 1200). Commercial software (pCLAMP 6) was used to control the amplifier, and collect and analyze the data. For measurement of I–V curves, voltages of -80 to 0 mV were applied in 10-mV steps, and the resulting steady-state currents were measured. To document excitability, cells were clamped at zero current and stimulated with successive current steps of 10–120 pA in increments of 10 pA and shown to respond with a fast membrane potential above 0 V. The result was identical to that obtained earlier [16]. The presence of gap junctions was evaluated by applying a 2-mV step to the electrode and measuring the time constant of the transient current [18]. The occurrence of long current decay was interpreted to show the presence of cell coupling by gap junctions.

The ability of the 5Y cells to respond to exogenous agents was established in several ways. When TEA or acetylcholine was added to the bath solution after patch formation (final concentration 100 mM and 100 μM, respectively), we routinely observed decreased current (in voltage clamp) and a membrane depolarization (zero current clamp). Addition of TTX and TEA blocked the Na^+ and K^+ currents, respectively, in the action potential.

Magnetic fields. Vertical magnetic fields were produced using a coil (average diameter, 12 cm) made from 907 turns of 18-gauge magnet wire. The coil was positioned on the microscope stage concentric with the petri dish containing the cells under study. The coil current was obtained from a wave generator (Wavetek) and power amplifier (Kron-Hite), and was controlled using pCLAMP; coil current was monitored continuously. The magnetic fields were measured using magnetometers whose sensing elements were about 1 cm^3 (Bartington, Bell). No harmonics could be detected in the magnetometer output (< 40 dB). The vertical and horizontal components of the geomagnetic field at the location of the cells were 280 and 286 mG, respectively. The geomagnetic field was not compensated, and therefore was present at all times in all experiments. The vertical

static (DC) fields reported include the vertical component of the geomagnetic field. Unless noted otherwise, all reported fields were measured at the location of the cells in the direction perpendicular to the plane containing them. The root mean square value of the applied time-varying fields is listed.

A trial consisted of exposure for 5 s to a field generated by the coil, followed immediately by a 5-s control period during which the only field present was the geomagnetic field. Our choice of exposure time was governed by two considerations: (i) the desire to test for the presence of acute effects as defined by the time scale for sensory transduction, and (ii) our experimental plan to record 100 trials thereby permitting each cell to serve as its own control. Transmembrane current in the voltage clamp mode was measured because of the inherent sensitivity of the amplifier in that mode. The current was sampled at 200 Hz. For display, the results of 100 trials were averaged to yield a tenfold increase in signal-to-noise ratio. In addition, for each cell, the average value of the current during the exposed and control epochs were compared using the Wilcoxon signed-rank test. Most experiments were performed with the membrane potential clamped at -50 mV (vicinity of zero current) to evaluate the effect of the field on the resting membrane potential. Some studies were done at -100 mV to evaluate the effect on transmembrane K^+ current.

Observations were made using DC fields, 60-Hz fields (the power frequency for North America), and combined DC and low-frequency fields, because all three kinds of fields have been reported to affect brain electrical activity [1–6].

Results

Resting transmembrane potential. The membrane potential of single 5Y cells was -55 ± 5.0 mV ($n = 10$). The I–V curve in the vicinity of the resting potential was linear (data not shown). Cells in aggregates exhibited a similar V_m (-54 ± 4.5 mV, $n = 5$, aggregates of 15–20 cells). The occurrence of cell aggregation raised the possibility of intercellular communication through gap junctions. That possibility was investigated because cells connected by gap junctions have been suggested to be more sensitive to EMFs than single cells [19, 20].

Gap junctions. A voltage step was applied to the electrode and the time constant (τ) of the resulting transient current was measured in single cells and in aggregates of 15–20 cells (fig. 1). The transient currents were fitted to a sum of two exponentials, and τ was chosen as the time constant associated with the larger term. For cells in aggregates, τ was 44.4 ± 7.4 ms, compared with 1.3 ± 0.6 ms for single cells ($n = 5$ in each group). The increased time constant could be explained only if the voltage step charged the membrane capacitance of many cells in the aggregate, which indicated that the cells were connected via gap junctions [18]. Because each cell in an aggregate that was patched was found to be electrically connected with adjacent cells, we could conclude that all aggregated cells were probably connected by gap junctions. The EMF experiments were performed on aggregates of 15–20 cells based on the assumption that gap junction intercellular communication might increase sensitivity to the field [19, 20].

Magnetic fields and transmembrane potential. In 14 separate experiments, cells were clamped at -50 mV and exposed to DC fields of 1, 5, and 75 G. The result from a typical measurement is shown in figure 2a. The presence of the field had no effect on cell membrane current in any of the cells measured (table 1). Studies were performed at 60 Hz, with a similar result. In ten independent experiments, neither fields of 1 nor 5 G affected the transmembrane current in the vicinity of zero current (fig. 2b, table 1).

A third series of experiments using combined fields was conducted to determine whether the membrane potential was affected by ionic resonant frequencies defined by $2\pi f = qB_0/m$, where B_0 is a static field, f is the frequency of a time-varying field having a root mean square value approximately equal to B_0, and q/m is the ionic charge-to-mass ratio [21, 22]. B_0 was chosen to be 1 G and was obtained by applying a DC field of 720 mG from the coil in an additive fashion with the vertical component of the geomagnetic field (280 mG). Pos-

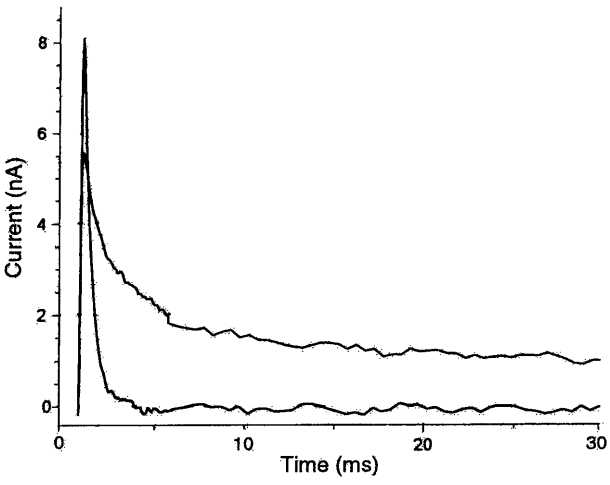

Figure 1. Transient current response to a step voltage. After the whole-cell configuration was established, a 2-mV step was applied to the electrode at time 0. Lower curve, single 5Y cell. Upper curve, patched cell in an aggregate of 17 cells. Curves were filtered with a 10-kHz, low-pass, four-pole Bessel filter; each curve was produced by averaging the current from ten voltage steps.

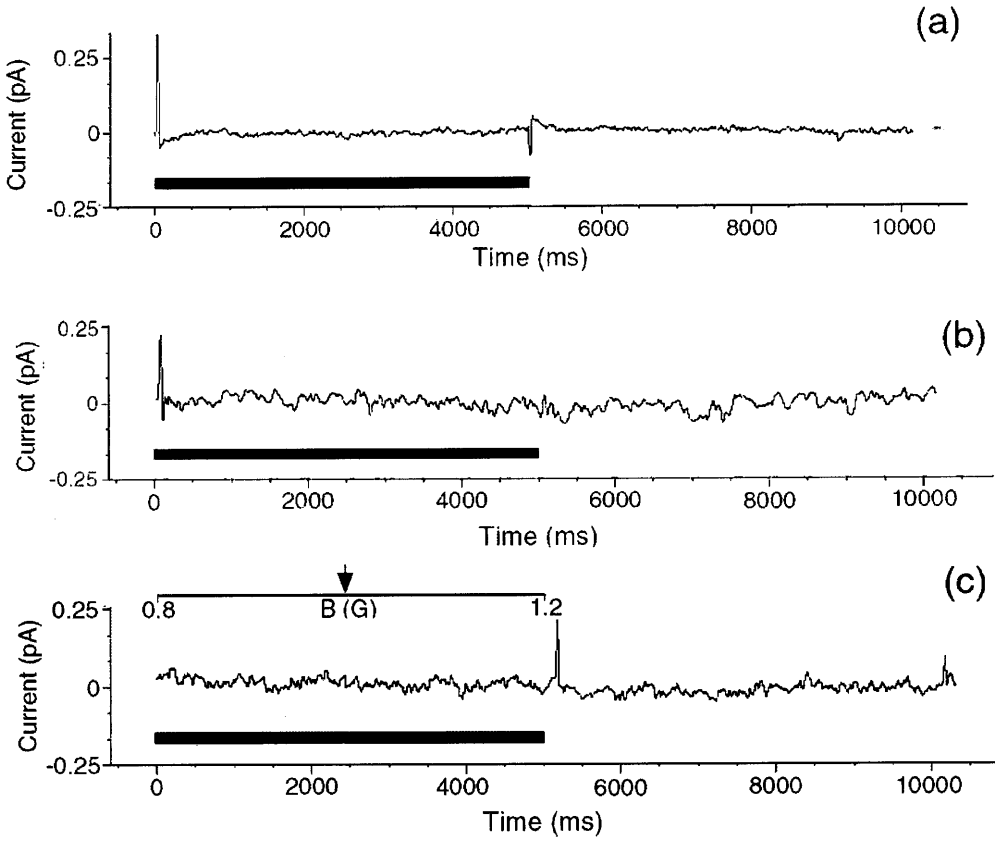

Figure 2. Effect of magnetic fields on cell transmembrane current with cells clamped at -50 mV: 5 G, DC (*a*); 5 G, 60 Hz (*b*); 0.8–1.2 G, ramp DC plus 1 G, 66.6 Hz (resonance condition for Na^+) (*c*). Solid bar indicates duration of field application. The spikes are switching artifacts. A trial consisted of a 5-s application of a field produced by the coil, followed by a 5-s control period in which the coil field was absent. Each curve was produced by averaging 100 trials. The additional abscissa in (*c*) indicates the total value of the vertical component of the static magnetic field (geomagnetic plus coil). The arrow indicates the point at which the resonance condition for Na^+ was satisfied. Each trace was obtained for one individual cell. The result for all cells is presented in table 1.

sible resonances of K^+, Ca^{2+}, Na^+, and H^+ (the ions likely to contribute to the transmembrane potential) were considered. The corresponding frequencies (determined by the ionic q/m values) were 39, 76.6, 66.6, and 1520 Hz. During the field epoch, the time-varying field (1 G in all cases) was applied, and the static field was simultaneously ramped linearly from 0.8 to 1.2 G. The resonance condition for each ion was satisfied at the mid-point of the exposure epoch (1.0 G). A typical result for Na^+ is shown in figure 2c. For all four ions (18 independent experiments), application of the combined time-varying and DC fields had no effect on cell membrane potential (table 1).

Rapid changes in transmembrane current and voltage were observed when TEA or acetylcholine were added to the bath solution, and the 5Y cells were shown to be capable of firing action potentials that could be inhibited by TTX and TEA (see Materials and methods). Thus, the ability of the cells to respond to exogenous active agents was established, indicating that the cells were viable.

Magnetic fields and transmembrane current. In a further series of experiments, we studied the effect of fields on the transmembrane current produced by voltage clamping at -100 mV. The current at this voltage was almost entirely due to K^+. Observations were made at static fields of 5 and 75 G, at 60 Hz (5 G), and using the resonance conditions for K^+ described above. A typical result is shown in figure 3. In three independent experiments for each field condition, the presence of the field had no detectable effect on the transmembrane current (Wilcoxon signed-rank test, $P > 0.05$).

Sensitivity. The apparent input cell membrane resistance in the vicinity of zero current was 1.3 ± 0.6 G ($n = 5$), as determined from the slope of I–V curves of patched cells in large aggregates. The resistance did not change during the course of the experiments within $\pm 10\%$ of the initial value. The smallest current change that could have been resolved in our system was approximately 0.02 pA (fig. 2). Consequently, we would have seen any EMF-induced effect on membrane potential as small as about 0.02 pA $\times$ 1.3 G $\cong 25 \pm 13$ μV.

Table 1. Effect of magnetic fields on transmembrane current under voltage clamp (−50 mV).

Treatment	Experiment no.	Transmembrane current (pA)	
		field	control
1 G, DC	1	0.003 ± 0.088	−0.005 ± 0.050
	2	−0.002 ± 0.057	0.004 ± 0.059
	3	−0.022 ± 0.067	0.018 ± 0.070
	4	−0.022 ± 0.110	0.023 ± 0.124
	5	−0.008 ± 0.119	0.009 ± 0.117
5 G, DC	1	−0.010 ± 0.036	0.010 ± 0.035
	2	−0.004 ± 0.009	0.003 ± 0.009
	3	−0.015 ± 0.046	0.010 ± 0.061
	4	−0.015 ± 0.050	0.024 ± 0.065
	5	0.005 ± 0.019	−0.007 ± 0.022
75 G, DC	1	−0.025 ± 0.044	−0.016 ± 0.031
	2	−0.024 ± 0.044	−0.018 ± 0.037
	3	−0.052 ± 0.103	−0.015 ± 0.055
	4	−0.061 ± 0.141	0.062 ± 0.058
1 G, 60 Hz	1	−0.606 ± 1.141	0.591 ± 0.637
	2	−0.014 ± 0.030	0.014 ± 0.042
	3	−0.008 ± 0.048	0.015 ± 0.074
	4	0.030 ± 0.076	−0.030 ± 0.092
	5	−0.344 ± 0.608	0.327 ± 0.482
5 G, 60 Hz	1	0.004 ± 0.019	−0.005 ± 0.017
	2	−0.011 ± 0.043	0.009 ± 0.033
	3	0.006 ± 0.025	−0.008 ± 0.024
	4	0.001 ± 0.042	−0.002 ± 0.040
	5	−0.001 ± 0.043	0.002 ± 0.054
Ca^{2+} resonance	1	0.064 ± 0.126	−0.058 ± 0.148
	2	−0.005 ± 0.029	−0.021 ± 0.043
	3	0.007 ± 0.028	−0.005 ± 0.032
	4	−0.055 ± 0.033	0.056 ± 0.035
	5	0.000 ± 0.043	0.028 ± 0.051
K^+ resonance	1	−0.020 ± 0.022	0.016 ± 0.019
	2	−0.002 ± 0.015	0.001 ± 0.017
	3	0.008 ± 0.018	−0.010 ± 0.022
	4	−0.010 ± 0.038	0.006 ± 0.035
	5	0.004 ± 0.036	−0.005 ± 0.034
Na^+ resonance	1	0.011 ± 0.016	−0.017 ± 0.022
	2	0.020 ± 0.042	0.010 ± 0.056
	3	−0.019 ± 0.042	0.019 ± 0.037
	4	0.005 ± 0.018	−0.009 ± 0.028
	5	0.029 ± 0.041	−0.038 ± 0.044
H^+ resonance	1	0.001 ± 0.010	0.005 ± 0.015
	2	−0.001 ± 0.032	0.007 ± 0.018
	3	0.001 ± 0.015	0.008 ± 0.025

Means ± SD for 100 exposed and 100 control epochs for the individual cells studied are listed. In each experiment, the respective means did not differ ($P > 0.05$) as determined using the Wilcoxon signed-rank test.

Discussion

Application of the magnetic fields did not result in detectable changes in cell membrane potential or transmembrane current. In both cases, the field could have affected component processes equally and oppositely resulting in the appearance of no effect. For example, the field could have increased K^+ current through open channels by an amount that was precisely compensated by an effect on the probability of the open state. We think this explanation is unlikely because there is no evidence of significant coordination between the current through a channel and its gating mechanism. Therefore, within the limitation imposed by the sensitivity of our system, the results are reasonably sufficient to exclude the possibility that EMF detection as reflected in changes in resting membrane potential or transmembrane current is a general property of excitable cells in culture.

Our conditions of measurement were such that we would have seen changes as small as about 38 μV, which is in the range of sensitivity of the electrosensory cells in fishes (10–100 μV) [23]. It appears, therefore, that the mechanism in the lower vertebrates that confers electrosensitivity is not present in 5Y cells. Consequently, if EMF sensitivity in mammals is a form of sensory transduction, as we hypothesized, then the ion channels responsible for the putative EMF-induced

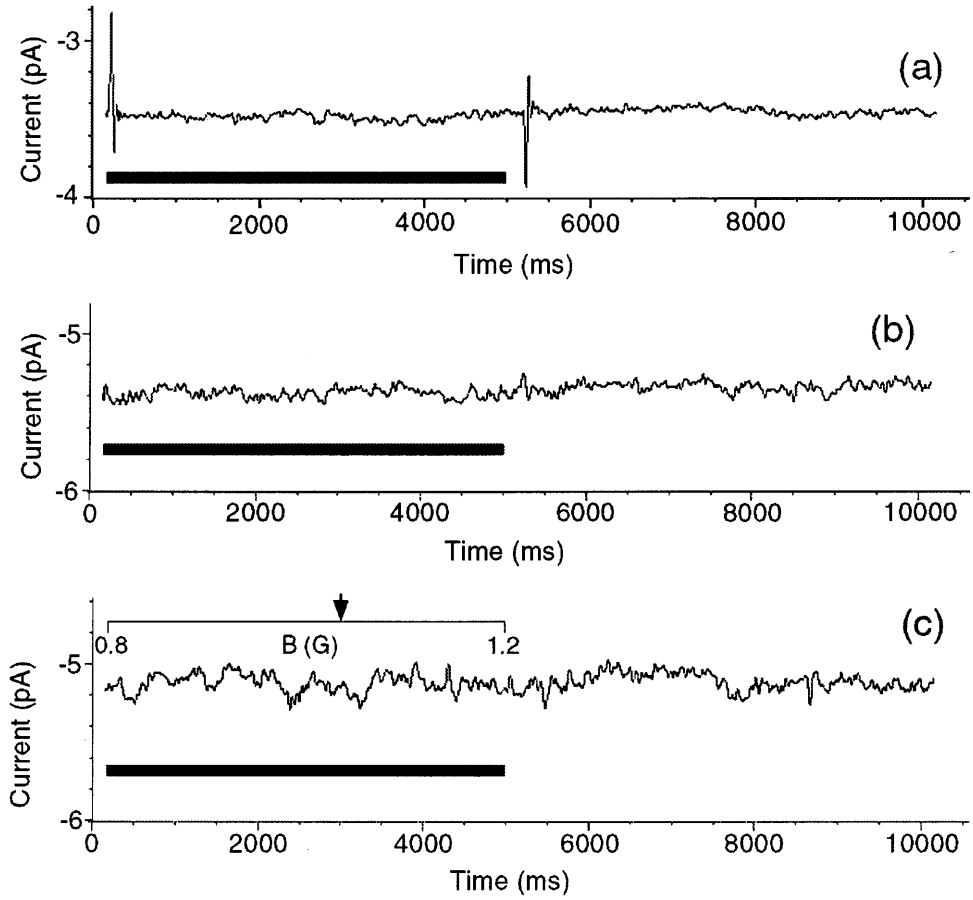

Figure 3. Effect of magnetic fields on cell transmembrane current with the cells clamped at −100 mV: 5 G, DC (*a*); 5 G, 60 Hz (*b*); 0.8–1.2 G, ramp DC plus 1 G, 39 Hz (resonance condition for K^+) (*c*). Solid bar indicates duration of application of the field. The spikes are switching artifacts. A trial consisted of a 5-s application of a field produced by the coil, followed by a 5-s control period in which the coil field was absent. Each curve was produced by averaging 100 trials. The additional abscissa in (*c*) indicates the total value of the vertical component of the static magnetic field (geomagnetic plus coil). The arrow indicates the point at which the resonance condition for K^+ was satisfied. Each trace was obtained for one individual cell.

changes in V_m are probably localized in specialized sensory neurons or neuroepithelial cells. Only a tiny fraction of mammalian sensory neurons are able to transduce sensory stimuli, and it is therefore not surprising that 5Y cells lack the receptor structures necessary for transducing EMFs.

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