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Evidence that transduction of electromagnetic field is mediated by a force receptor

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ABSTRACT

Low-strength magnetic fields triggered onset and offset evoked potentials, indicating that the detection process was a form of sensory transduction; whether the field interacted directly with an ion channel or indirectly via a signaling cascade is unknown. By analogy with electrosensory transduction in lower life forms, we hypothesized that the evoked potentials were initiated by a force exerted by the induced electric field on an ion channel in the plasma membrane. We applied a rapid magnetic stimulus (0.2 ms) and found that it produced evoked potentials indistinguishable in latency, magnitude, and frequency from those found previously when the stimulus was 50 times slower. The ability of the field-detection system in human subjects to respond to the rapid stimulus supported the theory that the receptor potentials necessary for production of evoked potentials originated from a direct interaction between the field and an ion channel in the plasma membrane that resulted in a change in the average probability of the channel to be in the open state.

The initial stages of sensory transduction include an interaction of the stimulus with plasma-membrane or intracellular structures in specialized cells, leading to changes in mean conductance of ion channels. For sound and touch, the ion channel is a force receptor that interacts directly with the stimulus; in other cases including light and some kinds of chemicals, ion channels are the effectors of a biochemical signaling cascade that results in a receptor potential (Fig. 1a–c). The electrical response of cells having force receptors in the membrane, hair cells for example, typically occurs 0.04–0.20 ms following application of the force [7,16]. Vertebrate photoreceptors, in contrast, have latencies (delay between photon absorption and change in channel conductance) about 100 times longer, consistent with the role of second-messengers in visual transduction [14,16].

The onset and offset of magnetic fields produced magnetosensory evoked potentials (MEPs) in human subjects, consistent with the view that the detection process was a form of sensory transduction [4,5]; the MEPs were observed using nonlinear analysis, but not by means of time averaging. The rise- and fall-times of the stimuli that produced the MEPs were about 10 ms, indicating that the system that mediated transduction (assumed to be based on one of the types of receptors shown in Fig. 1a–c) could respond to a stimulus at least as fast as 10 ms. The location of the human electroreceptor is unknown; animal studies suggest it is probably in the head [12], possibly the cerebellum [8].

Based on electrophysiological and modeling studies of the electroreceptor in the catfish *Kryptopterus bicirrhis*, a species for which the neuroanatomy of the electrosensory system is well known, we proposed that the catfish detected EMFs by means of their interaction with glyco groups attached to the gate of an ion channel, resulting in a force tending to open the gate [11]. Reasoning by analogy, it occurred to us that the electroreceptor responsible for the development of MEPs might also be a force detector responsive to the induced electric field (Fig. 1d). Under this assumption, using the patch-clamp technique [13], we measured single-channel properties of the field-sensitive channel in the catfish to gain insight into how rapidly we might expect the analogue human field-sensitive channel to respond to a field. After establishing that the channel being measured was the field-responsive membrane ion channel (data not shown), we recorded single-channel currents (Fig. 2). The average open time of the channel, a measure of the switching time between closed and open states, was about 0.2 ms. It could therefore be anticipated that 0.2-ms signals (and perhaps even more rapid, depending on signal intensity) might affect the probability of the force receptor to be in the open state.

To further explore the idea that the EMF transduction process responsible for MEPs involved a force receptor similar to that in *Kryptopterus*, we applied 0.2-ms magnetic stimuli to human subjects with the intent of interpreting observations of MEPs as evidence that the ion channel involved in the EMF transduction

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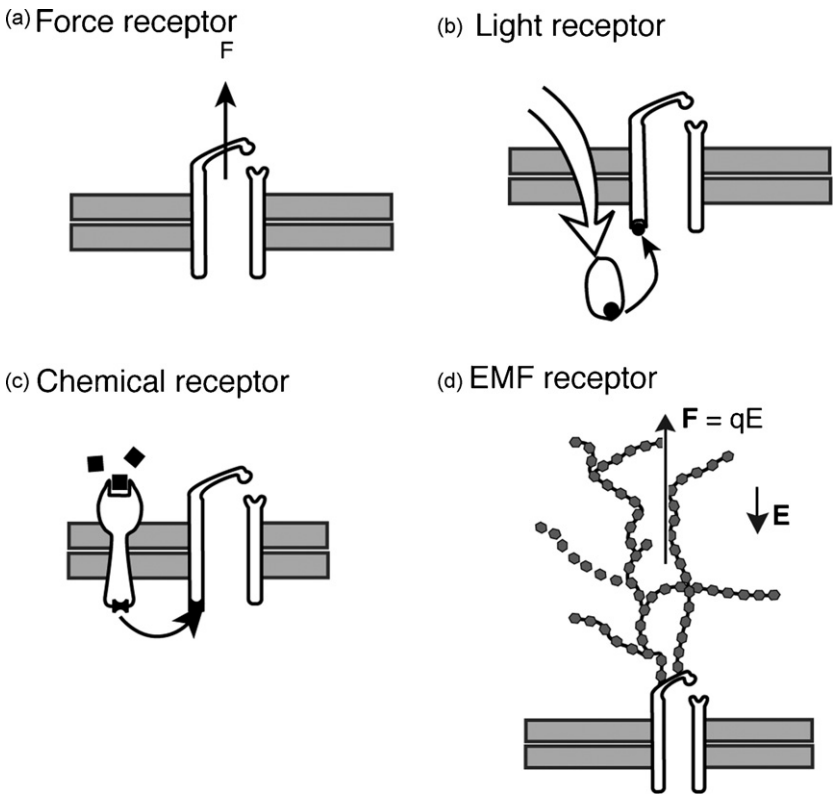

Fig. 1. Signal transduction in sensory receptors. (a) Some stimuli (sound, touch as examples) mechanically induce conformational change of an ion channel. In other cases such as detection of light (b) and chemicals (c), transduction is mediated by intracellular messengers released after the initial molecular events triggered by the stimulus. (d) Model for detection of electric fields. The glycocalyx consists of negatively charged oligosaccharide side chains covalently bound to ion channels [11]. An applied electric field **E** exerts a force **F** on the glycocalyx, thereby mechanically opening the channel gate.

process was a force receptor, recognizing that the evidence would not be direct proof.

Ten clinically normal subjects were studied (5 males, 24–61 years, and 5 females range 32–67 years). The subjects were informed of the goals, methods, and general design of the investigation, but were not told exactly when or for how long the field would be applied. Written informed consent was obtained for each subject prior to participation in the study. The review board for human research at our institution approved all experimental procedures.

To achieve precise control of the duration of the rise- and fall-times of the magnetic field we used dc (direct current) magnetic fields. A uniaxial dc magnetic field having a strength of 1 G (0.1 mT) uniform to within 5% over the region of the head was applied to the subjects in the coronal plane using a pair of coils (Fig. 3a); details of the experimental system are given elsewhere [3]. The field was applied for 2-s intervals, each separated by a 5-s interval during which there was no applied field. The geomagnetic field was 0.26 G, 59.9° below the horizontal (0.04 G along the direction of the applied field).

We showed previously that 94% (16 of 17 subjects) exhibited an onset MEP and 65% (11 of 17) exhibited an offset MEP when the rise- and fall-times were 10 ms [4]. We reproduced the rise-time as a positive control, and utilized a fall-time of 0.2 ms to assess whether the more rapid stimulus (by a factor of 50) would also result in offset MEPs (Fig. 3b). To quickly change the fall-time of the field, we used a NPN transistor (Fairchild TIP102) to switch off the coil current (the switching time was determined by the coil current and inductance, and the capacitance across the transistor leads).

The subjects sat in a comfortable wooden chair with their eyes closed; care was taken to insure the equipment controlling the coil current and recording the electroencephalogram (EEG) did not provide sensory cues. None of the subjects consciously perceived the field. Electroencephalograms ($V(t)$) were recorded continuously from O_1, O_2, C_3, C_4, P_3, and P_4 (International 10–20 system) referenced to linked ears, using gold-plated electrodes attached to the scalp with conductive paste. Electrode impedances (measured before and after each experiment) were <10 kΩ in all subjects. The signals were amplified (Nihon Kohden, Irvine, CA), filtered to pass 0.5–35 Hz, sampled at 300 Hz using a 12-bit analog-to-digital converter (National Instruments, Austin, TX), and analyzed offline. The signals were divided into consecutive 7-s trials with field onset

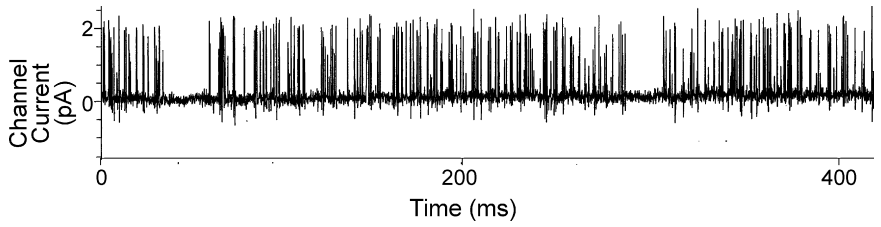

Fig. 2. Single channel current from a voltage-sensitive channel in an electroreceptor cell in *Kryptopterus bicirrhis*.

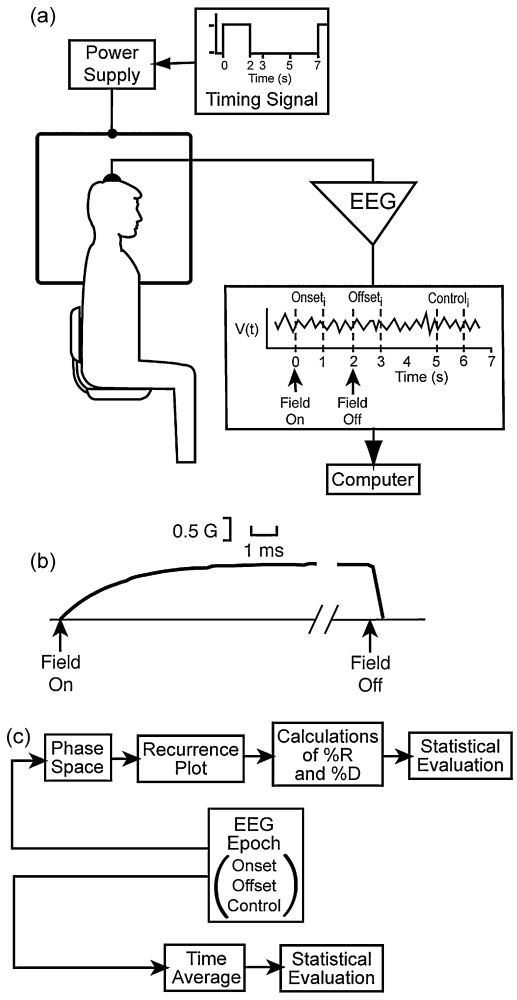

Fig. 3. Application of magnetic fields. (a) Schematic diagram of the exposure and EEG-detection systems (mid-sagittal view). (b) Onset and offset of the magnetic field. (c) Procedures for nonlinear and linear analysis.

beginning at $t = 0$, field offset beginning at $t = 2$ s, and a field-free period at $2 < t < 7$ s (Fig. 3a).

Each subject received two blocks of 80 trials. The magnetic field was applied in either the earlier or later block, as determined randomly from subject to subject. In the block where the field was not applied, the data was analyzed as a negative control (sham exposure). To help maintain alertness, 5 binaural 2-s 424-Hz tones were presented prior to each of the field and sham sessions. Trials containing artifacts as assessed by visual inspection [10] were discarded (<5% of all trials), and the artifact-free trials were digitally filtered between 0.5 and 35 Hz. All results were based on data from at least 50 trials.

The rise and the fall of the field each produced a spike in $V(t)$ that was broadened to 30 ms by the time constant of the EEG amplifier. In preliminary studies using electrical phantoms of the head, we established that the spikes arose from Faraday-type induction, and were unrelated to neuronal activity. Prior to analyzing $V(t)$, the spikes were removed by deleting the first 30 ms of data (10 points, see below) after presentation of the stimulus.

Details of our nonlinear method (Fig. 3c) were given elsewhere [3]. Briefly, the first 100 ms of each of the epochs of interest in $V(t)$ ($t = 0.03$–1 s, 2.03–3 s, and 5.03–6 s, corresponding to onset, offset, and control intervals, respectively) (Fig. 3a) were embedded in five-dimensional phase space, using a time delay of 5 points (17 ms), and the resulting trajectory was mapped to a two-dimensional recurrence plot by placing a point at (i,j) whenever the ith and jth state vectors in the trajectory were near (defined as within 15% of the maximum distance between any two states); distances were calculated using the Euclidean norm. The plots were quantified using two recurrence variables [18]: (1) percent recurrence (%R), defined as the ratio of the number of points in the plot to the total number of points in the recurrence matrix; (2) percent determinism (%D), defined as the fraction of points in the plot that formed diagonal lines consisting of at least 2 adjacent points. The process was repeated using a sliding window of 1 point in $V(t)$, yielding the time series $\%R(t)$, which was smoothed using a 100-ms, step-1 averaging window. The resulting time series, $\overline{\%R(t)}$ and $\overline{\%D(t)}$, were analyzed for the presence of evoked potentials. All calculations were performed using publicly available software [17], and verified using a custom Matlab code (Mathworks, Natick, MA).

Onset and offset of a magnetic field each produced an evoked potential [4]; we examined the same latency range to detect evoked potentials in the present study. Each of the 60 points in $\overline{\%R(t)}$ and in $\overline{\%D(t)}$ between 209 and 404 ms (which described the dynamical activity in $V(t)$ at 109–504 ms) were compared individually with the corresponding points in the control epochs using the paired t-test at a pair-wise significance level of $p < 0.05$ (identical results were found using the Wilcoxon signed rank test). In preliminary studies on baseline EEGs (no field) consisting of 2048 sets of 50 sham-field versus control comparisons, we found that the probability of observing $\geq$10 significant tests (out of 60) due to chance was about 0.04. We therefore planned to regard a comparison of a set of evoked-potential (onset or offset (Fig. 3a)) and control epochs from any particular electrode as significant if $\geq$10 tests were pair-wise significant at $p < 0.05$.

Filtering the EEG in the alpha band sometimes facilitated detection of an MEP, and the results depended on the nature of the filtering (sometimes filtering 9–12 Hz but not 8–10 Hz was effective, and sometimes conversely) [6]. In addition, although use of $\overline{\%R}$ and $\overline{\%D}$ often gave the same result, there were instances where only one of the quantifiers detected a field-induced change in the EEG [6]. Based on these prior observations, we systematically considered all conditions of analysis previously shown capable of revealing an MEP [6]. First, we analyzed $\overline{\%R(t)}$ in all 6 electrodes. If we found an evoked potential ($\geq$10 pair-wise significant tests within the expected latency interval) in at least 3 electrodes, no further analyses were conducted. If fewer than 3 evoked potentials were found, we analyzed $\overline{\%D(t)}$. If a total of 3 evoked potentials were still not detected, we filtered $V(t)$ prior to calculating $\overline{\%R(t)}$ and $\overline{\%D(t)}$ and continued the analysis until either 3 evoked potentials were detected or all the 6 predetermined conditions (combinations of recurrence variable and filtering conditions) were considered. The overall results did not depend on the order; for presentation, we chose the sequence $\overline{\%R(t)}$, $\overline{\%D(t)}$, $\overline{\%R(t)}$, after filtering the EEG at 8–10 Hz, $\overline{\%D(t)}$ after filtering at 8–10 Hz, $\overline{\%R(t)}$ after filtering at 9–12 Hz, $\overline{\%D(t)}$ after filtering at 9–12 Hz. Whenever tests were done to compare evoked-potential and control epochs, the conditions being evaluated were also applied to the sham data (sham evoked potential versus sham control). Thus, for example, when the experimental data was filtered at 8–10 Hz, so was the sham data. At the conclusion of the study we calculated the *a posteriori* false-positive rate (number of false-positive effects in the sham data divided by the total number of tests performed on the sham data), and used that error rate to estimate the family-wise error (P_{FW}) for the decision that a subject had detected the stimulus.

Prior to the study we were unaware of whether the probability of detection of evoked potentials would depend on the electrode derivation. We therefore computed the contributions to P_{FW} separately for the central, occipital, and parietal electrodes using the binomial formula, and the overall family-wise error rate for the occurrence of evoked potentials in each experiment was determined by the law of compound probability.

Table 1
Onset (a) and offset (b) evoked potentials in subjects exposed to a magnetic stimulus (1 G, DC) having a rise-time of 10 ms (onset potential) and a fall-time (offset potential) of 0.2 ms.

Subject	$\overline{\%R}$	$\overline{\%D}$	$\overline{\%R}$ (8–10 Hz)	$\overline{\%D}$ (8–10 Hz)	$\overline{\%R}$ (9–12 Hz)	$\overline{\%D}$ (9–12 Hz)	All Effects	No. Tests	P_{FW}
(a)									
S1	C3	X	C4	C4	–	–	C3 C4 C4	23	0.074
S2	P3	O1 P3	–	–	–	–	O1 P3 P3	12	0.005
S3	C3 P3	C3 P3	–	–	–	–	C3 C3 P3 P3	12	0.000
S4	O1 O2	O1 O2	–	–	–	–	O1 O1 O2 O2	12	0.001
S5	C3 C4 P3 P4	–	–	–	–	–	C3 C4 P3 P4	6	0.000
S6*	O2	O2	X	C3	–	–	O2 O2 C3	22	0.024
S7	C4	X	P4	C4 P4	X	–	C4 C4 P4 P4	23	0.012
S8	P3	P3	X	C3	–	–	C3 P3 P3	22	0.024
S9	X	X	X	P3	O1 P4	–	O1 P3 P4	30	0.057
S10	C4	X	P4	P4	–	–	C4 P4 P4	23	0.030
(b)									
S1	X	P4	P3	C4 P3	–	–	C4 P3 P3 P4	23	0.004
S2	O2 C3 P3	–	–	–	–	–	O2 C3 P3	6	0.001
S3	P3	P3	C3	–	–	–	C3 P3 P3	17	0.013
S4	P4	P4	C3	–	–	–	C3 P4 P4	17	0.013
S5	C4	X	X	X	X	X	C4	34	0.773
S6	X	X	X	X	O2 P3	O2 P3	O2 O2 P3 P3	36	0.025
S7	O2 C3	O2	–	–	–	–	O2 O2 C3	12	0.005
S8	X	X	O1	X	X	X	O1	35	0.785
S9	X	X	X	X	O1	O1	O1 O1	36	0.462
S10	P4	X	X	X	X	O1	O1 P4	34	0.234

Column heads indicate conditions of analysis. Effects in $\overline{\%D(t)}$ are shown in bold. X, evoked potentials not detected. Bars indicate conditions not analyzed. P_{FW}, family-wise error for the decision that the subject detected the field. *False-positive result found in the sham-field analysis.

$V(t)$ was also evaluated directly (no unfolding in phase space) by time averaging to detect linear evoked potentials, should they occur. The estimation of the *a posteriori* false-positive rate and the family-wise error for each of the two experiments in each subject was identical to the analysis used to evaluate the recurrence time series. We regarded a potential as nonlinear if it was detected by recurrence analysis but not by time averaging.

Using $\overline{\%R(t)}$, brain potentials were found in 9 subjects in response to field onset, and in 6 subjects in response to field offset (Table 1, first data column). In subject S2, for example, potentials occurred at P_3 due to field onset, and at O_2, C_3, and P_3 in response to field offset. Detailed results for P_3 (Fig. 4) illustrate the appearance of evoked potentials when assessed using recurrence variables; when the EEG signals were time averaged, evoked potentials were not detected (data not shown). A total of 120 statistical tests involving the $\overline{\%R(t)}$ time series were performed (2 stimuli × 6 derivations × 10 subjects), resulting in 23 evoked potentials (Table 1, first data column). When a subject exhibited fewer than 3 evoked potentials in response to either the onset or offset of the field, $\overline{\%D(t)}$ was computed and analyzed; onset potentials in S2 and offset potentials in S1 were found that had not been detected with $\overline{\%R(t)}$ (Table 1, second data column). Filtering the EEG to remove 8–10 Hz or 9–12 Hz prior to computing $\overline{\%R(t)}$ or $\overline{\%D(t)}$ revealed additional potentials (Table 1, data columns 3–6); all subjects detected field onset, and 6 subjects detected field offset. The *a posteriori* comparison-wise error rate (computed from the sham data) was 19 false-positive tests/439 tests = 0.043; thus $P_{FW} < 0.05$ for each of the 16 instances of field detection, except S1 onset ($P_{FW} = 0.074$) and S9 onset ($P_{FW} = 0.057$) (Table 1, last column). There was one instance of false-positive detection (Table 1, S6 onset).

Our main purpose was to test the hypothesis that the human magnetosensory system could respond to rapid stimuli (0.2 ms) with efficiency comparable to that for relatively slow stimuli (10 ms). The underlying idea was that a sensory system capable of responding to a 10-ms magnetic stimulus might be explainable on the basis of a second-messenger signal system in the electroreceptor cell, but that the quicker the field to which the system could respond, the more likely was the possibility that the field interacted directly with the ion channel, as in force transduction. The magnetic field, which had a 10-ms rise-time and a 0.2-ms fall-time, produced onset potentials in all subjects and offset potentials in 60% of the subjects (Table 1); the results were similar to those found when the rise- and fall-times were 10 ms (94%, 65%, respectively) [4].

For several reasons, the observed effects can be taken to have been a result of true post-transduction changes in brain electrical activity triggered by the magnetic stimuli. First, an alternate explanation that the effects were unrelated to neuronal activity but rather resulted from interactions between the field and the scalp electrodes can be ruled out because we showed in preliminary experiments involving phantoms of the human head that such interactions began instantaneously and lasted less than 30 ms after stimulus onset or offset. In contrast, the observed potentials occurred several hundred ms after initiation of the stimulus, which is a typical latency for evoked potentials. Second, the family-wise error rate for a decision that the subject detected each of the stimuli was sufficient to rule out the possibility that the effects were due to chance. Finally, the false-positive signal-detection rate as assessed during sham exposure ruled out the possibility that the effect could have arisen as a result of the analytical method used to analyze the EEG. For all these reasons, the observed changes in electrical activity were true MEPs.

It might be argued that the difference between the onset and offset response rates (100% versus 60%) was partly due to differences in the rise-times of the stimuli. However comparable response rate differences were observed previously (94%, 65%) when the rise-time of both stimuli was 10 ms. Further, onset evoked potentials due to auditory stimuli also occurred more frequently than offset potentials [2,9,15]. The likelihood is, therefore, that the difference in response rates observed here was not related to the difference in the rapidity of the stimuli.

In the catfish, the three-dimensional orientation of the electroreceptor cells and their afferent innervation (4–30 cells synapsed with a single neuron) help insure that the effect of the field on the probability of the channels to be in the open state is not averaged away across the combined cellular ensemble. If the proposed model (Fig. 1d) were applicable to human transduction of EMFs, structural ordering of the electroreceptor cells and afferent innervation would similarly be expected to insure that the response of the system was not averaged away. The geomagnetic field might also play a role,

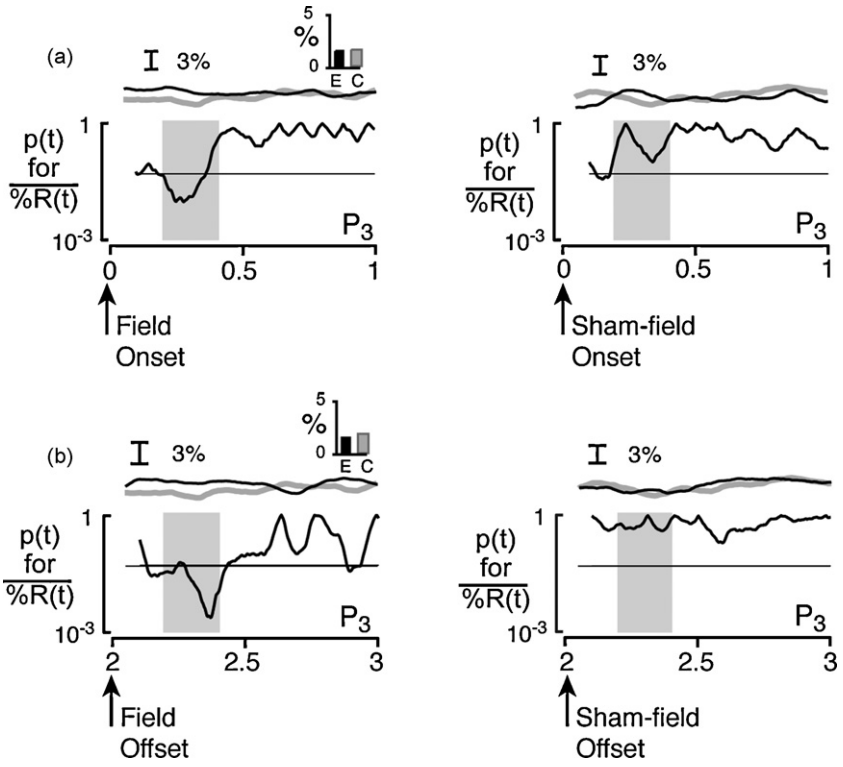

Fig. 4. Evoked potentials detected from P_3 in subject S2 using the nonlinear variable $\overline{\%R(t)}$. (a) Magnetic-field and sham-field onset (left and right panels, respectively). (b) Magnetic-field and sham-field offset (left and right panels, respectively). The curves at the tops of the panels show the average values of the stimulus (E) and control (C) epochs ($N \geq 50$ trials). The $p(t)$ curves are the probability that the difference between the means of the onset and control epochs at time t was due to chance. Bar graphs indicate the average value of $\overline{\%R}$ over the latency interval for which $p(t) < 0.05$ (horizontal line); the standard deviations are not resolved at scale shown. The stippled regions show the expected latency intervals.

possibly tipping ion-channel open-time probabilities one way or the other [1].

Several considerations suggested that the mechanism by which the magnetic field triggered the response involved the induced electric field. First, the strength of the induced field (determined from Fig. 1b, using Faraday's law) was theoretically capable of modifying the average open time of an ion channel [11], assuming the model depicted in Fig. 1d. Second, there is no published explanation regarding how the magnetic field used in this study could be detected by a sensory cell, because the small energy of interaction between the field and tissue (compared with thermal energy) has thus far defeated all proposed models except those that postulate the presence of ferromagnetic structures (which have not been observed in the human brain).

We conclude that the human EMF transduction system is capable of detecting signals that change at least as rapidly as about 0.2 ms (on-to-off), possibly indicating that the signal transduction process is directly initiated by a force receptor.

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