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Magnetosensory evoked potentials: Consistent nonlinear phenomena

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Abstract

Electromagnetic fields (EMFs) having strengths typically found in the general environment can alter brain activity, but the reported effects have been inconsistent. We theorized that the problem arose from the use of linear methods for analyzing what were actually nonlinear phenomena, and therefore studied whether the nonlinear signal-processing technique known as recurrence quantification analysis (RQA) could be employed as the basis of a reliable method for demonstrating consistent changes in brain activity. Our primary purpose was to develop such a method for observing the occurrence of evoked potentials in individual subjects exposed to magnetic fields (2 G, 30 and 60 Hz). After all conditions that affected the analysis of the EEG were specified in advance, we detected magnetosensory evoked potentials (MEPs) in all 15 subjects ($P < 0.05$ in each experiment). The MEPs, which occurred within the predicted latency interval of 109–504 ms, were independent of the frequency and the direction of the field, and were not detected using the traditional linear method of analysis, time averaging. When the results obtained within subjects were averaged across subjects, the evoked potentials could not be detected, indicating how real nonlinear phenomena can be averaged away when the incorrect method of analysis is used. Recurrence quantification analysis, but not linear analysis, permitted consistent demonstration of MEPs. The use of nonlinear analysis might also resolve apparent inconsistencies in other kinds of brain studies.

Keywords: Evoked potentials; Nonlinear; Recurrence analysis; Magnetic fields

1. Introduction

Electromagnetic fields (EMFs) having strengths typically found in the general environment produced a broad range of electrophysiological, neurochemical, behavioral, and health-related effects (Presman, 1970; Marino and Becker, 1982; Carpenter and Ayrapetyan, 1994; Barnes and Greenebaum, 2006). We proposed that the fields were detected by specialized neurons, ultimately leading to the diverse observations (Marino and Becker, 1982; Marino, 1993; Sonnier and Marino, 2001). However, EMF bioeffects have characteristically been inconsistent, leading experts to reject the neuronal transduction theory (and all theories that rationalized biological consequences of EMF-tissue interactions) on the basis that there were no real phenomena to be explained (Beem, 1985; World Health Organization, 1993; Park, 1995; Stevens, 1997; International Commission on Non-Ionizing Radiation Protection, 2004).

Nonlinear systems (those governed by nonlinear differential equations) can appear to be random when studied using linear methods (Mees, 2001). Results of EMF animal metabolic studies appeared random when analyzed using linear methods, but were shown to be deterministic when the data were analyzed using appropriate methods (Webber and Zbilut, 1994; Marino et al., 2000). Newly developed phase-space methods

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(Zbilut and Webber, 1992; Webber and Zbilut Retrieved Feb. 1, 2007) permitted us to show that fields produced nonlinear changes in brain activity that could not be detected using linear methods (Carrubba et al., 2007a,b).

In light of the discovery that at least some of the effects of EMFs on brain activity are nonlinear in origin (Carrubba et al., 2007a,b), it became necessary to reevaluate how the basic scientific requirement of reproducibility should be formulated because, in distinction to linear systems, consistency in the magnitude or direction of a stimulus–response relationship are not general properties of nonlinear systems. Our primary purpose was to develop and describe a reliable method for demonstrating the consistent occurrence of changes in evoked potentials in individual subjects exposed to a magnetic field, and to evaluate the role of the stimulus frequency and vector direction in determining the response.

2. Experimental procedure

2.1. Subjects

Fifteen clinically normal subjects were studied: seven males (age range 21–54 years) and eight females (29–51 years). The subjects were informed of the goals, methods, and general design of the investigation, but were not told exactly when during the session that the field would be applied. Written informed consent was obtained from each subject prior to participation in the study. The Institutional Review Board at the LSU Health Sciences Center approved all experimental procedures.

2.2. Magnetic field

Uniaxial magnetic fields, 2 FG rms (200 μT), 30 and 60 Hz, uniform to within 5% in the region of the head, were generated by passing current (California Instruments, San Diego, CA) through coaxial coils; details of the apparatus are given elsewhere (Carrubba et al., 2006). We used two frequencies to evaluate the possibility that the subjects might have been conditioned by the pervasive presence of 60-Hz fields in the environment. The magnetic stimulus was applied for 50 ms (Fig. 1a), with an inter-stimulus period of 2.95 s; the field strength (comparable to that of environmental fields) was below the threshold for awareness. The field was applied in the coronal (Table 1, S1–S10) or sagittal (S11–S15) plane while the subjects were seated (with their eyes closed) in an isolation chamber (to reduce the presence of random ambient stimuli). All electrical equipment was located outside the chamber to avoid the possibility of uncontrolled sensory cues; their absence was verified by interviewing each subject at the end of the experimental session. The background 60-Hz magnetic field (the field continuously present during the experimental session) was 0.5 mG; the geomagnetic field was 261.5 mG, 59.9° below the horizontal (component along the direction of the applied field, 35.6 mG). All field measurements were performed using a triaxial magnetometer (Bartington, MAG-03, GMW, Redwood City, CA).

After an acclimation period, there were two periods during which a magnetic field was presented and an intervening period during which no stimulus was applied (sham field); the 60- and 30-Hz fields were each presented first in alternate subjects. To help maintain the subject's attentiveness during the experimental session, a binaural 424-Hz tone (65 dB) was substituted for the magnetic stimulus in three brief sets of trials during the session (Fig. 1b).

2.3. EEG recording

EEGs were recorded from O1, O2, C3, C4, P3, and P4 (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 below 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 off-line.

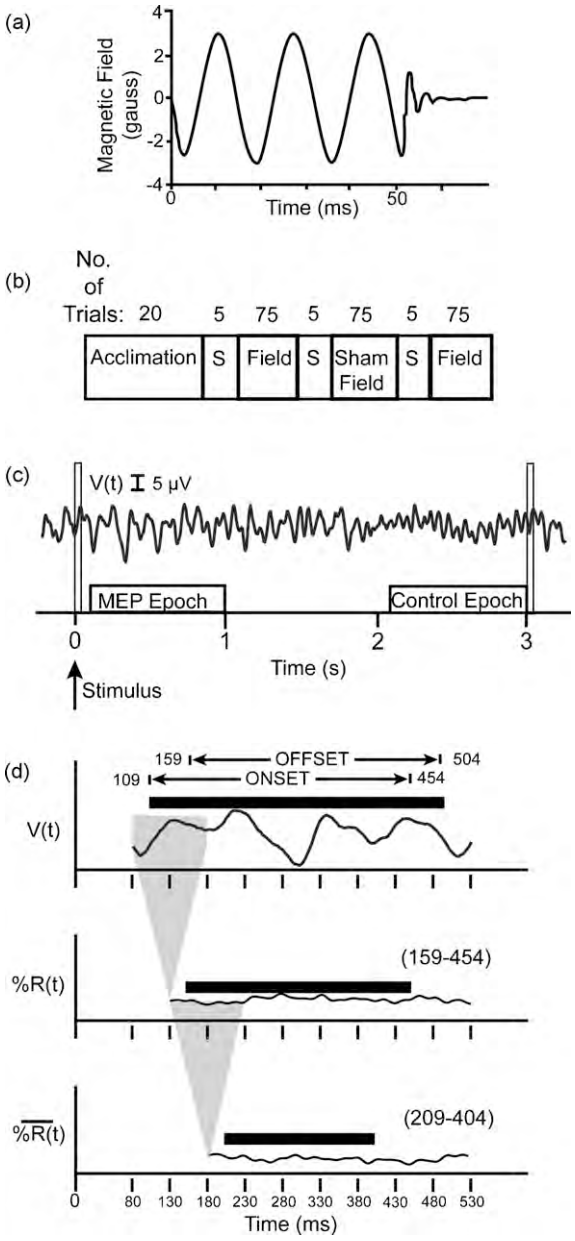

Fig. 1. Experimental design and procedure. (a) Applied magnetic stimulus (60 Hz shown). (b) Organization of trials in an experimental session. S, sound stimulus. (c) An EEG trial showing the locations of the epoch containing the magnetosensory evoked potential (MEP) and the corresponding control epoch. (d) Convention for synchronizing the graphical representation of related time series. The bar depicts the expected latency range for the MEP (superposition of the onset and offset MEPs). The stippled regions indicate the relation between the deterministic behavior in one time series and where that behavior was represented after analysis.

The signal from each electrode was divided into consecutive 3-s intervals (trials) consisting of a 50-ms stimulus and a 2.95-s inter-stimulus interval; trials containing artifacts (as assessed by visual inspection) were discarded (<5% of the trials). Termination of the field produced a voltage spike in the EEG signal of about 30 ms (Carrubba et al., 2007a); the first 80 ms of the signal (0–50 ms recorded during application of the field and 51–80 ms that contained the spike) were deleted and the trials were digitally filtered between 0.5 and 35 Hz. All results were based on data from at least 50 trials.

Table 1
Magnetosensory evoked potentials from indicated electrode derivations in 15 subjects

Subject	Stim. (Hz)	$\overline{\%R}$	$\overline{\%D}$	$\overline{\%R}$ (8-10Hz)	$\overline{\%D}$ (8-10Hz)	$\overline{\%R}$ (9-12Hz)	$\overline{\%D}$ (9-12Hz)	All Effects	†No. Tests	P_{FW}
S1 (30F)	60	O1, C4, P4						O1, C4, P4	2/4	0.001
	30	O2, C3	**O2, C3**					O2,**O2**,C3,**C3**	4/8	0.001
S2 (54M)	60	O2	**O2, P3**					O2, **O2**, **P3**	4/8	0.004
	30	O1	X	C4	**O1**			O1, **O1**, C4	7/16	0.022
S3 (23M)	60	X	**C4, P3**	X	**O1**			**O1**, **C4**, **P3**	8/14	0.047
	30	P3	**P3**	O2, C4				O2,C4,P3,P3	6/11	0.004
S4 (22M)	60	O1	**O1**	C4				O1, **O1**, C4	5/12	0.009
	30	C3	**C3**	O1				01, C3, **C3**	5/12	0.025
S5 (51F)	60	X	X	O1	**O1**	C3		O1, **O1**, C3	9/20	0.042
	30	O1, P3	**P3**					O1, **P3**, P3	4/8	0.01
S6 (23M)	60	C4	**C4**	X	X	P4		C4, **C4**, P4	10/17	0.14
	30	X	**O1**	X	X	O2, P3, P4		**O1**,O2,P3,P4	10/19	0.017
S7 (29F)	60	X	X	O1,O2,C4,P3,P4				O1,O2,C4,P3,P4	6/12	0.001
	30	C4	**C4**	C3				C3, **C4**, C4	6/11	0.046
S8 (45F)	60	X	**O1**	O2	**O2**			**O1**, O2, **O2**	7/16	0.003
	30	X	X	P4	**P4**	O2		O2, **P4**, P4	10/19	0.09
S9 (25M)	60	C4	X	O1	**O1**			O1, **O1**, C4	8/14	0.028
	30	X	X	O1	**O1, C4**			O1, **O1**, **C4**	8/16	0.03
S10 (21M)	60	O2	**P3**	C4				O2, C4, **P3**	5/12	0.025
	30	O2	**O2**	P3				O2, **O2**, P3	5/12	0.009
S11 (31F)	60	C3, C4, P4						C3, C4, P4	2/4	0.002
	30	O2	**O2**	X	**C4**			O2, **O2**, **C4**	6/16	0.017
S12* (51F)	60	X	X	X	**P4**	C4, P4		C4, **P4**, P4	10/26	0.251
	30	O2, P3, P4						O2, P3, P4	2/4	0.001
S13 (31F)	60	X	X	O1, O2	**O2, C3**			O1,O2,**O2**,**C3**	8/16	0.003
	30	O1, O2, P3						O1, O2, P3	2/4	0
S14 (24F)	60	X	X	O2, P3	X	X	X	O2, P3	11/23	0.286
	30	P3	**C3, P3**					**C3**, P3, **P3**	4/8	0.018
S15 (36M)	60	O2	**O2, C3**					O2, **O2**, **C3**	4/8	0.004
	30	X	X	X	**P3**	O2	**O2**	O2, **O2**, **P3**	12/23	0.15

Column heads indicate conditions of analysis. Effects found in $\overline{\%D}$ are shown in bold. *False-positive result ($P = 0.251$). X, MEPs not detected. Bar indicates conditions not analyzed. P_{FW}, family-wise error. †Occipital/nonoccipital. The stimulus was applied coronally to subjects S1–S10, and sagittally to subjects S11–S15.

2.4. Recurrence quantification analysis

The EEG voltage signal, $V(t)$, was analyzed by recurrence quantification analysis (RQA), which is an analytical method capable of detecting the presence of nonlinear deterministic activity in a time series (Webber and Zbilut Retrieved Feb. 1, 2007). We chose RQA because, unlike most nonlinear analytical methods, it is not restricted to stationary signals, and nonstationarity is a fundamental property of all biological times series including the EEG. A portion of the signal recorded during the interstimulus period served as the control (Fig. 1c). The regions of interest in each trial (0.08–1.0 s, 2.08–3.0 s, corresponding to the magnetosensory evoked potential (MEP) and control epochs, respectively) were embedded in separate phase spaces, and a series of recurrence plots were generated; such plots are useful devices for revealing patterns not detectable by conventional analysis (Eckmann et al., 1987). Briefly, the first 100 ms of each epoch (30 points) was embedded in a five-dimensional phase space using a time delay of 5 points (17 ms) (Jeong et al., 2001). The embedding parameters were chosen empirically because there was no reason to believe that the methods used for choosing embedding parameters for nonlinear equations (Abarbanel, 1994) were applicable to biological time series. The recurrence plot was produced by plotting a point in two-dimensions at the location addressed by (i,j) whenever the ith and jth state vectors were near. Two states were defined as near if they were within 15% of the maximum distance between any two states; for calculating the distances we used the Euclidean norm. The plot was quantified using percent recurrence, $\%R$ (the number of recurrent points in the plot divided by the total number of points in the recurrence matrix), and percent determinism, $\%D$ (the number of recurrent points that form diagonal lines in the recurrence plot) (Webber and Zbilut Retrieved Feb. 1, 2007). Percent recurrence is a measure of the extent to which the EEG is correlated with itself in phase space, and $\%D$ is a measure of the extent to which specific structures (diagonal lines) are formed in the plot (Webber and Zbilut Retrieved Feb. 1, 2007). Computation of $\%D$ requires the specification of the minimum number of points in the recurrence plot that will be taken to count as a line; we chose a line of four points. The computational process was iterated using a sliding window of one point in $V(t)$, thereby yielding the time series $\%R(t)$ and $\%D(t)$. The calculations were performed using publicly available software (Webber, 2007), and verified using a custom Matlab code (Mathworks, Natick, MA). The series were smoothed using a 100-ms, step-1 averaging window, and the resulting time series, $\overline{\%R(t)}$ and $\overline{\%D(t)}$, were analyzed for the presence of evoked potentials. All adjustable parameters in the analysis were defined previously (Marino et al., 2004; Carrubba et al., 2007a), except that we used a line parameter of 4 (not 2), because the results obtained were marginally better.

To synchronize the graphical representation of the various time series, we adopted the convention that computed points were plotted at the time corresponding to the middle of the interval in the time series from which they were computed. For example, the value of $\%R(t)$ or $\%D$ determined by the 100-ms interval in $V(t)$ beginning at $t = 80$ ms appeared in a plot of $\%R(t)$ or $\%D$ at

$t = 130$ ms; when that point was the first in the 100-ms averaging window for $\overline{\%R(t)}$ or $\overline{\%D(t)}$, it was plotted at $t = 180$ ms. Thus, $\overline{\%R(180)}$ reflected the dynamical activity that occurred in $V(t)$ within 80–280 ms (Fig. 1d).

2.5. Experimental design

Field onset and offset each produce an MEP with a latency between 109 and 454 s (Carrubba et al., 2007a). We therefore expected a combined onset/offset MEP with a latency of 109–504 ms (Fig. 1d). Each of the 60 points in $\overline{\%R(t)}$ 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 3-s trials, we found that the probability of observing ≥ 10 significant tests (out of 60) due to chance was about 0.04. We therefore planned to regard a comparison of an MEP and control epoch from any particular electrode as significant if ≥ 10 tests were pair-wise significant at $P < 0.05$.

We previously showed on the basis of empirical analysis that filtering the EEG in the alpha band sometimes facilitated detection of an MEP, and that the results depended on the nature of the filtering (sometimes removing 9–12 Hz did not lead to detection of an MEP whereas removing 8–10 Hz did so, and conversely) (Carrubba et al., 2007a). 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 (Marino et al., 2004). Based on these prior observations, we systematically considered all conditions of analysis previously shown capable of revealing an MEP. First, we analyzed $\overline{\%R(t)}$ in all six electrodes. If we found an MEP in at least three electrodes, no further analyses were conducted. If fewer than 3 MEPs were found, we analyzed $\overline{\%D(t)}$. If a total of 3 MEPs 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 MEPs were detected or all the predetermined 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 MEP and control

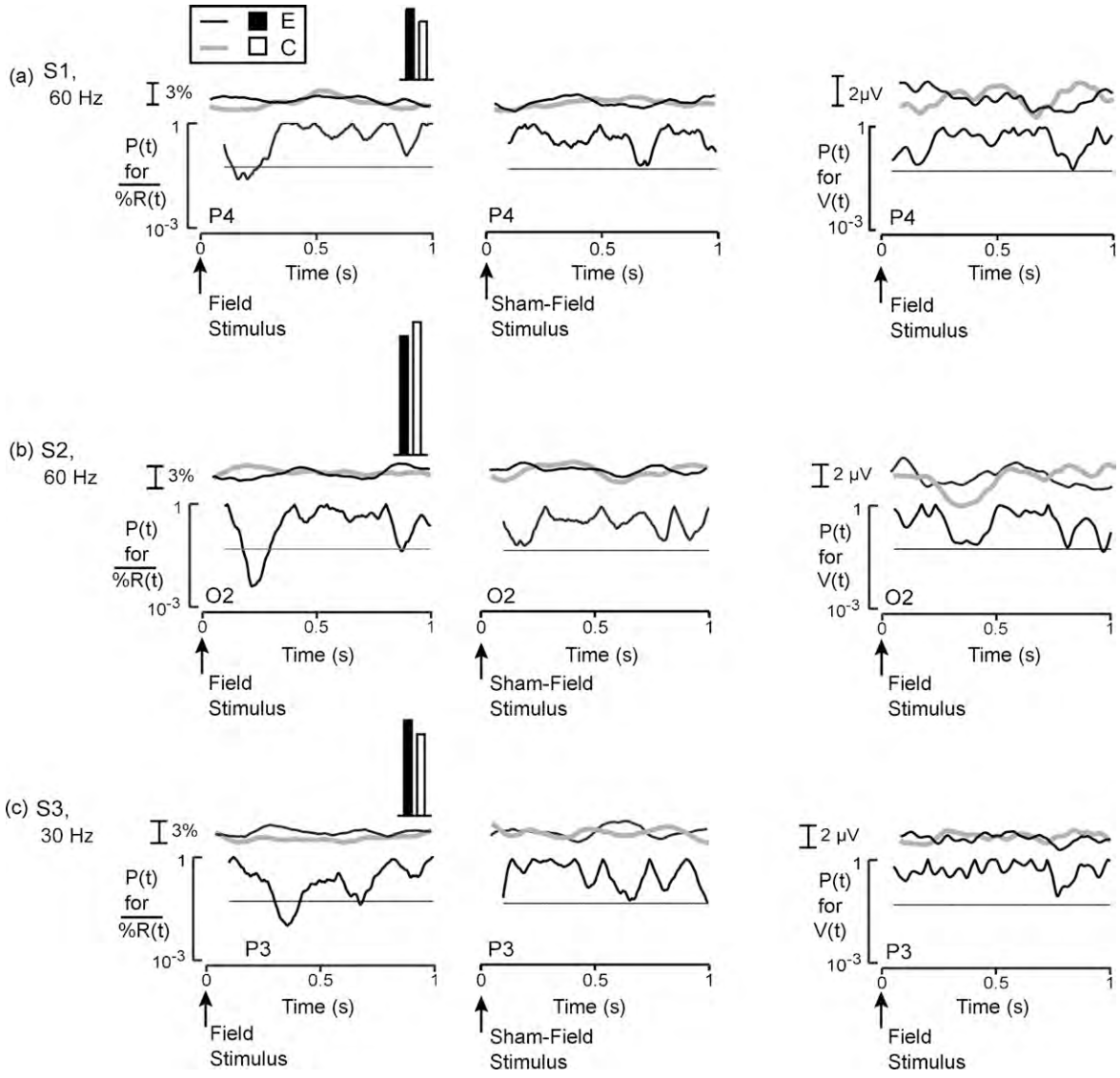

Fig. 2. Magnetosensory evoked potentials detected in three subjects using the recurrence analysis variable $\overline{\%R(t)}$. (a, b, c) Subjects S1, S2, S3, respectively. First column, field-stimulus trials; second column, sham-stimulus trials; third column, EEG from which $\overline{\%R(t)}$ in the field-stimulus trials was computed. The curves at the top of each panel show the average values of the exposed (E) and control (C) epochs for the respective time series ($N \geq 50$ trials). $P(t)$, probability of a difference in means at time t. Bar graphs (drawn to the indicated scale) indicate the average value of $\overline{\%R}$ over the latency interval for which $P(t) < 0.05$ (the standard deviations are not resolved at scale shown). The field stimulus was applied for 50 ms.

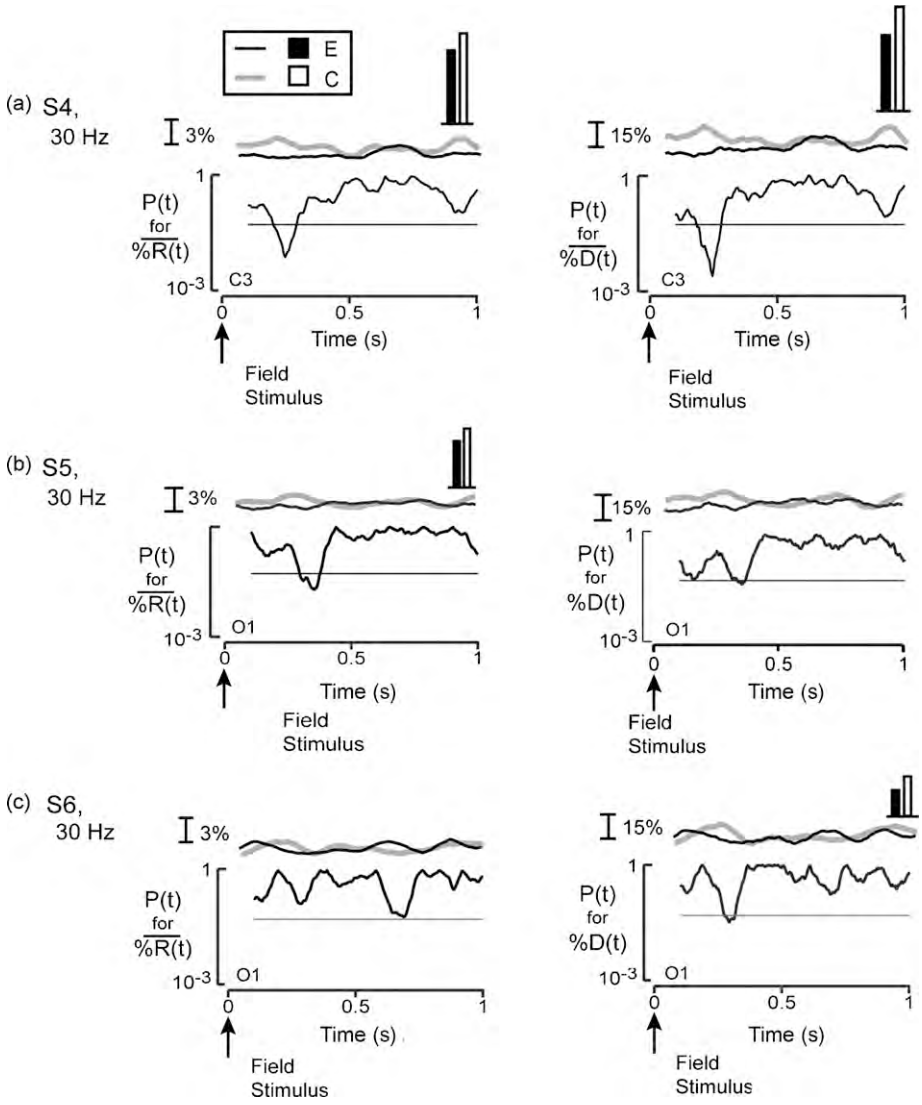

Fig. 3. Use of percent recurrence ($\overline{\%R(t)}$) and percent determinism ($\overline{\%D(t)}$) to detect magnetosensory evoked potentials. (a, b, c) Subjects S4, S5, S6, respectively. The curves at the tops of the panels show the average values of the exposed (E) and control (C) epochs for $\overline{\%R(t)}$ (left) and $\overline{\%D(t)}$ (right) ($N \geq 50$ trials). $P(t)$, probability of a difference in means at time t. Bar graphs (drawn to the indicated scale) show the average value of $\%R$ over the latency interval for which $P(t) < 0.05$ (the standard deviations are not resolved at scale shown).

epochs the conditions being evaluated were also applied to the sham data (sham MEP 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), and used that error rate to estimate the family-wise error for the 60-Hz and the 30-Hz experiments in each subject.

Brain potentials evoked by magnetic fields are more likely to be detected in the occipital electrodes, compared with the central and parietal electrodes (Carrubba et al., 2007a). We therefore computed the contributions to the family-wise error (P_{FW}) separately for the occipital and non-occipital electrodes using the binomial formula, and the overall family-wise error rate for the occurrence of MEPs in each experiment was determined by the law of compound probability.

$V(t)$ was evaluated directly (no unfolding in phase space) by time averaging to detect linear MEPs, 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.

3. Results

When subject S1 was exposed to the 60-Hz stimulus, a magnetosensory evoked potential (MEP) ($\geq$10 pair-wise significant tests between the exposed and control epochs) that had the expected latency was observed using $\overline{\%R(t)}$ computed from the EEG measured at P4; no difference was found in $\overline{\%R(t)}$ from P4 in the sham-exposure trials (Fig. 2a). A difference in $\overline{\%R(t)}$ was also found (from O2) in S2 when the 60-Hz stimulus was applied (from 0 to 2), but not during sham exposure (Fig. 2b). In S3, the 30-Hz stimulus but not sham-exposure produced an effect in $\overline{\%R(t)}$ from P3 (Fig. 2c). In each case evoked potentials were not observed in the EEG from which $\overline{\%R(t)}$ had been computed (Fig. 2, last column). Magnetosensory evoked potentials (MEPs) were observed in one or more electrodes in 14 of 15 subjects

using the RQA variable $\overline{\%R(t)}$ (first data column in Table 1, Fig. 2).

If a subject exhibited fewer than 3 MEPs in a particular experiment as assessed using $\overline{\%R(t)}$, $\overline{\%D(t)}$ was computed from the EEG and analyzed for the presence of MEPs. In some cases MEPs were found that mirrored those found using $\overline{\%R(t)}$ (Fig. 3a). However, there were instances where this was not true (Fig. 3b), and other instances where evoked potentials not detected with $\overline{\%R(t)}$ were detected with $\overline{\%D(t)}$ (Fig. 3c). In seven subjects (S2, S3, S6, S8, S10, S14, S15), MEPs that had not been observed when the EEG was analyzed using $\overline{\%R(t)}$ were observed using $\overline{\%D(t)}$ (second data column in Table 1, Fig. 3).

Filtering the EEG to remove 8–10 Hz or 9–12 Hz prior to computing $\overline{\%R(t)}$ or $\overline{\%D(t)}$ produced additional MEPs (Table 1, Figs. 4 and 5). For example, at 60 Hz, MEPs were not detected in S7 using $\overline{\%R(t)}$ or $\overline{\%D(t)}$. When the 8–10 Hz energy was removed from the EEG signals, however, previously undetected MEPs that had the expected latency were found in the EEGs from five of the six electrodes (Fig. 4). Overall, 8–10 Hz filtering permitted observation of MEPs in one or more electrodes in 10 subjects (S2, S3, S4, S5, S7, S8, S9, S10, S13, S14, third data column in Table 1).

The results produced by filtering depended on which alpha frequencies were removed. For example, in S11, analysis of $\overline{\%R(t)}$ revealed MEPs in three additional electrodes: C3, C4, P4 (Fig. 5). Filtering at 8–10 Hz increased detection sensitivity for the signal from C3 and confirmed the negative result from O1. However, removal of 9–12 Hz revealed an MEP in O1 and obscured the MEP in C3 (Fig. 5). Filtering at 9–12 Hz resulted in the observation of MEPs in five subjects (S5, S6, S8, S12, S15) that had not been detected after filtering at 8–10 Hz (fourth and fifth data columns in Table 1).

In the sham-exposure studies a total of 373 different comparisons were made between sets of sham-exposed and control epochs, and a false-positive effect ($\geq$10 significant pair-wise differences) occurred in 18 instances; the *a posteriori* false-positive error rate, 18/373 = 0.048, was used to compute P_{FW}. For example, in the experiment in which S5 was exposed to a 60-Hz stimulus, 3 MEPs occurred after nine tests had been performed for signals derived from the occipital electrodes and 20 tests for signals from the central and parietal electrodes. The probability of at least 2 significant differences out of 9 tests each at an error rate of 0.048 is 0.066, and the probability of 1 significant difference out of 20 tests is 0.63. Therefore the probability of both events occurring is (0.066)(0.63) = 0.042. In the case of the 30-Hz stimulus the corresponding probabilities were 0.18 (1/4 occipital) and 0.053 (2/8 non-occipital), yielding $P_{FW} = 0.01$. P_{FW} was less than 0.05 in 25 experiments; the exceptions were S6 at 60 Hz ($P_{FW} = 0.14$), S8 at 30 Hz ($P_{FW} = 0.09$), S12 at 60 Hz ($P_{FW} = 0.251$), S15 at 30 Hz ($P_{FW} = 0.15$), and S14 at 60 Hz, which did not detect the stimulus (significant differences in only two electrodes) (Table 1). Subject 12 was the only instance of false-positive detection of MEPs (occurrence of 3 of the 18 significant pair-wise comparisons in the sham-exposed data). The *a posteriori* probability that an observed MEP occurred in the occipital electrodes was 0.44, compared with 0.27 and 0.28 for the central and parietal electrodes, respectively (Table 1).

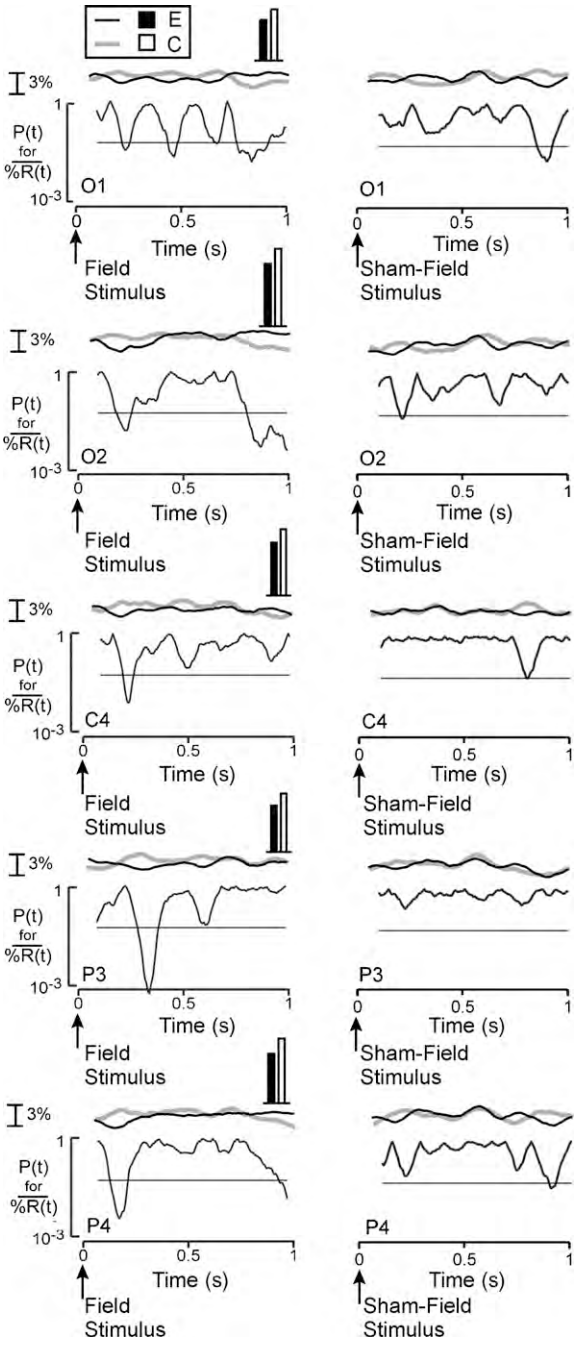

Fig. 4. Results obtained after filtering the EEG signals from subject S7 exposed to a 60-Hz stimulus using $\overline{\%R(t)}$. The EEGs had been digitally filtered to remove the 8–10 Hz components. The curves at the top of the panels show the average values of the exposed (*E*) and control (*C*) epochs for $\overline{\%R(t)}$ in the exposed (left) and sham-exposed (right) trials ($N \geq 50$ trials). $P(t)$, probability of a difference in means at time t. Bar graphs (drawn to the indicated scale) show the average value of $\overline{\%R}$ over the latency interval for which $P(t) < 0.05$ (the standard deviations are not resolved at scale shown). MEPs were not detected in the unfiltered EEG (Table 1).

Magnetosensory evoked potentials having the expected latency were detected in all subjects; the family-wise error (P_{FW}) was less than 0.05 in 25 of 30 experiments (last data column in Table 1). Neither the average latency nor the duration of the MEPs varied with the stimulus frequency, gender, or electrode derivation (Table 2).

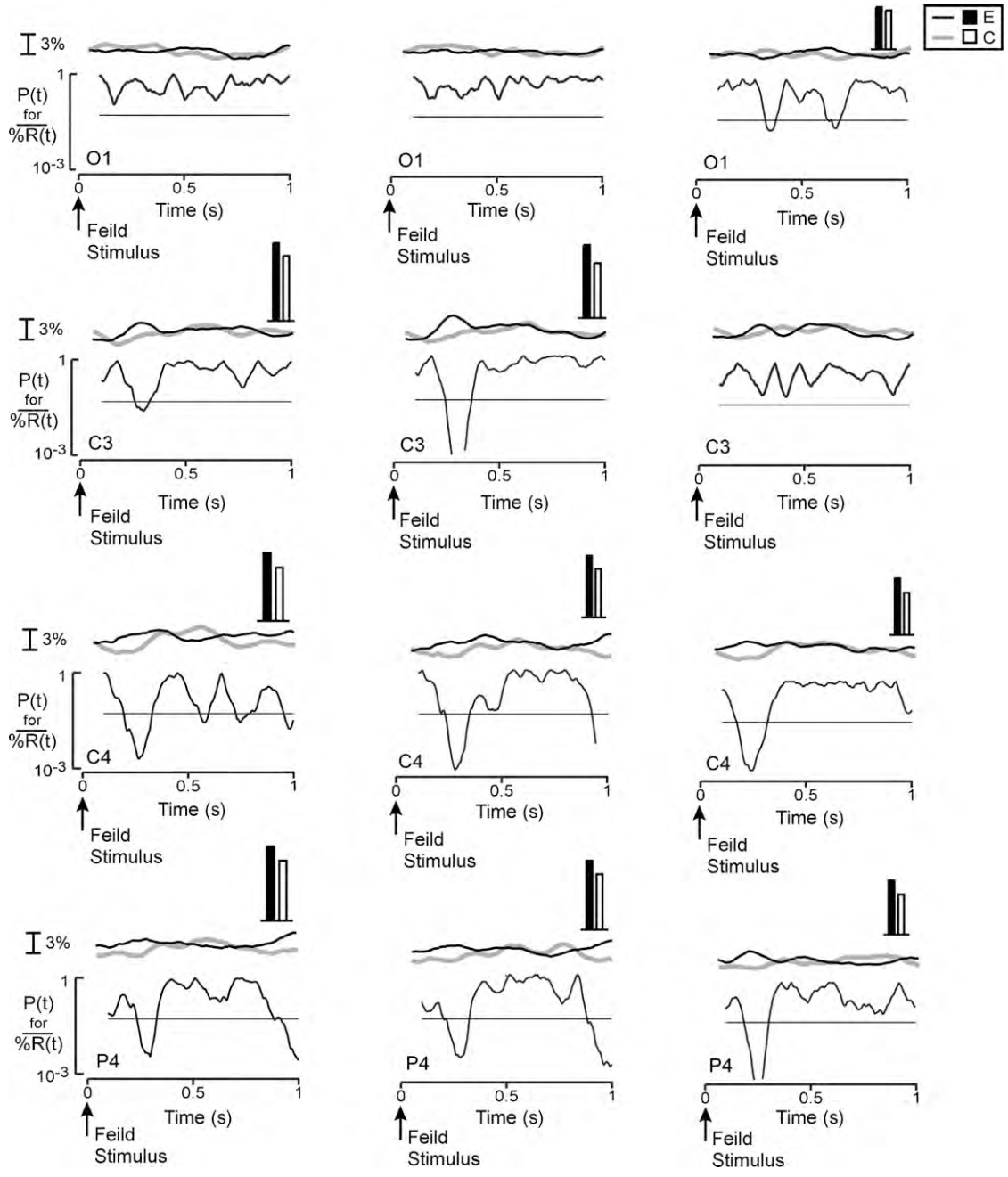

Fig. 5. Effect of filtering the EEG from four electrode derivations in subject S11 (60 Hz) on the detection of MEPs using $\overline{\%R(t)}$. Left, no filtering; center, 8–10-Hz components digitally removed; right, 9–12-Hz components digitally removed. The curves at the tops of the panels show the average values of the exposed (*E*) and control (*C*) epochs in the field-exposed trials ($N \geq 50$ trials). $P(t)$, probability of a difference at time t. The bar graphs (drawn to the indicated scale) show the average value of $\overline{\%R}$ over the latency interval for which $P(t) < 0.05$ (the standard deviations are not resolved at scale shown).

Table 2
Latency and duration of MEPs stratified by stimulus frequency, gender, and electrode derivation

	Stimulus (Hz)		Gender		Electrode	
	30	60	Male	Female	Occipital	Non-occipital
Latency (ms)	313 ± 57	308 ± 57	311 ± 55	309 ± 58	304 ± 56	322 ± 55
Duration (ms)	266 ± 31	259 ± 29	263 ± 31	264 ± 30	265 ± 33	262 ± 28
N	48	47	44	51	43	52

N: number of MEPs; total (unstratified) *N*: 95 (Table 1).

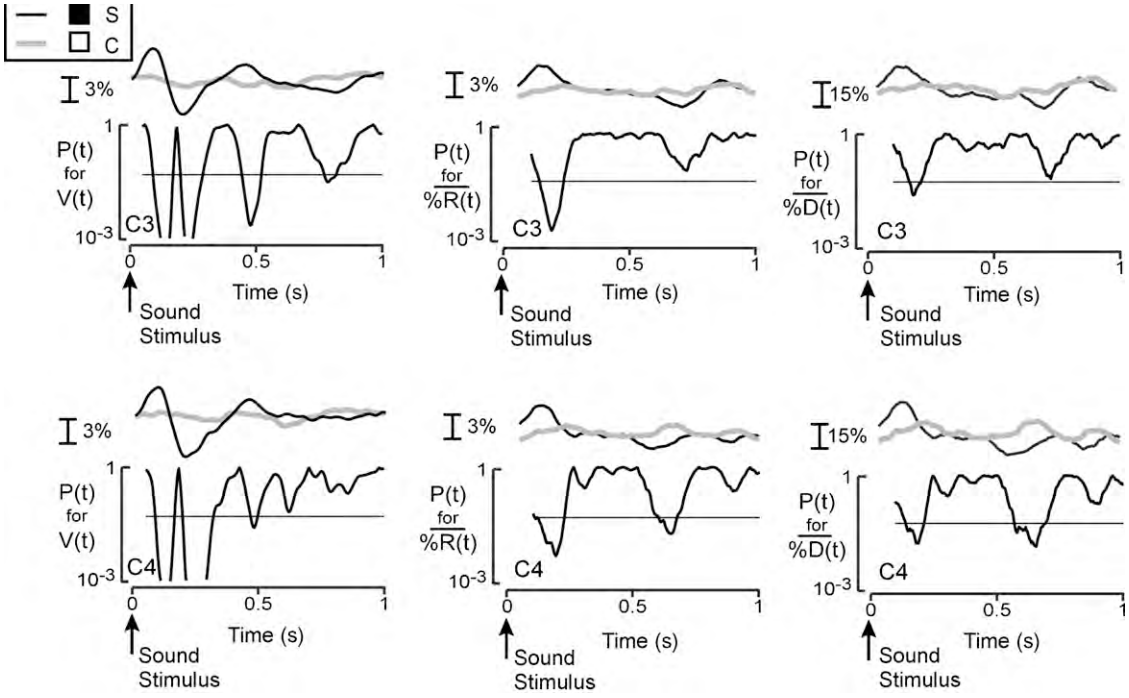

Fig. 6. Auditory evoked potentials in subject S5 from C3 (top row) and C4 (bottom) detected by time averaging (left column) and recurrence analysis (center and right). The curves at the tops of each panel show the average values of the sound (*S*) and control (*C*) epochs ($N \geq 50$ trials). $P(t)$, probability of a difference at time t. The bar graphs (drawn to the indicated scale) show the average value of the indicated variable over the latency interval for which $P(t) < 0.05$ (the standard deviations are not resolved at scale shown).

MEPs were not detected in any subject based on an analysis of the EEGs using time averaging with or without alpha filtering (data not shown). The *a posteriori* false-positive rate in the EEG (>10 pair-wise significant tests) was 16/180 = 0.089. The corresponding P_{FW} was less than 0.05 in 2 of the 30 experiments, but there were 3 false-positive effects in the shams, indicating that analysis of the EEG did not furnish evidence of any MEPs. In contrast, auditory evoked potentials (AEPs) could be routinely observed in the EEG. For example, AEPs were prominent in the signals in the central derivations from subject S5 (Fig. 6).

When the individual recurrence time series were averaged over the time interval for which the point-wise comparison with the control was statistically significant, the mean value of the recurrence variable was sometimes less than the corresponding control, and sometimes greater (Figs. 2–5). The direction of the changes in the variables (expressed as a percent of the average of the sum) was not correlated with the frequency of the stimulus, the electrode derivation, or the recurrence parameter (data not shown). The overall results, summarized without respect to these factors, are given in Fig. 7, which shows the magnitude of each MEP listed in Table 1; they consisted of both increases and decreases in the recurrence parameters with an average absolute value of 29%.

We previously found that unfolding the measured signal in a five-dimensional space using a time delay of five points was optimal for detecting the effect of a magnetic stimulus on the EEG (Carrubba et al., 2007a). The same dimension and the delay were therefore used in the present study. To examine our assumption that these conditions would again be optimal, for three subjects we also unfolded the EEG under other conditions (Table 3). In six experiments (S1, S5, S8 at 60 and 30 Hz), MEPs were detected in five cases when a five-dimensional phase space with a time delay of five points was used, but in only two to three experiments when the EEG was unfolded in the other phase spaces (Table 3).

The sensitivity of RQA for detection of known deterministic signals depended partly on the nature of the dynamical changes

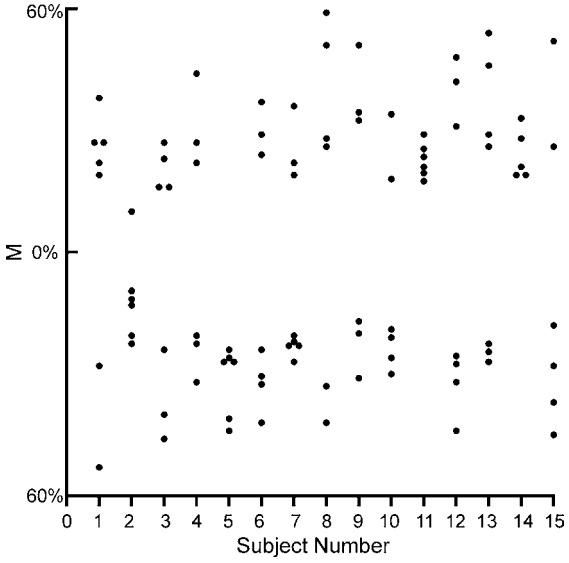

Fig. 7. Magnitude (M) of magnetosensory evoked potentials (MEPs) as determined by recurrence analysis. For each MEP (Table 1), $M = 100(E - C)/0.5(E + C)$. Identical values from different electrodes in a given subject are shown side-by-side.

Table 3
Effect of phase-space parameters on the sensitivity of detection of magnetosensory evoked potentials

Stim. (Hz)	Subject 1			Subject 5			Subject 8		
	Ed, τ	All effects	P_{FW}	Ed, τ	All effects	P_{FW}	Ed, τ	All effects	P_{FW}
60	*5, 5	O1, C4, P4	0.0012	5, 5	O1, **O1**, C3	0.042	5, 5	**O1, O2**, O2	0.003
	5, 3	O1, C3, P4	0.0012	5, 3	**O2**, C3, C4	0.037	5, 3	**O1, C3**	0.286
	5, 1	O1, O2, C3	0.0061	5, 1	**O1, O2, P3**	0.036	5, 1	P3	0.663
	3, 5	O1, **C3**, P4	0.0097	3, 5	C4, **O2**, P4	0.1	3, 5	**O1, O2, C3**	0.056
	3, 3	O1, O2, P4	0.0004	3, 3	**O2, C4**, P4	0.094	3, 3	**O1, C4, P3**	0.01
	3, 1	O2, **O2**, C3	0.009	3, 1	C4, P4	0.251	3, 1	O1, P3	0.297
30	*5, 5	O2, **O2**, C3, **C3**	0.0005	5, 5	O1, P3, **P3**	0.01	5, 5	O2, P4, **P4**	0.091
	5, 3	O1, O2, C3, P4	0.00003	5, 3	P3, **P3**	0.288	5, 3	O2, P4, **P4**	0.052
	5, 1	O1, C3, **P4**	0.076	5, 1	**O1, O2**	0.067	5, 1	**O1, O2**, P3	0.014
	3, 5	O1, O2, C3, P4	0.00003	3, 5	**P3**	0.68	3, 5	**O1**, C3, **P4**	0.129
	3, 3	O1, **O2**, C3, **C3**	0.001	3, 3	**O1**, C3	0.265	3, 3	**C4, P3**, P3	0.078
	3, 1	O1, **O1**, P4	0.003	3, 1	**O1**	0.390	3, 1	P3, **P4**	0.297

P_{FW}: computed using a comparison-wise error rate of 0.0483; Ed: embedding dimension; τ: number of points in the time delay (3.3 ms/point); *embedding parameters used in this study.

that occurred in the control epochs (Fig. 8). To explore the limitations of RQA, we studied its use in a model system consisting of the addition of nonlinear signals to background EEG. We randomly selected 50 300-ms segments of one of the solutions to a set of nonlinear equations (Fig. 8a) and added one segment at $t = 0.3$–0.6 s to each of 50 7-s EEG trials (Fig. 8b). As expected, the average EEG did not reveal the presence of the added segments (E compared with E_0, Fig. 8c). However, the

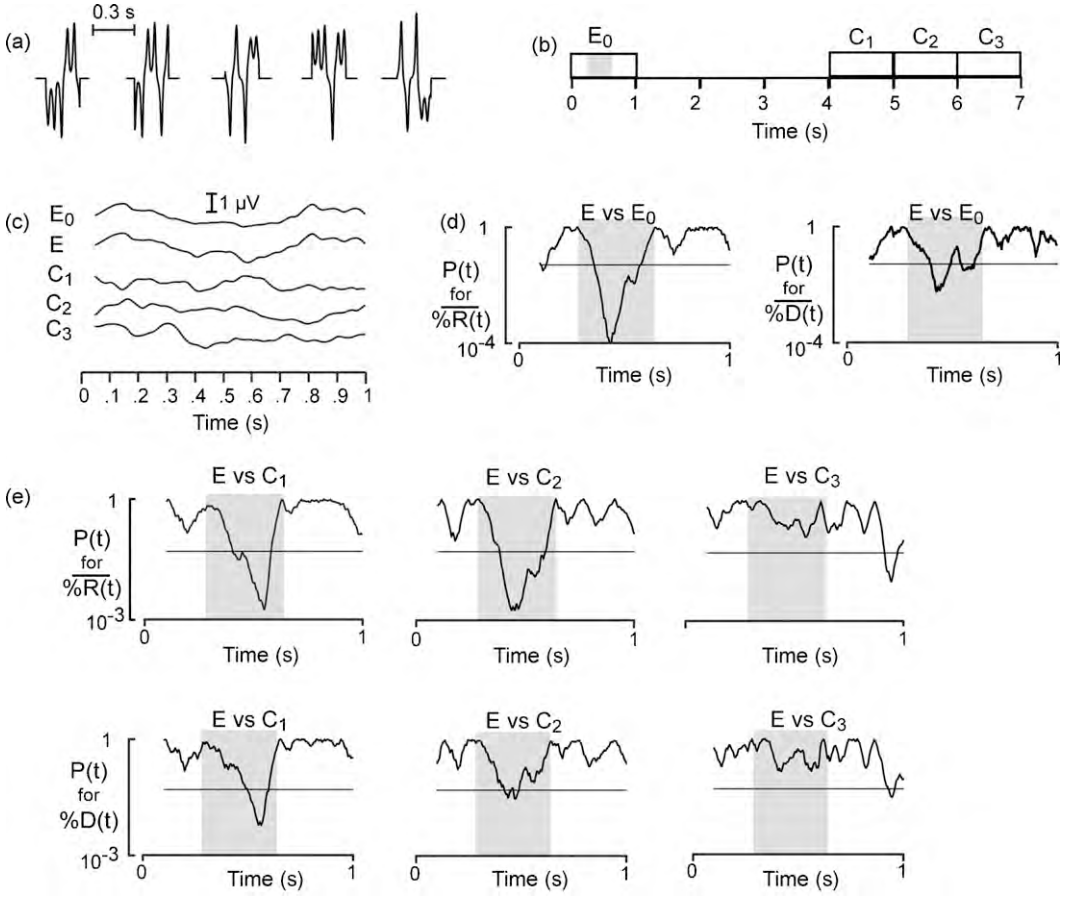

Fig. 8. Detection of known nonlinear activity present in the EEG. (a) Typical examples of segments of nonlinear signals that were added to the E_0 epoch (at $t = 0.3$–0.6 s), after which it is designated as the E epoch (rms of each added segment was equal to that of the EEG epoch to which the segment was added). The segments were selected randomly from a solution to the Lorenz equations (Abarbanel, 1996) operating in the chaotic mode ($\sigma = 16$, $r = 45.92$, $b = 4$). (b) Definition of experimental (E) and control (C1, C2, C3) epochs within a trial. Stippled region ($\tau = 0.3$–0.65) indicates location of the added Lorenz signal. (c) Average of the indicated time series. (d) Probability of a difference in means between E_0 and E_1, assessed using $\overline{\%R(t)}$ and $\overline{\%D(t)}$. (e) Effect of the choice of the control epoch on the ability to detect the added Lorenz determinism by means of recurrence analysis.

added determinism was detected by RQA (Fig. 8d). In an actual experiment, the presence of a putative signal must be detected on the basis of a comparison with a suitable control epoch because the EEG that would have been measured in the absence of the added signal is unknowable, unlike the model system (Fig. 8d). The ability to detect the added signals was sometimes affected by the choice of the control (Fig. 8e).

4. Discussion

Attempts to understand the effects of low-strength EMFs on brain activity have foundered on the consistent inconsistency of each of the various types of studies, leading to a pessimism bordering on despair (Crasson, 2003; D'Andrea et al., 2003). Inconspicuous experimental errors or hidden variables such as personality or laterality could account for a portion of this pattern of inconsistency (Cook et al., 2006), but a more global explanation is that it is artifactual and stems from the common use of inapplicable methods of analysis. Essentially all studies of EMF-induced effects on brain activity used linear methods and were thus unable to reliably detect nonlinear stimulus–response patterns. To support the concept that EMF-induced changes in brain electrical activity are both consistent and nonlinear in origin, evidence is required that the changes can be reliably demonstrated only if nonlinear methods of analysis are used. Our specific purpose was to describe and validate a procedure capable of reliably demonstrating nonlinear magnetosensory evoked potentials (MEPs).

Using RQA, changes from baseline brain electrical activity associated with presentation of a magnetic stimulus were found in all subjects (Table 1). Several considerations indicated that the changes were true MEPs. First, the analysis incorporated appropriate protection against comparison- and family-wise error. Second, comparable changes were not observed in the sham data. Third, the changes occurred several hundred milliseconds after the field had been switched off; this observed latency ruled out the possibility that the changes could have been generated by a field–electrode interaction but was consistent with the inference that they arose from brain processing of afferent signals that resulted from transduction of the field. We conclude, therefore, that the changes were true magnetosensory evoked potentials. There were no gender-related differences in MEP response, which supported our original finding that the ability to detect magnetic fields is a basic property of human beings (Carrubba et al., 2007a).

Magnetosensory evoked potentials were not detected when the EEGs were analyzed by time averaging, indicating that the evoked potentials were nonlinear in origin. Our observation that the changes in recurrence parameters could be either an increase or a decrease (Fig. 7) further confirmed the nonlinearity of the response, because only nonlinear systems can exhibit such behavior. One might intuitively expect an increase in $\%R$, but the addition of completely deterministic signals to a baseline EEG can result in a decrease in $\%R$ (Carrubba et al., 2006). The physical meaning of the bidirectional changes in $\%R$ in the EEG remain unclear. If the data in Fig. 7 were averaged across all the subjects, the average percent change would be less than 1%, highlighting the importance of using each subject as its own control and explicitly indicating how real nonlinear phenomena can be averaged away.

Even though magnetic fields are vectors, the electrophysiological consequences of stimulus transduction did not depend on whether the stimulus (which was in the horizontal plane) was applied coronally (S1–S10) or sagittally (S11–S15) (Table 1). This might mean that the MEP was independent of the angle between the field and the biological structure that mediated transduction. Alternatively, if there was an angular dependence, the results could mean that the spatial distribution of the structures within the nervous system exhibited no preferred orientation in the horizontal plane. Studies showing that comparable MEPs were produced when the stimulus was applied in the vertical and horizontal planes would strengthen the idea that MEPs are not dependent (or at least not strongly dependent) on the vector nature of the stimulus. The MEPs also did not depend on whether the stimulus frequency was 30 or 60 Hz, indicating that they could not be explained on the basis of conditioning by the pervasive presence in the environment of 60-Hz fields from the North American power system. In a study involving cell phone EMFs (10^{10} Hz), effects comparable to those reported here were observed in rabbits (Marino et al., 2003), suggesting that MEPs may be independent of frequency over a wide range.

When an MEP was observed, it was almost twice as likely to have been measured from an occipital electrode compared with either a central or parietal electrode. However, both the latency and magnitude of the MEPs were essentially the same, regardless of the electrode derivation. This observation could be explained if a primary contributor to the MEP were a brain region that was closest to the occipital locations, which would make electrotonic propagation of the signal to the scalp electrodes more efficient.

Filtering within the alpha band was frequently necessary for detection of the MEP by RQA. The rationale for removing alpha energy was that it did not contribute to the response and therefore that its removal increased sensitivity for detection of MEPs by removing noise from the system (Marino et al., 2003; Carrubba et al., 2007a). This might mean that sources of alpha activities, which are usually associated with consciousness or other high-level brain functions (Shaw, 2003), were not crucial in the brain processing that gave rise to the MEPs. The fact that magnetic stimulus was below the level of consciousness is consistent with the idea that MEPs and alpha activities originate in different areas.

Percent recurrence and $\%D$ were sufficient for detection of MEPs using RQA; other recurrence variables (Webber and Zbilut Retrieved Feb. 1, 2007) were therefore not required. It remains unclear whether $\%R$ and $\%D$ actually captured something different regarding the field-induced dynamical changes in brain activity, or whether the results obtained merely indicated that one variable or the other was more sensitive, given the random fluctuations that occurred in the control epoch. Random fluctuations in the control epoch sometimes affected the results in the model system (Fig. 8), and a similar

effect occurred following stimulation: approximately 5% of the overall results (Table 1) occurred at different electrodes when $t = 1$–2 s was used as the control epoch (data not shown). It is clear, however, that the choice of the phase-space embedding conditions can affect the sensitivity for detecting an effect due to the stimulus (Table 3). The relative importance of individual RQA variables or of particular phase-space conditions in the context of other stimulus–response systems remains to be evaluated.

Recurrence analysis has strengths and weaknesses, both of which are formidable. On the one hand, RQA permitted the discovery of the effect of low-strength EMFs on brain electrical activity in human subjects, which is an important class of biological phenomena that had previously been unrecognized. This, in turn, raises the possibility of gaining a deeper understanding regarding the amazingly diverse range of biological phenomena that have been attributed to EMFs (Presman, 1970; Marino and Becker, 1982; Carpenter and Ayrapetyan, 1994; Barnes and Greenebaum, 2006). Additionally, MEPs detected using RQA may prove useful as tools for studying cognitive activity. For example, where cognitive processes have been localized, studies of the interaction of the two stimuli may provide basic information regarding brain dynamics.

On the other hand, little about nonlinear analysis is intuitive, and the nonlinear quantifiers have no known relationship to familiar physiological or cellular properties. They can characterize the system, but what part or aspect of the system that they characterize remains unclear, and the physiological meaning of changes in the parameters remains undefined. In linear systems, changes in biological parameters are usually interpreted as beneficial or harmful depending on their magnitude and direction. Such an interpretation is generally not possible with nonlinear quantifiers. Finally, and perhaps most disturbing, the best that can be said for RQA of nonlinear systems is that it makes it possible to say something truthful about the system, not ‘‘the’’ truth, but simply ‘‘a’’ truth. It is possible, for example, that an analysis involving the recurrence of the recurrence time series might contain information regarding the response of the subjects to the stimulus that was not apparent based on the present analysis.

In summary, MEPs may be reliably detected in individual subjects by embedding the digitally sampled EEG in a five-dimensional phase space and analyzing the associated recurrence plot using the variables $\overline{\%R(t)}$ and $\overline{\%D(t)}$ to detect stimulus-induced changes occurring with a latency of 109–504 ms.

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