Direct Recording of Theta Oscillations in Primate Prefrontal and Anterior Cingulate Cortices

Toru Tsujimoto,1,3 Hideki Shimazu,2,5 and Yoshikazu Isomura1,4,6
1Department of Integrative Brain Science and 2Department of Neurology, Graduate School of Medicine, Kyoto University, Kyoto; 3Center for Brain Experiment, National Institute for Physiological Sciences, Okazaki; 4Department of System Neuroscience, Tokyo Metropolitan Institute for Neuroscience, Tokyo; 5Department of Neurology, Graduate School of Medicine, Tokushima University, Tokushima; and 6Neural Circuit Theory, RIKEN Brain Science Institute, Saitama, Japan

Submitted 11 July 2005; accepted in final form 31 January 2006

Tsujimoto, Toru, Hideki Shimazu, and Yoshikazu Isomura. Direct recording of theta oscillations in primate prefrontal and anterior cingulate cortices. J Neurophysiol 95: 2987–3000, 2006. First published February 8, 2006; doi:10.1152/jn.00730.2005. Recent evidence has suggested that theta-frequency (4–7 Hz) oscillations around the human anterior cingulate cortex (ACC) and frontal cortex—that is, frontal midline theta (Fm theta) oscillations—may be involved in attentional processes in the brain. However, little is known about the physiological basis of Fm theta oscillations because invasive study in the human is allowed in only limited cases. In the present study, we developed a monkey model for Fm theta oscillations and located the generators of theta waves using electrodes implanted in various cortical areas. Monkeys were engaged in a self-initiated hand-movement task with a waiting period. The theta power in area 9 (the medial prefrontal cortex) and area 32 (the rostral ACC) was gradually increased from a few seconds before the movement and reached a peak immediately after the movement. When the movement was rewarded, the theta power attained a second peak, whereas it swiftly decreased in the unrewarded trials. Theta oscillations in areas 9 and 32 were coherent and phase locked together. This theta activity may be associated with “executive attention” including self-control, internal timing, and assessment of reward. It is probably a homologue of associated with “executive attention” including self-control, internal timing, and assessment of reward. It is probably a homologue of.

INTRODUCTION

Theta-frequency (4–7 Hz) oscillations in the human brain are observed in a large variety of different situations (for review, see Schacter 1977). Although our present knowledge is insufficient to form a generalized theory, recent electrophysiological studies have suggested that some theta oscillations play important roles in the neural processes of memory, cognition, and attention in humans and animals (for review, see Basar et al. 2001; Kahana et al. 2001; Klimesch 1999). It has been established that frontal midline theta (Fm theta) oscillations are involved in attentional processes (for review, see Inanaga 1998). They have been recorded around the frontal midline region of the scalp by electroencephalogram (EEG) (Ishihara and Yoshii 1972) and are observed in various conditions, including problem solving (Arellano and Schwab 1950; Ishihara and Yoshii 1972), continuous arithmetic operations (Ishihara and Yoshii 1972; Sasaki et al. 1994, 1996c), verbal and spatial tasks (Gevins et al. 1979), working memory tasks of verbal and spatial modalities (Gevins et al. 1997; Grunwald et al. 1999; McEvoy et al. 2001; Smith et al. 1999), Stroop tasks (Yamada 1998), video game operation (Laukka et al. 1995; Slobounov et al. 2000; Smith et al. 1999; Yamada 1998), time-measuring tasks (Sasaki et al. 1996b), meditation (Aftanas and Goloubeikine 2001; Sasaki et al. 1996a), error monitoring (Luu et al. 2001, 2003, 2004), and the peek-a-boo game of infants (Stroganov et al. 1998). Individual differences in the appearance of Fm theta activity have been reported. Subjects with higher Fm theta activity tend to be less anxious, less neurotic, and more extrovert (Mizuki et al. 1984), and have higher cognitive ability (Gevins and Smith 2000). Higher Fm theta activity in patients with major depression predicts a better response to treatment (Pizzagalli et al. 2001). These findings suggest that Fm theta oscillations may play an important role in attentional functions.

However, the physiological basis of Fm theta oscillations is still unclear. Because invasive study in the human subject is allowed in only limited cases, it would be rather difficult to investigate their neural substrate and functional mechanisms in the human brain. If a monkey model for Fm theta oscillations was available, it would be possible to take advantage of many techniques, such as single-cell recording, ablation, electrical stimulation, and pharmacological methods. Consequently, we developed a monkey model as follows: human Fm theta oscillations have been observed mostly in tasks requiring intellectual ability, which are too difficult for the monkey. As a substitute, we chose a self-initiated hand-movement task with a waiting period. The task required the monkey to wait for a fixed interval after movement before carrying out the next movement. This would require the monkey brain to be loaded with the executive attention (Posner and Rothbart 1998) of self-control, internal timing, readiness for action, and assessment of result, successively in the course of task execution. We examined the feasibility of the model by recording the cortical field potentials and showed that this task successfully induced theta oscillations in the frontal cortex. We identified the source of the currents that generated the oscillatory field potentials in the theta frequency range using electrodes arranged in pairs, one at the surface and one deeper in the cortex, and confirmed that the theta activity recorded was not a product of volume conduction from a remote source. Recording from the mesial
wall of the hemisphere and the inside of the sulci, as well as the
dorsolateral convexity of the brain, we investigated the nature
of the theta activity, including the source distribution, fre-
cquency, task-related power modulation, intercortical correla-
tions, and phase relations to the task. The homology to human
Fm theta oscillations was then evaluated.

METHODS

Subjects

Three adult Japanese monkeys (4–6 kg, two female and one male)
were used in the study, as approved by the institutional ethics
committee. All experimental procedures were carried out in accord-
dance with the National Institutes of Health/Institute of Laboratory
Animal Resources Guide for the Care and Use of Laboratory Animals.

Behavioral task

During the experiment, the monkeys were seated in a primate chair
equipped with a hand lever, a reward dispenser, and a head holder
(Fig. 1A). From 24 h before each experimental day, the water supply
was restricted to half the amount of their average daily free drink. The
task was to lift a lever with the hand in the monkey’s own time
without external cues. When the monkey kept holding the lever in the
resting position for more than a fixed waiting period before lifting the
lever, the movement was rewarded with a drop of water (~0.2 ml,
delivered 0.6 s after the onset of movement). The holding of the lever
by the monkey was monitored by an electrostatic touch sensor. If the
monkey released the lever or made a premature movement, it initial-
ized the timer for the waiting period. Starting from 2 s, the waiting
period was gradually increased in the early training phase. As the
waiting period increased, the task was considered to be more difficult.
The waiting period was finally set to 6 s, and the monkeys were
trained for several weeks after they had reached a steady state. This
waiting period was determined so that the monkeys succeeded in
about two thirds of the trials.

Recordings

Electrodes were chronically implanted under pentobarbital sodium
anesthesia (initial dose more than 35 mg/kg, administered intrave-
nously, followed by additional injections as needed). The electrodes
for recording cortical field potentials (silver needles of 0.20 mm
diameter, insulated by Teflon except at their pointed tip) were ar-
anged in pairs, with one of each pair at the surface and the other at
a depth of 2.5–3.5 mm at various cortical sites (Fig. 1B), and were
fixed to the skull with acrylic resin and screws. Electrodes for the
electrooculogram (EOG) were buried in the orbital bone and control
electrodes were set in the bone marrow behind the ear on both sides.
The linked control electrodes served as the reference for the cortical
field potentials. After a recovery period of ~1 mo, retraining and
recording sessions were initiated. Data were recorded in the task and
resting (Rest) conditions. In the latter, the monkey was awake and
seated, but not engaged in any particular task. The cortical field
potentials and EOGs were processed by amplifiers at a 5-s time
constant and with a 100-Hz high-cut filter, digitized at 250 Hz, and
stored in a computer.

Data analysis

The main origin of the cortical field potential is synaptic activity on
the apical dendrites of pyramidal neurons (Fig. 2Aa) (for review, see
FIG. 2. Outline of data analyses. A: generation of cortical field potentials. a: extracellular currents (arrows) caused by synaptic activity generate a field potential in the extracellular volume conductor. Figure is an example of cases in which excitatory synaptic input arrived at the distal portion of the apical dendrite of a pyramidal neuron. b: when populations of pyramidal neurons are synchronously activated at the distal portion of the apical dendrite, the contribution from the synaptic activity can overlap and produce a measurable surface-negative and depth-positive electric field within the cortex. Alternatively, when the proximal portion of the apical dendrites is activated by excitatory synaptic input, a surface-positive and depth-negative field potential will be produced.

B: recording field potentials with S and D electrodes. a: if oscillatory field potentials are generated at the location of the recording electrodes, they are recorded as antiphase signals by the S and D electrodes. Because the S and D signals have opposite polarities, the amplitude of the signals becomes bigger in surface minus depth (S/H11002D) potentials. b: if field potentials originate from a remote source, they are probably recorded as in-phase signals by the S and D electrodes. Amplitude of such activities is canceled and reduced in S/H11002D potentials because the S and D signals have the same polarities.

C: phase relative to epoch indicates the relative location of oscillatory activity within an epoch (see text). D: time series data of S/H11002D potentials were segmented into epochs with \( t \) moving in 0.1-s steps, and converted to Fourier series data by discrete Fourier transform (DFT). Power spectra and the Rayleigh statistic \( R \) for the phase difference, which were calculated from the Fourier series data of X and Y in the same event-related manner as in D. X and Y can be the S and D potentials of the same recording site or 2 S/H11002D potentials of different areas.
Mitzdorf 1985). When populations of apical dendrites in a cortical region are synchronously activated at approximately the same location along their length, the contribution from the synaptic activity can overlap and produce a measurable electric potential gradient along the surface-depth direction within the cortex (Fig. 2a,b). The paired surface (S) and depth (D) electrodes were arranged to measure the dipole component of such an electric field (Fig. 2b). If oscillatory field potentials are generated between the S and D electrodes, the S and D potentials are recorded in opposite polarities and the phase difference should be around π (Fig. 2b). Conversely, in a situation where the field potentials originate from a remote source, the S and D signals probably have a common polarity and the phase difference should be around zero (Fig. 2b). Therefore by calculating the surface minus depth (S – D) potential, we are able to reduce the signal components of remote origins. This analysis has already been established as a useful method for assessing the source localization (e.g., Hashimoto et al. 1981; Sasaki et al. 1981, 1982). In this study, we used S – D potentials to evaluate regional activity, and examined the phase difference between S and D to assess whether a signal component found in the S – D potentials was generated at the recording site.

The data from the task condition were segmented into overlapping artifact-free epochs of 1,024 ms (256 points), giving a resolution of 0.98 Hz using discrete Fourier transform (DFT). The data from Rest were consecutively segmented into nonoverlapping artifact-free epochs of 1,024 ms. The epoch data were processed by the removal of DC bias and linear trend, application of a window function (a Kaiser window with the adjustable parameter set to 4π), and DFT. To calculate event-related spectra at a specified latency, denoted by t, from the onset of an event (movement), the operation was carried out on the set of epochs whose centers were T(n) + t (n = 1, 2, . . ., N), where n is the trial number, N is the total trial number, and T(n) is the onset time of an event in nth trial (Fig. 2, D and E). The transition of spectra was obtained by moving t in 0.1-s steps. To stabilize the variance, we applied a logarithmic transformation to the power spectra before calculating the mean and other statistical values (Halliday et al. 1995). Exponential transformation was applied to return the data to the original space before displaying the power spectra.

To analyze phase locking between oscillations and external events (movement and reward), we calculated the phase relative to epoch for the S – D potentials (Fig. 2c). Phase relative to epoch is defined as the angle φ, where the complex number of the DFT product at a specified frequency is expressed in the polar coordinate form of Ae^iφ. The null hypothesis of uniformity for the distribution of φ was tested using the Rayleigh statistic

\[ R = \sqrt{\frac{\sum \cos \phi^2 + \sum \sin \phi^2}{N}} \]

The summation in the preceding expression covered the set of epochs whose centers were T(n) + t (n = 1, 2, . . ., N) (Fig. 2d). The value of R ranges from 0 to 1, and R = 1 indicates complete phase locking.

To examine phase locking between two potentials X and Y, we calculated the Rayleigh statistic R for the phase difference φ_d = φ_X – φ_Y, where φ_X and φ_Y are the phase relative to epoch of the two potentials (Fig. 2e).

When we needed to confirm that an elevation in the coherence or Rayleigh statistic between two potentials, X and Y, was a true effect and not caused by a systematic error hidden in the experimental arrangement, we calculated the control statistic between the unmatched pairs of shuffled data, X(1)–Y(2), X(2)–Y(3), X(3)–Y(4), . . ., (X(N – 1))–Y(N), and Y(1)–X(N) where X(n) and Y(n) stand for the potentials X and Y in the nth trial and N is the total trial number. These statistical values were compared with the 95% confidence limits that were theoretically computed (Halliday et al. 1995).

Recording sites

After electrophysiological investigations, the monkeys were deeply anesthetized with an overdose of pentobarbital sodium and were perfused through the heart with 10% formaldehyde neutral buffer solution. The brains were cut into 50-μm-thick sections and stained for Nissl bodies. The penetration of recording electrodes was identified and photographed using a microscope. After confirming that degenerative tissue changes did not extend beyond the region adjacent to the electrodes, the recording sites were plotted on a standard brain map showing the positions in relation to the morphological landmarks of the major sulci and corpus callosum. The map was adopted from the Stereotaxic Atlas of the Brain of Macaca fascicularis (Kusama and Masbuchi 1970) and was complemented with a mesial view of our own brain specimen of similar dimensions. The cortical subdivisions were drawn at the estimated positions by consulting previous studies (Ongur and Price 2000).

RESULTS

Modulation of theta oscillations

We noted characteristic theta oscillations, in particular cortical regions (e.g., anterior cingulate cortex; Fig. 1, C–E), in all three monkeys. Figure 3 shows a representative record of 686 trials in Monkey A. In Fig. 3a, a segment of the raw data is shown with a timing chart of the task. The traces S and D are the raw cortical field potentials recorded at the surface and at a 3-mm depth in Walker’s area 9 ipsilateral to the moving hand (i.e., left in this case). Sinusoidal waves at about 5 Hz often appeared in the raw traces in opposite polarities between S and D. Part of the data are expanded in the inset. With waxing and waning, the amplitude of the theta waves sometimes measured up to about 200 μV (peak-to-peak) in the S – D trace.

The behavioral performance is shown as a histogram of the onset of the preceding and the following movements (Fig. 3b). Of 686 trials 195 were unrewarded because the monkey had not held the lever for a sufficient duration during the waiting period (Fig. 3b, red). The remaining 491 of 686 trials were rewarded (Fig. 3b, black).

A time–frequency analysis showed that the theta power of the S – D potential in area 9 was modulated in relation to the task (Fig. 3c). The power in the theta band is plotted in Fig. 3d. It was gradually increased from a few seconds before the movement and reached a peak immediately after the movement in both the rewarded and unrewarded trials. In the rewarded trials, it reached a second peak after the reward delivery, whereas it rapidly decreased in the unrewarded trials. The modulation was noted throughout the whole theta band and was most prominent at 4.9 Hz in this monkey.

Coherence and phase

The coherence between S and D in the theta band was significant at the 95% confidence level throughout the trials, for both the rewarded and the unrewarded cases (the solid and broken lines in Fig. 3e). Control coherence was calculated between the unmatched pairs of S(1)–D(2), S(2)–D(3), S(3)–D(4), . . ., S(N – 1)–D(N), and S(N)–D(1), where S(n) and D(n) stand for the S and D potentials in the nth trial and N is the total trial number. The control coherence (the dotted lines in the lower part of Fig. 3e) was not significant at the 95% confidence level and showed no clear event-related modulation, which together indicate that the significant coherence...
between the matched pairs (the solid and broken lines in Fig. 3E) cannot be attributed to a systematic error hidden in the recording arrangement.

The phase angle of S as referenced by D was 0.97π ± 0.03π (mean ± SD at 3.9 Hz), 0.97π ± 0.02π (4.9 Hz, plotted in Fig. 3F), 0.98π ± 0.01π (5.9 Hz), and 0.99π ± 0.01π (6.9 Hz) during the time region [-10 s, 10 s].

This theta activity was probably generated from the cortical region between the S and D electrodes and was not a result of a current spread from remote sources because the phase angle between S and D was around π (Figs. 3F and 2B).

**Statistical evaluation**

For quantitative and statistical evaluation of changes in spectra, the time regions of interest—R1 = [-3.7 s, -2.8 s], R2 = [-2.0 s, -1.1 s], R3 = [-0.3 s, 0.6 s], and R4 = [1.2 s, 2.1 s]—were set as marked in Fig. 3D. R1, R2, and R3 were arranged to analyze the gradual increase in the theta power from the premovement base to the postmovement peak. R4 was adjusted to compare the difference between the rewarded and unrewarded trials. In Fig. 3G, the mean power spectra are calculated for each time region and Rest. Rest included 620 epochs, which indicates that the modulation of power spectra was maximal in the same theta frequency in both the premovement increase and the transient rise after reward.

For statistical evaluation of the gradual increase in the theta power, the theta power in each of R1, R2, and R3 was calculated on a per trial basis for the rewarded trials, collecting the epochs whose center was included in the respective time regions (Fig. 2D), and compared by one-tailed paired Student’s t-test. The increase in the theta power at 4.9 Hz was statistically significant for the comparison both between R1 and R2 (t = 7.47, P < 2e-13) and between R2 and R3 (t = 8.27, P < 7e-16).

For statistical assessment of the difference in theta power between the rewarded and unrewarded trials, the theta power in R4 was calculated on a per trial basis and compared using two-tailed two-sample Welch’s t-test. The difference was statistically significant (t = 11.6, df = 483, P < 9e-28).

The theta power in Rest was lower than in the task condition (Fig. 3G) and was compared with the theta power in R1 calculated on a per trial basis for the rewarded trials using two-tailed two-sample Welch’s t-test. The difference was statistically significant (t = 7.86, df = 1061, P < 9e-15).

**FIG. 3.** Representative data of 686 trials recorded in Monkey A are shown. A: segment of the raw waveform recorded from the S and the D electrodes in the left area 9 (Fig. 6: Monkey A, a) is shown with the time markers of lever movement and reward delivery. S – D potential is the arithmetic difference between the S and D potentials. Scale of 200 μV is for S, D, and S – D potentials. S and D potentials in the dotted rectangle of 1-s duration are expanded in the top. B: onset of the preceding and following movement is shown as a stacked histogram for the rewarded (n = 491) and unrewarded trials (n = 195) in 100-ms bins. Time-axis zero is set to the onset of movement. C: temporal change in the power spectra is calculated for the S – D potential, separately for the rewarded and the unrewarded cases. Dotted lines in the bottom of the figure are the control coherence calculated from the unmatched pairs of data for the rewarded (black) and the unrewarded (red) cases. Dotted lines in the bottom of the figure are the control coherence calculated from the unmatched pairs of data for the rewarded (black) and the unrewarded (red) cases (see text). Control coherence is calculated for 3.9, 4.9, 5.9, and 6.9 Hz, separately for the rewarded and the unrewarded cases. E: time course of the coherence between S and D is plotted, separately for the rewarded and the unrewarded cases (solid and broken lines). Horizontal broken lines denote the theoretical 95% confidence limit for significant coherence for the rewarded (black) and the unrewarded (red) cases. Dotted lines in the bottom of the figure are the control coherence calculated from the unmatched pairs of data for the rewarded (black) and the unrewarded (red) cases (see text). Control coherence is calculated for 3.9, 4.9, 5.9, and 6.9 Hz, separately for the rewarded and the unrewarded cases. F: phase angle of S as referenced by D is plotted at 4.9 Hz. Line width of the plot represents the 95% confidence limit. G: mean power spectra are compared between R1, R2, R3, and R4 time regions as indicated in D and Rest. For R1, R2, and R3, the spectra are of the rewarded cases. For R4, they are compared between the rewarded and the unrewarded cases. Rest included 620 epochs.
Other examples and population results

The same kind of modulation of theta oscillations was observed in several recording sites in areas 9 and 32 of the three monkeys. Representative data recorded from Monkey B and Monkey G are presented in Fig. 4, A–D. The same time regions of interest for Monkey A were set for Monkeys B and G for further analysis (Fig. 4, C and D). The modulation was also recognizable in the grand mean power spectra in these areas (Fig. 4, E and F). When all the power spectra obtained from the recording sites in area 32 were averaged across the monkeys, the resulting grand mean spectra showed essentially the same modulation in the theta band as in Fig. 3G (Fig. 4E). This was also the case for area 9 (Fig. 4F). These results indicate that theta modulation may be a common feature of these areas.

Cortical distribution

To determine the cortical distribution of such theta activity, both types of theta power modulation, i.e., the gradual increase preceding the movement and the difference between the rewarded and unrewarded trials, were statistically tested in all three monkeys across all of the recording sites. The analysis was based on the records of 491 + 195, 545 + 196, and 555 + 285 (rewarded + unrewarded) trials, each taken in a day, for Monkeys A, B, and G, respectively. It was carried out at the frequency with the peak power in the theta band for the individual monkeys (4.9 Hz for Monkey A; 5.9 Hz for Monkeys B and G; see Fig. 6).

The gradual increase in theta activity was determined by the following criteria: Criterion 1, the power in R2 was significantly higher than in R1 (one-tailed paired Student’s t-test; P < 0.05); Criterion 2, the power in R3 was significantly higher than in R2 (one-tailed paired Student’s t-test; P < 0.05); Criterion 3, the coherence between S and D was significant (P < 0.05) and the phase angle between S and D potentials was in antiphase within the range [0.5π, 1.5π] in more than half of the epochs in both R2 and R3. The theta power increase was regarded as significant only when all three criteria were met.

FIG. 4. Representative data recorded from Monkey B (A and C) and Monkey G (B and D) and the grand mean spectra (E and F). A–D: task performance (A and B) and the time course of the theta power (C and D) are displayed in the same formats as in Fig. 3, B and D. Recording sites are Monkey B, a and f and Monkey G, a and d in Fig. 6. E and F: grand mean power spectra in areas 32 and 9 were calculated across the monkeys. For area 32 (E), power spectra obtained from the total 4 recording sites (2 in Monkey B and 2 in Monkey G) were averaged. For area 9 (F), data from the total 16 sites (4 in Monkey A, 8 in Monkey B, and 4 in Monkey G) were combined (see Fig. 6 for their location). Mean power spectra in R1, R2, R3, and R4 were displayed as values relative to the mean power spectra in Rest. Analysis was based on the record of 620, 747, and 612 epochs in Rest and 491 + 195, 545 + 196, and 555 + 285 (rewarded + unrewarded) trials in the task condition for Monkeys A, B, and G, respectively.
The difference in theta power between the rewarded and unrewarded trials was assessed by the following criteria: Criterion A, the theta power in R4 in the rewarded trials was significantly higher than in the unrewarded trials (two-tailed two-sample Welch’s t-test, \( P < 0.05 \)); Criterion B, the coherence between S and D was significant (\( P < 0.05 \)) and the phase angle between S and D potentials was in antiphase within the range \([0.5\pi, 1.5\pi]\) in more than half of the epochs in R4 in the rewarded trials; Criterion C, the power modulation was observed mainly in the theta band (4–7 Hz).

The cumulative result is plotted in Fig. 5. Eleven recording sites out of the total 99 sites were determined as significant generators of the gradual increase; eight of 11 were found in Walker’s area 9 and the remaining three were in area 32. These corresponded to 50% of the total 16 sites in area 9 and 75% of the total four sites in area 32. There was no significant theta increase in areas 24, 46, 12, 8B, 6, 7, the primary motor area (MI), or the primary somatosensory area (SI). With respect to the difference between the rewarded and unrewarded trials, 12 sites were determined as significant sites (seven in area 9, four in area 32, and one in area 24). These mostly overlapped with the significant sites for the gradual increase. The spectra are

![Diagram of brain areas with theta modulation](http://jn.physiology.org/)

**Fig. 5.** Sites of significant theta modulation. Theta power changes were statistically tested for 2 properties: 1) the gradual increase preceding the hand movement and 2) the difference between the rewarded and unrewarded trials (see text). Black marks indicate the significant theta generators. Left part of the mark indicates whether the gradual increase in theta power was significant, and the right part shows whether the theta power was significantly higher in the rewarded trials than in the unrewarded trials. Marks on the opened sulci represent the electrode pairs in the banks of sulci as shown in Fig. 1Bd, S3–D1 and S3–D2. Laterality is relative to the moving hand (ipsi and contra). Graphs show the numbers of the recording sites in which theta power change was significant or not significant. Abbreviations: SMA, supplementary motor area; CMAr, rostral cingulate motor area; MI, primary motor area; SI, primary somatosensory area.
FIG. 6. Mean power spectra are shown in the same format as in Fig. 3G for all the sites of significant theta modulation and for some of the surrounding sites. Spectra in the dotted rectangles (2.9–7.8 Hz) are expanded in the insets. Same marks as in Fig. 5 are used. Spectra were obtained from the sites with the same alphabetical labels on the map. Laterality is relative to the moving hand (ipsi and contra).
plotted in Fig. 6 and the scores for the criteria for all the significant theta generators and some of the surrounding sites are given in supplemental materials (Tables S1 and S2). The significant theta generators showed essentially the same spectral modulation in the theta band as in Fig. 3G. Figure 6 shows that both the theta modulations preceding the movement and after the reward were very similar in their frequency and cortical distribution.

Some sites in the dorsal part of area 46 (46d) showed monotonic increase of the theta power through the time regions of R1, R2, and R3. However, theta oscillations in these sites were in phase between the S and D. They passed Criterion 1 and 2, but were rejected by Criterion 3 [e.g., supplemental materials (Table S1); Fig. 6: Monkey A, b and e]. This theta activity may be caused by current spread from remote sources in areas 9 and/or 32 (Fig. 2Bb). Some sites in area 46 passed Criterion A and B, but showed a power modulation in a broader frequency range than the theta band [e.g., supplemental materials (Table S2); Fig. 6: Monkey A, f, Monkey G, i]. These were rejected by Criterion C. In area 46d, the power in the alpha and beta bands (>8–30 Hz) often increased through the time regions of R1, R2, and R3, and was higher in the rewarded trials than in the unrewarded trials in the time region of R4 (e.g., Fig. 6: Monkey A, b and e; Monkey B, i; and Monkey G, b and i). These results indicate that the oscillatory activity in area 46 has a profile different from that in areas 9 and 32.

When we simply repeat the statistical test across multiple recording sites, some sites may be falsely judged as significant generators by chance. To overcome this problem of multiple comparisons, we applied the same analysis to data taken on different days. This second analysis was based on the records of 378 + 230, 558 + 160, and 459 + 155 (rewarded + unrewarded) trials for Monkeys A, B, and G, respectively. All the significant theta generators in Fig. 5 were again identified as significant generators except for one. The exception was the site in area 24 (Fig. 6: Monkey G, f) in which Criterion A was not met (t = 0.81, df = 213, P > 0.4); therefore the theta generator in area 24 was not confirmed. The significant theta generators in areas 9 and 32 were confirmed not to be false positive by chance.

To ensure that the changes in theta power were not contaminated by EOG, we examined the power spectra of EOG and found no corresponding activity in the theta band.

**Task condition versus Rest**

As shown in Fig. 4, E and F and Fig. 6, there was a tendency that the theta power in Rest was lower than that in the task condition. The difference in the theta power was statistically tested by two-tailed two-sample Welch’s t-test across all the significant theta generators identified in the preceding analysis (eight sites in area 9 and four in area 32). The analysis was carried out at the frequency of the peak in the theta band for individual monkeys and based on 620, 747, and 612 epochs in Rest and 491 + 195, 545 + 196, and 555 + 285 (rewarded + unrewarded) trials in the task condition for Monkeys A, B, and G, respectively. The power in the task condition was calculated on a per trial basis.

For all the significant theta generators, the difference between Rest and R1 in the rewarded trials was statistically significant (P < 0.05).

With respect to the difference between Rest and R4 in the unrewarded trials, it was statistically significant (P < 0.05) in six sites (four in area 9 and two in area 32; Fig. 6: Monkey A, a and d; Monkey G, d, e, g, and h), but not significant in the remaining six sites (four in area 9 and two in area 32; Fig. 6: Monkey B, a, f, g, i, and j; Monkey G, a). In the cases where there was a significant difference, the theta power was lower in Rest than in R4 in the unrewarded trials.

The results indicate that the theta power in Rest was lower than that in the premovement period and that the theta power in R4 in the unrewarded trials sometimes decreased nearly to the level in Rest.

**Intercortical coupling**

Theta oscillations in areas 32 and 9 in both hemispheres showed marked intercortical coupling (Fig. 7). Coherence was maximal at the theta frequency between these areas as shown in the contour plots (Fig. 7B). Coherence and the Rayleigh statistic R between these areas were significantly high at the theta frequency throughout the trial (Fig. 7C). The phase angles were generally in phase within the range [-0.5π, 0.5π] and almost constant throughout the trial (Fig. 7C). The relative phase in the ipsilateral area 9 as referenced by the contralateral area 9 was 0.02π ± 0.01π (mean ± SD) during the time region [-10 s, 10 s] in Monkey A, -0.09π ± 0.01π in Monkey B, and -0.05π ± 0.01π in Monkey G. The relative phase in area 32 as referenced by area 9 in the same hemisphere was -0.04π ± 0.02π in Monkey B and -0.28π ± 0.02π in Monkey G. Because the control coherence and R (dotted lines in Fig. 7) calculated between the unmatched pairs of potentials were not significant at the 95% confidence level, the significant coherence and R between the matched pairs cannot be attributed to a systematic error contained in the recording arrangement. The results indicate that theta oscillations in areas 9 and 32 are significantly correlated and synchronized.

**Latency of peak theta power**

The latency of peak theta power was measured across the significant theta generators identified above. The latency of the first peak after the movement onset was 0.5 ± 0 s (mean ± SD, n = 2) for Monkey A, 0.6 ± 0.05 s (n = 4) for Monkey B, and 0.4 ± 0.1 s (n = 5) for Monkey G. The latency of the second peak after the reward delivery was 0.7 ± 0 s (n = 2) for Monkey A, 0.8 ± 0.04 s (n = 5) for Monkey B, and 1.1 ± 0.1 s (n = 5) for Monkey G. The analysis was based on 491, 545, and 555 rewarded trials for Monkeys A, B, and G, respectively.

**Phase relations between theta oscillations and external events**

Phase locking of the theta activity to the external events (movement onset and reward delivery) was assessed by the Rayleigh statistic R. Although R transiently rose above the 95% confidence limit (with the Bonferroni correction for multiple comparisons) at the moments of the movement and the reward delivery (Fig. 8), the rise was noted in a relatively broader frequency band (>1–20 Hz; data not shown). In other
time regions, $R$ was not significantly elevated throughout the analyzed frequencies (1–125 Hz). The results indicate that the phase of the premovement theta oscillations is not locked to the movement onset and the phase of the postreward theta oscillations is not locked to the reward delivery.

**DISCUSSION**

The main findings of the present study are as follows: The sources of the current generating the oscillatory field potentials in the theta frequency range were identified in areas 9 and 32 while the monkeys performed the self-initiated hand-move-
The frequency of oscillations identified in the present study is associated with executive attention or attention processes in general, and that the task in the present study loaded the monkey with executive attention or attention processes in general, and that the monkey activity was associated with the attentional load of the task. Compatible with such requirement of attention, the theta activity during the premovement periods in the task condition was significantly higher than that in the resting condition (Rest) in the present experiments. Although we should investigate this interpretation further, these findings suggest that the theta oscillations identified in the present study are associated with attentional processes, similar to human Fm theta oscillations. Theta oscillations dependent on attentional processes can also be observed in a task in which hand movement is triggered by warning and imperative stimuli: the theta power in areas 9 and 32 was higher in the warning-imperative interval than in the prewarning period (Tsujimoto et al. 2003).

2) Frequency of oscillation. The peak power of human Fm theta oscillations has been reported at about 5–7 Hz (Asada et al. 1999; Gevins et al. 1997; Inouye et al. 1988; Iramina et al. 1996; McEvoy et al. 2001; Sasaki et al. 1996a, c; Slabounov et al. 2000; Smith et al. 1999; Yamada 1998). The present theta frequency (4.9 Hz for Monkey A and 5.9 Hz for Monkeys B and G) shows a good correspondence with that of human Fm theta oscillations.

3) Dependency on attentional processes. The dependency on attentional processes is one of the significant characteristics of human Fm theta oscillations. Although human Fm theta oscillations are observed in apparently diverse circumstances (see INTRODUCTION), it has been established that they are involved in common neural processes for attentional functions (Inanaga 1998; Ishihara and Yoshii 1972). In the present experiment, we observed two phases of theta modulation in areas 9 and 32, i.e., a gradual increase in theta power preceding the movement and a transient rise in response to the reward. Because no direct cues for timing were given in the task, the monkey had to time the duration internally and to modulate the internal cue criterion retroactively, depending on whether the reward was acquired successfully. The first phase of gradual increase may be related to self-control, internal timing, and readiness for action. The second phase of transient rise may be involved in the assessment of reward. This reward-related theta modulation may also be associated with the process of success/error judgment (Falkenstein et al. 1991; Gehring et al. 1993; Gemba et al. 1986; Luu et al. 2003). According to Posner et al. (1994, 1998), it seems that “executive attention” is actively involved in the present experimental paradigm, which requires a sequential occurrence of self-control, internal timing, readiness for action, assessment of reward, and success/error judgment. Therefore it is most likely that the task in the present study loaded the monkey with executive attention or attention processes in general, and that the theta activity was associated with the attentional load of the task.

A monkey model for human Fm theta oscillations was developed and its validity has been tested in the present study. We propose that this model is likely to represent the monkey counterpart of human Fm theta oscillations and would be useful for studying executive functions in the frontal cortex for the following three major reasons: First, the source distribution identified in the model is compatible with that of human Fm theta oscillations (Source distribution). The second is the correspondence in the frequency (Frequency of oscillations). The third and last reason is that the present theta activity in the monkey is generated in a manner strongly dependent on attentional processes, similar to human Fm theta oscillations (Dependency on attentional processes). We give a more detailed explanation for these reasons below.
On these grounds, we propose that the theta oscillations identified in areas 9 and 32 may serve as a relevant model for human Fm theta oscillations.

Functional localization and theta oscillations

Several lines of evidence indicate that, in both the monkey and the human, the ACC is involved in executive processes, including attention allocation, motivated attention, assessment of motivational content, drive, emotion, error detection, motor control, and cognition (for review, see Carter et al. 1999; Ingvar 1994; MacLeod and MacDonald 2000; Mesulam 1981; Paus 2001; Posner et al. 1988, 1990, 1998; Vogt et al. 1992). It is also accepted that the ACC is functionally not a homogeneous area. Activation of area 32 is more often associated than area 24 with difficult tasks (Paus et al. 1998). As a relative tendency, the rostral ACC is involved in affective functions, whereas the caudal ACC is related to cognitive functions (for review, see Bush et al. 2000; Devinsky et al. 1995; Drevets and Raichle 1998; Paus 2001). The behavior of the theta oscillations found in the present study is in accordance with such functional localization in the rostral ACC and area 32. Two topics that have been independently studied in the human, i.e., the executive function of the rostral ACC, as revealed mainly by functional neuroimaging and neuropsychology, and Fm theta oscillations, as investigated by EEG and MEG, may be two facets of the same phenomenon.

Theta oscillations in Walker’s area 9 are another important finding. The functional role of area 9 is still under debate, although its involvement in executive monitoring within working memory has been suggested by a lesion study (Petrides 2000). The present results suggest that area 9 may be involved in attentional processes in cooperation with the rostral ACC. The coherence and synchronization of theta oscillations between areas 9 and 32 revealed by the present study suggest close functional coupling of these areas. Anatomically, area 9 of the monkey is situated in a pivotal position to interface with both the ACC and the dorsolateral frontal cortex, such as Walker’s area 46 and the premotor area, interconnecting them through reciprocal cortico-cortical projections (Barbas et al. 1999; Carmichael and Price 1996; Morris et al. 1999; Passingham et al. 2002). There is supportive evidence by positron emission tomography for the involvement of areas 9 and 32 in executive functions: in both areas 9 and 32, the regional cerebral blood flow is correlated to the supposed willingness of the monkey to the task (Tsujimoto et al. 2000).

Interaction with other activities

If theta oscillations interact with external events such as movement or reward delivery, there may be phase locking between the theta oscillations and the external events. The increase of the Rayleigh statistic $R$ was noted only at the moments of the movement and reward delivery (Fig. 8), however, and was not limited to the theta band. In time domain, it probably corresponds to transient-evoked potentials after the movement and reward delivery. With respect to the theta oscillations during the premovement gradual increase and the transient rise after reward, we obtained negative evidence for phase locking. This does not necessarily mean that the oscillatory phase is not important or that there is no interaction. Because the interaction between external events and areas 9 and 32 is presumably not direct but mediated by other regions in the brain, the phase relations may be blurred during such polysynaptic relays.

The present results pose a question as to how the theta rhythms in areas 9 and 32 are generated and interact with other activities in the brain. The common anatomical features of areas 9 and 32 are the connections with the thalamic dorsomedial (MD) and ventral anterior (VA) nuclei, caudate nucleus, hypothalamus, and hippocampal/parahippocampal regions (Barbas et al. 1991, 1995; Goldman-Rakic and Porrino 1985; Morris et al. 1999; Ray and Price 1993; Rempel-Clower and Barbas 1998; Yeterian and Pandya 1991). There may be an interaction between these structures and areas 9 and 32 through theta oscillations.

The hippocampus may be particularly important in this context. It is thought that theta oscillations in the hippocampus and their interaction with the neocortex have essential roles in the neural system (for review, see Buzsáki 2002; Vanderwolf 1988; Vertes and Kocsis 1997; Vinogradova 1995). In rodents, the activity of the prefrontal neurons is correlated with and phase locked to hippocampal theta oscillations (Hyman et al. 2005; Siapas et al. 2005). In monkeys, a well-practiced self-initiated hand movement is preceded and accompanied by desynchronization of low-frequency rhythmic potentials in the hippocampus, suggesting that the hippocampus may also be involved in the initiation and control of voluntary movement in the primate (Arezzo et al. 1987). The prefrontal–hippocampal interaction should be investigated further.

In conclusion, we have developed a monkey model for human Fm theta oscillations. A homologue of human Fm theta oscillations was identified in areas 9 and 32. The findings suggested that theta oscillations in areas 9 and 32 may play an important role in attentional processes. The model may be useful for studying executive functions of the frontal cortex.

Acknowledgments

We thank Prof. T. Ishihara for the kind suggestions, Dr. N. Kato for thoughtful guidance, Dr. N. Hatanaka for expert histological advice, Prof. S. Kawaguchi and Pres. K. Sasaki for support, and O. Ichikawa, I. Yokoi, and S. Sato for technical assistance.

Grants

This research was partly supported by grants from the Uehara Memorial Foundation, the Casio Science Promotion Foundation, and a Grant-in-Aid for Scientific Research 10780498 from the Ministry of Education, Science and Culture of Japan.

References


