Lukatch, Heath S. and M. Bruce MacIver. Physiology, pharmacology, and topography of cholinergic neocortical oscillations in vitro. J. Neurophysiol. 77: 2427–2445, 1997. Rat neocortical brain slices generated rhythmic extracellular field [microelectroencephalogram (m-EEG)] oscillations at theta frequencies (3–12 Hz) when exposed to pharmacological conditions that mimicked endogenous ascending cholinergic and GABAergic inputs. Use of the specific receptor agonist and antagonist carbachol and bicuculline revealed that simultaneous muscarinic receptor activation and γ-aminobutyric acid-A (GABA_A)-mediated disinhibition were necessary to elicit neocortical oscillations. Rhythmic activity was independent of GABA_B receptor activation, but required intact glutamatergic transmission, evidenced by blockade or disruption of oscillations by 6-cyano-7-nitroquinolinic acid-2,3-dione and (±)-2-amino-5-phosphonovaleric acid, respectively. Multisite mapping studies showed that oscillations were localized to areas 29d and 18b (Oc2MM) and parts of areas 18a and 17. Peak oscillation amplitudes occurred in layer 2/3, and phase reversals were observed in layers 1 and 5. Current source density analysis revealed large-amplitude current sinks and sources in layers 2/3 and 5, respectively. An initial shift in peak inward current density from layer 1 to layer 2/3 indicated that two processes underlie an initial depolarization followed by oscillatory activity. Laminar transactions localized oscillation-generating circuitry to superficial cortical layers and sharp-spike-generating circuitry to deep cortical layers. Whole cell recordings identified three distinct cell types based on response properties during rhythmic micro-EEG activity: oscillation-ON (theta-ON) and -OFF (theta-OFF) neurons, and transiently depolarizing glial cells. Theta-ON neurons displayed membrane potential oscillations that increased in amplitude with hyperpolarization (from −30 to −90 mV). This, taken together with a glutamate antagonist-induced depression of rhythmic micro-EEG activity, indicated that cholinergically driven neocortical oscillations require excitatory synaptic transmission. We conclude that under the appropriate pharmacological conditions, neocortical brain slices were capable of producing localized theta frequency oscillations. Experiments examining oscillation physiology, pharmacology, and topography demonstrated that neocortical brain slice oscillations share many similarities with the in vivo and in vitro theta EEG activity recorded in other brain regions.

INTRODUCTION

Rhythmic electroencephalographic (EEG) oscillations have been associated with both behaviorally relevant (Bland 1986; Vanderwolf 1969) and pathological (Schau 1990; Shinomiya et al. 1994; Steriade 1993) conditions. One well-studied class of oscillations is the 4- to 8-Hz theta EEG rhythm occurring in the hippocampus and associated limbic structures of rodents, cats, rabbits, and primates, including humans (Bland and Colom 1993). In freely moving rats, theta EEG activity, also termed rhythmic slow activity, encompasses a broader frequency range (3–12 Hz) (Lopes da Silva 1992; Whishaw and Vanderwolf 1973). The circuitry underlying hippocampal theta oscillations has yet to be fully elucidated; however, it has been shown that ascending medial septal cholinergic and GABAergic afferents are required for theta EEG generation (Colom et al. 1991; Smythe et al. 1992). Cholinergic inputs excite pyramidal neurons (Bernardo and Prince 1982; Cole and Nicoll 1984; Halliwell and Adams 1982; Krnjevic 1993; Madison et al. 1987). Medial septal GABAergic inputs selectively innervate inhibitory interneurons (Freund and Antal 1988), suggesting that theta-associated activation of GABAergic afferents results in localized hippocampal disinhibition (Bilkey and Goddard 1985). Pharmacological or anatomic deafferentation of medial septal inputs from the hippocampus results in loss of theta rhythmicity. Theta-like activity can be restored with the use of agents that mimic medial septal influences. Two pharmacological agents that together have been shown to elicit robust theta frequency oscillations both in vivo (Colom et al. 1991) and in brain slices (Konopacki and Golebiewski 1993) are carbachol, a cholinergic agonist, and bicuculline, a γ-aminobutyric acid-A (GABA_A) antagonist. Although pharmacologically induced theta frequency oscillations bear a striking resemblance to behaviorally relevant theta EEG activity (Konopacki 1996), their exact relationship with this activity remains unclear.

Some regions of rat neocortex also receive both cholinergic afferents (Lysakowski et al. 1989; Shute and Lewis 1967) and extrinsic GABAergic fibers that synapse preferentially onto local GABAergic interneurons (Freund and Gulyas 1991; Freund and Meskenaite 1992). This anatomy implies that neocortical circuitry may also be capable of generating oscillatory theta activity, as has been previously suggested (Landfield and McGaugh 1972; Yamaguchi et al. 1967). However, experimental findings in vivo have been controversial, with some groups observing an intrinsic neocortical theta generator within or near the posterior cingulate cortex (area 29) (Borst et al. 1987; Feenstra and Holsheimer 1979; Holsheimer 1982) whereas others have been unable to locate this generator (Colom et al. 1988; Gerbrandt et al. 1978). A confounding factor in these studies has been an inability to distinguish local neocortical theta EEG activity from volume conducted hippocampal theta activity (Bringmann 1995; Gerbrandt et al. 1978; Winson 1973). In the present study we used neocortical rat brain slices exposed to carbachol and bicuculline to determine whether intrinsic
neocortical circuitry has the capacity to generate oscillations within the theta frequency range, and to further examine the cellular mechanisms underlying synchronized oscillatory activity within the neocortex.

**METHODS**

**Slice preparation**

Experiments were performed on brain slices isolated from juvenile male Sprague-Dawley rats (80–120 g) obtained from Simonsen Laboratories (Gilroy, CA). Experimental protocols were approved by the Institutional Animal Care Committee at Stanford University and adhered to published guidelines of the National Institutes of Health, Society for Neuroscience, and American Physiological Society. Rats were anesthetized with diethyl ether and their brains were removed into cold (1–2°C) oxygenated artificial cerebrospinal fluid (ACSF). The ACSF had the following ionic composition (in mM): 151.25 Na⁺, 3.5 K⁺, 2.0 Ca²⁺, 2.0 Mg²⁺, 130.5 Cl⁻, 26 HCO₃⁻, 2.0 SO₄²⁻, 1.25 H₂PO₄⁻, and 10 glucose. Brains were sectioned in the coronal plane into 450-μm-thick slices with the use of a vibratome (Vibraslice Series 1000, Boston, MA). Before recording, slices were hemisected and placed on filter papers in a recovery chamber at the interface of a humidified carbogen (95% O₂-5% CO₂) gas phase and ACSF liquid phase. Slices from both hemispheres were allowed ≥1 h to recover from the slicing procedure before submersion in ACSF in a recording chamber. The ACSF was saturated with carbogen gas and perfused at a rate of 2.0 mL/min at room temperature (21–24°C). Rapid and accurate solution changes were made with the use of a ValveBank® computerized perfusion system (AutoMate Scientific, Oakland, CA).

**Micro-EEG recording**

The term “micro-EEG activity” describes extracellular field activity recorded with the use of microelectrodes, which measure electrical voltage of a small, well-localized neuronal population. This nomenclature was adopted from that used by previous authors (Bullock and Basar 1988; Putsche et al. 1984; Turbes 1993). In the present study, micro-EEG recording electrodes were low-resistance (<2 MΩ) glass microelectrodes filled with ACSF and placed in layer 2/3 of neocortex for most experiments (Fig. 1). Micro-EEG recording configuration was similar to that used for recording in vivo EEG activity: signals were amplified ×10,000–50,000 (model 210A, Brown-Lee Precision, San Jose, CA), filtered at 1–30 Hz band-pass, 60-Hz notch (Cyberamp 380, Axon Instruments, Foster City, CA), digitized at 512 or 2,048 Hz (DataWave Technologies, Longmont, CO), and stored on computer disk for further analysis.

**Spectral analysis**

Micro-EEG spectral quantification was accomplished with the use of fast Fourier transforms (FFTs) on 2.5-s epochs of data with the use of DataWave software. For each experiment, the frequency at peak power was extracted from averaged FFTs (n ≥ 5) of carbachol/bicuculline-induced oscillatory activity.

**Temperature studies**

An in-line Peltier temperature controller, provided by Dr. Keith Bley of Roche Bioscience (Palo Alto, CA), was used to control ACSF temperatures to an accuracy of 0.2°C. Temperature was stepped from 21 to 31°C in 0.3°C increments over 40 min (Fig. 3). Temperature was monitored in the perfusion chamber with the use of a calibrated minij probe (Yellow Springs Instrument, Yellow Springs, OH) placed near (<5 mm) the brain slice.

**Mapping studies**

Intraslice electrode position visualization and hard copy output was accomplished with a Wild dissecting microscope, Optronics charge-coupled device camera, and Sony video printer, provided by Technical Instrument (San Francisco, CA). Neocortical laminar boundaries in Figs. 7 and 8 were based on histological measurements from two independent laboratories (Beaulieu 1993; Lysakowski et al. 1989).

**Oscillation phase and amplitude analysis**

A roving field recording electrode was used to collect micro-EEG data at various laminar depths along a trajectory normal to the pial surface (n = 5 slices). Interevent oscillation homogeneity was concurrently monitored in layer 2/3 with the use of a reference electrode positioned ~200 μm from the pial surface and <25 μm medial to the roving electrode trajectory. For phase analysis, reference electrode oscillation peak positivities were assigned a phase of 0°. Relative phase shifts were calculated by time aligning the reference and roving recordings, then dropping a vertical line from the peak positivity of the reference trace to intercept the roving trace (Fig. 7A). Roving oscillation phase at the point of interception was termed the relative phase shift. For example, if the peak positivities of both traces aligned perfectly (in phase), then a phase shift of 0° was assigned to that roving electrode position. If reference trace peak positivities aligned exactly with roving trace peak negativities, then a phase shift of 180° was assigned to that roving electrode position.

To account for interslice amplitude variability, roving electrode amplitudes were normalized in the following manner

\[
\text{Normalized roving trace amplitude} = (\text{ROV/REF}) + \text{REF}_{\text{ave}}
\]

Where ROV is maximum peak-to-peak oscillation amplitude recorded with the roving electrode; REF is maximum peak-to-peak oscillation amplitude recorded with the reference electrode; and REFave is the average of maximum peak-to-peak oscillation amplitudes recorded with the reference electrode on the basis of 78 recordings, with one reference recording amplitude used per roving electrode position per slice (REFave = 198 ± 58 μV, mean ± SD).

**Current source density analysis and limitations**

A DC-coupled roving micro-EEG recording electrode was moved perpendicularly from the pial surface to the underlying white matter in 15 consecutive 80-μm increments (16 recording sites), while a stationary recording electrode monitored response homogeneity in neocortical layer 2/3 (Fig. 8). Each response was initiated by stimulating (6 V, 500 μs, 60–33 Hz) the underlying white matter and deep layer 6–100 μm medioventral to the deepest recording site. One-dimensional current source density (CSD) profile estimates were constructed from laminar field potentials with the use of a finite difference formula (Mitzdorf 1985; Nicholson and Freeman 1975). Cortical resistance along the axis was assumed to be constant (Mitzdorf and Singer 1980). Axum software (TriMetrix, Seattle, WA) was used for CSD calculations and for construction of three-dimensional surface and contour plots (Fig. 8).

CSD analysis accuracy depends on response homogeneity. In the present study, five separate CSD experiments were performed. All experiments yielded similar results, but for illustrative purposes the experiment displaying the greatest response homogeneity over the longest time period is displayed in Fig. 8. In this experiment, two methods were examined for reducing response heterogeneity at the stationary electrode recording site. The first method averaged four stationary electrode responses per roving electrode site, whereas the second method used phase matching to identify the
FIG. 1. Neocortical brain slices displayed oscillatory activity in the 3- to 12-Hz theta/rhythmic slow activity range when perfused with carbachol, a cholinergic agonist, and bicuculline, a $\gamma$-aminobutyric acid-A (GABA_A) antagonist. 

A: coronal brain slice shows the neocortical region in which most recordings were made (between · · ·). In many experiments an extracellular microelectroencephalogram (micro-EEG) recording electrode (EEG) was placed in layer 2/3, whereas whole cell patch-clamp recordings (WC) were obtained either from layer 5 or 2/3. In some experiments oscillations were electrically evoked with a stimulating electrode (STIM). B: 2-s recordings show oscillatory activity in the presence of carbachol (100 $\mu$M) and bicuculline (10 $\mu$M). Scale bars: 0.2 s, 100 $\mu$V. C: minislice was excised from the neocortex and recordings were made from layer 2/3. D: carbachol and bicuculline elicited oscillatory activity from the minislice, demonstrating the presence of an intrinsic generator in this neocortical region. Scale bars as in B. E: plot showing the averaged fast Fourier transform (FFT) of the traces in B. In this experiment peak power occurred at a frequency of 5.6 Hz. F: histogram plot shows the distribution of peak power frequencies for 123 slices. The peak power frequency distribution in vitro (6.0 ± 1.1 Hz, mean ± SD) was within the frequency ranges reported for in vivo theta/rhythmic slow activity.
single most consistent stationary response at each roving electrode position. The latter form of data analysis proved most effective and was used to construct CSD profiles displayed in Fig. 8.

The use of sequential depth measurements in the present study, as opposed to simultaneous 16-channel recordings, limits the degree of confidence that one can have in CSD analysis. In an attempt to quantify the error associated with CSD analysis in Fig. 8, B and C, an error analysis was performed on stationary electrode traces depicted in Fig. 8A. For each oscillation cycle, a 50-ms window (~1/4 of an oscillation) was used to encompass as many peak negativities as possible. All peak negativities falling outside of this 50-ms window were considered to be out of phase. The number of out-of-phase recordings was divided by the total number of recordings (16) to give an estimate of error associated with each oscillation cycle. This analysis yielded the following results: 0% error was associated with oscillation cycles 1 and 2 (all peak negativities fell within the 50-ms time window); 18.75% error was associated with oscillation cycles 3 and 4 (in each case 3 peak negativities fell outside the 50-ms time window); cycle 5 displayed 37.5% error, and cycle 6 displayed 50% error.

Although some degree of error was associated with calculating CSD analysis from sequential two-electrode recordings in the present study, employment of this technique in previous studies has demonstrated that useful CSD information can be derived from two-electrode measurements. Current sources and sinks associated with hippocampal theta EEG activity in vivo have been calculated with the use of repeated serial two-electrode recordings (Brankack et al. 1993; Buzsaki et al. 1986; Heynen and Bilkey 1994). Two-electrode data collection has also been used in neocortical brain slices to determine CSD profiles of epileptic (Hoffman et al. 1994) and nonepileptic (Cauller and Connors 1994) activity. In the present study, results from CSD analysis agreed well with results from both phase/amplitude analysis and laminar transection studies, suggesting that CSD error associated with response heterogeneity was minimal.

Single-cell recording

Whole cell (>1-GΩ seal) patch recording microelectrodes (4–8 MΩ) contained an internal solution composed of (in mM) 100 potassium glutonate, 10 ethylene glycol-bis(β-aminoethoxyether)-N,N,N',N'-tetraacetic acid, 5 MgCl₂, 40 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid free acid, 0.3 ATP, and 0.3 guanosine 5'-triphosphate, pH 7.2, osmolarity 280–290 mosM. Whole cell recordings were obtained from layer 2/3 and 5 neurons and glia in Oc2MM neocortex. Signals were amplified ×50–1000 (Cyberamp 380, Axon Instruments), low-pass filtered at <10 kHz (Axon Instruments), digitized at 10 kHz (DataWave Technologies), and stored on computer disk for further analysis.

Pharmacological agents

Carbamylcholine chloride (carbachol), (+)-tubocurarine chloride (curare), and atropine sulfate were obtained from Sigma (St. Louis, MO). (+)-Bicuculline methiodide, picrotoxin, muscimol HBr, (±)-2-amino-5-phosphonovaleric acid (APV), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were supplied by Research Biochemicals International (Natick, MA). CGP 35348 was provided by Ciba Geigy (Basel, Switzerland). All solutions were made up in spectrophotometric-grade water (Omnisolve) supplied by EM Science (Gibbstown, NJ). Chemicals for the ACSF and electrode solutions were reagent grade or better and were obtained from J. T. Baker (Philadelphia, PA).

RESULTS

Identification of neocortical micro-EEG oscillation generator

Neocortical brain slices perfused with carbachol (100 μM) and bicuculline (10 μM) exhibited trains of spontaneous rhythmic activity ranging in amplitude from 15 to 450 μV (Fig. 1, A and B). These oscillatory events began within 5–40 min of drug application, persisted for 1–4 h, appeared quasisinusoidal in nature, and were observed in both cortical hemispheres. To determine whether the oscillation generator was intrinsic to neocortex, a minislice of neocortex was dissected free (Fig. 1C) and exposed to carbachol (100 μM) and bicuculline (10 μM). The minislice displayed spontaneous rhythmic activity (Fig. 1D) similar both in form and frequency to the oscillations observed in intact brain slices. Averaging FFTs were performed on rhythmic activity, and the frequency at peak power was extracted for each slice (Fig. 1E). In each preparation, FFT analysis revealed a well-defined peak within the theta frequency range previously reported for rats (3–12 Hz). At room temperature, peak frequencies for 123 slices ranged from 3.8 to 9.5 Hz and followed a Gaussian distribution (χ² = 36.5) with a mean peak frequency of 6.0 ± 1.1 Hz (Fig. 1F).

Carbachol/bicuculline-induced epochs of oscillatory activity ranged from 3 to 9 s in duration and occurred from 0.5 to 2 times per minute (Fig. 2A). Oscillations of similar duration and frequency could also be evoked by repeated single shock stimulation (4–7 V, 500 μs, 0.033 Hz) of the underlying white matter or deep cortical layers 5 or 6 (Fig. 2B). Oscillatory activity was always preceded by a large-amplitude spike, similar to those recorded in the presence of bicuculline alone. DC-coupled recordings revealed that this initial spike was associated with a sustained extracellular negativity (335 ± 133 μV, mean ± SD; n = 12 slices) that lasted for the duration of each oscillatory epoch (Fig. 2, B and C). In experiments in which oscillations were electrically evoked, spontaneous oscillatory activity occasionally occurred between stimuli, usually resulting in a failure of electrical stimulation to evoke the next oscillatory epoch (see Fig. 2B, open arrow). DC-coupled recordings showed that sustained extracellular negativities were absent during these failures.

Oscillation temperature dependence

Oscillation peak frequency was temperature dependent, with a Q₁₀ of 2.2 from 21 to 31°C. Within this temperature range, oscillation peak frequencies increased from 5 to 11 Hz, whereas oscillation amplitude and waveform appearance remained relatively constant (Fig. 3). As temperature increased, rhythmic epoch duration decreased (<3 s at 31°C). Total number of oscillations per epoch remained roughly constant at all temperatures examined. Thus increased oscillation frequencies observed at higher temperatures were associated with shortened oscillatory epochs.

Neocortical oscillation pharmacology

GABAergic. Involvement of GABA in oscillatory activity was examined with the use of the GABA_A receptor antagonist
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FIG. 2. Multiple occurrences of carbachol/bicuculline-induced spontaneous and electrically evoked oscillatory activity are displayed on a slow time scale. A: in the presence of 100 μM carbachol and 10 μM bicuculline, spontaneous oscillatory epochs occurred 18 times in 10 min. B: under the same pharmacological conditions, trains of rhythmic oscillations were electrically evoked every 30 s by stimulation of deep cortical layer 6. B, top: AC-coupled micro-EEG recording. B, bottom: same data recorded in a DC-coupled configuration. Note that oscillatory epochs were associated with sustained extracellular negativities. Filled arrow: expanded rhythmic activity in C. Open arrow: failure of electrical stimulation to elicit oscillations. Failures occurred when spontaneous oscillations took place between electrical stimuli. C: expanded view showing that rhythmic micro-EEG oscillations ride on sustained extracellular negativities. A 2-s window (dashed box) represents the portion of an oscillatory epoch on which Fourier transform analysis was performed.

bicuculline, the chloride channel blocker picrotoxin, the GABA_{A} receptor agonist muscimol, and the GABA_{B} receptor antagonist CGP 35348. Bicuculline alone (10 μM, n = 15; 100 μM, n = 6; 500 μM, n = 5; or 1 mM, n = 3) elicited only large-amplitude (>150 μV) sharp spike activity in naive slices (Fig. 4A), similar to previously reported results in vivo (Mares et al. 1985) and in vitro (Chagnac-Amitai and Connors 1985; Connors 1984). The ability of these slices to produce oscillatory activity was confirmed by adding carbachol to the perfusate, which resulted in the appearance of oscillatory activity in all slices within 5–40 min. Application of muscimol (5 μM, n = 5) to rhythmically oscillating slices blocked oscillations (Fig. 4B), indicating that enhanced tonic GABA_{A}-mediated chloride flux and/or shunting inhibition can disrupt synchronous rhythmic activity.

Although oscillations could be electrically evoked in ACSF containing 100 μM carbachol and only 2.5 μM bicuculline, oscillatory activity was most robust in the presence of 10 μM bicuculline. Because bicuculline is a competitive antagonist that would block ~90% of GABA_{A}-mediated responses at this concentration (Chagnac-Amitai and Connors 1989; Zukin et al. 1974), it is possible that neocortical oscillations may require some intact inhibition. To determine whether GABA_{A}-mediated inhibition was required for oscillatory activity, experiments were performed with the use of either the channel blocker picrotoxin (100 μM, n = 3; Fig.
FIG. 3. Oscillation frequency was temperature dependent. A: micro-EEG traces obtained at progressively higher temperatures show that oscillation frequency increased with higher temperatures, whereas oscillation amplitude remained relatively constant. Scale bars: 100 μV, 0.2 s. B: oscillation frequencies (mean ± SD) are displayed at 0.3°C temperature increments between 21 and 31°C. The relationship between temperature and frequency was best fit with a 2nd-order polynomial curve that asymptoted near 12 Hz (---).

GABAergic. Because the majority of neocortical synapses utilize glutamate as an excitatory neurotransmitter (Douglas and Martin 1990), glutamatergic involvement was investigated. Glutamate alone (50 μM, n = 4; 500 μM, n = 3) or in combination with bicuculline (10 μM, n = 4) did not evoke oscillatory activity (Fig. 5, A and B). However, intact glutamate-mediated transmission was necessary to evoke oscillatory micro-EEG activity in neocortical brain slices.

GLUTAMATERGIC. Because the majority of neocortical synapses utilize glutamate as an excitatory neurotransmitter (Douglas and Martin 1990), glutamatergic involvement was investigated. Glutamate alone (50 μM, n = 4; 500 μM, n = 3) or in combination with bicuculline (10 μM, n = 4) did not evoke oscillatory activity (Fig. 5, A and B). However, intact glutamate-mediated transmission was necessary to evoke oscillatory micro-EEG activity in neocortical brain slices.

4°C) or high concentrations of bicuculline (100 μM, n = 3). Neither treatment had any effect on rhythmic activity, suggesting that these oscillations do not require GABA_A-mediated inhibition.

The use of 10 μM bicuculline to elicit oscillations should dis inhibit neocortical inhibitory interneurons as well as pyramidal cells, causing an increase in tonic GABA release. Although the GABA_A-mediated effects of increased GABAergic tone would be suppressed by 10 μM bicuculline, GABA_B-mediated effects should be amplified (Huguenard and Prince 1994). To test whether GABA_B receptor activation was involved in oscillation generation, the GABA_B receptor antagonist CGP 35348 was used. CGP 35348 (100 μM, n = 5) had no effect on oscillations (Fig. 4C), suggesting that activation of GABA_B receptors was not required for this rhythmic activity.

Cholinergic. To determine whether cholinergic receptor activation was sufficient to elicit rhythmic activity, the non-hydropizable cholinergic agonist carbachol was used in isolation. Carbachol alone (100 μM, n = 4; 500 μM, n = 3) or 1 mM, n = 3) did not evoke oscillatory activity (Fig. 4D). However, because carbachol produced oscillatory activity when applied in combination with bicuculline, cholinergic receptor subtype involvement in neocortical oscillation generation was investigated with the use of the nicotinic receptor antagonist curare and the muscarinic receptor antagonist atropine. Similar to results obtained in vivo (Bland 1986) and in vitro (Konopacki et al. 1988), curare (50 μM, n = 5) had no effect on carbachol/bicuculline-induced oscillations, whereas atropine (1 μM, n = 7) blocked oscillations, leaving only nonoscillatory activity resembling that observed with bicuculline alone (Fig. 4E). Thus simultaneous muscarinic receptor activation and GABA_A disinhibition were necessary to evoke oscillatory micro-EEG activity in neocortical brain slices.

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FIG. 4. GABA<sub>A</sub> receptor-mediated disinhibition and muscarinic receptor activation were necessary to elicit oscillations. A: control recordings show baseline micro-EEG activity in naïve slices. The GABA<sub>A</sub>-receptor antagonist bicuculline (10 μM and 500 μM) evoked only large-amplitude nonrhythmic activity in slices capable of producing oscillations when carbachol was subsequently added. B: the GABA<sub>A</sub> agonist muscimol (5 μM) eliminated both oscillatory and nonoscillatory activity. C: rhythmic oscillatory activity was unaffected by either picrotoxin (100 μM), a GABA<sub>A</sub> chloride channel blocker, or CGP 35348 (100 μM), a GABA<sub>B</sub> receptor antagonist. D: the cholinergic agonist carbachol (100 μM and 1 mM) evoked no micro-EEG response when applied alone. E: carbachol/bicuculline-induced oscillations were not affected by the nicotinic receptor antagonist curare (50 μM), but were blocked by the muscarinic receptor antagonist atropine (1 μM).

5D), suggesting that additional (non-NMDA-mediated) mechanisms underlie carbachol/bicuculline-induced rhythmic activity.

**Oscillation generator cortical topography and laminar distribution**

**MAPPING STUDIES.** The regional distribution of carbachol/bicuculline-induced neocortical oscillations was mapped with recordings from 51 slices across five consecutive coronal planes, each separated by 450 μm. On the basis of 249 recordings, oscillatory activity was localized to two bilaterally symmetric strips of cortex in the rostrocaudal plane (Fig. 6A). The medial-dorsal strip fanned out laterally at its posterior aspect, and, on the basis of previous histological measurements, included parts of four anatomically defined areas: 29d, 18b, 17, and 18a (Lysakowski et al. 1989), also known as the occipital association areas: Oc2MM, Oc2ML, Oc1M/Oc1B, and Oc2L, respectively (Zilles 1985). The lateral strip included areas of piriform cortex and entorhinal cortex, the latter of which has been shown to generate theta EEG activity in vivo (Dickson et al. 1994; Mitchell and Ranck 1980) and in vitro (Konopacki et al. 1992b) under similar pharmacological conditions. Neocortical areas not displaying theta frequency oscillations in the presence of carbachol and bicuculline exhibited sharp spike activity similar to that observed with bicuculline alone, large-amplitude delta activity (Lukatch and MacIver 1996), or no activity at all (Fig. 6B).

**LAMINAR ANALYSIS.** Phase, amplitude, and CSD analysis provided detailed interlaminar information regarding oscillatory activity in area Oc2MM. Phase analysis (see METHODS)
GLUTAMATERGIC TRANSMISSION

A  L-GLUTAMATE 50 μM  L-GLUTAMATE 500 μM  CARB 100 μM
              
B  L-GLUTAMATE 50 μM  L-GLUTAMATE 50 μM  BICUCCULINE 10 μM
              
C  CARB 100 μM  CARB / BIC  CNQX 8.6 μM  CARB / BIC  RECOVERY
        
D  CARB 100 μM  CARB / BIC  D-APV 100 μM  CARB / BIC  RECOVERY
        
E  LOW Mg++  LOW Mg++  D-APV 100 μM

FIG. 5. Glutamatergic transmission was necessary but not sufficient to evoke and maintain oscillations. A: application of L-glutamate (50 or 500 μM) did not elicit micro-EEG activity. B: glutamate (50 μM) in the presence of bicuculline (10 μM) elicited only nonoscillatory activity similar to that observed with bicuculline alone. C: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 8.6 μM) completely blocked carbachol/bicuculline-mediated oscillations. D: N-methyl-D-aspartate (NMDA) receptor antagonist (+)-2-amino-5-phosphonovaleric acid (APV, 100 μM) disrupted oscillation timing. E: nominally 0 mM magnesium elicited spontaneous slow oscillatory activity (note time scale) that was completely blocked by APV (100 μM). On washout of low magnesium and application of carbachol/bicuculline, spontaneous pharmacologically induced oscillations were observed.

performed on 78 paired micro-EEG recordings in five slices revealed that oscillations reversed phase twice (Fig. 7). One phase reversal occurred 97 μm from the pial surface, whereas a second reversal was observed 916 μm deep, corresponding to layers 1 and 5, respectively (Fig. 7B). Layer 1 phase reversals were always more abrupt than layer 5 phase reversals, with 80% of the phase reversal occurring over 166 μm in layer 1 versus 358 μm in layer 5. Amplitude analysis demonstrated that large-amplitude oscillatory activity occurred primarily in superficial cortical layers, whereas attenuated signals were observed in deep layers (Fig. 7C). The largest normalized (see METHODS) oscillations (203 ± 76 μV, mean ± SD) were observed in layer 2/3, 255 ± 29 μm from the pial surface. In addition to oscillation phase and amplitude varying with cortical depth, oscillation waveform appearance also varied. Sinusoidal patterns were observed primarily in superficial layers, whereas middle layers exhibited sharper activity. Rhythmic activity with well-defined peaks separated by wide troughs was observed in deep layers and in the most superficial 60 μm of layer 1 (Fig. 7A).
FIG. 6. Three-dimensional mapping studies revealed localized oscillatory micro-EEG activity within neocortex. A, right: 249 recordings in 51 slices map the presence (●) or absence (△) of theta-like oscillations throughout a 2.25-mm-long volume of neocortex. Rhythmic oscillations were observed in 2 bilaterally symmetric strips of cortex that ran rostrocaudally. A, left: schematized rat brain shows the region from which slices were taken. B: representative traces from 3 slices show simultaneously recorded activity from Oc2MM, sensorimotor (SM), and entorhinal (EC) cortices. Note that micro-EEG activity in each cortical area was independent of activity in other areas.
CSD analysis of neocortical oscillations revealed multiple current sources and sinks along the cortical axis (Fig. 8). Results from CSD analysis could be separated into three phases: 1) initial activation, 2) initial periodic response, and 3) sustained periodic response. In the initial activation phase, initial current flows (0- to 30-ms poststimulation) were confined to deep cortical layers and likely resulted from local responses to electrical stimulation of layer 6 neurons. For the initial periodic response, at 90-ms poststimulation, initial inward current density peaked in layer 1, while outward current density peaked simultaneously in layer 5. Recall that phase analysis revealed reversals in both layers 1 and 5 (Fig. 7B). Over the next 400 ms the largest outward currents remained in layer 5, whereas maximal inward currents shifted progressively and periodically from layer 1 to layer 2/3 (Fig. 8C). The significance of this shift was reflected in the finding that neocortical oscillation amplitudes peaked in layer 2/3 and not in layer 1 (Fig. 7C). For the next 1,100 ms a sustained periodic response was observed with maximal current sinks and sources occurring in layers 2/3 and 5, respectively. As expected, the periodicity of peak inward current densities (Fig. 8, B and C) corresponded to the frequency of oscillations from which the CSD analysis was derived. Thus peak current fluxes occurred at theta frequencies.

During early response stages, when inward currents were largest in layer 1, peak inward currents occurred nearly simultaneously with peak outward currents in layer 5. How-
FIG. 8. Current source density (CSD) analysis of oscillatory activity revealed large current sinks in superficial cortical layers and current sources in layer 5. A: traces show DC-coupled roving micro-EEG recordings at 16 laminar depths and their respective stationary micro-EEG reference traces. CSD analysis was performed on DC-coupled recordings. An electrical stimulus (● with dashed lines) was used to trigger oscillations. Vertical dotted lines allow for assessment of stationary micro-EEG response homogeneity. B: 3-dimensional plot of CSD data shows pronounced current sinks in layer 2/3 and sources in deeper cortical layers. C: contour plot showing laminar location of current sources and sinks. Solid lines: sinks. Dashed lines: sources. Initial current flow occurred in deep cortical layers near the stimulating electrode position. Vertical lines: 1st, 3rd, 5th, and 7th maximal inward currents associated with oscillations. Note that peak current density location shifts from layer 1 to layer 2/3 by the 3rd oscillation. Each contour = 22.5 mV/mm², with current densities of 0–12.5 mV/mm² omitted for clarity.

never, when inward current maxima shifted to layer 2/3, inward maxima led outward current peaks in layer 5 by progressively longer time periods with each additional oscillation (Fig. 8C). This CSD relationship suggests that during sustained oscillatory activity, layer 2/3 neuronal activity precedes deep layer activity, and it is possible that over time a dynamic interlaminar relationship is created that can sustain oscillatory activity at theta frequencies. To test this hypothesis, neocortical slices were transected in layer 4, and micro-EEG recording electrodes were placed in layers 2/3 and 5/6 (Fig. 9). After transection, oscillatory activity was localized to superficial cortical layers (n = 7), whereas deep layers produced only nonoscillatory sharp spike activity (6 of 7) or no activity at all (1 of 7). Thus deep layer columnar circuitry was not required for neocortical oscillation generation and maintenance.

Single-cell properties and theta frequency oscillations

OSCILLATION-ON (THETA-ON) NEURONS. Cellular events underlying theta frequency micro-EEG oscillations were examined with the use of whole cell patch-clamp recordings in layers 2/3 and 5. Of 38 cells examined, three different cell types were identified on the basis of their response properties during micro-EEG oscillations. The most common cell type observed in both layer 2/3 (16 of 21 cells) and layer 5 (13 of 17 cells) was termed an ‘oscillation-ON’ neuron. This neuronal subtype was characterized by the appearance of theta frequency membrane potential oscillations (MPOs, 2–15 mV) that ride on sustained depolarizations (4–25 mV) and were 180° out of phase with local oscillatory micro-EEG activity (Figs. 10A and 11A). In both layers 2/3 and 5, oscillation-ON neuron input resistances ranged from 100 to 400 MΩ. Oscillation-ON neurons could be either quiescent (24 of 29) or tonically active (5 of 29) at their resting membrane potential (RMP). For quiescent oscillation-ON neurons, average RMP was -60.1 ± 4.1 (SD) mV in layer 2/3 (n = 10) and -56.7 ± 4.3 mV in layer 5 (n = 14). RMPs of all tonically active oscillation-ON neurons were between -40 and -50 mV. In the absence of carbachol and bicuculline, RMPs ranged from -66 to -73 mV (mean = 70.9 ± 2.2 mV; n = 10), similar to previously reported
Neuronal circuitry in superficial cortical layers was sufficient to generate theta frequency micro-EEG oscillations. Control micro-EEG recordings from layers 2/3 and 5 demonstrate synchronous oscillatory activity in these cortical layers. For comparison purposes, recordings from layer 5 were amplified ×5 more than layer 2/3 recordings. After transection of superficial and deep cortical layers, oscillatory micro-EEG activity was confined to layer 2/3, whereas layer 5 displayed only sharp spike nonoscillatory events similar to those observed with bicuculline alone.

RMPs of neocortical neurons in vitro (Jones and Baughman 1988).

During rhythmic micro-EEG activity, quiescent oscillation-ON neurons frequently discharged action potentials at the peak positivities of intracellular MPOs (Fig. 11B). At RMP, action potential discharges did not accompany each theta cycle; rather, spike discharges occurred sporadically at MPO peak positivities throughout the theta field oscillation. Depolarizing neurons with injected direct current resulted in a higher probability of spike discharge at each MPO peak positivity, whereas neuronal hyperpolarization resulted in decreased discharge activity. By manipulating membrane potentials with constant current application, each neuron could be made to either fire a single action potential at each MPO peak positivity or to fire no action potentials throughout the course of an entire theta train. Tonically active oscillation-ON neurons always increased their discharge rates during oscillatory micro-EEG activity, frequently displaying multiple discharges during each oscillatory cycle (Fig. 10A). Thus the activity of these oscillation-ON neurons closely resembled the behavior of previously described ‘theta-ON’ neurons recorded in vivo during hippocampal theta activity (Konopacki et al. 1992a).

GLIAL CELLS AND OSCILLATION-OFF (THETA-OFF) NEURONS. The second most commonly encountered cell type in both layer 2/3 (5 of 21 cells) and layer 5 (3 of 17 cells) was glial cells. These cells had an average RMP of $-71.5 \pm 4.5$ mV, and input resistances of $<100 \, \Omega$, consistent with previously reported passive membrane properties for glial cells (O’Connor et al. 1994). In addition, depolarizing current pulses never elicited action potentials in this cell type. Glial cell responses during oscillatory micro-EEG activity were characterized by sustained depolarizations ($14.0 \pm 3.5$ mV, mean ± SD; Fig. 10B), which repolarized back to baseline RMP with a half-decay time of $4.8 \pm 0.9$ s after oscillation offset. Unlike oscillation-ON neurons, glia displayed either no MPOs or only small-amplitude MPOs ($<0.2$ mV).

The least common cell type observed during oscillatory micro-EEG activity was termed an ‘oscillation-off’ neuron. The defining characteristic of this cell type was a strong membrane potential hyperpolarization during rhythmic micro-EEG activity, which led to a brief cessation of ongoing
FIG. 10. Three cell types were observed during oscillatory micro-EEG activity. A: simultaneous micro-EEG and whole cell recordings show a tonically active “oscillation-ON” (theta-ON) neuron recorded in layer 5. During rhythmic micro-EEG oscillations this neuron depolarized and increased its firing rate. After oscillation cessation, a slight sustained membrane potential hyperpolarization (note ----) was accompanied by decreased neuronal discharge activity. B: glia cell response [note resting membrane potential (RMP)], time-aligned to the oscillatory micro-EEG activity in A, exhibited a rapid depolarization during rhythmic micro-EEG activity, followed by a slow depolarization back to RMPs after oscillations ceased. C: unlike the oscillation-ON cell displayed in A, a time-aligned “oscillation-OFF” (theta-OFF) cell ceased tonic firing and became hyperpolarized (note ----) during oscillatory micro-EEG activity. After termination of micro-EEG oscillations this neuron immediately returned to its preoscillation resting potential, unlike the cell types displayed in A and B.

**EXCITATORY CURRENTS APPEAR TO UNDERLIE OSCILLATION-ON** (THETA-ON) **NEURON ACTIVITY.** Intracellular membrane potential oscillations observed during rhythmic theta EEG activity in vivo have been associated with both excitatory postsynaptic potentials (EPSPs) (Fujita and Sato 1964; Nunez et al. 1987, 1990) and inhibitory postsynaptic potentials (IPSPs) (Leung and Yim 1986; Soltesz and Deschenes 1993; Ylinen et al. 1995). The nature of synaptic potentials underlying neocortical oscillation-ON neuron MPOs was investigated with the use of whole cell current injection. In 10 oscillation-ON neurons, membrane potentials were stepped from −30 to −90 mV, revealing maximal MPO amplitudes at the most hyperpolarized potentials and decreasing amplitudes with membrane depolarization (Fig. 11, A and B). Because neocortical EPSPs appear to reverse close to 0 mV (Sutor and Hablitz 1989), whereas chloride-mediated IPSPs reverse near resting potentials (Connors et al. 1988), this result suggests that EPSPs rather than IPSPs underlie MPO generation in oscillation-ON neurons. Although MPO amplitudes were quite sensitive to changes in membrane potential, MPO frequencies remained unchanged at all membrane potentials examined (Fig. 11). This voltage independence of MPO frequencies suggests that the primary drive for MPOs arises from network properties, rather than intrinsic properties of individual neurons (Ylinen et al. 1995).

**DISCUSSION**

Unambiguous identification of an intrinsic neocortical theta generator in vivo has been hampered by volume conducted hippocampal theta EEG activity (Bringmann 1995; Gerbrandt et al. 1978; Winson 1973). The present study
FIG. 11. Currents underlying oscillatory micro-EEG activity reversed polarity near 0 mV. A: simultaneous micro-EEG and whole cell current clamp recordings from a layer 2/3 oscillation-ON neuron demonstrated that membrane potential oscillation (MPO) amplitudes increased with hyperpolarization, whereas MPO frequency remained constant (---: RMP). Increased hyperpolarization also resulted in larger-amplitude, MPO-associated sustained depolarizing shifts. At depolarized potentials (-37 mV), single-cell tonic discharge activity did not register in micro-EEG recordings performed within 80 μm of the intracellular recording site, indicating that single-neuron discharge contributed relatively little to recorded micro-EEG activity. B: solid lines mark expanded micro-EEG activity and MPOs. A shorter time scale demonstrates that MPOs were ~180° out of phase with micro-EEG oscillations (-37 mV). MPO peak positivities were frequently associated with undershooting action potentials, some of which are marked with arrows. At depolarized potentials (-37 mV) only 1 in 5 oscillations evoked an action potential, perhaps owing to depolarization block of sodium channels at these potentials.

demonstrated that rat neocortical brain slices possess the requisite circuitry to generate synchronized oscillatory activity within the theta frequency range, consistent with earlier studies showing synchronized theta-like activity in brain slice preparations of hippocampus (Bland et al. 1988; Huerta and Lisman 1993; Konopacki et al. 1987; MacIver et al. 1986; MacVicar and Tse 1989) and entorhinal cortex (Konopacki et al. 1992b). It remains unclear, however, how representative in vitro neocortical oscillations are of theta EEG activity in vivo. To address this issue, in the present study we examined cellular and synaptic mechanisms underlying synchronous theta frequency neocortical oscillations.

Neocortical micro-EEG oscillation similarities to theta EEG activity

Neocortical oscillation physiology and pharmacology were similar to previously described theta EEG activity in the following ways. 1) Neocortical slice oscillation frequencies and amplitudes (6.0 ± 1.1 Hz, 15–450 μV; Fig. 1) were comparable with previously reported in vivo theta EEG frequencies and amplitudes in rats (3–12 Hz, 50–1000 μV) for both limbic (Bland 1986; Bland and Colom 1993; Whishaw and Vanderwolf 1973) and neocortical structures (Holsheimer 1982). 2) Neocortical oscillation frequency temperature dependence (Q10 = 2.2; Fig. 3) was similar to that reported for hippocampal theta EEG activity in vivo (Q10 = 1.9) (Whishaw and Vanderwolf 1971). 3) Rhythmic micro-EEG oscillations were observed in the presence of carbachol (100 μM) and bicuculline (10 μM), two agents that together evoke robust theta EEG oscillations in vivo (Colom et al. 1991; Smythe et al. 1992) and in brain slices (Konopacki and Golebiowski 1993). 4) Neocortical oscillations required muscarinic receptor activation (Fig. 4), as did theta EEG activity recorded from the hippocampus in vivo (Bland 1986) and from brain slices of hippocampal (Konopacki et al. 1988; MacVicar and Tse 1989) and entorhinal cortex (Konopacki et al. 1992b). 5) Localization of neocortical oscillations to areas adjacent to posterior cingulate cortex (Fig. 6) matched that of previously reported neocortical theta EEG activity in vivo (Borst et al. 1987; Bringmann 1995; Feenstra and Holsheimer 1979; Holsheimer 1982), and these oscillations were not observed in other neocortical regions. 6) Neocortical slices, like hippocampal slices (MacVicar and Tse 1989), required intact glutamatergic transmission for theta frequency oscillations (Fig. 5), whereas depressed GABA<sub>A</sub>-mediated transmission did not alter rhythmic activity in hippocampal or neocortical slices (Fig. 4). 7) Oscillation-on, oscillation-off, and glial cells described in the present study (Fig. 10) behaved similarly to theta-on, theta-off (Konopacki et al. 1992a), and glial cells (Osehobo and Andrew 1993) characterized during theta activity in vivo and in vitro, respectively. 8) Theta EEG activity in vivo undergoes a well-characterized transition to delta, burst suppression, and isoelectric activity when exposed to increasing concentrations of barbiturate anesthetics (Buhrer et al. 1992; MacIver et al. 1996). Neocortical slice theta frequency micro-EEG oscillations displayed similar shifts in patterned
activity when exposed to clinically relevant barbiturate concentrations (Lukatch and MacIver 1996).

**Oscillation similarities and differences with interictal epileptic EEG activity**

Previous studies have demonstrated that depression of GABA<sub>ₐ</sub>-mediated inhibition (Zia-Gharib and Webster 1991) or enhancement of muscarinic mediated cholinergic transmission (Cruickshank et al. 1994) can lead to epileptic-like spiking activity. Use of 10 μM bicuculline in the present study may account for the finding that carbachol/bicuculline-induced oscillatory epochs occurred with a frequency (Fig. 2) similar to that previously reported for bicuculline-mediated interictal spikes (Avalvi et al. 1995). In addition, the sharp spike micro-EEG negativity observed during neocortical oscillation commencement may represent the summed paroxysmal depolarizing shifts of a large neuronal population (Gutnick et al. 1982). It is possible that carbachol-induced muscarinic receptor activation gives rise to sustained plateau potentials by blocking rectifying K<sup>⁺</sup> conductances (Fraser and MacVicar 1996; Krnjevic 1993; Madison et al. 1987), some of which have been suggested to terminate epileptiform burst discharges (Traub et al. 1994). Although this mechanism may contribute to prolonged epileptic bursts (Kriegstein et al. 1983), hippocampal theta EEG activity has also been shown to require muscarinic receptor activation and GABA<sub>ₐ</sub> receptor suppression (Colom et al. 1991; Smythe et al. 1992). Thus it is not clear whether sustained rhythmic activity in neocortical brain slices should be thought of as a prolonged epileptic event, an abbreviated physiologically relevant oscillation, or some combination of both.

For the following reasons we argue that carbachol/bicuculline-induced neocortical oscillations are at least partially distinct from epileptic activity. 1) Bicuculline alone never evoked sustained theta frequency oscillations, nor did high concentrations of carbachol ever evoke epileptic-like spiking activity (Fig. 4). Rather, oscillations required concurrent muscarinic receptor activation and GABAergic disinhibition. 2) Antimuscarinic drugs have been shown to be ineffective at blocking seizure discharge activity in spontaneous models of epilepsy (De Deyn et al. 1992). Similarly, in the present study the muscarinic receptor antagonist atropine spared sharp spike discharge activity, but abolished all oscillatory activity (Fig. 4). 3) The NMDA receptor antagonist APV has been shown to block low-magnesium-induced seizure activity in this and previous studies (Zhang et al. 1994). APV disrupted, but did not block cholinergically induced neocortical oscillations (Fig. 5). 4) Previous studies have demonstrated that most, if not all, neocortical areas were susceptible to bicuculline-mediated seizure activity (Chagac-Amitai and Conners 1989; Chervin et al. 1988; Sutor et al. 1994). In contrast, theta EEG activity was localized to specific brain regions (Bland 1986; Feenstra and Holsheimer 1979; Holsheimer 1982). In the present study, theta frequency oscillations were confined to a narrow localized region of neocortex (Fig. 6), which included those regions shown to support theta activity in vivo. 5) Laminar transection studies demonstrated that neuronal populations underlying oscillatory and sharp spiking activity were anatomically separable (Fig. 9). and 6) Previous reports have demonstrated that virtually all neurons display large-amplitude (>25 mV) paroxysmal depolarizing shifts during epileptic activity (Gutnick et al. 1982). Neuronal membrane responses during neocortical cholinergic oscillations were varied; some neurons displayed moderate depolarizations (~20 mV), whereas others hyperpolarized or displayed small membrane potential depolarizations (<5 mV) and low-amplitude MPOs (Figs. 10 and 11).

**Laminar specific oscillation characteristics**

Because volume conducted EEG activity will not produce phase shifts or an increasing amplitude with increasing distance from a generator source (Holsheimer and Feenstra 1977), in vivo studies seeking to identify intrinsic theta generators in cortex have constructed oscillation amplitude and phase depth profiles (Feenstra and Holsheimer 1979; Heynen and Bilkey 1994; Ylinen et al. 1995). Criteria for a local generator in these studies has been based on finding a phase reversal and/or an amplitude maximum within the cortical region of interest. In the present study we adopted the same criteria for localizing neuronal generators. It was first determined that neurons and synchronizing circuits generating theta frequency oscillations must be intrinsic to neocortex because isolated minislices of Oe2MM cortex produced oscillatory activity (Fig. 1). Within this neocortical area, a phase reversal was observed in layer 5 (Fig. 7B), consistent with previously reported theta frequency oscillation phase reversals in layer 5 in vivo (Feenstra and Holsheimer 1979; Holsheimer 1982). Layer 5 phase reversals are evident in vivo and in vitro both occurred over ~350 μm. Neocortical brain slices also displayed a phase reversal in layer 1 that was not observed in vivo. In vivo studies likely did not detect this phase reversal because it occurred abruptly, and very close to the pial surface (~100 μm), at a depth not reported in vivo (Feenstra and Holsheimer 1979). Consistent with in vivo findings, neocortical brain slice oscillation phase reversals were associated with decreased oscillation amplitudes.

Neocortical slice oscillation amplitudes peaked in layer 2/3 (Fig. 7C), suggesting that layer 2/3 cells generated theta frequency oscillations. Consistent with this interpretation, results from CSD analysis demonstrated that sustained inward current flux peaked in layer 2/3 (Fig. 8), and laminar transection studies localized oscillatory activity to superficial cortical lamina (Fig. 9). During the first 250 ms of oscillatory micro-EEG activity, CSD analysis revealed large-amplitude current sinks and sources throughout the cortical axis, similar to those observed in neocortex exposed to 10 μM bicuculline alone (Hoffman et al. 1994). This initial CSD profile may reflect a bicuculline-mediated epileptic-like event that over time (~250 ms) is replaced in layer 2/3 by muscarinic-mediated synchronous oscillations resembling theta EEG activity. Similarly, previous studies have demonstrated that separate mechanisms underlie synchronized neuronal discharges and depolarizing shifts associated with hippocampal electrographic seizure activity (Swann et al. 1993). Further support for the idea that two separate processes underlie neocortical micro-EEG oscillations came from laminar transection experiments that demonstrated that
Superficial cortical layers were capable of supporting sustained oscillatory activity, whereas isolated deep cortical layers produced only sharp spike activity (Fig. 9). The observation of epileptiform activity in deep layers is consistent with previous studies that showed the lowest threshold for epileptiform discharge occurred in neocortical layers 4 and 5 (Connors 1984; Prince and Connors 1986). However, to our knowledge, localization of theta frequency micro-EEG oscillations to superficial neocortical layers is a novel finding.

Neocortical oscillation single-cell properties

Previous studies in vivo have shown that activation of ascending cholinergic fibers results in sustained neocortical neuron depolarizations (Metherate and Ashe 1993; Metherate et al. 1992). In the present study, similar intracellular depolarizations (4–25 mV) were observed during oscillatory activity (Figs. 10 and 11). These sustained depolarizations were accompanied by rhythmic MPOs that were 180° out of phase with local micro-EEG oscillations. Comparable MPOs have been observed during hippocampal theta activity, in vivo and in vitro, in which pyramidal neurons became depolarized by 10–20 mV and generated spontaneous MPOs of 5–25 mV, which were also 180° out of phase with respect to local EEG signals (Bland et al. 1988; Konopacki et al. 1992a; Nunez et al. 1987). Neurons involved in interictal spiking activity also demonstrate sustained depolarizing shifts, sometimes accompanied by MPOs (Hoffman et al. 1994). However, seizure-associated depolarizing shifts are usually significantly larger in amplitude (typically >30 mV), and MPOs are faster in frequency (typically >20 Hz), than those observed in the present study and those previously reported for theta activity in vivo. In addition, one hallmark of epileptogenesis, the simultaneous occurrence of large-amplitude paroxysmal depolarizing shifts in virtually every neuron, was not observed during oscillatory micro-EEG activity in neocortex; many oscillation-on neurons displayed only small depolarizing shifts (<10 mV), and some neurons hyperpolarized during rhythmic oscillations (Fig. 10). Thus, the behavior of these neurons more closely resembled previously reported theta-on and theta-off cell activity displayed by neurons during physiologically occurring theta EEG activity (Bland and Colom 1993).

Synaptic mechanisms of neocortical oscillations

MPOs associated with theta activity in vivo have been ascribed primarily to either EPSPs (Fujita and Sato 1964; Nunez et al. 1987, 1990) or IPSPs (Leung and Yim 1986; Soltesz and Deschenes 1993; Ylinen et al. 1995), and the exact nature of these cellular oscillations remains a matter of controversy. In the present study, neocortical micro-EEG oscillations were unaffected by GABA_A and GABA_B receptor antagonists, but were blocked by the glutamate receptor antagonist CNQX. This, together with the finding that oscillations decreased in amplitude as RMPs approached 0 mV, suggests that excitatory rather than inhibitory currents underlie these particular oscillations. Synchronized theta frequency oscillations were localized to superficial cortical layers, and peak oscillation amplitudes were observed in layer 2/3, suggesting that layer 2/3 neurons generated muscarinic-mediated rhythmic activity. Previous studies have demonstrated a population of cholinergic neurons intrinsic to layer 2/3 that may account for up to 30% of neocortical cholinergic fibers (House et al. 1985), and it is possible that these neurons may be involved in neocortical theta frequency oscillations in vivo. In addition, small groups of layer 2/3 neurons have been shown to be electrophysiologically coupled (Gutnick and Prince 1981), and this coupling could help sustain excitatory network reverberations that contribute to synchronous oscillation generation.

Oscillations and an ascending cholinergic synchronizing system

Cholinergically driven oscillations in neocortical brain slices augment a growing list of synchronized oscillatory activity currently under investigation. This list includes, but is not limited to, slow neocortical oscillations (Steriade et al. 1993b), thalamocortical oscillations (Steriade et al. 1993a), spindle activity in lateral geniculate slices (Bal et al. 1995; Kim et al. 1995), metachromatic receptor-mediated oscillations in hippocampal slices (Taylor et al. 1995), NMDA receptor-mediated synaptic activity in slices of caudal nucleus tractus solitarii (Tell and Jean 1993), and rhythmic slow activity (theta) in hippocampal brain slices (MacIver et al. 1986; MacIver and Tse 1989). Results from the present study fit a pharmacological and physiological profile of an ascending cholinergic synchronizing pathway that originates in the brain stem (Vertes et al. 1993) and projects via the hypothalamus to medial septum (Bland et al. 1994), hippocampus (Oddie et al. 1994), and midline neocortical structures (Feenstra and Holsheimer 1979; Holsheimer 1982), suggesting that many if not all limbic structures support theta EEG oscillations. Unlike the hippocampus, which receives its primary cholinergic afferents from the medial septum (Gaykema et al. 1990), medial neocortical areas generating theta frequency micro-EEG oscillations in the present study receive primary cholinergic innervation from the magnocellular basal nucleus (Luiken et al. 1987). Although the function of cholinergically mediated oscillations remains to be determined, there is growing evidence that theta frequency synchronizations are involved in arousal, sensory/motor processing, learning, and memory (Bland and Colom 1993; Huerta and Lisman 1993).

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REFERENCES


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