

# Slow Periodic Events and Their Transition to Gamma Oscillations in the Entorhinal Cortex of the Isolated Guinea Pig Brain

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**Dickson, Clayton T., Gerardo Biella, and Marco de Curtis.** Slow periodic events and their transition to gamma oscillations in the entorhinal cortex of the isolated guinea pig brain. *J Neurophysiol* 90: 39–46, 2003; 10.1152/jn.01063.2002. Slow (<1 Hz) periodic activity is a distinctive discharge pattern observed in different cortical and sub-cortical structures during sleep and anesthesia. By performing field and cellular recordings, we demonstrated that slow periodic events (0.02–0.4 Hz) are spontaneously generated in the entorhinal cortex of the in vitro isolated whole brain of the guinea pig. These events were characterized by gradually developing runs of low-amplitude (50–300  $\mu$ V), high-frequency (25–70 Hz) oscillations superimposed on a slow potential that lasted 1–3 s. Both slow and fast components showed a phase reversal in the superficial layers. In layer II-III entorhinal neurons, the slow periodic events correlated to a slowly developing depolarizing envelope capped by subthreshold membrane potential oscillations and action potential discharge. Slow periodic field events propagated tangentially across the entorhinal cortex and could be triggered by stimulation of superficial associative fibers, suggesting that they were generated by and propagated via network interactions in the superficial layers. Slow periodic events were reversibly abolished by muscarinic excitation elicited by carbachol (50  $\mu$ M) that promoted intracellular membrane potential depolarization associated with continuous fast oscillatory activity in the gamma frequency range. These results suggest that, as proposed in vivo, activity changes in the entorhinal cortex of the in vitro isolated guinea-pig brain reflect different activation states that are under cholinergic control.

## INTRODUCTION

The correlation of different levels of vigilance with particular patterns of synchronous, rhythmical activity in large populations of neurons has led to the concept of state-dependent EEG patterns (Berger 1929, 1930; Llinás and Paré 1991; Llinás et al. 1998; Penfield and Jasper 1954; Steriade 1997, 2000). The mechanisms of state-dependent shifts in field/EEG activity presumably underlie different processing features of the cerebral cortex across behavioral states. Recently, a slow periodic pattern (named “slow oscillation”) has been described, which exhibits frequencies lower than 1 Hz (Steriade 2000; Steriade et al. 1993b). In both animals and humans, this pattern of activity appears during spontaneous slow-wave sleep (Achermann and Borbely 1997; Amzica and Steriade 1997; Steriade et al. 1993b), as well as during anesthesia induced by a wide

variety of anesthetics (Lampl et al. 1999; Steriade et al. 1993e). These slow periodic events (SPEs) include delta and faster rhythms, such as sleep spindles and ultra-fast ripples (80–200 Hz) (Grenier et al. 2001; Steriade et al. 1993b,d) and may represent the K-complexes typically observed in slow sleep EEG (Amzica and Steriade 1997, 1998). Because of their behavioral correlates, SPEs have been suggested to be an objective marker for unconsciousness (Niedermeyer 1999). While studied extensively in the neocortex, SPEs have also been reported in diverse cortical and subcortical structures including the hippocampal formation, the entorhinal cortex, the perirhinal cortex, the neostriatum, and the amygdala (Buzsáki et al. 1992; Charpak et al. 1995; Chrobak and Buzsáki 1996; Collins et al. 2001; Wilson and Kawaguchi 1996).

At the neuronal level, SPEs reflect synchronous depolarization-hyperpolarization sequences (Lampl et al. 1999; Steriade et al. 1993e; Wilson and Kawaguchi 1996) generated at the level of the local cortical circuitry, since they are present in the *cerveau isolé* preparation (Steriade et al. 1993d), in isolated cortical slabs (Timofeev et al. 2000), and have more recently been shown in different in vitro preparations (Sanchez-Vives and McCormick 2000; Wu et al. 2002). Consistent with their state dependency, they are abolished by stimulation of ascending cholinergic or noradrenergic activating systems, which produces arousal in the behaving animal (Steriade et al. 1993a) and induces long-lasting depolarization in cortical cells (Metherate et al. 1992; Steriade et al. 1993a).

In this study, we show that spontaneously developing SPEs that bear resemblance to the slow (<1 Hz) oscillations described in the intact, sleeping, or anesthetized brain (Steriade et al. 1994) can be recorded in the entorhinal cortex of the isolated adult guinea pig brain maintained in vitro. We further demonstrate that SPE are reversibly abolished by muscarinic receptor stimulation, suggesting that they may reflect an activation state similar to that observed in the sleeping or anesthetized animal.

## METHODS

The isolation methods for the guinea pig brain preparation have been previously documented in detail (de Curtis et al. 1991, 1998; Llinás et al. 1981; Muhlethaler et al. 1993) and will be only briefly

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described here. Following deep barbiturate anesthesia (thiopental sodium 20 mg/kg ip), female Hartley guinea pigs (150–250 g; Charles River, Comerio, Italy) were perfused through the heart with a cold (4–10°C) carbogenated (95% O<sub>2</sub>-5% CO<sub>2</sub>) solution composed of (in mM) 126 NaCl, 3 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.3 MgSO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 15 glucose, 2.1 HEPES, 0.4 thiourea, 0.5 ascorbic acid, and 3% dextran (MW 70,000). Following cooling to 15°C, the brain was rapidly and carefully dissected out of the cranium and was submerged in a recording chamber filled with the same solution maintained at a temperature of 15°C. Internal perfusion through the existing brain vasculature at a rate of 5.5 ml/min was achieved by cannulating the basilar artery. Leaky arterial vessels were ligated, and the brain was gradually (0.2°C/min) warmed to a final temperature of 32°C. This protocol was reviewed and approved by the Committee on Animal Care and Use and by the Ethics Committee of the Istituto Nazionale Neurologico.

Extracellular field recordings were made with 1) saline-filled glass micropipettes with a 10 μm tip diameter 2) 16-channel linear silicon probes (50 μm contact separation, kindly provided by Jamille Hetke, of the Center for Neural Communication Technology, University of Michigan, Ann Arbor, MI), or 3) stainless steel microelectrode matrix arrays (1 × 4 or 4 × 4 with 410-μm tip separation; FHC, Bowdoinham, ME). All single-site recordings, unless otherwise noted, were made at a depth of 500 μm from the pial surface. Recordings at multiple sites were carried out simultaneously. Intracellular recordings were made with 1.5 M K<sup>+</sup>-acetate-filled micropipettes pulled to a final resistance of 80–100 MΩ. Neurons selected for analysis in this study had resting membrane potentials of at least -55 mV and an overshooting spike.

Field signals were amplified at a gain of 1,000 using an AC amplifier (Biomedical Engineering, Thornwood, NY), high-pass filtered at 0.2 Hz, and low-pass filtered at 1,000 Hz. Intracellular signals were amplified at a gain of 10 using a Neuro Data amplifier (IR-283, Cygnus Technology, Delaware Water Gap, PA) and low-pass filtered at 10 KHz. All signals digitized on-line using a National Instruments DAQ board (PCI-6071E) were acquired with customized software developed by Gerardo Biella and were stored on DAT tape (DTR 2602 Biologic, Claix, France) for off-line analysis. Waveforms were sampled on-line at a frequency of 2–20 kHz depending on the bandwidth of the signal. Single channel spectral analyses were conducted off-line using MATLAB (Mathworks, Natick, MA).

Intracortical stimulation was conducted using thin insulated tungsten bipolar electrodes (FHC). When metal electrodes were used for intracortical recording or stimulation, the location of the electrode tips were marked by small electrolytic lesions. Following completion of experiments, brains were fixed overnight in a 4% paraformaldehyde solution in a 0.1 M sodium phosphate buffer and sectioned by vibratome at 100 μm. Sections were mounted, stained with thionin, and inspected for the location of electrode sites.

Cholinergic agents such as CCh (25–50 μM) and atropine sulfate (ATSO<sub>4</sub>; 5 μM) were diluted in the perfusion solution. All salts were obtained from BDH (Poole, England) and all drugs were obtained from Sigma (St. Louis, MO). Dextran was obtained from SIFRA (Isola della Scala, Italy).

## RESULTS

In 47 of 59 experiments, baseline spontaneous field recordings in the medial entorhinal cortex (mEC) of the isolated whole brain demonstrated spontaneous activity occurring at <1 Hz that showed a variable periodicity and rate of occurrence especially at the onset of the experiment (SPE; Fig. 1). When stabilized, the frequency and duration of the occurrence of SPEs were on average  $0.11 \pm 0.11$  (SD) Hz and  $2.2 \pm 0.7$  s, respectively. SPEs were characterized by fast activity at  $43 \pm 13$  Hz riding on top of a slower biphasic potential (Fig. 1, A and

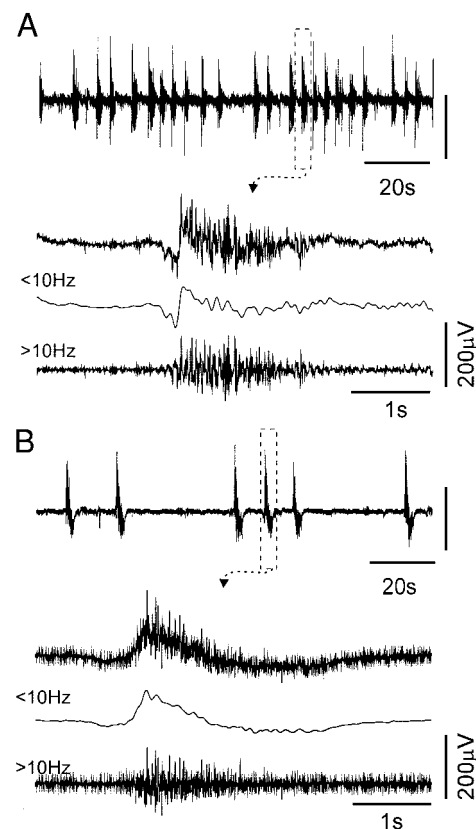


FIG. 1. Spontaneous periodic events (SPE) recorded in the isolated whole brain. Extracellular field recordings from the medial entorhinal cortex (mEC) in 2 different experimental preparations are shown. *A*: example of slow events occurring at an average periodicity of 0.19 per second (recorded at 200 microns depth). *B*: example of slow events occurring at a slower rate (0.04 Hz) recorded at 500 microns depth. Unfiltered, low-pass (<10 Hz) and high-pass (>10 Hz) filtered expanded traces are illustrated to show the low frequency and high-frequency components of the SPE.

*B*) that showed average amplitudes (peak-to-peak) of  $165 \pm 85$  and  $163 \pm 99$  μV, respectively.

To test whether SPEs were generated within the mEC, we performed simultaneous multi-site laminar recordings using 16-channel linear silicon probes with 50 μm inter-site spacing inserted orthogonal to the pial surface of the mEC. Phase reversals of both the fast and slow oscillatory components within single SPEs were observed in the superficial layers (Fig. 2, *B* and *C*). Histological analysis of probe tracts demonstrated that the depth of phase reversal corresponded to layer II (Fig. 2*A*;  $n = 10$ ). These results confirmed that SPE are generated locally within the mEC and suggest that the superficial network is critical for their generation. Further confirmation of this was gleaned by performing simultaneous field and intracellular recordings of superficial layer principal cells during SPE. As shown in Fig. 3, the occurrence of individual field events corresponded to a slowly developing depolarizing envelope recorded intracellularly ( $n = 31$ ). Riding on the intracellular depolarization were fast membrane potential oscillations and action potential discharges, which corresponded to the fast oscillations present in the field recordings (Fig. 3*B*). The long-lasting depolarizing envelope was dependent on the value of the membrane potential, becoming smaller and larger with membrane depolarization and hyperpolarization, respectively (Fig. 3*C*). Interestingly, as shown in Fig. 3*C*, the fast mem-

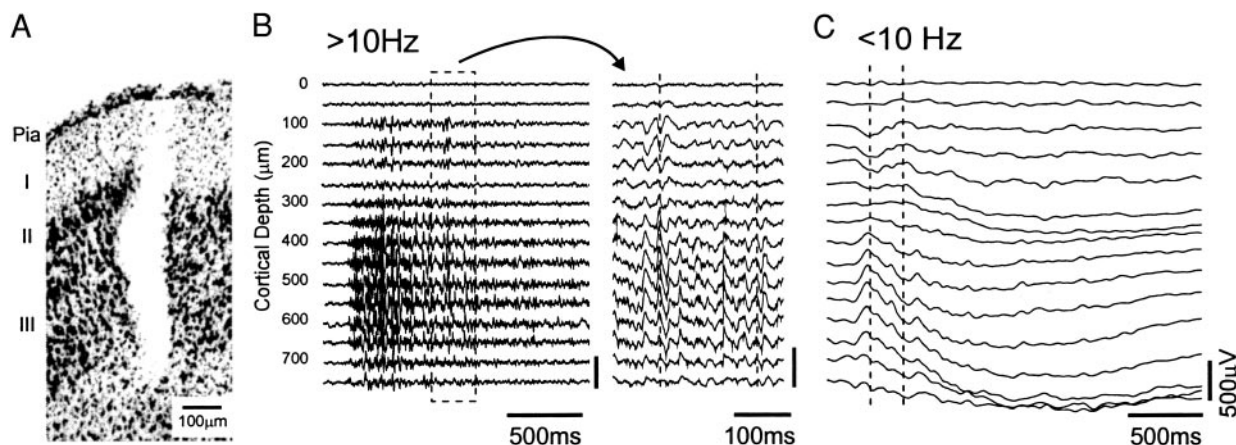


FIG. 2. Extracellular field profile of spontaneous SPEs. *A*: electrode tract made by the 16-channel silicon probe in the mEC. Cortical layers are numbered with roman numerals. *B*: high-pass ( $>10$  Hz) and (*C*) low-pass ( $<10$  Hz) filtered traces from the same SPE at corresponding depths from the pial surface. *Right column* in *B* shows the expansion of the area marked by the dotted rectangle. The irregular fast oscillatory activity as well as the slow component within the SPE demonstrated a phase reversal at a depth corresponding to layer II of the mEC.

brane potential oscillations during the slow depolarization decreased in amplitude or vanished with membrane hyperpolarization.

When recording SPEs with multiple single electrodes across the surface of the EC, it was frequently noted (10 of 16 cases) that the onset of activity at some sites preceded that at others, suggesting that such events were actively propagated tangentially across the cortex. To examine intra-EC propagation more systematically, a pair of multielectrode arrays, each consisting of an equally spaced row of four electrodes ( $1 \times 4$ ), was used to record field activity simultaneously across the surface of the EC ( $n = 8$ ). In all experiments, evidence of SPE propagation could be observed. In three additional experiments, we performed simultaneous field recordings with a  $4 \times 4$  (16 site) matrix electrode. Using this protocol we were able to consistently observe propagation of SPE with variable directions within the same experiment (Fig. 4).

The tangential propagation of SPE across the surface of the EC may occur through synaptic interactions between superficial neurons. We tested, therefore, whether electrical

stimulation of the superficial mEC layers, previously shown to elicit an AMPA-dependent evoked potential (Dickson et al. 2000a), could also evoke SPEs. In 8 of 12 experiments, stimulation that evoked a measurable evoked potential (*inset* in Fig. 5*C*) induced and entrained events (Fig. 5*C*) similar to those occurring spontaneously (Fig. 5*B*). As for SPE, laminar field recordings of these evoked events using multichannel silicon probes showed depth reversal patterns of both slow and fast components in the superficial mEC layers (data not shown).

One of the characteristics of the slow waves in vivo is that they are abolished by brain stem activating cholinergic and noradrenergic stimuli (Belardetti et al. 1977; Moruzzi and Magoun 1949; Steriade et al. 1993a). The mEC receives a prominent cholinergic input (Alonso and Köhler 1984) that appears to be necessary for the state-dependent expression of rhythmic oscillatory activity (theta and gamma) in vivo (Chrobak and Buzsáki 1998; Dickson 1994; Dickson et al. 1995; Jefferys 1995; Leung 1998; Mitchell et al. 1982). We have previously demonstrated that muscarinic receptor activa-

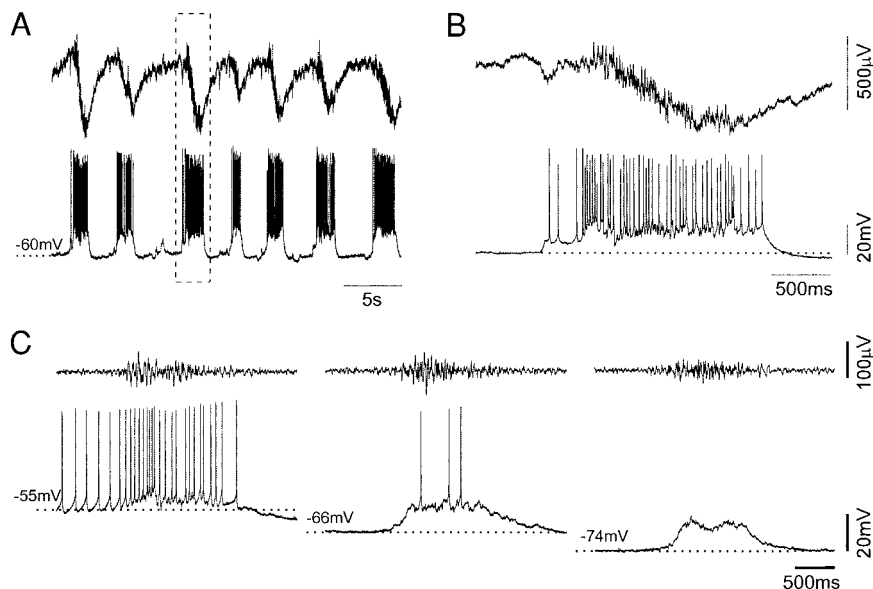


FIG. 3. Intracellular correlates of SPE in the mEC of the isolated whole brain. *A*: simultaneous field (*top*) and intracellular (*bottom*) recording from a mEC superficial layer cell. Field events were correlated with phasic depolarization and spiking activity. *B*: expansion of the traces in *A* (dotted rectangle), demonstrating a correspondence between fast oscillatory activity and high rate of firing in the cell. *C*: field and intracellular recording in another experiment. Membrane depolarization and hyperpolarization evoked by current injection decreased and increased, respectively, the amplitude of the intracellular depolarizing envelope correlated with SPE. Note that the amplitude of membrane potential oscillations associated with fast oscillations of the SPE showed the opposite effect. Field events were high-pass filtered ( $>10$  Hz). Resting membrane potential was  $-61$  mV.

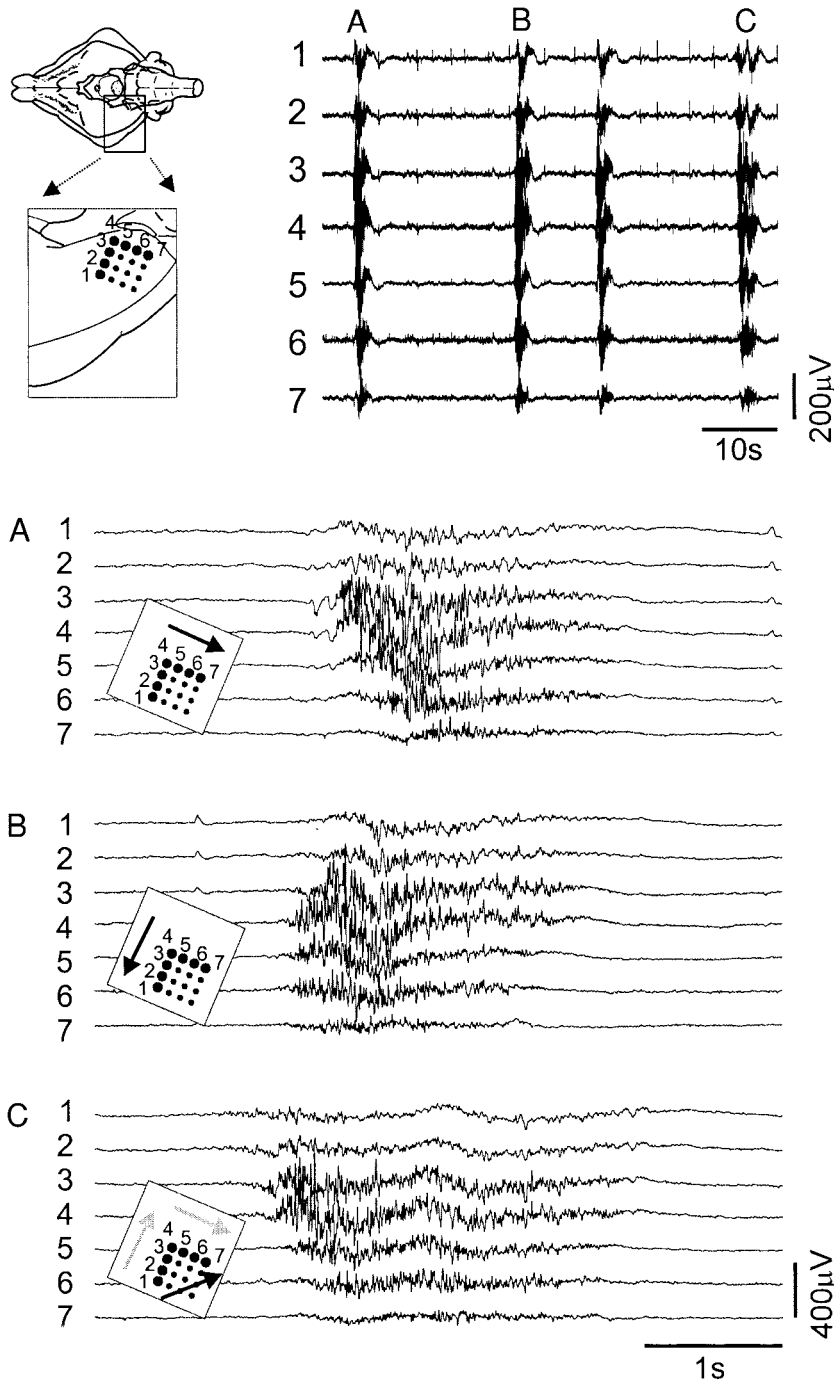


FIG. 4. Spontaneous SPE propagate across the surface of the mEC. A 16-channel matrix electrode ( $4 \times 4$ ) with a  $410\text{-}\mu\text{m}$  inter-electrode spacing was inserted in the mEC, as shown in the scheme in the *top left panel*. Field recordings with SPE are shown in the *right panel*. For clarity, only traces from the 7 labeled locations are shown. SPEs marked as A, B, and C are shown as the corresponding expansions in the *bottom* of the figure. At this time scale, the occurrence of SPE is not simultaneous across all electrodes and appears to propagate, although the propagation direction vary within the same experiment. In A, although the latency of events appears to occur simultaneously across electrodes sites 1, 2, 3, and 4, they appear to propagate from 4 to sites 5, 6, and 7. The main axis of propagation is shown in the *inset*. In the example in B, the latencies are progressively longer for the activity across sites 4–1. The main axis of propagation is shown in the *inset*. In C, a progressive delay was observed across all electrodes, from sites 1 to 7. These 2 axes of direction are shown in gray, whereas the main axis of propagation is shown in black.

tion in the isolated whole brain preparation induces continuous oscillatory activity at around 25–33 Hz (the gamma range at  $32^\circ\text{C}$ ) exclusively in the mEC (Dickson et al. 2000a; van der Linden et al. 1999), and we thus sought to test the activation of muscarinic receptors on slow periodic activity. In all cases examined ( $n = 19$ ), perfusion of carbachol ( $50\ \mu\text{M}$ ) gradually abolished SPE (Fig. 6). With increasing times of perfusion, the inter-SPE frequency increased and the amplitude of the events decreased until they eventually disappeared to be replaced by continuous fast oscillations (expanded trace on the right in Fig. 6A). Of special note, when laminar profiles were recorded for both SPE and gamma in the same experiment with 16-channel silicon probes, the fast oscillatory component of both types of

activity showed identical phase profiles with reversals localized to layer II (data not shown;  $n = 3$ ). In four further experiments, co-perfusion of CCh with the muscarinic antagonist, atropine sulfate ( $5\ \mu\text{M}$ ), abolished gamma activity and reinstated the SPE (Fig. 6B). Intracellular recordings made from superficial layer mEC cells during these manipulations ( $n = 3$ ) demonstrated that the gradual abolition of the slow field events by CCh coincided with a gradual depolarization of the membrane potential to a level at which the gamma oscillatory activity was expressed (Fig. 7, *middle*). Likewise, the reinstatement of the slow periodic activity by co-perfusion of CCh and atropine coincided with a return of the membrane potential toward control levels (Fig. 7, *right*).

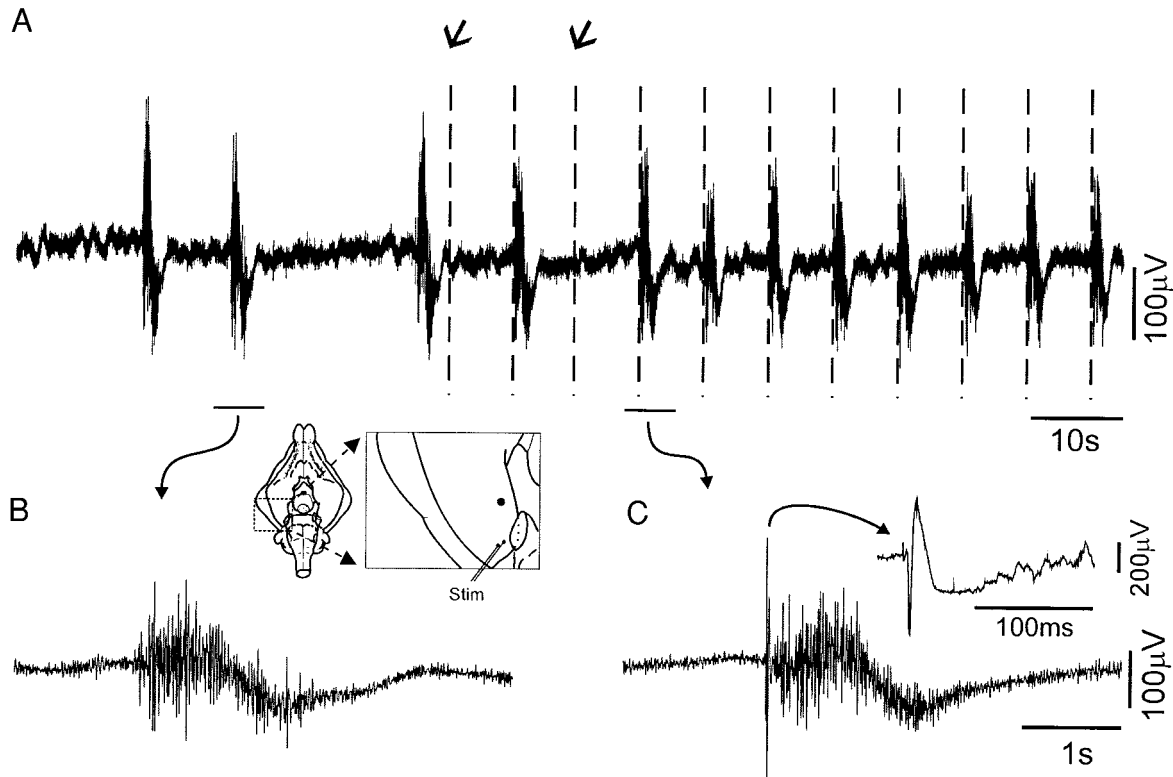


FIG. 5. Stimulation of superficial associational EC pathways can trigger spontaneous events. *A*: field traces demonstrating spontaneous activity prior and following stimulation of the superficial mEC every 7 s. Dotted vertical lines mark each stimulus and arrows mark the instances in which stimulation failed to trigger an event. *B* and *C*: expanded traces of spontaneous and stimulus-evoked events, respectively. The *inset* in *B* illustrates the sites of stimulation and recording. The *inset* in *C* is a further expansion of the evoked field potential that occurred just before the slow event onset. Recordings were performed at 600 microns depth.

#### DISCUSSION

The SPE observed in the EC of the isolated whole brain resemble periodic activity described *in vivo* in the EC of rats, cats, and guinea pigs (unpublished data; Charpak et al. 1995; Collins et al. 2001) and in other cortical and subcortical regions during deep anesthesia (Lampl and Yarom 1997; Steriade et al.

1993b,e; Wilson and Kawaguchi 1996). SPEs in the EC occurred with a variable periodicity, much lower (0.02–0.4 Hz) than that observed in the neocortex *in vivo* (0.7–1 Hz), but similar to recurrent slow events described in ferret neocortex and in the hippocampus both *in vitro* (Sanchez-Vives and McCormick 2000; Zhang et al. 1998) and *in vivo* (Penttonen et

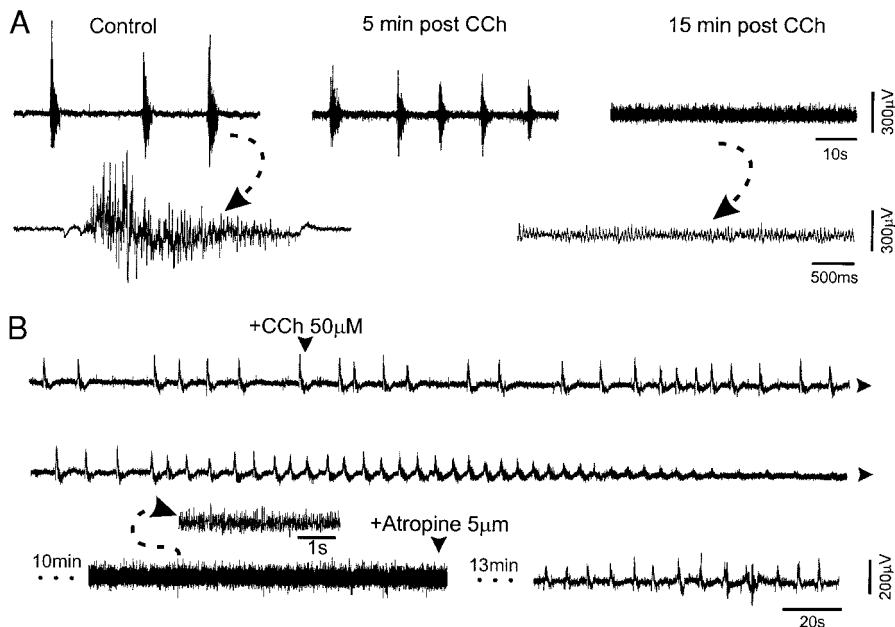


FIG. 6. Muscarinic receptor activation reversibly transforms slow periodic activity into continuous gamma activity. *A*: spontaneous field recordings before (control), 5 and 15 min after addition of 50  $\mu$ M carbachol (CCh) to the perfusate. SPEs (1 expanded *below left*) were replaced by gamma frequency (26 Hz) oscillations (see right expansion). *B*: continuous traces of spontaneous activity are shown prior and during a perfusion with carbachol. Following the addition of 50  $\mu$ M CCh to the perfusate (arrow), the frequency of SPE gradually increased and their amplitude gradually decreased until they were finally abolished. The disappearance of SPEs corresponded to the emergence of fast (gamma) oscillations at 27 Hz (see *inset*). Following the addition of 5  $\mu$ M of the muscarinic receptor antagonist atropine sulfate to the perfusate, gamma oscillations were abolished and SPEs were reinstated.

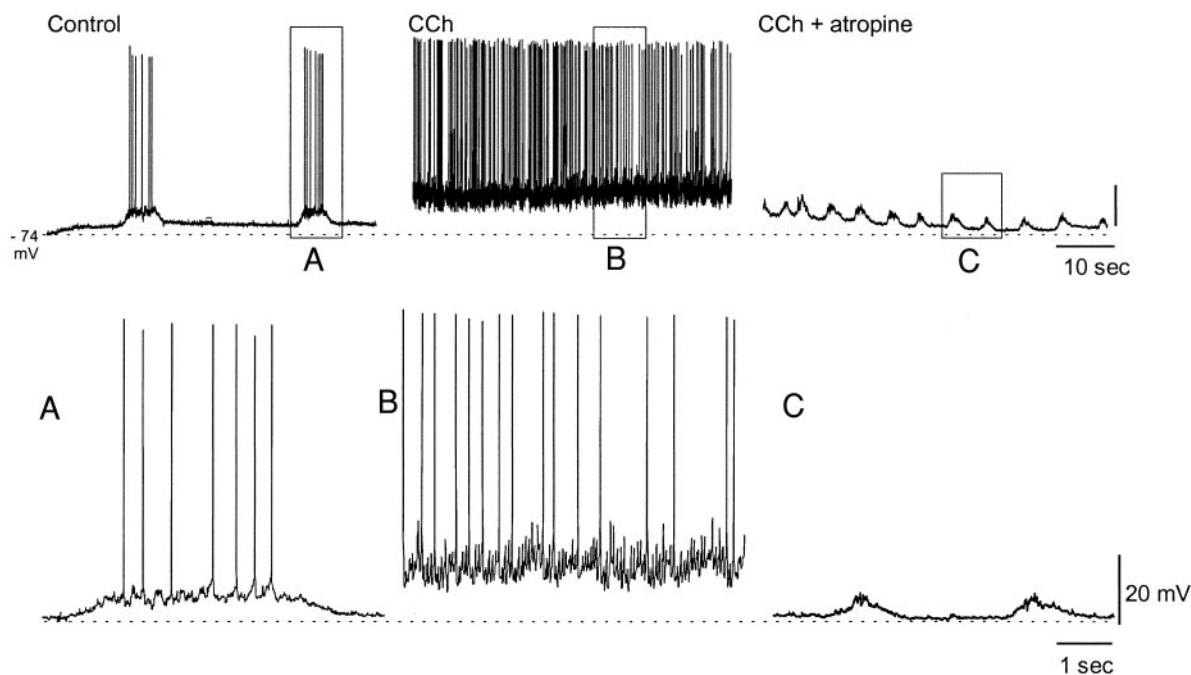


FIG. 7. Intracellular recording from a layer II neuron of the mEC, in control condition (*left*), after 15 min of 50  $\mu$ M CCh arterial perfusion (*middle*), and 10 min after co-perfusion of CCh with 5  $\mu$ M atropine sulfate (*right*). The areas marked by the dotted rectangles are expanded in the lower traces A, B, and C. Resting membrane potential =  $-74$  mV.

al. 1999). Such a difference in SPE frequency could be due to 1) the marked level of nervous system depression associated with the sensorial deprivation of the *in vitro* condition attained both in slices and in the isolated brain preparation, 2) the low temperature ( $32^{\circ}\text{C}$ ) utilized to substitute for pharmacological anesthesia in our experiments, and 3) a residual effect of the barbiturate anesthesia performed before brain isolation. Several clinical and experimental studies have demonstrated, indeed, that body temperature below  $32^{\circ}\text{C}$  considerably reduces brain metabolism to a functional state that correlates with unconsciousness or severe impairment of vigilance (Curley and Irwin 1991; Fischbeck and Simon 1981; FitzGibbon et al. 1984; Rosin and Exton-Smith 1964).

Our findings suggested that SPE in the EC are locally generated, since they showed a depth reversal in superficial layers. This is consistent with the observation that neocortical slow oscillations are local events spontaneously produced not only in the intact (*in vivo*) brain (Steriade et al. 1993e) but also in the undercut (deafferented) cortical slab (Timofeev et al. 2000) and in cortical slices maintained *in vitro* (Sanchez-Vives and McCormick 2000; Zhang et al. 1998). Even though the shaping of spontaneous slow neocortical activity *in vivo* involves a dynamic interaction with the thalamus (Timofeev et al. 1996), the above observations suggest that the necessary events subtending periodic slow oscillations are generated within the cortex itself in the absence of subcortical inputs. Such a conclusion is further strengthened by the finding that extensive thalamic lesions do not abolish the cortical slow rhythm (Steriade et al. 1993b) and that the slow oscillations are preserved in the *cerveau isolé* preparation (Belardetti et al. 1977; Steriade et al. 1993b).

*In vivo* studies have suggested that repetitive periodic slow activity in the neocortex correlates at the intracellular level with alternating “on” and “off” states. The “on” state is char-

acterized by threshold level plateau depolarizations topped with high-frequency neuronal firing and subthreshold oscillations. In contrast, the “off” state is characterized by membrane hyperpolarization. Both phases may be generated by active or disfacilitatory mechanisms, either synaptic or intrinsic (Amzica and Steriade 1995; Contreras et al. 1996; Timofeev et al. 2001). Such activity is similar to the two-state behavior observed in spiny neostriatal neurons *in vivo* (Wilson and Kawaguchi 1996), which is characterized by the sudden shift between two preferred membrane potential levels, presumably controlled by a nonidentified potassium conductance. The long-lasting time course of the hyperpolarized period observed between two SPEs in the mEC ( $\leq 20$  s) suggest that active inhibitory events, either synaptic or intrinsic, cannot account exclusively for the inter-SPE interval. Therefore, it is reasonable to hypothesize that the depolarization at the onset of SPE is not only mediated by the release of inhibition, but it is also driven by an active depolarization, possibly involving synaptic-mediated mechanisms. In support of this hypothesis, 1) intracellular depolarizing responses correlated to SPEs were increased in amplitude by injections of hyperpolarizing currents, 2) SPEs propagated tangentially across the surface of the EC possibly through excitatory associative connections, and 3) SPEs could be elicited by local EC stimulation that induces synaptic activation of fast glutamatergic neurotransmission mediated by intra-EC associative fibers (Dickson et al. 2000b). These observations are consistent with the study on *in vitro* slices (Sanchez-Vives and McCormick 2000), which demonstrated that generation and propagation of slow population events could be induced by pressure applications of glutamate and were blocked by antagonists of different synaptic excitatory receptor subtypes.

While the slow biphasic component of SPE was correlated with the slow depolarizing envelope recorded intracellularly in

superficial EC neurons, the fast oscillatory field activity of SPE correlated to the production of high-frequency spiking and membrane potential oscillations, as reported in vivo (Buzsáki 1992; Grenier et al. 2001; Steriade et al. 1993d). These fast rhythms are assumed to reflect local synchronization of cortical networks involving recurrent connections between excitatory principal neurons and inhibitory interneurons (Grenier et al. 2001), similar to those involved in the production of fast (gamma) rhythms in vitro (Dickson et al. 2000a; Fisahn et al. 1998; Traub et al. 1996). Interestingly, in the current study it was observed that the phase reversal of both the fast oscillatory activity during SPEs and gamma oscillations evoked by CCh occurred at the same depth, suggesting that they may be dependent on similar local network mechanisms.

As mentioned in the introduction, slow periodic patterns correlated with sleep and unconsciousness (Steriade et al. 1993b–e) can be disrupted by brain stem stimulation of cholinergic and noradrenergic nuclei (Moruzzi and Magoun 1949; Steriade and Contreras 1995; Steriade et al. 1993a) and by basal forebrain nuclei activation (Belardetti et al. 1977; Cape and Jones 1998). Even though the nonselective activation determined by in vivo brain stem and forebrain stimulation does not allow for a precise identification of the type of neurotransmitters involved, it has been suggested that cortical arousal is at least partially mediated via the cholinergic ascending pathways. Our findings demonstrate for the first time in an intact brain that slow periodic activity of cortical origin is disrupted by cholinergic activation, pharmacologically induced by exogenous application of a muscarinic agonist. Interestingly, the disappearance of SPEs coincides with the gradual development of a fast rhythm that is commonly associated with cortical activation (Cape and Jones 1998; Metherate et al. 1992; Steriade et al. 1991). During the cortical state transition induced by CCh, the amplitude of SPEs decreased and their frequency increased, while membrane potentials of principal cells progressively depolarized and fast oscillations in the gamma range gradually established. Previous reports from our group, indeed, have demonstrated that muscarinic activation with CCh consistently induces fast oscillations that are restricted to the medial part of the EC without involvement of other cortical regions, such as the lateral EC, the perirhinal cortex or the neocortex (Dickson et al. 2000a; van der Linden et al. 1999).

We conclusively demonstrate that the EC of the isolated brain preparation produces in resting conditions a periodic pattern similar to that observed in cortical brain structures in vivo and in vitro during spontaneous and during pharmacologically induced slow sleep. Furthermore, we demonstrate that these events are reversibly abolished by muscarinic receptor stimulation, suggesting that they are indeed representative of a deactivated state in the whole brain preparation. Since state-dependent shifts in the expression of cortical field activity are thought to reflect different functional modes of processing across different behavioral states, model systems for studying these shifts are extremely valuable. Our findings suggest that the isolated whole brain is a suitable preparation to study the mechanisms of generation and modulation of SPE.

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## REFERENCES

- Achermann P and Borbely AA.** Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience* 81: 213–222, 1997.
- Alonso A and Köhler C.** A study of the reciprocal connections between septum and entorhinal area using anterograde and retrograde axonal transport methods in the rat brain. *J Comp Neurol* 225: 327–343, 1984.
- Amzica F and Steriade M.** Short and long range neuronal synchronization of the slow (<1 Hz) cortical oscillation. *J Neurophysiol* 73: 20–38, 1995.
- Amzica F and Steriade M.** The K-complex: its slow (<1-Hz) rhythmicity and relation to delta waves. *Neurology* 49: 952–959, 1997.
- Amzica F and Steriade M.** Cellular substrates and laminar profile of sleep K-complex. *Neuroscience* 82: 671–686, 1998.
- Belardetti F, Borgia R, and Mancina M.** Proencephalic mechanisms of ECoG desynchronization in cerveau isole cats. *Electroencephalogr Clin Neurophysiol* 42: 213–235, 1977.
- Berger H.** Über das Elektrenkephalogramm des Menschen. *Arch Psychiat Nervenkr* 87: 527–570, 1929.
- Berger H.** Über das Elektrenkephalogramm des Menschen. Zweite Mitteilung. *J Psycho Neurol* 40: 160–179, 1930.
- Buzsáki G, Horváth Z, Urioste R, Hetke J, and Wise K.** High-frequency network oscillation in the hippocampus. *Science* 256: 1025–1027, 1992.
- Cape EG and Jones BE.** Differential modulation of high-frequency gamma-electroencephalogram activity and sleep-wake state by noradrenaline and serotonin microinjections into the region of cholinergic basal ganglia neurons. *J Neurosci* 18: 2653–2666, 1998.
- Chapak S, Pare D, and Llinás R.** The entorhinal cortex entrains fast CA1 hippocampal oscillations in the anaesthetized guinea-pig: role of the mono-synaptic component of the perforant path. *Eur J Neurosci* 7: 1548–1557, 1995.
- Chrobak JJ and Buzsáki G.** High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J Neurosci* 16: 3056–3066, 1996.
- Chrobak JJ and Buzsáki G.** Gamma oscillations in the entorhinal cortex of the freely behaving rat. *J Neurosci* 18: 388–398, 1998.
- Collins DR, Pelletier GJ, and Paré D.** Slow and fast (gamma) neuronal oscillations in the perirhinal cortex and lateral amygdala. *J Neurophysiol* 85: 1661–1672, 2001.
- Contreras D, Timofeev I, and Steriade M.** Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J Physiol* 494: 251–264, 1996.
- Curley FJ and Irwin RS.** Disorders of temperature control: hypothermia. In: *Intensive Care Medicine*, edited by Rippe J, Irwin RS, Alpert JS, and Fink MP, Boston, MA: Little, Brown and Company, 1991, p. 658–674.
- de Curtis M, Biella G, Buccellati C, and Folco G.** Simultaneous investigation of the neuronal and vascular compartments in the guinea pig brain isolated in vitro. *Brain Res Prot* 3: 221–228, 1998.
- de Curtis M, Pare D, and Llinás RR.** The electrophysiology of the olfactory-hippocampal circuit in the isolated and perfused adult mammalian brain in vitro. *Hippocampus* 1: 341–354, 1991.
- Dickson CT.** The extrinsic modulation of entorhinal cortex theta: field and unit activity studies. In: *Behavioral Neuroscience* (PhD thesis). Calgary: University of Calgary, 1994.
- Dickson CT, Biella G, and de Curtis M.** Evidence for spatial modules mediated by temporal synchronisation of carbachol induced gamma rhythm in medial entorhinal cortex. *J Neurosci* 20: 7846–7854, 2000a.
- Dickson CT, Biella GB, and de Curtis M.** Carbachol evoked gamma activity in medial entorhinal cortex reflects synchronisation of spatially localized superficial interneurons. *Eur J Neurosci* 12: S155, 2000b.
- Dickson CT, Kirk IJ, Oddie SD, and Bland BH.** Classification of theta-related cells in the entorhinal cortex: cell discharges are controlled by the ascending brainstem synchronizing pathway in parallel with hippocampal theta-related cells. *Hippocampus* 5: 306–319, 1995.
- Fisahn A, Pike FG, Buhl EH, and Paulsen O.** Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature* 394: 186–189, 1998.
- Fischbeck KH and Simon RP.** Neurological manifestation of accidental hypothermia. *Ann Neurol* 10: 384–387, 1981.

- FitzGibbon T, Hayward JS, and Walker D.** EEG and visual evoked potentials of conscious man during moderate hypothermia. *Electroencephalogr Clin Neurophysiol* 58: 48–54, 1984.
- Grenier F, Timofeev I, and Steriade M.** Focal synchronization of ripples (80–200 Hz) in neocortex and their neuronal correlates. *J Neurophysiol* 86: 1884–1898, 2001.
- Jefferys JGR.** Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol Rev* 75: 689–723, 1995.
- Lampl I, Reichova I, and Ferster D.** Synchronous membrane potential fluctuations in neurons of the cat visual cortex. *Neuron* 22: 361–374, 1999.
- Lampl I and Yarom Y.** Subthreshold oscillations and resonant behavior: two manifestations of the same mechanism. *Neuroscience* 78: 325–341, 1997.
- Leung LS.** Generation of theta and gamma rhythms in the hippocampus. *Neurosci Biobehav Rev* 22: 275–290, 1998.
- Llinás R, Ribary U, Contreras D, and Pedroarena C.** The neuronal basis for consciousness. *Philos Trans Roy Soc Lond Ser B* 353: 1841–1849, 1998.
- Llinás R, Yarom Y, and Sugimori M.** Isolated mammalian brain in vitro: new technique for analysis of electrical activity of neuronal circuit function. *Fed Proc* 40: 2240–2245, 1981.
- Llinás RR and Paré D.** Of dreaming and wakefulness. *Neuroscience* 44: 521–535, 1991.
- Metherate R, Cox CL, and Ashe JH.** Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J Neurosci* 12: 4701–4711, 1992.
- Mitchell SJ, Rawlins JN, Steward O, and Olton DS.** Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats. *J Neurosci* 2: 292–302, 1982.
- Moruzzi G and Magoun HW.** Brainstem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* 1: 455–473, 1949.
- Muhlethaler M, de Curtis M, Walton K, and Llinás R.** The isolated and perfused brain of the guinea pig in vitro. *Eur J Neurosci* 5: 915–926, 1993.
- Niedermeyer E.** Sleep and EEG. In: *Electroencephalography*, 4th ed., edited by Niedermeyer E and Lopes da Silva F. Baltimore: Williams and Wilkins, 1999, p. 174–188.
- Penfield W and Jasper HH.** *Epilepsy and the Functional Anatomy of the Human Brain*. Boston, MA: Little, Brown Publisher, 1954.
- Penttonen M, Nurminen N, Miettinen R, Sirvio J, Henze DA, Csicsvari J, and Buzsáki G.** Ultra-slow oscillation (0.025) triggers hippocampal after-discharges in Wistar rats. *Neuroscience* 94: 735–743, 1999.
- Rosin AJ and Exton-Smith AN.** Clinical features of accidental hypothermia with some observations on thyroid function. *Br Med J* 1: 16–30, 1964.
- Sanchez-Vives MV and McCormick DA.** Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci* 3: 1027–1034, 2000.
- Steriade M.** Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. *Cereb Cortex* 7: 583–604, 1997.
- Steriade M.** Corticothalamic resonance, states of vigilance and mentation. *Neuroscience* 101: 243–276, 2000.
- Steriade M, Amzica F, and Nunez A.** Cholinergic and noradrenergic modulation of the slow (0.3 Hz) oscillation in neocortical cells. *J Neurophysiol* 70: 1385–1400, 1993a.
- Steriade M and Contreras D.** Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. *J Neurosci* 15: 623–642, 1995.
- Steriade M, Contreras D, and Amzica F.** Synchronized sleep oscillations and their paroxysmal developments. *Trends Neurosci* 17: 199–208, 1994.
- Steriade M, Contreras D, Curró Dossi R, and Nuñez A.** The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci* 13: 3284–3299, 1993b.
- Steriade M, Curró Dossi R, Paré D, and Oakson G.** Fast oscillations (20–40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *Proc Nat Acad SciUSA* 88: 4396–4400, 1991.
- Steriade M, McCormick DA, and Sejnowski TJ.** Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262: 679–685, 1993c.
- Steriade M, Nuñez A, and Amzica F.** Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the EEG. *J Neurosci* 13: 3266–3283, 1993d.
- Steriade M, Nuñez A, and Amzica F.** A novel slow (<1 Hz) oscillation of neocortical neurons *in vivo*: depolarizing and hyperpolarizing components. *J Neurosci* 13: 3252–3265, 1993e.
- Timofeev I, Contreras D, and Steriade M.** Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. *J Physiol* 494: 265–278, 1996.
- Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, and Steriade M.** Origin of slow cortical oscillations in deafferented cortical slabs. *Cereb Cortex* 10: 1185–1199, 2000.
- Timofeev I, Grenier F, and Steriade M.** Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. *Proc Nat Acad SciUSA* 98: 1924–1929, 2001.
- Traub R, Whittington M, Colling S, Buzsáki G, and Jefferys J.** Analysis of gamma rhythms in the rat hippocampus *in vitro* and *in vivo*. *J Physiol* 493: 471–484, 1996.
- van der Linden S, de Curtis M, and Panzica F.** Carbachol induces fast oscillations in the medial but not in the lateral entorhinal cortex of the isolated guinea pig brain. *J Neurophysiol* 82: 2441–2450, 1999.
- Wilson CJ and Kawaguchi Y.** The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J Neurosci* 16: 2397–2410, 1996.
- Wu C, Shen H, Luk HP, and Zhang L.** A fundamental oscillatory state of isolated rodent hippocampus. *J Physiol* 540: 509–527, 2002.
- Zhang Y, Perez Velazquez JL, Tian GF, Wu CP, Skinner FK, Carlen PL, and Zhang L.** Slow oscillations (<1 Hz) mediated by GABAergic inter-neuronal networks in rat hippocampus. *J Neurosci* 18: 9256–9268, 1998.