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INCESSANT TRANSITIONS BETWEEN ACTIVE AND SILENT STATES IN CORTICO-THALAMIC CIRCUITS AND ALTERED NEURONAL EXCITABILITY LEAD TO EPILEPSY

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Résumé

La ligne directrice de nos expériences a été l'hypothèse que l'apparition et/ou la persistance des fluctuations de longue durée entre les états silencieux et actifs dans les réseaux néocorticaux et une excitabilité neuronale modifiée sont les facteurs principaux de l'épileptogenèse, menant aux crises d'épilepsie avec expression comportementale.

Nous avons testé cette hypothèse dans deux modèles expérimentaux différents. La déafférentation corticale chronique a essayé de répliquer la déafférentation physiologique du neocortex observée pendant le sommeil à ondes lentes. Dans ces conditions, caractérisées par une diminution de la pression synaptique et par une incidence augmentée de périodes silencieuses dans le système cortico-thalamique, le processus de plasticité homéostatique augmente l'excitabilité neuronale. Par conséquent, le cortex a oscillé entre des périodes actives et silencieuses et, également, a développé des activités hyper-synchrones, s'étendant de l'hyperexcitabilité cellulaire à l'épileptogenèse focale et à des crises épileptiques généralisées.

Le modèle de stimulation sous-liminale chronique (« kindling ») du cortex cérébral a été employé afin d'imposer au réseau cortical une charge synaptique supérieure à celle existante pendant les états actifs naturels - état de veille ou sommeil paradoxal (REM). Dans ces conditions un mécanisme différent de plasticité qui s'est exprimé dans le système thalamo-corticale a imposé pour des longues périodes de temps des oscillations continuelles entre les époques actives et silencieuses, que nous avons appelées des activités paroxysmiques persistantes.

Indépendamment du mécanisme sous-jacent de l'épileptogenèse les crises d'épilepsie ont montré certaines caractéristiques similaires : une altération dans l'excitabilité neuronale mise en évidence par une incidence accrue des décharges neuronales de type bouffée, une tendance constante vers la généralisation, une propagation de plus en plus rapide, une synchronie augmentée au cours du temps, et une modulation par les états de vigilance (facilitation pendant le sommeil à ondes lentes et barrage pendant le sommeil REM).

Les états silencieux, hyper-polarisés, de neurones corticaux favorisent l'apparition des bouffées de potentiels d'action en réponse aux événements synaptiques, et l'influence post-synaptique d'une bouffée de potentiels d'action est beaucoup plus importante par rapport à l'impacte d'un seul potentiel d'action. Nous avons également apporté des évidences que les neurones néocorticaux de type FRB sont capables à répondre avec des bouffées de potentiels d'action pendant les phases hyper-polarisées de l'oscillation lente, propriété qui peut jouer un rôle très important dans l'analyse de l'information dans le cerveau normal et dans l'épileptogenèse. Finalement, nous avons rapporté un troisième mécanisme de plasticité dans les réseaux corticaux après les crises d'épilepsie - une diminution d'amplitude des potentiels post-synaptiques excitatrices évoquées par la stimulation corticale après les crises - qui peut être un des facteurs responsables des déficits comportementaux observés chez les patients épileptiques.

Nous concluons que la transition incessante entre des états actifs et silencieux dans les circuits cortico-thalamiques induits par disfacilitation (sommeil à ondes lentes), déafférentation corticale (épisodes ictales à 4-Hz) ou par une stimulation sous-liminale chronique (activités paroxysmiques persistantes) crée des circonstances favorables pour le développement de l'épileptogenèse. En plus, l'augmentation de l'incidence des bouffées de potentiels d'actions induisant une excitation post-synaptique anormalement forte, change l'équilibre entre l'excitation et l'inhibition vers une supra-excitation menant a l'apparition des crises d'épilepsie.

Abstract

The guiding line in our experiments was the hypothesis that the occurrence and / or the persistence of long-lasting fluctuations between silent and active states in the neocortical networks, together with a modified neuronal excitability are the key factors of epileptogenesis, leading to behavioral seizures.

We addressed this hypothesis in two different experimental models. The chronic cortical deafferentation replicated the physiological deafferentation of the neocortex observed during slow-wave sleep (SWS). Under these conditions of decreased synaptic input and increased incidence of silent periods in the corticothalamic system the process of homeostatic plasticity up-regulated cortical cellular and network mechanisms and leaded to an increased excitability. Therefore, the deafferented cortex was able to oscillate between active and silent epochs for long periods of time and, furthermore, to develop highly synchronized activities, ranging from cellular hyperexcitability to focal epileptogenesis and generalized seizures.

The kindling model was used in order to impose to the cortical network a synaptic drive superior to the one naturally occurring during the active states - wake or rapid eye movements (REM) sleep. Under these conditions a different plasticity mechanism occurring in the thalamo-cortical system imposed long-lasting oscillatory pattern between active and silent epochs, which we called outlasting activities.

Independently of the mechanism of epileptogenesis seizures showed some analogous characteristics: alteration of the neuronal firing pattern with increased bursts probability, a constant tendency toward generalization, faster propagation and increased synchrony over the time, and modulation by the state of vigilance (overt during SWS and completely abolished during REM sleep).

Silent, hyperpolarized, states of cortical neurons favor the induction of burst firing in response to depolarizing inputs, and the postsynaptic influence of a burst is much stronger as compared to a single spike. Furthermore, we brought evidences that a particular type of neocortical neurons - fast rhythmic bursting (FRB) class - is capable to consistently respond

with bursts during the hyperpolarized phase of the slow oscillation, fact that may play a very important role in both normal brain processing and in epileptogenesis. Finally, we reported a third plastic mechanism in the cortical network following seizures - a decreasing amplitude of cortically evoked excitatory post-synaptic potentials (EPSP) following seizures - which may be one of the factors responsible for the behavioral deficits observed in patients with epilepsy.

We conclude that incessant transitions between active and silent states in cortico-thalamic circuits induced either by disfacilitation (sleep), cortical deafferentation (4-Hz ictal episodes) and by kindling (outlasting activities) create favorable circumstances for epileptogenesis. The increase in burst-firing, which further induce abnormally strong postsynaptic excitation, shifts the balance of excitation and inhibition toward overexcitation leading to the onset of seizures.

Foreword

The following thesis is presented in the form of a collection of scientific articles - published, accepted, or submitted for publication. The general introduction describes the theoretical context and the experimental strategies we used in the presented studies. A review of the results and a general discussion finalizes the thesis. The bibliography used for both introduction and discussion is presented at the very end of the manuscript, while the bibliography for each chapter follows the text of the corresponding paper, with a formatting of the reference list in accordance to the rules of the journal where the paper was published.

In order to preserve the coherence of the present thesis I have chosen to include only six from more than twelve papers to which I contributed during my thesis. The core of the thesis is represented by four scientific articles which focus on the cortical epileptogenesis in two different models of epilepsy - cortical deafferentation and kindling; and on the general characteristics of neocortical seizures and their modulation by the different states of vigilance. Two more studies have also been included, one related to the integrative properties of FRB neurons which may play a very important role in maintaining the intracortical dialogue during the cortical slow-oscillation thus being able to promote seizures, and a second study which reported a plastic change in the excitatory output of the neocortical neurons following seizures, which may account for the neurological impairments observed in epileptic patients.

I would like to use this opportunity to express my deep gratitude to my thesis supervisor - Prof. Mircea Steriade, whom I consider my mentor that I highly admire and continue to love. Beyond the fruitful discussions, authoritative critics and support throughout the progression of my thesis without which the present studies would not have been possible, I will always remember his innate charisma and the strict, coherent value system of hard working, motivation, dedication, and perseverance to which he was committed and which he shared with his students. More than a mentor however, I consider him a friend whose support and advice had a decisive influence in my life.

I also would like to express my gratitude to the co-director of my thesis - Prof. Igor Timofeev for his distinctive presence during all these years, for his help during the complex

and laborious experimental proceedings, some of which I would have considered unattainable before.

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Equally, I would like to thank the organisms which provided financial support for the development of these projects: Canadian Institute for Health Research, Human Frontier Research Program, National Institute of Neurological Disorders and Stroke, and Fonds de la Recherche en Santé de Québec.

Following, is the chronological list of scientific articles in which I have participated during the progress of my PhD program in the Anatomy and Physiology Department of Medical School in Laval University:

1. Hyperpolarization rectification in cat lateral geniculate neurons modulated by intact corticothalamic projections. Nita DA, Steriade M, Amzica F, J Physiol. (Lond), 552, pp. 325-332 (2003).

2. Non-neuronal origin of CO2-related DC EEG shifts: an in vivo study in the cat. Nita DA, Vanhatalo S, Lafortune FD, Voipio J, Kaila K, Amzica F, J Neurophysiol., 92(2), pp. 1011-1022 (2004).

3. Cholinergic action on cortical glial cells in vivo. Seigneur J, Kroeger D, Nita DA, Amzica F, Cereb. Cortex, 16(5), pp. 655-68 (2006).

4. Increased propensity to seizures following chronic cortical deafferentation in vivo. Nita DA, Cissé Y, Timofeev I, Steriade M, J Neurophysiol., 95(2), pp. 902-13 (2006).

5. Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. Nita DA, Cissé Y, Timofeev I, Steriade M, Cereb. Cortex, 17(2), pp. 272-83 (2007).

6. State-dependent outlasting activities following neocortical kindling in cat. Nita DA, Cissé Y, Timofeev I, (submitted to Experimental Neurology)

7. Cortical and thalamic components of outlasting activities following neocortical kindling in cat. Nita DA, Cissé Y, Timofeev I, (submitted to Experimental Neurology)

8. Callosal responses of fast-rhythmic-bursting neurons during slow oscillation in cat. Cissé Y, Nita DA, Steriade M, Timofeev I, Neuroscience, 147(2), pp. 272-6 (2007).

9. EPSPs' depression following neocortical seizures. Nita DA, Cissé Y, Timofeev I (submitted to Epilepsia)

10. Immunohistochemical evidences for the loss of cortical interneurons following cortical deafferentation in vivo. Avramescu S, Nita DA, Timofeev I, (in preparation)

Some of the original eperimental results from the previous mentioned papers were discussed in a recently published book chapter:

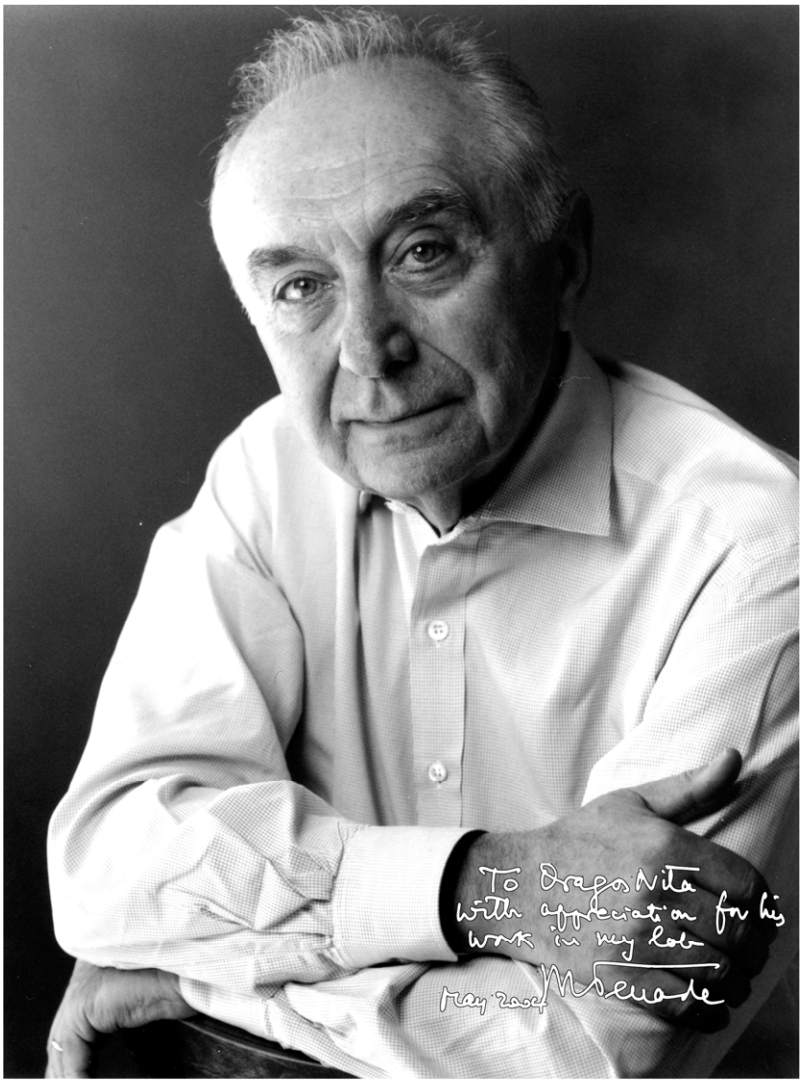
11. Incessant transitions between active and silent states in thalamocortical circuits lead to epilepsy. Nita DA and Timofeev I, in "Mechanisms of Spontaneous Active States in the Neocortex" (Ed. Timofeev I), 2007 (in press)

Equally, I collaborated with my colleagues from the Neurophysiology Laboratory, Medical School of "Carol Davila" University (Bucharest, Romania) on the methodology, critics and discussions of two of their published studies:

12. Delayed ischemic electrocortical suppression during rapid repeated cerebral ischemia and kainate-induced seizures in rat. Ilie A, Spulber S, Avramescu S, Nita DA, Zagrean AM, Zagrean L, Moldovan M, Eur J Neurosci, 23(8), pp. 2135-44 (2006).

13. Endogenous activation of adenosine A(1) receptors accelerates ischemic suppression of spontaneous electrocortical activity. Ilie A, Ciocan D, Zagrean AM, Nita DA, Zagrean L, Moldovan M, J Neurophysiol., 96(5), pp. 2809-14 (2006).

Last but not least, I would like to thank my family for their love, especially my mother, Viorica, for exposing me to fundamental research and for advocating the role of research in medical practice; and my father, Vasile, for his continuous encouragements and support.



In memoriam Mircea Steriade (1924-2006)

To all those who generously taught me their knowledge, shared with me their experience and creatively advocated for their beliefs.

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List of abbreviations

ACh	acetylcholine
AS	acute seizures
AHP	afterhyperpolarizing potential
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate
CT	corticothalamic
EEG	electroencephalogram
EMG	electromyogram
EOG	electro-oculogram
EPSP	excitatory postsynaptic potential
EPSC	excitatory postsynaptic current
EThG	electrothalamogram
FRB	fast-rhythmic-bursting
FPP	fast prepotential
FS	fast-spiking
EThG	electrothalamogram
GABA	gamma-amino butyric acid
Hz	Hertz
I_{AHP}	calcium dependant potassium current
I_H	H-potassium current
$I_{Na(p)}$	persistent sodium current
I_T	low-threshold calcium current
IB	intrinsically-bursting
IPSP	inhibitory postsynaptic potential
IS	interictal spike
LDT	laterodorsal tegmentum
LG	lateral geniculate
LTD	long-time depression
LTP	long-time potentiation
LTS	low-threshold spike
MG	medial geniculate
NB	nucleus basalis
NMDA	N-methyl-D-aspartate
NREM	non-REM sleep
OA	outlasting activity
PDS	paroxysmal depolarizing shift
PPT	pedunculopontine tegmentum
PSW	poly-spike-wave
RE	thalamic reticular
REM	rapid eye movement
RS	regular-spiking
STDP	spike-timing dependent plasticity
SW	spike-wave
SWS	slow-wave sleep
TC	thalamocortical

TTX	tetrodotoxin
VL	ventrolateral
VP	ventro posterior
V_m	membrane potential
VM	ventral medial

1 Introduction

1.1 Active and Silent States of the Brain

The brain is a complex system of numerous self-governed oscillators, which are internally generated and extend the timing of activation/deactivation processes to different portions of the brain linked by reciprocal intrinsic connections. The reciprocal interactions between cortical and subcortical structures in the control of the sleep-wake states involve the thalamus, the hypothalamus, the forebrain and the brainstem.

1.1.1 The Corticothalamic Circuit

Corticothalamic (CT), thalamocortical (TC) and thalamic reticular (RE) neurons are the key players in a network that generates the main EEG sleep rhythms: slow oscillation, delta and spindles, under the lilt of the modulatory systems originating in the brainstem and basal forebrain; jointly to other oscillatory patterns (infraslow oscillations, beta, gamma, ripples) which may occur during all states of vigilance and pathological developments. The aim of this chapter is to briefly present the main cellular types and synaptic articulations in the corticothalamic circuitry operating under the influence of the brainstem cholinergic and basal forebrain modulatory systems, which control the neuronal excitability during brain activated states of wakefulness and rapid eye movement (REM) sleep, as well as during the sleep cycle.

1.1.1.1 Neocortical Neurons

There are two main types of neocortical neurons: pyramidal-shaped long-axoned (excitatory) neurons and non-pyramidal neurons. A part of non-pyramidal neurons, stellate aspiny or sparingly spiny, are inhibitory neurons. Each group of these two major neuronal types presents a great variability regarding their targets structures, electrophysiological characteristics and firing patterns related to behavioral states of vigilance.

The targets of the axons from the small pyramidal neurons located in cortical layers II-III are represented by ipsi- and contra-lateral cortical areas; layer V big pyramidal neurons project to intralaminar thalamic nuclei, basal ganglia, superior colliculus, and spinal cord; while layer VI pyramidal neurons project to claustrum and different specific thalamic

nuclei. The neurotransmitter used by the pyramidal-shaped neurons is either glutamate, or aspartate.

Local inhibitory interneurons that form 20-25% of all cortical neurons have a remarkably great variety. This was originally described in morphological studies several decades ago (Jones, 1975) and was recently substantiated by studying the chemical codes of different local-circuitry neurons and their electrophysiology (Hendrickson et al., 1981; Hendry et al., 1987; Kisvarday et al., 1990; Gonchar and Burkhalter, 1997). Inhibitory interneurons release gamma-amino butyric acid (GABA) as transmitter and some of them also express parvalbumin, somatostatin, cholecystokinin and vasointestinal peptides (Somogyi et al., 1983; Somogyi et al., 1985). They target various parts of the somato-dendritic membrane of pyramidal neurons or other local-circuitry neurons.

Basket cells are located in all cortical layers, but mainly layers III and V/VI, their axons contact the soma and the proximal dendrites of pyramidal neurons, and they autoregulates through autapses. Chandelier neurons are mainly located in layers II/III and their main target is the initial segment of pyramidal cell axons, thus disposing of a strategic location in preventing the latter to communicate with other neurons. Double-bouquet dendritic neurons are concentrated in cortical layers II/III and their vertical axons contact pyramidal cells and local interneurons. The smaller interneurons, the neurogliaform cells are found in superficial layers, mainly layer I, and have a very dense axonal arbor. Martinotti cells are found in deep layers, mainly layer VI (reviewed in Steriade and McCarley, 2005).

Neocortical neurons from different cortical layers reveal several firing patterns in response to depolarizing current pulses injected to soma both *in vitro* experiments (Connors et al., 1982; McCormick et al., 1985), and *in vivo* anesthetized (Nunez et al., 1993; Gray and McCormick, 1996) or chronically non-anesthetized animals (Baranyi et al., 1993a; Baranyi et al., 1993b; Steriade et al., 2001; reviewed in Steriade, 2004). By their response type they classify in four classes: regular-spiking (RS), intrinsically-bursting (IB), fast-rhythmic-bursting (FRB) and fast-spiking (FS) (Fig. 1-1). While RS, IB and FRB firing patterns are usually associated with excitatory pyramidal-shaped neurons, cells with FS firing pattern are presumably small inhibitory neurons. However, there were reported local-circuit, sparsely spiny or aspiny neurons discharging with a FRB pattern (Steriade et al., 1998b), or

some local inhibitory interneurons discharging like RS or IB cells (Thomson et al., 1996), therefore the neuronal cell classes should be rather considered as flexible entities (Steriade, 2004).

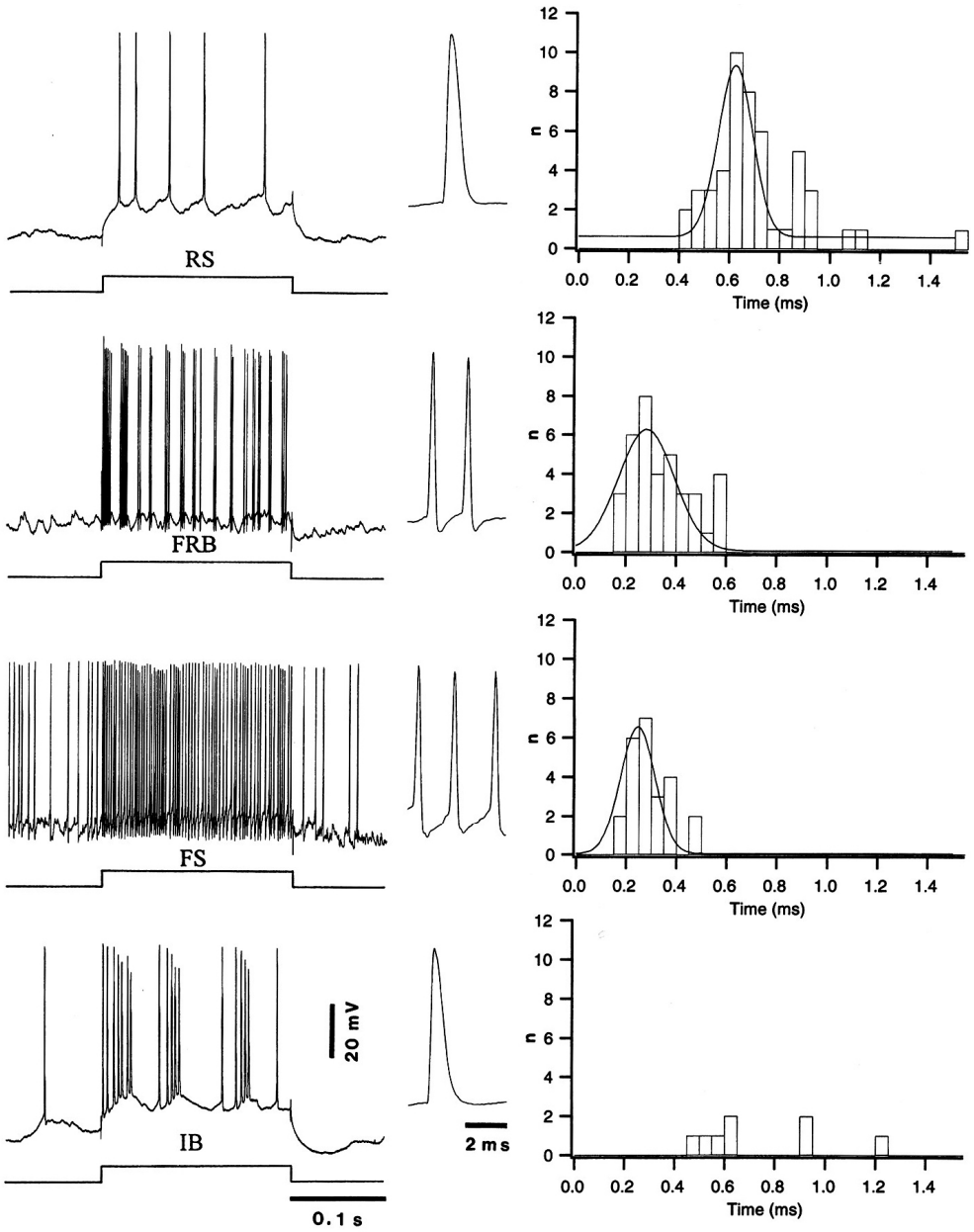

Figure 1-1 Electrophysiological identification of different cell classes. Left: responses of regular-spiking (RS), fast-rhythmic-bursting (FRB), fast-spiking (FS), and intrinsically-bursting (IB) neurons from cortical area 4 to depolarizing current pulses (0.2 s, 0.8 nA). At right of each depolarizing current pulse, action potentials of each cell classes. Right panels: histograms of the duration of action potentials (at half-amplitude). (From Steriade et al., 2001)

RS neurons constitute the majority of cortical pyramidal neurons. They display trains of single spikes that can adapt quickly or, more often, slowly to the direct stimulation. FRB neurons generate high-frequency (300-600 Hz) spike-bursts recurring at fast rates (30-50 Hz). Some of these neurons are found both in the superficial and deep cortical layers and could be antidromically activated from the thalamus, other are local circuit neurons (Steriade et al., 1998a). IB neurons generate clusters of action potentials with spike inactivation. FS neurons fire thin action potentials and sustain tonically very high firing rates without frequency adaptation (Nunez et al., 1993; Steriade, 2001).

In behaving animals, the duration of intracellularly recorded action potential is 0.6-1 ms in RS neurons, with slightly longer spikes fired by IB neurons. In contrast, FRB and FS neurons fire much shorter action potentials with modes at ~0.3 ms (Steriade et al., 2001).

The spike-burst in IB neurons develop from a depolarizing after-potential as is also the case for FRB neurons. The depolarizing after-potential of IB neurons is related to the activation of the persistent Na^+ current ($I_{Na(p)}$) as it is sensitive to TTX and QX314 (Nishimura et al., 2001). The high-frequency spike-bursts and very short action potential fired by FRB neurons are probably due to voltage-gated K^+ currents of the Kv3 subfamily, which are characterized by very fast deactivation rates, property that underlies the fast repolarization of action potentials (Rudy and McBain, 2001). Other ionic currents described in cortical pyramidal neurons include a hyperpolarization-activated cation current (I_H) (Solomon et al., 1993), a low-threshold Ca^{2+} current de-inactivated by hyperpolarization (Kawaguchi, 1993; de la and Geijo-Barrientos, 1996), high-threshold Ca^{2+} currents (Brown et al., 1993) and several K^+ currents (Schwindt et al., 1988a; Schwindt et al., 1988b; Schwindt et al., 1989).

1.1.1.2 Thalamic Neurons

Thalamus contains three types of neurons: excitatory glutamatergic TC neurons, inhibitory GABA-ergic neurons from the reticular thalamic nucleus (RE) that form a thin sheet that surrounds the lateral and rostral surfaces of the thalamus, and GABA-ergic local-circuit neurons whose axons remain within the limits of the dorsal thalamic nuclei.

The distinction between TC neurons is linked mainly to their soma size, large neurons projecting to deep and middle cortical layers, whereas small neurons projecting to

superficial layers (Jones, 1985). The highest degree of specificity and organization characterize the sensory nuclei, in particular the lateral geniculate (LG), medial geniculate (MG), and ventroposterior nuclei (VP). Ventrolateral (VL) and ventromedial (VM) motor relay nuclei receive afferents from the cerebellum and other structures related to motor functions. Other thalamic nuclei such as pulvinar and lateroposterior, project to wider cortical areas and are implicated in association processes. Distinctly from relay nuclei that receive specific information from sensory or motor pathways, intralaminar nuclei receive afferents from a variety of heterogeneous sources, including fibers from pain pathways, motor center and brainstem systems implicated in the control of the states of vigilance. The posterior intralaminar nuclei project mainly to the striatum and hippocampal formation, while the anterior intralaminar nuclei project not only to the striatum but also to superficial as well as deep layers of widespread cortical areas cortex.

Neurons from the thalamic reticular nucleus have axons that collateralize within the nucleus, and also project to the dorsal thalamic nuclei, but not to the cortex. Contrary to TC neurons that can communicate only through intermediary reticular or neocortical neurons, RE neurons form an interconnected network which is particularly well suited for the generation of some sleep oscillatory types which can occur even in the deafferented reticular nucleus (Steriade et al., 1987; Bazhenov et al., 1999). Many pairs of RE neurons are electrotonically coupled (Landisman et al., 2002; Fuentealba et al., 2004) fact that may play an important role in the synchronization of their activity. There is a reciprocal excitatory circuit between glutamatergic neocortical and TC neurons and a recurrent inhibitory loop between TC neurons and GABA-ergic reticular neurons.

Local circuit GABA-ergic thalamic interneurons constitute 25-30% of neurons in all thalamic nuclei of cats and other primates, but they are virtually absent in other nuclei (Jones, 1985). They receive afferents from the axon terminals of ascending pathway and target both TC neurons and dendrites of other local interneurons in thalamic synaptic islands (called glomeruli) encapsulated by glial cell, at the end of ascending afferent fibers. This specific architecture has a very important functional consequence: it generates typical EPSPs with a very short duration that allows the relay cells to transfer with high fidelity signals recurring at very fast rates (even 300Hz). The short duration of the EPSPs in TC

neurons is explained by a cut-off phenomenon mediated by the local circuit GABA-ergic neurons. IPSPs derived from these neurons immediately cut off each EPSP that occurs in the TC neurons, and these IPSPs are themselves immediately removed by a GABA-to-GABA inhibitory projection (Hirsch and Burnod, 1987; Crunelli et al., 1988; Pare et al., 1991; Curro et al., 1992; Steriade and McCarley, 2005).

The intrinsic properties of thalamic neurons allow them to function in two different modes, in two distinct behavioral states: a tonic discharge pattern during both EEG-activated states (wakefulness and REM sleep), accompanied by enhanced and accurate synaptic transmission of incoming information from the outside world during the adaptive waking state; and a bursting mode associated with depressed transfer function during EEG-synchronized sleep (Hirsch et al., 1983; see for review Steriade et al., 1997).

The electrophysiological properties of TC neurons are mediated mainly by a transient Ca^{2+} current (I_T) de-inactivated by hyperpolarization and underlying low-threshold-spikes (LTS) crowned by rebound spike-bursts (Llinas and Jahnsen, 1982; Steriade and Llinas, 1988), high-threshold Ca^{2+} currents (Hernandez-Cruz and Pape, 1989), a hyperpolarization-activated cation current (I_H) that produces a depolarizing sag (McCormick and Pape, 1990a; McCormick and Pape, 1990b; Leresche et al., 1991), a persistent Na^+ current ($I_{Na(p)}$) (Llinas and Jahnsen, 1982; Jahnsen and Llinas, 1984a; Jahnsen and Llinas, 1984b), and different types of K^+ currents (Jahnsen and Llinas, 1984a; Jahnsen and Llinas, 1984b; McCormick, 1991). These intrinsic properties are important in the generation and synchronization of thalamic oscillations (Fig. 1-2).

Reticular neurons operate similar to TC neurons: tonic discharges during brain-active states and rhythmic spike-bursts during slow-wave sleep (SWS). The spike-bursts are much longer (30-80 ms but up to 1 second when followed by a tonic tail) than in TC neurons (5-15 ms), and the bursts of the reticular cells display an accelerando-decelerando pattern, different from the progressively increasing inter-spike intervals in TC cells (Domich et al., 1986; Steriade et al., 1986). The graded bursting behavior of reticular neurons supports their role as generator and synchronizer of spindle rhythmicity in vivo (Steriade et al., 1985).

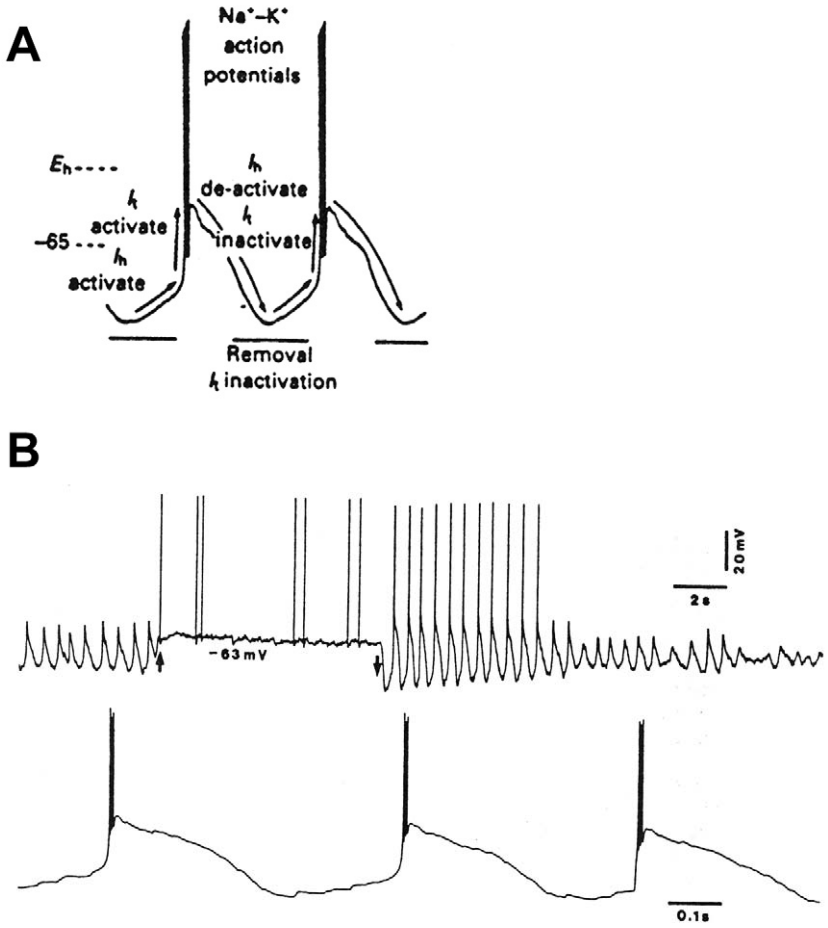

Figure 1-2 Clock-like delta oscillation (1-4 Hz) in TC neurons. A) The specific property of TC neurons is the interplay between I_H and I_T that generates the clock-like delta oscillation. (Modified from McCormick et Pape, 1990a) B) Neuron from the thalamic LP nucleus recorded in vivo under urethane anesthesia. At rest the neuron oscillated spontaneously at 1.7 Hz. A 0.5 nA depolarizing current (between arrows) prevented the oscillation, and its removal set the cell back in the oscillatory mode. Three cycles after removal of depolarizing current in top trace are expanded below to show high-frequency spike bursts crowning LTSs. (Modified from Steriade et al., 1991)

1.1.1.3 Corticothalamic Loop

The TC network is organized in a loop. The main gateway of the thalamocortical system is the dorsal thalamus, which receives specific inputs from ascending sensory pathways and from the brainstem modulatory systems. TC neurons send their excitatory glutamatergic axons both to the cerebral cortex and to the RE thalamic nucleus (Bazhenov and Timofeev, 2006). The axons of TC neurons terminate in the middle layers of neocortex (primarily layer IV). In the visual system of cat the synapses of TC neurons form approximately 5-6 %

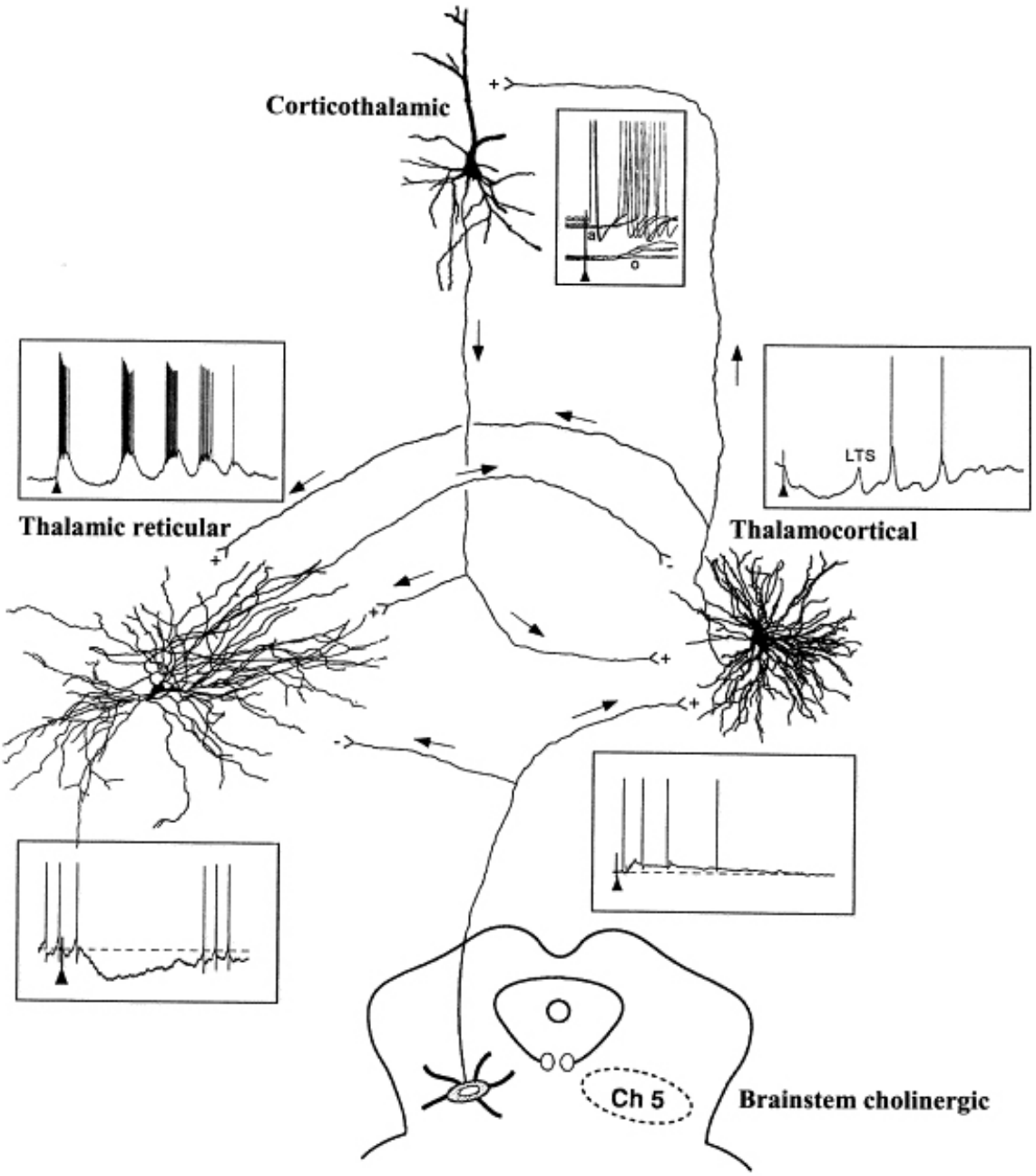

Figure 1-3 Neuronal loops in corticothalamic networks implicated in coherent oscillations and their control by brainstem cholinergic neurons. The CT neuron (spikes truncated) from area 7 responded to thalamic stimulation of centrolateral intralaminar nucleus with antidromic (a) and orthodromic (o) action potentials (top superimposition, at a membrane potential of −55 mV). At more hyperpolarized levels (bottom superimposition, at −64 mV), the antidromic response failed but the orthodromic response survived as subthreshold EPSPs. In addition to such closed loops, in which the cortical neuron is excited from a given thalamic nucleus and projects back to the same nucleus, cortical neurons may project to thalamic nuclei that are different from those representing the input source for the cortex. Such cases provide the substrate for distribution of activities beyond the site of their generation in the cerebral cortex. The RE GABAergic neuron (recorded from the rostrolateral district of the nucleus) responded to motor cortical stimulation with a high-frequency spike-burst, followed by a sequence of spindle waves on a depolarizing envelope (membrane potential −68 mV). Spindle waves occur

spontaneously, with a frequency of 7–14 Hz in animals (12–14 Hz in humans) during light sleep. In this case, spindles are elicited by cortical stimulation. The TC neuron (recorded from the VL nucleus) responded to motor cortex stimulation with a biphasic IPSP, leading to an LTS and a sequence of hyperpolarizing spindle waves (membrane potential −70 mV). For the sake of simplicity, local-circuit inhibitory neurons in the cortex and thalamus are not illustrated. Shown below are the dual effects of brainstem cholinergic neurons, namely hyperpolarization of the RE neuron and depolarization of the TC neuron. (From Steriade, 2000)

of the total number of synapses on layer IV neurons (Peters and Payne, 1993; Ahmed et al., 1994). The major sources of afferents to the RE thalamic nucleus are the collaterals of TC and CT fibers, all of which pass through the RE nucleus (Steriade et al., 1997). The excitatory influence of CT fibers on RE neurons is much larger than their influence on TC neurons due to the specific distribution of glutamate receptors (Golshani et al., 2001a) (Fig. 1-3).

Axons arising from RE neurons, after giving one or two collaterals in the nucleus enter the underlying dorsal thalamus and terminate (Scheibel and Scheibel, 1966b; Scheibel and Scheibel, 1966a). A distinct feature of the connection inside the reticular nucleus is the presence of gap junctions, which couple electrotonically RE neurons (Fuentealba et al., 2004; Landisman et al., 2002). An ensemble of these factors suggests that synchronous cortical volleys generated during sleep or paroxysmal activity through a primary synaptic relay in GABA-ergic thalamic RE neurons, may overcome the direct excitation of TC neurons. The TC neurons would remain in prolonged hyperpolarizing states and would not actively function during cortical synchronous discharges.

1.1.2 Oscillators in the TC network

The oscillatory activity is an emerging property of the thalamo-cortical system. There are two different types of mechanisms mediating the rhythms generated in the thalamocortical system: the intrinsic mechanisms based on the interplay between specific intrinsic currents, and the extrinsic or network mechanisms, which require the interaction of excitatory and inhibitory neurons within a population. Intrinsic and extrinsic mechanisms can work alone (e.g., thalamic delta oscillations depend on the intrinsic properties of thalamic relay cells, cortical slow oscillation depends on network properties) or in combination (e.g., spindles depend on the interaction between thalamic relay and RE neurons as well as on their

intrinsic properties). The patterns and the dominant frequencies of thalamocortical oscillations depend on the functional state of the brain.

The slow-wave sleep is dominated by slow-activities in the 0.1-15 Hz range, which includes the cortical slow-oscillation (0.2-1 Hz), thalamic and cortical delta generated oscillations in the range of 1-4 Hz, spindles (7-15 Hz), and theta which is generated in the limbic system and is not discussed here. The fast (beta, gamma – 20-60 Hz) and ultra-fast activities (ripples 100-600 Hz) may be present in various states of vigilance including sleep and frequently coexist with slower rhythms (e.g., fast gamma oscillations may be found during depolarized phases of slow sleep oscillations) but they are more specific for the waking state and therefore will be described later. Infraslow oscillations occur during all states of vigilance and equally during the pathological developments of normal brain activities and are closely related to ionic homeostasis.

1.1.2.1 Cortical Slow Oscillation

The cortical slow oscillation (<1Hz, generally 0.5-1 Hz) was initially described by Steriade et al. in 1993 and was recorded in cortical pyramidal and non-pyramidal neurons, and in all four major classes of neocortical neurons identified by their electrophysiological properties (Steriade et al., 1993a; Steriade et al., 1993b; Steriade et al., 1993d; Steriade et al., 1993e).

This oscillation is made up by a prolonged depolarizing phase, followed by a long-lasting hyperpolarization. The duration of the depolarizing phase slightly varies: is 0.8-1.5 seconds in urethane anesthesia, 0.3-0.5 seconds under ketamine-xylazine anesthesia and its duration is further shorter during natural slow-wave sleep (SWS) when the frequency of oscillation rises to 1 Hz or even slightly beyond it (Steriade et al., 1993e).

The cortical nature of the slow oscillation was demonstrated by its survival in the cerebral cortex after thalamectomy (Steriade et al., 1993d), the presence in isolated large cortical slabs *in vivo* (Timofeev et al., 2000a) or cortical slices maintained *in vitro* (Sanchez-Vives and McCormick, 2000), its absence in the thalamus of decorticated animals (Timofeev and Steriade, 1996), and the disruption of its long-range synchronization after disconnection of intracortical synaptic linkages (Amzica and Steriade, 1995).

The depolarizing phase is primary mediated by NMDA and non-NMDA excitatory postsynaptic potentials (EPSPs), fast prepotentials (FPPs), a voltage-dependent persistent Na^+ current, and fast inhibitory postsynaptic potentials (IPSPs) reflecting the action of synaptically coupled GABA-ergic local-circuit cortical cells; and could be engaged by a variety of intrinsic currents, including $I_{Na(p)}$. The hyperpolarized phase of the slow oscillation in neocortical neurons was demonstrated to be due to disfacilitation (removal of synaptic, mainly excitatory, inputs in intracortical and thalamocortical networks) rather than active inhibition (Timofeev et al., 1996; Contreras et al., 1996), and to some K^+ currents (Timofeev et al., 2001b). Moreover, neurons identified electrophysiologically as fast-spiking cells and morphologically as basket cells, during anesthesia (Contreras and Steriade, 1995) and natural non-REM sleep (Grenier et al., 2001), behave in phase with regular-spiking pyramidal-shaped neurons, being silent during the hyperpolarizing phase.

Intracellular recordings with Cs^+-filled pipettes reduced and often abolished the hyperpolarizing phase of the slow oscillation (Timofeev et al., 2001b) leading to the conclusion that the hyperpolarizations during the slow oscillation are, at least partially, produced by a series of K^+ currents, including leak currents. In support of this, the stimulation of cholinergic afferents blocks selectively the prolonged hyperpolarizations, even without changes in membrane potential (Steriade et al., 1993a); and acetylcholine (ACh) increases the excitability of cortical neurons by reducing some voltage-and Ca^{2+} dependent K^+ currents (Krnjevic et al., 1971; McCormick, 1992a).

The disfacilitation might be explained by a progressive depletion of $[Ca^{2+}]_o$ during the depolarizing phase of the slow oscillation (Massimini and Amzica, 2001), which would produce a decrease in synaptic efficacy (Crochet et al., 2005).

1.1.2.2 Delta Waves: Thalamic and Cortical Components

There are two types of delta oscillation which overlaps on the frequency range of 1-4 Hz, that are distinct as to their site of origin and underlying cellular mechanisms: a cortically generated delta activity and an intrinsic thalamic-generated oscillation.

Little is know about the neuronal mechanisms involved in the generation of cortical activity in the delta band frequency. However, the isolation of the neocortex from the thalamus either by thalamectomy or by surgical individualization of small neocortical islands significantly enhanced delta activity (Ball et al., 1977; Houweling et al., 2005). Depth-negative (surface-positive) delta waves are correlated with an increased neuronal firing probability and one working hypothesis proposed that they are expression of the discharging pattern of IB neurons (Amzica and Steriade, 1998). However, intracellular recordings of IB neocortical neurons during NREM sleep shown rather long-lasting hyperpolarizing than depolarizing potentials (Steriade et al., 2001; Timofeev et al., 2001b). Depth positive waves are associated with a summation of long lasting after-hyperpolarizations produced by a variety of K^+ currents in cortical neurons (Schwindt et al., 1988a; Schwindt et al., 1988b).

The thalamic component of delta waves has a clock-like pattern and depends on two inward currents interplaying in TC neurons: the hyperpolarization-activated current, I_H, carried by Na^+ and K^+, which is expressed as a constant depolarizing sag of membrane potential toward rest; and a transient Ca^{2+} current, I_T, underlying the low-threshold spike (LTS). The mechanisms of generation and synchronization of this thalamic oscillation were revealed using intracellular studies *in vitro* (Leresche et al., 1990; McCormick and Pape, 1990a; Leresche et al., 1991) and *in vivo* (Steriade et al., 1991; Curro Dossi et al., 1992).

Spindles and delta waves occur at different membrane potentials of TC neurons. Around -60 mV, TC neurons display spindles, whereas at more negative membrane potentials spindles progressively decrease in amplitude and oscillations are within the delta frequency range. Therefore, a progressive hyperpolarization of TC cells with the deepening of NREM sleep, attributable to a progressive decrease in firing rates of corticothalamic, mesopontine cholinergic neurons with thalamic projections and some monoaminergic nuclei could explain the transition from spindles to low-frequency oscillations with the deepening of sleep (see the recent monography of Steriade and McCarley, 2005).

On the contrary, at the transition from NREM sleep towards REM sleep, when TC neurons become partially depolarized because of increased firing rates of excitatory mesopontine

cholinergic neurons which precede the onset of REM sleep (Steriade et al., 1990), spindles are more obvious than during preceding epochs of deep NREM sleep.

However, it the intact brain the interplay between the two ionic currents (I_H and I_T) can be disrupted and the generation of intrinsic clock-like delta oscillation in TC neurons obscured, since I_H is strongly modulated by both neuromodulators, such as serotonin, norepinephrine or adenosine (McCormick and Pape, 1990a; Pape, 1992); and synaptic drive from ascending pathways (Nunez et al., 1992) or corticothalamic projections (Nita et al., 2003).

Intrinsic delta oscillation in TC neurons is not synchronized and thus it is unlikely that it can induce detectable EEG patterns (Timofeev and Steriade, 1996). Furthermore, the hyperpolarization-activated depolarizing sag in thalamic neurons is prevented during the active, up-state, phases of sleep-like slow oscillation by synaptic activity arising in cortex and the expression of I_H is unveiled only during spontaneous disfacilitation periods or K^+-induced depression of cortical activity (Nita et al., 2003). The periodic activation of the cortical network during the depolarizing phase of the slow oscillation would disrupt any possible sequences of clock-like delta cycles in TC neurons since the duration of the hyperpolarizing phase of the slow oscillation ranges from 0.3 to 0.7 s and it is too short to allow more than one cycle of the fastest ~4 Hz delta activity (Amzica and Steriade, 1998).

In spite of this, I_H could still play a role during conditions associated with prolonged loss of consciousness such as coma or burst suppression, during which the cortical activity is depressed. The usefulness of the I_H under such conditions could rely on its ability to maintain a minimal neuronal activity in the thalamus with negligible energetic impact on target structures.

1.1.2.3 Spindles

This sleep rhythm is termed so because of the spindle shape of the oscillation envelope. It has a frequency of 7-14 Hz in cats, and 12-15 Hz in humans, and consists of sequences that recur rhythmically every 2 to 5 seconds. Slightly different frequency of this rhythm reported previously in clinical EEG (~12 Hz localized more anteriorly and ~14 Hz posteriorly) (Gibbs and Gibbs, 1952; Zygierewicz et al., 1999) could be explained by

different behaviors of the TC neurons: low-frequency spindles are due to long-lasting hyperpolarizations (lasting 100-150 ms) followed by rebound spike-bursts, whereas relatively shorter hyperpolarization-rebound sequences (70-100 ms) account for faster spindles. The possibility is open that TC neurons projecting to anterior cortical fields in humans display longer hyperpolarizations during spindles, thus accounting for lower-frequency of spindles.

A significant difference has been observed in the synchronization patterns during spindles between *in vivo* or *in vitro* experimental approaches. While *in vivo* it was reported a nearly simultaneous appearance of spindle sequences during natural NREM sleep on the cortex surface (Contreras et al., 1996; Contreras et al., 1997), *in vitro* slices from the visual thalamus it was described a systematic propagation of spindles (Kim et al., 1995). Since following decortication spindle sequences are no longer simultaneous in the thalamus in intact brain preparations (Contreras et al., 1996; Contreras et al., 1997), it is acceptable to presume that neocortex play an extremely important role in synchronizing spindle oscillations generated in the thalamus.

Spindles are generated by the interactions between the GABA-ergic thalamic RE neurons and the TC neurons. During the early phase of spindles, the reticular nucleus single-handedly drives the spindle oscillation via intrinsic mechanisms. The rhythmic spike-bursts of RE neurons induce IPSPs in target TC neurons and these IPSPs de-inactivate a transient low-threshold Ca^{2+} current that promotes burst firing which is further transferred to cortical neurons where it induces rhythmic EPSPs and, occasionally, action potentials. Additionally, cortical firing contributes to spindle synchronization via CT neural firing, thereby imposing simultaneous excitation of RE and TC relay neurons. This cooperation between RE, TC and CT neurons maintain the spindle (Timofeev et al., 2001a).

CT neurons also intervene in the process of terminating individual spindle sequences. This is due to the depolarizing actions of CT neurons over TC neurons that lead to different duration of spindle-related IPSPs, with the consequence of different times at which postinhibitory rebound spike-bursts are fired. Indeed during the first 2 to 4 IPSPs composing the spindles, TC neurons do not display rebound spike-bursts, thus they do not return signals to RE neurons (Timofeev et al., 2001a). Another, factor accounting for the

termination of spindles may be the Ca^{2+}-induced up-regulation of the hyperpolarization-activated depolarizing current (I_H) (Bal and McCormick, 1996; Luthi and McCormick, 1998). However, a computational study that investigated the contribution of network (corticothalamic) versus intrinsic (I_H) factors in terminating spindle sequences showed that the reticular-thalamocortical isolated network oscillated infinitely and up-regulation of I_H alone was not sufficiently strong to terminate spindling in the absence of the corticothalamic feedback (Timofeev et al., 2001a).

The assumption that RE neurons are pacemakers of spindles is based on two experimental observations: spindles disappear in TC systems after disconnection from RE neurons (Steriade et al., 1985), and they are preserved within the RE nucleus disconnected from the thalamus and the cerebral cortex (Steriade et al., 1987). These experimental data were corroborated in different types of computational models of isolated RE neurons, which displayed oscillations within the frequency range of spindles (Wang and Rinzel, 1993; Golomb et al., 1994; Destexhe et al., 1994; Bazhenov et al., 1999).

It is also known that cortical volleys act as the most powerful trigger for spindle induction in the thalamus. The synchronous pattern of discharge of neurons during the cortical slow oscillation in NREM sleep induces brief sequence of spindles after each cycle of the slow oscillation (Contreras and Steriade, 1995; Timofeev and Steriade, 1996; Amzica and Steriade, 1997).

1.1.2.4 Coalescence of Sleep Rhythms

While simple circuits generate pure highly-regular rhythms within distinct and often very specific frequency bands, complex circuits, as the living brain during the various states of vigilance, do not generally display separate oscillations, but exhibit a summation of different slow-oscillations and fast rhythms. The coalescence of the sleep rhythms represents the remarkable attribute of the cortical slow oscillation (0.5-1 Hz) which is able to group other sleep rhythms within complex wave-sequences (Steriade and Amzica, 1998).

For example, during the depolarizing envelope of the cortical slow oscillation, the synchronous firing of CT neurons impinges upon thalamic RE neurons, thus creating conditions for formation of spindles, which are further transferred to the TC neurons. This

reciprocal connectivity explains why a brief sequence of spindles in TC neurons as well as in the cortical EEG is triggered by a cycle of the slow oscillation in anesthetized and naturally sleeping animals, as well as in the EEG recordings from human non-REM sleep (Steriade et al., 1993d; Amzica and Steriade, 1997; Steriade et al., 2001).

The grapho-element resulting from the combination between the slow and spindle oscillation is called the K-complex and is a reliable sign for stage 2 of human sleep, even if it is apparent in all stages of NREM sleep (Amzica and Steriade, 1997).

The effects of slowly oscillating CT neurons lead to the grouping of other sleep oscillations, generated in the thalamus. The depolarizing component of the cortical slow oscillation can group thalamic spindles, thalamic clock-like delta waves, and cortical delta waves (Steriade et al., 1993d; Steriade and Amzica, 1998). In addition, the depolarizing phase of the cortical slow sleep oscillation is associated with the presence of voltage-dependent fast (Steriade et al., 1996b) and ultra-fast (Grenier et al., 2003) rhythms.

1.1.2.5 Electrical Activities of Activated States

The activated states of the brain – the waking state and the REM sleep are best described by the presence of fast activities over 20 Hz (beta and gamma rhythms). However, studies performed using DC EEG amplifiers (Aladjalova, 1957; Aladjalova, 1964a; Aladjalova, 1964b) also identified infra slow oscillations, which will be described in this section, together with the fast electrical activities occurring during the activated states of the brain, since infra slow oscillations can synchronize faster activities, and modulate cortical excitability (Vanhatalo et al., 2004).

1.1.2.5.1 Fast Activities during Activated States

There are two very different types of fast-oscillation which can be observed during the activated states of the neocortex: the beta/gamma rhythms and the ripples.

Beta and Gamma Rhythms

Wake and REM sleep are characterized by a low correlation of activities at the cellular level (Noda and Adey, 1970) and the expression in the EEG of fast frequencies in the beta (15-30 Hz) and gamma (30-60 Hz) ranges (Freeman, 1991). They are generally associated

with attentiveness (Bouyer et al., 1981), focused arousal (Sheer, 1989), sensory perception (Gray et al., 1989), and fine movement (Murthy and Fetz, 1992). The fast oscillations are generated in interacting cortical and thalamic networks under the control of activating ascendant pathways, depending on the neuronal depolarization. Fast rhythms can also occur during deep anesthesia, natural SWS and REM sleep (Steriade et al., 1996a; Steriade et al., 1996b), synchronized between very-neighboring sites, when consciousness is either suspended or bizarre. Transition between gamma and beta oscillations was simulated by alternating excitatory coupling between pyramidal neurons and by change in K^+-conductances (Traub et al., 1999). The resulting network gamma oscillation demonstrated in computational model shares all of the properties of gamma oscillations and shows critical dependence on multiple spiking in FRB cells.

Ripples

Ripples are very fast oscillations (80-200 Hz, sometimes up to 600 Hz) described in CA1 hippocampal area and perirhinal cortex associated with bursts of sharp potentials (Ylinen et al., 1995; Chrobak and Buzsaki, 1996; Collins et al., 1999), in neocortex in sensory-evoked potentials (Jones and Barth, 1999), during high-voltage spike-wave discharges in rat (Kandel and Buzsaki, 1997) and during natural states of vigilance in cats (Grenier et al., 2001). They were generally more prominent during the depolarizing component of the cortical slow oscillation during SWS, than during waking or REM sleep (Grenier et al., 2001). Around epileptic foci in humans (Bragin et al., 2002) and cats (Grenier et al., 2003) the amplitude of ripples is dramatically enhanced. The high-frequency field potential oscillations during ripples are phase-locked with neuronal firing and dependence of ripples on neuronal depolarization was shown by their increased amplitude in field potentials in parallel with progressively more depolarized values of the membrane potential of neurons. As ripples can be generated within small isolated slabs of cortex, neocortical networks seem to be sufficient for their generation (Grenier et al., 2003).

1.1.2.5.2 Infra-Slow Activities

Infra-slow oscillation occurs as slow DC potential shifts with time-periods from tens of seconds to minutes range. Classically, they were often attributed to dipoles along the apical

dendrites of cortical neurons (Speckmann and Elger, 1999). Beside neuronal generators, several studies have demonstrated glial cells involvement in such slow-potential during spreading depression (Somjen, 1973; Somjen and Trachtenberg, 1979), sleep (Amzica and Neckelmann, 1999) and seizures (Somjen, 1973; Caspers et al., 1987). A recent study *in vivo* focused on the role of the brain-blood-barrier as source of slow potential shifts (Nita et al., 2004). The relative contributions and interactions of non-neuronal and neuronal/glial generators to slow potential shifts seen in both invasive and non-invasive recordings of brain activity are not fully elucidated. Presumably these infra-slow oscillations appears as an expression of pH-dependant ionic steady potential gradients across blood-brain barrier and are important for the brain functioning since they can influence neuronal excitability in the cortex during seizures and sleep (Vanhatalo et al., 2004).

1.1.3 States of vigilance in the TC networks

1.1.3.1 Functional cortical deafferentation during SWS

The distinctive feature that defines the transition from wakefulness to slow-wave sleep is the occurrence of spindles associated with long periods of hyperpolarization and increased membrane conductance in TC neurons. This initial step has as consequence a blockade of afferent messages at the thalamic level and a deprivation of the cerebral cortex of external signals (Steriade et al., 1969). Following the appearance of these initial signs, other oscillatory types, especially the cortical slow-oscillation, mark the late stage of slow-wave sleep and they further deepen the inhibition of thalamic and cortical cells. Therefore, the neuronal substrates of brain disconnection during slow-wave sleep should be searched in the two major cell-classes of the thalamus: TC glutamatergic neurons and thalamic reticular GABA-ergic neurons.

All CT axons, acting both on reticular neurons and TC cells are glutamatergic, but the outcome of artificial or natural cortical volleys is opposite on each of these two thalamic neuronal types. Natural synchronous cortical volleys, occurring during slow-wave sleep when neurons exhibit highly coherent activity, or electrical stimuli produce excitation and rhythmic spike-bursts over a depolarizing envelope in reticular neurons. Simultaneously, TC neurons display rhythmic and prolonged IPSPs, occasionally followed by rebound excitations. Thus, the bi-synaptic inhibition of TC neurons, induced by cortex through a

prior synaptic relay in GABA-ergic RE neurons, overcomes the direct excitation of TC neurons (Steriade et al., 1993c).

This experimental observation was substantiated by studies showing that the numbers of glutamate receptor subunits GluR4 are 3.7 times higher at corticothalamic synapses in RE neurons, compared to TC neurons, and that the mean peak amplitude of CT excitatory postsynaptic currents (EPSCs) is about 2.5 higher in RE, than in TC, neurons (Golshani et al., 2001b). This differential effect exerted by corticofugal neurons on RE and TC neurons is important because natural phenomena occurring especially during slow-wave sleep depend on different functional states of RE neurons, which diverse consequences on TC neurons.

During slow-wave sleep, and even more during some types of electrical seizures that develop from sleep, the rhythmic, high-frequency, spike-bursts fired by RE neurons induce greater postsynaptic inhibitory responses in TC neurons than those elicited by single-spikes discharge of the same cells during waking or REM sleep. Such potent IPSPs, associated with increased membrane conductance, account for the disconnection of TC networks from the outside world during slow-wave sleep and for the loss of consciousness during spike-wave seizures (Steriade and Contreras, 1995). Furthermore, the highly-synchronous activity of CT neurons during slow-wave sleep is effective in reinforcing thalamically generated spindles.

By contrast, cholinergic brainstem core systems obliterate SWS oscillations and beside the conventional neuronal mechanisms underlying the onset of sleep, which operate on relatively short time scales, an important role is supposed to be played by the humoral factors and their modulatory actions upon critical brain structures.

There are several arousal systems which maintain the waking state through the actions of chemical neurotransmitters. Among these, noradrenaline-, histamine- and orexin-containing neurons fire during wake, decrease firing during SWS and cease firing during REM sleep. Although maintained by multiple arousal systems, wakefulness falters if orexin, orexin receptors, or orexin neurons are deficient; and narcolepsy may result with hypersomnolence or sudden onset of REM sleep (Jones, 2005; Lee et al., 2005). By contrast, acetylcholine-

containing neurons discharge during waking, decrease firing during SWS and fire at high rates during REM sleep in association with fast cortical activity, obliterating SWS oscillations and promoting patterns that characterize brain-activated states of waking and REM sleep (see for review Jones, 2005). The effect of acetylcholine is due, on one hand, to the inhibitory action (hyperpolarization with increased membrane conductance) exerted by brainstem cholinergic neurons on thalamic RE GABA-ergic neurons (Hu et al., 1989), and on the other hand, the same mesopontine cholinergic neurons exert prolonged depolarizing (muscarinic-mediated) actions on TC neurons (Curro Dossi et al., 1991); thus bringing them to membrane potential levels at which low-threshold spike-bursts during spindles are prevented, and they also block the hyperpolarization-activated intrinsic thalamic delta waves (Steriade et al., 1991).

Moreover, sleep and wakefulness are regulated to occur at appropriate times that are in accordance with our internal and external environments. Avoiding danger and finding food, which are life-essential activities, as well as emotional stimuli, reward and energy balance implies circuitry involving the cerebral cortex, thalamus, amygdale and the hippocampus (Saper et al., 2005; Saper, 2006; Sakurai, 2007).

1.1.3.2 Brain activation during REM sleep

Activation can be defined as a state of readiness in the cortical networks associated with a depolarized level of the membrane potential, which brings neurons closer to the firing threshold, ensuring in this way an accurate transmission of information and an increased responsiveness to either external stimuli during waking, or internal drives during REM sleep (Steriade, 1991; Steriade et al., 2001). Waking and REM sleep shows virtually identical electrophysiological characteristics and very similar patterns of neuronal activity in the cortical and thalamic neurons. Only few neuronal types, particularly the serotoninergic and noradrenergic neurons in the upper brainstem and histaminergic neurons in the posterior hypothalamus, are active in waking and quiet during REM sleep (Hobson et al., 1975; McCarley and Hobson, 1975; McGinty and Harper, 1976; Aston-Jones and Bloom, 1981; Vanni-Mercier et al., 1984).

Cholinergic and glutamatergic neurons that give rise to ascending projections toward thalamus and cortex are similarly active during wakefulness and REM sleep, while they are

much less active in SWS. Two parallel pathways from pedunculopontine and laterodorsal tegmentum (PPT/LDT) activate the thalamus and cerebral cortex. The dorsal path uses ACh as neurotransmitter to TC neurons that, in turn, release glutamate at the cortical level. The ventral projection activates the neurons from nucleus basalis (NB). Since ACh inhibit NB cells (Khateb et al., 1997), PPT/LDT neurons activate NB cells through glutamate (Rasmusson et al., 1996) that is co-localized with ACh in many PPT/LDT cells (Lavoie and Parent, 1994). The direct PPT/LDT projection to TC neurons in felines and primates (Steriade et al., 1988; Pare et al., 1988) produces a powerful excitation at their targets. The muscarinic component of this prolonged depolarization is associated with an increase in input resistance (Curro Dossi et al., 1991), which explains the increased responsiveness of TC neurons during brain-activated states of waking and REM sleep (Glenn and Steriade, 1982). PPT/LDT neurons exhibit increased activity before the first EEG signs of waking and REM sleep (Steriade et al., 1990). All these features make mesopontine cholinergic nuclei the best candidates to generate activation processes in TC systems.

However, non-cholinergic neurons are much more numerous than the cholinergic ones in the brainstem reticular core and some of them are glutamatergic (Jones, 2000). Glutamate-induced actions at the level of the TC neurons is excitatory, resulting from a direct depolarization associated with an increased input resistance, similarly to the effects exerted by acetylcholine, as both these neurotransmitters block a "leak" K^+ current (McCormick, 1992b).

Activation processes at the level of TC neurons consist therefore in the obliteration of thalamic sleep spindles and intrinsic clock-delta oscillation by two mechanisms: an inhibition of the spindle pacemaker (the RE nucleus), and a direct depolarization of TC neurons that bring TC neurons at a membrane potential where the clock-like delta rhythm can not be generated.

At the cortical level, activation means the disruption of the cortically-generated slow oscillation by blocking the hyperpolarizing phase of this oscillation, even without changes in membrane potential, following PPT/LDT or NB nuclei stimulation (Metherate et al., 1988; Steriade et al., 1993a), and generation of voltage-dependent 20-60 Hz fast-oscillations (Llinas et al., 1991; Gutfreund et al., 1995) (Fig. 1-4).

Someone may think that a decreased input resistance of neocortical neurons would be observed in parallel with increased synaptic inputs during active states of waking and REM sleep when many ionic conductances are open due to inputs from other cortical and TC cells, as well as generalized activating systems. However, the membrane resistance of neocortical neurons exhibit higher values during wake compared to the depolarizing phase of the cortical slow-oscillation (Steriade et al., 2001), which can be related to the increased release of ACh in cortex during brain activation (Celesia and Jasper, 1966) and the fact that ACh released during brain-active states increases the membrane resistance of cortical neurons (Krnjevic et al., 1971; McCormick, 1992b). An increased membrane resistance makes neurons more responsive to synaptic input, since even smaller excitatory synaptic ionic currents will be able to reach the threshold to generate action potentials.

There are some important monoaminergic systems that exert excitatory actions on given thalamic neuronal types and inhibitory on others, as well as differential effects on various types of cortical neurons, located in various cortical layers. Serotonin released by the neurons of the dorsal raphe nucleus depolarizes thalamic RE neurons, hyperpolarizes TC neurons in all investigated thalamic nuclei, and may increase the excitability of cortical pyramidal neurons through a reduction of various K^+ currents (McCormick, 1992a). Norepinephrine released in the thalamus and cerebral cortex by locus coeruleus neurons depolarizes thalamic RE neurons, and in the very few investigated dorsal thalamic nuclei it depolarizes also the TC neurons and enhances a hyperpolarization-activated cation current through β-adrenoreceptors (McCormick and Pape, 1990a). Histamine, released by neurons in the tuberomammillary nucleus of the posterior hypothalamus, produces slow-depolariza–tions of TC neurons in dorsal geniculate slice preparations, associated with an increase in the input resistance due to a decrease in a leak K^+ current (McCormick and Williamson, 1991). In the cortex histamine activates pyramidal neurons through inhibition of a K^+ current I_{AHP}.(Haas and Greene, 1986; McCormick, 1992a). In addition, all these activating systems target not only neocortical neuronal networks, but also cortical glial cells and have important vasoactive properties modulating the blood supply. Since neuronal activity can be influenced by both cerebral blood flow (Lauritzen, 2001) and reciprocal neuro-glial interactions (Amzica, 2002), the behavior of cortical structures during active states should be considered as the outcome of a more complex relation between neocortical neurons,

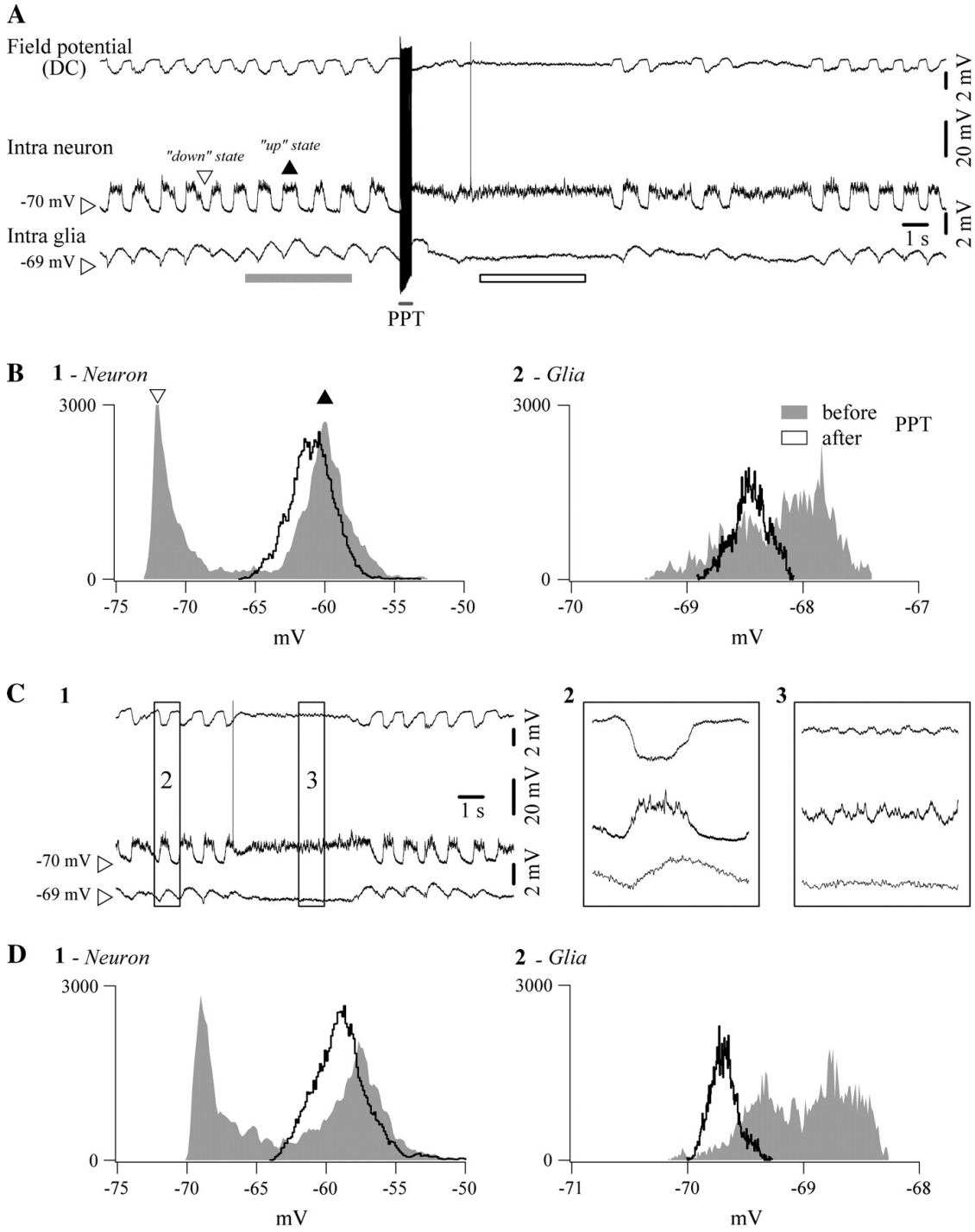

Figure 1-4 Electrically elicited and spontaneously occurring activation in a pair of simultaneously impaled neuron and glia. A) Sequence of slow (<1 Hz) oscillation followed by PPT stimulation. Depolarizing phases of the slow oscillation are indicated with a black arrowhead (up state), while hyperpolarizing phases are marked with an empty arrowhead (down state). After activation, the sustained neuronal depolarization is accompanied by glial hyperpolarization. B) Histograms of neuronal (panel 1) and glial (panel 2) Vm during the slow oscillation (in gray) and during activation (black trace) calculated on the corresponding epochs in A). Note the bimodal histograms during sleep and the unimodal histogram after activation, as well as the opposite evolution of glial and neuronal

Vm.. C) Similar pattern of activity in the same double intracellular recording during a period of spontaneous activation of the EEG. Periods within squares are expanded at right (panels 2 and 3). D, Histograms from equivalent time periods before and after activation. (From Seigneur et al., 2006)

neocortical glia and blood vessels (Seigneur et al., 2006); in which the common link might well be of ionic nature, of which K^+ may play a central role, but also may rely on neurotransmitters such as glutamate.

1.1.3.3 Abnormal development of brain rhythms

Two abnormal patterns can develop from slow-wave sleep oscillations: the electrographic paroxysms that accompany different types of clinical seizures, and, less frequently, the EEG pattern of burst-suppression.

Paroxysmal electrical activities represent the most frequent abnormal development of a normal brain rhythm. Both low-frequency rhythms, cortical slow-oscillation (0.5-1 Hz) and spindles (7-15 Hz) can degenerate in paroxysmal discharges, with various clinical expressions (recently reviewed in Steriade, 2003; Timofeev and Steriade, 2004). Cortically generated seizures occur preferentially during SWS, while REM sleep is a state in which seizures are not usually observed. The reciprocal interrelation between sleep and seizures, and the modulation of seizures by the state of vigilance will be discussed later on.

The spontaneous progression to spike-wave (SW) seizures from the normal slow-oscillation is a continuous process at the neuronal level in which the difference between the active "up-state" and the silent hyperpolarized "down-state" of the membrane potential is constantly increasing, up to a level where the action potentials are partially inactivated and the afterhyperpolarizing potentials (AHPs) are obscured, while the depolarizing shift from one membrane state to the other is becoming more and more steeper (Steriade et al., 1998a) (Fig. 1-5). At the EEG level these correspond to the expression of SW complexes at 1.5-3 Hz, eventually followed by episodes of fast runs at 10-20 Hz. The end of the seizure is associated with a post-ictal depression seen in the intracellular recordings as a silent period of hyperpolarization. Generalized "atypical spike-wave" discharges can be seen in symptomatic or secondary generalized epilepsies, such as the epileptic encephalopathy of the Lennox-Gastaut syndrome, where 1.5–3 Hz, more irregular SW complexes usually arises from an abnormally slow background EEG.

There is increasing evidence that SW seizures arises also from normal sleep spindle oscillation, since both involve the same circuitry, occur in the same sleep stage and have an inverse relationship to each other in frequency of occurrence (Kellaway, 1985; Kostopoulos, 2000). Clinically, the typical SW discharges are the 3–4 Hz generalized seizures as seen in typical childhood absence epilepsy. They consists of large amplitude 200–300 μV surface-negative EEG slow-waves alternating with single or double surface-negative spikes, seen in a bilateral and widespread distribution, with an abrupt onset and end, a highly regular rhythm, and a variable degree of impaired consciousness.

A variant, especially seen in the juvenile myoclonic epilepsy is the polyspike-wave discharge, in which a brief series of two to three spikes alternates with slow waves in a widespread, frontally predominant distribution, sometimes at a slightly higher frequency (3.5–5 Hz) than in typical absence.

During SW seizures in the intact thalamocortical circuits only a minority of TC neurons fires low-threshold spike-bursts, while the vast majority is steadily hyperpolarized and exhibit phasic IPSPs by the over-excited GABA-ergic neurons from the RE nucleus (Steriade and Contreras, 1995; Steriade and Contreras, 1998; reviewed in Crunelli and Leresche, 2002). The minimal substrate for the generation of SW paroxysmal activities is the neocortex, sometimes some very circumscribed pools of cortical neurons without reflection in the cortical layers (Steriade, 1974), since they exist in thalamectomized preparations where spindles are also absent (Marcus et al., 1968; Marcus and Watson, 1968; Steriade and Contreras, 1998); or in neocortical slabs (Timofeev et al., 2000a).

The very high frequency oscillations called ripples (80-200 Hz) are also subtly intricated with the mechanisms of paroxysmal activities generation, since there is an increased correlation between the neuronal excitation and the intensity of ripples, they are present at the transition between normal slow-oscillation and seizures, and both ripples and paroxysmal activities are antagonized by halothane (Grenier et al., 2003).

Burst-suppression appears in cortex following trauma associated with cerebral anoxia or major ionic homeostasis impairment (Siemkowicz and Hansen, 1981; Nita et al., 2004), during coma (Stockard et al., 1974; Bauer and Niedermeyer, 1979), but appears also in

deep anesthesia (Swank and Cammermeyer, 1949). It consists in long periods of electrical silence punctuated by high amplitude paroxysmal discharges. At intracellular level the membrane potential of cortical neurons hyperpolarizes by approximately 10 mV before any major EEG change, anticipating the transition from the normal cortical slow-oscillation to burst-suppression (Steriade et al., 1994).

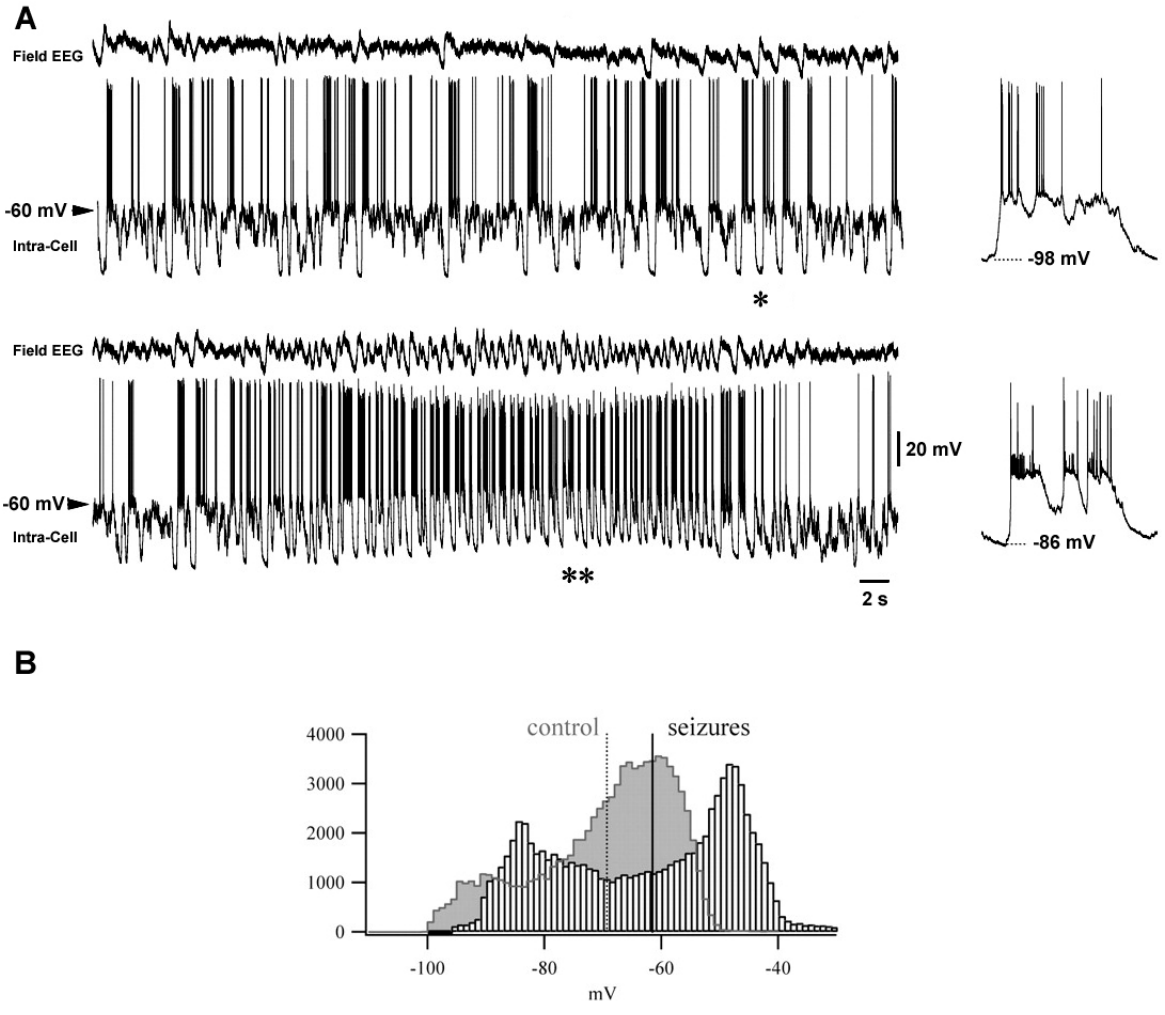

Figure 1-5 Continuous development of the cortical slow-oscillation into seizures. A) Top panel contains an intracellular recording of a cortical RS neuron during normal slow-oscillation. Bottom panel depicts the development of a spontaneous seizure from the slow-oscillation. Insets at right: details from the intraneuronal recordings, corresponding to the epochs marked by simple and double asterisks. Note higher-amplitude paroxysmal depolarizing shifts during epileptic discharges associated with action potential inactivation. B) Histograms of neuronal membrane potential comparing the seizure-free top trace with slow oscillations (in gray), with equivalent periods with epileptic discharges (black). (Modified from Nita et al., 2004)

During the suppressed periods with flat EEG the apparent input resistance decreases and neuronal responsiveness to synaptic volleys is dramatically decreased. In contrast with the consistent behavior of the cortical neurons, only 60% of thalamic relay neurons cease firing during periods of flat EEG activity; the remaining TC neurons displaying clock-like delta oscillations (Steriade et al., 1994). Hypothetically, burst-suppression pattern is achieved through a complete disruption of the brain circuits, since revival of normal EEG and neuronal activities follows stimulation or restoration of cortical and thalamic network connectivity.

1.2 From seizures to epilepsy

Epilepsy is the name of a brain condition characterized by recurrent and unpredictable interruptions of normal brain function, called epileptic seizures. Epilepsy is not a singular disease entity but a variety of disorders reflecting underlying brain dysfunctions that may result from many different causes. However, despite the fact that epilepsy consists of about 40 different clinical syndromes, apparently, many of them have to share common mechanisms (Jacobs et al., 2001).

1.2.1 Definitions: seizures, epilepsy, epileptogenesis

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (International League Against Epilepsy / International Bureau for Epilepsy definition, Fisher et al., 2005). An epileptic seizure is “transient”, demarcated in time, with a clear start and finish. Termination of a behavioral epileptic seizure may be less evident than the onset, since symptoms of the postictal state can blur the end of the seizure. Status epilepticus is a special circumstance with prolonged or recurrent seizures.

The clinical presentation of seizures depends on the location of onset in the brain, on the patterns of propagation, maturity of the brain, co-existing conditions, medication, sleep-wake cycle, and a variety of many other factors. Seizures can affect sensory, motor, and autonomic functions, consciousness, emotional state, memory cognition or behavior. Not all seizures affect all of these factors, but all influence at least one.

Cognitive deficits during seizures can express themselves as problems with perception, attention, emotion, memory, execution, praxis or speech. Memory distortions can be either negative or positive, in the sense of disruption of memory formation or retrieval as a negative symptom, or intrusion of inappropriate memories, giving raise to déjà vu and other forced memories as a positive symptom.

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition. The definition requires the presence of at least one epileptic seizure (International League Against Epilepsy / International Bureau for Epilepsy definition, Fisher et al., 2005). Therefore, a predisposition, as determined for example by a family history or by the presence of epileptiform EEG changes, is not sufficient to determine epilepsy. Furthermore, the definition requires in addition to at least one seizure, the presence of an enduring alteration in the brain which increases the likelihood of further seizures.

The diagnosis of epilepsy under this concept would not require two seizures; it would require only one epileptic seizure in association with an enduring disturbance of the brain capable of giving rise to other seizures. Equally, multiple epileptic seizures due to multiple different causes in the same patient would not be considered to be epilepsy (Fisher et al., 2005).

Epileptogenesis is defined as the cascade of dynamic biological events altering the balance between excitation and inhibition in neural networks (Clark and Wilson, 1999). The term applies to any of the progressive biochemical, anatomic, and physiologic changes leading up to recurrent seizures. Progressive changes are suggested by the existence of a so-called silent interval, often years in duration, between CNS infection (Marks et al., 1992), head trauma (Salazar et al., 1985; Marks et al., 1995), or febrile seizures and the later appearance of epilepsy. Understanding these changes is key to preventing the onset of epilepsy (Jacobs et al., 2001).

There are many possible proposed mechanisms of epileptogenesis ranging from the molecular level of organization (e.g., altered gene expression) to the macrostructural level

(e.g., altered neural networks). Some of them that are related to the experimental approaches we used will be discussed further in this introduction.

1.2.2 Forms of seizures

The development of a rational treatment and prognosis in epileptic patients are often based on a specific profile of seizure events, rather than on the neurobiological mechanisms. Although a classification based on the neurobiological knowledge would be highly desirable, the present state of information on the anatomical, pathophysiological, genetic, and biochemical mechanisms and substrates of epileptic seizures and epilepsies still make such a task rather difficult in clinic (Seino, 2006).

The International League Against Epilepsy (ILAE) Commission on Classification and Terminology proposed in 2001 a diagnostic scheme divided into five axes: axis 1 – ictal phenomenology, axis 2 – seizure type, axis 3 – syndrome, axis 4 – etiology, and axis 5 – impairment. However, since EEG remains the primary technique in the clinical diagnosis, characterization and localization of seizures on one hand, and on the other hand EEG is the major physiological parameter recorded in our experimental studies, a classification of seizures based on the aspect of the EEG and on the localization of seizure is preferred.

1.2.2.1 Partial Seizures

The ILAE defines focal seizures as “seizures whose initial semiology indicates, or is consistent with, initial activation of only part of one cerebral hemisphere”. The EEG findings and ictal semiology are varied and depend on the site of focal onset (see for review Verma and Radtke, 2006). Here are the most frequently observed EEG patterns of partial seizures:

1.2.2.1.1 Interictal epileptiform discharges (IIEDs)

IIEDs are rare in individuals without epilepsy and the most common ones observed include centro-temporal spikes, generalized spike-wave discharges, and photoparoxysmal discharges. The unifying feature of these discharges is that they all have a strong genetic component. It is easy to conceive of an individual who has never had a seizure but who carries the genetic trait that would manifest itself with such an EEG pattern.

1.2.2.1.2 Focal slow activity

Focal slow activity can express either as polymorphic delta activity (PDA) or as temporal intermittent rhythmical delta activity (TIRDA). PDA is commonly seen in patients with partial epilepsy, being associated with underlying structural abnormalities and has a poor predictive value for epilepsy. However if no structural abnormality exist to explain the slowing, continuous focal PDA is associated with seizures in about 50% of patients. TIRDA has a strong association with temporal lobe epilepsy. It occurs in runs lasting 3 to 20 seconds and is commonly seen in association with ipsilateral IIEDs (Reiher et al., 1989; Normand et al., 1995).

1.2.2.1.3 Periodic lateralized epileptiform discharges (PLEDs)

PLEDs are prominent moderate to high amplitude sharp wave discharges that usually occur with a frequency of 0.5 to 2 Hz. PLEDs are usually seen in the setting of an acute destructive lesion and commonly resolve over days to weeks. However, up to 70% to 80 % of patients with PLEDs on EEG exhibit overt clinical seizures and 3% to 66% subsequently develop epilepsy (Markand and Daly, 1971; Walsh and Brenner, 1987).

1.2.2.1.4 Mesial temporal lobe epilepsy (MTLE)

MTLE consists in anterior temporal spikes with maximal amplitude in the anterior temporal or temporal basal electrodes, frequently in patients with IIEDs during sleep. The clinical seizure onset is followed 10 to 40 seconds later by lateralized rhythmical theta or alpha activity (Risinger et al., 1989).

1.2.2.1.5 Neocortical temporal lobe epilepsy (NTLE)

NTLE differentiate from MTLE by a more broadly distribution of IIEDs and ictal rhythmic activity, a slower frequency, less stable in terms of both frequency and amplitude.

1.2.2.1.6 Focal lobar epilepsies

Frontal lobe epilepsy exhibits interictal EEG more frequently normal. Frontal lobe seizures more commonly show no definite correlate on scalp EEG as compared to seizures of temporal lobe onset.

Parietal lobe epilepsy and **occipital lobe epilepsy** are both difficult to evaluate and localize with scalp EEG. In one study centroparietal IIEDs appeared localized in only 5%

to 15% of patients with parietal lobe epilepsy (Salanova et al., 1995). Occipital ictal patterns may be seen in 15% to 20% of patients with occipital lobe epilepsy (Salanova et al., 1992).

1.2.2.2 Generalized Seizures

The purpose of this part is to briefly describe the interictal and ictal EEG patterns associated with a select group of generalized seizures, including absence seizures, myoclonic seizures as seen in juvenile myoclonic epilepsy, idiopathic generalized tonico-clonic seizures, infantile spasms; and atypical absence, tonic and atonic seizures associated with Lennox-Gastaut syndrome. A detailed description of the clinical manifestations of the seizures is beyond the scope of this presentation.

1.2.2.2.1 Absence seizures

Ictal EEG findings in both simple and complex absence seizures consist in bursts of generalized 3 Hz spike-wave activity, which generally last less than 30 seconds in duration. Clinical manifestations of bursts lasting less than 2.5 seconds in duration are difficult to detect, although specialized measurements of reaction times in patients with 3 Hz spike-wave activity have shown that, regardless of its duration, 3 Hz spike-wave activity impairs performance (Mirsky and Vanburen, 1965; Browne et al., 1974). Thus, in the clinical setting, the number of absences occurring daily is often difficult to determine, and a precise count requires the use of long term video EEG monitoring.

The background activity in the interictal periods is usually normal, although some degree of slowing of the background rhythms may appear in up to one third of the patients (Sato et al., 1983). Paroxysms of rhythmic slow-wave activity with a frequency of 2.5 to 3.5 Hz may occur in a generalized fashion or may be restricted to the occipital derivations (occipital rhythmic intermittent delta activity – ORIDA), generally symmetric and usually blocked by eyes opening (Holmes et al., 1987).

The interictal EEG in patients with absence seizures typically demonstrates brief bursts of 3 Hz spike-waves activity, that are bilaterally synchronous and symmetric (Penry et al., 1975; Holmes et al., 1987). During sleep, the SW complexes become more fragmented and bursts of poly-spike-wave (PSW) activity may appear. The total amount of epileptiform activity

increases with the onset of slow-wave sleep, and then waxes and wanes in close relationship with the sleep wake cycle. In most patients, REM sleep is associated with a cessation of SW activity (Kellaway and Frost, 1983).

1.2.2.2.2 Myoclonic seizures from Juvenile Myoclonic Epilepsy (JME)

The myoclonic jerk seen in JME is typically associated with a burst of 3 to 4 Hz PSW activity (Janz, 1985). The spikes are followed by slow-waves occurring at a frequency of 2.5 to 5 Hz. The PSW pattern, which often persists beyond the termination of the myoclonic jerk, may last up to several seconds. The myoclonic jerks may occur in isolation or repetitively. The arousal mechanism is a potent activator of myoclonic seizures in JME (reviewed in Hrachovy and Frost, Jr., 2006).

As with other idiopathic generalized epilepsies the background EEG activity is usually normal. SW / PSW bursts may occur with a frequency which tends to be more irregular varying between 3 and 5 Hz (Janz, 1985). During sleep the SW / PSW complexes may decrease, particularly during SWS, and the bursts are absent / markedly diminished during REM sleep. As mentioned above, arousal from sleep is an extremely potent activator of SW / PSW discharges and in some patients this may be the only time epileptic activity is seen during routine EEG recording.

1.2.2.2.3 Idiopathic generalized tonico-clonic seizures

In the usual clinical setting the background EEG activity is obscured by high voltage myogenic artifacts during a primary generalized tonico-clonic seizure. High frequency filtering of muscle artifacts allows adequate visualization of the EEG activity. However, the most complete data concerning the ictal EEG changes has been obtained from patients paralyzed with muscle relaxants. Seizure consists in SW / PSW complexes associated with myoclonic jerks, further on the EEG shows generalized voltage attenuation with superimposed fast activities (20-40 Hz) known as fast runs, a tonic phase and slower activity thereafter which decreases in frequency and become mixed with SW / PSW complexes. Following the last burst the EEG shows a generalized voltage attenuation lasting for seconds called post-ictal depression. The time required by the EEG to return to the baseline state is highly variable from patient to patient (Gastaut and Broughton, 1972).

In all patients with idiopathic generalized tonico-clonic seizures the interictal background EEG is usually normal, and EEG may show brief bursts of 2.5 to 3.5 SW / PSW complexes. In most patients these bursts last less than 2.5 seconds and are frequently referred as abortive SW activity. Only half of the patients with idiopathic generalized tonico-clonic seizures will demonstrate SW activity on a routine EEG. The bursts become more frequent with the onset of SWS.

1.2.2.2.4 Infantile spasms

Infantile spasm is a specific type of seizure seen in an epilepsy syndrome of infancy and early childhood known as West Syndrome. The onset is predominantly in the first year of life, typically between 3-6 months. The typical pattern of infantile spasms is a sudden bending forward and stiffening of the body, arms, and legs; although there can also be arching of the torso. Infantile spasms usually stop by age 5, but are often replaced by other seizure types. West Syndrome is characterized by infantile spasms, hypsarrhythmia (abnormal, chaotic brain wave patterns), and mental retardation. Other neurological disorders, such as cerebral palsy, may be seen in 30-50% of those with infantile spasms.

Video/EEG monitoring studies have identified 11 different ictal EEG patterns that occur with infantile spasms (Kellaway et al., 1979; Donat and Wright, 1991; Fusco and Vigevano, 1993; Wong and Trevathan, 2001). These include: (1) a high voltage, frontal dominant, generalized slow wave transient; (2) a generalized sharp and slow wave complex; (3) a generalized sharp and slow wave complex followed by a period of voltage attenuation; (4) a period of voltage attenuation only (electrodecremental episode); (5) a generalized slow wave transient only; (6) a period of voltage attenuation with superimposed fast activity; (7) a generalized slow wave transient followed by a period of voltage attenuation with superimposed fast activity; (8) a period of attenuation with rhythmic slow activity; (9) fast activity only; (10) a sharp and slow wave complex followed by a period of voltage attenuation with superimposed fast activity; and (11) a period of voltage attenuation with superimposed fast activity followed by rhythmic slow activity. The most common ictal pattern seen is a period of generalized voltage attenuation. The duration of the ictal events can range from less than a second to more than 100 seconds. There is no close correlation between specific types of clinical events (e.g., flexor, extensor, or mixed

spasms) and specific ictal EEG patterns. Also, as noted above, episodes of voltage attenuation frequently occur during slow wave sleep in the absence of clinical spasms. Also, no significant correlation has been found between the various ictal EEG patterns and underlying cause, response to therapy, or long term developmental outcome (Haga et al., 1995a; Haga et al., 1995b).

The most common interictal EEG pattern associated with infantile spasms is hypsarrhythmia. This pattern consists of generalized high voltage, generally asynchronous, slow waves mixed with random high voltage multifocal spikes and sharp waves. At times, the spikes and sharp waves occur in a generalized fashion, but they do not occur in rhythmic, repetitive sequences such as the slow spike slow wave discharges seen in the Lennox Gastaut Syndrome (Gastaut and Remond, 1952; Kellaway, 1952; reviewed in Hrachovy and Frost, Jr., 2006). However, in many infantile spasms patients, variations of this prototypical pattern are seen and may express as: (1). Hypsarrhythmia with increased interhemispheric synchronization, (2) Asymmetric hypsarrhythmia, (3) Hypsarrhythmia with episodes of generalized or lateralized voltage attenuation, (4) Hypsarrhythmia with a consistent focus of abnormal discharge, (5) Hypsarrhythmia with little or no spike or sharp wave activity. In addition to these variants, transient alterations occur in the hypsarrhythmic pattern throughout the day. During slow wave sleep, the voltage of the background activity usually increases and often there is a grouping of the multifocal sharp wave and spike activity, resulting in a quasi-periodic pattern (Hrachovy et al., 1981; Hrachovy et al., 1984; Hrachovy and Frost, Jr., 2003). As noted previously, episodes of generalized voltage attenuation frequently occur during slow wave sleep. Conversely, during REM sleep there is a marked reduction or total disappearance of the hypsarrhythmic pattern (Hrachovy et al., 1981; Hrachovy et al., 1984).

1.2.2.2.5 Lennox-Gastaut Syndrome

Lennox-Gastaut syndrome is a severe form of epilepsy. Seizures usually begin before 4 years of age. Seizure types, which vary among patients, include tonic (stiffening of the body, upward deviation of the eyes, dilation of the pupils, and altered respiratory patterns), atonic (brief loss of muscle tone and consciousness, causing abrupt falls), atypical absence (staring spells), and myoclonic (sudden muscle jerks). There may be periods of frequent

seizures mixed with brief, relatively seizure-free periods. Most children with Lennox-Gastaut syndrome experience some degree of impaired intellectual functioning or information processing, along with developmental delays, and behavioral disturbances. Lennox-Gastaut syndrome can be caused by brain malformations, perinatal asphyxia, severe head injury, central nervous system infection and inherited degenerative or metabolic conditions. In 30-35 percent of cases, no cause can be found.

Atypical absence seizures are accompanied by bursts of high amplitude generalized 1.5 to 2.5 Hz activity basically indistinguishable from that seen interictally (Markand, 1977). Less commonly, bursts of fast activity (10-20 Hz) have been reported to accompany atypical absence seizures (Markand, 2003). In general, the impairment of consciousness that occurs with atypical absence seizures is progressive and not abrupt like that which occurs with 3 Hz spike-and-wave activity.

The EEG during tonic seizures may show generalized voltage attenuation or so-called electrodecremental change, bursts of rhythmic fast activity (15-25 Hz), or attenuation followed by rhythmic fast activity. A generalized slow SW complex may precede these ictal patterns.

The characteristic interictal EEG pattern in the Lennox Gastaut syndrome is the slow spike-and-wave discharge. Although the frequency of this discharge may vary from 1 to 4 Hz, the typical frequency is 1.5 to 2.5 Hz (Markand, 1977; Markand, 2003; Niedermeyer and Lopes da Silva, 2005). The frequency, amplitude, distribution, and morphology often vary between bursts and during bursts of slow SW activity. Shifting asymmetries of the discharge are common. If patients have large unilateral hemispheric lesions, the slow SW activity is generally higher over the good hemisphere with corresponding suppression of the background activity over the abnormal hemisphere (Markand, 1977; Markand, 2003).

1.2.3 Cellular correlates of seizures

This part will briefly describe the intrinsic neuronal properties and the network operations underlying three types of seizures: interictal spikes, seizures with SW complexes at ~3 Hz and Lennox-Gastaut seizures. The cellular correlates of the seizures from NTLE are not discussed here, since NTLE probably originate in a different neuronal circuit, and,

moreover, patients display hippocampal sclerosis and pathological changes in structures adjacent to hippocampus (entorhinal cortex and amygdala), making difficult to determine if seizures result after brain damage, or if lesions in this system generate seizures.

1.2.3.1 Cellular basis of EEG interictal spikes

Interictal spikes (ISs) on the EEG are sharp, large amplitude (> 50 μV), relatively brief (80-150 ms) potentials, sometimes followed by slow-waves, which occur periodically on a background of otherwise normal EEG activity, or during short epochs which separate repetitive full-blown seizures. In humans, neuromagnetic recordings have shown that ISs may originate from a single source and distribute with an orderly field pattern and a fixed chronological sequence of discharges (Barth et al., 1984). Experimental studies also demonstrated that the synchronicity of the ISs does not necessarily imply simultaneity, as ISs can propagate both vertically and tangentially across neocortical layers (Albowitz and Kuhnt, 1995). The cellular mechanisms underlying ISs have been investigated in the neocortex, thalamus and hippocampus, both in vitro and in vivo. The intracellular correlate of an IS is an overt depolarization, termed paroxysmal depolarizing shift (PDS). Usually the depolarization is so large than it leads to a partial inactivation of the action potentials. PDSs can be generated by an imbalance between excitation and inhibition, such as impairing inhibition through application of $GABA_A$ receptor antagonists, by increasing the excitability of neuronal networks through an increased extracellular potassium concentration, and by reduction of K^+ currents using application of 4-aminopyridine. Many PDS are formed by a primary spike-burst which lasts for 50-100 ms, followed by a secondary discharge that is probably dependent of the activating effect of the primary burst on other components of the network.

Two apparently contrasting approaches tried to explain PDSs in the hippocampus or neocortex as either a sum of synchronous EPSPs generated in large neuronal pools in which increased excitation and/or depressed inhibition take place (Matsumoto and Marsan, 1964; Ayala et al., 1973; Johnston and Brown, 1981), or, alternatively, resulting from intrinsic neuronal properties (Prince, 1968a; Prince, 1968b). However, these hypotheses are not mutually exclusive as both factors contribute to the slow envelope of the PDS (Schwartzkroin and Prince, 1978; Schwartzkroin and Prince, 1980). Moreover experimental

evidences suggest an active role played by $GABA_A$-mediated potentials in the maintenance and termination of these prolonged epileptiform events (Lopantsev and Avoli, 1998).

By difference with the hippocampal CA3 pyramidal neurons which possess special properties in both soma and dendrites allowing them to generate spike-bursts even during normal spontaneous activity and more so in seizure prone states, neocortical neurons are less susceptible to generate PDSs. These differences consist of a lower propensity to generate slow-depolarizations and spike bursts, less prominent Ca^{2+} conductances, and inward rectification mediated predominately by Na^+, whereas both Na^+ and Ca^{2+} mediate the inward rectification during depolarization of hippocampal pyramidal cells (reviewed in Prince, 1983). Although neocortical neurons are less prone to epileptiform discharges than hippocampal pyramidal neurons IB and FRB neurons can generate high frequency bursts discharges. IB cells have been proposed to promote PDS generation (Connors, 1984; Chagnac-Amitai and Connors, 1989) and FRB cell from either deep or supragranular layers are predominantly implicated in generating repetitive PDSs during the fast-runs of Lennox-Gastaut-type seizures (Timofeev et al., 1998).

1.2.3.2 Seizures with spike-wave complexes at ~3 Hz

Seizures with spike-wave or polyspike-wave complexes at ~3 Hz occur in petit-mal or absence epilepsy. However, SW complexes should not be equated with absence epilepsy since they occur also in other types of epilepsy, in a focal or generalized manner, as previously described.

Clinical and experimental studies pointed out that, instead of being “suddenly generalized and bilateral synchronous” as conventionally regarded according to the “centrencephalic” hypothesis, many SW seizures are progressively built up within cortico-cortical and cortico-thalamic networks. Multiple, independent cortical foci and asymmetrical SW or PSW complexes have been described in patients and also found in animal experiments, Thus, some SW/PSW seizures display focal paroxysmal activity confined to one cortical area or to few contiguous cortical fields. This is in line with the concept that SW/PSW seizures originate in the neocortex and are disseminated through mono-, oligo- and multi-synaptic intracortical circuits, before they spread to the thalamus and exhibit generalized features (Neckelmann et al., 1998).

In experiments on acutely prepared or chronically implanted animals, seizures with SW/PSW complexes at ~3 Hz originated in the neocortex (even in athalamic preparations), and, in intact brain preparations, such seizures spread to the thalamus after their cortical initiation. Experiments using multisite, field potential, extracellular and intracellular recordings of spontaneously occurring seizures demonstrate the intracortical synchronization of SW/PSW paroxysm (Topolnik et al., 2003a; Topolnik et al., 2003b; Nita et al., 2006; Nita et al., 2007). The degree of synchrony among simultaneously recorded cortical neurons increases progressively from the pre-seizure period to the early stage of SW/PSW seizure and further to the late stage of the seizure. The increase of long-range synchrony during seizures was associated with seizure termination (Topolnik et al., 2003a). The absence of SW seizures in cortical slices is probably due to the absence of long-range connections that synchronize paroxysmal activity.

During cortically generated SW seizures RE GABA-ergic neurons and TC cells undergo opposite influences: the former follow each PDS of SW complexes, whereas the majority of the latter (~60-90% in different experimental conditions) are steadily hyperpolarized and display phasic IPSPs that do not de-inactivate the Ca^{2+}-dependant low-threshold spikes and thus do not transfer spike-bursts to the cortex. This is due to the greater power of excitatory cortical projections on reticular neurons compared to thalamo-cortical cells. However, the remaining thalamo-cortical cells may reinforce the coherence of seizures activity arising in the neocortical areas (Timofeev and Steriade, 2004).

Although clinical absences can only be detected in the waking state, the electrographical correlates of these seizures (i.e. SW complexes at ~3 Hz) preferentially occur during SWS and are absent or dramatically decreased during REM sleep (Sato et al., 1973; Kellaway, 1985; Shouse et al., 2000).

1.2.3.3 Patterns of Lennox-Gastaut syndrome

As previously mentioned the Lennox-Gastaut syndrome is characterize by intractable seizures and mental retardation, associated with an complex EEG pattern consisting of SW/PSW complexes at 2-3 Hz or lower frequencies (1.5-2.5 Hz), and fast-runs at 10-20 Hz. The major distinction between infantile spasms and Lennox-Gastaut syndrome reside

in the age of the onset; otherwise “no separating line is drawn” (Niedermeyer and Lopes da Silva, 2005).

Such seizures occur preferentially during SWS. The neuronal bases of neocortical and thalamic events implicated in SW/PSW complexes in Lennox-Gastaut syndrome are similar to those exposed above for pure SW/PSW seizures at 3 Hz. Multi-site extracellular and intracellular recordings from the neocortex and the thalamus used to investigate the neuronal substrates of these seizures showed that, comparing to the pre-seizure epoch, the slope as well as the amplitude of the shift in the membrane potential from the depolarized to the hyperpolarized state continuously increased. During the pre-seizure epochs the shape of the action potentials and the fast AHP remained usually unchanged. At the beginning of the seizure the actions potentials are partially inactivated and the AHP disappears. Seizures contains periods of fast runs characterized by EEG spikes at 10-20 Hz and, at the cellular level, a tonically depolarized membrane potential with smaller superimposed depolarizations. The end of these seizures is associated with a hyperpolarization of the neurons during the post-ictal depression. (Steriade et al., 1998a)

As to the fast runs at 10-20 Hz they demonstrate changes in frequency during different epoch of the same seizure and the absence of the perfect synchrony between different cortical populations (Timofeev et al., 1998), which explains why CT inputs does not elicit synchronous fast oscillations in the thalamus during this type of seizures.

Whereas RS neurons discharge single action potential or spike-doublets during either fast runs or PSW complexes, FRB cells fire more action potentials during each depolarizing component of fast runs as well as during each of the multiple EEG spikes composing the PSW complexes (Steriade et al., 1998a). Because of the high-frequency, fast repetitive spike-bursts that characterize FRB neurons, it was hypothesized that their impact on local networks could be sufficient to initiate seizures focally, and possibly, in the target structures.

1.3 Seizures in a multi-system oscillatory machine: defining the objectives

1.3.1 Fluctuations between active and silent states in cortico-thalamic circuits

Complex systems, and especially living systems, are distinguished in nature by their ability to maintain stable, ordered states in far-from-equilibrium conditions, and, furthermore, they *require* such conditions of inherent parameters fluctuations and dissipation of energy in order to maintain self-organization and growth (Morowitz, 1955). Here we address the question if the key factor of epileptogenesis, leading to manifest seizures, may be represented by the occurrence and / or the persistence of long-lasting far-from-equilibrium fluctuations between *silent* and *active* states in the neocortical networks.

A recent study using both *in vivo* and *in computo* approaches, performed in cortical slabs isolated from thalamic and cortical inputs, as well as in a larger isolated gyrus, revealed that activity was sparse and irregular in small slabs, consisting in short active periods separated by long periods of silence, but became progressively more similar to the slow oscillations observed in the intact cortical tissue as the size of the slab was increased concluding that the level of activity and properties of neurons in a neocortical slab depend on the number of elements in the network (Timofeev et al., 2000a; Timofeev et al., 2000b). Therefore, we can assume that *silence* represents the distinctive state in a completely disconnected cortex and that the sparse bursts of activity seen in cortical slabs and the more coherent slow-oscillation observed in bigger networks depict the ability of the cortex to self-organize and to fluctuate between *active* and *silent* states.

At the level of the whole brain, during wake the cortex receives a vast amount of information from the exterior world by the ascending pathways, but during SWS the cortex is functionally deafferented by a blockade at the thalamic level, due to the hyperpolarization of the TC cells. Under these conditions the neocortex exhibits a highly synchronous activity pattern - slow-oscillation which may develop without discontinuity into spike-wave seizures.

Nevertheless, in order to prove our hypothesis, the neocortex should be able to generate fluctuations between *active* and *silent* states not only in chronic condition associated with a reduced synaptic input (when homeostatic plasticity mechanisms up-regulate processes leading to increased excitability (Turrigiano et al., 1998), and additional reorganization in the intracortical synaptic network results in a significant shift of the balance between excitation and inhibition toward increased excitation), but also in conditions associated with an increased external synaptic drive.

Repeated induction of focal seizure discharges in many brain sites leads to a progressive, highly reliable, permanent increase in epileptic response to the inducing agent, usually electrical stimulation. This remarkably general phenomenon is called kindling and it acts both as a chronic animal model of epileptogenesis and as a form of neuroplasticity. One of our objectives was to identify if cortical kindling may be related to transitions between *active* and *silent* states, and which may account for epileptogenesis.

1.3.2 Experimental approaches

1.3.2.1 Decreased cortical input following cortical deafferentation

Decreased synaptic pressure on the cortical neuronal network can occur either in relative simple preparation with a limited number of constitutive elements, a completely regular connection topology and sparsely connectivity - like in vitro cortical slices; in disfacilitated or seriously impaired in vivo complex network systems with a big number of elements interlinked in complex networks; or in a middle-size network that lies somewhere between these two extremes both in terms of the number of elements and the network complexity. We have chosen for our experiments this middle-size network represented by a cortical gyrus (suprasylvian) given that the particularly significant property of this network size is that it can be "rewired" to introduce increasing amounts of disorder that can completely change its output (Watts and Strogatz, 1998) that could translate at the functional level in epileptogenesis leading to seizures.

Undercut is a well established model of epileptogenesis *in vitro* (Prince and Tseng, 1993; Hoffman et al., 1994) and *in vivo* (Topolnik et al., 2003a; Topolnik et al., 2003b). Acute experiments performed *in vivo* showed that partially deafferented neocortex displays

increased local cortical synchrony in areas surrounding the undercut cortex, leading to paroxysmal activity that occurs 2-3 h after the undercut and arises from enhanced intrinsic and synaptic neuronal responsiveness, increased incidence of intrinsically-bursting neurons, and slight reduction of inhibitory influences (Topolnik et al., 2003a; Topolnik et al., 2003b). In view of these data, we built a gradual experimental approach starting with recordings in chronic anesthetized animals and continuing in naturally awake and sleeping animals in order to study the evolution of electrical paroxysms induced by cortical deafferentation, up to 3 months following the undercut.

In all cases the undercut was performed in the white matter below the suprasylvian gyrus (covering 13-15 mm postero-anteriorly and 3–4 mm medio-laterally) and transected the thalamo-cortical afferences. A standard blade (3 mm width and 14 mm long) was inserted in the posterior part of suprasylvian gyrus perpendicular to its surface for a depth of 3–4 mm, then rotated 90° and advanced rostrally along the gyrus parallel to its surface for a total distance of about 14 mm, then moved back, rotated 90° and removed from the same place where it was entered. The use of a custom-designed knife ensured a similar extension of the lesion. Thus, the anterior part of the undercut cortex was relatively intact and the white matter below the posterior part of the gyrus was transected, creating conditions of partial cortical deafferentation.

We investigated the spatiotemporal development of post-traumatic seizures with respect to the initial cortical insult, quantified at different stages the transformation from the normal slow oscillation to paroxysmal patterns, and determined how epileptic activities are modulated by the three major states of vigilance: waking, SWS and REM sleep.

1.3.2.2 Increased cortical input by cortical kindling

Repeated induction of focal seizure discharges in many brain sites leads to a progressive, highly reliable, permanent increase in epileptic response to the inducing agent, usually electrical stimulation (Goddard et al., 1969; reviewed in Racine, 1978). Since its discovery more than three decades ago, this kindling phenomenon has been widely studied both as a chronic animal model of epileptogenesis and as a form of neuroplasticity. Kindling is a remarkably general phenomenon (Barnes and Pinel, 2001). First, it has been demonstrated in a wide variety of species including frogs, mice, gerbils, rats, rabbits, cats, dogs, rhesus

monkeys, baboons (reviewed in McNamara et al., 1980) and several authors have claimed kindling-like phenomena in humans (Morrell, 1985; Sato et al., 1990; Coulter et al., 2002); second, kindling has resulted from the stimulation of many, but not all, brain sites (Goddard et al., 1969); and finally, in addition to focal electrical stimulation, kindling-like phenomena can be produced with the periodic administration of most, if not all, convulsive agents (electroconvulsive shock, intracranially or systemically administered convulsant drugs, etc.) (McNamara et al., 1980).

Despite the fact that kindling is the most used model of epileptogenesis and several reviewers have considered it as a model for clinical epilepsy (McNamara et al., 1980; Racine and Burnham, 1984; Sato et al., 1990; McNamara, 1994; Loscher, 1997) spontaneous motor seizures, other than those triggered by stimulation, have been observed in relatively few kindled animals (Wada et al., 1974; Wada and Osawa, 1976). Therefore, at variance with previous kindling protocols, we focused on finding the most favorable moment of cortical seizures induction between the different states of vigilance of the animal, and on choosing the most effective frequency of stimulation. Firstly, the supra-threshold cortical stimulation was applied during different states of vigilance (SWS, REM sleep, wake, and transition from SWS to wake), in order to identify the most efficient moment to induce brief, focal, afterdischarges. Secondly, different stimulation frequencies were tested and only the frequency which gives a progressive growing evoked response was selected as the best frequency susceptible to induce paroxysmal discharge events in the animal.

One of the findings consistently observed in all experimental animals, was the expression of long-lasting highly-rhythmic activities, named here “outlasting activities”, at the end of each convulsive generalized acute seizure (AS). These highly rhythmic paroxysmal activities expressed themselves following the postictal depression, from the very first AS which was triggered by electrical stimulation in kindled cats. We aimed to characterize these outlasting activities, to describe the influence of the natural states of vigilance on the occurrence or expression of paroxysmal activity patterns following kindling, and at studying the relative contribution of the thalamus and of the cerebral cortex in the genesis of outlasting activities.

1.3.3 Mechanisms of epileptogenesis

It is widely accepted that the development of epileptiform activity results from a shift in the balance between excitation and inhibition towards excitation (Dichter and Ayala, 1987; Galarreta and Hestrin, 1998). The shift is produced either by an obstruction of inhibition or by increased excitation, both representing experimental approaches used to elicit seizures (reviewed in Timofeev and Steriade, 2004). Another conditions for seizure generation is the functional heterogeneity of cortical networks, such as the presence of two or more different cortical regions with relatively high and low levels of synaptic activity (Timofeev and Bazhenov, 2005). The mechanism of epileptogenesis following cortical deafferentation and kindling may be similar but they will be discussed separately, with emphasis on the current state of knowledge.

1.3.3.1 Epileptogenesis in deafferented cortex

1.3.3.1.1 Acute epileptogenesis

Several factors may account for the increased propensity to seizures following the cortical insult produced by undercut. While acute epileptogenesis due to increased $[K^+]_o$ (Moody et al., 1974) may be partially explained by K^+-mediated increase in the hyperpolarization-activated depolarizing current (I_H) that leads to paroxysmal activity in neocortical networks (Timofeev et al., 2002), the chronic epileptogenesis following undercut would not favor the same mechanism, since homeostatic mechanisms are supposed to regulate the $[K^+]_o$ on a long time scale. The same reasoning may apply to changes in extracellular glutamate which is increased following cortical trauma and was reported to promote epileptogenesis (Sakowitz et al., 2002).

1.3.3.1.2 Synaptic mechanisms in chronic undercut

The reasoning for disfacilitation through deafferentation as an important factor eliciting seizures is congruent with two lines of evidences (see for review Timofeev and Bazhenov, 2005). First, seizures occur most often during SWS or during transition from waking to SWS (see Steriade, 2003). SWS is characterized by the presence of periods of disfacilitation during the cortical slow-oscillation associated with neuronal hyperpolarization (Steriade et al., 2001; Timofeev et al., 2001b). During periods of disfacilitation synapses are likely released from a steady depression and the synaptic

transmission is strengthen, fact that may contribute to the onset of seizures (Galarreta and Hestrin, 1998). The latter one is that penetrating wounds or acute experimental cortical deafferentation have been described as strong epileptogenic factors (Prince et al., 1997; Topolnik et al., 2003a; Topolnik et al., 2003b). In such conditions, a part of axons impinging onto postsynaptic neurons is not functioning as they are damaged, which creates a partial deafferentation that in turn enhances the effectiveness of remaining incoming synaptic inputs.

Transitory or persistent reduction in synaptic activity within some cortical foci would increase the sensitivity of cortical neurons in those foci and in surrounding areas (Abbott et al., 1997); therefore, the synaptic inputs from cortical regions exhibiting moderate or high levels of activity would lead to an increased responsiveness in those cortical areas where the sensitivity is increased. This fits in well with injury-induced enhanced responsiveness of corticospinal neurons following their axotomy (Tseng and Prince, 1996) and with the increased synaptic and intrinsic responses of cultured cortical neurons during chronic absence of spontaneous activity (Turrigiano et al., 1998; Desai et al., 1999a).

Thus, both factors, the sleep-related disfacilitation and the traumatic deafferentation, increase the probability of seizures via the same mechanism of an increased effectiveness of synaptic transmission (Crochet et al., 2005; Li et al., 2005). The increased effectiveness of synaptic transmission depends on higher levels of extracellular Ca^{2+} concentration, as reported during silent periods of network activities (Massimini and Amzica, 2001; Crochet et al., 2005), and on synaptic facilitation that follows periods of neuronal silence(Galarreta and Hestrin, 1998). Increased levels of extracellular Ca^{2+} increase the intrinsic excitability of cortical neurons and may convert some of them to burst firing.

In chronic undercut, after a few days of pharmacological blockade of activity in cortical cell cultures, the amplitudes of mini-EPSCs and EPSCs in pyramidal cells increase (Turrigiano et al., 1998; Watt et al., 2000) as well as the quantal release probability (Murthy et al., 2001). Synaptic scaling occurs in part postsynaptically by changes in the number of open channels (Turrigiano et al., 1998; Watt et al., 2000), although all synaptic components may increase (Murthy et al., 2001), including the numbers of postsynaptic glutamate receptors (Rao and Craig, 1997; Lissin et al., 1998; O'Brien et al., 1998; Liao et

al., 1999). There is a similar activity-dependent regulation of NMDA currents (Watt et al., 2000). Interestingly, mIPSCs are scaled down with activity blockade, in the opposite direction to excitatory currents. This effect is reversible (Rutherford et al., 1997) and is accompanied by a reduction in the number of open $GABA_A$ channels and $GABA_A$ receptors clustered at synaptic sites (Kilman et al., 2002).

These observations suggest that some homeostatic mechanism may regulate the average levels of neuronal activity. These processes, collectively termed "homeostatic plasticity mechanisms" (Turrigiano, 1999), also occurring in vivo (Desai et al., 2002), may up regulate the already increased neuronal excitability in the deafferented cortex leading to seizures (Houweling et al., 2005). They are discussed in detail in section 1.3.4.

1.3.3.1.3 Neuronal intrinsic mechanisms in chronic undercut

Not only synaptic but also intrinsic excitability is regulated by activity. After chronic activity blockade, Na^+ currents increase and K^+ currents decrease in size, resulting in an enhanced responsiveness of pyramidal cells to current injections (Desai et al., 1999b).

The changes in intrinsic neuronal properties following cortical injury or undercut, leading to seizures, have been first studied in vitro and some of these results have been corroborated in vivo. Prince and Tseng (1993) compared layer V neurons of epileptogenic slices with those in control slices and found no significant differences in action potential characteristics and resting V_m, but the value of input resistance was more than double in injured than in control slices. Similar results were obtained in vivo following the deafferentation of the suprasylvian gyrus in chronic cats (Avramescu and Timofeev, in preparation). The increased membrane resistance of neurons recorded from epileptogenic slices is likely behind the increased intrinsic and synaptic responsiveness found in neurons recorded from the relatively intact suprasylvian cortex, at which level seizures are initiated following acute deafferentation in vivo (Topolnik et al., 2003b). Furthermore, the high membrane resistance shown to characterize neurons in epileptogenic slices (Prince and Tseng, 1993), is probably a factor promoting seizures by favoring transformation of regular-spiking into intrinsically-bursting neurons whose incidence is much higher in disconnected cortical slabs in vivo (Timofeev et al., 2000a) and in cortical slices in vitro (Nishimura et al., 2001) than in the intact cortex (Steriade et al., 2001).

1.3.3.1.4 Loss of inhibiton

Several lines of evidence have suggested that decreases in postsynaptic inhibition may have a role in epileptogenesis in cortical structures. A number of studies have suggested that GABA-ergic neurons might be selectively vulnerable to some forms of injury such as hypoxia (Sloper et al., 1980; Romijn et al., 1988), status epilepticus induced by convulsant agents (Franck and Schwartzkroin, 1985; Ashwood and Wheal, 1986; Obenaus et al., 1993) or excessive electrical stimulation (Sloviter, 1987; Sloviter, 1991), and neocortical isolations (Ribak and Reiffenstein, 1982).

Using immunohistochemical staining with GAD 65&67 and anti-GABA antibodies, we detected a reduction in the number of stained GABA-ergic neurons in disconnected cortical areas (unpublished data).

1.3.3.2 Epileptogenesis in kindled cortex

Several reviews have considered kindling and/or status epilepticus as models for clinical epilepsy (McNamara et al., 1980; Racine and Burnham, 1984; Sato et al., 1990; McNamara, 1994; Loscher, 1997). Some of the effects of kindling appear to be related to a long-term potentiation-like reorganization of the circuitry mediating the behavior (Adamec and Young, 2000), while other behavioral effects, particularly following status epilepticus, are likely due to the damage induced by excessive seizure discharge or to the paroxysmal activity that occurs when the seizures become spontaneous. With a few exceptions (e.g. Cavazos and Sutula, 1990; Sutula et al., 1994), kindling is still widely accepted as a functional epilepsy model in which the altered neuronal response develops in the absence of gross morphological damage, such as that seen in many other epilepsy models. However, a limit between kindling model and experimental models of status epilepticus is difficult to be traced since the mechanisms of epileptogenesis in both are based on the unremitting recurrence of seizures.

Several mechanisms may account for epileptogenesis during kindling (see for review Morimoto et al., 2004): enhanced activation in existing excitatory systems (glutamate receptor activation, synaptic potentiation); new circuitry formation (including sprouting, synaptogenesis, neurogenesis and astrogliosis); and failure of inhibitory systems (by

neuronal loss, impairment of GABA release and uptake, and reorganization of inhibitory synapses).

1.3.3.2.1 Enhanced activation in excitatory systems

A number of experimental epilepsy studies have focused on the enhancement of transmission in excitatory systems, particularly glutamatergic systems, as an epilepsy mechanism. Repeated focal microinjection of NMDA into the amygdala produces a kindling effect (blocked by co-injection of a selective NMDA receptor antagonist AP-7), that lasts at least 1 month, and shows significant facilitation of subsequent electrical kindling (Croucher et al., 1995). If NMDA is applied at a non-convulsive dose prior to each electrical stimulation, amygdala kindling is facilitated (Croucher et al., 1997).

A role for NMDA in electrical kindling is supported by demonstrations that amygdala kindling is significantly retarded by pretreatment with selective NMDA receptor antagonists, such as the competitive antagonists AP-5, CPP, and CGS19755 (Holmes et al., 1990; Morimoto et al., 1991), the non-competitive antagonist MK-801 (McNamara et al., 1988; Morimoto et al., 1991), and the allosteric glycine site antagonist 7-CK (Croucher and Bradford, 1990).

As most of the ionotropic receptor action at baseline appears to be generated at AMPA receptors, AMPA antagonists might be expected to block both kindling development and fully kindled seizures. NBQX, a selective antagonist of AMPA receptors, has potent inhibitory effects on amygdale kindling development (Namba et al., 1994). Systemic administration of NBQX for 14 days profoundly suppressed the growth of AS. After drug withdrawal, kindling proceeded normally. Moreover, NBQX and another selective antagonist, YM90K, could block kindling at doses that had no effect on monosynaptic evoked responses in the piriform cortex and hippocampal dentate gyrus nor on the induction of long-term potentiation (LTP) in anesthetized rats (Kodama et al., 1999).

The potency of the glutamate receptor blockers in shutting down kindling indicates that glutamate synapses play an important role in the development of kindling. It is commonly held that AMPA receptors are critical for the induction of seizure discharges, while NMDA receptors are critical for inducing the trans-synaptic alterations that underlie permanent

kindled epileptogenesis. The role of the NMDA receptor is most clearly seen in models of synaptic plasticity such as LTP.

As to synaptic potentiation, kindling has been shown to produce a substantial increase in the amplitude of population EPSPs in limbic system pathways (Racine et al., 1972; Douglas and Goddard, 1975; Maru and Goddard, 1987; de Jonge and Racine, 1987). Kindling-induced potentiation can persist for months (Douglas and Goddard, 1975; Maru and Goddard, 1987; de Jonge and Racine, 1987), is observed in widespread limbic brain sites (Racine et al., 1983), and thus must be considered as a potential mechanism contributing to the developing epileptogenesis.

Kindling-induced potentiation resembles LTP, except that it is induced by epileptogenic stimulation. LTP induction in the perforant path input to hippocampal dentate granule cells requires NMDA receptor activation (Morris et al., 1986; Morimoto et al., 1991), and repeated induction of LTP in limbic pathways significantly facilitates subsequent limbic kindling (Racine et al., 1975; Sutula and Steward, 1987). NMDA blockers suppress kindling-induced potentiation and retard kindling development. (Gilbert and Mack, 1990).

1.3.3.2.2 New circuitry formation

It has been known for some time that collateral axon sprouting can be induced in adult brain pathways in response to damage. These demonstrations included the damage induced by epileptogenic treatments such as local or systemic application of kainate (Nadler et al., 1980; Tauck and Nadler, 1985). The pathway most thoroughly studied is the mossy fiber pathway that runs from the dentate gyrus granule cells to the area CA3 pyramidal cells. Kindling induces changes in the pattern of staining of mossy fiber terminals in the inner molecular layer of the dentate gyrus (Sutula et al., 1988), and, also, altered staining patterns of axonal arborizations have also been reported in the stratum oriens of area CA3 following amygdala kindling (Represa and Ben-Ari, 1992).

The sprouting of local CA1 pyramidal cell axon collaterals into the stratum oriens and the alveus is consistent with an increased recurrent excitation via newly formed synaptic, and possibly autaptic, contacts with pyramidal cell dendrites. Using fluorescent tracers

evidences were found for sprouting between CA1 and CA3, between CA1 sites and other CA1 sites, and between the subiculum and hippocampus in rats (Lehmann et al., 2001).

However, now it seems unlikely that mossy fiber sprouting is a major contributor to kindled epileptogenesis. Amygdala kindling, for example, can reach the standard stage five criterion well before much mossy fiber reorganization has taken place (Ebert and Loscher, 1995). No reliable mossy fiber sprouting was observed in response to amygdala kindling in the Sprague–Dawley rat strain, unless kindling continued well beyond the usual criterion (Osawa et al., 2001). With a rapid kindling procedure, in fact, stage five can be achieved in 1–2 days (Ebert and Loscher, 1995), while kindling-induced mossy fiber sprouting does not become apparent before about 4 days (Cavazos et al., 1991). Also, mossy fiber sprouting requires several months to reach maximal levels (Cavazos et al., 1991).

Neurogenesis and astrogliosis are other possible mechanisms. Neurogenesis occurs in the subventricular zone in the adult rat following repetitive seizures in pilocarpine-induced status epilepticus model (Parent et al., 2002), producing precursor cells that migrate to the olfactory bulb. Kainate- or pilocarpine-induced seizures increased the migration of neuroblasts to the hilar - CA3 border (Scharfman et al., 2000). Kindling also resulted in a prominent hypertrophy of astrocytes, which was accompanied by a reorganization of the astrocytic cytoskeleton, was seizure-intensity dependent, occurred early in the kindling process, and persisted for weeks following the last seizure. In addition to hypertrophy, there was an increase in proliferation of astrocytes in the hippocampus, amygdala and piriform cortex following kindling (Khurgel and Ivy, 1996).

1.3.3.2.3 Failure of inhibition

Similar to glutamate activation, GABA deactivation can produce epileptiform discharges and, if repeated, a kindling effect. For example, repeated application of the selective $GABA_A$ receptor antagonists picrotoxin (Cain, 1987) or bicuculline (Uemura and Kimura, 1988) produces a kindling-like response. Bicuculline-kindled seizures are blocked by co-injection with GABA agonists, such as GABA or muscimol (Uemura and Kimura, 1988). There are also positive interactions between $GABA_A$ antagonist kindling and electrical kindling (Cain, 1987; Uemura and Kimura, 1990). Fully kindled seizures, for example,

were induced by injection of a single, normally nonconvulsant dose of bicuculline after completion of electrical kindling (Uemura and Kimura, 1988).

Evidences for a decreased level of inhibition in the kindling model are contradictory. For example, although a first study reported a persistent reduction of GABA immunoreactivity in the CA1 region (Kamphuis et al., 1987), data were not replicated in a later study (Lehmann et al., 1996). A long-lasting reduction of the synthesis of GABA-transaminase was found following amygdala kindling (Itagaki et al., 1986), while there was only a transient decrease in immunoreactivity of GAD (Babb et al., 1989) or no change in GAD mRNA expression (Shinoda et al., 1989). Subsequently, it was found that amygdala kindling led to a lasting decrease in GABA-immunoreactive neurons in the bilateral basolateral amygdala and ipsilateral central piriform cortex (Lehmann et al., 1998).

However, an impairment of GABA release and uptake is demonstrated by increased K^+-evoked, Ca^{2+}-dependent GABA release in hippocampal slices one day after kindling (Liebowitz et al., 1977). This increase was found to be persistent in CA1 (Kamphuis et al., 1990) and amygdala (Kaura et al., 1995) slices. Rapid kindling, in contrast, resulted in a persistent decrease in K^+-evoked Ca^{2+}-dependent GABA release in the CA1 region (Kapur et al., 1989).

1.3.3.3 Homeostatic plasticity

1.3.3.3.1 Homeostasis in the nervous system

The observation that biological systems remain stable by continuously changing and evolving led Cannon to develop the concept of homeostasis to encompass all the set of mechanisms that change continuous, in order to maintain the constancy of the internal environment (Cannon, 1932). In the particular case of the nervous system, incessant changes in neuronal circuits permit network output to be refined by experience (Hebb, 1949; Shatz, 1990), and constancy allow neurons to stabilize their excitability while retaining relative differences in strength among synapses.

Nevertheless, since synaptic connections in the nervous system are highly plastic this may challenge the stability of the network. Experimental efforts focused on identifying the substrates of experience-based plasticity. Most of the attention has been paid to Hebbian

plasticity, in which correlated presynaptic and postsynaptic activities interact to induce long-lasting changes in synaptic strength, while uncorrelated firing leads to synaptic weakening (Hebb, 1949; Bi and Poo, 2001; Malinow and Malenka, 2002). However, Hebbian plasticity encounters a problem created by the nature of the correlation-based rules themselves - instability due to positive feedback (inputs correlated positively would increase to their limit, while inputs correlated negatively would decrease to zero, finally leading to a loss of network selectivity) (Bienenstock et al., 1982; Miller, 1996).

In the last decade, experimental work has begun to uncover a set of mechanisms that promote stability and functionality in neuronal networks subjected to Hebbian synaptic change, all grouped under the notion of "homeostatic plasticity" (Turrigiano, 1999; Abbott and Nelson, 2000; Davis and Bezprozvanny, 2001). Plasticity is now comprehended as two contrasting but complementary forces: one changing neuronal circuits gradually by creating selective variations between elements, and another one adjusting circuit's properties in order to stabilize the overall activity of the network.

1.3.3.3.2 Mechanisms of homeostatic plasticity

There are four main mechanisms that could provide stability to neurons and networks: synaptic scaling, spike-timing dependent plasticity, synaptic redistribution and the Bienenstock-Cooper-Munro synapse.

Synaptic scaling is a biological mechanism that globally modifies synaptic strengths by balancing up or down the quantal amplitude of all synapses onto a postsynaptic neuron in response to long-lasting changes in neuronal activity. Synaptic scaling has been proven in vitro as neocortical pyramidal neurons up-regulate the strengths of their excitatory synapses when firing rates are low, and down-regulate them when firing rates are high (Turrigiano et al., 1998), and in vivo following a variant of a classic manipulation of monocular deprivation (Desai et al., 2002).

Synaptic scaling seems to depend primarily on the postsynaptic firing rate rather than on correlations between pre- and postsynaptic activity, therefore it is a non-Hebbian form of plasticity. However, Hebbian forms of plasticity can also regulate total levels of synaptic drive, but require a delicate balance between LTP and long term depression (LTD) (Bell et

al., 1997; Magee and Johnston, 1997; Feldman, 2000). The sensitivity of synaptic plasticity to the timing of postsynaptic action potentials, also called spike-timing dependent plasticity (STDP) can provide a mechanism for establishing and maintaining this balance (Abbott and Nelson, 2000). STDP is based on the interplay between the dynamics of NMDA receptor activation and the timing of action potentials back-propagating through the dendrites of the postsynaptic neuron (Magee and Johnston, 1997; Linden, 1999; Sourdet and Debanne, 1999). Although the long-term synaptic changes induced by STDP vary in different preparations, generally it assures that only presynaptic spikes that arrive in the range of time in which a neuron integrates its inputs are potentiated. Therefore, STDP weakens inputs that fire shortly after a postsynaptic action potential and have no contribution to evoking it, and also randomly occurring presynaptic spikes (Feldman, 2000).

The mechanism of synaptic redistribution is consistent with a form of LTP that acts presynaptically by increasing the probability of transmitter release. This raises the chance of transmission occurring early in a sequence of presynaptic action potentials, but also reduces the availability of existing vesicles for transmission later in the sequence. The overall effect is to enhance the average transmission amplitude for presynaptic action potentials occurring after a period of inactivity, but also to increase the onset rate of synaptic depression (Tsodyks and Markram, 1997). This type of network mechanism produce occasional spontaneous bursts during otherwise quiet periods, but prolonged periods of high activity are suppressed (O'Donovan and Rinzel, 1997; Tsodyks and Markram, 1997).

The Bienenstock-Cooper-Munro synapse was initially proposed as a model for competition in neuronal networks (Bienenstock et al., 1982). In this type of homeostatic regulation individual excitatory synapses can undergo both potentiation and depression; all depending upon whether postsynaptic activity is above or below a threshold. Synaptic input that drives postsynaptic firing to high levels increases synaptic strength, whereas input that produces only low levels of postsynaptic firing results in a decrease (Kirkwood et al., 1996). The threshold firing rate is itself a slow function of postsynaptic activity, modifying to make potentiation more likely when average activity is low and less likely when it is high (Abraham and Bear, 1996; Holland and Wagner, 1998; Wang and Wagner, 1999).

1.3.3.3.3 Homeostatic plasticity and paroxysmal activity

The balance between excitation and inhibition in cortical networks can critically influence the level and the type of spontaneous activity (Kriegstein et al., 1987; Chagnac-Amitai and Connors, 1989), information transfer through the network (Sillito, 1975; Somers et al., 1995), and experience-dependent synaptic plasticity (Kirkwood and Bear, 1994; Hensch et al., 1998). For highly recurrent cortical networks, the extensive positive feedback connections between excitatory pyramidal neurons are regulated by feedback and feedforward inhibition mediated by networks of inhibitory interneurons. Apparently, there are important differences between the mechanisms by which chronic changes in activity regulate inhibition and those that regulate excitation. Although inhibition, like excitation, is modified through changes in quantal amplitude and receptor clustering, there is also a marked reduction in the number of functional inhibitory synapses (Kilman et al., 2002). Inhibition and excitation onto pyramidal neurons are therefore regulated in opposite directions, and in fundamentally different ways, by activity blockade and this has profound implications in understanding the mechanisms of trauma-induced seizures. Furthermore, it has been shown that this process has limits and that after prolonged periods of reduced activity HSP may increase network excitability, which may finally lead to the development of paroxysmal activity (Houweling et al., 2005).

Homeostatic plasticity may also contribute to generation of paroxysmal activities in other conditions with prolonged periods of reduced activity, such as in the GABA withdrawal syndrome (GWS) (Brailowsky et al., 1988), after removal of prolonged pharmacological blockade (Turrigiano et al., 1998) or sensory deprivation (Pierson and Swann, 1988).

It is possible that additional factors contribute to the epileptogenesis in chronically injured neocortex, such as increased input resistances (Prince & Tseng, 1993) and axonal sprouting (Salin et al., 1995) of layer 5 pyramidal cells with the formation of new synapses. However, it is possible that axonal sprouting and the formation of new synapses is a secondary effect induced by paroxysmal activity (McNamara, 1999). In fact, axonal sprouting of corticostriatal neurons after ischemic cortical lesions appears to depend on synchronous neuronal activity in perilesioned neocortex (Carmichael & Chesselet, 2002).

1.3.3.4 Progression of epileptogenesis

Independently by the accountable agent the progression of epileptogenesis should follow the same steps (described in Morimoto et al., 2004). We assume that stimulation (during kindling) or changes in the synaptic/intrinsic neuronal properties and/or extracellular milieu (following cortical deafferentation) produce elevation of glutamate and GABA release from presynaptic terminals into the synaptic cleft. Glutamate stimulates postsynaptic AMPA receptors, but this depolarization is immediately reduced by $GABA_A$ receptor-mediated recurrent inhibition. If the stimulus intensity is above threshold, inhibition failure occurs along with continuous AMPA receptor activation, resulting in burst firing and synchronization. This prolonged depolarization secondarily activates NMDA receptors, which facilitates Ca^{2+} influx in a voltage-dependent manner.

The activation of AMPA and particularly NMDA receptors should trigger intracellular cascades that lead to functional changes and eventual synaptic reorganization in both glutamate and GABA systems. Many of these changes are just activity-dependent or compensatory and not directly related to the epileptogenesis.

In the late stage when focal seizure activity is prolonged and generalized, morphological changes such as neurogenesis, axonal sprouting, synaptogenesis, and astrogliosis appear, which result in the remodeling of excitatory and inhibitory systems and production of abnormal patterns of neurotransmission.

In the interictal period of epileptogenesis, neurons become increasingly burst-prone, leading, eventually, to spontaneous interictal discharges. The increased glutamate release is attributable to a number of factors, such as an increased number of new terminals, enhanced release, and the loss of presynaptic glutamate autoreceptors. Focal seizure activity can propagate via multiple reorganized brain circuits involving perirhinal and motor cortices, thalamus, basal ganglia, and brainstem.

Recurrent spontaneous seizures can emerge, possibly with sclerosis-like neuropathology. A loss of inhibition due to the loss of selective subclasses of interneurons may become present if kindling continues beyond the standard endpoint, or following cortical deafferentation.

1.3.4 Objectives of the thesis

The guiding line of the experiments was the hypothesis that the occurrence and / or the persistence of long-lasting far-from-equilibrium fluctuations between *silent* and *active* states in the neocortical networks if the key factor of epileptogenesis, leading to manifest seizures. We tested this hypothesis in two different experimental models, one associated with a decreased synaptic input to the cortex – the undercut model - and corresponding to the physiologically deafferented cortex during SWS; and the second one associated with an increased synaptic drive – the kindling model – corresponding to the cortex during alert states (wake / REM).

In both circumstances we aimed at understanding the mechanisms of epileptogenesis. Seizures arising in heterogeneous networks with different levels of excitability inflicted by plasticity mechanisms are built on a neuronal hyper-synchrony promoted by the neuronal switch from normal activity patterns towards a bursting behavior at a cellular level.

Independently of the underlying mechanism of epileptogenesis, we aimed at identifying the common temporo-spatial patterns of synchronizations of these seizures and the modulation of seizures by the state of vigilance, reflected by the level of synchronization of the cortical network.

2 Increased propensity to seizures after chronic cortical deafferentation in vivo

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2.1 Résumé

Les traumatismes corticaux peuvent engendrer des crises d'épilepsie. Nous avons investigué les caractéristiques et la transformation de l'oscillation corticale lente en activités paroxystiques du type complexe pointe-onde à 2-4 Hz à la suite de la déafférentation partielle du gyrus suprasylvian. Des expériences ont été effectuées chez des chats anesthésiés, à différents intervalles de temps (de 1 à 5 semaine, W1-W5) après la déafférentation corticale. L'activité électrique de ces aires a été enregistrée extracellulairement par des potentiels de champ et des neurones, ainsi que par des enregistrements intracellulaires. Les composantes EEG de l'oscillation lente ont augmenté en amplitude et ont dégénéré en des activités de type paroxystique, manifestées par une fréquence de décharge neuronale augmentée et des déchargés des potentiels d'action ayant l'aspect de bouffées. L'incidence des crises d'épilepsie de type pointe-onde a augmenté parallèlement avec la transition de stade semi-aigu (1ere semaine) au stade chronique (semaines 2 à 5) subséquemment à la déafférentation corticale. La vitesse de propagation des activités de basse fréquence a diminué de semaine 1 à semaine 5, pendant les oscillations lentes et les crises épileptiques de type pointe-onde. Les crises étaient initiées dans les aires corticale relativement intactes (aire 5 dans la partie antérieure du gyrus), qui avoisinaient les régions déconnectées, et se propageaient vers les aires sous lesquelles la section était faite; fait démontré par les corrélations croisées des potentiels de champ des différentes aires corticales et par les enregistrements intracellulaires simultanés dans les parties antérieure et postérieure du gyrus. L'amplitude croissante de l'oscillation lente et des crises de type pointe-onde, conjointement avec la synchronie augmentée de potentiels de champ exprimée par des vitesses de propagation de plus en plus rapides, sont attribuable aux changements des propriétés neuronales intrinsèques et à la desinhibition potentielle qui suit à la déafférentation corticale.

2.2 Abstract

Cortical injury may lead to clinical seizures. We investigated the changing patterns of the sleep-like slow oscillation and its tendency to develop into paroxysmal activity consisting of spike-wave (SW) complexes at 2-4 Hz following partial deafferentation of the suprasylvian gyrus. Experiments were carried out in anesthetized cats, at different time-intervals (week 1 to week 5, W1-W5) after cortical undercut. Multi-site field potentials and single or dual intracellular recordings from the whole extent of the deafferented gyrus were used. The field components of the slow oscillation increased in amplitudes and were transformed into paroxysmal patterns, expressed by increased firing rates and tendency to neuronal bursting. The incidence of SW seizures was higher with transition from semi-acute (W1) to chronic (W2-W5) stages following cortical undercut. The propagation delay of low-frequency activities decreased from W1 to W5, during both the slow oscillation and seizures. The initiation of seizures took place in territories contiguous to the relatively intact cortex (area 5 in the anterior part of the gyrus), as shown by cross-correlations of field potentials from different sites and simultaneous intracellular recordings from the anterior and posterior parts of the gyrus. The increased amplitudes of both slow oscillation and SW seizures, and their enhanced synchrony expressed by shorter time of propagation, are ascribed to changes in intrinsic neuronal properties and potential disinhibition following cortical undercut.

2.3 Introduction

Acute cerebral cortical trauma may lead to paroxysmal activities. Within 24 hours up to 80 % of patients with penetrating wounds display clinical seizures (Dinner 1993; Kollevold 1976). Ten to 15 years after the trauma about 50 % of patients with penetrating cranial wounds would develop epilepsy characterized by recurring seizures (Marcikic et al. 1998; Salazar et al. 1985). In experimental animals, chronic neuronal hyperexcitability and epileptogenesis have been demonstrated in isolated neocortical islands with intact pial circulation *in vivo* (Burns 1951; Sharpless 1969; Sharpless and Halpern 1962) and in neocortical *in vitro* slices after chronic cortical injury (Hoffman et al. 1994; Li et al. 2005; Li and Prince 2002; Prince et al. 1997; Prince and Tseng 1993).

The origin of these seizures is unclear. Computational models of posttraumatic epileptogenesis in isolated cortical islands concluded that paroxysmal discharges are possibly due to changes in intrinsic properties of pyramidal cells and enhanced excitatory synaptic conductances without altering synaptic inhibition (Bush et al. 1999; Houweling et al. 2005). *In vitro* experimental studies on models of acute and chronic cortical deafferentation revealed an increased synaptic and intrinsic neuronal responsiveness following decrease in input signal (Prince et al. 1997; Prince and Tseng 1993; Turrigiano et al. 1998), which may favor the development of epileptogenesis. After a few days of pharmacological blockade of activity in cortical cell cultures, the amplitudes of mEPSCs and EPSCs in pyramidal cells increase (Turrigiano et al. 1998; Watt et al. 2000) as well as the quantal release probability (Murthy et al. 2001). Synaptic scaling occurs in part postsynaptically by changes in the number of open channels (Turrigiano et al. 1998; Watt et al. 2000), although all synaptic components may increase (Murthy et al. 2001), including the numbers of postsynaptic glutamate receptors (Liao et al. 1999; Lissin et al. 1998; O'Brien et al. 1998; Rao and Craig 1997). There is a similar activity-dependent regulation of NMDA currents (Watt et al. 2000). Interestingly, mIPSCs are scaled down with activity blockade, in the opposite direction to excitatory currents. This effect is reversible (Rutherford et al. 1997) and is accompanied by a reduction in the number of open $GABA_A$ channels and $GABA_A$ receptors clustered at synaptic sites (Kilman et al. 2002). Not only synaptic but also intrinsic excitability is regulated by activity. After chronic activity

blockade, Na^+ currents increase and K^+ currents decrease in size, resulting in an enhanced responsiveness of pyramidal cells to current injections (Desai et al. 1999b). These observations suggest that homeostatic mechanism may regulate the average levels of neuronal activity. Some of these processes, collectively termed `homeostatic plasticity' (Turrigiano 1999), may also occur *in vivo* (Desai et al. 2002).

Acute experiments performed *in vivo* showed that partially deafferented neocortex displays increased local cortical synchrony in areas surrounding the undercut cortex, leading to paroxysmal activity that occurs 2-3 h after the undercut and arises from enhanced intrinsic and synaptic neuronal responsiveness, increased incidence of intrinsically-bursting neurons, and slight reduction of inhibitory influences (Topolnik et al. 2003a, b). We have now investigated the evolution of electrical paroxysms induced by cortical deafferentation up to 5 weeks following the undercut, to determine the spatiotemporal development of post-traumatic seizures with respect to the initial cortical insult, and to quantify at different stages the transformation from the normal sleep-like slow oscillation (0.5-1 Hz) to increased amplitudes of phases building up this cortical rhythm, eventually reaching the level of paroxysmal activity.

2.4 Materials and methods

2.4.1 Animals and cortical deafferentation

Experiments were performed on 29 adult cats of both sexes. Surgical procedures were carried out in sterile condition, following a premedication with acepromazine (0.3 mg/kg i.m.), butorphanol (0.3 mg/kg i.m.), atropine (0.05 mg/kg i.m.) and ketamine (20 mg/kg i.m.), under isoflurane anesthesia (1-2%). The level of anesthesia was continuously monitored by the EEG, heart rate, oxygen saturation of the arterial blood (aiming over 90%), and end-tidal CO_2 (~3.5%). Isoflurane concentration was adjusted correspondingly in order to maintain a cardiac frequency at 90-110 beats/min. General surgical procedures included: cephalic vein canulation for systemic liquid delivery (lactated Ringer's solution 5-10 ml/kg/h) and lidocaine (0.5%), infiltration of all pressure points or incision lines. Body temperature was maintained at 37–39°C with a heating pad.

Craniotomy was used to expose the cerebral cortex and a large undercut of the white matter below the suprasylvian gyrus (13-15 mm postero-anteriorly and 3–4 mm medio-laterally) was used to produce partial cortical deafferentation (Fig. 2-1). A custom-designed knife was inserted in the posterior part of suprasylvian gyrus perpendicular to its surface for a depth of 3–4 mm, then rotated 90° and advanced rostrally along the gyrus parallel to its surface for a total distance of 13-15 mm, then moved back, rotated 90° and removed from the same place where it was entered. Thus, the anterior part of the undercut cortex was relatively intact and the white matter below the posterior part of the gyrus was transected, creating conditions of partial cortical deafferentation. The skull was reconstituted using acrylic dental cement and the skin of the scalp sutured. Animals were kept under observation up to full recovery and they received analgesic medication (anafen 2 mg/kg s.c.) for the next 48-72 hours.

2.4.2 Semichronic experiments

At different time intervals after the initial surgery, the different subgroups of animals were anesthetized with ketamine & xylazine (10-15 mg/kg and 2-3 mg/kg, respectively, i.m.) and recorded at one week from the undercut (W1, n = 5), 2 weeks (W2, n = 5), 3 weeks (W3, n = 5), 4 weeks (W4, n = 5), and 5 weeks (W5, n = 5). In addition, electrophysiological experiments were done on 2 cats at W1 and 2 cats at W5 anesthetized with sodium pentobarbital (30 mg/kg). EEG and heart rate were monitored continuously to maintain the anesthesia, and additional doses of anesthetic were given at the slightest tendency toward an activated EEG pattern or accelerated heart rate. All pressure points to be incised were infiltrated with lidocaine (0.5%). Muscle paralysis was induced with gallamine triethiodide and artificial ventilation (20-30 cycles/min) maintained the end-tidal CO_2 concentration around 3.5% (±0.4). The craniotomy holes exposed the cerebral cortex and allowed the insertion of recording electrodes. Cisternal drainage, hip suspension, pneumothorax, and filling of the hole in the skull with a 4% solution of agar were used to enhance the stability of the intracellular recordings. Body temperature was maintained at 37–39°C and glucose (5% solution) was administered i.v. every 3–4 h during experiments. At the end of experiments, the cats were given a lethal dose of sodium pentobarbital (50 mg/kg, i.v.). After experiments brains were removed and the extension of the undercut was verified on Nissl stained (thionine) 80-µm brain sections (Fig. 2-1B). All experimental procedures

were approved by the committee for animal care of Laval University and in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

2.4.3 Extracellular recordings

Field potential (EEG) recordings were obtained by means of an array of 8 tungsten electrodes (impedance 8–12 MΩ), approximately 2.5 mm apart, placed on the whole length of the suprasylvian gyrus (Fig. 2-1A). Because the anterior limit of the undercut was not visually noticeable, we positioned the array of electrodes in such manner as to have 2 electrodes in the relatively intact cortex, 2 electrodes over the area corresponding to the anterior limit of the undercut, and 4 electrodes in the deafferented cortex.

2.4.4 Intracellular recordings

Intracellular recordings in the partially deafferented area 21 and relatively intact area 5 of the suprasylvian gyrus were obtained with glass micropipettes (tip diameter <0.5 μm) filled with potassium acetate (3 M, in situ impedance 35-50 MΩ). Only stable recordings with resting membrane potentials more negative than -60 mV, overshooting action potentials and input resistances >20 MΩ were accepted for analysis. The intracellular signals were passed through a high-impedance amplifier with an active bridge circuitry (bandwidth DC to 9 kHz). All signals were digitized (20 kHz sampling rate) and stored for off-line analysis.

2.4.5 Data analysis

To estimate changes in slow wave activities following cortical undercut, the power in the 0-4 Hz range was quantified by the area under the graph of the fast Fourier transform (FFT) of field EEGs. The EEG amplitude was estimated from 1 min artifact-free periods as a difference between the most positive and most negative values. Means of comparative data were statistically evaluated with paired Student's t-test. Differences between means were considered significant at $p < 0.05$. Auto- and cross-correlograms of different EEG channels were computed from periods of 2-3 minutes of stable activity and averaged for each different group (see Fig. 2-7B). The electrode where the seizures first occurred was taken as time reference.

Histograms of membrane potential (V_m) distribution (see Fig. 2-9C) were created for successive periods of 10 s by counting the number of samples with bins of 1 mV. The peaks of distribution that corresponded to the most probable mode of V_m were taken as the level of V_m. Time-delay histogram (as in Fig. 2-9D) was obtained from differences in time between the two intracellular recordings of the points corresponding to 10% of the amplitude of the depolarization between the V_m during hyperpolarizing states of the SW and the V_m at the onset of burst.

2.5 Results

2.5.1 Changes in slow oscillation and development of seizures in the undercut cortex

The normal slow cortical oscillation (<1 Hz) comprises two different activity levels: an active ("up") state in which cortical neurons are depolarized and tonically fire action potentials, and a silent ("down") state in which cortical neurons are hyperpolarized by a disfacilitation process (Steriade et al. 1993; Contreras et al. 1996).

All cats with chronic cortical deafferentation displayed a paroxysmal pattern of the slow oscillation. More than 90% of animals anesthetized with ketamine-xylazine (23 out of 25) displayed low-frequency SW and polyspike-wave (PSW) complexes (3-4 Hz), intermingled with fast runs (10-20 Hz), which resemble the hypsarrhythmia pattern of the Lennox-Gastaut syndrome (Fig. 2-2A). One of the 2 cats that did not develop seizures was part of W1 group and the other part of the W2 group.

The FFT of EEG showed a unimodal distribution with a peak of activity corresponding to the frequency of the slow oscillation, 0.5-1 Hz (Fig. 2-2B, W1). EEG recordings of the slow oscillation in the undercut cortex, performed from W1 to W5, showed that, while the frequency was similar at different electrodes, the EEG amplitude and the peak of FFT activity were higher for electrodes placed over the anterior limit of the undercut, at the border between the intact and deafferented cortex (EEG3), compared with posterior electrodes, in the most deafferented cortex (EEG8) (Fig. 2-2B). The higher EEG amplitude and peak of FFT activity were also observed during SW/PSW seizures. Following cortical deafferentation, the power of slow activities markedly augmented with time, mainly by the

increase in the 0-4 Hz frequency domain and the change from a unimodal to a multimodal distribution (compare W1 with W5 in Fig. 2-2B).

It is known that ketamine-xylazine anesthesia favors development of seizures (Steriade and Contreras 1995; Steriade et al. 1998); however, the proportion of anesthetized animals with acute undercut (Topolnik et al. 2003b) and chronic undercut (present study) displaying seizures is much higher. To completely avoid the influence of ketamine-xylazine anesthesia on the development of paroxysmal activities, we recorded electrical activities from 4 cats with cortical undercut, anesthetized with barbiturates (two on them at W1 and the other two at W5). Barbiturates enhance GABA-ergic inhibition via prolongation of time of opening of Cl^- channels (Twyman et al. 1989). Similarly to our previous study (Topolnik et al. 2003b), we did not expect to see the development of full-blown seizures in those experiments, but we expected to see an enhanced activity in areas surrounding the undercut cortex. This was indeed the case in W1 animals (not shown). W5 animals revealed some different patterns (Fig. 2-3). (a) During the early phases of anesthesia, in the relatively intact anterior part of suprasylvian gyrus, spindle activity was prominent, while it was absent in the partially deafferented areas. In the partially deafferented areas field potentials demonstrated aperiodic, asynchronous EEG "spikes" (see arrowheads in expended parts of Fig. 2-3A). Five-to-eight hours later, when the level of anesthesia decreased, the activity in the relatively intact areas was dominated by 2-3 Hz paroxysmal discharges that merged with spindle oscillations (Fig. 2-3B). Probably, the barbiturate-related enhancement of inhibition prevented propagation of paroxysmal activities into partially deafferented cortical areas. Isolated EEG spikes still persisted in the partially deafferented cortex (Fig. 2-3B, EEG 7).

The quantification of data on EEG amplitude in cats under ketamine-xylazine anesthesia is shown in the Fig. 2-4. During the slow oscillation, the maximal EEG amplitudes grew up in different electrodes as a function time following the undercut, suggesting the increase in local synchrony. The maximal values of amplitude were recorded around electrode EEG 3, i.e. the electrode that in most cases was located between relatively intact and partially deafferented areas. During electrographic seizures, the EEG amplitude was higher than

during slow oscillation at all studied time samples and statistically significant differences were mainly found between W1 and W5 (Fig. 2-4).

To quantify the power spectra of EEG recorded from 8 electrodes placed over the undercut suprasylvian gyrus (see Fig. 2-1A, EEG1-relatively intact cortex through EEG8-partially deafferented cortex), the area under the FFT graph for 0-4 Hz domain was computed for each period ranging from W1 to W5 (Fig. 2-5 A). The increased power of slow activities in time, following deafferentation, was observed for both the slow oscillation and SW/PSW seizures. During seizures, the power of slow activities was consistently higher than during the slow oscillation and the electrodes in the anterior part of the undercut (mainly EEG3) contained more slow activities than electrodes placed in the posterior part of the undercut (EEG8), suggesting that the occurrence of seizures is initiated at the border between the relatively intact and the deafferented cortex. Fig. 2-5B presents comparatively the evolution of power spectra, from W1 to W5, in EEG activity recorded from posterior electrode 8 (in black) and from electrode with highest levels of slow activities, usually the electrode 3 (in red), for both the slow oscillation and the SW/PSW seizures, together with the standard deviation (SD) in each group. For the slow oscillation, statistical difference between W1 and W5 was obtained for both anterior and posterior parts of the undercut, while the difference between anterior and posterior electrodes was significant only at W5. As to seizure activities, a significant difference was found between the anterior and posterior electrodes starting from W3 to W5, and from the same electrode from W1 and W5 (Fig. 2-5B).

As illustrated above (see Fig. 2-2B), the increase in power spectra of slow oscillation evolved in time from the peak around 1 Hz (at W1, ampler in anterior electrode EEG3 than in the posterior EEG8) to the expression of multiple frequencies in the FFT and the widening of the peaks over the whole 0-4 Hz domain (at W5).

Out of 38 neurons recorded intracellularly, 12 were recorded from the anterior part of the undercut, before the occurrence of overt SW/PSW seizures. The intracellular recording in the chronic deafferented cortex (Fig. 2-6) contained normal periods of slow oscillation (expanded in left panel) alternating with periods of paroxysmal slow activities. During paroxysmal-like activities the Vm of neurons revealed slight (2.6±1.4 mV) depolarization

calculated from the maximum of membrane potential distribution (Fig. 2-6D). This depolarization was accompanied with increased firing rates and reduction in action potential amplitudes. The other two distinct features were the presence of brisk hyperpolarizing waves (100-300 ms) and spontaneous bursts action potential generated by regular-spiking neurons (see inset in Fig. 2-6). The cell's bursts were revealed by short intervals (5-10 ms) in the inter-spike histogram (Fig. 2-6C) and were absent during normal spontaneous activities (Fig. 2-6B).

2.5.2 Spatiotemporal properties of seizures in the deafferented cortex

In all cases the seizures started, and their amplitudes were most conspicuous, in the anterior part of the suprasylvian gyrus (electrodes EEG1 to EEG4), and propagated to the deafferented cortex (Fig. 2-7A). Usually, seizures started with SW/PSW complexes at 2-3 Hz, followed by episodes of fast runs at 10-20 Hz. Cross-correlation between activities recorded through different electrodes indicated a distinctive time-lag range between the electrodes during slow oscillation, as compared to SW/PSW discharges (Fig. 2-7B). The time-lags between activities recorded from different cortical sites ranged from ~6 to ~190 ms and were shorter during SW/PSW seizures than during propagation of the cortical slow oscillation (Fig. 2-7B). Pooled data from all animals (5 cats in each week, from W1 to W5) showed that the velocity in activity propagation for 0-10 Hz frequencies in the undercut cortex increased from W1 to W5, both during the slow oscillation (from 0.063±0.004 m/s to 0.3257±0.0404 m/s between EEG4 and EEG8) and seizures (from 0.1182±0.006 m/s to 0.7751±0.3107 m/s between EEG4 and EEG8); and the time-lag of propagation was persistently shorter during SW/PSW seizures compared with that during the slow-oscillation (Fig. 2-8). The level of correlation between the electrodes followed the same rule: it increased for both condition from W1 to W5 and it was steadily greater during SW/PSW seizures than during the slow oscillation (not shown).

Dual intracellular recordings (n = 9) from neurons located in the area around the anterior (relatively intact) area 5 and the posterior (deafferented) area 7, in conjunction with field EEGs recorded over the whole extent of the suprasylvian gyrus (with a space resolution of 1.5 mm) were used to analyze the pattern of synchrony during seizures and their propagation. Such simultaneous intracellular data showed that neurons in the anterior part

of the suprasylvian gyrus, in the relatively intact cortex, fired consistently before the neurons in the posterior part of the undercut (Fig. 2-9). Time detection was made at 10% amplitude between the resting Vm and the firing threshold of the first spike (Fig. 2-9B and D). Histograms of the Vm of two neurons are depicted in Fig. 2-9C. Both displayed a bimodal distribution with peaks corresponding to the resting Vm and to the value of the depolarizing plateau. The mean membrane potential of neurons in relatively intact and partially deafferented areas was not statistically different, but the membrane potential of neurons had tendency to be more hyperpolarized during silent network states ("up" states in relatively intact area 56.4±2.2 mV, "down" states in relatively intact area 69.4±3.9 mV; "up" states in partially deafferented area 53.5±4.2 mV, "down" states in partially deafferented area 74.3±4.8 mV).

2.6 Discussion

We found that the chronic stages of cortical undercut are characterized by (a) increase in amplitudes of field potentials that build up both the slow oscillation and SW/PSW seizures; (b) increased velocity of low-frequency activity propagation from W1 to W5, during both the slow oscillation and seizures; and (c) initiation of seizures in territories contiguous to the relatively intact cortex, as shown by both field potentials and intracellular recordings.

The ketamine-xylazine anesthesia used in this study induced a sleep-like slow oscillation, which is similar to that recorded during natural sleep in cats and humans (Achermann and Borbély 1997; Amzica and Steriade 1997; Massimini et al. 2004; Mölle et al. 2002; Steriade et al. 1993). Under this type of anesthesia, 20-30% of animals can spontaneously develop seizures with SW/PSW complexes (Steriade and Contreras 1995; Steriade et al. 1998). However, the present and previous data rule out the main contribution of ketamine-xylazine anesthesia in the generation of seizures that followed cortical deafferentation, based on: (a) the much higher percentage (over 90%) of seizures after the undercut, compared to the incidence of such paroxysms previously observed in the non-lesioned cortex (20-30%); (b) the presence in all recorded animals of a peculiar pattern of slow oscillation, containing alternating periods of normal activity and periods with paroxysmal oscillation in the 0-4 Hz domain (Fig. 2-2A, W5), which was never observed in previous experiments conducted under the same anesthesia, but without chronic cortical

deafferentation; and (c) the highly increased amplitudes and sharp waves following cortical undercut in animals under barbiturate anesthesia (Fig 2-3), while barbiturates normally prevent the occurrence of seizures by enhancing GABA-ergic inhibitory processes. The transformation of the slow oscillation into SW/PSW seizures was shown by the preferential occurrence of these paroxysms during slow-wave sleep and by similar relations between field and intracellular activities during slow oscillation and epochs with SW/PSW seizures (see also Fig. 2-4).

Several factors may account for the increased propensity to seizures following the cortical insult produced by undercut. While acute epileptogenesis due to increased $[K^+]o$ (Moody et al. 1974) may be partially explained by K^+-mediated increase in the hyperpolarization-activated depolarizing current (I_H) that leads to paroxysmal activity in neocortical networks (Timofeev et al. 2002), the progressively increased power of seizures over time, up to five weeks following undercut (present data) would not favor the same mechanism. The same reasoning may apply to changes in extracellular glutamate that is increased following cortical trauma and was reported to promote epileptogenesis (Sakowitz et al. 2002). The changes in intrinsic neuronal properties following cortical injury or undercut, leading to seizures, have been first studied in vitro and some of these results have been corroborated in vivo. Prince and Tseng (1993) compared layer V neurons of epileptogenic slices with those in control slices and found no significant differences in action potential characteristics and resting Vm, but the value of input resistance (Rin¬) was more than double in injured than in control slices. The increased Rin of neurons recorded from epileptogenic slices is likely behind the increased intrinsic and synaptic responsiveness found in neurons recorded from the relatively intact suprasylvian cortex, at which level seizures are initiated following acute deafferentation in vivo (Topolnik et al. 2003a). This result fits in well with injury-induced enhanced responsiveness of corticospinal neurons following their axotomy (Tseng and Prince 1996) and with the increased synaptic and intrinsic responses of cultured cortical neurons during chronic absence of spontaneous activity (Desai et al. 1999a; Turrigiano et al. 1998). Trauma induced chronic hyperexcitability and focal epileptogenesis could occur when homeostatic plasticity mechanisms up regulate processes leading to increased excitability (Houweling et al. 2005). The high Rin, shown to characterize neurons in epileptogenic slices (Prince and Tseng

1994), is probably a factor promoting seizures by favoring transformation of regular-spiking into intrinsically-bursting neurons whose incidence is much higher in disconnected cortical slabs in vivo (Timofeev et al. 2000) and in cortical slices in vitro (Nishimura et al. 2001) than in the intact cortex (Steriade et al. 2001). Besides the above-mentioned changes in intrinsic properties, a shift in the balance between inhibitory and excitatory processes may be changed toward excitation as, in parallel experiments using immunohistochemical methods, we detected a reduction by about 40% in GABA-ergic neurons in disconnected cortical areas (unpublished data). The presence of normal inhibitory connectivity in more intact cortical areas may favor spatial localization of excitatory neuronal networks and thus prevent generalized epileptogenesis (Traub and Wong 1982).

The above changes explain, at least partially, the increased synchrony and shorter time-delay propagation of low-frequency (slow oscillation and seizure) activities following cortical undercut. High-density EEG recordings of the slow sleep oscillatory waves in the human cortex indicate a wide range of conduction velocities, 1.2 to 7 m/s (Massimini et al. 2004). The present data indicate that, following deafferentation, the slow oscillation propagates with progressively shorter time-lags from W1 to W5 (see Fig. 2-7B). Multi-site field potential and intracellular recordings have shown that cortically generated SW seizures propagate from one to another area in the suprasylvian gyrus through mono-, oligo- and multi-synaptic linkages (Amzica and Steriade 1995; Neckelmann et al. 1998). Following cortical undercut, the propagation of paroxysmal activity is also progressively shorter from W1 to W5 (Fig. 2-7B).

In summary, following partial deafferentation the neocortex displays progressively increased signs of paroxysmal activity, reflected in paroxysmal-like patterns of the slow oscillation and progressively enhanced amplitudes and synchrony of SW/PSW seizures, which are however relatively localized within disconnected territories adjacent to the relatively intact cortex.

2.7 Acknowledgements

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2.9 Figures

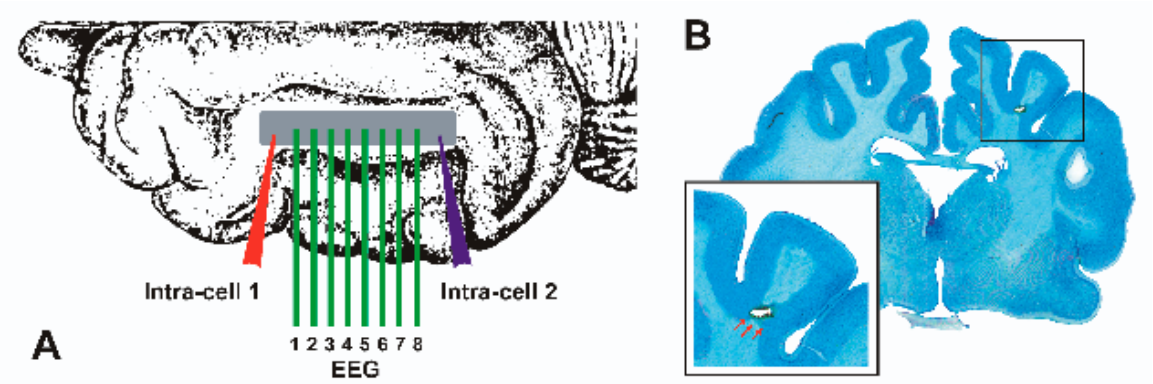

Figure 2-1 Experimental paradigm. *A*, array of EEG electrodes and of two intracellular pipettes over the left suprasylvian gyrus. Area in grey represents cortical deafferentation. Cortical deafferentation was performed from posterior to anterior. *B*, frontal section of cat brain (Nissl staining). The trace left by the knife in the white matter is expanded in the inset (red arrows).

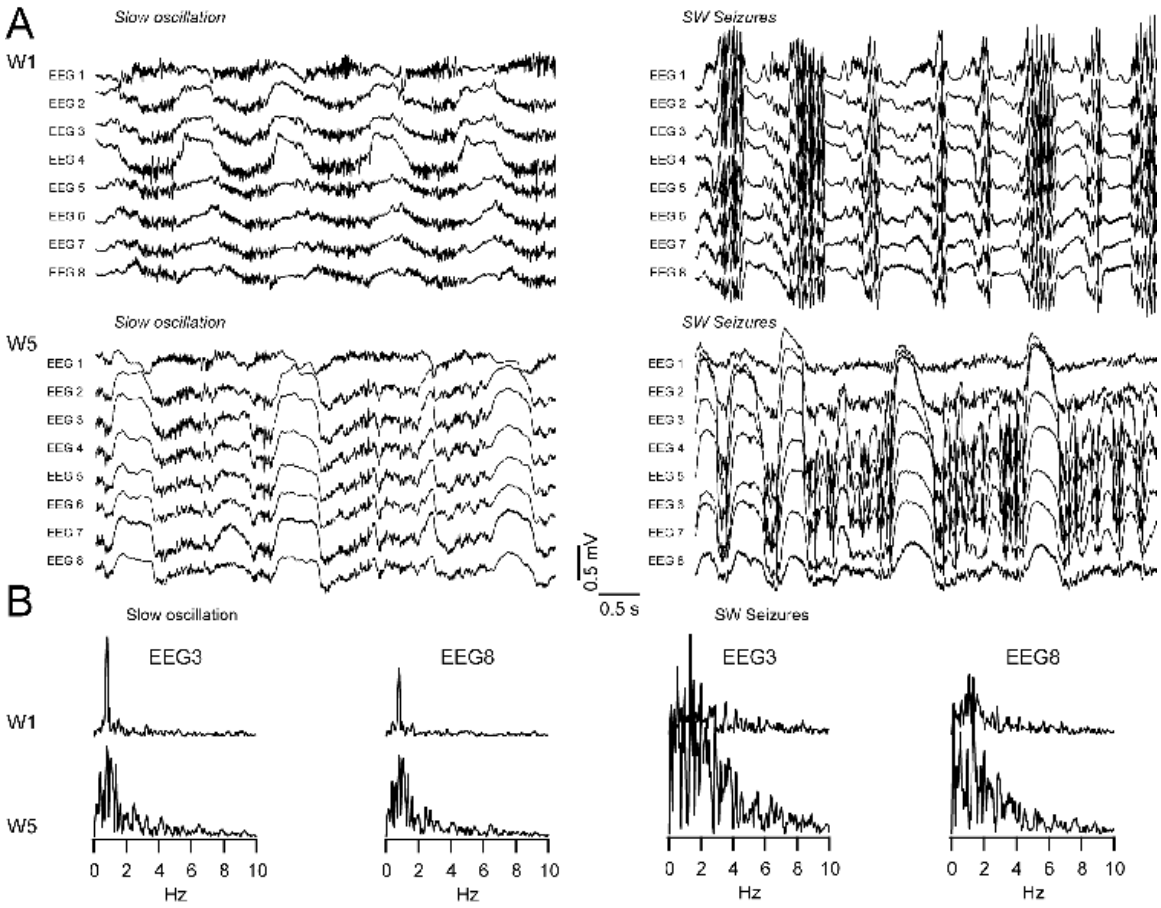

Figure 2-2 Patterns of slow oscillation and electrical seizures one week (W1) and 5 weeks (W5) after cortical deafferentation. Ketamine-xylazine anesthesia. A, EEG field potentials during slow oscillation and SW/PSW seizures at W1 and W5 after cortical deafferentation. B, FFT of EEG activity shows an increased power of slow activities (less than 4 Hz) in anterior electrodes (EEG3) compared to posterior ones (EEG8), and in time from W1 to W5, both during slow oscillation and SW seizures.

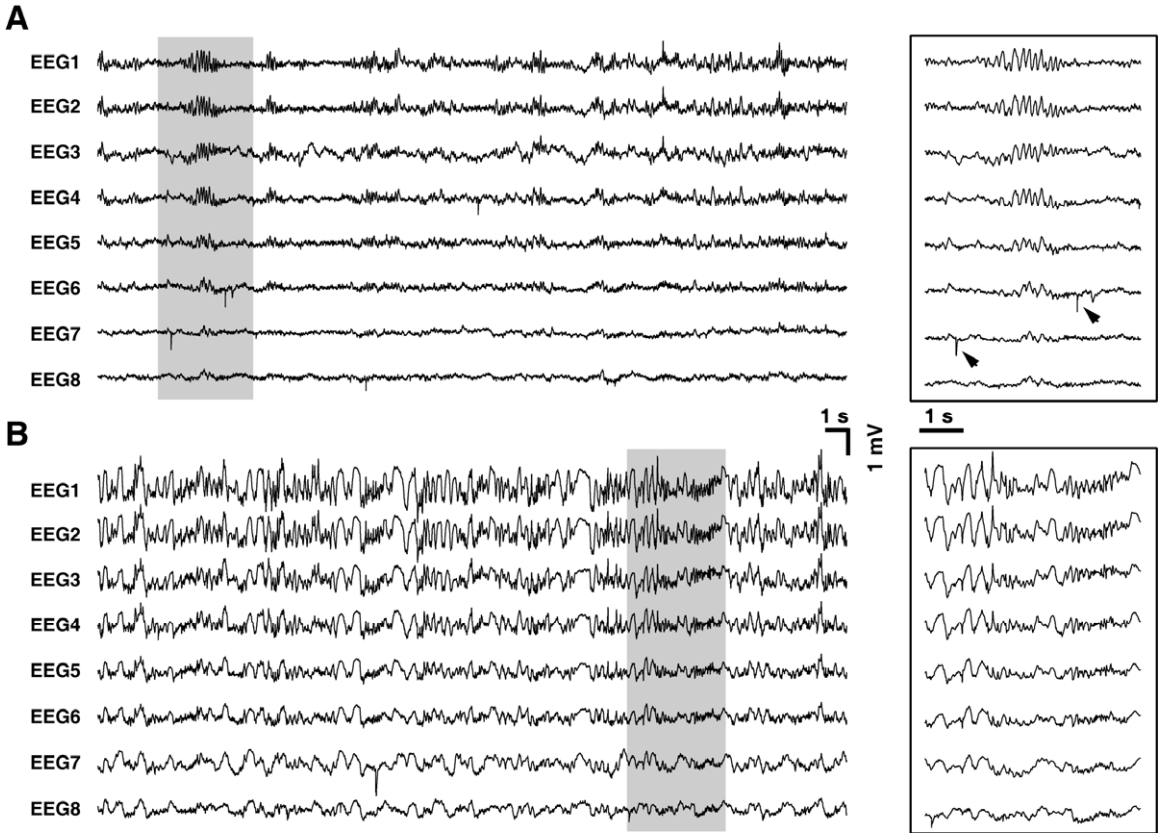

Figure 2-3 Patterns of electrographic activities in barbiturate-anesthetized cat 5 weeks after the undercut. *A*, 8 EEG recordings from suprasylvian gyrus 1 hour after the beginning of anesthesia. The position of electrodes as in Fig. 2-1. The period indicated by a grey rectangular is expanded at right. Note the presence of spindles in the anterior, relatively intact cortex and the presence of fast EEG spikes (see arrowheads) in the posterior, partially deafferented cortex. *B*, the same experiment as in *A*, but 7 hours after the induction of anesthesia. Note the presence of paroxysmal discharges, merged with spindles in the relatively intact part of undercut cortex.

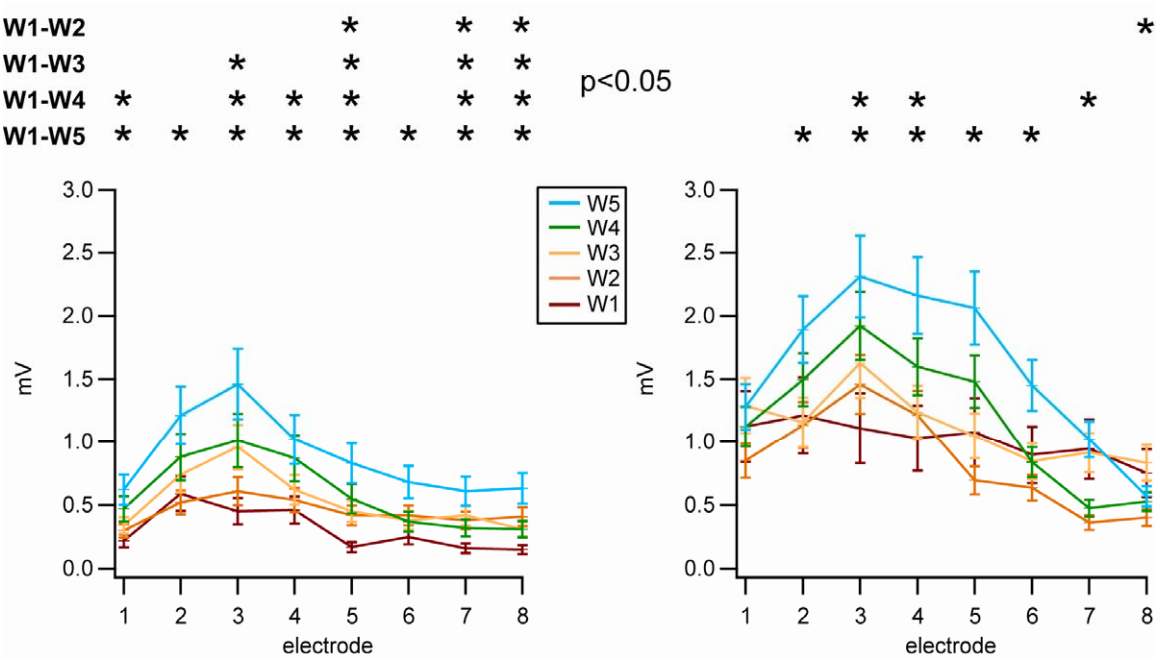

Figure 2-4 EEG amplitudes in the undercut cortex during slow oscillation (left) and paroxysmal discharges (right) in ketamine-xylazine anesthetized cats. The mean amplitude of EEG traces obtained from different animals (*n* = 5 in each group) with electrodes placed in the positions indicated in Fig. 2-1. The highest EEG amplitudes were found around EEG 3 in animals 1-5 weeks after the undercut. Stars above plots indicate statistically significant difference in the EEG amplitude.

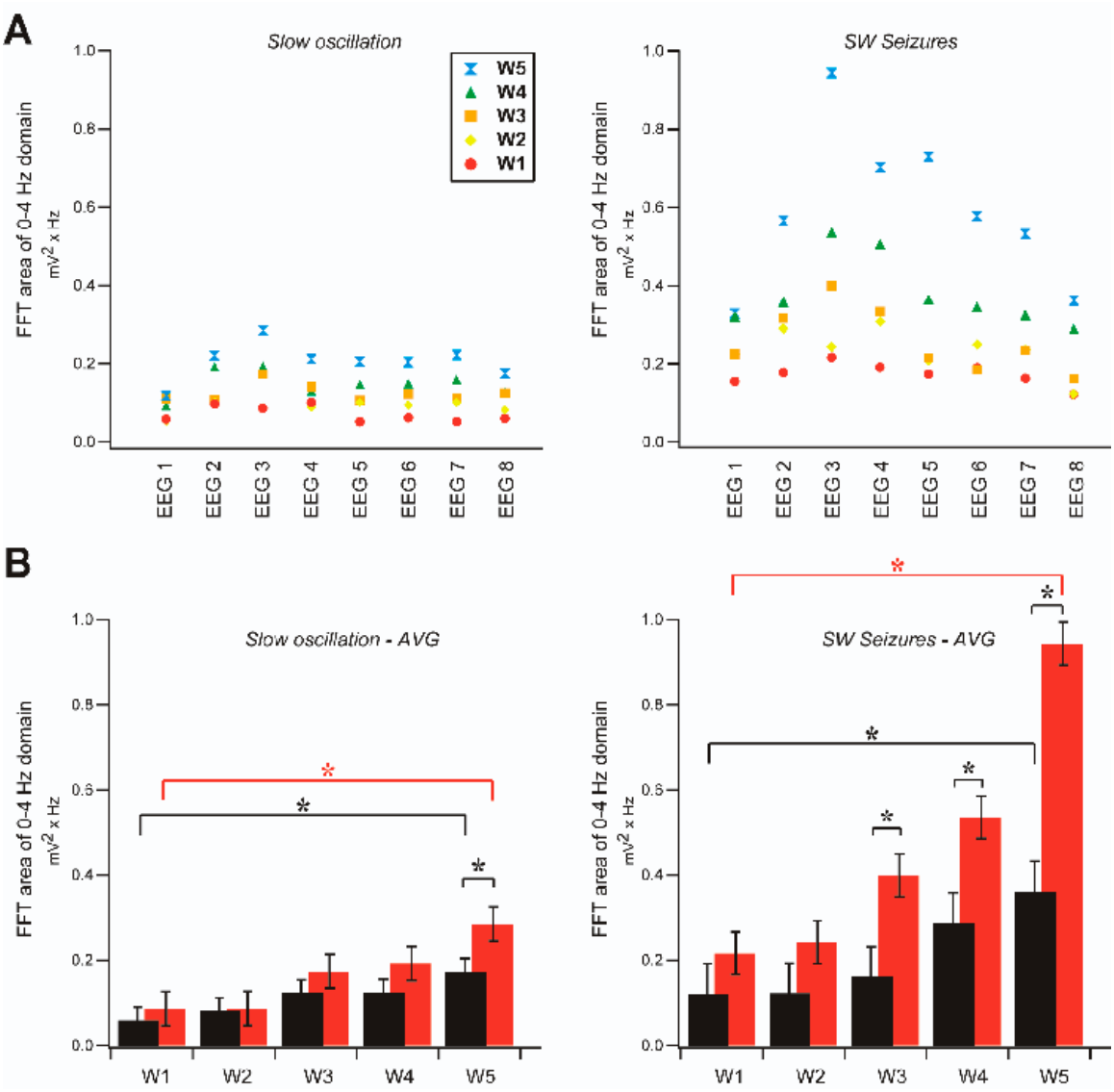

Figure 2-5 Quantification of EEG power in the 0-4 Hz domain. Ketamine-xylazine anesthesia. *A*, FFT mean amplitude per EEG electrode from W1 to W5 during slow oscillation and seizures. *B*, quantification of the EEG power in the 0-4 Hz domain for EEG8 (most posterior electrode, in black) and EEG3 (most anterior electrode from where activities propagates, in red). Significant statistical differences ($p < 0.05$) are marked with *.

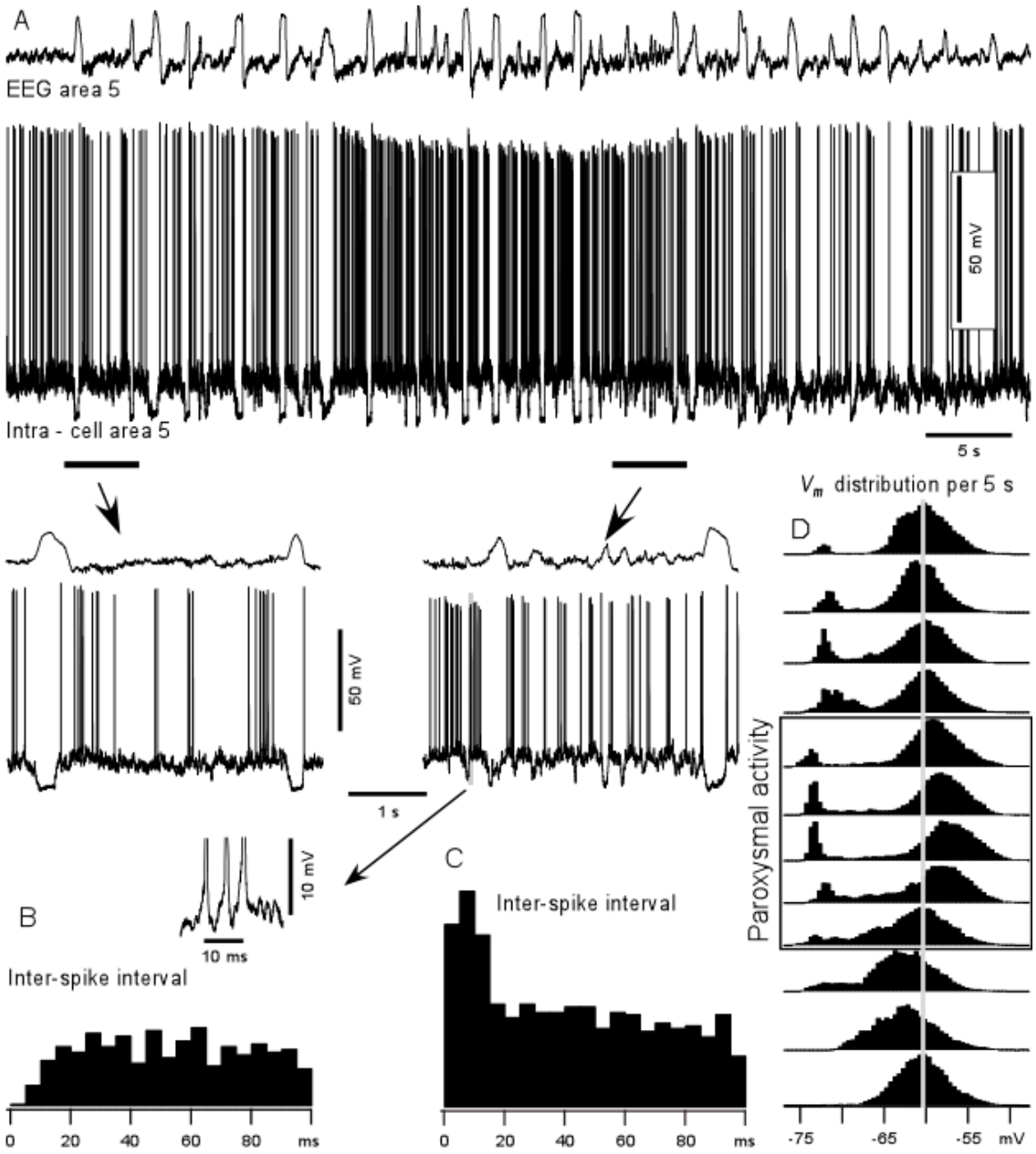

Figure 2-6 Intracellular recording in the anterior part of the undercut cortex during slow oscillation, 3 weeks after deafferentation. Ketamine-xylazine anesthesia. Underlined epochs in *A* are expanded in *B* and *C*. The slow oscillation contained normal periods (*B*) alternating with paroxysmal periods (starting in the middle of top trace, *A*), during which the neuron displayed a tendency toward bursting (*C*), sometimes induced by brisk hyperpolarizing events or spontaneously occurring during the active periods. The burst spotted in grey is expanded in the inset. FFT of the field EEG and inter-spike interval histograms from intracellular activity are displayed for the normal (*D*) and paroxysmal slow oscillation (*E*).

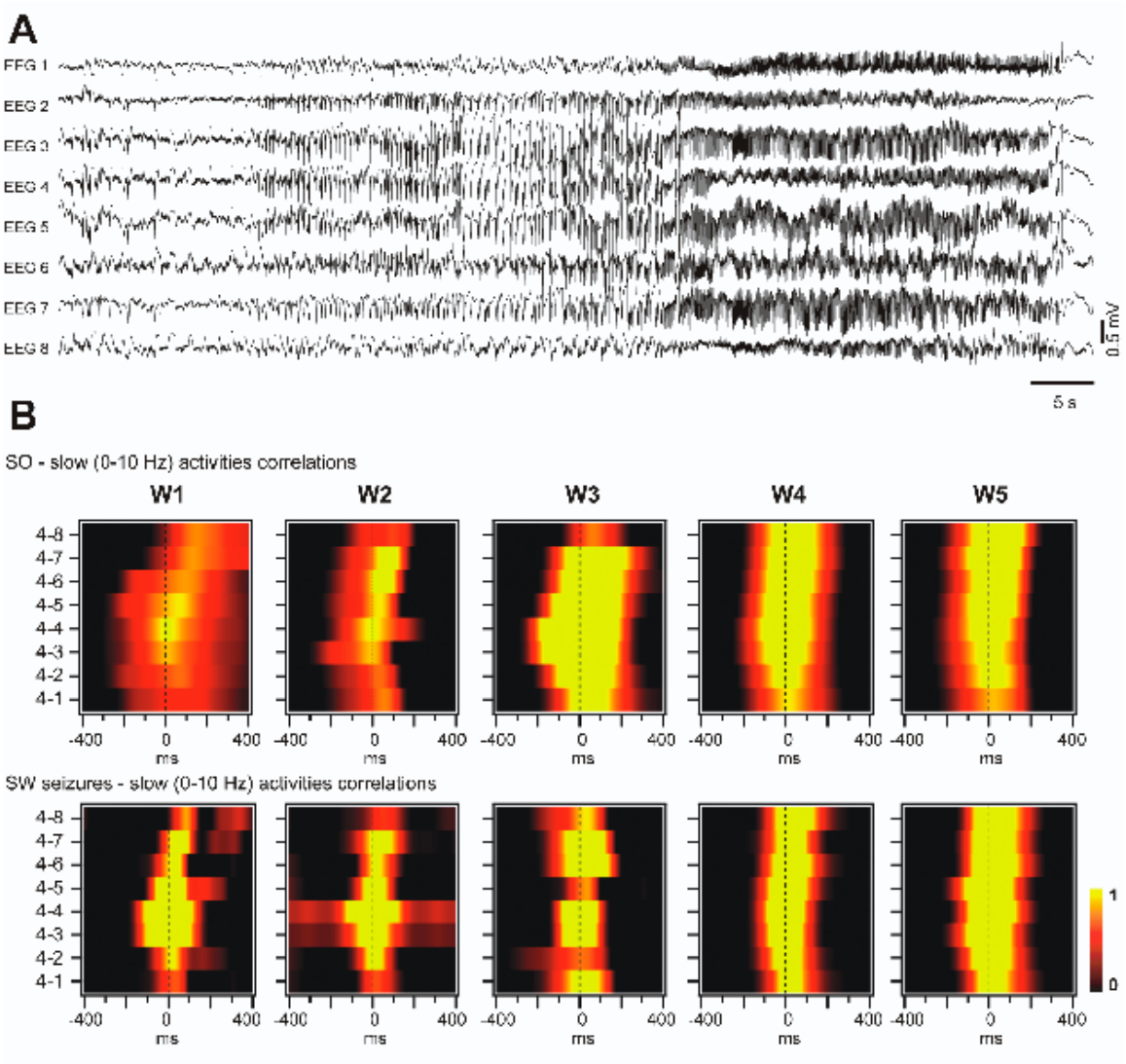

Figure 2-7 Time correlations across different cortical leads and from W1 to W5 during slow oscillation and seizures. Ketamine-xylazine anesthesia. *A*, development of seizure during W4 consisting of SW/PSW complexes and finishing with fast runs over electrodes EEG1 to EEG8. Note that seizure started in anterior leads (EEG1 to EEG4) and then propagated more posteriorly. *B*, spatio-temporal properties of the slow oscillation (SO) and SW/PSW seizures in the deafferented cortex. One cat is depicted for each week (W1 to W5). Cross-correlations were performed between the most anterior electrode where the seizures occur first (EEG4) and the rest of the electrodes placed over the suprasylvian gyrus. Signal was filtered in the 0-10 Hz range. For both the paroxysmal slow oscillation and the SW/PSW seizures the time-lags diminished from W1 to W5, and the propagation was faster during seizures compared with the paroxysmal slow oscillation (during W1 to W4).

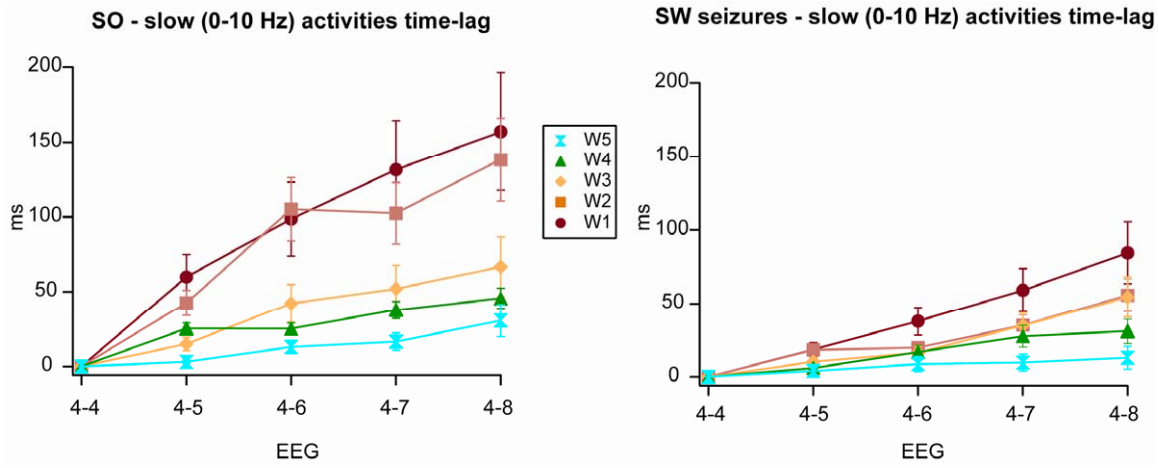

Figure 2-8 Quantification of time-lags during paroxysmal slow oscillation (SO) and SW/PSW seizures. Pooled data from 5 cats in each week (W1 to W5). The bottom axis represents the electrode from the anterior to posterior.

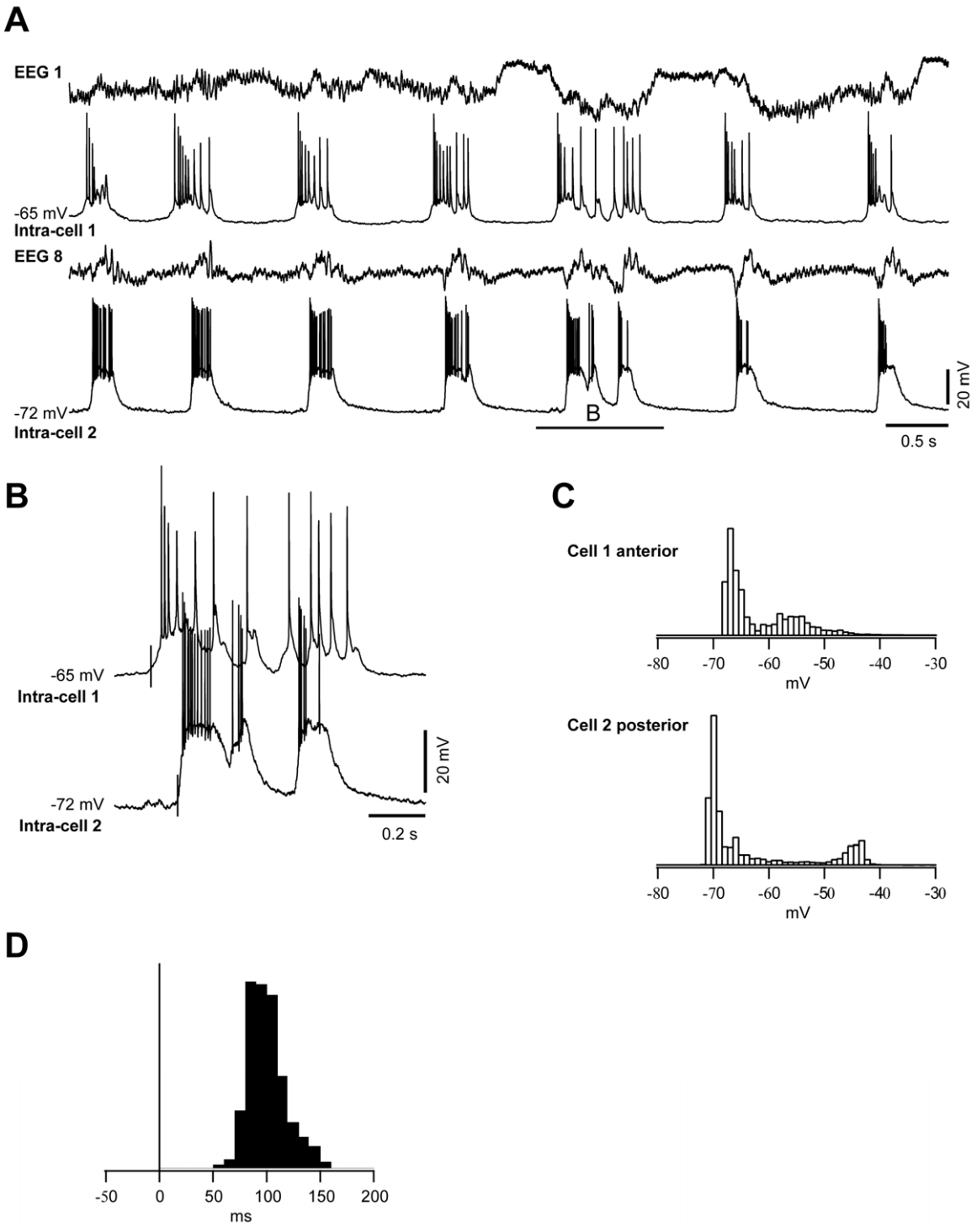

Figure 2-9 Dual intracellular recordings from the relatively intact (Intra-cell 1) and deafferented (Intra-cell 2) cortex, one week after undercut (*A*) in ketamine-xylazine anesthetized cat. Neuron 1 fired before neuron 2 (expanded in *B*). Histogram of time-lags at 10% of the amplitude of depolarization between the two neurons (with anterior one as reference) is presented in *D*. In *C*, histogram of the V_m of the two neurons.

3 Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation

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3.1 Résumé

Nous avons étudié la modulation des crises épileptiques de type pointe-onde produites suite à une déafférentation corticale par les états naturels de vigilance chez des chats avec des électrodes EEG chroniquement implantés. Des enregistrements ont été réalisées une jusqu'à douze semaines après la section de la substance blanche sous-jacente au gyrus suprasylvian. L'activité électrique du gyrus suprasylvian et du gyrus marginal a été enregistrée extracellulairement par des potentiels de champ et des neurones, ainsi que par des enregistrements intracellulaires. Des activités paroxystiques se sont développées dans les jours qui suivent la deafferentation corticale sous la forme des complexes de type pointe-onde à 3-4 Hz présentes continuellement pendant l'état de veille (et reliés aux mouvements oculaires), avec une incidence accrue pendant le sommeil à ondes lentes, virtuellement absents pendant le sommeil paradoxal. Au niveau intracellulaire, des hyperpolarisations prolongées ont été vues non seulement pendant le sommeil à ondes lentes (qui est le cas chez les animaux normaux) mais également pendant l'état de veille et le sommeil paradoxal, ayant ainsi comme résultat une distribution bimodale du potentiel de membrane dans chacun des trois états normaux de vigilance. La synchronie augmentée des potentiels de champ EEG exprimée par des délais de plus en plus courts de la propagation de l'activité paroxystique sur la surface corticale, et la tendance vers la généralisation des crises épileptiques, sont attribuables aux changements des propriétés neuronales intrinsèques et à la disinhibition potentielle qu'accompagne la déafférentation corticale.

3.2 Abstract

We investigated the dependency of electrical seizures produced by cortical undercut upon behavioral states of vigilance in chronically implanted cats. Experiments were performed one to 12 weeks after white matter transection. Multi-site field potentials and intracellular activity were recorded from suprasylvian and marginal gyri. Paroxysmal activity developed within days and consisted in spike-wave complexes at 3-4 Hz occurring during the waking state (correlated with eye movements), being enhanced during slow-wave sleep (SWS), and blocked during REM sleep. Prolonged hyperpolarizing events were seen not only during SWS (which is the case in normal animals) but also during both waking and REM, thus resulting in bimodal distribution of the membrane potential in all three natural states of vigilance. The increased synchrony of field potential activity expressed by shorter time of propagation over the cortical surface, and the tendency toward generalization, are ascribed to changes in intrinsic neuronal properties and potential disinhibition following cortical undercut.

3.3 Introduction

Acute seizures following cortical trauma produced by penetrating wounds attain up to 80% of patients in the first 24 hours (Dinner, 1993), and chronic posttraumatic epilepsy was reported in over 50% of cases (Salazar et al., 1985). Trauma elicits partial neocortical deafferentation and, consequently, decreases input signals, which can result in enhanced intrinsic and synaptic excitability of individual neurons (Turrigiano et al., 1998; Desai et al., 1999a). Chronic neuronal hyperexcitability and epileptogenesis have been experimentally demonstrated in isolated cortical islands in vivo (Burns, 1951; Sharpless and Halpern, 1962); and in neocortical slices maintained in vitro after cortical injury (Prince and Tseng, 1993; Hoffman et al., 1994; Prince et al., 1997; Li and Prince, 2002). After isolation, the neocortex becomes progressively more excitable and may develop prolonged ictal events within several weeks. Computational models of posttraumatic epileptogenesis suggested that paroxysmal discharges are due to changes in intrinsic properties of pyramidal cells and enhanced NMDA synaptic conductances (Bush et al., 1999; Houweling et al., 2005).

Acute experiments performed under anesthesia, in vivo, showed that partially deafferented neocortex displays changes in long-distance synchrony and paroxysmal activity that occurs 2-3 h after cortical undercut and arises from enhanced intrinsic and synaptic neuronal responsiveness, increased incidence of spontaneously bursting neurons, and slight reduction in inhibitory processes (Topolnik et al., 2003a; Topolnik et al., 2003b). Recordings under anesthesia in a model of chronic partial cortical deafferentation showed that, following the undercut, the neocortex displays progressively increased signs of paroxysmal activity, expressed by progressively enhanced amplitudes and synchrony of spike-wave/polyspike-wave (SW/PSW) complexes (Nita et al., 2005).

The aim of the present study was to investigate the relations between paroxysmal activity in the deafferented cortex and natural states of vigilance in chronically implanted cats, with the hypothesis that the incidence and widespread feature of such electrical seizures would be mainly expressed during slow-wave sleep (SWS). It was indeed shown that cortical seizures with SW complexes prevalently occur during early SWS stages in behaving monkeys (Steriade, 1974) and that the slow sleep oscillation (0.5-1 Hz) may develop,

without discontinuity, into electrographic seizures in cats (Steriade et al., 1998), probably due to highly synchronous activities in corticothalamic systems. Clinically too, SWS is considered as a potent activator of epileptiform discharges (Gigli et al., 1992; Dinner, 2002; Niedermeyer and Lopes da Silva, 2004) whereas REM sleep, with its asynchronous cellular discharge pattern, is resistant to the propagation of epileptic EEG potentials (Shouse et al., 2000). We then used field potentials and intracellular recordings in chronic experiments in vivo to study the evolution of electrical paroxysms induced by cortical deafferentation, up to 3 months following the undercut, to determine the spatiotemporal development of post-traumatic seizures with respect to the initial cortical insult, to quantify at different stages the transformation from the normal slow oscillation to paroxysmal patterns, and to determine how epileptic activities are modulated by the three major states of vigilance, waking, SWS and REM sleep.

3.4 Materials and methods

3.4.1 Animal preparation

Experiments were performed on 6 chronic cats of both sexes. Surgical procedures were carried out in sterile condition, following a pre-medication with acepromazine (0.3 mg/kg i.m.), butorphanol (0.3 mg/kg i.m.), atropine (0.05 mg/kg i.m.) and ketamine (20 mg/kg i.m.), under barbiturate anesthesia (30 mg/kgc i.v.). The level of anesthesia was continuously monitored by the aspect of the electroencephalogram (EEG) and cardiac frequency (aiming 90-110 beats/min). Oxygen saturation of the arterial blood and end-tidal CO2 were also monitored. General surgical procedures included: cephalic vein canulation for systemic liquid delivery (lactated Ringer's solution 5-10 ml/kg/h) and lidocaine (0.5%) infiltration of all pressure points or incision lines. Body temperature was maintained between 37–39°C with a heating pad.

Craniotomy was used to expose the cerebral cortex and a large undercut of the white matter below the suprasylvian gyrus (13-15 mm postero-anteriorly and 3–4 mm medio-laterally) was used to produce partial cortical deafferentation by transecting the thalamo-cortical projections. A standard blade (3 mm width and 14 mm long) was inserted in the posterior part of suprasylvian gyrus perpendicular to its surface for a depth of 3–4 mm, then rotated 90° and advanced rostrally along the gyrus parallel to its surface for a total distance 14 mm,

then moved back, rotated 90° and removed from the same place where it was entered (see Fig. 3-1, A-C). The use of a custom-designed knife ensured a similar extension of the lesion. Thus, the anterior part of the undercut cortex was relatively intact and the white matter below the posterior part of the gyrus was transected, creating conditions of partial cortical deafferentation.

Coaxial bipolar macroelectrodes (with the tip in the cortical depth at about 0.8-1 mm and the ring placed at the cortical surface) were placed in various cortical areas in different configurations. In 3 cats a recording chamber allowing intracellular penetrations of micropipettes was placed over the intact dura, above the anterior part of the suprasylvian gyrus. Additional pairs of electrodes were placed around the orbit and neck muscles to monitor the states of vigilance by recording the electro-oculogram (EOG) and electromyogram (EMG). The calvarium was reconstituted using acrylic dental cement and a few bolts were placed in the cement to allow non-painful fixation of the cat's head in a stereotaxic frame. Animals were kept under observation up to the full recovery and they received analgesic medication (anafen 2 mg/kg s.c.) for the next 48-72 hours.

After a recovery period (2–3 days), cats were trained to stay in the frame for 1–2 h/day. After a few days of training, cats started to sleep in the frame and they displayed clearly identifiable states of waking, SWS, and REM sleep. Criteria used to distinguish between different waking states were: the level of the tonic EMG activities (high during wake, decreased during SWS and very low during REM sleep) and the occurrence of eye movements during wake and REM.

3.4.2 Electrophysiological recordings

EEG recordings started 3–5 days after surgery, were performed 2-5 times per week and lasted up to 3 months. Intracellular recordings of cortical neurons were obtained with glass micropipettes (tip diameter <0.5 μm) filled with potassium acetate (3 M, in situ impedance 35-50 M∧) after small perforations in the dura were carefully made. The chamber was filled with warm sterile solution of 4% agar in order to enhance the stability of the recordings. Only stable recordings with resting membrane potentials more negative than -60 mV, overshooting action potentials, and input resistances >20 MΩ were kept for

analysis. The intracellular signals were passed through a high-impedance amplifier with an active bridge circuitry (bandwidth DC to 9 kHz). All signals were digitized (20 kHz sampling rate) and stored for off-line analysis.

As a rule for intracellular recordings, two to three recording sessions, each lasting for 2-3 h, were performed daily, and usually contained periods of waking, SWS and REM. The cats were not deprived of sleep between recording sessions, and during recordings they could move their limbs and make postural adjustments. During recording session animals were monitored using a night-shoot video surveillance camera.

Behavioral seizures were considered an "end limit point" of these experiments, as consented with the local ethic committee. Three cats displayed small localized muscular jerks and abnormal tail wagging during waking state and 1 cat displayed generalized seizures. At the end of experiments or at the first sign on clinically manifest seizures, the cats were given a lethal dose of intravenous sodium pentobarbital (50 mg/kg). After experiments, the brains were removed and the extension of the undercut was verified on Nissl stained (thionine) 80-mm brain sections (Fig. 3-1, A-C). All experimental procedures were performed in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals, with the proceedings and politics of the local committee for animal protection, and all experimental procedures were approved by the committee for animal care of Laval University.

3.4.3 Data analysis

Wave-triggered averages (WTAs) were calculated taking as reference the segment with the steepest deflection in the EOG during eyes movements and averaging equal windows around that point from both EEG and EOG channels (see Fig. 3-2B). EEG power in the 3-5 Hz range was quantified by the area under the FFT graph of field EEGs (see Fig. 3-5C). Means of comparative data were statistically evaluated with paired Student's t-test. Differences between means were considered significant at $p<0.05$.

Autocorrelograms were generated on successive windows of 1 sec length (Fig. 3-6B) and the area under the autocorrelation graph on a 100 ms time-window (shifted from the zero line with 250 ms corresponding to the dominant 4 Hz frequency) was measured and

displayed as in Fig. 3-6A. Cross-correlograms of different EEG channels (Fig. 3-3B & 3-7B) were computed on 30 sec of stable recording and the time-shifts measured and averaged between different individuals. In Fig. 3-3B electrode 5 was taken as reference for all cross-correlograms between pairs of EEG electrodes placed over intact cortical areas, while for the undercut the reference was the electrode 12 since previous studies in chronic undercut under anesthesia shown a propagation of seizures from the more intact to the more deafferented cortex (Nita et al., 2005).

Histograms of the time duration of ictal events and inter-ictal periods (Fig. 3-4A) were obtained from epochs of 3 min of stable and clearly identifiable waking EEG recordings and displayed with a 1 sec bin width. Histograms of membrane potential distribution (Fig. 3-8C) were created for successive periods of 10 sec by counting the number of samples with bins of 1 mV. The peaks of distribution that corresponded to the most probable mode of membrane potential were taken as the level of membrane potential. The peri-spike histograms (Fig. 3-8E) were obtained over 5 min epochs, taking as time reference each action potential in the intracellular recording and counting the level of the membrane voltage with a bin width of 5 ms.

3.5 Results

3.5.1 Development of seizures and spatiotemporal properties of paroxysmal activities

Six hours following acute partial cortical deafferentation, recordings performed under anesthesia showed paroxysmal-like field potentials in the anterior part of the suprasylvian gyrus, consisting in high-amplitude slow waves with the morphological features of interictal spikes and with ripples superimposed on their depth-negative phase (Fig. 3-1D). Their amplitude was at least twice as high as the normal slow oscillation recorded in the contralateral hemisphere, and the dominant frequency was around 1 Hz as revealed by the FFT power spectra (Fig. 3-1E, left panel).

Several days later, the EEG recorded during natural SWS from the anterior part of the undercut cortex still displayed a slow oscillation with a larger amplitude, compared to the contralateral cortical areas (Fig. 3-1D), but two clearly distinct frequency peaks appeared in

the power spectra (Fig. 3-1E, right panel). The first peak was centered around 1 Hz, similarly to the activity observed during the acute period, while the second peak represented a faster rhythm in the 3-4 Hz domain (marked with * in Fig. 3-1D).

These ictal events were observed in all recorded animals and consisted in patches of ample 3-4 Hz SW complexes occurring intermittently during the waking state (Fig. 3-2A) when it was related with eye movements (Fig. 3-2B), and continuously during SWS. Deflection on EOG recordings appeared at the beginning and/or the end of the ictal events (see lead 14 in Fig. 3-2A; and Fig. 3-2B), in a similar manner with the eye movements clinically observed in absence epileptic seizures in humans (Penfield and Jasper, 1954; Bickford and Klass, 1964).

Polygraphic recordings by means of EEG electrodes placed over multiple areas in the deafferented cortex as well as in the contralateral cortex were performed from day 5 up to day 120 to study the distribution of ictal events with respect to the original site of trauma. This evolution is depicted during the waking state in Fig. 3-3A. Initially, paroxysmal activities were exclusively present over areas around the undercut cortex, especially in the marginal gyrus of the ipsilateral hemisphere (leads 5, 7 and 9 during days 5 and 10 in Fig. 3-3A) but also in some homotopic marginal foci of the contralateral hemisphere (lead 10 on day 5, Fig. 3-3A). Besides the marginal gyrus, paroxysmal activity was also detected around the undercut, within the most posterior part of the suprasylvian gyrus (see lead 4 in Fig. 3-5). During later stages, all cats exhibited a tendency toward generalization over the whole cortical surface, and the deafferented suprasylvian gyrus displayed only after 30-40 days this type of generalized paroxysmal activity (day 45 in Fig. 3-3A). Average time delays and peak level of cross-correlograms computed between different cortical EEG electrodes from day 5 to day 90 (Fig. 3-3B) indicated that in the undercut the propagation of 4 Hz activities regularly have an antero-posterior pattern, while in the intact cortex seizures start around the deafferented cortex (electrodes 7 and 9) with a more heterogeneous dynamics. A tendency toward faster propagation (smaller time-lags between electrodes) and increased correlation, both in the intact and the undercut cortex, was observed with time from day 5 to day 90.

The quantification of the number and time-duration of ictal events during the waking state was computed based on 3 min recordings from day 4 up to day 120 (Fig. 3-4A). While the duration of individual ictal events remained quasi-constant (around 5 sec), their number showed a plateau in the first 2 weeks; thereafter, it significantly decreased up to 30% of the original value and then increased again up to 75% of the initial number, remaining constant afterwards for long periods of time (Fig. 3-4B). This behavior was clearly observed in 4 over 6 experimental animals which displayed easily identifiable ictal episodes.

3.5.2 Sleep modulation of seizures

Polygraphic recordings during natural waking and sleep states showed that the 4 Hz ictal events occurred during waking, became quasi-continuous during SWS, whereas they were completely absent during periods of REM sleep (Fig. 3-5A). This behavior was also reflected in the peak of the field EEG power spectra which was increased three times during SWS, compared to the waking state, and disappeared during REM sleep (Fig. 3-5B). However, low-amplitude slow-waves were occasionally present during REM sleep, which is not the case in the intact cortex. Quantifications were performed by measuring the area corresponding to the 3-5 Hz window in the power spectra from 10 successive recordings between day 5 and 15 in each of the 6 chronically implanted animals (Fig. 3-5C). Statistically significant differences (t-Student test, $p<0.05$) were observed in the amount of 4 Hz activities between waking and REM state as well as between SWS and REM, while the 30% increase of power during SWS compared with waking did not reach statistical significance level.

Auto-correlograms of EEG activity (Fig. 3-6) indicated a dominant frequency oscillation ~4 Hz (220-250 ms width of the main peak in the auto-correlogram) during both SWS and wake, more reliable in time during SWS compared to wake. This was replaced during REM sleep by an uneven correlogram containing both fast activities and some slow-waves (Fig. 3-6B). Measuring the area under each single auto-correlogram corresponding to sequential 1-sec epochs of recording, we determined that paroxysmal activities were increased during SWS compared to wake, and strikingly diminished during REM sleep (Fig. 3-6A).

Seizures started successively in different EEG leads during the waking state (with a delay decreasing with time, from day 1 to day 120), while during SWS they occurred with much shorter delays (Fig. 3-7A; electrodes 5-6: 45.21±15.24 in waking vs. 35.02±3.89 ms in SWS; electrodes 5-7: 872.48±315.14 vs. 189.4517±72.45 ms; electrodes 5-8: 912.35±478.12 vs. 213.35±65.14 ms). They originated at the border between the intact and the deafferented suprasylvian gyrus, generally displayed highest amplitudes in the marginal gyrus, and propagated ipsilaterally as well as to the contralateral marginal gyrus. Occipital (posterior marginal) areas were involved afterwards, first on the lesion side, then contralateraly. Correlations between activities recorded by different EEG electrodes over marginal gyri (Fig. 3-7B) were used to compute the time-lag of SW propagation during wake and SWS and to quantify the time delays in all recorded experimental animals (Fig. 3-7C). In all cases a quasi-simultaneous occurrence of SW complexes occurred during SWS (electrodes 5-6: 1.25±0.85 ms; electrodes 5-7: 1.04±0.97 ms; electrodes 6-8: 1.04±0.89 ms; electrodes 7-8: 1.2609±0.93 ms), while paroxysmal activities required a longer time during waking (electrodes 5-6: 5.02±3.89 ms; electrodes 5-7: 13.21±4.01 ms; electrodes 6-8: 9.4512±4.12 ms; electrodes 7-8: 1.2672±3.97 ms).

3.5.3 Intracellular correlates of seizures

Intracellular recordings were performed in the anterior part of the suprasylvian gyrus from day 14 to day 21 after cortical deafferentation. Of 23 recorded neurons, 7 underwent a transition from one state of vigilance to another. Four neurons were recorded during all three states of vigilance (Fig. 3-8A), and all of them were identified as regular-spiking neurons following depolarizing current pulses applied to the soma. At variance with our previous intracellular recordings in naturally awake and sleeping, non-epileptic cats, in which rhythmic hyperpolarizations within the frequency of the slow oscillation exclusively occurred during SWS (Steriade et al., 2001), the present data showed hyperpolarizing events during all states of vigilance. They were manifest during both SWS and waking (overall incidence: 1.64±0.7/sec and 1.26±0.6/sec, respectively), but short-duration hyperpolarizations also occurred during REM sleep (Fig. 3-8B). The incidence of these events pointed out a bimodal distribution of the values of the membrane potential in all three states of vigilance (Fig. 3-8C).

Based on their time duration calculated at the half of their amplitude, these hyperpolarizing events were grouped in two clusters: short-length (<150 ms) and long-length (>150 ms). While the first ones displayed a quite constant incidence during all states of vigilance: SWS: 0.85±0.17/sec; REM: 0.51±0.15/sec; waking 0.83±0.15/sec, the latter resembled the periods of long-lasting disfacilitation that normally takes place during the cortical slow sleep oscillation and were present during waking (0.43±0.13/sec), enhanced during SWS (0.78±0.14/sec), and absent during REM sleep (0.02±0.01/sec) (Fig. 3-8D).

Furthermore, all recorded neurons displayed an enhanced excitability consisting in a sharp transition between the "up" and the "down" states during SWS and waking. This was related to the occurrence of spike-bursts. Indeed, peri-spike histograms of intracellular activities indicated a preference toward bursting behavior of cortical neurons during SWS and waking, whereas neurons discharged tonically during REM sleep (Fig. 3-8E).

3.6 Discussion

The main findings reported in the present study are as follows: (i) following partial deafferentation of suprasylvian gyrus, recurrent seizures with 4-Hz SW complexes, first localized in territories contiguous to trauma, thereafter generalized over the whole cortical surface, were observed in all experimental animals; (ii) the incidence of ictal events was modulated by the state of vigilance, occurring during the waking state, being enhanced during SWS, and absent during REM sleep; (iii) the propagation of seizures increased during SWS, compared to waking; and (iv) all cortical neurons recorded in this study expressed a bimodal distribution of Vm values, due to the presence of hyperpolarizations during all states of vigilance, more obvious during SWS and waking.

Several factors may account for the increased propensity of seizures following the cortical insult produced by undercut. While both an acute raise of $[K^+]o$ (Moody et al., 1974) triggering a K^+-mediated increase in the hyperpolarization-activated depolarizing current (I_H) leading to cortical paroxysmal activities (Timofeev et al., 2002) and an increase in the concentration of the extracellular glutamate leading to seizures (Sakowitz et al., 2002) could not be envisaged in chronic conditions, trauma-induced chronic hyperexcitability and

focal epileptogenesis could be explained by the homeostatic plasticity mechanisms up-regulating the neuronal excitability (Houweling et al., 2005).

The progressive development of seizures in the partially deafferented cortex is in line with earlier studies showing that the chronically isolated neocortex develops hyperexcitability and focal epileptogenesis (Echlin and Battista, 1963). Although spontaneous bursts appear a few days after isolation, they occur more frequently during subsequent days and weeks (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962; Burns and Webb, 1979). Chronic activity blockade enhances Na^+ currents and reduces K^+ currents, resulting in enhanced responsiveness of pyramidal neurons to current injection (Desai et al., 1999b). The isolated cortex develops an increased susceptibility to epileptiform activity that is similar to the disuse supersensitivity in some structures deprived of afferent inputs (Sharpless, 1969). Some additional mechanisms for an increased synaptic drive following axotomy could also be envisaged, such as axonal regeneration and sprouting of axonal arborizations of pyramidal cells onto neighboring neurons (Chen et al., 2002).

The initial and prevalent occurrence of paroxysmal activity in cortical territories located outside the most deafferented (suprasylvian) cortex, namely the marginal gyrus and parts of the suprasylvian gyrus posterior to the cortical undercut (see Figs 3-2 & 3-3), may be explained by the fact that only relatively intact neurons, which are found in the vicinity of the isolated cortex, are expected to promote paroxysmal activity. This may partially arise from a transformation of regular-spiking into intrinsically-bursting neurons due to K^+ accumulation (Jensen and Yaari, 1997). Synaptic inputs to neurons with an enhanced intrinsic excitability, such as bursting neurons, may promote the initial paroxysmal discharges in sites outside the disconnected cortex.

The activating properties of SWS on epileptiform discharges should be ascribed to highly synchronized oscillations in reciprocal thalamocorticothalamic systems, which characterize this sleep stage (Steriade and Contreras, 1995). Neocortical, thalamocortical and thalamic reticular neurons are interconnected in networks that generate the three main EEG sleep rhythms: spindles, delta, and slow oscillation. The priming role of the neocortex in generating the slow oscillation which groups the other SWS rhythms (reviewed in Steriade, 2003), as well as some types of SW seizures (which may develop without

discontinuity from the slow oscillation; see Introduction) is demonstrated by several data. The slow oscillation is generated in cortex even after extensive thalamic lesions (Steriade et al., 1993) and was also recorded in cortical slices (Sanchez-Vives and McCormick, 2000) and isolated cortical slabs (Timofeev et al., 2000). On the other hand, SW seizures occur in neocortex of thalamectomized animals (Steriade and Contreras, 1998) and thalamocortical neurons are steadily hyperpolarized during cortically elicited SW seizures, via excitation of thalamic reticular neurons that faithfully follow paroxysmal depolarizing shifts of cortical neurons (Steriade and Contreras, 1995; reviewed in Crunelli and Leresche, 2002).

Although SWS is the behavioral state during which SWS seizures occurred most often in the present study, which is in line with clinical studies (see Introduction), these seizures were also observed during wakefulness (Figs 3-2, 3-3 & 3-5), but the time-lags of SW propagation was much longer in waking than in SWS. The seizures during waking may be related to the presence of hyperpolarizations after cortical deafferentation (Fig. 3-8), which stands in contrast with absence of such events during waking of non-epileptic cats (Steriade et al., 2001). The waking-related hyperpolarizations detected in the present experiments on animals with cortical undercut could facilitate the expression of I_H and the Ca^{2+}-activated rebound depolarization is known to promote paroxysmal activity with SW patterns (Timofeev et al., 2002).

One of the characteristics of the 3-4 Hz SW complexes occurring during waking state was their relationship with the eye movements. Indeed, the EOG displayed deflections at the beginning and/or at the end of the ictal events matching the important manifestations of eye movements in the clinical expression of absence epileptic seizures in humans; which range from minimal changes of fixation, or prolonged fixation (staring, centering) to a large variety of lateral or oblique concomitant nystagmoid jerks and, in some instances, tonic conjugate deviation (Bickford and Klass, 1964). Beside clinical observations, there are only few studies dealing with ocular motility during absence seizures. The eye movements could reflect a propagation of the ictal discharge to the brainstem (Bogacz et al., 2000), since a previous study demonstrated a marked dependence of saccadic parameters on global EEG oscillations (Skrandies and Anagnostou, 1999).

In summary, our results support the conclusion that alterations in the intracortical synaptic network result in a significant shift of the balance between excitatory and inhibitory inputs on pyramidal neurons toward increased excitation. Both epileptogenesis and the propagation of seizures are modulated by the state of the cortical network. Highly synchronous activity in corticothalamic systems promoted epileptogenesis and seizures propagation during SWS sleep and waking following cortical deafferentation, while paroxysmal activities were obliterated during REM sleep. Epileptogenesis during waking and SWS was also supported by the presence of hyperpolarizing events at the level of the cortical neurons, favouring the occurrence of bursts in the activity pattern of the cortical neurons.

3.7 Acknowledgements

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3.9 Figures

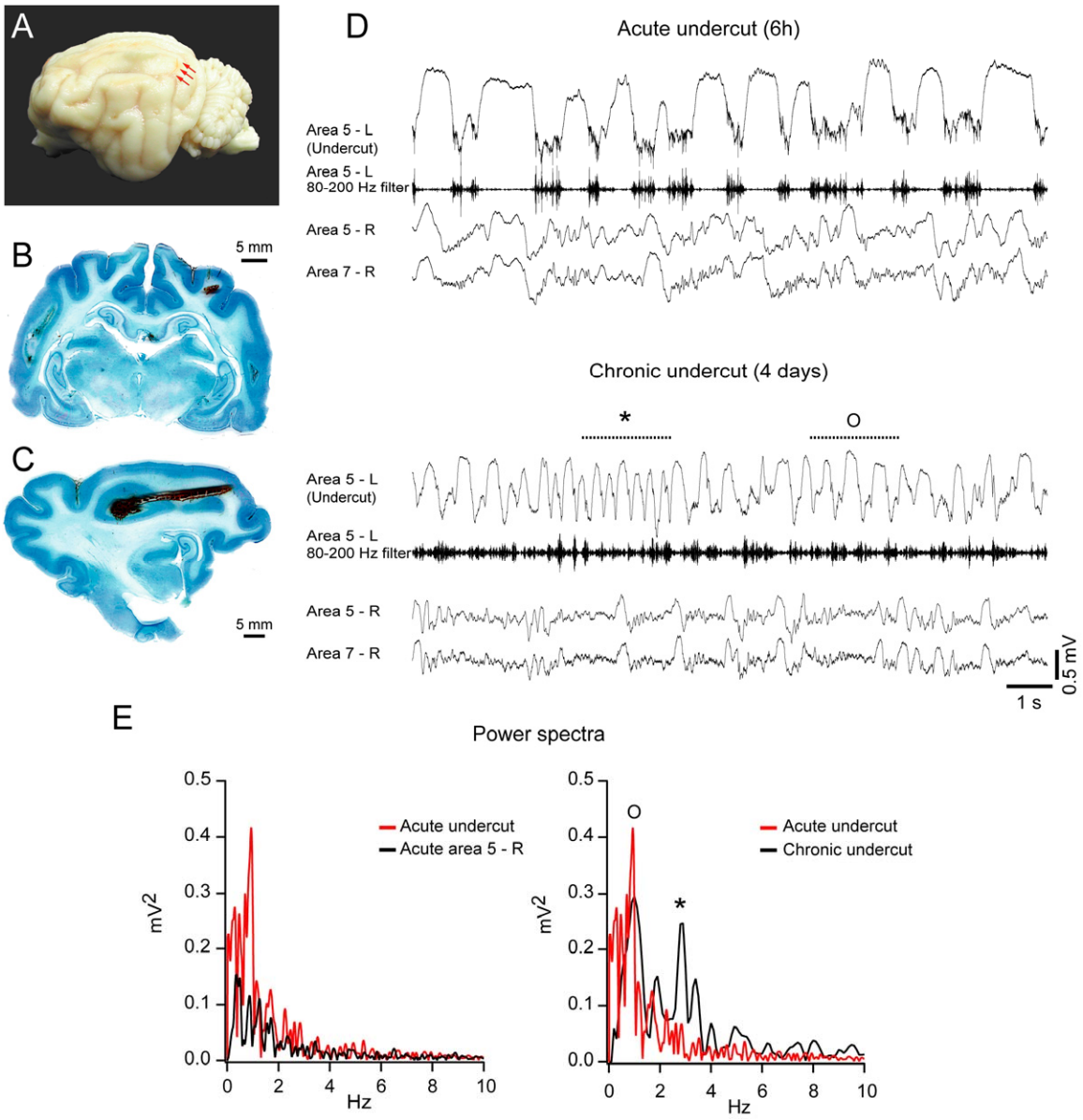

Figure 3-1 Experimental paradigm. (A) Lateral view of the left cerebral hemisphere. Experiments were performed on chronically implanted cats with complete transections of white matter below the suprasylvian gyrus. Red arrows indicate the place where the knife used to perform the undercut entered the cortex. (B) Frontal and (C) sagittal brain sections in Nissl staining used to ascertain the extent of cortical deafferentation. (D) EEG recordings in the deafferented (undercut) and intact cortex (areas 5 and 7 on the right side) 6 hours after surgical proceeding (under anesthesia) and 4 days later (during SWS). Activity in the acutely partially deafferented cortex (area 5) is dominated by highly increased amplitude of the cortical slow oscillation (~ 1Hz), compared to normal slow oscillation in the opposite hemisphere (see also the left panel in E). (E) Fast Fourier Transformation (FFT) power spectra. Four days later, during slow-wave sleep, EEG in the undercut consists in a mixture of ~1Hz activity (O) and a faster activity of about 3-4Hz (*).

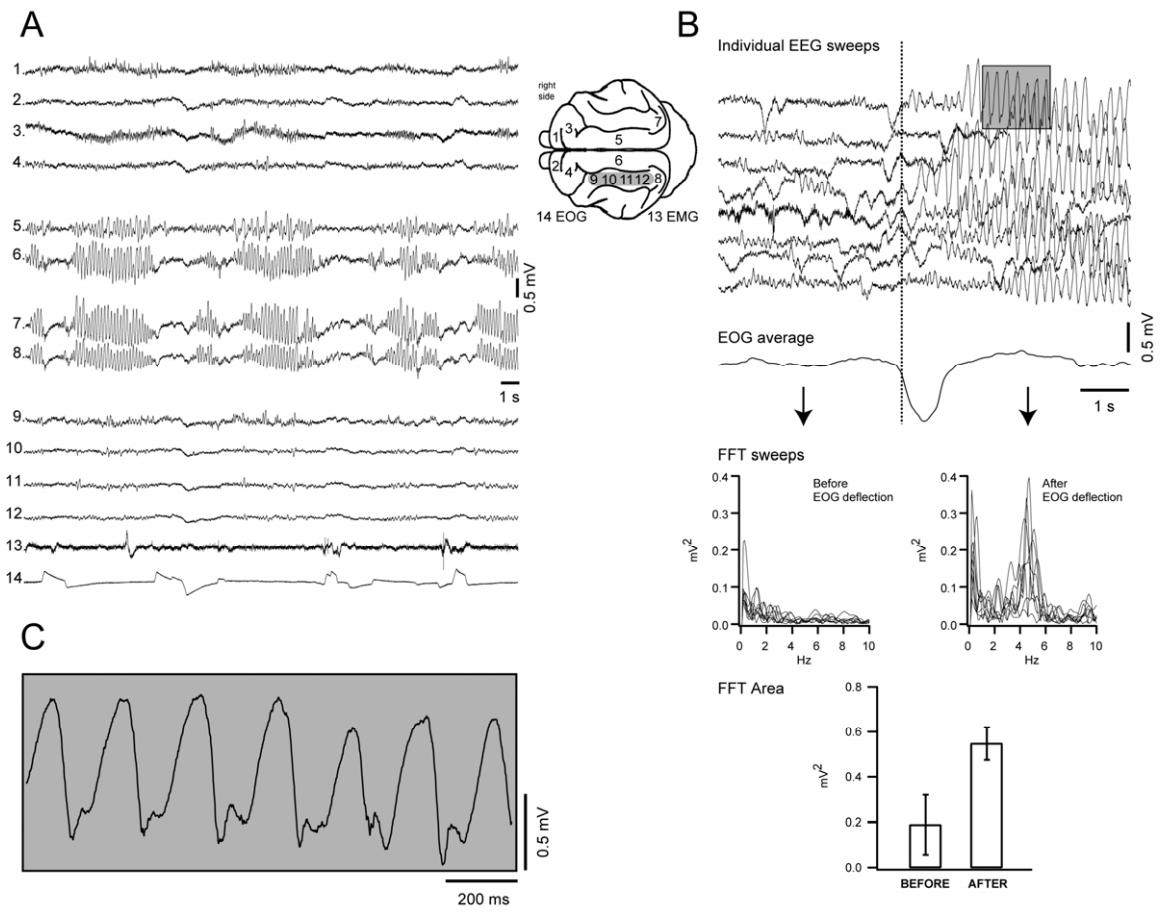

Figure 3-2 Relation of ictal events with eye movements. (A) Polygraphic recordings in a chronically implanted cat with a cortical undercut on the left size (depicted as a grey box on the schema) during wake. Ictal events with frequencies in the 3-4 Hz time domain are associated with ocular movements at the beginning and at the end of the seizure. (B) Multiple EEG individual sweeps obtained taking as reference the steepest drop in the EOG deflection. Spectral decompositions of single EEG trials (on 4 seconds windows before and after the EOG deflections) point out the occurrence of seizures after the eye movements both by the expression of a 4 Hz peak in the FFT and by the increase in the FFT area over the 2-6 Hz domain. (C) Ictal events expanded from the underlined grey box in (B) show typical spike-wave complexes.

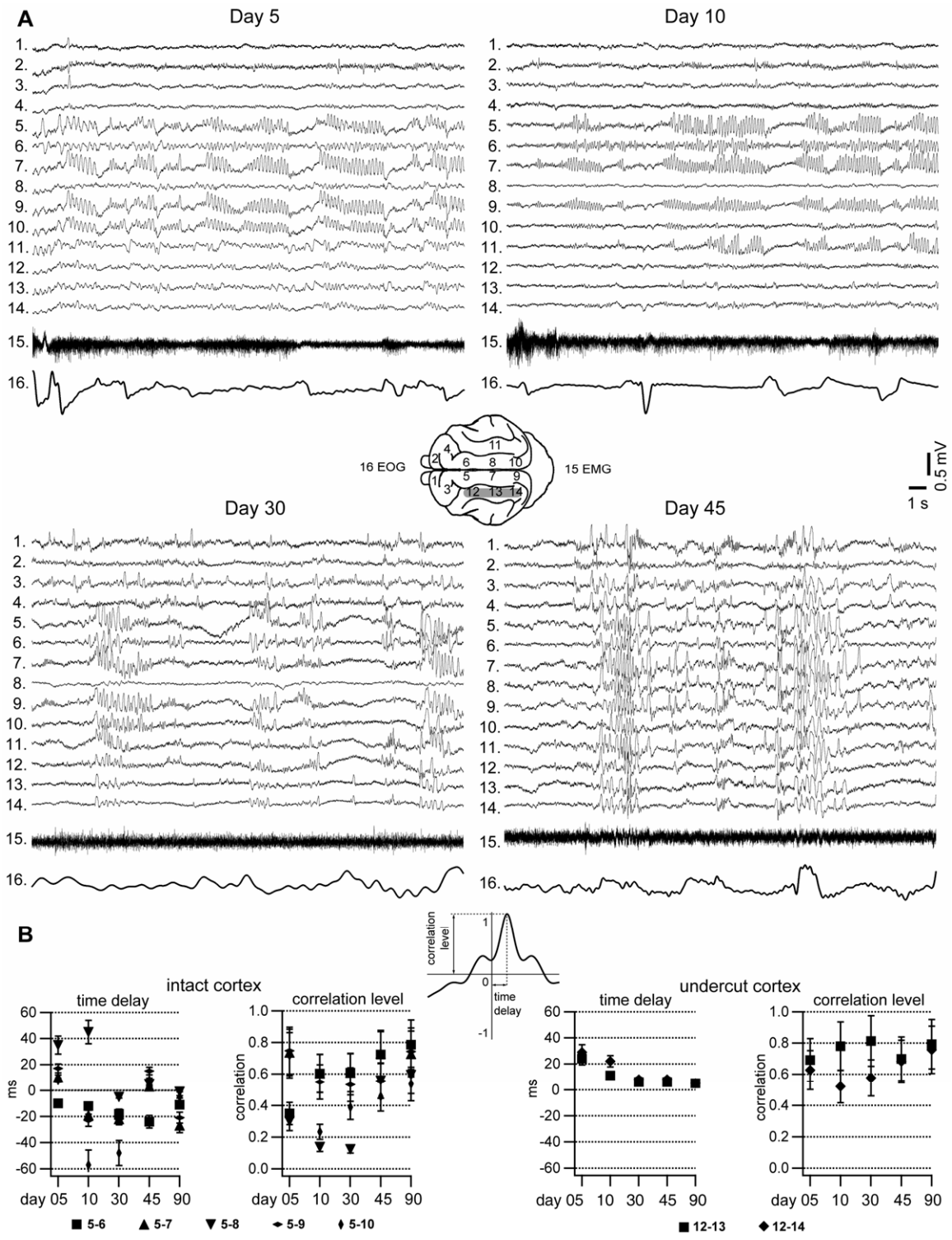

Figure 3-3 Topographic distributions of ictal events over the cortical surface from day 5 to day 45 during wake. (A) Polygraphic recordings in chronic cat with deafferented left suprasylvian gyrus (depicted as a grey box) indicate that paroxysmal activities are present initially in the cortical areas adjacent to the deafferented cortex, and they display a tendency toward generalization in time over the whole cortical surface. (B) Average time delays and peak level of cross-correlograms performed between different cortical EEG electrodes from day 5 to day 90 in 3 different experimental animals. In

the intact cortex electrode 5 was taken as reference while in the undercut the reference was the electrode 12. In the undercut the propagation of 4 Hz activities regularly displayed an antero-posterior pattern, while in the intact cortex seizures started around the deafferented cortex (electrodes 7 and 9). Data indicate a tendency toward faster propagation and increased correlation with time both in the intact and the undercut cortex.

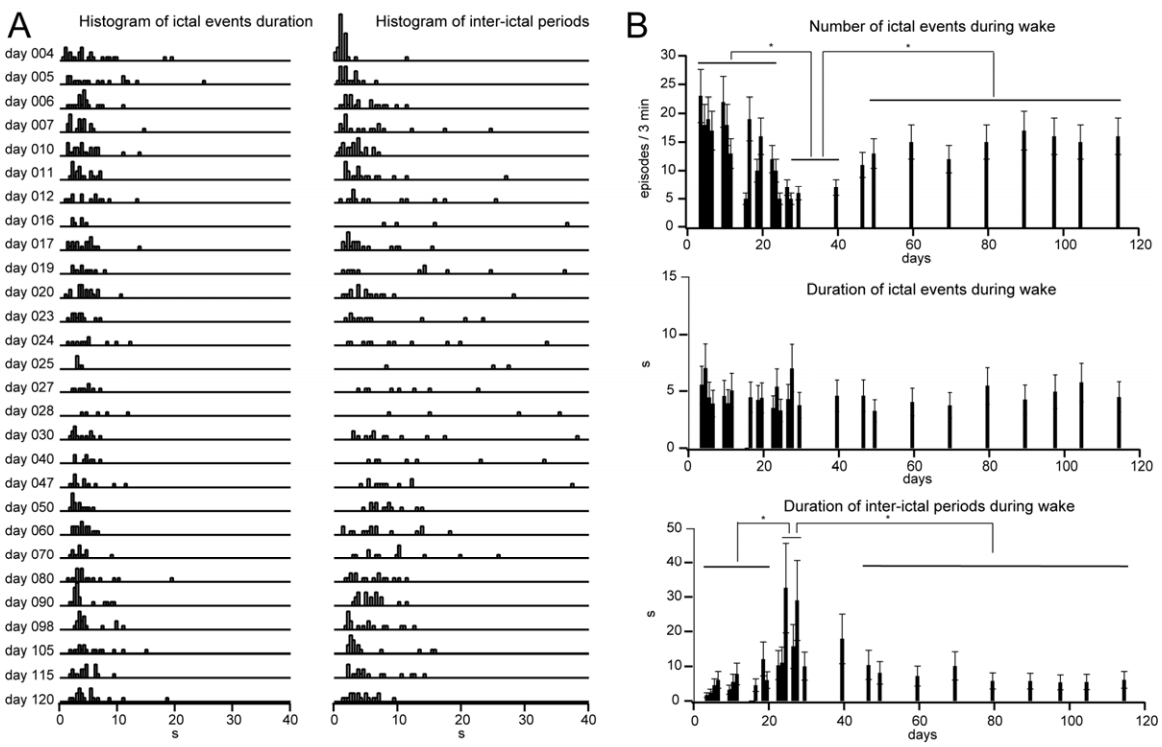

Figure 3-4 Incidence and duration of ictal events. (A) Histograms of ictal event duration and of the length of inter-ictal periods in a chronically implanted cat during waking state, in the left marginal gyrus. (B) Variation in time of the number of ictal events, of the duration of ictal events and of the length of the inter-ictal during wake from recording day 3 to day 120. While the duration of ictal event remained unchanged in time, the number is significantly diminished from day 25 to day 40, and it increase later up to a stable rate. Statistical differences (t-Student test, $p<0.05$) are indicated with stars. This behavior was observed in 4/6 experimental animals.

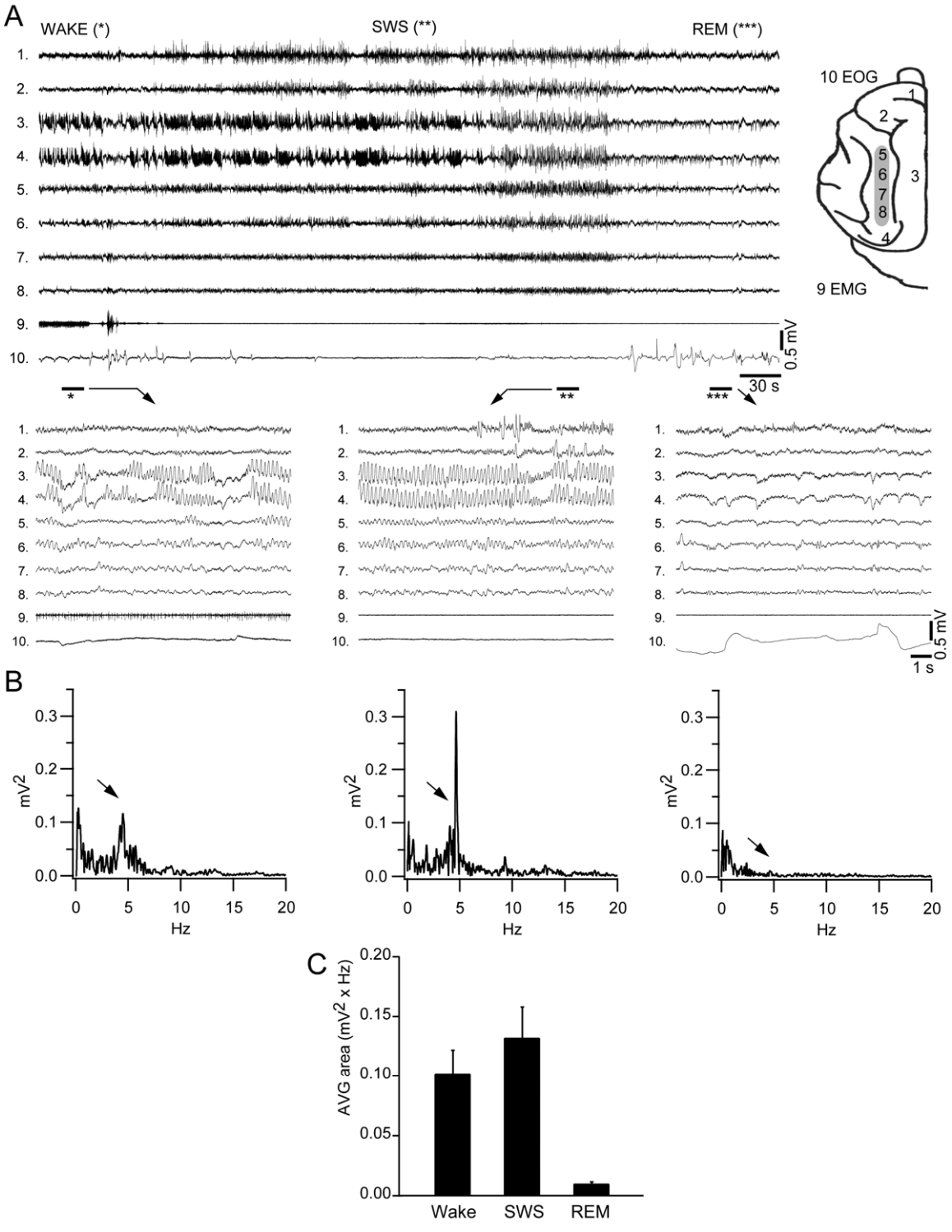

Figure 3-5 Sleep modulation of paroxysmal activities. (A) Top panel: Polygraphic recording in a chronically implanted, naturally awake and sleeping cat, 10 days after the undercut. The placement of recording electrodes is depicted on right scheme and the undercut is represented as a grey box. Bottom panels: occurrence of paroxysmal (3-4 Hz) activities was consistently found during wake, quasi-continuous during slow-wave sleep (SWS) and completely abolished during periods of REM sleep. (B) FFT power spectra of the field EEG from the left anterior marginal gyrus (electrode 3) during wake,

SWS and REM sleep. The peak of 4 Hz indicated by arrows is increased during SWS compared to wake and disappears during REM sleep. (C) Pool data from 6 chronic cats of the FFT area in the 3-5 Hz domain, corresponding to electrode 3, from recordings performed between day 5 and day 15.

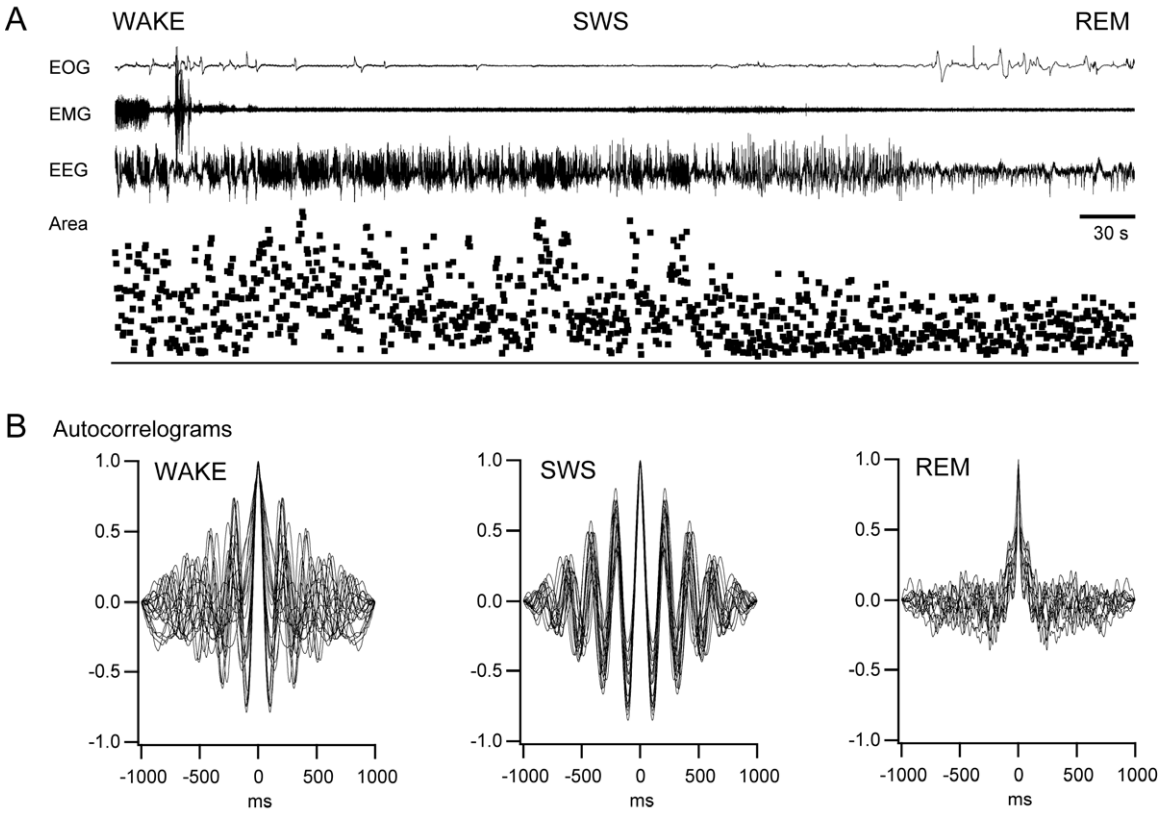

Figure 3-6 Quantification of sleep modulation of seizures. (A) Modulation by the state of vigilance of the area under successive auto-correlograms measured over the window corresponding to the dominant frequency. (B) Auto-correlograms indicate a dominant frequency oscillation in the 4 Hz range during SWS and wake, more evident during SWS, and abolished during REM sleep.

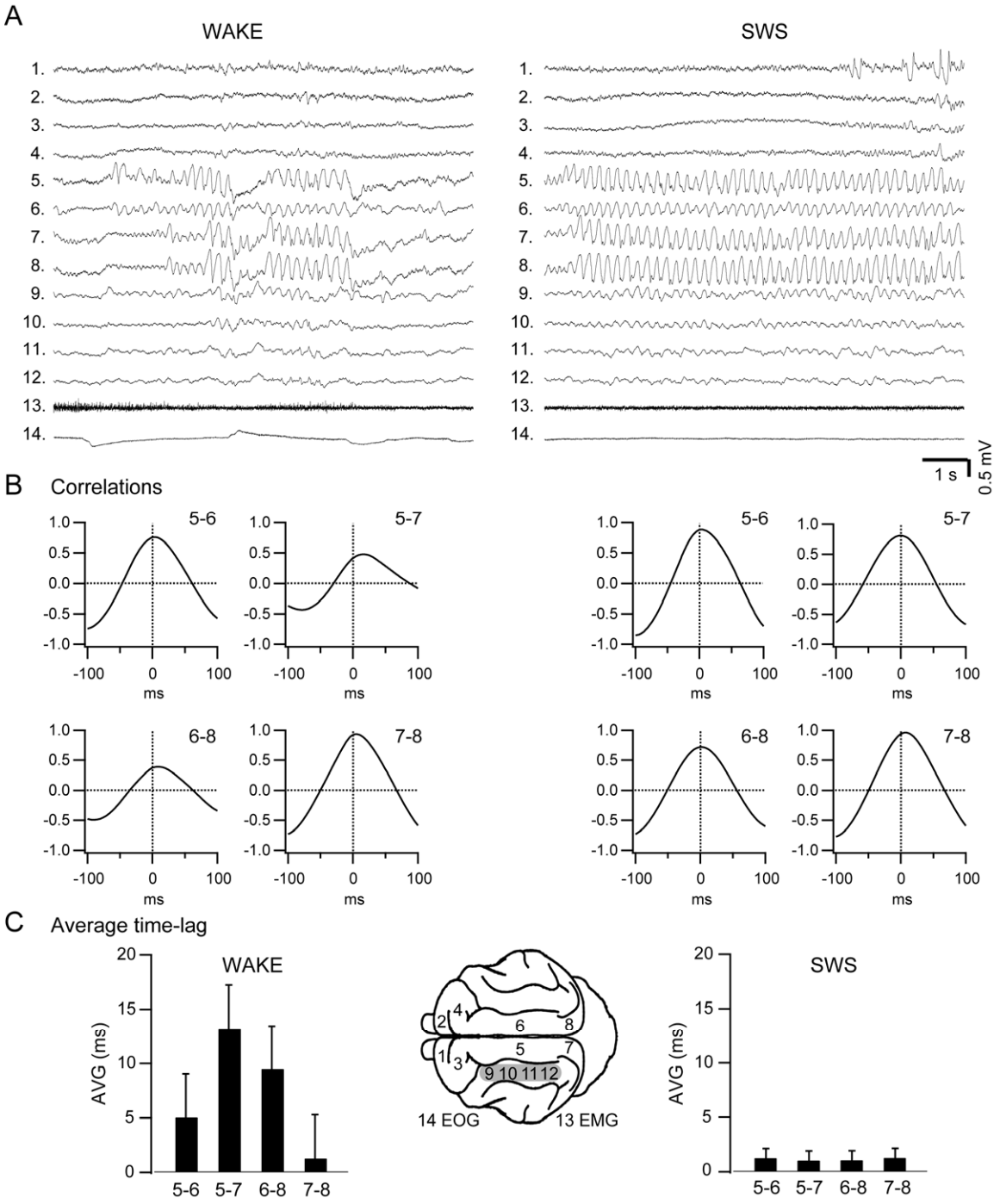

Figure 3-7 Spatio-temporal characteristics of ictal events. (A) Propagation of paroxysmal activities on the cortical surface during wake and SWS in a chronic cat with a deafferented left suprasylvian gyrus in day 15 after the cortical undercut. The position of the recording electrodes is depicted on right scheme and the undercut is represented as a grey box. While during wake the ictal events appear consecutively in different EEG channels, during SWS they display a tendency toward a simultaneously occurrence. (B) Activities occurring in four EEG channels (anterior marginal left and right gyrus, and posterior suprasylvian left and right gyrus) were correlated over 30 seconds and depicted as average correlations. (C) Average correlation time-lag in 4 different chronic cats.

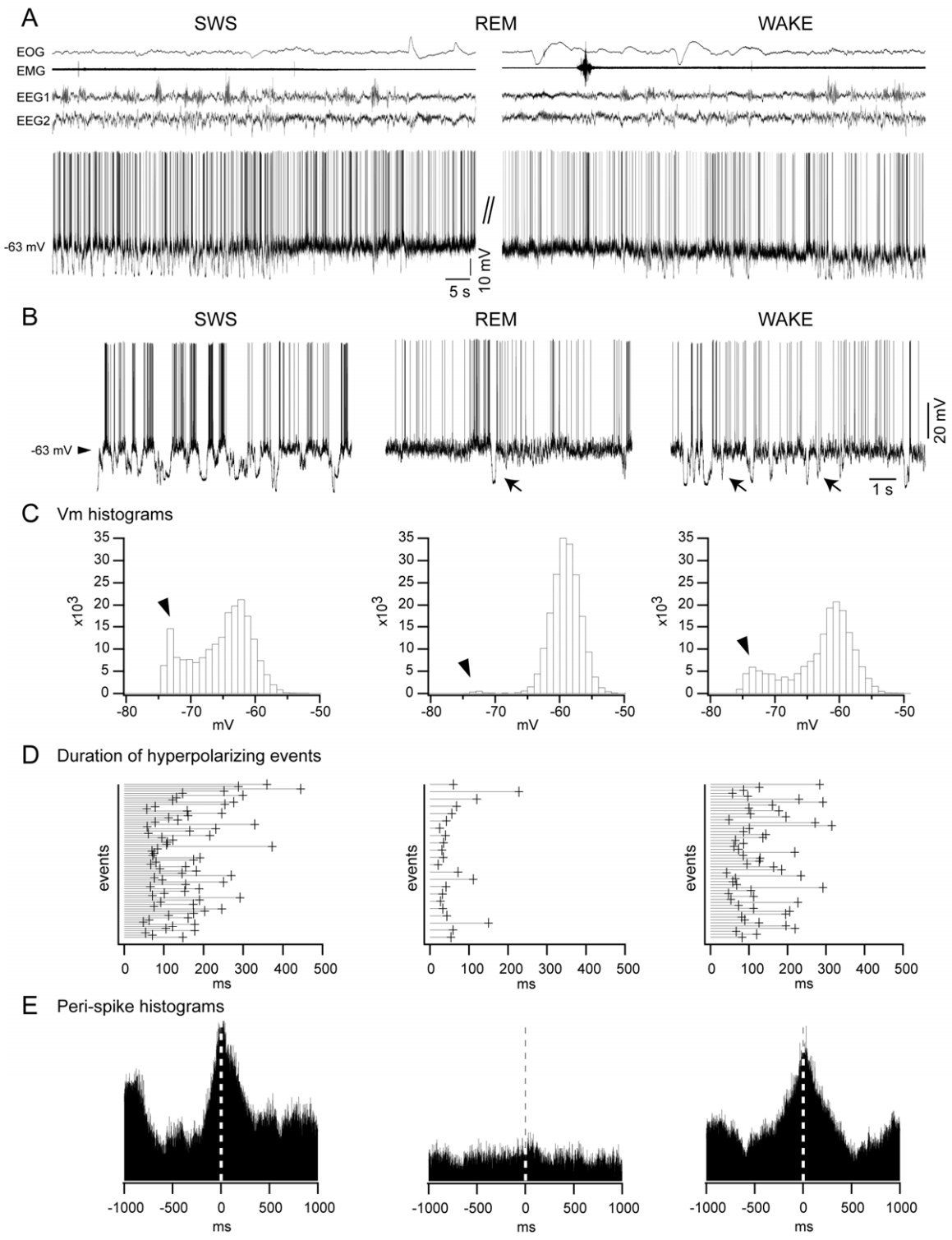

Figure 3-8 Intracellular correlates of seizures. (A) EEG in the marginal gyrus (EEG1) and local field (EEG2), EMG, EOG and intracellular recording of a regular-spiking neuron in a chronically implanted, naturally awake and sleeping cat, with a cortical undercut over the left suprasylvian gyrus, 15 days after deafferentation. (B) Patterns of neuronal activity during SWS, REM and wake. Note the presence of hyperpolarizing events during both wake and REM sleep (pointed by arrows); which are

expressed as the minor peak in the histograms of membrane potential (C). (C) Histograms of membrane potential distribution during SWS, REM and wake. (D) Duration of the hyperpolarizations in the intracellular recording, during SWS, REM and wake. These hyperpolarizing events are grouped in two clusters: short-length (< 100 ms) and long length (around 200-300 ms) and their occurrence is modulated by the state of vigilance. (E) Changes in the pattern of discharge of cortical neurons during various states of vigilance. Peri-spike histograms indicate a predilection for bursting behavior during SWS and wake, while during REM sleep neurons display a tonic firing pattern.

4 State-dependent outlasting activities following neocortical kindling in cat

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Submitted to Experimental Neurology.

[†] Both authors equally contributed to the present paper.

4.1 Résumé

Certaines formes d'épilepsie sont générées au niveau du réseau cortical. L'épilepsie secondaire à la stimulation sous-liminale chronique (« kindling ») du cortex cérébral du chat ne produit pas des convulsions généralisées dans la plupart des cas. Toutefois, nous avons développé une nouvelle méthode de stimulation, au-dessus du seuil, et nous avons étudié la synchronisation des potentiels du champ et les activités neuronales au niveau intracellulaire chez des chats non-anesthésiés avec des implants chroniques d'électrodes pendant les états de vigilance et pendant les crises épileptiques induites par la stimulation électrique prolongée (20-60 s). Chez les chats qui présentaient un cycle éveil-sommeil naturel, la plus grande probabilité d'induire des crises épileptiques aiguës a été observée pendant la transition entre le sommeil à ondes lentes (SOL) et l'état d'éveil. Les crises aiguës ont eu généralement un aspect clonique avec des composantes toniques, et ont été suivies par des dépressions postictales et par des activités paroxysmiques persistantes (ARP) (~1.5 Hz) avec une durée de jusqu'à 2 heures, rapportées ici pour la première fois. L'incidence des ARPs était la plus grande pendant le SOL, suivie de l'état d'éveil, alors qu'elles étaient virtuellement absentes pendant le sommeil REM. Les ARPs étaient initiées dans les aires corticales qu'avoisinaient l'électrode de stimulation, et se généralisaient sur tout le cortex suite à la stimulation chronique. Les enregistrements extra- et intracellulaires des neurones pendant les ARP ont montré des doublets de potentiels d'action par sommation des potentiels excitateurs post-synaptique ou des pré-potentiels rapides, montrant une excitation dendritique accrue. Les résultats suggèrent que la persistance des activités rythmiques hyper-synchrones dans le réseau cortico-thalamique est un des facteurs importants de l'épileptogenèse.

4.2 Abstract

Some forms of electrographic seizures are generated at the level of cortical network. The neocortical kindling exhibits a resistance to produce generalized convulsive seizures, and therefore, it was rather difficult to use it to study the cortical epileptogenesis. Here, using supra-threshold cortical kindling, we report electrophysiological patterns of field-potential synchronization and intracellular activities in chronically implanted non-anesthetized cats, during different states of vigilance, and during acute seizures elicited by prolonged (20-60 s) electrical stimulation. Acute seizures were easily elicited during transition from SWS to waking state. The seizures were mainly clonic accompanied with tonic components followed by prolonged postictal depression. Delayed rhythmic outlasting activities (OA) at ~1.5 Hz, first time reported here, followed the postictal depression and lasted up to 2 hours. These activities were clear during waking state, slightly reduced during SWS and completely absent during REM sleep. They started focally and following daily stimulations generalized over the whole cortical surface. Extra- and intracellular neuronal recordings during OA spike doublets, built on the summation of successive excitatory postsynaptic potentials and fast-prepotentials, entailing an increased dendritic excitation. Our results suggest that such rhythmic long-lasting oscillatory activity outlasting seizures are the key factor of epileptogenesis, leading to epilepsy.

4.3 Introduction

Repeated induction of focal seizure discharges in many brain sites leads to a progressive, highly reliable, permanent increase in epileptic response to the inducing agent, usually electrical stimulation (Goddard GV, 1967); also see review of (Morimoto K et al., 2004). The kindling phenomenon has been widely studied both as a chronic animal model of epileptogenesis and as a form of neuroplasticity. Kindling is a remarkably general phenomenon (Barnes SJ and JP Pinel, 2001). First, it has been demonstrated in a wide variety of species including frogs, mice, gerbils, rats, rabbits, cats, dogs, rhesus monkeys, baboons (reviewed in McNamara JO et al., 1980) and several authors have claimed kindling-like phenomena in humans (Coulter DA et al., 2002; Morrell F, 1985; Sato M et al., 1990); second, kindling has resulted from the stimulation of many, but not all, brain sites (Goddard GV et al., 1969); and finally, in addition to focal electrical stimulation, kindling-like phenomena can be produced with the periodic administration of most, if not all, convulsive agents (electroconvulsive shock, intracranially or systemically administered convulsant drugs, etc.) (McNamara JO et al., 1980).

Despite the fact that kindling is the most used model of epileptogenesis and several reviews have considered it as a model for clinical epilepsy (Loscher W, 1997; McNamara JO, 1994; McNamara JO et al., 1980; Racine RJ and WM Burnham, 1984; Sato M et al., 1990) spontaneous motor seizures, other than those triggered by stimulation, have been observed in relatively few kindled animals (Wada JA and T Osawa, 1976; Wada JA et al., 1974).

It was previously shown that the sleep-wake cycle significantly influences the expression of seizures in chronic models of trauma related epilepsy (Nita DA et al., 2007). The changes in the level of different neuromodulatory systems during sleep, together with the functional deafferentation of cortical neurons induced by the thalamic blockade of afferent inputs, embodied by the occurrence of disfacilitating periods during the cortical <1 Hz slow-oscillation modulate neuronal excitability (Contreras D et al., 1996; Steriade M, A Nuñez et al., 1993; Timofeev I et al., 1996; Timofeev I et al., 2001).

In this study we tested and confirmed the hypothesis that the transition between different states of vigilance is associated with a change of excitatory-inhibitory balance, creating

conditions for the rapid development of acute seizures. In addition, we describe a secondary rhythmic paroxysmal activity named “outlasting activity” (OA) which follows the initial elicited seizure, its electrophysiological patterns of synchronization during different states of vigilance, its intracellular correlates, and the epileptogenic processes triggered by these seizures.

4.4 Materials and methods

4.4.1 Animal preparation

Experiments were performed on 5 cats of both sexes. Surgical procedures were carried out in sterile condition, following a pre-medication with acepromazine (0.3 mg/kg i.m.), butorphanol (0.3 mg/kg i.m.), atropine (0.05 mg/kg i.m.) and ketamine (20 mg/kg i.m.), under barbiturate anesthesia (30 mg/kg i.v.). The level of anesthesia was continuously monitored by the aspect of electroencephalogram (EEG) and cardiac frequency (aiming 90-110 beats/min). Oxygen saturation of the arterial blood and end-tidal CO2 were also monitored. General surgical procedures included: cephalic vein canulation for systemic liquid delivery (lactated Ringer’s solution 5-10 ml/kg/h) and lidocaine (0.5%) infiltration of all pressure points or incision lines. Body temperature was maintained between 37–39°C with a heating pad.

Coaxial bipolar macroelectrodes (FHC Inc., USA) (with the inner pole in the cortical depth at about 0.8-1 mm and the outer pole placed at the cortical surface) were placed bilaterally in the motor cortex, anterior and posterior associative cortex, auditory cortex, primary and secondary visual cortex (areas 3, 4, 5, 7, 17, 18, 21, and 22). In 3 cats a recording chamber, enabling intracellular and extracellular unit recordings was placed over the intact dura, above the anterior part of the suprasylvian gyrus. Additional pairs of electrodes were placed around the orbit and neck muscles in order to monitor the states of vigilance by recording the electro-oculogram (EOG) and electromyogram (EMG). The Ag/AgCl reference was placed in the temporal muscle in all animals. The skull was reconstituted using acrylic dental cement and a few bolts were placed in the cement to allow non-painful fixation of the cat’s head in a stereotaxic frame. Animals were kept under observation up to the full recovery and they received analgesic medication (anafen 2 mg/kg s.c.) for the next 48-72 hours. All drugs were obtained from Sigma, Canada.

After a recovery period (2–3 days), cats were trained to stay in the frame for 2–4 h/day. After a few days of training, cats started to sleep in the frame and they displayed clearly identifiable states of waking, SWS, and REM sleep. The field potentials were recorded for at least 4 hours per day.

All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Committee for Animal Care of Laval University.

4.4.2 Kindling procedure

In all cats the electrode used for stimulation was placed in the left associative cortex (area 5), and its position was adjusted in a way that its corresponding homotopic point in the contralateral site must evoke response elicited by electrical stimuli applied to this electrode. The kindling stimuli finally consisted of 40-60 s of a 50 Hz rectangular pulse-train with stimulation intensity in the range of 0.5-1.5 mA. This superpose on the frequency range used in the majority of studies on kindling model.

In one cat electrical stimulation was randomly applied during each phase of sleep (i.e., SWS, REM, W) and transition from SWS to W, 3 times per day. Once we identified the SWS-W transition as the most seizure-prone moment of sleep-wake cycle we stimulated the subsequent animals only during this period (n=4), 3 times per day, and acute seizures elicited by electrical stimulation appear after 5-7 days of kindling. Daily electrical stimulations continued up to the end of experiments.

Here we provide only data coming from animals in the stage VI - generalized convulsion (Fernandez-Guardiola A et al., 1999) with an afterdischarge lasting for about 30-200 s depending to the animal (see Fig. 4-5D), observed after the cessation of stimulation.

Corresponding behavioral responses evoked by the stimulation were monitored using a night-shoot video surveillance camera. The animals were kept for 1 to 4 months to observe the behavioral changes. Generalized behaviorally manifest seizures appearing outside the stimulating session were considered an “end limit point” of these experiments, as consented with the local ethic committee. On 79th day one cat developed spontaneous status

epilepticus and it was euthanatized. However, cats frequently displayed localized muscular jerks and abnormal tail wagging during silent waking state. At the end of experiments or at the first sign on clinically manifest seizures, the cats were given a lethal dose of intravenous sodium pentobarbital (50 mg/kg).

4.4.3 Intracellular recordings

Intracellular recordings of cortical neurons, as described in (Steriade M et al., 2001; Timofeev I et al., 2001) were obtained with glass micropipettes (tip diameter <0.5 μm) filled with potassium acetate (2.5 M, in situ impedance 35-50 M∧) after small perforations in the dura were carefully made. The recording chamber was filled with warm sterile solution of 4% agar in order to enhance the stability of the recordings. Only stable recordings with resting membrane potentials more negative than -60 mV, overshooting action potentials, and input resistances >20 MΩ were kept for analysis. The intracellular signals were passed through a high-impedance amplifier with an active bridge circuitry (bandwidth DC to 9 kHz). All signals were digitized (20 kHz sampling rate) and stored for off-line analysis. After the end of the recording session 25 mg/ml solution of 5-fluorouracil was applied into recording chamber to prevent dural scarring (Spinks RL et al., 2003).

4.4.4 Data analysis

Average amplitudes of 5 steady consecutive evoked potentials from the beginning (4th to 8th) and from the end of the pulse-train (Fig. 4-1C) during wake, SWS and SWS – wake transition were computed from all stimulation episodes in cat 1. FFT quantifications (Fig. 4-4C) were performed by averaging the area under the FFT graph on the 1-2.5 Hz window, in all 5 experimental animals, from 5 different epochs. All statistical significant differences were considered for $p<0.05$ (paired Student t-test). Autocorrelograms (Fig. 4-5A) were generated on successive windows of 5 sec length. The maximal values of auto-correlation in the range of 400-700 ms (corresponding to the frequency of OA) were interpolated using a polynomial fit (Fig. 4-5B). The end of OA was considered when the interpolation reached 30% of the maximum value (Fig. 4-5C). The cross-correlations for each state (Fig. 4-6 & Fig. 4-7) were calculated from 45-50 consecutive periods in which a maximum of depth negativity recorded from one of the EEG electrodes was chosen as zero time and the total duration of segments was ±2000 ms. The histogram of membrane potential distribution

(Fig. 4-8D) was created by counting the number of samples with bins of 1 mV. The peaks of distribution that corresponded to the most probable mode of membrane potential were taken as the level of membrane potential. The peri-event spike histograms (Fig. 4-8E) were obtained over 5 min epochs, taking as time reference the maximal depth-negativity of EEG field, and by counting each action potential in the intracellular recording with a bin width of 10 ms.

4.5 Results

4.5.1 Induction of acute seizures is related to the states of vigilance

The first 3-4 stimuli of the electrical pulse-train applied to the cortical tissue evoked augmenting responses (Timofeev I et al., 2002) followed by constant amplitude responses if stimulation was performed during steady SWS or waking states (Fig. 4-1A & Fig. 4-1B panels 1-2), and progressively increasing responses if applied during the transition from SWS to wake (Fig. 4-1A & 1B panel 3). In the first cat we quantified the amplitude of five steady consecutive evoked potentials (4th to 8th) induced by the electrical stimulation at the beginning and at the end of each pulse-train. Averaged amplitude of response at the beginning of the pulse-train was 0.17±0.11 mV during SWS, 0.15±0.09 mV during wake and 0.18±0.1 mV at the transition from SWS to wake. The average amplitudes of the last five evoked potentials from 40-60 sec trains, recorded during SWS or wake, remained similar (0.15±0.08 mV [SWS], 0.18±0.08 mV [wake]), while when applied during the transition from SWS to wake the mean amplitude evoked potentials significantly increased to 0.46±0.09 mV ($p<0.01$, paired t-test, n=25) (Fig. 4-1C).

We found that 95.5% of acute seizures followed a cortical stimulation characterized by a progressive increase in the evoked field potentials amplitudes during the repetitive pulse-train, and this occurred when the stimulation was applied in the early periods (~20 s) of the transition from SWS to wake. Only 4.5% of seizures followed constant amplitude responses, 3.37% occurring when stimulating during steady SWS and 1.13% while steady wake. Therefore our data indicate that the highest probability to induce generalized convulsive seizures occurs when stimulations is applied during the transition from SWS to wake.

In the remaining experimental animals (n=4), five to seven days after the beginning of the kindling protocol, electrical stimulations applied during the transition from SWS to wake developed into generalized convulsive seizures after the cessation of the electrical stimulation. There were periods of time when the kindled cats manifested a resistance to develop generalized convulsive seizures, independently to the state of vigilance (see cat 1 in Fig. 4-5D), or the duration of acute seizures stopped to increase with the progression of kindling (see cat 2 in Fig. 4-5D). However, resistance to induced seizures was observed only in the first experimental animal which was mainly used to identify the most effective state for stimulation, and which received randomly stimulations during the sleep-wake cycle, (the half of train-pulses not being followed by acute seizures), fact that dramatically impeded on the development of kindling in this cat. Similar observations were previously reported (Goddard GV, 1967; Herberg LJ and PJ Watkins, 1966), but in those experiments resistance could last for days or weeks and were animal dependent.

4.5.2 Outlasting activities and their modulation by the state of vigilance

Probably the most interesting finding, consistently observed in all experimental animals, was the expression of long-lasting rhythmic activities, named here "outlasting activities", at the end of each convulsive generalized acute seizure. Following the postictal depression these highly rhythmic paroxysmal activities expressed themselves, from the very first seizures triggered by electrical stimulation in kindled cats (Fig. 4-2A expanded in Fig. 4-2B panel 2).

OA were characterized by a reduced amplitude compared with the initial AS (Fig. 4-2B), and an oscillating frequency of 1.5-2 Hz, slightly slower than the frequency of AS which consisted in 2 Hz spike-wave complexes (Fig. 4-2B panel 1), and faster than the 0.5-0.9 Hz cortical slow-oscillation observed during SWS (Fig. 4-2C).

OAs appeared localized in the vicinity of the stimulating electrode at the beginning of kindling protocol (Fig. 4-2A), but after subsequent days of stimulation they were recorded from all implanted electrodes (Fig. 4-3). However, the generalization of OA over the whole cerebral surface followed more the pattern of a primary-focal secondary generalized seizure, than the pattern of primary generalized seizure, since even at advanced stages in

the kindling protocol OA started in the vicinity of stimulating electrode hundreds of milliseconds to seconds before generalizing to other cortical areas (Fig. 4-3).

OA were recorded for up to two hours and observed throughout the sleep-wake cycle. At the beginning of OA (first tens of seconds) some cats displayed eye blinking with frequency of OA. Later on during OA in the waking state the cats were conscious, and could eat and walk when removed from the recording frame. Moreover, during quiet wake state accompanied with OA the cats displayed isolated, localized, or generalized muscular jerks and abnormal tail wagging. However during active wake (walking, eating, etc.) no abnormal muscular activity were seen (see supplemental material).

Once elicited by the initial acute seizures these activities were strongly modulated by the state of vigilance the animal undergone (Fig. 4-4A). They were obvious during waking state, slightly reduced during SWS and were completely abolished during REM sleep, as revealed by FFT power spectra (Fig. 4-4B). A quantification of the modulation of OAs power during different states was performed by averaging the area under the FFT graph over a window (1-2.5 Hz) centered on their mean frequency. During early periods of kindling procedure, in electrodes which displayed OA, this area was significantly decreased ($p<0.05$) during REM sleep compared to both wake and SWS, to 4.86±0.71 mV^2 x Hz vs. 22.19±4.65 mV^2 x Hz and 18.80±2.81 mV^2 x Hz respectively. Statistical significant differences were also obtained in the surface of the area between electrodes with and without OA during wake and SWS (22.19±4.65 mV^2 x Hz vs. 5.1±0.75 mV^2 x Hz during wake, and 18.80±2.81 mV^2 x Hz vs. 12.62±1.37 mV^2 x Hz respectively during SWS, $p<0.05$). During late stages of kindling, when outlasting activities were present in all electrodes, the electrophysiological distinction between SWS and waking states was not always clear. However, during REM sleep (identified by muscle atonia and ocular saccades) OA were always absent.

The duration of these rhythmic activities were quantified by measuring the peak correlation amplitude in the time range of 400-700 ms from 5 sec consecutive periods (Fig. 4-5A) during rhythmic activities with frequency about 2 Hz. This peak was high (Fig. 4-5 A, B) and if the rhythmicity was impaired the peak was low. The end of OA was considered when the polynomial interpolation of auto-correlation peaks (Fig. 4-5B) reached 30% of the

initial maximum value. The extinction of OAs in different electrodes took place gradually at the level of EEG, OAs being slowly replaced by the background activity. However, the polynomial interpolation crossed the limit we considered as threshold for OA relatively simultaneous for all electrodes (Fig. 4-5C). Both generalized convulsive seizures and OA increased their duration with the progression in time of the electrical stimulation (Fig. 4-5D).

We tested whether the coherence of OA was modified as kindling protocol progressed. Coherence of OAs within cerebral cortex continuously increased with time being relatively low at the beginning, increasing with time and reaching maximum at one month from the beginning of stimulating protocol (Fig. 4-6A). At the same moment of time from the beginning of kindling procedure the correlation over the same hemisphere was always higher as compared to the correlation between hemispheres (Fig. 4-6B).

Dynamic positive or negative peaks of cross-correlations between different EEG electrodes were in the range 0.4-0.6 during SWS, 0.4-0.7 during seizures, 0.4-0.6 during focal OA, increasing to 0.65-0.95 when OA became generalized (Fig. 4-7). The maximal phase shift between electrodes was observed during the SWS (0.3 s) but varied from cycle to cycle. During acute elicited seizures the phase shift was either minimal, progressively shifted, or it jumped from cycle to cycle. Finally, during generalized OA the phase shift was normally minimal 0-0.1 s and remained stable for the given pair of electrodes (Fig. 4-7C). Thus, out of investigated brain states (wake, SWS, electrically elicited seizures, focal and generalized outlasting activities, the coherence was highest during generalized outlasting activities.

4.5.3 Intracellular correlates of OA

Intracellular recordings were performed in the anterior part of the suprasylvian gyrus in the vicinity of stimulating electrode. From more than 50 recorded neurons in 3 different animals, 39 were impaled in animals undergoing OA accompanied with body jerks, and 5 neurons responded to the quality criteria described in the methods. They were all identified as regular-spiking neurons following depolarizing current pulses applied to the soma, had similar pattern of discharge during OA, and were located in infragranular layers (below 1 mm from cortical pia). Intracellular oscillations during paroxysmal OAs consisted of

silence during depth-positive EEG waves and neuronal discharges during the depth-negative waves (Fig. 4-8A), built on the summation of successive EPSPs and fast-prepotentials (FPPs), entailing an increased dendritic excitation (Fig. 4-8, B-C).

At variance with the bimodal distribution of the membrane potential observed during SWS (see Fig. 5 in Steriade M et al., 2001; and Fig. 2 in Timofeev I et al., 2001), here the neurons displayed a unimodal distribution (Fig. 4-8D) since the silent periods, virtually deprived of spontaneous synaptic events, were not accompanied by remarkable hyperpolarizations.

The peri-event spike histograms (Fig. 4-8E) showed that neurons fired in phase with field potential from the same hemisphere, in concordance with the high level of correlation observed in the cortical EEG when OAs were generalized.

Enhanced neuronal excitability in kindled animals during normal SWS and waking states was reflected by the presence of doublets or bursts of action potentials in 4/5 stable intracellularly recorded regular-spiking neurons (Fig. 4-9A) and in 70 % (n=35) extracellular unit recordings (Fig. 4-9B). Since the bursting pattern was not elicited by somatically applied current pulses, we assume that the origin of these bursts was dendritic. This observation is congruent with previous studies showing the presence of spontaneous bursts in neocortical neurons during trauma induced epileptogenesis in acute (Nita D et al., 2006; Topolnik L et al., 2003) and chronic animals (Nita DA et al., 2007).

4.6 Discussion

The major findings reported in the present study are: (1) acute seizures following cortical electrical stimulation during the transition from SWS to waking state were easily elicited, (2) the acute convulsive seizures were followed by the prolonged (tens to hundreds of minutes) rhythmic non-convulsive “outlasting activities” with frequency 1.5-2 Hz, lasting up to two hours, (3) during REM sleep the OA were absent, (4) following acute seizure the OA were local and they spread over all cortical areas in 2-4 weeks from the beginning of kindling procedure, and (5) similar to SWS, the membrane potential of cortical neurons uncovered the presence of active and silent states, but distinct from SWS, the distribution of the membrane potential was unimodal.

4.6.1 Major features of neocortical kindling

In the majority of previous studies on kindling the parameters of electrical stimulation were in the order of seconds as time duration, and in the order of hundreds of micro-amperes as intensity. However, in the present study as well as in the first studies reporting kindling phenomena the pulse-train duration was in order of 1 min (Goddard GV, 1967). Using standard kindling protocol (1 s, hundreds of micro-amperes) the first generalized convulsive seizures could be triggered by amygdala stimulation in 2-4 weeks and by cortical stimulation in 3-8 weeks (Racine R, 1978; Racine RJ and WM Burnham, 1984). In our study acute seizures were elicited by cortical stimulation after 5-7 days from the beginning of the stimulating protocol.

Despite multiple studies carried out on kindling models, very few of them successfully reported recurrent spontaneous paroxysmal events, even thought, it require a long period (months) of observation (Wada JA and T Osawa, 1976; Wada JA et al., 1974). In our present work, we did observe spontaneous paroxysmal events following the first acute seizures induced by electrical stimulation, with a rhythmic activity at 1.5-2Hz, which we called “outlasting activities”. They appear when the first generalized convulsive AS was triggered by the electrical stimulation of the cortex. Furthermore, OAs were never before observed following acute electrical stimulation of the cerebral cortex under anesthesia (neither barbiturate, nor ketamine-xylazine), though this manipulation constantly elicited seizures (reviewed in Timofeev I and M Steriade, 2004).

The membrane potential of neurons during outlasting activities revealed the presence of active and silent states, but the silent states were not accompanied with remarked hyperpolarizations, and in fact the membrane potential distribution of neurons during outlasting discharges was similar to the one recorded with Cs+ containing pipettes during sleep (see Fig. 2 in Timofeev I et al., 2001). Since most of K^+ currents are blocked by acetylcholine (McCormick DA, 1992) and acetylcholine act on the same channels as intracellular Cs+ (Metherate R et al., 1992) it is easily predictable that the neuronal silence is not accompanied with large amplitude hyperpolarizing potentials, because the major hyperpolarizing currents are blocked in awaked animals as in the present study, and the membrane potential for neurons remain virtually unchanged. This observation stands in

difference with experiments involving cortical undercuts (Nita DA et al., 2007). There, all the fibers, including the cholinergic ones were cut and the periods of neuronal silence could be found during all states of vigilance and the silent periods were accompanied with the large amplitude hyperpolarizing potentials.

4.6.2 States of vigilance and neocortical epileptogenesis

Clinically, SWS is considered as a potent activator of epileptiform discharges (Dinner DS, 2002; Gigli GL et al., 1992; Niedermeyer E and F Lopes da Silva, 2005) whereas REM sleep, with its asynchronous cellular discharge pattern, is resistant to the propagation of epileptic EEG potentials (Shouse MN et al., 2000). Similarly results were recently reported in a chronic model of trauma related epilepsy (Nita DA et al., 2007).

We report that the highest probability to induce acute seizures was observed during the transition from SWS to wake state. One of the factors which may account for this phenomena is that during this transitory period basal forebrain cholinergic neurons projecting topographically to entire cerebral cortex release a large quantity of acetylcholine (Sarter M and JP Bruno, 2000; Semba K, 1991; Steriade M, 2004) and during this state, cortical acetylcholine is at its highest level (Celesia GG and HH Jasper, 1966); leading to an activation of the cortex and thalamus, and possibly a hyperexcitability and unbalance between the excitatory and inhibitory components, facilitating the generation of seizures. The transitions from wake to SWS and from SWS to REM sleep were not tested in the present study since they are difficultly predictable.

The sleep-wake cycle significantly influences the expression of seizures. Cortical seizures with SW complexes prevalently occur during early SWS stages in behaving monkeys (Steriade M, 1974) and the slow sleep oscillation (0.5-1 Hz) may develop, without discontinuity, into electrographic seizures in cats (Steriade M et al., 1998). The activating properties of SWS on epileptiform discharges should be ascribed to highly synchronized oscillations in reciprocal thalamo-cortico-thalamic systems, which characterize this sleep stage (Steriade M and D Contreras, 1995), but also to the changes in neuronal excitability due to the level of different neuromodulators systems. Several studies, focused on the relationship of epileptic seizures to sleep stages in humans, have noted that seizures are

more common during sleep (Bazil CW and TS Walczak, 1997; Crespel A et al., 1998; Minecan D et al., 2002). Furthermore, it has been previously reported in a chronic model of trauma epilepsy in cats that that most seizures occurs during slow-wave sleep and are completely blocked during REM sleep (Nita DA et al., 2007). OA reported here followed the same pattern: they were obvious during waking state, slightly reduced during SWS and were completely abolished during REM sleep.

4.6.3 Common features and mechanisms of neocortical seizures onset

Epilepsy consists of about 40 different clinical syndromes and apparently many of these have to share common mechanisms (Jacobs M et al., 2001). At the level of neocortical neurons, the major feature of SWS is the presence of large amplitude hyperpolarizing potentials, accompanied with neuronal silence, which contributes to the generation of large amplitude depth-positive EEG waves (Contreras D and M Steriade, 1995; Mukovski M et al., 2007; Steriade M et al., 2001; Timofeev I et al., 2001). The hyperpolarized states of some regular-spiking cortical neurons favor the induction of burst firing in response to depolarizing inputs (Steriade M, 2004; Steriade M, F Amzica et al., 1993; Timofeev I et al., 2000; Wang Z and DA McCormick, 1993) and the postsynaptic influence of the burst is much stronger as compared to a single spike (Lisman JE, 1997; Timofeev I et al., 2000). Moreover, extracellular recordings with ion-sensitive electrodes demonstrated that interictal spikes determine both a rapid increase in extracellular potassium concentration and an extracellular alkalinization, phenomena that further increase neuronal excitability (de Curtis M et al., 1998).

It is also known that the repetition of seizures, whether spontaneous or evoked, leads to lowering down the threshold to elicit following seizures (McNamara JO, 1994; Morimoto K et al., 2004). Although the seizures are very powerful and strongly synchronized events, usually they lasted for only tens of seconds. Thus unlikely they could induce long-lasting modifications of synaptic or intrinsic efficiency that would affect the threshold for the onset of seizures. It is very likely that some long-lasting brain process could be much more efficient in the promotion of paroxysmal events.

In the present study we found the presence of long-lasting OA that maintained an alternation of active and silent states for tens of minutes or hours. Similarly, during trauma induced epileptogenesis, in the areas affected by trauma (Nita DA et al., 2007), we recently reported the presence of silent periods during both SWS and waking state, and to lesser extent during REM sleep.

A long-lasting increase or decrease in neuronal excitation triggers the onset of homeostatic process, which normally should lead to decreased or increased neuronal sensitivity to incoming signals, that would apparently maintain a certain level of network excitability (Desai NS et al., 1999; Turrigiano GG, 1999; Turrigiano GG et al., 1998). However, the homeostatic mechanisms are not always able to stabilize the network in a state of "normal" excitability that leads to the development of different forms of paroxysmal activities (Houweling AR et al., 2005). Therefore, we suggest that silent network states induced by disfacilitation (sleep), deafferentation (cortical trauma) and by kindling (outlasting activities) increase the number of burst-firing neurons, which further induce abnormally strong postsynaptic excitation, which shifts the balance of excitation and inhibition toward overexcitation leading to the onset of seizures. Neocortical kindling induces dynamic modulation of the amount of apical and basilar dendritic spine density, length and branching (Teskey GC et al., 1999), but after several weeks of kindling procedure these parameters return to control values (Racine R et al., 1975; Teskey GC et al., 2001). In addition, studies on piriform cortex (PC) or amygdala-PC slice preparations from kindled rats showed long-lasting changes in synaptic efficacy in the ipsilateral PC, including spontaneous discharges and enhanced susceptibility of PC neurons to evoked burst responses (Bloms-Funke P et al., 1999).

We conclude that the transitions between states of vigilance, accompanied by changes of neuronal excitability create conditions in which the balance of excitation and inhibition is not always tuned and these conditions favor the onset of seizures.

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4.9 Figures

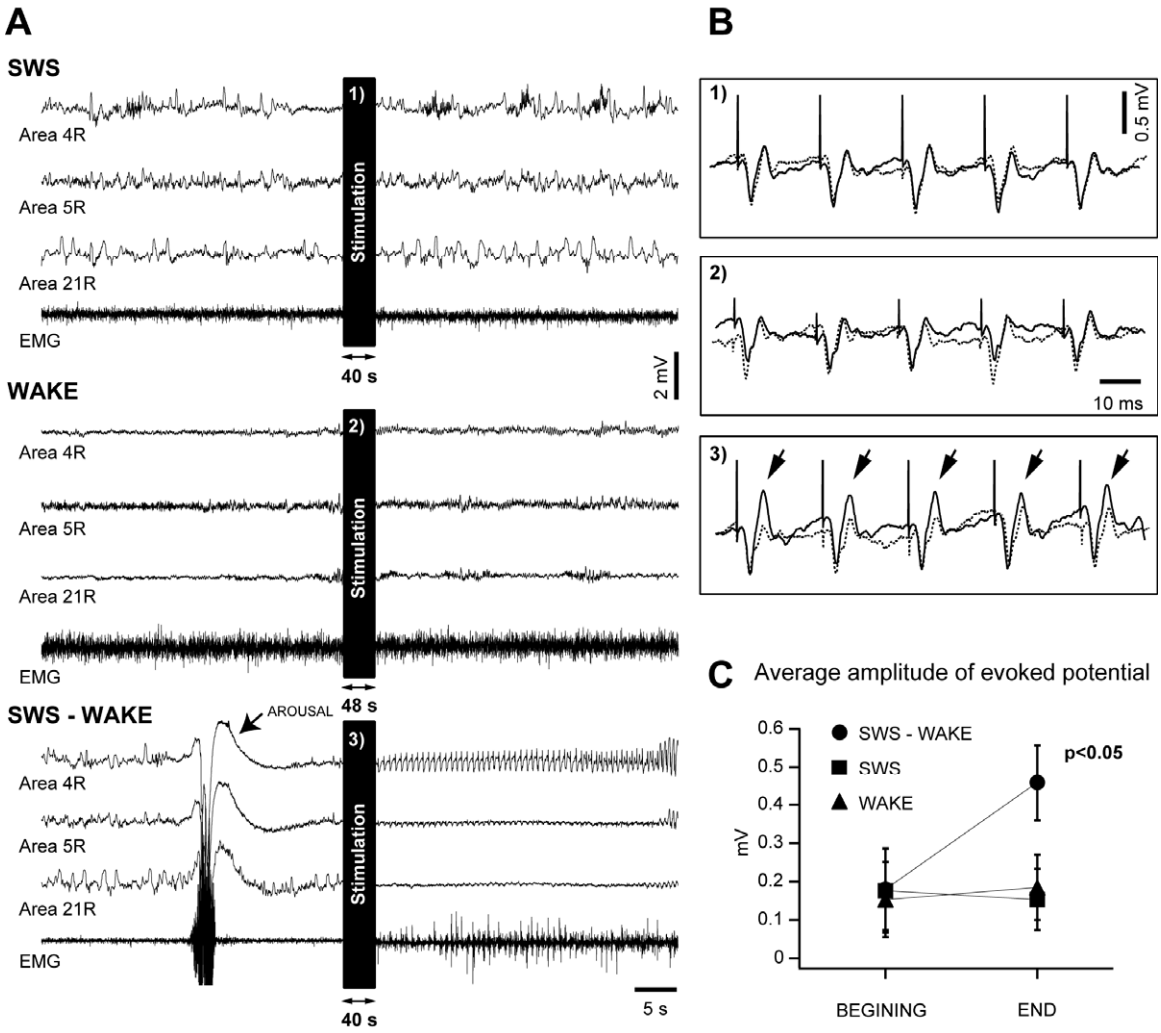

Figure 4-1 State dependency of acute seizures induction susceptibility. A, Field potential recordings from different cortical areas during Slow Wave Sleep (SWS), wake and the transition from SWS to wake, in a kindled cat (at day 8). The electrical stimulation (50 Hz) is indicated by black rectangles on top of the traces. Field potential activities recorded from motor cortical area 4 right (Area 4R), associative 5 right (Area 5R) and visual area 21 right (Area 21R) are depicted. A clear development of neocortical paroxysmal discharge is observed during the transition from SWS to wake in the top EEG trace (Area 4R). EMG stands for electromyogram. B, superposition of five consecutive evoked potentials (stimuli 4-8) from the beginning of the pulse train (dotted line) and last five (solid line) evoked potentials (Area 4R), from the corresponding stimulation period in A. Note the increased amplitude of the last evoked potential in the train of stimuli when applied at the transition from SWS to wake. C, Average amplitudes of five consecutive responses from the beginning and the end of the train pulse during wake, SWS and SWS-wake transition.

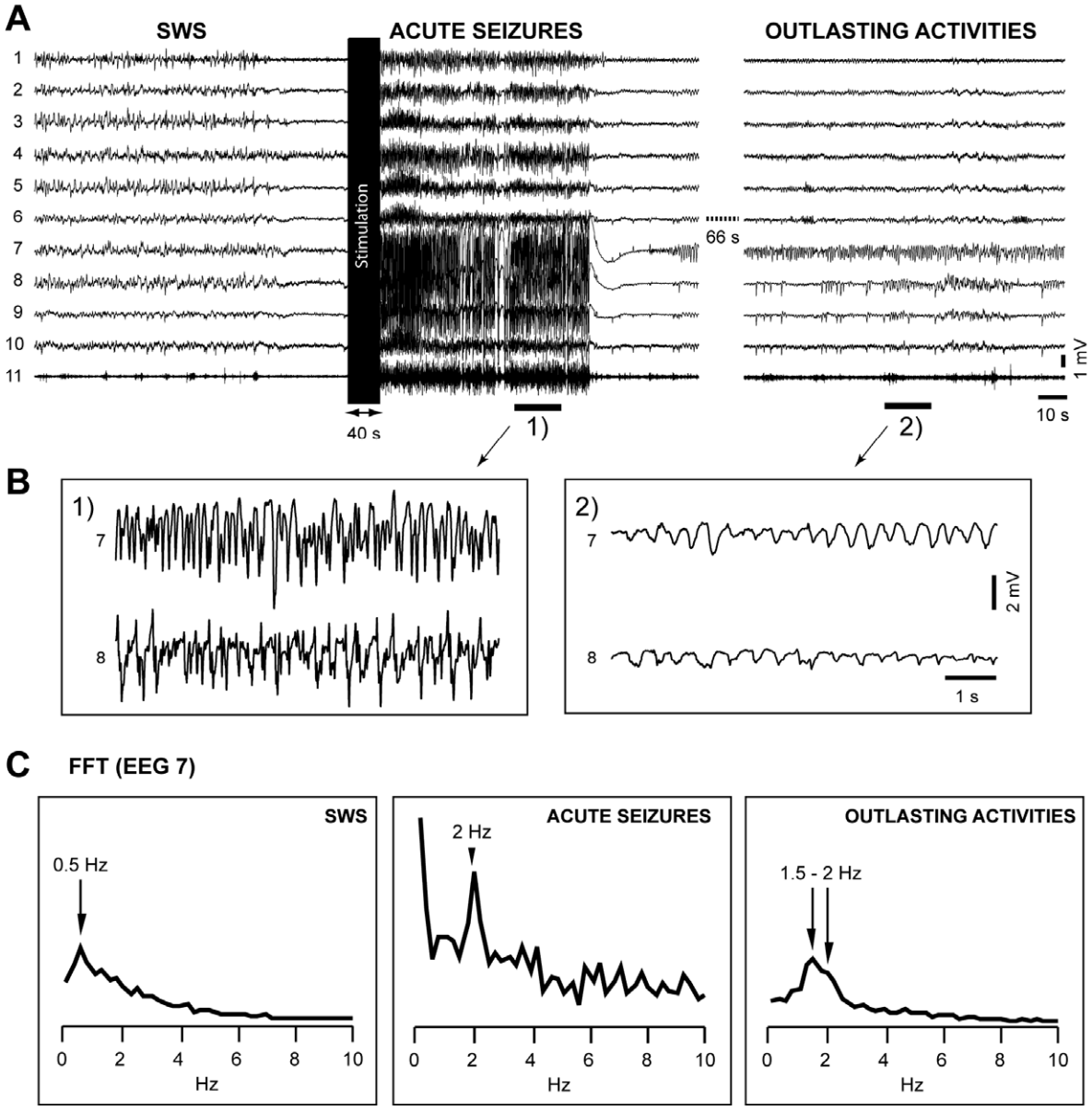

Figure 4-2 Acute seizure followed by outlasting rhythmic activity. A, EEG recordings show a development of acute primary generalized convulsive seizures following electrical stimulation, and localized outlasting activity (EEG field 7) in a kindled cat (day 10). Depicted field potential recordings were obtained from: 1. motor cortex (area 4 right); 2. somatosensory cortex (area 3 right); 3. anterior associative cortex (area 5 right); 4. auditory cortex (area 22 right); 5. middle associative cortex (area 7 right); 6. posterior associative cortex (area 21 right); 7. auditory cortex (area 22 left); 8. posterior associative cortex (area 21 left); 9. primary visual cortex (area 17 left); 10. primary visual cortex (area 17 right); 11. EMG. B, contains the expanded details of the periods underlined with horizontal bars in panel A. C, depicts the Fast Fourier Transformation (FFT) of EEG 7 during SWS (left), induced seizures (middle) and outlasting activity (right). Note the peaks at 1.5 and 2 Hz, which characterized the oscillatory frequency of OA discharges.

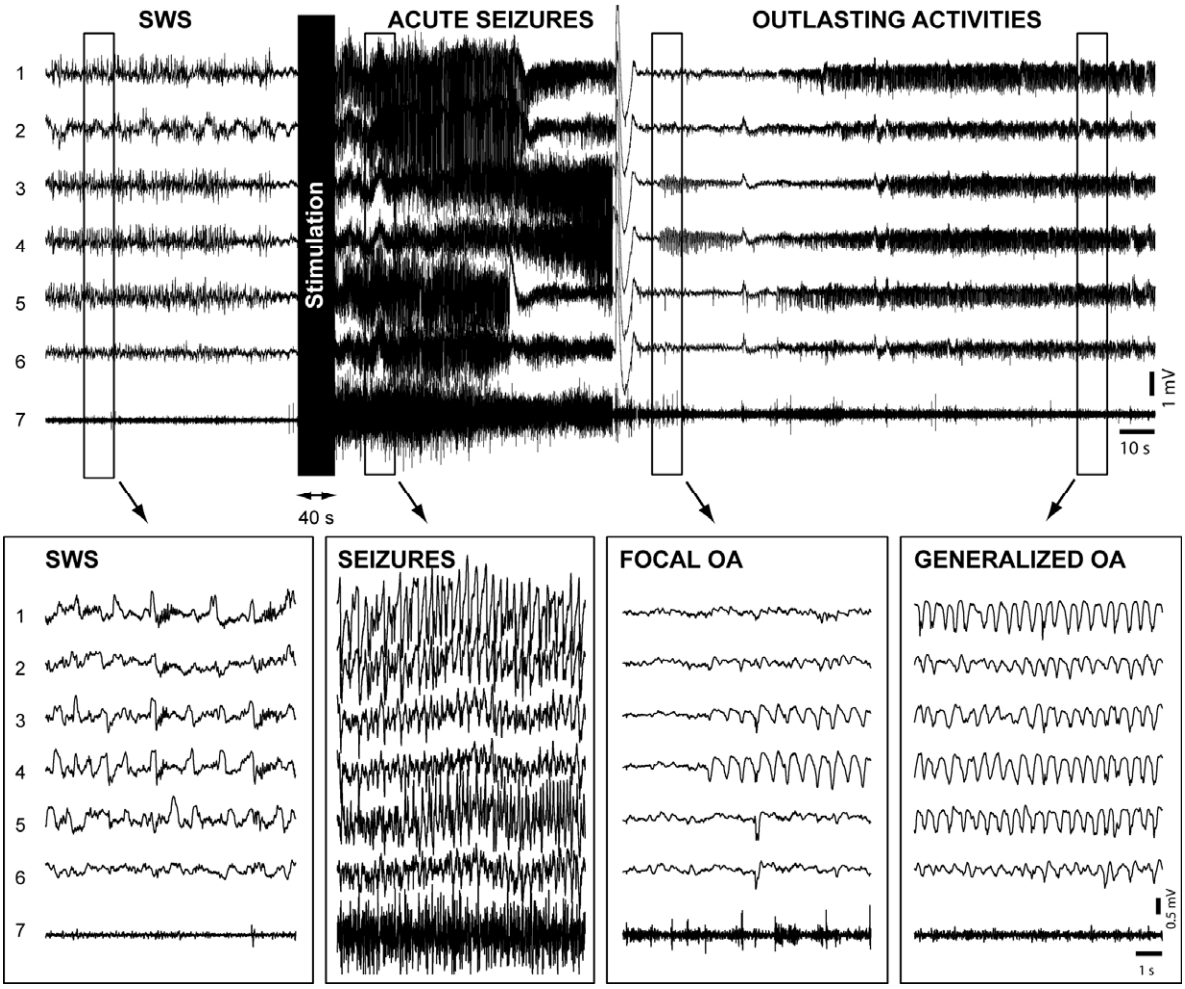

Figure 4-3 Generalization of outlasting activity discharges in a kindled cat (day 21). Top panel, same animal and same stimulation conditions as in Figure 4-2 in the order: slow-wave sleep (SWS), acute elicited seizure, focal and generalized outlasting activity (OA). Electrical stimuli were applied ~10 s after the onset of wake state. Depicted field potential recordings were obtained from: 1. motor cortex (area 4 left); 2. motor cortex (area 4 right); 3. auditory cortex (area 22 right); 4. auditory cortex (area 22 left); 5. primary visual cortex (area 17 left); 6. primary visual cortex (area 17 right); 7. EMG. Bottom panels shows expanded recordings from the corresponding periods in the top panel.

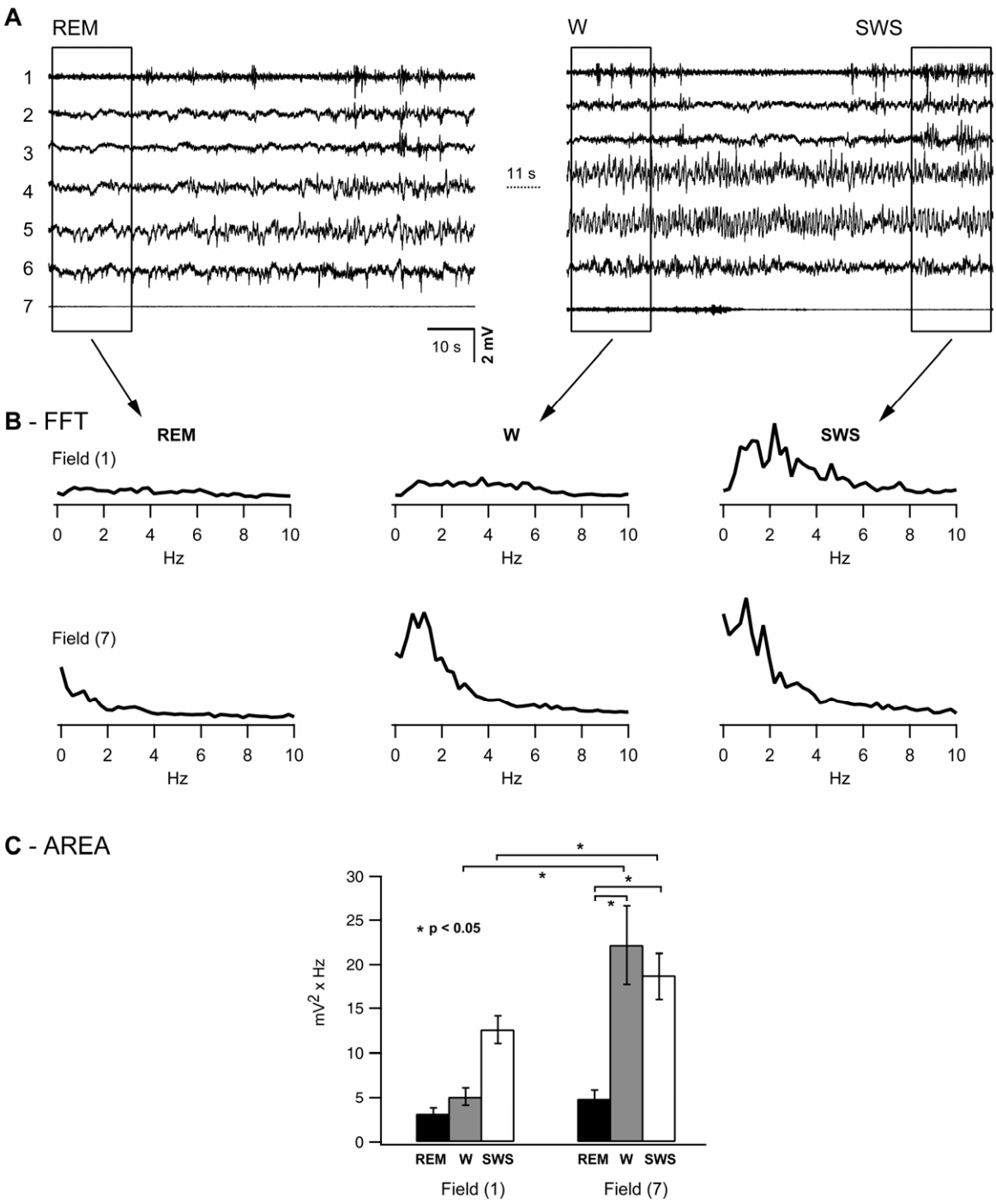

Figure 4-4 The occurrence of outlasting activity (OA) in relation to different states of vigilance in a kindled cat (day 10). A, left panel - EEG recordings during rapid eye movement (REM) sleep and right panel - another period of the same recording showing the transition from wake to slow-wave sleep (SWS). Note the clear presence of OA discharges on electrodes 4 and 5. Numbers on the left of the EEG recordings represent: 1. motor cortex (area 4 right); 2. somatosensory cortex (area 3 right); 3. middle associative cortex (area 7 right); 4. auditory cortex (area 22 left); 5. posterior associative cortex (area 21 left); 6. posterior associative cortex (area 21 right); 7. EMG. B, FFT during the states of vigilance for an electrode not affected by OA (EEG 1) and another electrode displaying outlasting activity (EEG 5). Note the absence of outlasting activity during REM sleep and its slight diminution during SWS. C,

Average areas under the FFT graph on the 1-2.5 Hz window, for the situations depicted in B. * indicate a statistical significant difference (paired Student's t-test, $p<0.05$).

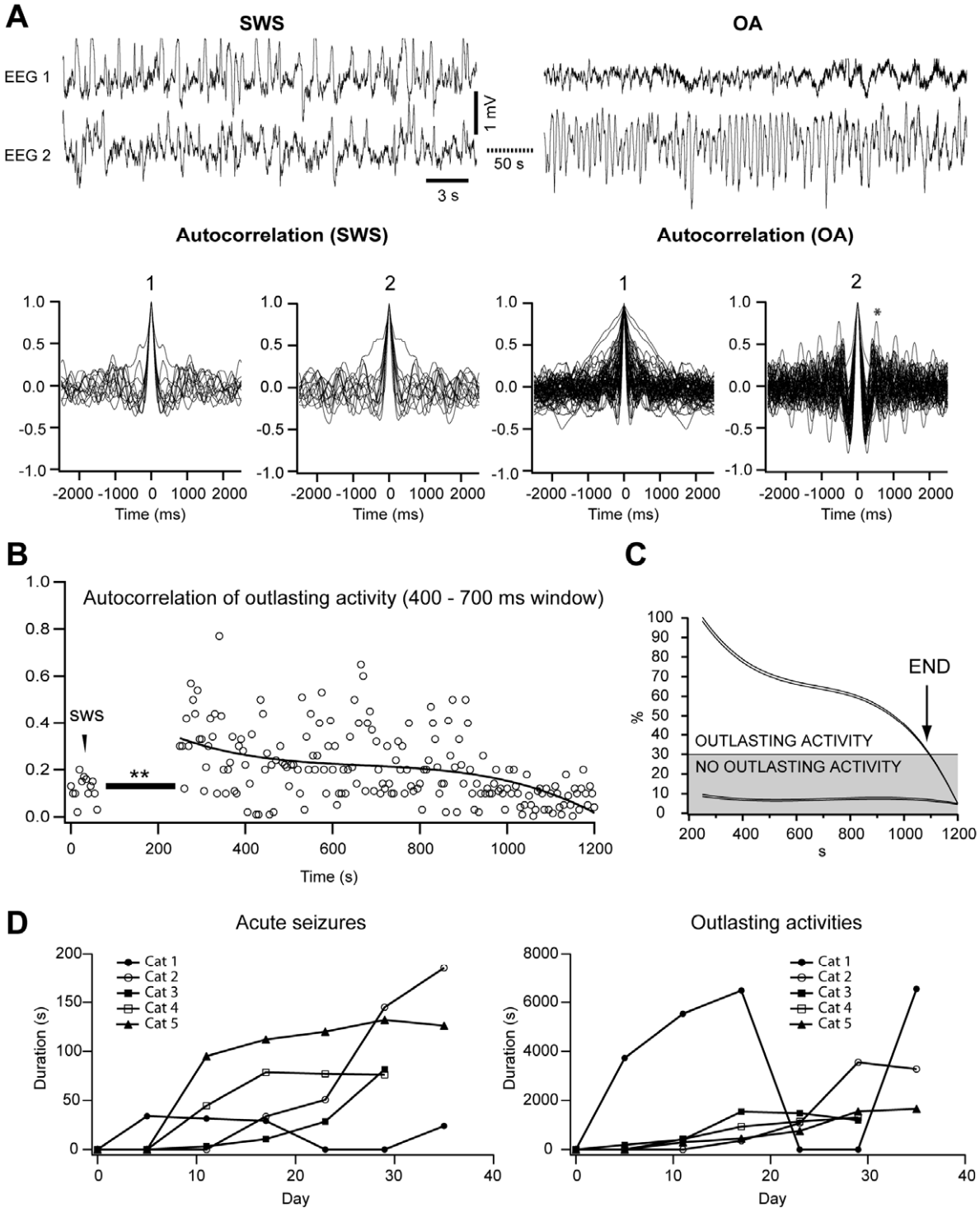

Figure 4-5 Estimation of the duration of outlasting activity (OA) and duration of seizures. A, top panels, left - EEG recordings during slow-wave sleep (SWS) and right - EEG recordings of two EEG leads during wake with (1) and without (2) OA discharges (day 8). EEG 1 – motor cortex (area 4, right), EEG 2 – auditory cortex (area 22, left). Middle panel, left, the plots of auto-correlation during SWS for electrodes (1 and 2). Right, the plots of auto-correlation during wake for electrodes (1 and 2). The auto-correlations were obtained from consecutive 5 sec periods. (*) Indicate the peak of the oscillatory

activity frequency of OA around (400-700 ms). B, Panel depicts the evolution of maximal values of auto-correlation in the range of 400-700 ms window before the stimulation (during SWS) and during OA. (**) with thick horizontal line indicate electrical stimuli and paroxysmal afterdischarge. Thin line – polynomial fitting. C, Polynomial interpolation of the maximum auto-correlation values in the specified window of two EEG recordings showing OA (area 22, left [shown in A] and area 21, left [not shown]), and other two EEG field recordings without OA (area 4, right [shown in A] and area 21, right [not shown]). The end of outlasting activities was considered when the interpolation reaches 30% of the maximum value, and was simultaneous for all EEG field recordings with OA. Values were normalized for the maximum initial amplitude in the auto-correlation level. D, left - the duration of acute seizures elicited by cortical stimulation in most of cats progressively increased with time; right - similar progressive increases were seen for the duration of the OA. Note the failure or resistance of acute seizures induction in the interval days of (20-30) for cat 1.

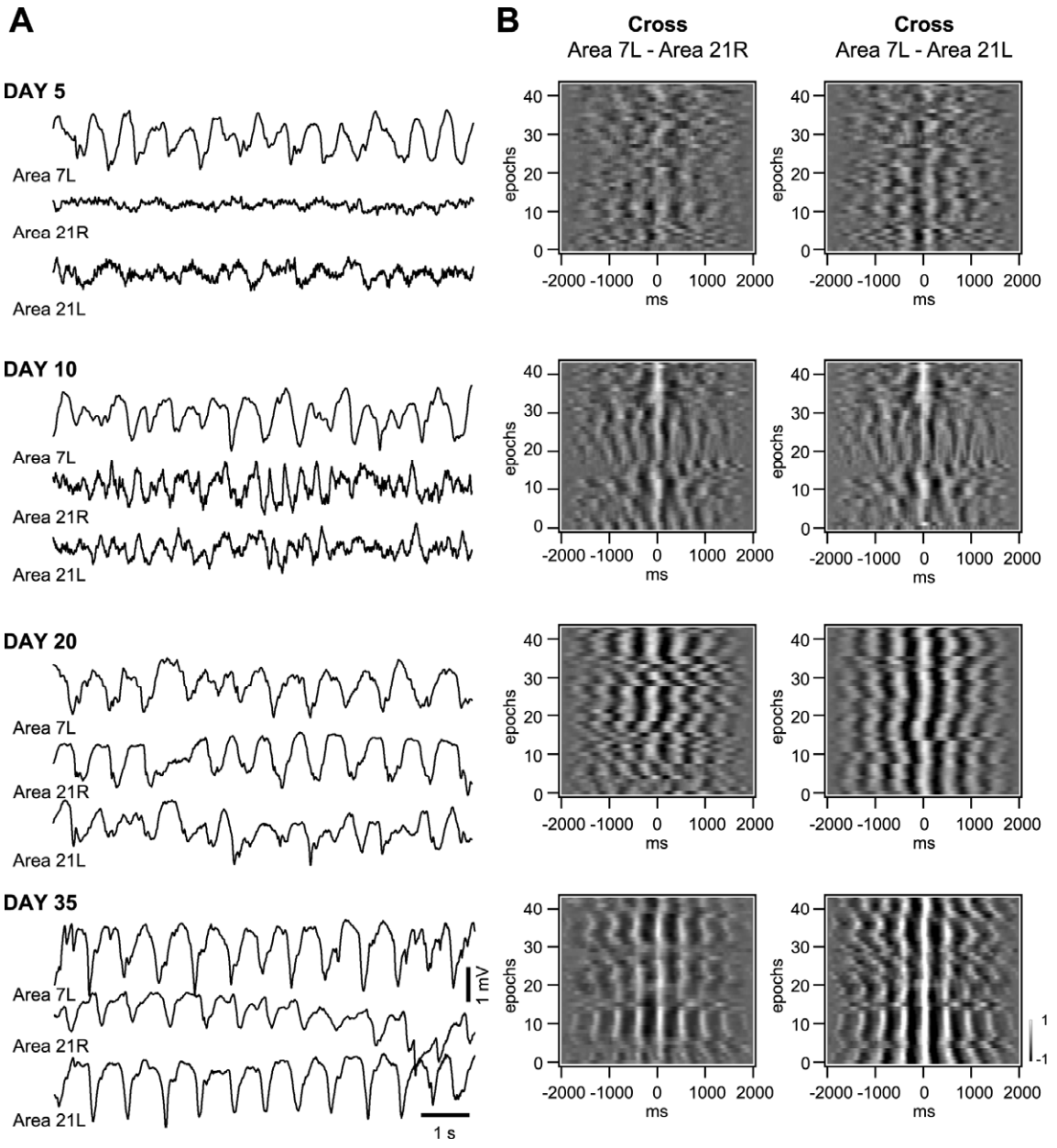

Figure 4-6 Temporo-spatial correlation of outlasting activity (OA). A, panel depicts field recordings of OA from day 5 to day 35 in a chronically kindled cat. Numbers on the left of the EEG represent: 1. middle associative cortex (area 7 left - ipsilateral to stimulation); 2. posterior associative cortex (area 21 right); 3. posterior associative cortex (area 21 left). B, cross-correlations of traces in panel A calculated from 45 consecutive 4-seconds windows indicates an increased coherence in time with stimulation from day 5 to day 35, both in the ipsilateral and contralateral hemisphere.

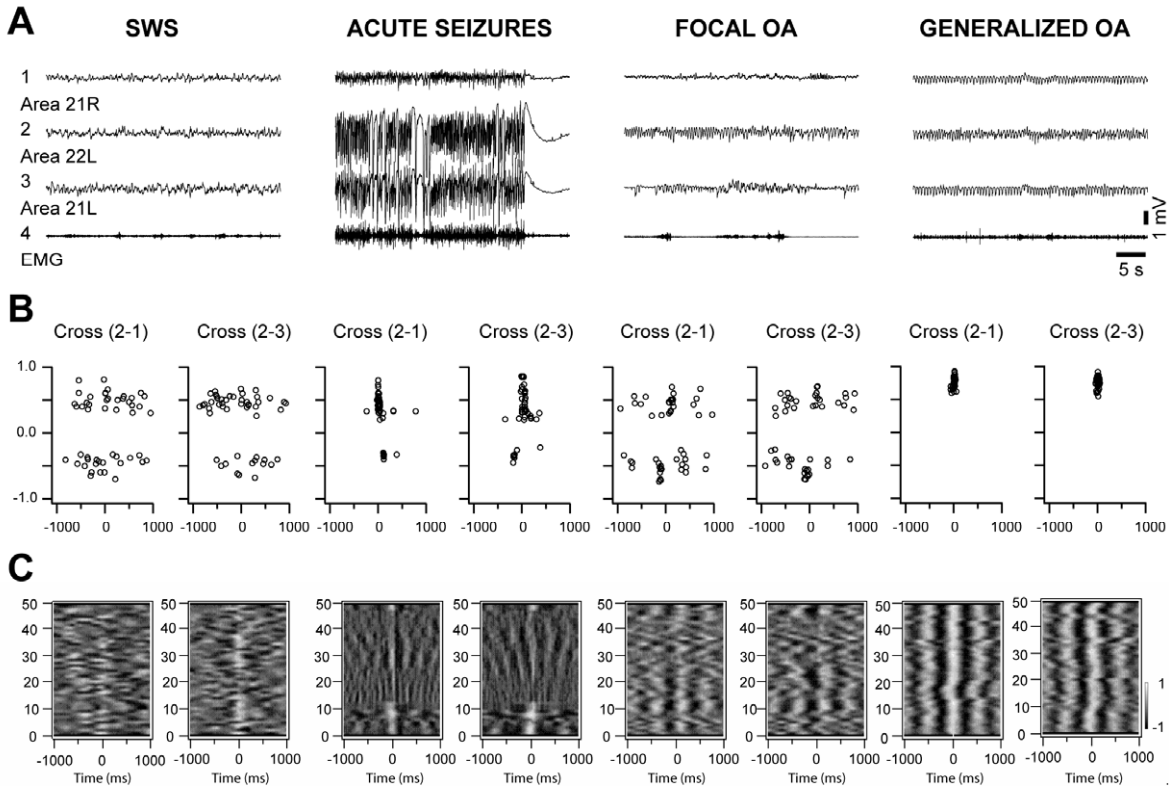

Figure 4-7 Synchronization patterns during slow-wave sleep (SWS), elicited seizures, focal and generalized outlasting activities (OA). A, EEG recordings during SWS, acute seizures, focal (day 5) and generalized (day 17) OA respectively. Scatter plots in the B show the maximal values of cross-correlation amplitude (y-axis) and the delays (x-axis) during different network states. The cross-correlations in each state were calculated from 50 consecutive periods in which a maximum of depth negativity on one of the channels was chosen as zero time and the total duration of segments was ±2000 ms. C, depicts the cross-correlations for all the segments (from 1 to 50).

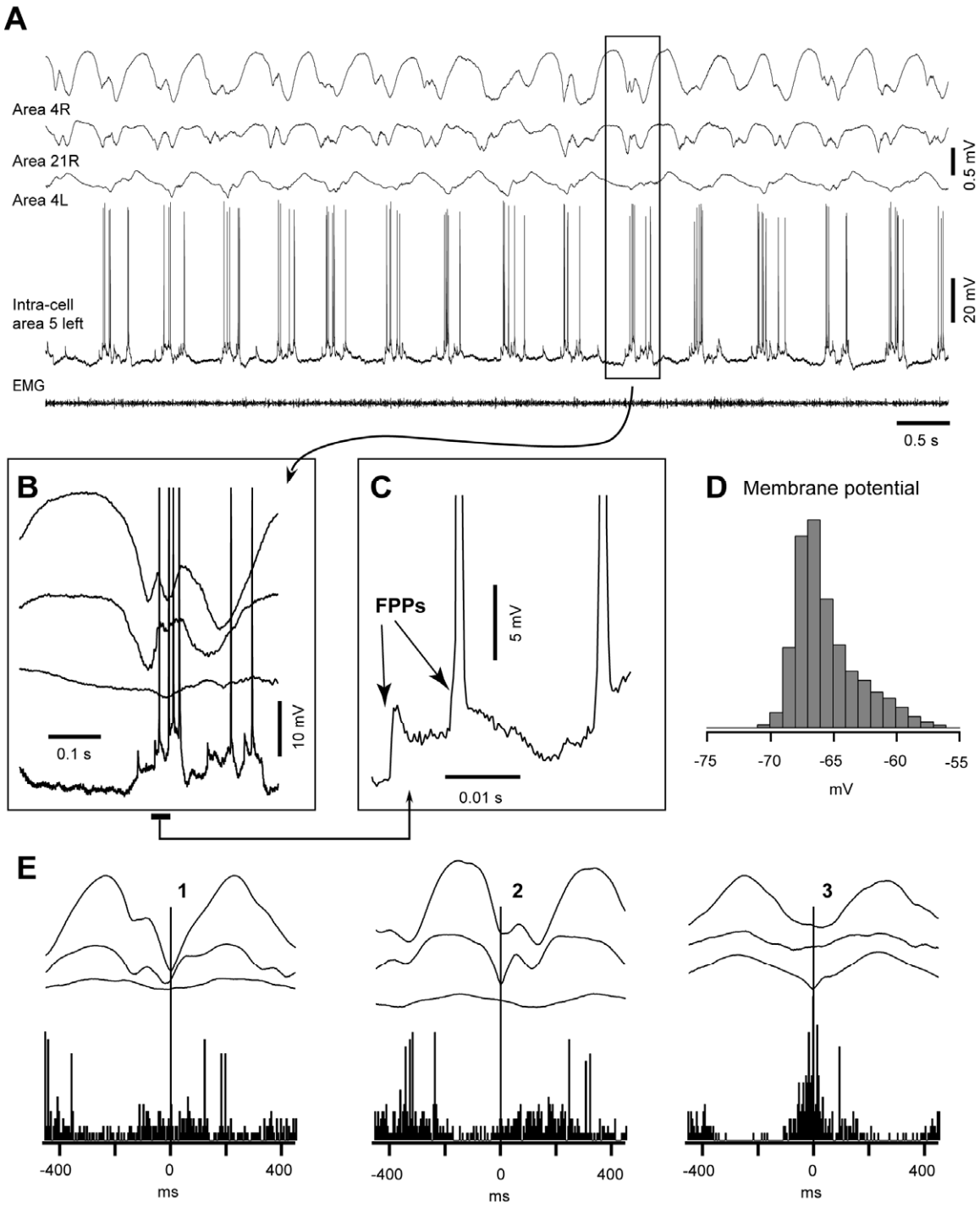

Figure 4-8 Intracellular oscillations during outlasting activities (OA) in a kindled cat (day 10) during wake. A, intracellular recording during OA in area 5. Electrodes were placed in: 1. motor cortex (area 4 right); 2. posterior associative cortex (area 21 right); 3. motor cortex (area 4 left). B, expanded fragment of one oscillatory cycle shown in A. C, further expansion of intracellular trace showing the presence of fast-prepotentials (FPPs). D, Histogram of membrane potential distribution. Note unimodal distribution. E, peri-event spike histograms. (1) maximal depth-negativity of EEG area 4 right is

reference time, (2) EEG area 21 right is reference time and (3) EEG area 21 left is reference time. In day 10 neuron is firing in phase only with field potential from the same hemisphere.

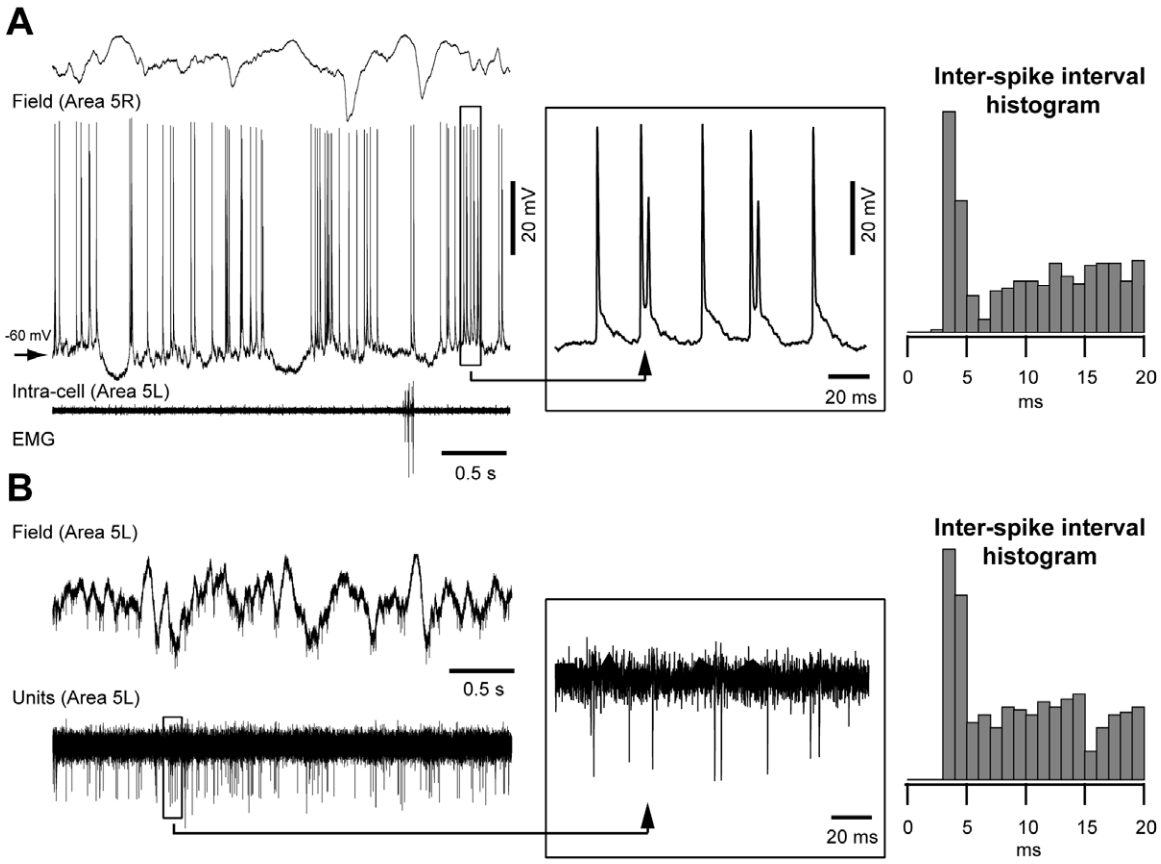

Figure 4-9 Cellular activities of neocortical neurons during outlasting activities in a kindled cat (day 23). A, Intracellular, field recording and EMG during wake. The action potentials are expanded as indicated by arrow. Note the frequently occurring doublets. Histogram of the inter-spike intervals in the right panel indicates an increased incidence of high-frequency bursts. B, Upper trace - local field potentials in area 5 left. Lower trace – unit activity obtained by filtering (0.5-10 kHz) of local field potential displayed in the upper trace. The indicated period is expanded as point out by arrow, and the right panel shows the histogram of the inter-spike intervals in the extracellular unit recording.

5 Cortical and thalamic components of outlasting activities following neocortical kindling in cat

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5.1 Résumé

La stimulation sous-liminale chronique (« kindling ») du cortex cérébral représente un paradigme essentiel utilisé également comme modèle d'épileptogenèse et de neuroplasticité dans le système nerveux central. Nous avons déjà rapporté chez des chats non-anesthésiés avec des implants chroniques d'électrodes l'expression des activités paroxysmiques persistantes (1-2 Hz) à la suite des crises épileptiques aigues induisent par la stimulation électrique corticale. Dans la présente étude, nous avons analysé la synchronisation de l'EEG cortical (de l'aires corticales 4, 5, 7, 21, 17, 18, 22) et de l'EEG thalamique (noyau latéral postérieur ventral), et l'influence des systèmes modulateurs ascendants provenant du tegmentum pedunculo-pontine (PPT) et du locus coeruleus (LC) sur la décharge des neurones thalamiques. Nous nous sommes interrogés si les activités paroxysmiques persistantes observées dans le neocortex des animaux soumis à une stimulation électrique chronique peuvent être expliquées par une décharge des neurones thalamiques de type bouffée de potentiels d'action. La synchronie des potentiels de champ de l'EEG a prouvé que pendant les crises aiguës, les composantes corticales précédaient les composantes thalamiques, mais pendant les ARPs les composantes thalamiques apparaissaient en premier. Pendant les ARPs les neurones thalamiques ont régulièrement déchargé des bouffées de potentiels d'action en synchronie avec les phases négatives de l'ARP. La stimulation électrique du PPT et du LC pendant les ARP a diminué la probabilité de décharge en bouffée des neurones thalamiques et l'amplitude des ARP dans l'EEG au niveau thalamique, mais ni l'un ni l'autre n'a été capable d'abolir complètement les ARPs. Suite à la stimulation du PPT/LC le déphasage fixe existant au préalable entre les activités unitaires neuronales et la phase négative des ARP dans l'EEG au niveau thalamique a été perturbée et les neurones ont déchargé toniquement pendant les deux phases, négative et positive, de l'EEG avec une probabilité égale. Puisque pendant les crises d'épilepsie d'origine corticale les neurones du thalamus sont fortement inhibées par les neurones GABA-ergiques du noyau réticulaire, montrant des potentiels post-synaptiques inhibiteurs phasiques, nous supposons que les ARPs sont engendrées par les neurones thalamo-corticaux une fois la pression inhibitrice des neurones réticulaires enlevée et suite à une

modification dans leur décharge et dans leurs courants intrinsèques apparue pendant la stimulation électrique chronique.

5.2 Abstract

Kindling is an essential operating paradigm of the nervous system extensively used both as a model of epileptogenesis and neuroplasticity. We previously reported in chronically implanted non-anesthetized kindled cats long lasting oscillatory patterns (1.5-2 Hz) called outlasting activities (OA) following the acute seizure (AS) induced by cortical stimulation. Here, we address the question if OA observed in the neocortex of kindled animals are generated exclusively by the cortical networks or if they also rely on the burst firing of thalamic neurons. We analyzed the electrophysiological patterns of synchronization of cortical EEG (areas 4, 5, 7, 21, 17, 18, 22) and thalamic field (EThG) (ventral posterior lateral nucleus - VPL), and the influence of modulatory systems originating in the pedunculo-pontine tegmentum (PPT) and locus coeruleus (LC) on the discharge pattern of thalamic neurons during OA. Synchrony analysis of field recordings showed that during AS cortical activity preceded thalamic activity, while during OA this sequential order was reversed. During OA thalamic neurons regularly discharged bursts with the frequency of OA. The electrical stimulation of either PPT or LC during OA decreased both the probability of bursts in thalamocortical neurons and the amplitude of OA. Yet, neither of them was able to completely block the expression of OA. Following PPT/LC stimulation the previously locked phase-shift between units and the depth negative phase of thalamic EEG waves was disrupted and neurons discharged tonically during both the depth negative and positive phase of EEG waves with an equal probability. We conclude that thalamus is involved in the generation of OA but that it does not play an exclusive role.

5.3 Introduction

Kindling is an essential operating paradigm of the nervous system, extensively used both as a model of epileptogenesis and neuroplasticity. In kindling, repeated administration of a weak stimulus that initially evokes no behavioral response produces gradually increasing paroxysmal EEG patterns and behavioral seizures over the course of time, ultimately leading to generalized tonico-clonic seizures {Goddard, 1967 21481 /id}. Once the effect of kindling is established, the induced change is persistent so that even administration of a weak stimulus elicits generalized seizures (Goddard et al., 1969; McNamara, 1984). Kindling can be produced by administering various electrical or chemical convulsive agents to different regions of the nervous system (Goddard et al., 1969; McNamara et al., 1980; McIntyre et al., 2002), and several reviews have considered kindling as a model for clinical epilepsy (McNamara et al., 1980; Racine and Burnham, 1984; Sato et al., 1990; McNamara, 1994; Loscher, 1997). Another particularity of kindling is that, with a few exceptions (e.g. Cavazos and Sutula, 1990; Sutula et al., 1994), it is still widely accepted as a functional epilepsy model in which the altered neuronal response develops in the absence of gross morphological damage, such as that seen in many other epilepsy models.

Among the wide range of mechanisms that may account for epileptogenesis during kindling synaptic potentiation and new circuitry formation play a major role (reviewed in Morimoto et al., 2004). However, by difference with previous studies demonstrating kindling-induced morphological changes at the dendritic and synaptic level in the hippocampus (Geinisman et al., 1988; Sutula, 1990; Hawrylak et al., 1993; Jiang et al., 1998) and amygdala (Nishizuka et al., 1991; Okada et al., 1993), there has been a lack of evidence of dendritic variations in the neocortex following kindling (Teskey et al., 1999). In the single documented attempt, Racine and coworkers found no changes in dendritic branching or spine density in the anterior cortex of cortically kindled rats (Racine et al., 1975). Thus, there is no evidence at the structural level that would support the hypothesis of an extensive role of neocortex in epileptogenesis during cortical kindling. Furthermore, neocortical kindling is characterized by a relatively high threshold of elicited acute seizure (AS), unstable seizure development, and difficulty in establishing a stable generalized convulsive seizure state (reviewed in Wake and Wada, 1976; Majkowski et al., 1981; Okamoto, 1982).

Therefore, is it reasonable to address the question if the paroxysmal long-lasting patterns of oscillatory activities observed in the neocortex of kindled animals following the initial AS, called outlasting activities (OA) (Timofeev et al., 2005; Nita et al., 2006), is rather generated by thalamic than cortical networks. Corticothalamic (CT) neurons are reciprocally interconnected with thalamocortical (TC) neurons from different dorsal thalamic nuclei, and also project to reticular (RE) neurons. This circuit generates both the physiological EEG rhythms occurring during natural states of vigilance (Steriade et al., 1993), and the pathological developments of the normal brain oscillations into seizures (reviewed in Steriade, 2003; Timofeev and Steriade, 2004).

During cortically-generated spike-wave (SW) seizures in the intact thalamocortical circuit only a minority of TC neurons fires low-threshold spike-bursts during SW seizure, while the vast majority is steadily hyperpolarized and exhibits phasic inhibitory post-synaptic potentials (IPSPs) induced by over-excited GABA-ergic reticular neurons (Steriade and Contreras, 1995; Pinault et al., 1998; Timofeev et al., 1998; Steriade and Timofeev, 2001; reviewed in Crunelli and Leresche, 2002). However, some recent studies in kindling and pilocarpine models of epilepsy reported that the development of spontaneous recurrent seizures in the cortico-thalamic system is associated with increased T-type calcium currents in thalamic neurons, which may underlie the bursts of action potentials participating in SW activity (Bertram et al., 2001; Su et al., 2002).

In the present study we aim at understanding the differential contribution of the neocortex and thalamus in the generation of OAs, using EEG and multiunit recordings in kindled cats in vivo.

5.4 Materials and methods

5.4.1 Animal preparation

Experiments were performed on 5 cats of both sexes. Surgical procedures were carried out in sterile condition under barbiturate anesthesia (30 mg/kg i.v.), following a pre-medication with acepromazine (0.3 mg/kg i.m.), butorphanol (0.3 mg/kg i.m.), atropine (0.05 mg/kg i.m.) and ketamine (20 mg/kg i.m.). The level of anesthesia was continuously monitored by the aspect of electroencephalogram (EEG) and cardiac frequency (aiming 90-110

beats/min). Oxygen saturation of the arterial blood and end-tidal CO2 were also monitored. General surgical procedures included: cephalic vein canulation for systemic liquid delivery (lactated Ringer's solution 5-10 ml/kg/h) and lidocaine (0.5%) infiltration of all pressure points or incision lines. Body temperature was maintained between 37–39°C with a heating pad.

Coaxial bipolar EEG electrodes (FHC Inc., USA) (with the inner pole in the cortical depth at about 0.8-1 mm and the outer pole placed at the cortical surface) were bilaterally placed in the motor cortex, anterior and posterior associative cortex, auditory cortex, primary and secondary visual cortex (cortical areas 3, 4, 5, 7, 17, 18, 21, and 22); and a bundle of electrodes used to record the neuronal firing pattern was stereotaxically lowered in the thalamic ventral posterior lateral (VPL) nucleus. For extracellular unit recordings we used tungsten microelectrodes with tip impedances between 9.3-11.4 M∧ (FHC Inc., USA). Additional pairs of recording electrodes were placed around the orbit and neck muscles in order to monitor the states of vigilance by recording the electro-oculogram (EOG) and electromyogram (EMG). In all animals, three Ag/AgCl references were implanted: two of them along the skull in the region of the external auditory canals on both sides, and one over the nasium in the frontal bone (see schema in Fig. 5-1A).

In all cats the electrode used for stimulation was placed in the left associative cortex (area 5). The kindling stimuli consisted in 40-60 s of a 50 Hz rectangular pulse-train with stimulation intensity in the range of 0.5-1.5 mA. Our stimulating parameters lie within the range of values used in the majority of kindling studies. Kindling procedure consisted in stimulating the cortical site 5 times per day up to occurrence of AS followed by OAs (after 5-7 days of kindling). After this moment, we applied 1-2 electrical stimulation per day up to the end of experiments.

Bipolar concentric stimulating electrodes (FHC Inc., USA) were placed into the locus coeruleus (LC) nucleus (stereotaxic coordinates: 1 mm posterior, 3 mm lateral and -1 mm depth) and peduculopontine tegmental (PPT) area (stereotaxic coordinates: 1 mm anterior, 3 mm lateral and -2.5 mm depth) (Reinoso-Suarez, 1961). The correct placement of electrodes was tested by passing stimulating current pulses through the electrodes (0.1 ms

pulse duration, 100 Hz at 0.1–1.0 mA for 2 seconds) seeking an activating effect in the EEG, and was confirmed by electrolytic lesions on Nissl (thionine) stained sections.

The skull was reconstituted using acrylic dental cement and a few bolts were placed in the cement to allow non-painful fixation of the cat's head in a stereotaxic frame. Animals were kept under observation up to the full recovery and they received analgesic medication (anafen 2 mg/kg s.c.) for the next 48-72 hours. After the recovery period (2–3 days), cats were trained to stay with the head restrained in a stereotaxic frame and the body suspended in a rubber bag which allowed free body movements for 2–4 h/day, and usually in less than a week they were fully conditioned displaying clearly identifiable states of waking, SWS, and REM sleep, and being able to stay in the frame for several hours.

Behavioral responses evoked by the stimulation were monitored using a night-shoot video surveillance camera. Generalized behaviorally manifest seizures appearing outside the stimulating session were considered an "end limit point" of these experiments, as consented with the local ethic committee.

At the end of experiments, or at the first sign of clinically manifest seizures outside the experimental protocol, animals received a lethal dose of barbiturate. All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Committee for Animal Care of Laval University. Every effort was made to minimize the number of animals used and their suffering.

5.4.2 Data analysis

Extracellular EThG consisted in both unit and multiunit recordings. Analysis was performed only on data originating from individual units. In the case of a multiunit recording the extraction of putative single units from extracellular recording traces was performed in three steps: i) signal preprocessing, ii) event extraction, and iii) spike sorting. All analysis was performed in Matlab (The MathWorks, Natick, MA) with a custom designed algorithm. For this process, each electrode was treated separately since no time-locked events on several recording sites were observed. i) The extracellular traces

(sampling rate 20 kHz) were digitally filtered (Butterworth filter, 10th order, cut-off frequencies [400 Hz, 5000 Hz], Matlab) to remove the slow frequency signal component not due to action potential firing. ii) The filtered signal was subjected to a manually chosen threshold (negative threshold of minus five standard deviations of the extracellular trace) to extract presumed spikes. Each time the threshold was crossed, a corresponding 2 ms waveform snipped was extracted and stored as a vector (length: 40 samples). Events that were clearly artifacts based on their amplitude were manually excluded. iii) Putative single units were found with a spike sorting algorithm (adapted from Fee et al., 1996).

The spike sorting was a two-step process. First, the waveform vectors were grouped into an overly large number of subclusters with the K-means clustering algorithm. The number of subclusters was manually set to value around ten times as high as the number of expected single units to be found. Subclusters that consisted of less than twenty waveform vectors were excluded from the subsequent analysis. Second, subclusters were iteratively merged to form clusters that represent putative single units. Linkage analysis of the subcluster centroids was used as a guide for manual merging of subclusters. Finally, spike sorting results were assessed by testing for refractory period violations and by visual inspection of resulting waveform clusters in a reduced two-dimensional space determined by principal component analysis. Waveform stability was evaluated by plotting the first principal component of all spikes as a function of time.

Inter-spike intervals <5 ms were considered bursts and were counted on 5 epochs of 5 minutes each during waking state. Averaged values from all experimental animals for spontaneous burst incidence were normalized to the incidence observed in the last day of the kindling protocol. Cross-correlograms between cortical and thalamic electrodes were performed on 1-second windows with 50% overlap, color coded, and successively displayed in dynamic cross-correlogram graphs (Fig. 5-2D). FFT quantifications (Fig. 5-3C and 5.4C) were performed by averaging the area under the FFT graph on the 0-4 Hz window in all 5 experimental animals from 5 different epochs. The average EEG amplitude was computed as a mean of the amplitudes of voltage deflection between successive positive and negative EEG peaks on a 1 minute window. Autocorrelograms (Fig. 5-5B and 5-6B) were generated on successive windows of 5 sec length. Threshold for statistical

significance was $p<0.05$ (paired Student t-test). The probability of discharging during the negative or positive phase of the OA was calculated for each presumed single unit on a 10-second window before the stimulation of PPT or LC and on a 10-second window that followed the stimulation.

5.5 Results

Acute elicited seizures consisting in spike-wave (SW) complexes at 1-2 Hz (Fig. 5-1B) were evoked after 4-5 days of suprathreshold electrical stimulation of the neocortex. As we previously reported this initial AS is followed by a postictal depression and, afterwards, by a pattern of long-lasting paroxysmal EEG oscillation with a frequency of ~2 Hz, called "outlasting activities" (Timofeev et al., 2005; Nita et al., 2006). OA proved to be dramatically influenced by the natural occurring states of vigilance in cortical and thalamic networks; being apparent during waking state and slow-wave sleep (SWS), and completely abolished during rapid eye movements (REM) sleep (Nita et al., 2006).

Since CT and TC neurons are tightly interconnected in a circuitry that generates the main EEG rhythms and mediates the pathological developments of normal brain oscillations into seizures (Steriade et al., 1993), we aimed at understanding the involvement of TC neurons in the expression of OAs. As we previously showed (Timofeev et al., 2005; Nita et al., 2006), the main frequency of OA (1.5-2 Hz) matches the frequency of intrinsic delta oscillation of the TC neurons (McCormick and Pape, 1990b). Therefore we envisaged that TC neurons could mediate the generation of OA. Extracellular multiunit recordings performed during waking state in VPL nucleus revealed the occurrence of bursts of action potentials in the firing pattern of TC neurons (Fig. 5-1C and D) with an inter-spike interval of 4-5 ms. The gradual increase in incidence of spontaneously occurring bursts over the course of the kindling procedure is presented in Fig. 5-1D as incidence frequency normalized to the frequency of bursts observed on the 30th day after the occurrence of the first AS.

To further study the role of the thalamus in the generation of OA we correlated the EThG from VPL nucleus with the EEG from the target cortical areas during the SWS, during both the initial and final periods of the AS, and during OA (Fig. 5-2A and B), starting from day

20 of the kindling protocol when OAs were generalized on the whole cortical surface. During SWS the EEG of the cerebral cortical area 5 and VPL nucleus showed a high level of correlation (average peak level of 0.86±0.07) and a minimal time shift of few milliseconds. At the onset of the stimulation-evoked cortical AS the EThG recording from the VPL nucleus did not display highly synchronous activities by difference with the cortical EEG. The average correlation coefficient was decreased to 0.43±0.17. As the AS developed thalamus became gradually involved and, sometimes, EThG recordings displayed sharp spike-waves at the end of the AS. In these cases the average coefficient of correlation increased to 0.72±0.12 and the peak in the cross-correlogram was shifted to 71.34±9.21 ms, showing a correspondingly delay of thalamic activities. When OA expressed after the postictal depression the average correlation level was 0.67±0.16 but the peak in the correlogram was now shifted in the opposite direction by an average of -94.85±14.32 ms. These observations demonstrate that during OA activation of the thalamus is prior to the involvement of the cortex on each cycle of the paroxysmal oscillation.

Since thalamus seemed to play a major role in the generation of the OA by both showing an increased incidence of bursting neurons, which further can recruit cortical neurons in the oscillatory pattern of OA (Fig. 5-1), and by preceding the target cortical areas in the expression of OA (Fig. 5-2), we attempted to block the generation of OA by electrical activation of two modulatory brainstem systems: the cholinergic pedunculo-pontine tegmantal area (PPT) and noradrenergic locus coeruleus (LC), which both depolarize TC neurons and thus reduce intrinsic bursting (McCormick and Pape, 1988; Pape and McCormick, 1989; Curro Dossi et al., 1991; McCormick, 1992a; McCormick, 1992b).

The stimulation of PPT during SWS induced an EEG activation (Fig. 5-3A) revealed by a decrease in the amplitude of cortical EEG from 1.27±0.16 mV to 0.14±0.15 mV and of EThG from 1.32±0.07 mV to 0.18±0.09 mV ($p<0.05$). This activation was accompanied by an amplitude diminution of oscillations in the 1-4 Hz range (Fig. 5-3E). The power in the 0-4 Hz band of the power spectrum significantly decreased ($p<0.05$) from 2.11±0.32 mV^2 x Hz to 1.21±0.34 mV^2 x Hz for cortical electrodes, and from 1.81±0.17 mV^2 x Hz to 1.21±0.16 mV^2 x Hz for thalamic electrodes (Fig. 5-3D). Both variation were statistical significant ($p<0.05$). However, when PPT electrical stimulation was applied during OA

(Fig. 5-3B) the average amplitude of cortical components of OA only slightly decreased from 1.61±0.21 mV to 1.53±0.19 mV (not statistically significant), while the amplitude of the OA recorded in the thalamus decreased from 2.71±0.29 mV to 1.93±0.3 mV ($p<0.05$)(Fig. 5-3E). The diminution of the amplitude of the 2 Hz FFT peak of thalamic component of OA was reflected in a decrease in the area of the 0-4 Hz frequency band from 3.11±0.2 mV^2 x Hz to 2.65±0.18 mV^2 x Hz ($p<0.05$), while the corresponding frequency band for cortical electrodes did not significantly change (from 1.96±0.25 mV^2 x Hz to 1.99±0.27 mV^2 x Hz - Fig. 5-3C, D).

A similar behavior of the EEG amplitude and of the components of the power spectra for cortical and thalamic EEG electrodes was observed following the electrical stimulation of LC (Fig. 5-4, A-C). In control condition, during SWS, LC stimulation decreased the amplitude of the cortical EEG from 1.25±0.14 mV to 0.18±0.16 mV ($p<0.05$) and of the thalamic EThG from 1.31±0.08 mV to 0.23±0.11 mV ($p<0.05$)(Fig. 5-4E). The frequency band measuring slow rhythms decreased in power from 1.66±0.31 mV^2 x Hz to 1.03±0.28 mV^2 x Hz in cortical electrodes ($p<0.05$), and from 1.31±0.15 mV^2 x Hz to 0.23±0.12 mV^2 x Hz in thalamic electrodes ($p<0.05$, Fig. 5-4D). During OA the amplitude of the cortical EEG slightly changed from 1.5±0.24 mV to 1.52±0.23 mV (not statistically significant), and the thalamic EThG amplitude decreased from 2.5±0.28 mV to 1.7±0.25 mV ($p<0.05$) (Fig. 5-4E). The area under the 2 Hz FFT peak of OA was not significantly changed in the power spectrum of cortical EEG (1.44±0.21 mV^2 x Hz vs. 1.39±0.26 mV^2 x Hz) but it was significantly diminished in the power spectrum of thalamic electrodes (2.91±0.3 mV^2 x Hz vs. 2.17±0.29 mV^2 x Hz, $p<0.05$) (Fig. 5-4D).

The main frequency peak in the power spectra during OA was not shifted by either PPT or LC electrical stimulation, and remained stable at a frequency of ~2 Hz (Fig. 5-3C and 5-4C).

Furthermore, the dynamic cross-correlation between cortical and thalamic field EEG before and after the electrical stimulation of cholinergic (Fig. 5-5A) and noradrenergic (Fig. 5-6A) modulatory systems showed a desynchronization in the phase shift of the correlogram peak amplitude triggered by the stimulation. Before the stimulation of either PPT (Fig. 5-5A) or LC (Fig. 5-6A), the peak of the correlogram remained stable at ~ -95 ms indicating a fixed

delay between the generation of OA waves in the thalamus and the involvement of the cortex. Following the activation of thalamus, the fixed delay faded away and the position of the peak in the correlogram changed from sweep to sweep. These variations in the synchrony of the thalamocortical system were probably elicited by the changes of the firing pattern of thalamic neurons depicted by the auto-correlograms of thalamic extracellular unit recordings before and after the stimulation of the PPT (Fig. 5-5B) and LC (Fig. 5.6B). In both cases activation through PPT/LC stimulation was accompanied by a diminution in the probability of burst-firing from ~40% to 20%.

The mechanism which may account for the decreased amplitude of the thalamic EEG component of OA and for the desynchronization of OA between thalamus and cortex following the activation of cholinergic and noradrenergic modulatory systems could be based on the spike timing of thalamic neurons relative to the phase of the OA. Since both PPT and LC neurons depolarize TC relay neurons and cause switching from a synchronous bursting pattern to a tonic desynchronized discharge, we further investigated the phases of spikes relative to the OA oscillation in the thalamus.

From multiunit recordings in the thalamic VPL nucleus we extracted individual units based on their extracellular action potential waveform (Fig. 5-7A). We counted every occurrence during the positive or negative phases of OA for each putative single unit (Fig. 5-7B). The probability of firing for 10 isolated units obtained from extracellular recordings in the VPL nucleus during OA, before and after the stimulation of the PPT, is depicted in Fig. 5-7C. During the OA the vast majority of units discharged during the negative phase of the OA (average probability for firing on the negative half-cycle was $p=0.74$). The depolarization of thalamic neurons and the change in the firing pattern from bursting to tonic firing described above decreased the probability of firing during the negative phase of the OA to 0.51, a value similar to the average probability of firing during the positive phase (0.49). Thus, activation of neuromodulatory systems indeed disrupted the grouping of thalamic action potentials by the OA oscillatory pattern at the single unit level.

5.6 Discussion

The present study reports some important findings regarding the mechanisms of generation of OA induced by kindling and the effects of modulatory systems on OA. First, we showed a continuous increase in the incidence of spontaneous burst firing of TC neurons from VPL nucleus during cortical kindling. Second, we found that cortex and thalamus are sequentially involved in the generation of seizures in kindled cats. As previously reported in anesthetized animals (Steriade and Contreras, 1995; Polack and Charpier, 2006) cortical paroxysmal activities precede thalamic activities during the AS. However, thalamic discharges precede cortical discharges during OA. Third, despite the fact that PPT/LC stimulation showed a strong activating effect when applied during naturally occurring SWS, activation via PPT/LC during OA did not succeed in stopping the paroxysmal discharges. However, it diminished the amplitude of OA at thalamic but not at cortical level without changing the mean frequency of the OA. Finally, we determined that the synchrony between cortical and thalamic oscillations was impaired following PPT/LC stimulation as TC neurons changed their firing pattern from bursting to tonic and the fixed phase-lock between cellular activities and field oscillation was lost. Overall our study suggests that thalamus contributes to the generation of OA.

OA represent a spontaneous paroxysmal event with a rhythmic activity at ~2 Hz that follows the AS induced by electrical stimulation. OA has never before been observed following acute electrical stimulation of the cerebral cortex under anesthesia (neither barbiturate, nor ketamine-xylazine), although this manipulation constantly elicits seizures (reviewed in Timofeev and Steriade, 2004). Therefore, it is reasonable to presume that OA represent the outcome of some plastic mechanisms occurring following repetitive seizures and that the activity of neuromodulatory systems may play an important role.

Both in humans and in experimental animals, repetitive seizure activity as it occurs during kindling or status epilepticus results in a large number of plastic changes (Coulter and DeLorenzo, 1999; Sloviter, 1999; Ben-Ari, 2001). These changes in the neuronal function may consist either in modified synaptic function or in intrinsic membrane properties adjustments. Up to now, a multitude of structural and functional synaptic changes have been shown to follow repetitive seizures, such as reorganization of excitatory axons (Sutula

et al., 1989), altered function of excitatory neurotransmitter receptors (Turner and Wheal, 1991; Lothman et al., 1995), or changes in GABAA receptor-mediated inhibition (Gibbs, III et al., 1997; Cossart et al., 2001).

Much less is known about the changes in intrinsic neuronal properties. A study in which status epilepticus induced by pilocarpine progressed to a chronic epileptic condition resembling human temporal lobe epilepsy (Turski et al., 1983) suggested that such alterations may be highly significant, and a more recent study found a marked up regulation of intrinsic neuronal bursting associated with the development of temporal lobe epilepsy (Sanabria et al., 2001). Although almost all pyramidal neurons in the hippocampal CA1 area are regular spiking cells in normal conditions (Jensen et al., 1994), ~50% of CA1 pyramidal cells in hippocampal tissue removed from rats that experienced repetitive seizures were found to be intrinsically burst-firing cells. Moreover, the upregulation of intrinsic bursting seemed to result from the de novo appearance of Ca^{2+}-dependent bursting that is not ordinarily seen in this class of principal hippocampal neurons (Azouz et al., 1996). The density of T-type Ca^{2+} currents, but not of other pharmacologically isolated Ca^{2+} current types, was upregulated in CA1 pyramidal neurons after status epilepticus (Su et al., 2002).

Thalamus is a hub for information processing in the brain and is interconnected with neurons from thalamic reticular nucleus and from different cortical areas in a circuit which generates both the physiological EEG rhythms occurring during natural states of vigilance (Steriade et al., 1993), and the pathological developments of the normal brain oscillations into seizures (reviewed in Steriade, 2003; Timofeev and Steriade, 2004). Human neuroimaging studies in patients with temporal lobe epilepsy have shown changes both in ictal and interictal perfusion and in metabolism in the thalamus (Henry et al., 1993; Yune et al., 1998; Blumenfeld et al., 2004) suggesting an involvement in SW seizures synchronization. Also, reduced thalamic volume was described (Margerison and Corsellis, 1966; DeCarli et al., 1998), which may possibly occur as a degenerative consequence of repetitive seizures. Therefore changes in the intrinsic neuronal properties of TC neurons may play an important role in the synchronization of SW seizures and in the generation of OA. Some recent studies reported increased T-type calcium currents in thalamic neurons,

which may underlie the bursts of action potentials participating in SW activity (Bertram et al., 2001; Su et al., 2002), and also increased thalamic T-type calcium channel gene expression in a pilocarpine model of epilepsy (Graef et al., 2006). Such intrinsic changes of the T-type calcium channel may account for the bursting discharge pattern of TC neurons (Fig. 5-1) and may be associated with the development of spontaneous recurrent seizures in the corticothalamic system.

TC neurons function in two different modes: either tonically discharging at depolarized membrane potentials or slow-bursting at hyperpolarized levels of the membrane potentials (see for review Steriade et al., 1997). The existence of these two different firing regimes result from their two main intrinsic electrophysiological currents: i) a transient Ca^{2+} current (I_T) that deinactivates for hyperpolarized membrane potentials and mediates low-threshold-spikes (LTS) crowned by rebound spike-bursts (Llinas and Jahnsen, 1982; Steriade and Llinas, 1988), and ii) a hyperpolarization-activated cation current (I_H) that produces a depolarizing sag (McCormick and Pape, 1990a; McCormick and Pape, 1990b; Leresche et al., 1991). Neuromodulators can depolarize TC cells and switch the intrinsic activity pattern from bursting to tonic firing.

Thus, TC neurons operate under the lilt of modulatory systems originating in the brainstem and basal forebrain (Steriade et al., 1997). The direct PPT projection to thalamocortical neurons in felines and primates (Pare et al., 1988; Steriade and Llinas, 1988) produces a powerful excitation at their targets. The muscarinic component of this prolonged depolarization is associated with an increase in input resistance (Curro Dossi et al., 1991), which explains the increased responsiveness of TC neurons during brain-activated states of waking and REM sleep (Glenn and Steriade, 1982). Norepinephrine released in the thalamus by locus coeruleus neurons depolarizes thalamic RE neurons, and in the very few investigated dorsal thalamic nuclei it depolarizes also the thalamocortical neurons and enhances a hyperpolarization-activated cation current through β-adrenoreceptors (McCormick and Pape, 1990a).

We tested here the effect of PPT/LC stimulation on the expression of OA since REM sleep is the sleep stage most resistant to the propagation of epileptic EEG activities based on the

clinical experience (Shouse MN et al., 2000), whereas SWS is considered to facilitate the epileptiform discharges (Dinner DS, 2002; Gigli GL et al., 1992; Niedermeyer E and F Lopes da Silva, 2005). LC neurons typically discharge during active waking, decrease their firing during SWS and are silent during REM sleep (McCarley and Hobson, 1975; Rudolph and Antkowiak, 2004). They either excite or inhibit their target neurons in the brain and spinal cord, depending on the type of adrenoreceptors (in general, α1-adrenoreceptors are associated with depolarization through closing K^+ channels, and α2-adrenoreceptors are associated with hyperpolarization by opening K^+ channels) (see for review Jones, 2005). On the other hand, cholinergic pontomesencephalic neurons discharge during waking, decrease firing during SWS and increase firing during REM sleep and facilitate cortical activation through nicotinic and muscarinic receptors (Curro Dossi et al., 1991; McCormick, 1992b). In our experiments the stimulation of neither of these structures was able to completely block the occurence of OA. Yet, we succeeded in activating the cortical EEG and waking up the animals (Fig. 5-3 and 5-4). This may suggest that the plastic changes in the intrinsic properties of thalamocortical neurons following kindling overcome the physiological mechanisms regulating thalamic excitability by neuromodulators.

We were not able to completely investigate the behavioral seizures since the limiting point of our experiments was the occurrence of behavioral seizures in kindled cats, as agreed with the local committee for animal protection. Ethical considerations also limited us in providing definitive evidence for the role of the thalamus in the generation of OA. We predict that OA is preserved in isolated thalamus and is absent from all other target structures, including the cerebral cortex, but complete isolation of these structures in vivo in non-anesthetized cats cannot be envisaged. However, in two experiments, we kindled cats that underwent cortical deafferentation of the suprasylvian gyrus - an experimental model that we currently use to study epilepsy related to cortical deafferentation. The amplitude of the OA was much diminished in the deafferented cortex in those experiments (data not shown). This observation further supports a contribution of extracortical structures to the generation of OA. These data are not extensively discussed here since it is difficult to distinguish between epileptogenesis of deafferentation-related seizures (Timofeev and Bazhenov, 2005) and epileptogenesis during kindling.

Our results demonstrate plastic changes in the firing pattern of thalamic neurons following kindling and differential roles for the cortex and the thalamus in the generation of seizures following kindling (Fig. 5-8). The initial AS that follows cortical stimulation probably has a cortical origin, and cortical volleys impinging on RE inhibitory thalamic neurons induce strong IPSPs in TC neurons. As a result, TC neurons do not display SW complexes at the beginning of the AS, while at the end of the AS TC neurons may display rebound spike-bursts. Cortical SW complexes precede thalamic SW activity. During OA, however, thalamic neurons released from inhibition by reticular neurons may fire bursts of action potentials in the frequency range of intrinsic delta oscillation (1-4 Hz) with thalamic components of OA preceding cortical components. The involvement of thalamus in OA generation is further supported by the fact that PPT/LC stimulation decreases the amplitude of OA at thalamic but not at cortical level. We found that neuromodulation changes the pattern of neuronal firing from bursts to tonic firing and desynchronizes the relation between thalamic cellular discharges and the phase of OA. The persistence of OA at cortical level suggests that neocortex is able to sustain these oscillations even in the absence of an active contribution of the thalamus.

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5.9 Figures

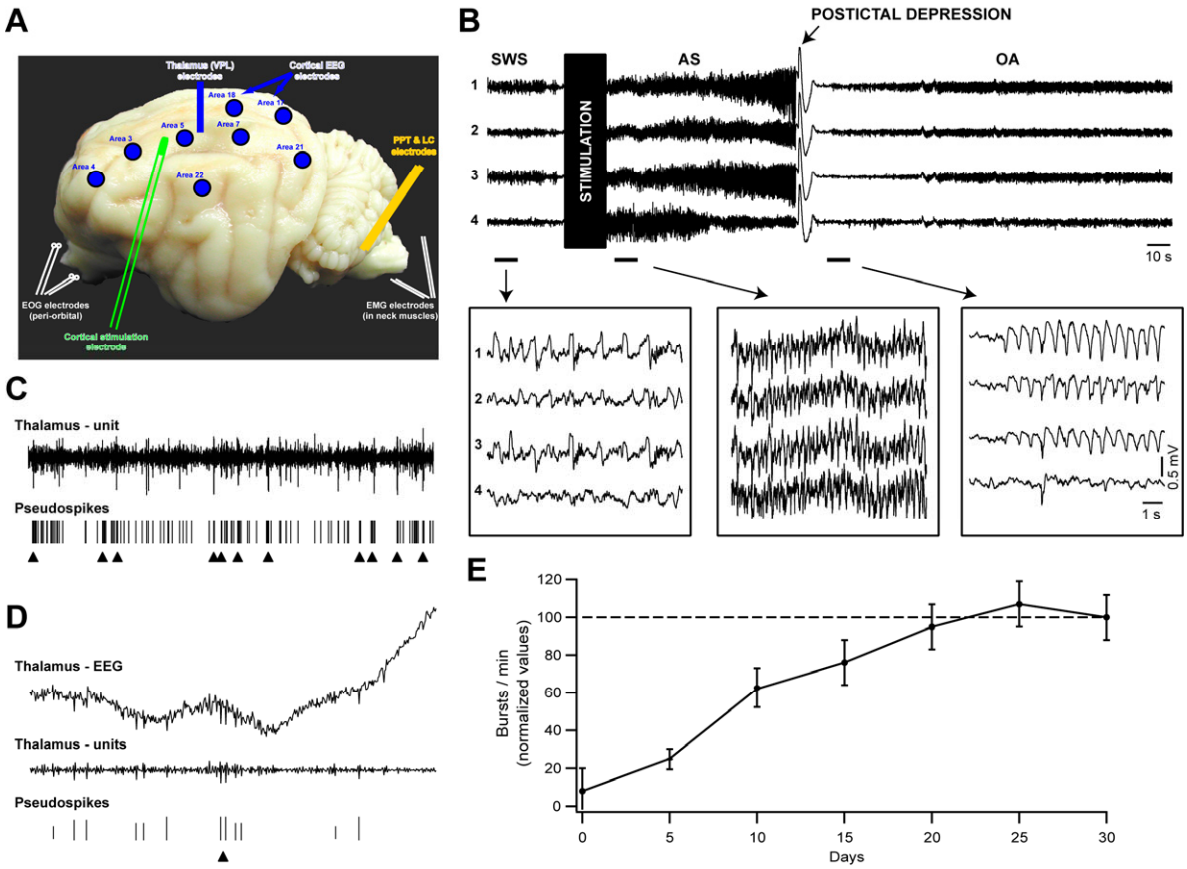

Figure 5-1 Development of burst firing in the thalamus during kindling. A) Experimental paradigm. EEG electrodes (circles) are depicted in blue together with the corresponding cortical area, the cortical stimulation electrode in green, periorbital EOG and the EMG electrodes placed in neck muscles are indicated in white, while the electrodes used for PPT/LC stimulation are depicted in orange. B) Top panel contains four cortical EEG traces illustrating the occurrence of outlasting activities (OA) following the postictal depression which comes after the initial acute seizure (AS) induced by cortical electrical stimulation. Insets depict the expansions from the underlined periods in upper panel. Numbers on the left of the EEG recordings stand for: 1. left motor cortex (area 4), 2. left anterior associative cortex (area 5), 3. left posterior associative cortex (area 7), 4. left visual cortex (area 21). C) Extracellular unit recordings in the thalamus and identification of spikes. Bursts of action potentials (interspike interval < 5 ms) are indicated by black triangles. D) Expanded example of burst recorded in the thalamus (indicated by a black triangle) depicted as field EEG, filtered unit recording and pseudospikes. E) Average frequency of spontaneously occurring bursts in the thalamus during kindling procedure. Bursts were counted on 5 different epochs of 5 minutes of wake and are presented as normalized values with respect to the last day of experiment.

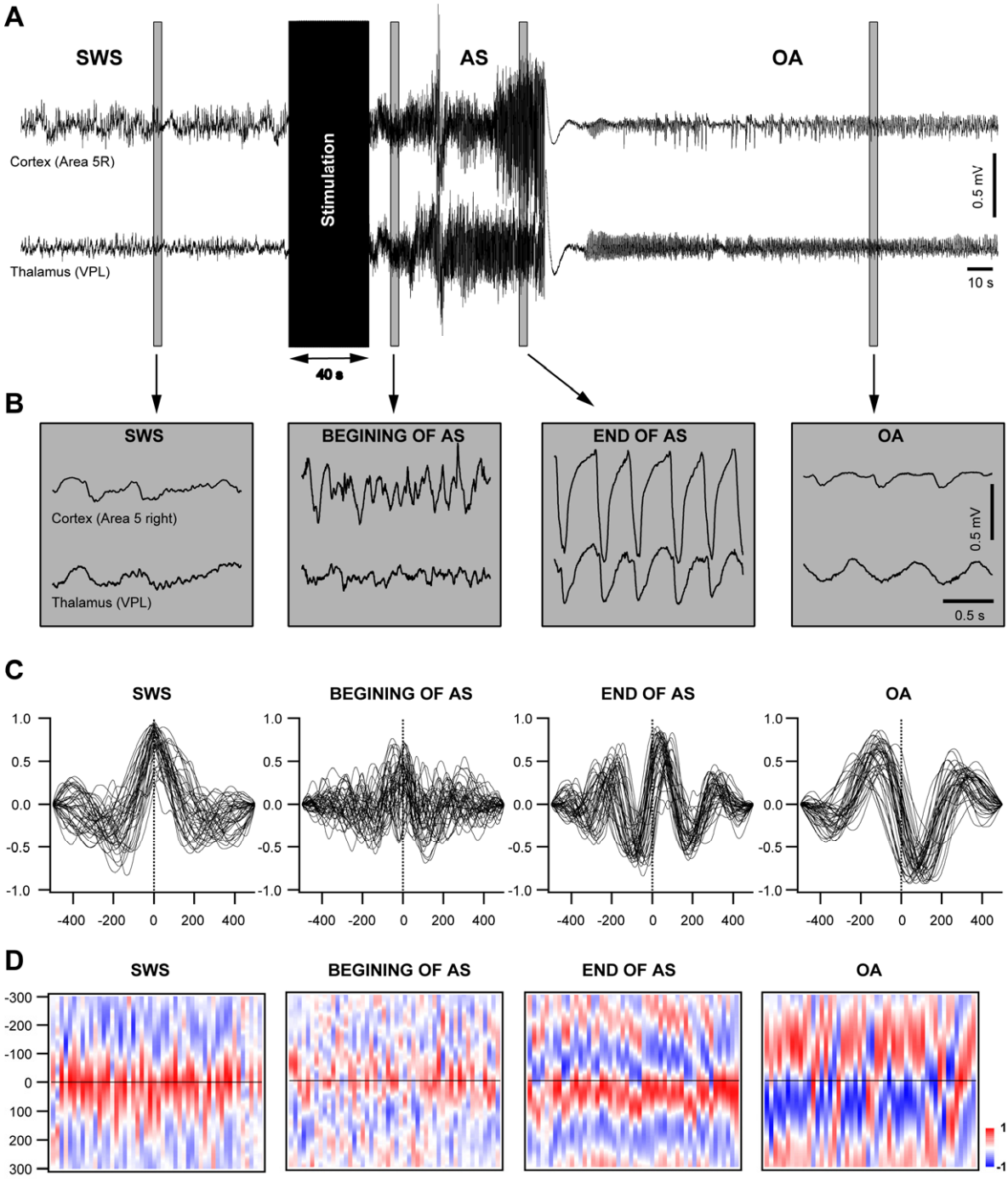

Figure 5-2 Cortical and thalamic components during slow-wave sleep (SWS), acute seizures (AS) and outlasting activities (OA). A) Simultaneous field recording of cortical area 5 right and VPL thalamic nucleus during SWS, AS, and OA. B) Expansions of periods shaded in grey in panel A. C) Sequential cross-correlograms between cortical and thalamic field computed on 1-second windows. D) Same cross-correlograms as in panel C plotted as a function of time. Each vertical bar corresponds to a color-coded version of a cross-correlogram in panel C. During SWS cortical and thalamic activities appear quasi-simultaneous, during AS the cortical activities precede thalamic activities while during OA this sequential order is reversed and thalamus leads cortex.

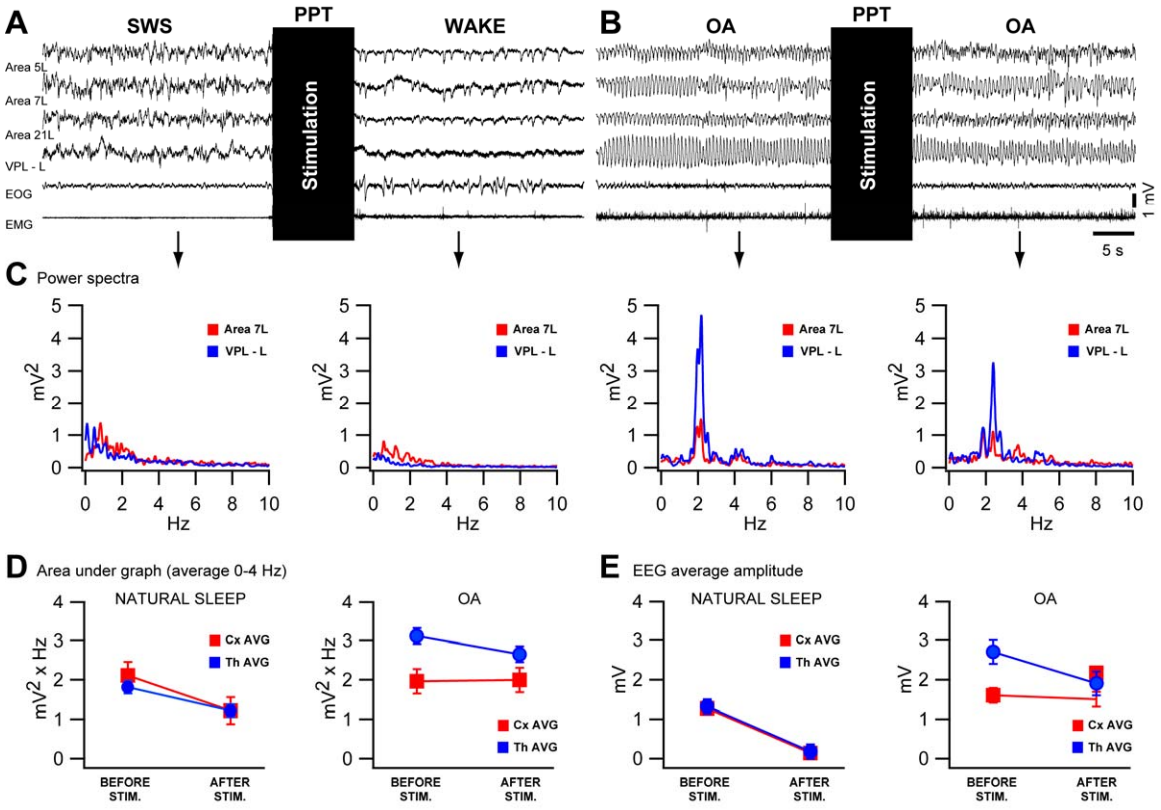

Figure 5-3 Effect of pedunculo-pontine tegmentum (PPT) activation on sleep oscillations and outlasting activity (OA). A) Electrical stimulation of PPT during slow-wave sleep awakes the cat and activates the EEG. Negative deflections in the EEG during wake are associated with eye movements. B) Electrical stimulation during OA does not completely block the expression of OA, but diminishes the amplitude of thalamic EThG. C) Fluctuations in power spectra (FFT) related to the conditions depicted in panels A and B. The correspondences are indicated by arrows. D) Quantification of area under the FFT graph (on 0-4 Hz domain) before and after the PPT stimulation during natural sleep and OA. E) Variation of the average amplitude of EEG and EThG before and after the PPT stimulation during natural sleep and OA.

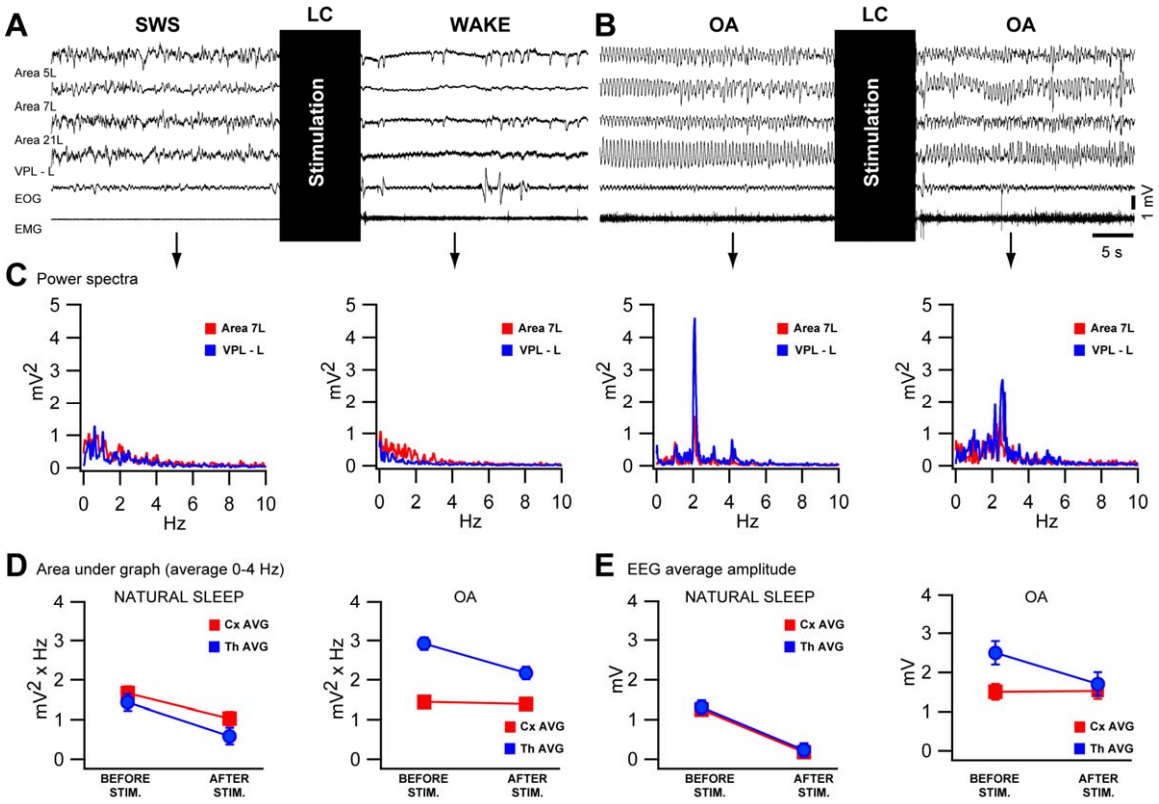

Figure 5-4 Effect of locus coeruleus (LC) activation on sleep oscillations and outlasting activity (OA). A) Electrical stimulation of LC during slow-wave sleep awakes the cat and activates the EEG. B) Electrical stimulation during OA does not completely block the expression of OA in the cortical EEG, but, similar to PPT stimulation, it diminishes the amplitude of EThG. C) Fluctuations in power spectra (FFT) corresponding to the conditions depicted in panels A and B. Similar arrangement as in Fig. 5-3. D) Quantification of area under the FFT graph (on 0-4 Hz domain) before and after the PPT stimulation during natural sleep and OA. E) Variation of the average amplitude of the EEG and EThG before and after the LC stimulation during natural sleep and OA.

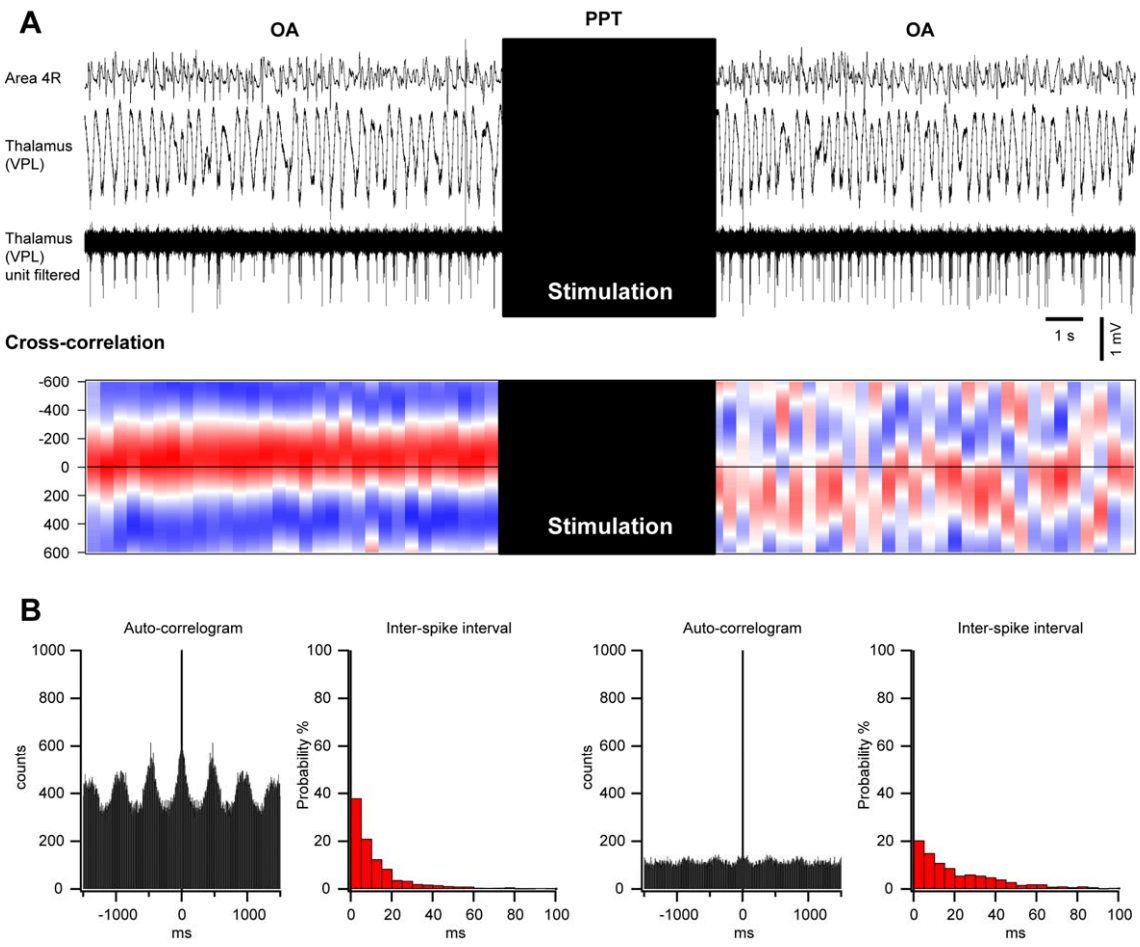

Figure 5-5 Influence of pedunculo-pontine tegmentum (PPT) activation on the cellular thalamic activities during OA. A) Top panel depicts a cortical field EEG recording together with thalamic field EEG and unit activity during OA. Stimulation of PPT is indicated as a black rectangle. The sequential cross-correlogram between cortical and thalamic field EEG is presented in the bottom panel. Note the loss of stable shift in the peak of the correlation following PPT stimulation. B) Spike-triggered auto-correlograms of thalamic unit recording and histograms of inter-spike interval probability before and after stimulation. PPT activation switches the shape of the auto-correlogram from oscillatory to tonic discharge, and reduces the probability of burst discharges.

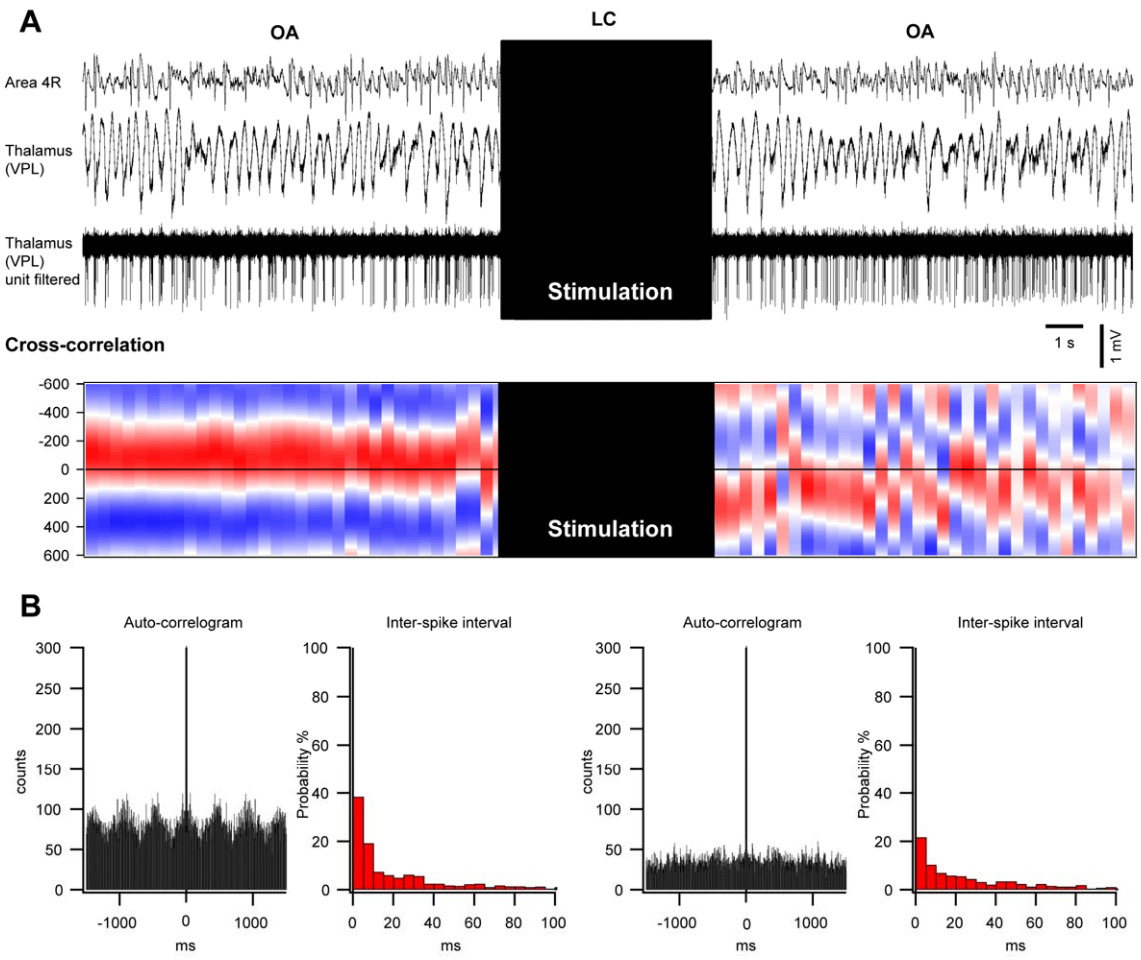

Figure 5-6 Influence of locus coeruleus (LC) activation on the cellular thalamic activities during OA. A) Top panel depicts a cortical field EEG recording together with thalamic field EEG and unit activity during OA. Stimulation of LC is indicated as a black rectangle. The sequential cross-correlogram between cortical and thalamic field EEG is presented in the bottom panel. Note the loss of stable shift in the peak of the correlation following LC stimulation. B) Spike-triggered auto-correlograms of thalamic unit recording and histograms of inter-spike interval probability before and after stimulation. LC activation switches the shape of the auto-correlogram from oscillatory to tonic discharge, and reduces the probability of burst discharges.

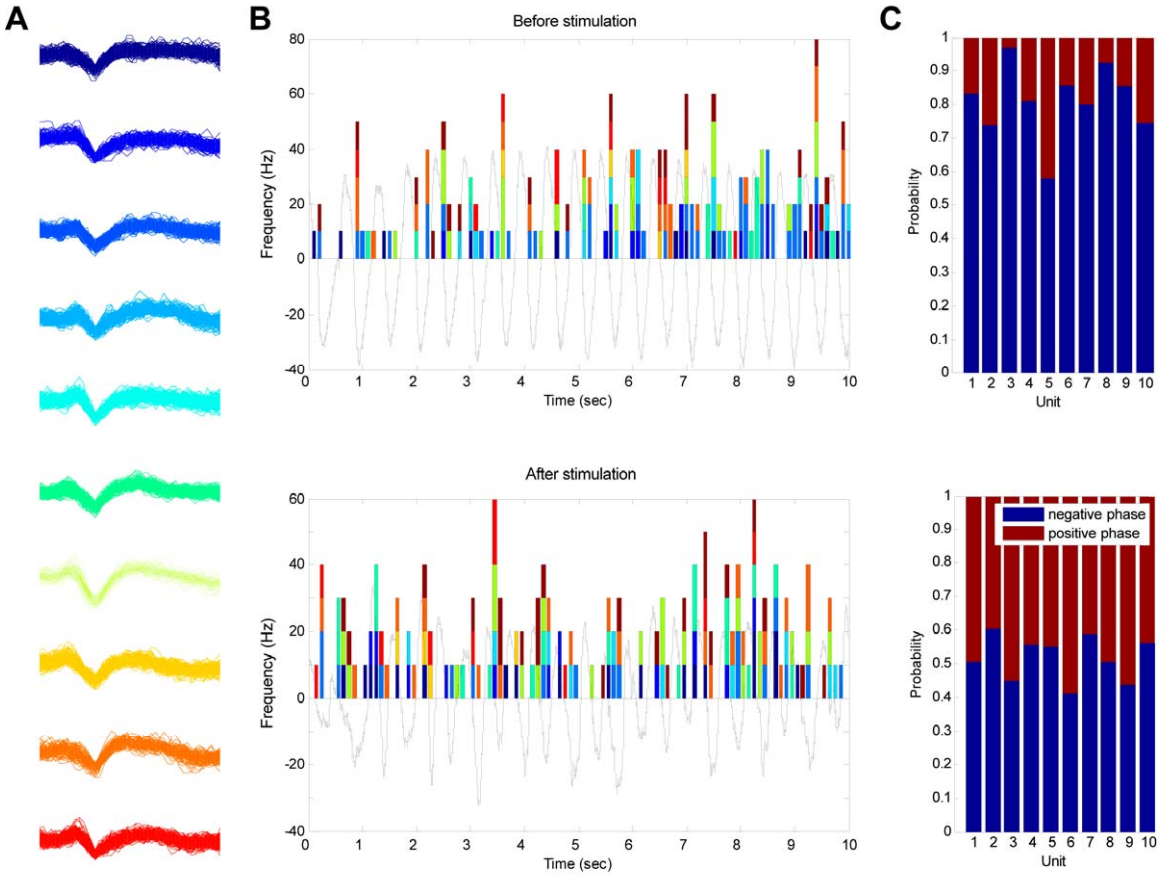

Figure 5-7 Disruption of thalamic firing patterns during OA following stimulation of PPT. A) Raw waveforms of the ten isolated units from a multiunit recording. Individual units are color-coded. B) Spike histogram and field potential before and after stimulation. Same color-code for units as in panel A. C) Fraction of spikes per unit during the negative (blue) and the positive (red) phases of the field potential before and after stimulation. Stimulation of PPT abolished phase preference of thalamic spikes.

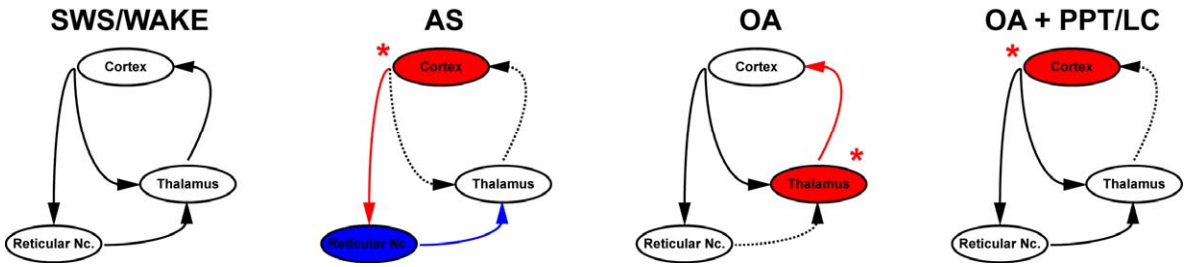

Figure 5-8 Proposed model for thalamocortical interactions during acute seizures/afterdischarges (AS) and outlasting activities (OA). During natural states of vigilance the three structures (cortex, reticular nucleus and thalamus) cooperate to generate the EEG rhythms. During cortically generated AS cortical neurons stimulate GABAergic reticular neurons and thus strongly inhibit thalamus. Once released from reticular neuron's inhibition during the postictal depression thalamic neurons generate rhythmic OAs which further propagates to cortex. However, when stimulating PPT/LC during OA the persistence of unaltered OA at cortical level suggests that neocortex is able to self-sustain these oscillations. Asterisks mark the leading structure.

6 Callosal responses of FRB neurons during slow oscillation in cats

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6.1 Résumé

L'oscillation lente corticale se compose des phases hyperpolarisantes liées aux ondes positives observées sur l'EEG et des phases dépolarisantes au niveau des neurones accompagnées par des ondes négatives dans l'EEG. C'est déjà reconnu que pendant les hyperpolarisations prolongées le transfert d'information à partir des voies pre-thalamiques au neocortex est altéré, tandis que le dialogue intracortical est maintenu. Pour étudier les facteurs qui peuvent expliquer la persistance du transfert de l'information au niveau intracortical pendant les phases hyperpolarisantes, nous avons effectué des enregistrements intracellulaires dans les aires corticale associatives 5 et 7 chez des chats anesthésiés, et nous avons étudié la réponse synaptique des neurones de type FRB, RS et FS à des stimulations des aires corticales controlatérales. Pendant les périodes d'hyperpolarisation la stimulation transcallosalle a généré des potentiels post-synaptiques excitateurs de grand-amplitude couronnés par des potentiels d'action dans les neurones de type FRB, mais pas dans des neurones RS ou FS. Nos données prouvent que les neurones de type FRB peuvent décharger pendant les deux phases de l'oscillation corticale lente, et que leur habilité de décharger pendant les hyperpolarisations prolongées leur confère un rôle majeur dans la transmission et l'analyse de l'information dans les réseaux corticaux pendant le sommeil à ondes lentes.

6.2 Abstract

The cortically generated slow oscillation consists of long-lasting hyperpolarizations associated with depth-positive EEG waves and neuronal depolarizations accompanied by firing during the depth-negative EEG waves. It has previously been shown that, during the prolonged hyperpolarizations, the transfer of information from prethalamic pathways to neocortex is impaired, whereas the intracortical dialogue is maintained. To study some of the factors that may account for the maintenance of the intracortical information transfer during the hyperpolarization, intracellular recordings from association areas 5 and 7 were performed in anesthetized cats, and the synaptic responsiveness of fast-rhythmic-bursting, regular-spiking and fast-spiking neurons was tested using single pulses to the homotopic sites in the contralateral areas. During the long-lasting hyperpolarizations callosal volleys elicited in fast-rhythmic-bursting neurons, but not in regular-spiking or fast-spiking neurons, large-amplitude EPSPs crowned by single action potentials or spike clusters. Our data show that callosal volleys excite and lead to spiking in fast-rhythmic-bursting neurons during prolonged hyperpolarizations, thus enabling them to transmit information within intracortical networks during slow-wave sleep.

6.3 Introduction

Cortically generated slow oscillation (<1 Hz) is characterized by sequences of depolarized (active) and hyperpolarized (silent) phases in anesthetized and naturally sleeping animals (Steriade et al., 1993, Steriade et al., 2001, Timofeev et al., 2001), in humans (Achermann and Borbely, 1997), and in cortical slices (Sanchez-Vives and McCormick, 2000). During cortical EEG depth-positive waves both cortical and thalamocortical neurons are hyperpolarized, far from the firing threshold (Contreras and Steriade, 1995, Contreras et al., 1996, Rosanova and Timofeev, 2005). However, the intracortical dialogue during silent phases of the slow oscillation is maintained (Timofeev et al., 1996, Shu et al., 2003, Reig et al., 2006). The mechanism of response maintenance during the hyperpolarization of cortical neurons remains unknown. Here we asked is there a particular type of cortical neurons that could maintain intracortical interactions during hyperpolarized phase of the slow oscillation? Using contralateral (callosal) cortical stimulation, we demonstrate that fast-rhythmic-bursting (FRB) neurons respond with action potentials during both phases of the cortical slow-oscillation, whereas regular-spiking (RS) and fast-spiking (FS) neurons respond with spikes only during the depolarizing phase of the slow oscillation.

6.4 Materials and methods

Experiments were conducted on 20 adult cats anesthetized with ketamine-xylazine (10-15 and 2-3 mg/kg i.m., respectively). Animals were paralyzed with gallamine triethiodide (20 mg/kg) after EEG showed typical signs of deep general anesthesia, essentially consisting of slow oscillation (0.5-1 Hz). Supplementary doses of anesthetics were administered at the slightest changes toward activated EEG patterns. The cats were ventilated artificially with the control of end-tidal CO2 at 3.5-3.7%. Body temperature was maintained at 37-38o C and heart rate was ~90-100 beats/min. The stability of intracellular recordings was ensured by drainage of cisterna magna, hip suspension, bilateral pneumothorax, and filling the hole made for recordings with a solution of 4% agar. At the end of experiences, a lethal dose of barbiturate was administered. All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Committee for Animal Care of Laval University.

The details of experimental design and intracellular recordings are the same as described previously (Cisse et al., 2003, Cisse et al., 2004). The duration of callosal stimuli was 0.2 ms. For each neuron, we established the threshold intensity for evoked EPSPs and then use the doubled intensity for stimulation through the experiment. Electrical stimulation was applied for 15-20 minutes, every 2-3 s, to evoke EPSPs. The stimuli felt on either depolarizing or hyperpolarizing phases of the slow oscillation. At least 50 callosally evoked responses were taken randomly during each phase of the slow oscillation to detect and count the number of spikes in order to determine the percentage of spike discharge incidence in each phase.

6.5 Results and Discussion

All recorded neurons were intracellularly stimulated with depolarizing current pulses to identify their electrophysiological class, including the FRB category (Connors and Gutnick, 1990, Gray and McCormick, 1996, Steriade et al., 1998). Out of more than 120 neurons recorded intracellularly in areas 5 and 7, 48 neurons were retained for analysis because they responded to the stimulation of the contralateral cortex. Of those, 25 were RS neurons (located at depths between 0.7 and 1.5 mm), 15 were FRB neurons (1-1.6 mm), and 8 were FS neurons (0.8-1.1 mm). Only one intrinsically-bursting cell was analyzed but callosal stimuli did not elicit responses of that neuron during the depolarizing phase of the slow oscillation.

Intracellular recording of an FRB neuron and corresponding depth-EEG recording together with the electrophysiological identification of the neuron are presented in Fig. 6-1A, B. Spontaneously hyperpolarization-activated depolarizing sags occurring during the slow oscillation are indicated by arrows. Examples of FRB neuron's responses to callosal stimuli during the hyperpolarizing phase of slow oscillation and during 50-150 ms that preceded the onset or followed the end of the hyperpolarizing phase are depicted in Fig. 6-1C. The half amplitude transitions from active to silent states and from silent to active states were taken as reference points. Before the onset of the hyperpolarizing phase the neuron responded with EPSPs, which in 31 % of cases led to spike generation. Similar relations were found during the active period that followed silent states, but the incidence of spikes was higher (Fig. 6-1D). During the hyperpolarizing phase of slow oscillation, that neuron

responded with large-amplitude depolarizing responses which were invariably accompanied by spikes (Fig. 6-1C, D). Thirteen of 15 intracellular recorded FRB neurons showed similar relations, namely, more than 60% of contralateral stimuli applied during the hyperpolarizing phase of the slow oscillation elicited firing; the percentage of stimuli eliciting action potentials was lower during the subsequent depolarization; and during the active period that preceded the onset of the hyperpolarizing phase the probability of callosally evoked spiking was lowest, a difference that was statistically significant (t-test, $p<0.01$) (Fig. 6-1E).

The same analyses were performed on 25 RS neurons (Fig. 6-2A, B) and 8 FS neurons, and demonstrated striking differences between them and FRB neurons. Stimuli applied during the hyperpolarizing phase of the slow oscillation elicited depolarizing responses, but none led to spike generation (Fig. 6-2C). The ability to generate more spikes before the onset and after the end of the hyperpolarizing phase compared to that silent period (Fig. 6-2D), was statistically significant (t-test, $p<0.001$) in RS neurons. Similar results to those obtained in RS neurons, but distinctly different from those in FRB cells, were obtained in the sample of FS neurons (Fig. 6-2E, F).

The variant responses of FRB vs. RS and FS neurons were probably due to higher amplitude EPSPs elicited in FRB neurons by callosal volleys and to a different set of intrinsic currents (see below). Indeed, the majority (13 out of 15) of FRB neurons spontaneously displayed depolarizing sags following the initial hyperpolarizations that were associated with EEG depth-positive waves (Fig. 6-1A). All those FRB neurons fired single action potentials or clusters of spikes in response to callosal stimulation during the phase of neuronal hyperpolarization of the slow oscillation. We were unable to elicit depolarizing sags of comparable amplitude with intracellularly applied pulses in most FRB neurons (not shown).

The increased ability of FRB neurons to generate action potentials in response to callosal volleys during the hyperpolarizing phase of the slow oscillation could be related to the observed depolarizing sag. The depolarizing sag in response to hyperpolarizing inputs usually leads to rebound excitation and is associated with the presence of I_H¬ (Pape, 1996). Since we did not observe similar depolarizing sag during somatically applied

hyperpolarizing current pulses, we hypothesize that the depolarizing sag in FRB neurons originates in dendrites. In layer V pyramidal neurons the number of h channels increases exponentially with distance from soma (Kole et al., 2006). It is still not clear whether or not I_H is expressed in FRB neurons.

Other voltage-dependent intrinsic currents of cortical neurons could lead to the enhancement of responsiveness during hyperpolarizing phases of slow oscillation (Llinas, 1988, Schwindt and Crill, 1995, de la Pena and Geijo-Barrientos, 1996, Huguenard, 1996, Pape, 1996, Pare et al., 1998, Hutcheon and Yarom, 2000). This enhancement could be particularly important for FRB neurons that demonstrated threefold larger amplitudes of EPSPs compared to other neuronal types (Cisse et al., 2003). The presence of large-amplitude EPSPs could be due to convergence of callosal inputs onto FRB neurons from multiple contralateral sources. The high input resistance of cortical neurons during the hyperpolarizing phase of the slow oscillation (Contreras et al., 1996, Pare et al., 1998, Steriade et al., 2001) and higher driving force of EPSPs at hyperpolarized voltages amplify EPSPs' amplitudes. However, this mechanism works equally for all neuronal types (not only for FRB neurons), and data from rat barrel cortex suggest that differences in input resistance (Waters and Helmchen, 2006) may be species- or modality- dependent. Thus, we propose that a unique set of intrinsic neuronal currents, which characterizes FRB firing pattern is also responsible for the enhancement of FRB neuron responses during hyperpolarizing phases of slow oscillation.

The FRB neurons which are located in both superficial and deep cortical layers (Timofeev et al., 2000) could play an important role in sustaining intracortical communication during both active and silent states of the cortical network, via callosal volleys, that represent a pure cortical mechanism. Furthermore, their ability to generate bursts make synaptic efficacy more reliable (Lisman, 1997). The capacity of FRB neurons to fire spikes during hyperpolarizing phases of slow oscillation could be particularly important for sensory processing during sleep oscillations. Animal and human studies have demonstrated that peripheral somatosensory stimuli reach neocortex during all phases of slow sleep oscillation (Massimini et al., 2003, Rosanova and Timofeev, 2005) and intracortical whisker-evoked sensory responses in the barrel cortex are stronger during hyperpolarizing

phases of cortical oscillation as compared to depolarizing phases (Petersen et al., 2003, Sachdev et al., 2004). In these examples the FRB neurons, in particularly those receiving direct thalamic inputs (Steriade et al., 1998), could play a critical role in the generation of intracortical dialogues, while the other neurons just reflect activity of FRB neurons.

In this study we demonstrated that during silent network states, the FRB neurons, unlike RS and FS cells, fire action potentials in response to callosal volleys. Therefore, the FRB neurons may be the only type of cortical neurons that could efficiently maintain the intracortical dialogue during all phases of slow sleep oscillation.

6.6 Acknowledgements

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6.7 References

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6.8 Figures

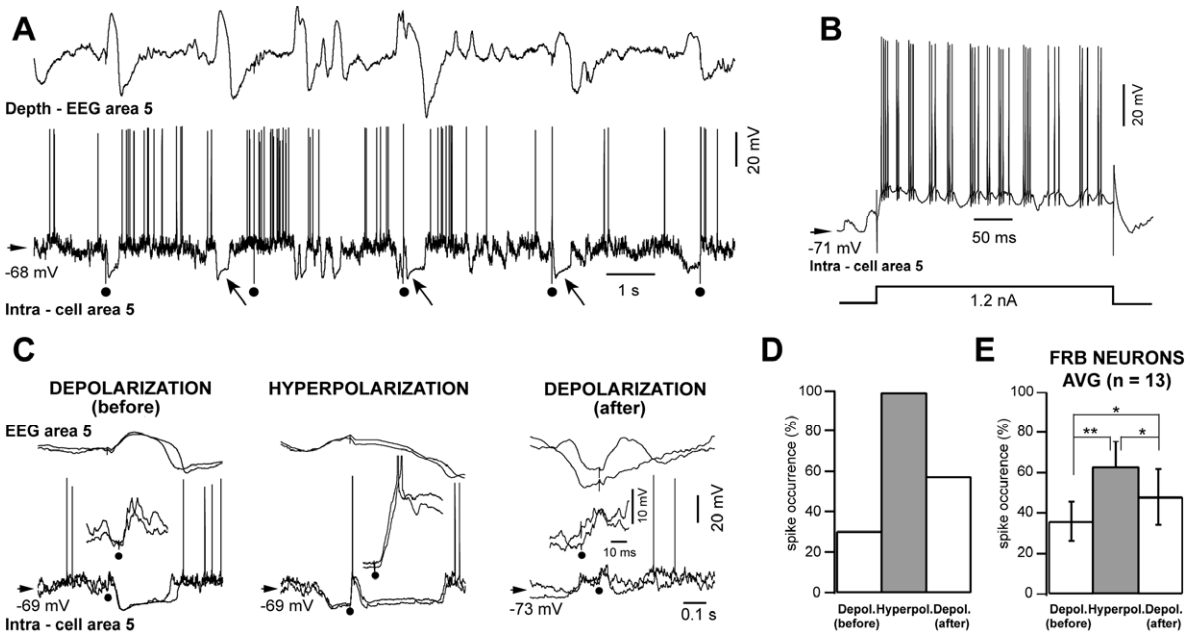

Figure 6-1 Responsiveness of FRB neurons to callosal stimulation during different phases of the slow oscillation. (A) The two traces depict depth-EEG and spontaneous intracellular activity of a FRB neuron in area 5 of left hemisphere and 5 stimuli, indicated by filled circles, applied every 3 s to the homotopic site in the right hemisphere. Arrows indicate spontaneously occurring hyperpolarization-activated depolarizing sags in the FRB neuron. (B) The identification of FRB neuron by depolarizing current pulse. (C) Two superimposed traces of the depth-EEG and spontaneous intracellular activity from the neuron depicted in (A), in each condition, illustrate responses during the periods preceding the hyperpolarizing phase of the slow oscillation (before), during the hyperpolarization, and during the following period (after). Stimulation artifacts are indicated by closed circles. (D) Histogram with the incidence of spikes elicited in the FRB neuron depicted in (A) by contralateral stimulation. (E) Histogram with population data obtained from 13 FRB neurons. Statistically significant differences are indicated (t-test, *p<0.05; **p<0.01).

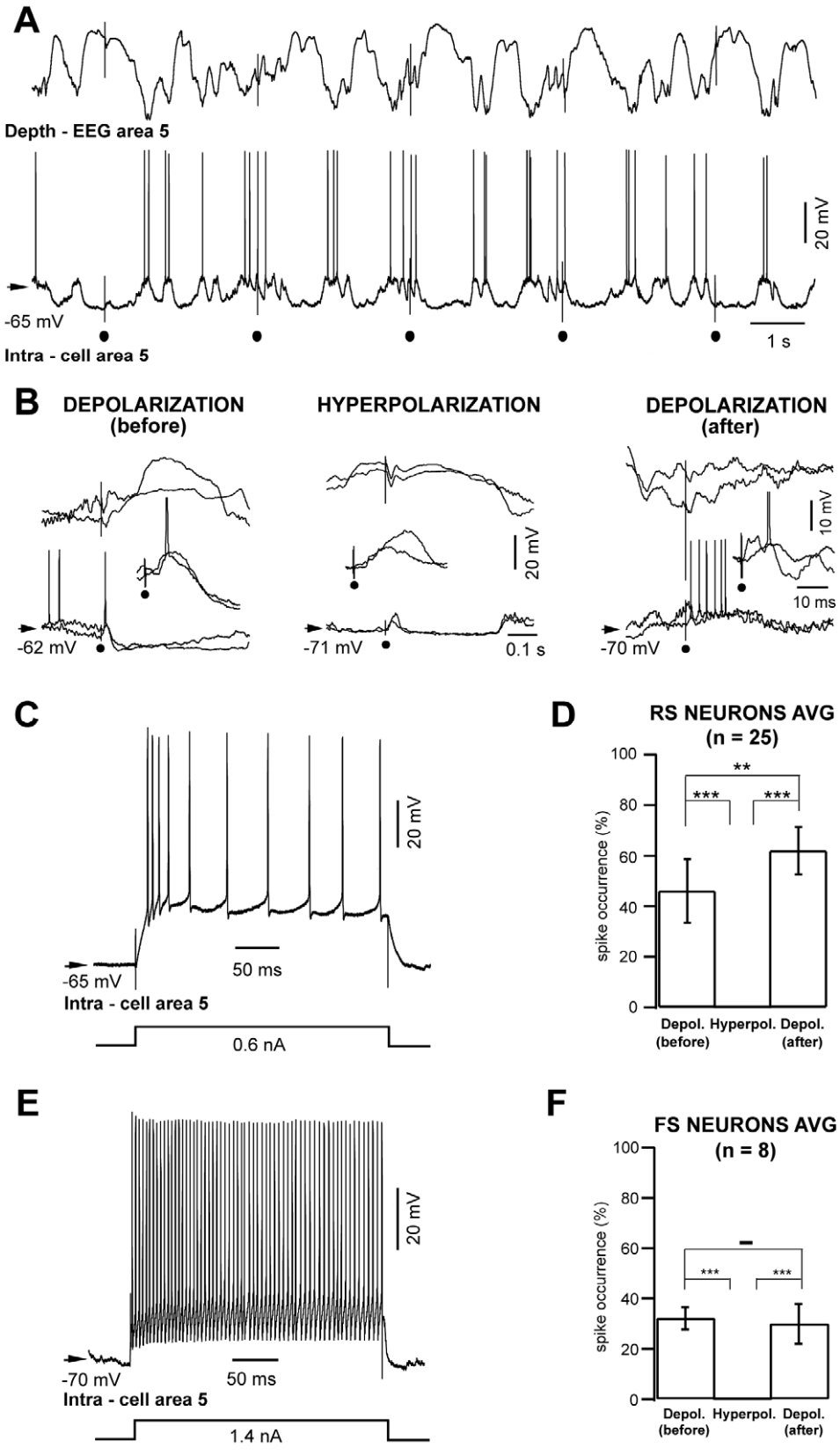
A
Depth - EEG area 5
20 mV
-65 mV
1 s
Intra - cell area 5
B
DEPOLARIZATION
(before)
HYPERPOLARIZATION
DEPOLARIZATION
(after)
20 mV
10 mV
10 ms
-62 mV
-71 mV
0.1 s
-70 mV
C
20 mV
50 ms
-65 mV
Intra - cell area 5
0.6 nA
D
RS NEURONS AVG
(n = 25)
spike occurrence (%)
Depol.
(before)
Hyperpol.
Depol.
(after)
E
20 mV
-70 mV
50 ms
Intra - cell area 5
1.4 nA
F
FS NEURONS AVG
(n = 8)
spike occurrence (%)
Depol.
(before)
Hyperpol.
Depol.
(after)

Figure 6-2 Responsiveness of RS and FS neurons to callosal stimulation during different phases of the slow oscillation. (A) The two traces depict depth-EEG and spontaneous intracellular activity of a RS neuron in area 5 of left hemisphere and 5 stimuli, indicated by filled circles, applied every 2 s to the homotopic site in the right hemisphere. (B) Two superimposed traces in each condition illustrate the behavior of the RS neuron presented in (A) to callosal stimulation during cortical slow-oscillation. FS neurons have a similar behavior therefore are not illustrated here. (C) Electrophysiological identification of RS neurons. (D) Histogram with the average incidence of elicited spikes in the population of RS neurons (n=25). (E) Electrophysiological identification of FS neurons. (F) Histogram with the average incidence of elicited spikes in the population of FS neurons (n=8). Statistically significant differences are indicated (t-test, **p<0.05; ***p<0.001).

7 EPSPs depression following neocortical seizures

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7.1 Résumé

Nous avons étudié les mécanismes possibles que pourraient expliquer la diminution de la responsivité néocorticale suite à des crises épileptiques en enregistrant intracellulairement les potentiels postsynaptiques excitatrices (EPSPs) des neurones corticaux évoquées par la stimulation corticale ipsilatérale avant et après les crises d'épilepsie spontanées ou induites par stimulation électrique. Des neurones avec décharge régulière des potentiels d'action (n = 32) ont été intracellulairement enregistrés dans l'aire corticale d'association 5 chez des chats sous anesthésie à l'aide de ketamine-xylazine ou des barbituriques. Les EPSPs corticaux évoqués ont été caractérisés par une amplitude diminuée après les crises épileptiques électrographiques par rapport aux amplitudes des réponses control. Les composantes synaptiques et intrinsèques des réponses neuronales ont été mesurées en appliquant des stimulations électriques extracellulaires suivis par des pulses de courant hyperpolarisants intracellulaires. La résistance membranaire des neurones est diminuée pendant les crises épileptiques mais elle retourne rapidement au niveau control après les paroxysmes, tandis que l'amplitude des EPSPs évoqués demeure plus basse après les crises, généralement pendant 2 à 12 minutes, suggérant que la diminution de l'amplitude des EPSPs n'était pas due à une altération des propriétés neuronales intrinsèques mais à un mécanisme de réseau. Les données suggèrent que la diminution de la responsivité néocorticale suite à des crises épileptiques puisse être un des mécanismes responsables de déficits observés chez les patients épileptiques.

7.2 Abstract

To study the possible mechanism(s) underlying unresponsiveness following neocortical seizures, we recorded excitatory postsynaptic potentials (EPSPs) of cortical neurons evoked by ipsilateral cortical stimulation before and after spontaneously or elicited seizures. Regular-spiking neurons (n = 32) were intracellularly recorded in association area 5 of cats under ketamine-xylazine or barbiturate anesthesia. Compared with control responses, cortically evoked EPSPs were characterized by decreased amplitude after electrographic seizures. Synaptic responses and intrinsic properties were measured by applying extracellular electrical stimuli followed by and intracellular hyperpolarizing current pulses. The input resistance decreased during seizures but quickly recovered to control level after the paroxysms, whereas the amplitude of evoked EPSPs remained lower following seizures, generally for 2 to 12 min, suggesting that the decreased EPSPs were not due to an alteration of intrinsic response. Data suggest that decreased synaptic responsiveness following seizures may underlie behavioral disturbances and cognitive deficits observed in patients following seizures.

7.3 Introduction

Electrographic seizures consisting of spike-wave (SW) complexes ~3 Hz or SW and polyspike-wave (PSW) complexes at lower frequency (1.5-2.5 Hz), intermingled with fast-runs (10-20 Hz), are often observed in animal experiments in vivo. Such seizures are generated intracortically as they have been recorded after thalamectomy (Steriade & Contreras, 1998; Timofeev et al., 1998), and the majority of thalamocortical neurons are inhibited during cortically generated SW/PSW seizures (Steriade & Contreras, 1995; Pinault et al., 1998; Crunelli & Leresche, 2002). The frequencies of SW/PSW complexes are similar to the EEG patterns of the Lennox-Gastaut syndrome (see for review Steriade, 2003).

We aimed at understanding the cellular mechanisms underlying behavioral disturbances and cognitive deficits observed in patients following seizures (Smith et al., 1986; Jokeit & Ebner, 2002; Meador, 2002; Kanner et al., 2004), by intracellularly testing the changes in the synaptic responsiveness of cortical neurons, during and after seizures, in vivo, in anesthetized cats. In the present study, we used ipsilateral cortical stimulation during spontaneously occurring and electrically induced seizures, and recorded from regular-spiking (RS) neurons. The major finding was that RS neurons consistently decreased their EPSPs' amplitudes for up to 20 min after seizures.

7.4 Materials and methods

Experiments were conducted on 8 adult cats under barbiturate anesthesia (pentobarbital sodium, 25 mg/kg, i.p.) and 15 adult cats under ketamine-xylazine anesthesia (10-15 and 2-3 mg/kg i.m., respectively). The animals were paralyzed with gallamine triethiodide (20 mg/kg i.v.) after the EEG showed typical signs of deep general anesthesia, essentially consisting of sequences of spindle waves (7-14 Hz) under barbiturate anesthesia and slow oscillation (0.5-1 Hz) under ketamine-xylazine anesthesia. Supplementary doses of anesthetics were administered at the slightest changes toward activated EEG patterns. The cats were ventilated artificially with the control of end-tidal CO2 at 3.5-3.7%. The body temperature was maintained at 37-38o C and the heart rate was ~90-100 beats/min. Stability of intracellular recordings was ensured by the drainage of cisterna magna, hip suspension, bilateral pneumothorax, and filling the hole made for recordings with a solution

of 4% agar. At the end of experiences, a lethal dose of barbiturate was administered (pentobarbital, 50 mg/kg, i.v.). All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Committee for Animal Care of Laval University. Every effort was made to minimize the number of animals used and their suffering.

Intracellular recordings from left suprasylvian association area 5 were performed using glass micropipettes filled with a solution of 3 M potassium-acetate. The distance between the stimulating electrode and the recording micropipette in area 5 was less than 2 mm (usually 1-1.5 mm). The threshold of stimulation intensity that elicited cortically evoked EPSPs was in the range of 0.02-0.3 mA, and we used the double of the minimum intensity to investigate EPSPs' amplitudes before and after seizures. Stimulus duration was 0.2 ms in all cases. Concentric bipolar EEG electrodes (with the inner pole in the cortical depth at about 0.8-1 mm and the outer pole at the cortical surface) were bilaterally placed in the cortical areas 5 and 7. High-frequency (50-100 Hz) rhythmic pulse-trains (0.2 ms duration and intensity of 0.5 mA), applied for 10-30 seconds, were employed to induce electrographic seizures. Seizures were induced each 4 hours (1-2 per experiment), but in some animals (n=12) we induced successive seizures before the complete recovery of the EPSPs amplitude (at 60-75 minutes) to test a summative effect.

Statistical analysis included a preliminary assessment of data distribution based on Levine's test for variation. Since EPSPs amplitudes variances were equal we further used the paired Student t-test (2-tails, equal variance). Results are presented as mean ± standard deviation, and statistical significant differences were considered for $p<0.05$. EPSPs amplitudes for the first elicited or occurring seizure were normalized in the whole population of recorded neurons (Fig. 7-1E) to the control average amplitude of EPSPs in the first minute before the seizure (n=23).

7.5 Results

Seizures reported here consisted in rapidly generalizing SW/PSW complexes, frequently intermingled with runs of fast-activities (10-20 Hz), and constantly followed by post-ictal

depressions, resembling the patterns of Lennox-Gastaut syndrome. There was no correlation between the duration or intensity of stimulation and the duration of the elicited acute seizure.

We retained for analysis 32 RS neurons that could be recorded for periods of time from 20 to 40 minutes (n=8 under barbiturate anesthesia; n=24 under ketamine-xylazine anesthesia) with membrane potential (Vm) during silent states (without current injection) of -74±0.6 mV, and slow Vm fluctuations that did not exceed 1-2 mV over the whole recording. These neurons were characterized as RS by their responses to depolarizing current pulses and were recorded in cortical layers II-VI in epileptic loci closed to stimulating electrode.

Synaptic responsiveness during control periods and following spontaneously occurring or electrically induced seizures was tested using single pulses applied every 2 seconds in the vicinity of intracellularly recorded neurons. In 30 out of 32 neurons, recorded under either ketamine-xylazine or barbiturate anesthesia, cortically evoked EPSPs displayed decreased amplitudes during the post-ictal period, compared to control responses (Fig. 7-1). Of those 30 neurons 25 were recorded before and following stimulation-evoked seizures; and 5 neurons were recorded before and following spontaneously occurring seizures that only occurred under ketamine-xylazine anesthesia. The possible factors accounting for the relatively high incidence of spontaneous seizures under ketamine-xylazine anesthesia are discussed elsewhere (Steriade et al., 1998).

The post-seizure period started when paroxysmal depth-EEG waves stopped. This was associated at the intracellular level either with the arrest of neuronal spiking followed by a post-ictal depression and reinstatement of the normal slow-oscillation (see Fig. 7-1A); or, in other instances, with neuronal repolarization after the overt depolarization observed during the paroxysm (see Fig. 7-2A). The ending point of the post-seizure period was considered the time when both the depth-EEG and neuronal activity recovered the pre-seizure aspect.

The major finding was that, at a similar Vm as in control, RS neurons consistently display decreased EPSPs' amplitudes following seizures (Fig. 7-1B,C). Under ketamine-xylazine anesthesia, the amplitude of EPSPs decreased in average from 12.8±0.7 mV (control) to

7.5±0.9 mV (in the first minute of the post-seizure), a depression of about 40%. This depression was similar in both spontaneous and electrically-induced seizures. Under barbiturate anesthesia, the EPSPs' amplitudes decreased in average from 11.3±0.6 mV to 8.8±0.8 (about 22%). The EPSPs' depression lasted up to 20 minutes (namely: 0.6-5 minutes in 15 neurons, 5-10 minutes in 12 neurons, and 10-20 minutes in 3 neurons). The effect was summative in the sense that seizures occurring before the complete recovery of EPSPs amplitude further decreased the amplitude of subsequently EPSPs (Fig. 7-1D). In the normalized average of all recorded neurons the diminution in amplitude of evoked EPSPs attained 36% in the first minute following seizures (Fig. 7-1E).

To determine whether this depression depended upon an intrinsic property of RS neurons or a network mechanism, we tested both synaptic and intrinsic responses by applying every 2 seconds synaptic volleys and hyperpolarizing pulses (delayed by 300 ms) during the control period, throughout the seizure, and the post-seizure period (Fig. 7-2A, n=10). Both EPSP's amplitude and Rin decreased throughout the seizure. However, Rin quickly (<1 min) recovered to control level and then remain unchanged, whereas the EPSP's amplitude remained depressed for periods as long as 15 min (Fig. 7-2B,C).

7.6 Discussion

The major finding in the present study was the decreased amplitudes of cortically evoked EPSPs after seizures that occurred spontaneously or were induced by rhythmic pulse-trains stimulation. Extracellular evoked potentials (field EPSPs) in the dentate gyrus of developing gerbils showed decreased responsiveness during the post-seizure period that lasted 12 seconds (Buckmaster & Wong, 2002), shorter than detected intracellularly in the present study on adult cats.

The evolution of neocortical neuronal responsiveness prior, during and following neocortical paroxysms associated with SW/PSW electrographic patterns similar to those occurring in Lennox-Gastaut syndrome is as follows: (a) progressively decreased latencies and increased depolarization slope of oligosynaptic responses preceding the seizure, followed by paroxysmal depolarizing shifts elicited during the "wave"-related hyperpolarization of the SW complex, but not during the "spike" (Steriade & Amzica,

1999); and (b), present results, showing decreased synaptic responsiveness following seizures, generally from 1 to 10 min, but up to 20 min in few neurons. These changes in synaptic responsiveness are associated with increased membrane conductance during the seizure (Matsumoto et al., 1969), with maximal conductance increase during the "spike" of SW/PSW complexes, and recovery to control values during the 10-15 seconds following the end of the seizure (Neckelmann et al., 2000; Timofeev et al., 2002) (also Fig. 7-2B, right panel, in the present study). While the decrease in Rin progressively recovered to control level within 1 minute and then remain unchanged, the EPSPs amplitude did not recover to control level for long periods (generally 5-10 min). This supports the hypothesis that synaptic depression after seizures depends on synaptic and not intrinsic alterations. Nevertheless, we cannot exclude that other intrinsic properties apart the membrane resistance are modified during seizures, for instance at the dendritic level, but this compartment cannot be targeted intracellularly in vivo.

The extent of EPSPs depression was similar in various subpopulations of neurons recorded in different cortical layers, and was not significantly dissimilar in the deep cortical layers that promote seizure development in absence epilepsy models (Pinault et al., 2006; Polack et al., 2007), compared to other layers.

During seizures many active molecules are released and extracellular ionic concentrations are altered both in normal and epileptic brains. Extracellular potassium concentration is increased during seizures but returns to baseline rapidly after seizure termination, while extracellular calcium concentration decreases with the progression of seizure and returns to baseline in few seconds (Heinemann et al., 1977; Pumain & Heinemann, 1985). Therefore, a calcium-dependent drop in the neurotransmitter release following seizure could explain the decrease in the amplitude of EPSPs only for a short period of time during the post-ictal depression, but not for longer time intervals, of tens of minutes, as reported in the present study.

To sum up, the present study reports a long lasting reduction of EPSPs amplitudes following acute seizures, and a summation of successive EPSP's depressions that may be some of the factors responsible for the post-ictal behavioral disturbances and cognitive deficits observed in patients with epilepsy (Binnie, 2003; Motamedi & Meador, 2003).

7.7 Acknowledgements

We dedicate this paper to the memory of Mircea Steriade, and we are very grateful to him for many stimulating discussions and continuous support. We would like to thank P. Giguère for his excellent technical assistance. This research was supported by grants (MOP-67175 and MOP-37862) from Canadian Institutes of Health Research. I.T. is scholar of Canadian Institutes of Health Research. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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7.9 Figures

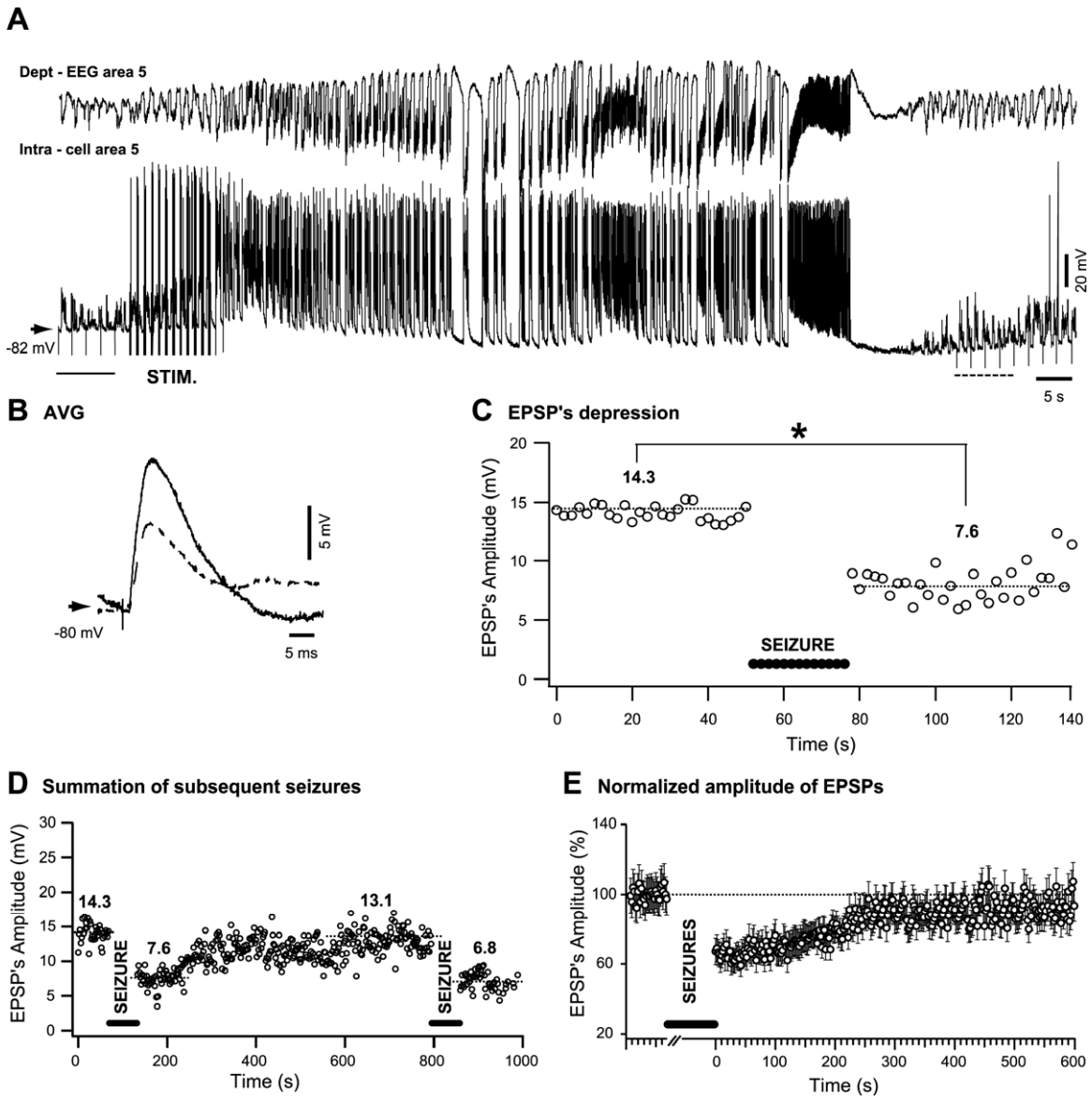

Figure 7-1 Depression of cortically evoked EPSPs after electrically induced seizures in ketamine-xylazine anesthesia. A) Intracellular and depth-EEG recording from associative area 5. Single stimuli were applied every 2 seconds to evoke EPSPs and rhythmic pulse-trains (5 stimuli at 100 Hz) were delivered to elicit seizure (STIM). B) Averages of last five control EPSPs (continuous line) and first five EPSPs following seizures (dotted line) identified in panel A) by solid and dotted lines. C) Variation of the amplitudes of evoked EPSPs following seizures. Dotted lines indicate mean amplitude of corresponding points. The statistical significant difference (paired Student t-test, $p<0.05$) is marked with *. D) Illustration of the summating effect of successive seizures on the amplitudes of evoked EPSPs. Numbers indicate average EPSPs amplitudes during the underlined periods. E) Amplitude of elicited EPSPs following seizures normalized to the average amplitude of last 30 control EPSPs (1 minute) recorded before the induction of seizures.

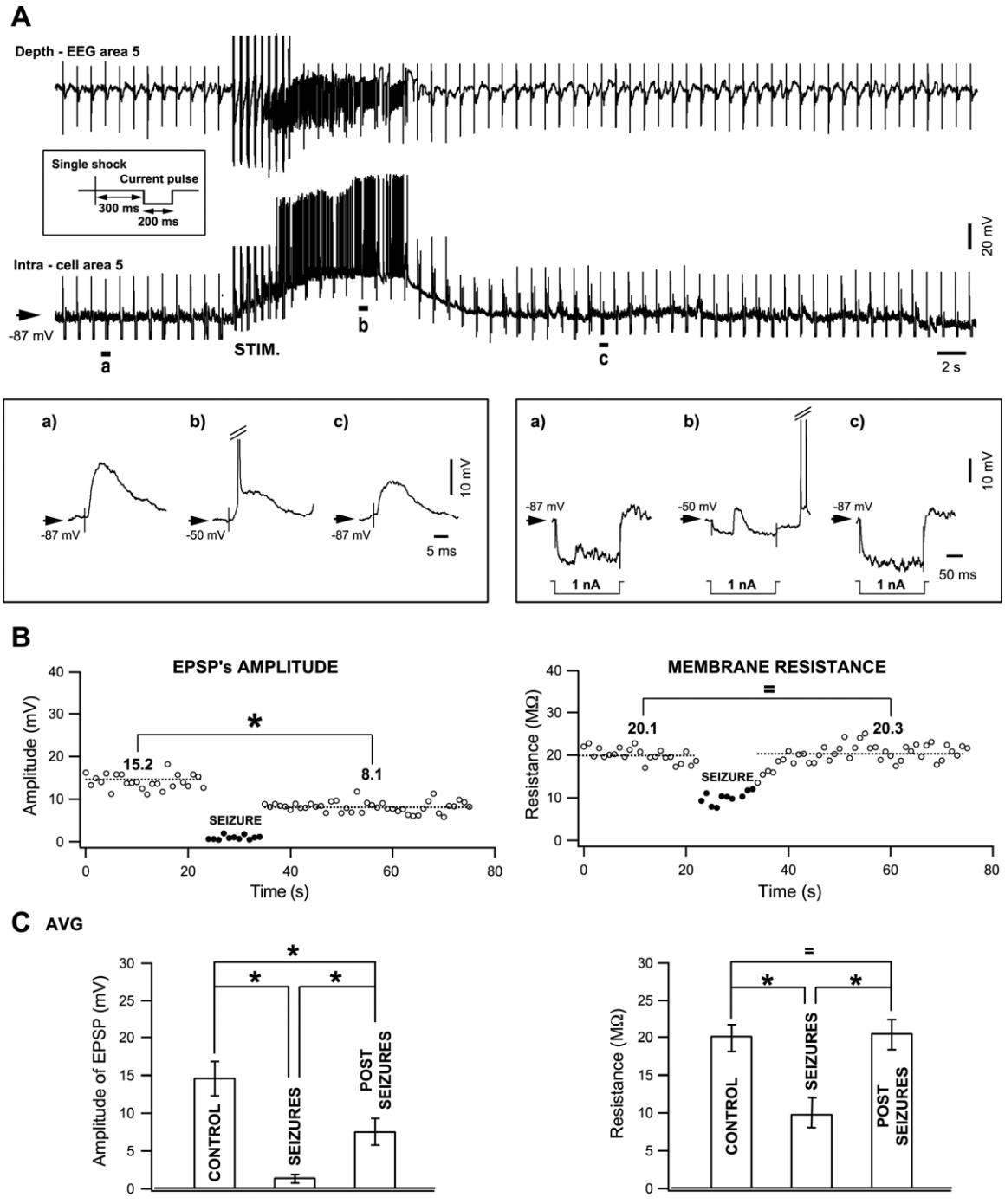

Figure 7-2 EPSPs' depression and apparent input resistance of a RS neuron during electrically induced seizure under barbiturate anesthesia. A) Top panel depicts an intracellular and depth-EEG recording from associative area 5. Synaptic volleys combined with hyperpolarizing current pulse were applied every 2 (see pattern in the inset). The seizure was elicited by cortical stimulation (STIM) consisting in 9 pulse-trains at 100 Hz. Epochs underlined in the top panel are expanded in the bottom insets as follows: (a) control; (b) seizure; and (c) post-seizure. Left inset shows the EPSPs evoked by cortical stimulation in the above conditions, while right inset depicts the input resistance (similar arrangement as in left). B) The variation of EPSP's amplitude in the neuron showed above during control, seizure, and post-seizure period is presented in the left panel. The right panel presents the corresponding variation of the input resistance. Dotted lines represent the average of the points during the identical period. C)

Statistical quantification of data depicted in panel B in the population of recorded neurons (n=10). * indicates statistical significant differences (paired Student t-test, $p<0.05$).

8 Conclusions

8.1 Review of the results

The results of the studies included in the present thesis can be summarized as follows:

1) The first study showed that in anesthetized animals the chronic stages of deafferented suprasylvian gyrus are characterized by (i) increase in amplitudes of field potentials that build up both the slow oscillation and SW/PSW seizures; (ii) increased velocity of low-frequency activity propagation from week 1 to week 5, during both the slow oscillation and seizures; and (iii) initiation of seizures in the territories contiguous to the relatively intact cortex, as shown by both field potentials and intracellular recordings. This study demonstrated an increased intrinsic and synaptic excitability following cortical deafferentation.

2) The main findings reported in the second study in chronic behaving animals subjected to partial cortical deafferentation were: (i) recurrent seizures with 4-Hz SW complexes, first localized in territories contiguous to trauma, thereafter generalized over the whole cortical surface, were observed in all experimental animals following the partial deafferentation of suprasylvian gyrus; (ii) the incidence of ictal events was modulated by the state of vigilance, occasionally occurring during the waking state, being enhanced during SWS, and absent during REM sleep; (iii) the propagation of seizures increased during SWS, compared to waking; and (iv) all cortical neurons recorded in this study expressed a bimodal distribution of Vm values, due to the presence of hyperpolarizations during all states of vigilance, more obvious during SWS and waking. Overall, in this study we described an increased incidence in the frequency of ictal events in chronic stages of deafferented cortex and the modulation of the ictal events by the states of vigilance.

3) In the third study on chronic kindled cats we reported: (i) an increased probability to induce paroxysmal afterdischarges occurring during the transition from slow-wave sleep to waking state, (ii) the generalized convulsive seizures followed by the prolonged (tens to hundreds of minutes) rhythmic OA with frequency 1.5-2 Hz, (iii) that during REM sleep the OA were absent, (iv) following initial acute seizure the OA were local and they spread over all cortical areas in 2-4 weeks from the beginning of kindling procedure, and (v) that

the membrane potential of cortical neurons uncovered the presence of active (depolarized) and silent (but not hyperpolarized) states producing a unimodal distribution of the membrane potential. The main finding of the present study was the expression of oscillatory patterns outlasting seizures that altered the normal brain rhythms for hours. We supported the idea that OA is efficient in the promotion of epileptogenesis and seizures via an up-regulation of synaptic efficacy.

4) The fourth study on the mechanisms of generation of OA and the effects of modulatory systems on kindling epileptogenesis reported: (i) a continuous increase in the incidence of spontaneous burst firing in the VPL nucleus during cortical kindling in waking state, (ii) we found that cortex and thalamus are sequently involved in the generation of seizures in kindled cats, cortical paroxysmal activities preceding thalamic ones during the initially stimulation-evoked AS and thalamic discharges foregoing cortical ones during OA, (iii) despite the fact that PPT/LC stimulation showed a strong effect when applied during naturally occurring SWS (easily waking up the animal) the activation via PPT/LC during OA did not succeed to stop the paroxysmal discharges; however, it diminished the amplitude of OA at thalamic but not at cortical level, without changing the mean frequency of the OA, (iv) we showed that following PPT/LC stimulation the synchrony between cortical and thalamic oscillations is impaired as thalamocortical neurons change their firing pattern from bursting to tonic, and the fixed phase-lock between cellular activities and EThG oscillation is lost. Overall this study suggests that thalamocortical neurons contribute to the generation of OA when the strong inhibitory impingement of reticular neurons occurring during the first part of the AS is released.

5) The fifth study on the neuronal responsiveness during cortical slow-oscillation demonstrated that during silent network states, the FRB neurons, unlike RS and FS cells, fire action potentials in response to synaptic volleys; therefore, the FRB neurons may be the only type of cortical neurons that could efficiently maintain the intracortical dialogue during both active and silent states of the cortex.

6) Finally, in the sixth study on the impact of repetitive seizures on the properties of the cortical network we found decreased amplitudes of cortically evoked EPSPs after seizures;

which may be one of the factors responsible for the behavioral deficits observed in patients with epilepsy.

8.2 Technical considerations

All the results presented here come from *in vivo* experiments, where the structure and connectivity of the central nervous system are preserved intact (except the experimental paradigms as the cortical deafferentation); and, moreover, the ionic milieu, which is of utmost importance for any electrophysiological study, is not affected by the method. The valuability and viability of this preparation is further sustained by the occurence of spontaneously autosustained, reverberant, network activities in all brain regions, and especially in the corticothalamic circuits, which were of major importance for our studies.

Beyond the experimental complexity related to animal preparation and conditioning, to the fact that some structures of the brain are difficult to be intracellularly investigated, and ethical concerns, *in vivo* it is very difficult or not possible to study in detail the ionic composition of both synaptic and intrinsic neuronal responses; or to determine the specific properties of the receptors involved in the transduction cascades. Investigations by optic techniques and voltage-sensitive dyes are nowadays still limited to the superficial cortical layers but their use could be envisaged in further experiments.

Thus, in vivo preparation have a highly qualitative value, since they provide physiological conditions for manipulations; however their impact of high resolution subcellular elements and mechanisms is poor, given that they cannot shed light on many ionic and receptor cascade mechanisms.

8.3 Active and silent states in corticothalamic circuits and their relation with seizures

TC, RE and CT neurons are interconnected in a circuit which generates both the physiological EEG rhythms occurring during natural states of vigilance, and the pathological developments into seizures of the normal brain oscillations. While individual oscillators generate pure regular rhythms, complex circuits, as the living brain during the various states of vigilance, do not generally display separate rhythms, but spontaneous activities in which the frequencies of different oscillators interact.

The cortical slow oscillation (0.5-1 Hz) built on a recurring alternation between *active* and *silent* periods in the cortical networks has not only the remarkable ability of being able to group other sleep rhythms within complex wave-sequences, but, furthermore, it is the brain rhythm that may continuously develop into seizures.

Neocortical neurons may be in three different states: they can either display a depolarized level of the membrane potential as during wake or REM sleep with steady *active* periods and action potentials discharges, either be hyperpolarized as observed in cortical suppression / burst-suppression following major ionic homeostasis impairment or deep anesthesia where the *silent* periods predominate, or incessantly alternate between *active* and *silent* periods as during SWS or seizures.

The guiding line in our experiments was the hypothesis that the occurrence and / or the persistence of long-lasting fluctuations between *silent* and *active* states in the neocortical networks, together with a modified neuronal excitability are the key factors of epileptogenesis, leading to behavioral seizures.

We addressed this hypothesis in two different experimental models. The chronic cortical deafferentation (undercut) replicated the physiological deafferentation of the neocortex observed during SWS. Under these conditions of decreased synaptic input and increased incidence of *silent* periods in the corticothalamic system the process of homeostatic plasticity up-regulated cortical cellular and network excitability contributing to the developpement of seizures. Therefore, the deafferented cortex was not *silent*, but it was able to organize its activity and to oscillate between *active* and *silent* for long periods of time and, furthermore, to develop highly synchronized activities, ranging from cellular hyperexcitability to focal epileptogenesis and generalized SW seizures.

On the other hand, with the kindling model we imposed to the cerebral cortex an increased synaptic drive compared to the one naturally occurring during the *active* states (wake / REM / or depolarized epochs during SWS). Under these conditions a different plasticity mechanism occurring in the thalamo-cortical system imposed long-lasting oscillatory pattern between *active* and *silent* epochs, entitled OA.

Independently of the model we used and the mechanism of epileptogenesis seizures showed some analogous characteristics: alteration of the neuronal firing pattern, similar temporo-spatial patterns of synchronizations towards generalization and increased coherence, and modulation by the state of vigilance. To summarize:

1) Seizures arising in heterogeneous networks with different levels of excitability inflicted by plasticity mechanisms were built on neuronal hyper-synchrony promoted by the neuronal switch from normal activity patterns towards a bursting behavior at a cellular level as identified by intracellular and extracellular unit recordings.

2) Both the paroxysmal activities following cortical undercut and OA following kindling started focally and showed a constant tendency toward generalization on the whole cortical surface, faster propagation and increased synchrony with time.

3) We reported that both paroxysmal 4-Hz ictal events observed following cortical deafferentation and the OA following kindling were overt during SWS and quiet wakefulness; and were completely abolished during REM sleep.

The homeostatic mechanisms are not always able to stabilize the network in a state of "normal" excitability, which leads to the development of different forms of paroxysmal activities. A *silent*, hyperpolarized state of cortical neurons favors the induction of burst firing in response to depolarizing inputs, and the postsynaptic influence of a burst is much stronger as compared to a single spike. Furthermore, we brought evidences that a particular type of neocortical neurons - FRB class- is capable to consistently respond with bursts during the hyperpolarized phase of the slow oscillation, fact that may play a very important role in both normal brain processing and a leading role in epileptogenesis. Finally, we reported a third plastic mechanism in the cortical network following seizures - a decreasing amplitude of cortically evoked EPSPs following seizures - which may be one of the factors responsible for the behavioral deficits observed in patients with epilepsy.

We conclude that incessant transitions between *active* and *silent* states in cortico-thalamic circuits induced either by disfacilitation (sleep), cortical deafferentation (4-Hz ictal episodes) and by kindling (outlasting activities) create favorable circumstances for

epileptogenesis. The increase in burst-firing, which further induce abnormally strong postsynaptic excitation, shifts the balance of excitation and inhibition toward overexcitation leading to the onset of seizures.

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