SINZIANA AVRAMESCU

CELLULAR AND HOMEOSTATIC NETWORK MECHANISMS OF POSTTRAUMATIC EPILEPSY

Thèse présentée
à la Faculté des études supérieures de l'Université Laval
dans le cadre du programme de doctorat en neurobiologie
pour l'obtention du grade de Philosophiae Doctor (Ph.D.)

DÉPARTEMENT D'ANATOMIE ET PHYSIOLOGIE
FACULTÉ DE MÉDECINE
UNIVERSITÉ LAVAL
QUÉBEC

2008

Résumé

Suite aux traumatismes crâniens pénétrants, le cerveau devient graduellement hyperexcitable et génère des activités paroxystiques spontanées. Les mécanismes qui sous-tendent l'épileptogénèse demeurent cependant peu connus. La ligne directrice de nos travaux consiste en l'hypothèse que la diminution de l'activité corticale engendrée par la déafférentation déclenche des mécanismes homéostatiques agissant tant au niveau cellulaire qu'au niveau du réseau cortical, et qui mènent à une excitabilité neuronale accrue culminant en crises d'épilepsie.

Nous avons testé cette hypothèse chez des chats adultes, lors de différents états de vigilance ou sous anesthésie, ayant subits une déafférentation partielle du gyrus suprasylvien. Nous avons évalué les effets de la déafférentation corticale aigue et chronique sur la survie des neurones et des cellules gliales et nous avons investigué comment la privation chronique d'afférences neuronales pourrait modifier les propriétés du réseau cortical et déclencher des crises d'épilepsie.

Après la déafférentation du gyrus suprasylvien, les neurones situés dans les couches corticales profondes, en particulier les neurones inhibiteurs GABAérgiques, dégénèrent progressivement et parallèlement à une fréquence croissante des activités paroxystiques, notamment pendant le sommeil à ondes lentes. La privation chronique d'afférences neuronales et la perte de neurones activent les mécanismes homéostatiques de plasticité qui favorisent une plus grande connectivité neuronale, une efficacité plus élevée des connexions synaptiques excitatrices et des changements des propriétés neuronales intrinsèques. Ensemble, ces facteurs favorisent une excitation accrue du réseau cortical. L'activité corticale spontanée, mesurée par les taux moyens de décharge, augmente progressivement, en particulier pendant le sommeil à ondes lentes, caractérisé par des périodes silencieuses alternant avec des périodes actives. Ceci soutient, en outre, notre hypothèse concernant la participation des mécanismes de plasticité homéostatique. La dégénération des neurones des couches corticales profondes produit des changements importants dans la distribution laminaire de l'activité neuronale, qui est déplacée vers les couches plus superficielles, dans la partie déafferenté du gyrus. Ce changement dans la distribution de profils de profondeurs de décharges neuronales modifie également le

déclenchement de l'activité corticale spontanée. Dans le cortex normal et dans la partie relativement intacte du gyrus suprasylvien, l'activité corticale est générée dans les couches corticales profondes. Pourtant, dans le cortex chroniquement déafferenté, l'oscillation lente et les activités ictales sont générées dans les couches superficielles et puis diffusent vers les couches plus profondes. Le traumatisme cortical induit également une importante gliose réactive et une altération de la fonction normale des cellules gliales, ce qui cause l'enlèvement dysfonctionnel du K^+ extracellulaire et qui augmente l'excitabilité des neurones favorisant ainsi la génération d'activités paroxystiques.

En conclusion, les mécanismes de plasticité homéostatique déclenchés par le niveau diminué d'activité dans le cortex déafferenté produisent une hyperexcitabilité corticale incontrôlable et génèrent finalement les crises d'épilepsie. Dans ces conditions, l'augmentation de l'activité corticale plutôt que la diminution avec des médicaments antiépileptiques pourrait être salutaire pour empêcher le développement de l'épileptogenèse post-traumatique.

Abstract

After penetrating cortical wounds, the brain becomes gradually hyperexcitable and generates spontaneous paroxysmal activity, but the progressive mechanisms of epileptogenesis remain virtually unknown. The guiding line of our experiments was the hypothesis that the reduced cortical activity following deafferentation triggers homeostatic mechanisms acting at cellular and network levels, leading to an increased neuronal excitability and finally generating paroxysmal activities.

We tested this hypothesis either in anesthetized adult cats, or during natural sleep and wake, using the model of partially deafferented suprasylvian gyrus to induce posttraumatic epileptogenesis. We evaluated the effects of acute and chronic cortical deafferentation on the survival of neurons and glial cells and how long-term input deprivation could shape up the properties of neuronal networks and the initiation of spontaneous cortical activity.

Following cortical deafferentation of the suprasylvian gyrus, the deeply laying neurons, particularly the inhibitory GABAergic ones, degenerate progressively in parallel with an increased propensity to paroxysmal activity, mainly during slow-wave sleep. The chronic input deprivation and the death of neurons activate homeostatic plasticity mechanisms, which promote a gradual increased neuronal connectivity, higher efficacy of excitatory synaptic connections and changes in intrinsic cellular properties favoring increased excitation. The spontaneous cortical activity quantified by means of firing rate augments also progressively, particularly during slow-wave sleep, characterized by periods of silent states alternating with periods of active states, which supports furthermore our hypothesis regarding the involvement of homeostatic plasticity mechanisms. The degeneration of neurons in the deep cortical layers generates important changes in the laminar distribution of neuronal activity, which is shifted from the deeper layers to the more superficial ones, in the partially deafferented part of the gyrus. This change in the depth profile distribution of firing rates modifies also the initiation of spontaneous cortical activity which, in normal cortex, and in the relatively intact part of the deafferented gyrus, is initiated in the deep cortical layers. Conversely, in late stages of the undercut, both the cortical slow oscillation and the ictal activity are initiated in the more superficial layers and

then spread to the deeper ones. Cortical trauma induces also an important reactive gliosis associated with an impaired function of glial cells, responsible for a dysfunctional K^+ clearance in the injured cortex, which additionally increases the excitability of neurons, promoting the generation of paroxysmal activity.

We conclude, that the homeostatic plasticity mechanisms triggered by the decreased level of activity in the deafferented cortex, generate an uncontrollable cortical hyperexcitability, finally leading to seizures. If this statement is true, augmenting cortical activity rapidly after cortical trauma rather than decreasing it with antiepileptic medication, could prove beneficial in preventing the development of posttraumatic epileptogenesis.

Foreword

The following thesis is presented in the form of a collection of scientific articles - published 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 or submitted.

As Carl Sagan liked to say, "Science is a way of thinking much more than it is a body of knowledge", and I feel honored to be part of a group of privileged people who do science and think science.

I am grateful to my fiancé, Dragos, for teaching me to "never stop exploring", for making everyday look brighter and for just being there every time I needed.

Last but not least, I would like to thank my family for their affection and continuous support - my mother, Emilia, for giving me the courage to dream, but also to fulfill my dreams; my father, Victor, who taught me the meanings of determination and self-esteem; and my brother, Radu, from whom I learned that adults are allowed to be kids and that braking the rules once in a while makes you feel better.

"There is no scientific study more vital to man than the study of his own brain. Our entire view of the Universe depends on it."
Francis Crick (Scientific American, 1979)

Table of contents

List of tables

List of figures

List of abbreviations

AED	antiepileptic drugs
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AQP4	aquaporin-4
CNS	central nervous system
DSWS	deep slow-wave sleep
EC	extracellular
ECS	extracellular space
EEG	electro-encephalogram
EMG	electro-myogram
EOG	electro-oculogram
EPSP	excitatory postsynaptic potential
FFT	Fast Fourier Transformation
FRB	fast-rhythmic-bursting
FS	fast-spiking
GABA	gamma-amino butyric acid
GAD	glutamate decarboxylase
HP	homeostatic plasticity
IB	intrinsically-bursting
IC	intracellular
IEA	inter-ictal epileptiform activity
I_h	H-potassium current
ILAE	International League Against Epilepsy
$I_{Na(p)}$	persistent Na^+ current
IPSP	inhibitory postsynaptic potential
IR	input resistance
ISF	interstitial fluid
IS	interictal spikes
ISI	inter-spike interval
$[K^+]_o$	extracellular K^+ concentration
$[K^+]_i$	intracellular K^+ concentration
KCC2	K^+/Cl cotransporter
Kir	inward rectifying K^+ channels
LSWS	light slow-wave sleep
mEPSC	mini excitatory postsynaptic current
NE	network excitation
NI	network inhibition
NKCC1	$Na^+/K^+/Cl^-$ cotransporter
NMDA	N-methyl-D-aspartate
PDS	paroxysmal depolarizing shift
PSW	poly spike-waves
PTE	posttraumatic epilepsy
PTS	posttraumatic seizures
REM	rapid-eye movements
RS	regular-spiking

SD	spreading depression
STA	spike-triggered averages
SW	spike-waves
SWS	slow-wave sleep
TBI	traumatic brain injury
TC	thalamo-cortical
TLE	temporal lobe epilepsy
W	wake

Introduction

1 Seizures and Epilepsy

1.1 Definitions

Modern epileptology emerged from John Hughlings Jackson's 1870 paper "A study of convulsions" in which he spectacularly pointed out that seizures do not equal epilepsy: "A convulsion is but a symptom, and implies only that there is an occasional, an excessive and a disorderly discharge of nerve tissue on muscles. This discharge occurs in all degrees; it occurs with all sorts of conditions of ill health, at all ages, and under innumerable circumstances" (Jackson, 1870).

According to the latest consensus of the International League Against Epilepsy (ILAE) and of the International Bureau for Epilepsy, an epileptic seizure is a clinical, transient event, with clear start and finish, characterized by an abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005).

Epilepsy is not one condition, but a diverse family of disorders, having in common an abnormally increased predisposition to seizures. The definition of epilepsy requires the occurrence of at least one unprovoked epileptic seizure (Fisher et al., 2005). Therefore, a predisposition, as determined for example by a family history or by the presence of epileptiform activities on the electroencephalogram (EEG), is not sufficient to determine epilepsy. 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 (Fisher et al., 2005).

Epileptogenesis refers to the cascade of biological events altering the balance between excitation and inhibition in the brain and leading to the development of an epileptic disorder (Clark and Wilson, 1999). It has been shown both in animal models of epilepsy (Cavalheiro et al., 1991; Mello et al., 1993) and in patients (Annegers et al., 1980; Marks et al., 1992) that there is a latent period between the induction of a localized cerebral insult and the appearance of a chronic epileptic condition. The progressive pathological processes occurring during the latent period, such as neuronal loss and abnormal synaptic

reorganization (Mello et al., 1993; Leite et al., 1996; Mathern et al., 1996), lead to abnormally increased excitability and synchronization and eventually to the generation of spontaneous seizures (Cavalheiro et al., 1991; Isokawa and Mello, 1991). This latent period may offer a therapeutic window for the prevention of epileptogenesis and the development of unprovoked seizures and epilepsy (Herman, 2002).

Even after its development, epilepsy should not be viewed as a random succession of seizures but as a dynamic process which results in both ictal phenomena and interictal functional and structural abnormalities in the brain (Rodin, 1972). Patients who develop chronic intractable epilepsy demonstrate progression in both the number of seizures and in seizure-related neurological symptoms such as cognitive and behavioral disorders (Elwes et al., 1984; French et al., 1993; Williamson et al., 1993; Cole, 2000). In the view of these data, the challenge we are facing is to fill in the gaps of the basic mechanisms that induce epilepsy, so that we will be able to prevent epileptogenesis and the progression of disease.

1.2 Diagnostic overview

The ILAE Commission on Classification and Terminology proposed in 2001 a revised diagnostic scheme intended to provide the basis for a standardized description of individual patients, consisting of five levels, or Axes. The Axes are organized to facilitate a logical clinical approach to the development of hypotheses necessary to determine the diagnostic studies that need to be performed, and the therapeutic strategies to be undertaken.

Axis 1 – Ictal semiology – includes the description of the ictal event, without reference to etiology, anatomy, or mechanisms. It can be very brief or extremely detailed, as required for clinical or research purposes, because the communication among clinicians, and among researchers, would be greatly enhanced by the establishment of standardized terminology for describing ictal semiology.

Axis 2 – Epileptic seizure type – indicates the type of seizure derived from a list of accepted seizure types which represent diagnostic entities with etiologic, therapeutic, and/or prognostic implications (Table 1). Localization within the brain should be specified when this is appropriate, and in the case of reflex seizures, the specific stimulus will also be specified here.

Table 1. EPILEPTIC SEIZURE TYPES AND PRECIPITATING STIMULI FOR REFLEX SEIZURES
[Modified from: ILAE (Epilepsy, 1989)]

<u>Self-limited seizure types</u>
Generalized seizures
- Tonic-clonic seizures
- Clonic seizures
  - o Without tonic features
  - o With tonic features
- Typical absence seizures
- Atypical absence seizures
- Myoclonic absence seizures
- Tonic seizures
- Spasms
- Myoclonic seizures
- Massive bilateral myoclonus
- Eyelid myoclonia
  - o Without absences
  - o With absences
- Myoclonic atonic seizures
- Negative myoclonus
- Atonic seizures
- Reflex seizures in generalized epilepsy syndromes
- Seizures of the posterior neocortex
- Neocortical temporal lobe seizures

Focal seizures
- Focal sensory seizures
  - o With elementary sensory symptoms
  - o With experiential sensory symptoms
- Focal motor seizures
  - o With elementary clonic motor signs
  - o With asymmetrical tonic motor seizures
  - o With typical (temporal lobe) automatisms
  - o With hyperkinetic automatisms
  - o With focal negative myoclonus
  - o With inhibitory motor seizures
- Gelastic seizures
- Hemiclonic seizures
- Secondarily generalized seizures
- Reflex seizures in focal epilepsy syndromes

<u>Continuous seizure types</u>
Generalized status epilepticus
- Generalized tonic-clonic status epilepticus
- Clonic status epilepticus

- Absence status epilepticus
- Tonic status epilepticus
- Myoclonic status epilepticus

Focal status epilepticus
- Epilepsia partialis continua of Kojevnikov
- Aura continua
- Limbic status epilepticus (psychomotor status)
- Hemiconvulsive status with hemiparesis

Precipitating stimuli for reflex seizures
- Visual stimuli
  - o Flickering light -color to be specified when possible
  - o Patterns
  - o Other visual stimuli
- Thinking Music
- Eating
- Praxis
- Somatosensory
- Proprioceptive
- Reading
- Hot water
- Startle
- Precipitating stimuli for reflex seizures

Axis 3 – Epilepsy syndrome – the syndromic diagnosis is also derived from a list of accepted epilepsy syndromes (Table 2), although it is understood that a syndromic diagnosis may not always be possible.

Axis 4 – Etiology – specifies the etiology when this is known. The etiology could consist of a specific disease derived from a classification of diseases frequently associated with epileptic seizures or syndromes, a genetic defect, or a specific pathological substrate.

Axis 5 – Degree of impairment – this axis is optional and designs the degree of impairment caused by the epileptic condition. Classification of impairment will be derived from the World Health Organization's International Classification of Impairments, Disabilities and Handicaps (ICIDH-2) which is currently in preparation.

Next, I will discuss in detail only those epileptic syndromes and types of seizures characterized by Spike-Waves/Poly-Spike-Waves (SW/PSW) and/or fast runs pertinent to my experimental approach.

_____

Table 2. EPILEPTIC SYNDROMES AND RELATED CONDITIONS
[Modified from: ILAE (Epilepsy, 1989)]

EPILEPTIC SYNDROMES
- Benign familial neonatal seizures
- Early myoclonic encephalopathy
- Ohtahara syndrome
- Migrating partial seizures of infancy[*]
- West syndrome
- Benign myoclonic epilepsy in infancy
- Benign familial infantile seizures
- Benign infantile seizures (non-familial)
- Dravet's syndrome
- Hemiconvulsions-Hemiplegia syndrome (HH syndrome)
- Myoclonic status in nonprogressive encephalopathies
- Benign childhood epilepsy with centrotemporal spikes
- Early onset benign childhood occipital epilepsy (Panayiotopoulos type)
- Late onset childhood occipital epilepsy (Gastaut type)
- Epilepsy with myoclonic absences
- Epilepsy with myoclonic-astatic seizures
- Lennox-Gastaut syndrome
- Landau-Kleffner syndrome
- Epilepsy with continuous spike-and-waves during slow-wave sleep
- Childhood absence epilepsy
- Progressive myoclonus epilepsies
- Idiopathic generalized epilepsies with variable phenotypes
  - Juvenile absence epilepsy
  - Juvenile myoclonic epilepsy
  - Epilepsy with generalized tonic-clonic seizures only
- Reflex epilepsies
  - Idiopathic photosensitive occipital lobe epilepsy
  - Other visual sensitive epilepsies
  - Primary reading epilepsy
  - Startle epilepsy
- Autosomal dominant nocturnal frontal lobe epilepsy
- Familial temporal lobe epilepsies
- Generalized epilepsies with febrile seizures plus[*]
- Familial focal epilepsy with variable foci[*]
- Symptomatic (or probably symptomatic) focal epilepsies
  - Limbic epilepsies

- Mesial temporal lobe epilepsy with hippocampal sclerosis
- Mesial temporal lobe epilepsy defined by specific etiologies
- Other types defined by location and etiology
o   Neocortical epilepsies
- Rasmussen syndrome
- Other types defined by location and etiology

[*] Syndromes in development

CONDITIONS WITH EPILEPTIC SEIZURES THAT DO NOT REQUIRE A DIAGNOSIS OF EPILEPSY

-      Benign neonatal seizures
-      Febrile seizures
-      Reflex seizures
-      Alcohol withdrawal seizures
-      Drug or other chemically-induced seizures
-      Immediate and early post traumatic seizures
-      Single seizures or isolated clusters of seizures
-      Rarely repeated seizures (oligo-epilepsy)

---

2   Epileptic syndromes characterized by SW/PSW complexes and/or fast runs

2.1 Absence seizures

Absence seizures are broadly divided into (a) typical absences of mainly idiopathic generalized epilepsy with generalized, greater than 2.5 Hz spike or polyspike-and-slow waves, and (b) atypical absences of symptomatic or cryptogenic epilepsies with slower, less than 2.5 Hz generalized discharges (International League Against Epilepsy (Commission on Epidemiology and Prognosis, 1981).

2.1.1 Typical absences

Typical absences are brief, generalized epileptic seizures with sudden onset and termination. They have two essential components: clinically the impairment of consciousness (absence) and EEG generalized 3 Hz to 4 Hz spike and slow wave discharges (International League Against Epilepsy (Commission on Epidemiology and Prognosis, 1981). Impairment of consciousness may be severe moderate, mild, or inconspicuous (the detection of which may require special cognitive testing). The discharge

SW frequency varies from onset to termination. It is usually faster and unstable in the opening phase (first second), becomes more regular and stable in the initial phase (first 3 seconds), and slows down towards the terminal phase (last 3 seconds).

2.1.2 Atypical absences

Atypical absences are associated with a high incidence of changes in postural tone. The beginning and end are usually difficult to identify because they are more progressive and because this type of seizure affects children whose mental function is altered. It is, therefore, difficult to determine the duration that ranges from 5 seconds to 20 seconds. The axial tone is affected, and this may cause the patient to fall. Eyelid clonus, mild tonic or autonomic features, or automatisms may also be observed. There is, therefore, a whole spectrum of clinical manifestations varying from typical absence to mild manifestations (Loiseau et al., 1995). Because the clinical features may be mild in an intellectually impaired child, it is often difficult to count such seizures, even with video EEG monitoring. Attention may decrease seizure frequency, whereas drowsiness may increase it. The frequency of atypical absences varies from a few a day to nearly continuous. The frequency of generalized SW discharge on EEG is less than 3Hz (Panayiotopoulos, 1997). Only an EEG can identify this type of seizure. Many patients have, in addition to clinical seizures, subclinical discharges. Counting the seizures is, therefore, an unresolved challenge since isolated clinical observation omits subclinical discharges that can affect cognition. Counting EEG discharges would ignore differences in the impact of clinical versus subclinical discharges, and video EEG is reliable only if permanent clinical observation is also used to determine whether the discharge is clinical or subclinical. Such observation would alter the frequency of seizures, though, since it would raise the vigilance of the child and, therefore, prevent the occurrence of absences and discharges. However, the practical implications of this difficulty are moderate because the aim of treatment, including those used in clinical trials, should be the disappearance of seizures and of spikes on EEG (Dulac, 2003). Atypical absences may be combined with tonic seizures and slow spike waves, and this combination defines the Lennox-Gastaut syndrome, which is usually symptomatic and may follow West syndrome in 40% of the cases. Atypical absences may be atonic or tonic. They may occur as the only type of seizure in a patient who exhibits continuous SW in slow wave sleep (SWS). In such patients, the absences are mainly atonic. Atypical absences

may be combined with generalized tonic-clonic and myoclonic seizures in myoclonic-astatic epilepsy. In this condition, the absences are also mainly atonic (for review see Panayiotopoulos, 2002c).

2.2 Absence status epilepticus

Absence status epilepticus is a prolonged, generalized absence seizure, which is defined as lasting more than half an hour, but usually lasts for hours and even for days. It is associated with typically regular and symmetrical generalized discharges of 1 Hz to 4 Hz spike or multiple spike-and-slow wave complexes. Though the sharing symptom of absence status epilepticus is impairment of cognition, this is often associated with other clinical manifestations that may be syndrome-related. It should be emphasized that absence status epilepticus, like the brief absence seizure, is not one but many types of a prolonged, generalized, absence seizure. The cardinal symptom shared by all cases of absence status is the altered content of consciousness in a patient who is usually fully alert. Memory and higher cognitive intellectual functions such as abstract thinking, computation, and personal awareness are the main areas of disturbance. This varies from extremely mild to extremely severe with intermediate states of severity occurring more often (for review see Andermann and Robb, 1972; Guberman et al., 1986; Agathonikou et al., 1998; Panayiotopoulos, 2002a).

The pathophysiology of absence status (typical or atypical) is unknown. It is likely that the generating mechanisms of absence seizures and absence status are the same because their clinico-electroencephalographic features show marked syndrome-related similarities (Agathonikou et al., 1998). The difference is in the duration of the discharge, which may also perpetuate more severe cognitive impairment. Absence seizures last for only a few seconds, whereas absence status epilepticus is prolonged for hours and days (Panayiotopoulos, 2002b).

2.3 Electrical status epilepticus during sleep

A disorder in which sleep induced an EEG pattern characterized by "subclinical" SW occurring almost continuously during SWS and appearing every night for a variable length of time in children was reported in 1971 by Patry and coworkers under the title of

"subclinical electrical status epilepticus induced by sleep in children" (Patry et al., 1971). The disorder was later termed "electrical status epilepticus during sleep" (Tassinari et al., 1985).

The clinical manifestations of this syndrome include: (1) a heterogeneous epileptic disorder, (2) a deterioration of neuropsychological functions associated with or independent from the epileptic disorder and (3) a deterioration of motor functions. The typical EEG pattern of continuous SW during SWS is also an essential and absolute feature for the recognition of the syndrome (Tassinari et al., 1999).

The seizure types occurring in the disorder can be both partial and generalized. They include unilateral or bilateral clonic seizures, generalized tonic-clonic seizures, absences, partial motor seizures, complex partial seizures or epileptic falls. They may occur during wakefulness or sleep. Tonic seizures, however, never occur. The first seizure is reported to be nocturnal and of unilateral type in almost one half of the cases reported. At onset, the frequency of seizure attacks is low. At the time of discovery of the typical nocturnal EEG pattern, however, the epileptic seizures frequently change in severity and frequency. Absences and epileptic falls herald the appearance of continuous SW during SWS and seizure frequency increases, both during wakefulness and sleep (Tassinari et al., 1985; Tassinari et al., 1999). Most researchers assert that more than 85% of SWS is occupied by SW discharges; however, quantitative studies of different sleep stages and of temporal evolution of this EEG disturbance have not been carried out (Jayakar and Seshia, 1991). The typical EEG changes appear 1 year to 2 years after the first seizure and are associated with behavioral deterioration. Focal and generalized interictal spikes (ISs) occur before this time and persist during wakefulness and REM sleep after the appearance of continuous SW during SWS (Tassinari et al., 1999).

After the seizures appear, but before the continuous SW during SWS develop, the EEG during wakefulness may show both diffuse SW, sometimes in bursts, and focal abnormalities such as spikes and slow spikes, with or without associated slow waves, which usually involve the fronto-temporal or the centro-temporal region. Sleep EEG performed at an early stage shows an increase of the aforementioned abnormalities without the features of the continuous SW during SWS (Tassinari et al., 1985).

2.4 The Lennox-Gastaut syndrome

An EEG with a 2 Hz ("slow") SW pattern was first described by Gibbs in 1939 (Gibbs et al., 1939). It was associated with a special type of absence seizure characterized by incomplete loss of consciousness. By contrast, classic petit mal absence seizures had been known to be associated with rhythmic 3 Hz SWs. The term "petit mal variant" was therefore used to describe the EEG pattern and the clinical seizure complex. Lennox and Davis were the first to correlate the slow SW EEG pattern with a distinctive group of clinical manifestations (e.g., mental retardation and specific seizure types), including myoclonic jerks, atypical absences, and astatic seizures (drop attacks) (Lennox and Davis, 1950). Thereafter, Gastaut and his colleagues described the clinical manifestations and EEG patterns of 100 patients with slow SW (Gastaut et al., 1966). They called this syndrome "Lennox syndrome" or "childhood epileptic encephalopathy with diffuse slow SW." Finally, the term "Lennox-Gastaut syndrome" first appeared in the literature in 1969 (Niedermeyer, 1969).

The Lennox-Gastaut syndrome may result from a variety of diffuse encephalopathies. It is characterized by the clinical triad of diffuse slow spikes-and-waves on EEG, mental retardation, and multiple types of generalized seizures, including especially atypical absences and tonic and atonic seizures (Aicardi and Levy Gomes, 1992; Dulac and N'Guyen, 1993). The Lennox-Gastaut syndrome is, with rare exception, a condition of children. The age of onset is between 2 and 8 years in most cases. Boys are affected more frequently than girls. Symptoms can appear de novo without apparent cause (cryptogenic Lennox-Gastaut syndrome) or result from obvious brain insult (symptomatic Lennox-Gastaut syndrome). Cryptogenic cases start later than symptomatic cases do (for review see Dulac and Engel, 2003).

In young children, the Lennox-Gastaut syndrome usually begins with episodes of sudden falls. In the school-age group, behavioral disturbances may be the heralding signs, along with drop attacks. This is soon followed by frequent seizures, episodes of status epilepticus, progressively deteriorating intellectual functions, personality disturbances, and chronic psychosis (Roger et al., 1989).

Tonic seizures occur in most affected children. They are usually brief, lasting only seconds. Depending on the extent and groups of muscles involved, they may appear as axial (characterized by flexor movements of the head and trunk), axial rhizomelic (characterized by elevation and adduction of proximal upper limbs, stiffening of posterior neck muscles, elevation of shoulders, opening of the mouth, upward deviation of the eyes and brief apnea), or global, leading to sudden falls if the patient is in an upright position (Gastaut et al., 1966).

Atypical absence seizures occur in approximately two-thirds of patients. Both the onset and the termination are gradual in contrast to the strikingly sudden lapses in typical absence. Whereas typical absences are usually brief (less than 10 seconds) and consciousness returns immediately and completely afterward, atypical absences are usually longer and are often followed by some postictal cognitive impairment. During the atypical absence there is "clouding" rather than loss of consciousness so that patients can continue their activity to some degree (Gastaut et al., 1966). Associated manifestations are more common in atypical than in typical absences and include eyelid or perioral myoclonus, progressive flexion due to loss of postural tone, and localized motor phenomena, such as neck-stiffening or head-nodding. Other kinds of seizures, including atonic, partial and generalized tonic-clonic seizures, are less frequent (Roger et al., 1989).

Most patients with the Lennox-Gastaut syndrome have one or more episodes of status epilepticus (Dulac and N'Guyen, 1993). A variety of forms of status epilepticus occur that represent a continuum from absence status, consisting of an insidious confused state that can last for days or weeks, to pure tonic status epilepticus, which is more often seen in adolescents or adults than in children.

The slow SW consists of a blunt, slow spike (approximately 150 ms), followed by a slow wave (approximately 350 ms). The amplitude ranges from 200 μV to 800 μV. When 2 spikes to 3 spikes precede the slow wave, they constitute a PSW complex. The frequency (1.5-2.5 Hz) is relatively slow and arrhythmic compared to that of classical absence seizures (rhythmic 3 Hz). Occasionally, bursts of rapid spike-and-waves at 3 Hz or even 4 Hz may occur in combination with the slow SW (Gastaut et al., 1966). Generalized PSW discharges or lower voltage fast activity (generalized paroxysmal fast activity), lasting 1

second or more without obvious clinical correlates, are common during SWS. Relaxation, drowsiness, sleep, and hyperventilation facilitate the appearance of slow SW. During sleep, the abnormal patterns become more prominent, symmetrical, and synchronous, with even slower SW or PSW complexes. Photic stimulation, on the other hand, has no effect on these EEG events (Markand, 1977). When evolution in EEG patterns occurs in patients previously affected by infantile spasms, the direction is from hypsarrhythmia to multifocal interictal spikes to generalized spike discharges to slow SW, with the last representing a stable pattern that characterizes children with the Lennox-Gastaut syndrome (Kotagal, 1995).

The EEG correlate of tonic seizures consists of a 10 to 13 Hz recruiting rhythm, usually followed by high amplitude slow activity rather than postictal EEG depression. Although the EEG during absence seizures often shows irregular SW discharges, these patterns are not necessarily different from interictal EEG discharges and do not clearly demarcate the occurrence of an ictal event. Similarly, there is no characteristic pattern of absence status; this prolonged confused state may be associated with an increase in slow SW activity or with irregular slowing resembling hypsarrhythmia (Dulac and N'Guyen, 1993).

2.5 The West syndrome

West syndrome comprises a triad of spasms in clusters, mental retardation, and diffuse and profound paroxysmal EEG abnormalities. The onset is insidious in either an otherwise normal or an already handicapped infant. It is an age-dependent epilepsy syndrome that begins in infancy, mostly between 4 months and 6 months of life, before the age of 12 months in over 90% of cases (Kellaway et al., 1979).

Infantile spasms are characterized by usually symmetrical, bilateral, brief, and sudden contractions of the axial muscle groups. The features of the seizures depend on whether the flexor or extensor muscles are predominantly affected and also on the number and distribution of the muscle groups involved. Thus, spasms may vary from extensive contractions of all flexor or extensor muscles to contractions of only neck muscles or abdominal recti (Hrachovy and Frost, 1989).

In fact, the type of spasms, whether in flexion, extension, or mixed does not seem to be affected by etiology or the prognosis. In contrast, whether the spasms are symmetrical or not is important because asymmetry, consisting of lateral deviation of the head or eyes, contributes to indicate some kind of cortical brain damage (Fusco and Vigevano, 1993). Spasms tend to occur soon after awakening or on falling asleep. Most of the spasms occur in clusters, so the interval between successive spasms is less than 60 seconds. Usually the intensity of spasms in a given cluster will peak gradually and then decline (Hrachovy and Frost, 1989).

The usual EEG abnormalities consist of diffuse, high amplitude, asynchronous paroxysmal and slow wave theta and delta activity with loss of background features that is continuous when awake and fragmented in sleep. This hypsarrhythmic pattern may be symmetrical or asymmetrical because of additional foci, or unilateral. In other conditions, it consists of one or several spike foci when awake with secondary generalization in sleep (Dulac, 2003). The interictal EEG of infantile spasms is usually characterized by hypsarrhythmia with a continuous, irregular, random, ever-changing, and disorganized, high-voltage spike and slow wave activity. This is sufficiently characteristic to be easily identified, and the term "chaos" may be inappropriate from this point of view. It may be present during wakefulness and SWS sleep or may be present only during sleep (Watanabe et al., 1993).

2.6 Posttraumatic epilepsy

The posttraumatic seizures (PTS) are the seizures occurring after head trauma that are thought to be causally related to the trauma itself, while posttraumatic epilepsy (PTE) is defined as one or more unprovoked seizures after head trauma (Frey, 2003).

An unprovoked seizure is a seizure occurring in the absence of one or more precipitating factors. By contrast, a provoked (acute symptomatic) seizure is a seizure occurring in close temporal relation with an acute systemic, toxic, or metabolic insult (including traumatic brain injury - TBI), which is expected to be the underlying cause. Unprovoked seizures include events occurring in patients with antecedent stable (nonprogressing) insults of the central nervous system (CNS), including TBI (remote

symptomatic seizures) (Beghi, 2003). After TBI, the difference between provoked and unprovoked seizures is relevant to the assessment of the indications for and effects of treatment, as suggested by The Brain Injury Special Interest Group of the American Academy of Physical Medicine and Rehabilitation.

PTS are usually divided into three categories: "immediate" seizures, early seizures, and late seizures. Early seizures are those occurring while the patient is "still suffering from the direct effects of the head injury" (Annegers et al., 1980), a period commonly defined as 1 week after head injury. Approximately 90% of seizures occurring within the first 4 weeks after head injury will happen during this first week (Jennett, 1975). In general, seizures that happen at or minutes after impact – "immediate", "contact," or "concussive" seizures – are not included in studies of early PTS. The exact pathophysiology behind immediate seizures and their exact clinical significance therefore remains unclear. Late PTS are usually defined as seizures occurring >1 week after injury (for review see Frey, 2003).

By definition, seizures occurring within 24 h of TBI (immediate seizures) and seizures occurring between the first 24 h and the first 7 days after injury (early PTS) are provoked seizures, whereas seizures seen after 7 days (late PTS) are unprovoked seizures. Based on these definitions, the effects of antiepileptic drugs (AED) in patients with TBI must be assessed separately in terms of prevention and control of provoked seizures (which include immediate and early PTS) and prevention of subsequent unprovoked seizures (late PTS or PTE).

2.6.1 Epidemiology of PTE

In individuals younger than 45 years, injury is the primary cause of death in the developed nations and the general incidence of TBI in developed countries is frequently stated to be 200 per 100,000 population at risk per year (Bruns and Hauser, 2003).

The overall risk of seizures is as high as 53% after war injuries (Salazar et al., 1985) and ranges from 1.8% to 5% in general population (Jennett, 1975; Annegers et al., 1980), with a significant proportion of cases resulting in lifelong disability (Goldstein, 1990). Of mild head injuries, 10% are thought to result in permanent disability, as well as 66% of moderate head injuries and 100% of severe head injuries (Jallo and Narayan, 2000). The

higher incidence of PTSs following wartime injuries is explained by the correlation between the occurrence of PTSs and the severity of injury. Compared with series done in the civil sector, a consistently higher proportion of severe head injuries was seen in series of wartime injuries (Salazar et al., 1985). This disproportionate severity of wartime injuries is in large part due to a higher proportion of injuries that involve dural penetration and widespread brain damage in military patients, as mentioned earlier. For example, 40.9% of the veterans involved in the Vietnam Head Injury Study (Caveness et al., 1979) had head injuries with damage to multiple lobes of the brain, whereas only 7.1% of the civilian patients studied by Annegers et al. (Annegers et al., 1980) qualified for a diagnosis of severe brain injury. It only makes sense, then, that these series of wartime injuries, with their higher proportion of severe head injuries, would show greater PTS frequencies than would civilian series.

2.6.2 Frequency of PTE

2.6.2.1 Early seizures
The incidence of early seizures (usually within 1 week of injury) ranges from 2.1 to 16.9% and, in general, is correlated with the distribution of head-injury severity within the specific group being studied. Of the patients in one series, 10% were in status epilepticus, a presentation more common in children (Jennett, 1975).

2.6.2.2 Late seizures
Depending on the series, the incidence of late seizures ranges from 1.9% to more than 30%. Like the incidence of early PTSs, the variability in this finding is likely due to variability within the patient populations being studied, especially with respect to injury severity. In general, most late PTSs occur during the first year after injury (Jennett, 1975; Annegers et al., 1998), although they can also occur for many years afterward. Exactly how long the increased risk of seizures persists after head injury remains still unclear. Investigators for the Vietnam Head Injury Study concluded that, in their population, PTSs incidence does not reach that of the general uninjured population until well after 15 years after injury (Weiss et al., 1986). However, with extended follow-up of the same population of head-injured patients, other authors concluded that even unprovoked seizures occurring more

than 10 years after a severe traumatic brain injury can be attributed in large part to the initial injury (Annegers et al., 1998).

2.6.3 Risk factors for PTE

The risk factors that have been found to be significant for the development of both early and late PTSs are described below.

2.6.3.1 Early seizures

The most consistent risk factor is the presence of an intracerebral blood collection, which confers approximately 30% increase in the risk of early PTSs, regardless of the patient's age or other features of their injury (Jennett, 1975; Desai et al., 1983). This increased risk is especially true for subdural hematomas in children (Hahn et al., 1988). In this same age group, intraparenchymal and epidural hematomas do not confer the same risk of early seizure development, presumably because of the lesser degree of direct cortical irritation from blood products (Hahn et al., 1988).

The second most predominant risk factor for the occurrence of early PTSs is a higher injury severity. It has been reported a five-year cumulative probability of developing PTSs of 0.7% in patients with mild injuries, 1.2% in those with moderate injuries, and 10.0 percent in those with severe injuries. Moreover, the 30-year cumulative incidence of seizures was 2.1% for mild injuries, 4.2% for moderate injuries, and 16.7% for severe injuries (Annegers et al., 1998).

In many studies, early seizures occurred 50–100% more frequently in children than they did in adults with comparable injuries (Kollevold, 1979; Annegers et al., 1998). Nevertheless, younger age is not an independent risk factor for the occurrence of early PTSs if the incidence is adjusted for the increased risk of seizures that exists for this population, regardless of antecedent head injury (Annegers et al., 1998).

Other risk factors that likely influence the occurrence of early PTSs include diffuse cerebral edema (in children), intracranial metal-fragment retention, residual focal neurologic deficits, and depressed or linear skull fractures (adults only) (Frey, 2003).

2.6.3.2 Late seizures

Although some studies state that the presence of early PTSs is the most consistently significant risk factor for the development of late PTSs (Hauser et al., 1996; Olafsson et al., 2000; Olsen, 2001), others found that the occurrence of early seizures was not an independent risk factor in multivariate analysis (Annegers et al., 1998), or even that there was no relationship whatsoever between early seizures and the incidence of late seizures (Temkin, 2001; Herman, 2002). Nevertheless, the relation between early PTSs and the development of late PTSs appears to vary based on age. Indeed, Jennett (Jennett, 1975) found that the risk of late PTSs is not significantly increased in children younger than 16 years who only have focal early seizures. In another study, no children with early PTSs were at increased risk of developing late seizures, regardless of their early seizure type (Annegers et al., 1980).

Intracranial hemorrhage is also a risk factor for the occurrence of late PTSs and may confer as much as a 10 fold increase in risk (Annegers et al., 1998). Subdural hematomas are likely responsible for most of this increased risk in both children and adults. The presence of a brain contusion was as strong of a predictor of late seizure occurrence as was the presence of a subdural hematoma (Frey, 2003).

Premorbid chronic alcoholism likely increases also the risk of the development of late PTSs (Kollevold, 1979; Hauser et al., 1984; Ng et al., 1988). In one study, a higher percentage of patients with chronic alcohol use had late PTSs than did comparable patients who did not drink (Kollevold, 1979). In addition, the same study suggested that chronic alcohol use may decrease the chance of seizure remission, although the data were not necessarily tested for significance. Conversely, patients with seizures after head injury have been shown to be at higher risk for both drug and alcohol abuse (Hauser et al., 1984). The relation between alcohol use and seizures (of any type) was studied more specifically by Ng et al. (Ng et al., 1988), who concluded that heavy alcohol use is an independent, dose-related risk factor for seizures.

Other risk factors for the development of late PTSs include metal-fragment retention, skull fracture, residual cortical neurologic deficits, a single CT lesion in the temporal or

frontal regions, and persistent focal abnormalities on EEG for more than one month after injury (Frey, 2003).

3 Sleep and epilepsy

Although it is obvious that 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., 1973a; Sato et al., 1973b; Kellaway, 1985; Shouse et al., 2000), suggesting a profound effect of sleep on epilepsy. Nevertheless, we should also acknowledge that epilepsy influences sleep in several ways, such as decreasing sleep efficiency and increasing sleep stage shifts (Malow, 2007).

3.1 Effect of sleep on epilepsy

The intimate relationship between epilepsy and sleep has been recognized since antiquity, when both Hippocrates and Aristotle observed the occurrence of epileptic seizures during sleep. However, the relationship between sleep and epilepsy was not investigated until the end of the 19th century when Gowers studied the effect of the sleep/wake cycle on grand mal epilepsy (Gowers, 1885). An early study looking at the time of occurrence of nocturnal seizures described two peaks, the first peak occurring approximately two hours after bedtime and the second between 4-5 am (Langdon-Down and Brain, 1929). Nevertheless, these reports were based on clinical observations alone since they were published before the discovery of the human electroencephalogram (EEG) (Berger, 1929), when the EEG was not yet incorporated into studies of sleep and epilepsy.

It was in 1947 when Gibbs and Gibbs first reported on the relationship of interictal epileptiform activity (IEA) and sleep using EEG (Gibbs and Gibbs, 1947). They observed an increase in the IEA during sleep compared to the frequency of the IEA recorded during the waking state.

Recognizing the close relationship that exists between sleep and epilepsy, many authors have classified seizures based on the time of occurrence of seizures with regard to

the sleep/wake cycle. Three distinct groups were created (Gowers, 1885; Langdon-Down and Brain, 1929; Janz, 1962):

1. Seizures restricted to sleep (sleep epilepsy)

2. Seizures occurring only when awake (waking epilepsy)

3. Seizures occurring in both the sleep and waking states (diffuse epilepsy)

In the early studies based on clinical observation alone, it was found that 42-45% of the patients experienced seizures that occurred predominately during the daytime, 19-24% experienced seizures that occurred only during nocturnal sleep, and 33-37% experienced seizures that occurred both during the daytime and nighttime hours (Gowers, 1885; Langdon-Down and Brain, 1929; Patry, 1931). It soon became apparent to investigators that the various types of seizure are affected differently by the circadian sleep-wake cycle, but also by the ultradian rapid-eye-movement/slow-wave-sleep (REM/SWS) cycle, and new studies emerged.

It appears that according to the kind of epilepsy, sleep may have a facilitating or precipitating effect on seizures or else a protective effect against epilepsy (Baldy-Moulinier, 1986). The protective effect is suggested by the occurrence of seizures after sleep deprivation. This effect was well established for the grand mal epilepsies of awakening defined by Janz (Janz, 1953).

The facilitating effect of sleep on seizure occurrence is suggested by:

1. The existence of sleep epilepsies, called 'morpheic epilepsies' (Passouant et al., 1951). A prospective study restricted to patients with partial seizures showed that overall, 20% of seizures occurred during sleep (Herman et al., 2001). Nevertheless, seizures that occur only during sleep may represent a distinct class with a particularly good prognosis, except in patients with a history of head trauma or central nervous system lesions (Yaqub et al., 1997; Park et al., 1998).

2. The increase of IED during sleep (Gibbs and Gibbs, 1947). More recently, Malow et al. compared interictal discharges in routine awake EEGs with overnight EEG recordings

in 24 patients with refractory temporal lobe epilepsy. All showed IEA in the overnight study, as opposed to only 46% during daytime recordings (Malow et al., 1999).

3. The relationship between the production of sleep spindles and epileptic discharges (Gloor, 1979; Kostopoulos et al., 1981). In these studies, electrophysiological data obtained in generalized penicillin epilepsy of the cat indicate that bilaterally synchronous SW discharge represents an abnormal response pattern of cortical neurons to afferent thalamocortical (TC) volleys normally involved in the elicitation of spindles.

The different stages of sleep may have a different effect on interictal discharge production. In SWS, particularly in stage 2, a synchronizing effect is shown by an increase in frequency and a spreading of seizures, while REM sleep prevented generalized discharges of generalized epilepsies (Bazil and Walczak, 1997; Herman et al., 2001). Furthermore, it has been shown that focal epileptiform spikes that occur during REM sleep are more accurate for seizure localization compared with other sleep states (Malow and Aldrich, 2000).

3.2 Effect of epilepsy on sleep

Reciprocally, epilepsy alters the organization and microarchitecture of sleep: firstly, by the acute effect of a seizure during sleep, disrupting continuity, and secondly, by the chronic effect of epilepsy, impairing the organization and altering the microarchitecture of sleep. These modifications differ according to the kind of epilepsy.

From an organizational point of view, epilepsies generate a decrease in total sleep time, wakefulness after sleep onset and sleep efficiency index, an increase in sleep onset latency, first REM delay and percentage of the stage 2 SWS rate with a decrease in the percentage of REM sleep rate have been reported (Touchon et al., 1991; Crespel et al., 1998; Crespel et al., 2000). Similarly, decreased sleep efficiency and increases in sleep stage shifts, entries to wakefulness, and the number and duration of awakenings were noted in 15 patients with recently diagnosed (less than 3 months) and untreated temporal lobe epilepsy (Touchon et al., 1987). Carbamazepine treatment improved sleep stability in these patients and this improvement could play a role in the therapeutic effect of the drug. Apparently, seizures themselves suppress REM sleep and increase SWS stage 1 sleep, as

reported in a study of 87 recordings in 21 patients that compared nights with seizures and seizure-free nights (Bazil et al., 2000). In this study, patients in an epilepsy monitoring unit were recorded with polysomnography under baseline conditions (seizure free) and compared with the same patients following complex partial or secondarily generalized seizures. With daytime seizures, there was a significant decrease in REM sleep the following night without significant changes in other sleep stages or in sleep efficiency. When seizures occurred at night, this decrease in REM was more pronounced and there were increases in stage 1 and decreases in sleep efficiency, and these effects were even more pronounced when seizures occurred early in the night (Bazil et al., 2000). This reciprocal acute and chronic action of sleep and epilepsy may constitute a vicious circle, enabling the facilitation of seizure and/or diurnal sleepiness by chronic sleep deprivation.

The physiopathological mechanisms that underlie this relationship between sleep and epilepsy remain unclear and hypothetical. An action of the inputs of the ascending neurotransmitter systems and their changes with sleep-waking states and the subcortical synchronizing systems or inhibitory systems may be involved. The TC volleys, the local conditions and the different degrees of responsiveness to sleep-regulating factors may play a role in this interrelationship between sleep and epilepsy, but future studies are needed in order to better understand this complex association.

4 Cortically generated seizures

In the light of the above information, one understands that although seizures with SW/PSW complexes at about 3 Hz are the signature of petit-mal or absence epilepsy, the SW complexes should not be equated with absence epilepsy since they occur also in other types of epilepsy (Niedermeyer, 1999).

Clinical and experimental studies pointed out that SW seizures are not "suddenly generalized and bilateral synchronous" as traditionally regarded according to the initial "centrencephalic" hypothesis (Jasper and Kershman, 1941). Instead it is now proven that they are locally generated and progressively built up within cortico-cortical and cortico-thalamic networks and then they propagate from one hemisphere to another with time-lags as short as 15 ms (Lemieux and Blume, 1986), which obviously cannot be estimated by

visual inspection. In another study, the investigation of apparently bilateral synchronous SW complexes in children with sleep-activated seizures similarly showed an inter-hemispheric time difference during SW activity of 12-26 ms (Kobayashi et al., 1994).

Indeed, even early studies in humans show focal and multi-focal clinical and EEG abnormalities in a significant percentage of patients with absence seizures associated with SW complexes at ~3 Hz (Gibbs and Gibbs, 1952). Some SW/PSW seizures display focal paroxysmal activity (O'Brien et al., 1959) confined to one cortical area or to few contiguous cortical fields, and this is in line with the concept that SW/PSW seizures originate in the neocortex and are thereafter disseminated through intra-cortical circuits, before they spread to the thalamus and exhibit generalized features (Neckelmann et al., 1998).

4.1 Evidence for cortical origin of SW seizures

Recent experimental studies strongly suggest that seizures characterized by an EEG aspect of SW complexes at ~3 Hz originate in the neocortex (for review see Steriade, 2003; Timofeev and Steriade, 2004). The findings that support this conclusion are:

1. The presence of such paroxysms in neuronal pools within the cortical depth, even without reflection at the cortical surface (Steriade, 1974), and in isolated cortical slabs *in vivo* (Timofeev et al., 1998; Timofeev et al., 2000).

2. Total hemithalamectomy did not prevent the occurrence of SW seizures elicited by local infusion of the gamma-amino butyric acid $GABA_A$-receptor antagonist, bicuculline, in the cortex (Steriade and Contreras, 1998).

3. Absence of SW seizures after intra-thalamic injections of bicuculline, which rather induced a pattern of highly synchronous slowed spindling in cat (Ajmone-Marsan and Ralston, 1956; Steriade and Contreras, 1998) or rat (Castro-Alamancos, 1999) thalamus *in vivo*, in ferret slices *in vitro* (Bal et al., 1995), but not seizures.

4. Most of the TC neurons are hyperpolarized and do not fire spikes during paroxysmal discharges recorded in corresponding cortical areas (Steriade and Contreras, 1995; Pinault et al., 1998; Timofeev et al., 1998; Timofeev and Steriade, 2004).

Frequently, the SW/PSW complexes on EEG correspond clinically with the clonic components of seizures, while the runs of fast spikes lasting longer than 5 seconds correspond to the tonic components (Niedermeyer, 1999).

4.2 Cellular correlates of SW complexes and fast runs

The neuronal mechanisms underlying the generation of electroencephalographic SW complexes are similar in seizures consisting of only SW and in those paroxysms resembling the EEG patterns of the clinical Lennox-Gastaut syndrome, in which SW complexes at slightly lower frequencies are intermingled with fast runs (Halasz, 1991; Yaqub, 1993; Niedermeyer, 1999; Timofeev and Steriade, 2004). During SW discharges the cortical neurons are depolarized and fire spikes during depth-negative (EEG spike) components and hyperpolarized during depth-positive (EEG wave) components. The typical seizure consisting of SW/PSW complexes recurring with frequencies 1-3 Hz and fast runs with frequencies of oscillations at 8-14 Hz is shown in the Figure 4-1. The seizure starts with SW/PSW discharges, which progressively increase in duration and the seizure displays a prolonged period of fast runs, followed again by PSW complexes and the seizure ends with SW discharges (Timofeev and Bazhenov, 2005).

The electrographic "spike"-components of SW complexes correspond intracellularly to paroxysmal depolarizing shifts (PDS) (for review see McNamara, 1994; Traub et al., 1996; Timofeev and Steriade, 2004). Interictal EEG "spikes" (IS) or PDS are similar to those that occur during SW seizures and firstly isolated PDS may progressively evolve into full-blown SW paroxysms (Figure 4-2) (Neckelmann et al., 1998; Timofeev and Steriade, 2004). Initially, PDS have been regarded as giant excitatory postsynaptic potentials (EPSP) (Johnston and Brown, 1981, 1984), enhanced by activation of voltage-gated intrinsic currents (Wong and Prince, 1978; Dichter and Ayala, 1987). Nevertheless, recent *in vivo* and *in vitro* data demonstrate the presence of inhibitory processes during different types of seizure activity (Traub et al., 1996; Esclapez et al., 1997; Prince and Jacobs, 1998) and the important inhibitory component of PDS (Cohen et al., 2002; Timofeev et al., 2002a; Fujiwara-Tsukamoto et al., 2003). This inhibitory component of PDS accounts, at least partially, for the diminished excitability of neocortical neurons to antidromic and synaptic volleys during the EEG "spike" component of SW complexes (Steriade and Amzica, 1999).

Therefore, while during the hyperpolarization following each PDS cortical stimuli reliably elicit PDS that are similar to the EPSP latency prior to seizure, during the EEG "spike" component of SW complexes the same stimuli are completely ineffective in eliciting an overt response (Figure 4-3) (Steriade and Amzica, 1999). The decreased responsiveness of cortical neurons during the EEG "spike" is complemented by a significant decrease in the apparent input resistance (IR) during this component of SW complexes (Matsumoto et al., 1969; Neckelmann et al., 2000; Timofeev et al., 2002a).
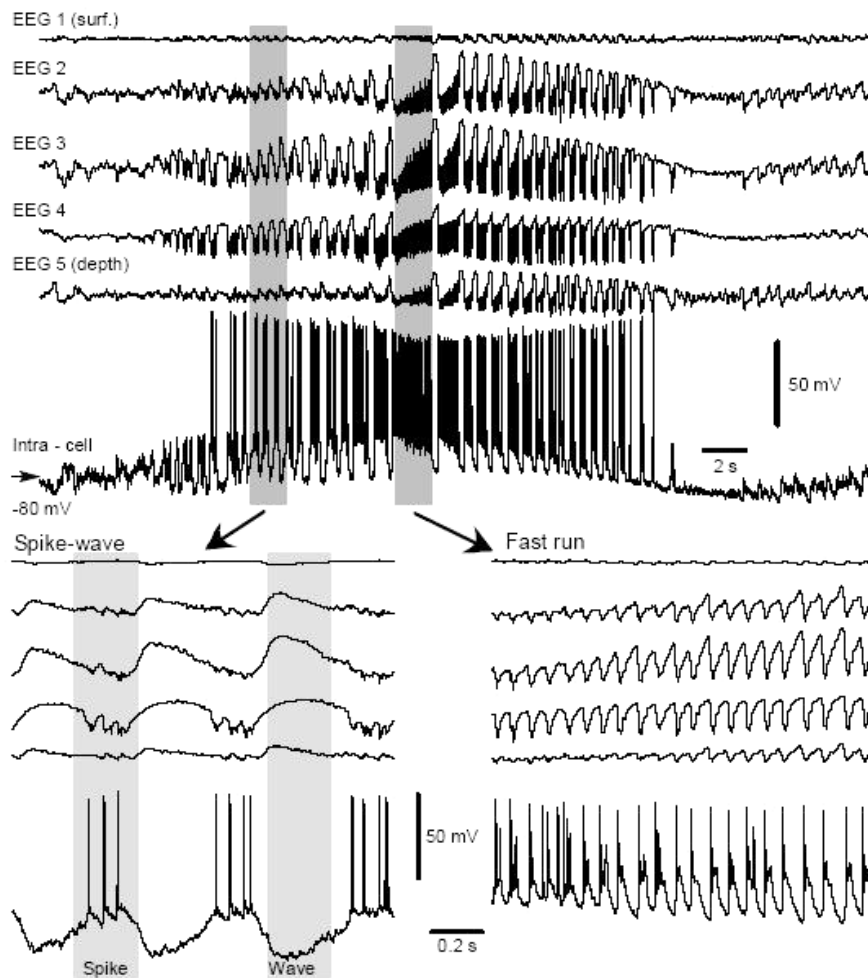
Figure 4-1. Field potential and intracellular features of ideopatic electographic seizure recorded from cortical area 5 of cat anesthetized with ketamine-xylazine. Upper panel, five upper traces are the local field potentials recorded with an array of electrodes from cortical surface and different depth. The distance between electrodes in the array was 0.4 mm. Lower trace, an intracellular recording from cortical regularspiking neuron located at 1 mm depth and ~0.5 mm lateral to the array. A period of spike-wave discharges and fast runs is expanded in the lower panels as indicated. In the left lower panel the EEG-spike and the EEG-wave components are marked by grey rectangulars. (From: Timofeev and Bazhenov, 2005)
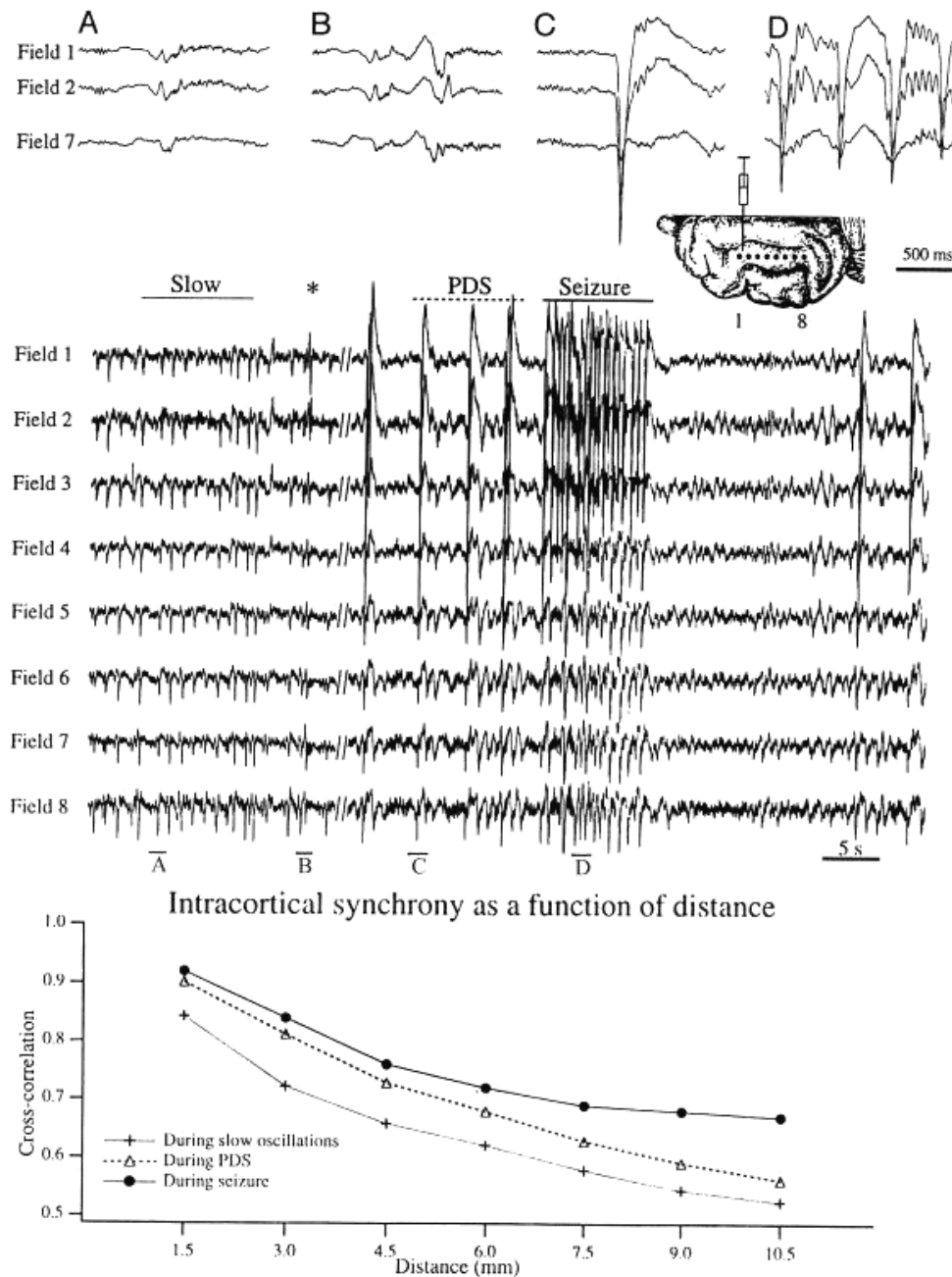
Figure 4-2. Evolution from slow oscillation to isolated PDSs and SW seizure. Cat under ketamine–xylazine anesthesia. Progressive changes in cortical activity and synchrony after placement of a syringe with 10 µl 0.2 mM bicuculline in area 5. Bicuculline was not injected, but leaked into cortex. Eight electrodes (1.5 mm apart) were inserted; the syringe was between electrodes 1 and 2 (see brain figurine). The left part shows slow oscillation (see detail A). A star marks the first paroxysmal EEG "spike" B. Later, the field potentials became dominated by recurrent EEG "spikes" that, intracellularly, correspond to PDSs C. eventually leading to a seizure with a PSW pattern D. Lower panel, sequential cross-correlation on 1-s windows between all electrode pairs. Peak amplitude of the cross-correlation function was averaged across all electrode pairs (see inter-electrode distance below the abscissa) for each of the three 10 s periods marked Slow, PDS and Seizure, and plotted as a function of inter-electrode distance. Similar voltage calibration in all panels. (From: Neckelman et al., 1998).
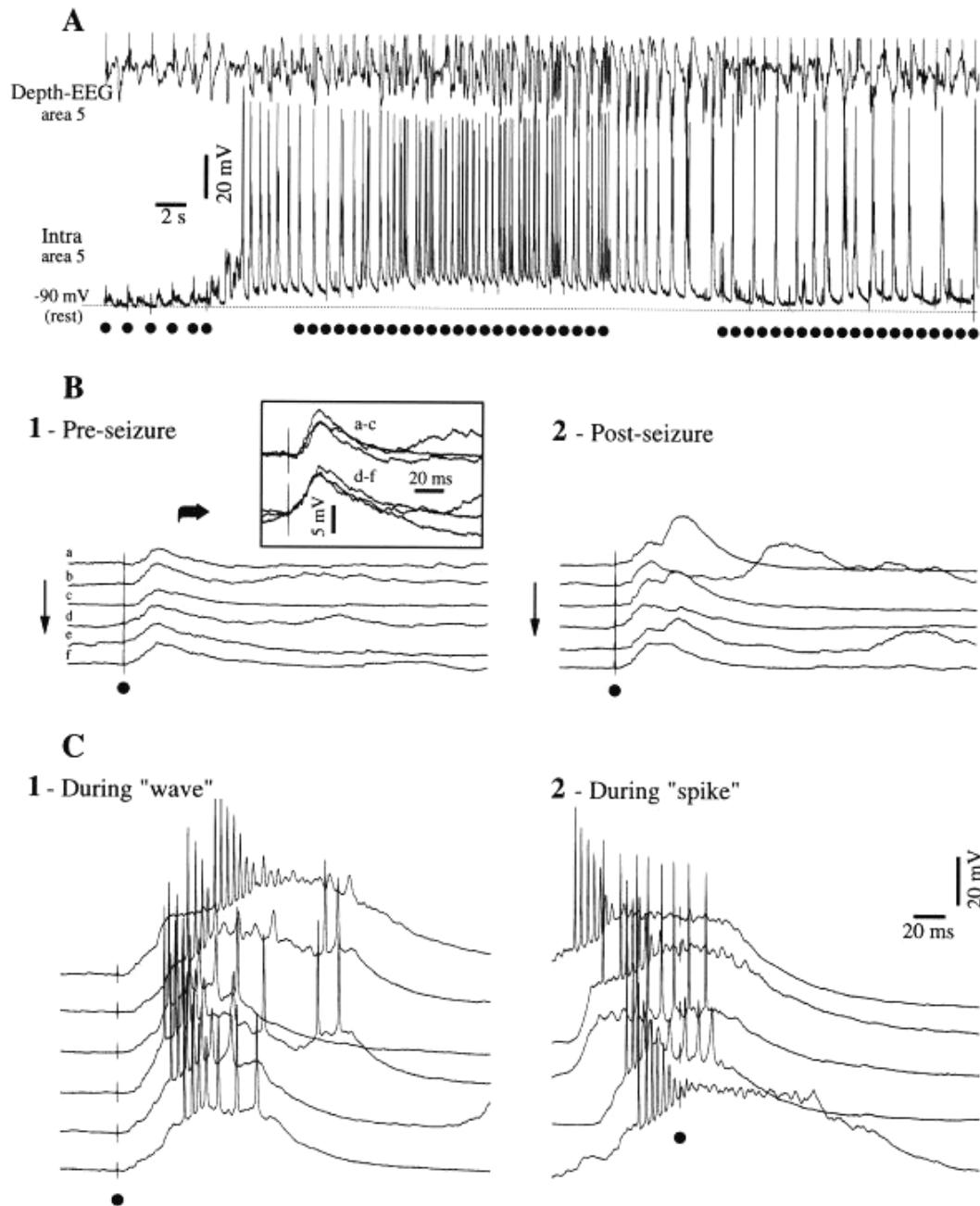
Figure 4-3. Synaptic excitability to cortical stimuli during the pre-seizure epoch, different components of the seizure consisting of SW complexes over a depolarizing envelope, and post-seizure epoch. Cat under ketamine–xylazine anesthesia. Intracellular and depth-EEG recordings from cortical area 5. (A) Stimuli applied to area 7 (marked by dots); stimulation frequency increased from 0.5 Hz to 1 Hz after the first five stimuli (see left part of the intracellular trace). (B) Expanded traces depicting cortically evoked EPSPs during the pre-seizure (B1) and post-seizure (B2) epochs. Inset in B1 depicts (higher gain) the evolution of the six EPSPs preceding the seizure (a–f); note progressively decreased latency and increased amplitude while approaching the seizure. (C) Cortically evoked PDSs during the EEG "wave" (C1) and "spike" (C2). During the "wave," the PDSs started at the same latency (approximately 3 ms) as the EPSPs prior to seizure, lasted for 120–150 ms, and were crowned by high-frequency (250–300 Hz) spike-bursts (C1). (From: Steriade and Amzica, 1999)).

During fast runs, typically seen in Lennox-Gastaut syndrome jointly with SW, it has been described a sustained depolarization of regular spiking (RS) neurons, which often lead to partial spike inactivation (Figure 4-4) (Steriade et al., 1998). This has been considered as being caused by the firing pause or very reduced discharges in the overwhelming majority of electrophysiologically characterized fast-spiking (FS) neurons (presumably inhibitory neurons) (Figure 4-5) (Timofeev et al., 2002a). This feature of FS neurons during fast runs probably arises from asynchronous synaptic bombardment that is not able to depolarize FS neurons to the firing threshold. Moreover, when FS neurons displayed several spikes during fast runs, the firing ratio FS/RS was the lowest during fast runs (Timofeev and Steriade, 2004). Recent data demonstrate changes in frequencies of fast runs during different periods of the same seizure episode and a deficient synchrony between cortical neuronal networks during different epochs of cortical seizures with fast runs (Boucetta et al., 2008). This might explain why corticothalamic inputs are not able to elicit synchronous fast oscillations in the thalamus during this type of seizure (Steriade, 2003).

The EEG "wave" component of SW was considered, by early studies, the reflection of summated IPSPs that were ascribed to GABAergic processes triggered in cortical pyramidal neurons by local-circuit inhibitory cells (Pollen, 1964; Giaretta et al., 1987). Even in more recent computational studies, the "wave" was similarly regarded as produced by active inhibitory processes as $GABA_B$-mediated IPSPs (Destexhe, 1998). If this were true, a decreased IR would be expected during the EEG "wave" component. Instead, the opposite was found *in vivo*, IR increases relative to the "spike" component, refuting the idea of a role played by GABA receptors in the generation of the hyperpolarization associated with the EEG "wave" component of SW complexes (Neckelmann et al., 2000; Timofeev et al., 2002a). Recent studies show that in fact disfacilitation, which prevents the cortical and TC neurons to fire, is the most important mechanism responsible for the "wave"-related hyperpolarization during cortically generated SW seizures (Charpier et al., 1999; Neckelmann et al., 2000). Some K^+ currents also appear to be related to the "wave" component of SW, since recordings with Cs^+-filled pipettes, to non-selectively block K^+ currents, showed that, during the "wave" component of SW seizures, pyramidal neurons displayed depolarizing potentials (Timofeev et al., 2002a; Timofeev and Steriade, 2004).
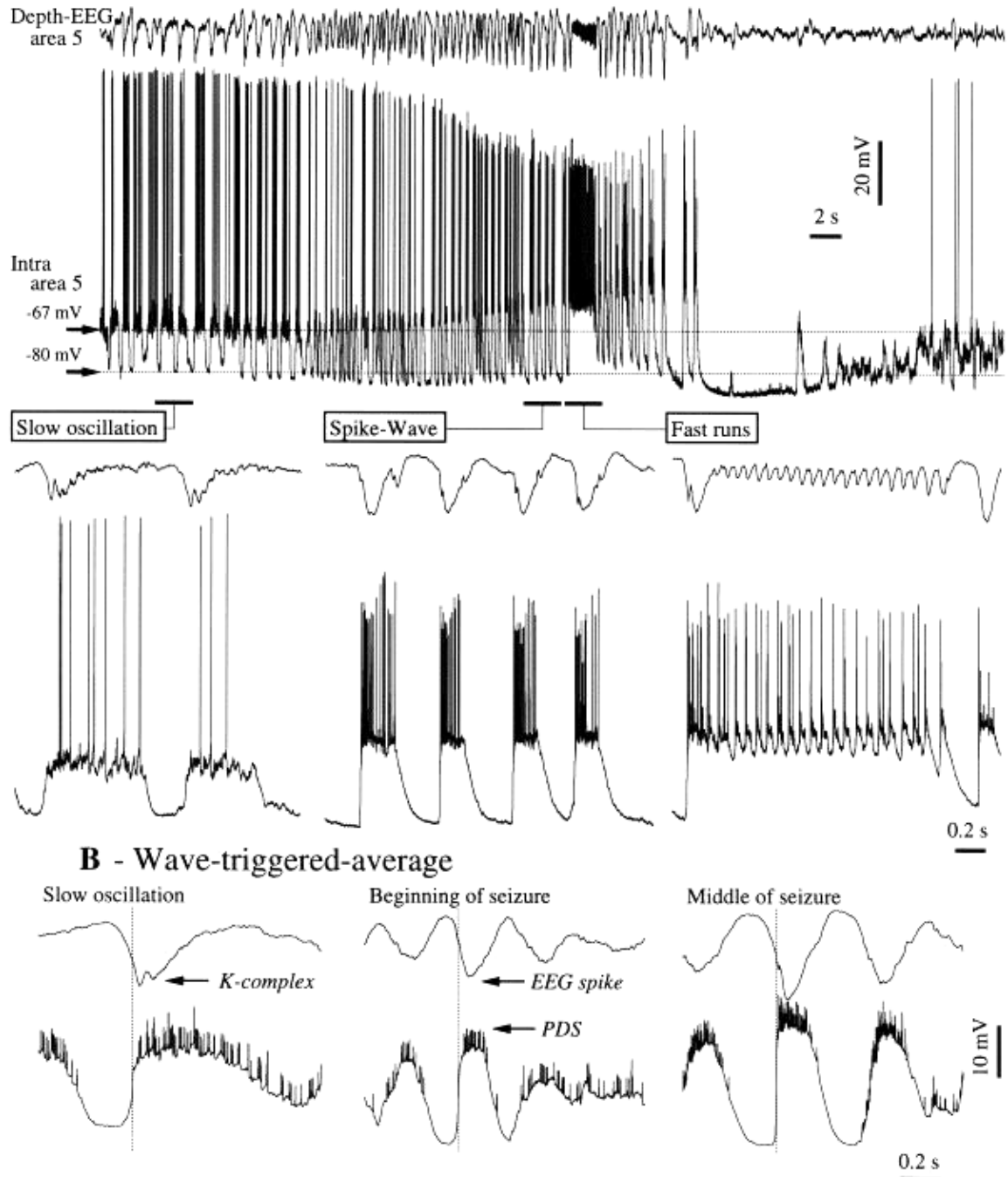
Figure 4-4. Spontaneously occurring seizure, developing without discontinuity from slow (sleep-like) oscillation. Cat under ketamine-xylazine anesthesia. Intracellular recording from RS area 5 neuron together with depth-EEG from the vicinity in area 5. (A) Smooth transition from slow oscillation to complex seizure consisting of SW complexes at approximately 2Hz and fast runs at approximately 15Hz. Epochs of slow oscillation preceding the seizure, SW complexes, and fast runs are indicated and expanded below. (B) Wave-triggered-average during the slow oscillation, at the beginning of seizure and during the middle part of seizure. Averaged activity was triggered by the steepest part of the depolarizing component in cortical neuron (dotted lines), during the three epochs. The depth-negative field component of the slow oscillation (associated with cell's depolarization) is termed K-complex. During the seizure, the depolarizing component reaches the level of a PDS, associated with an EEG "spike." (From: Steriade et al., 1998)).
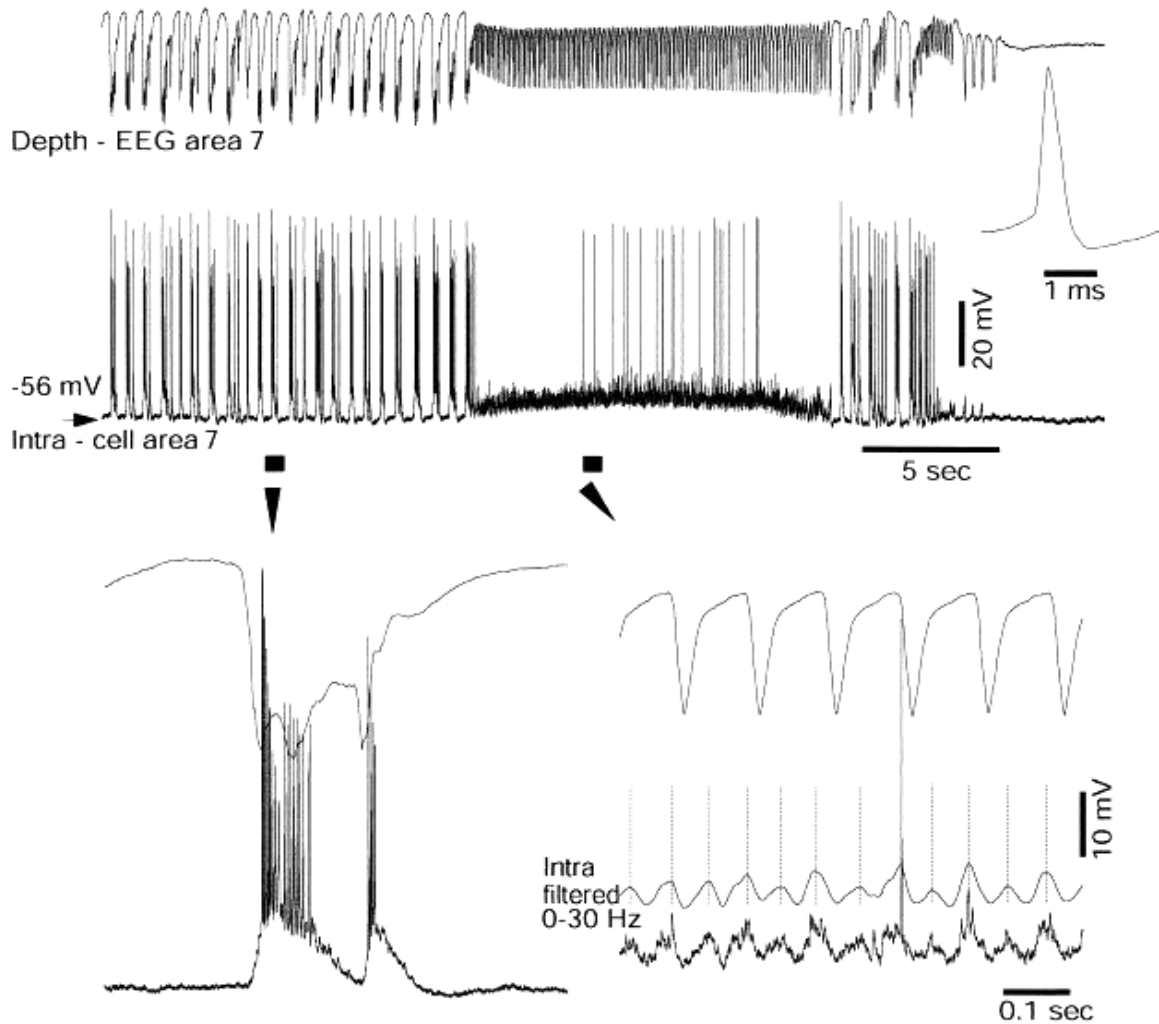
Figure 4-5. FS neuron receives asynchronous synaptic inputs and displays decreased firing rates during fast runs. Cat under ketamine–xylazine anesthesia. Spontaneous seizure. The neuron displayed conventional FS pattern (not shown). An action potential (0.35 ms at half-amplitude) is expanded at top right. Note the presence of numerous action potentials during the EEG "spikes" of PSW complex (bottom left) and their absence during most cycles of fast runs (bottom right). During the fast runs, the neuron received multiple asynchronous synaptic potentials (best seen at bottom right). (From: Timofeev et al., 2002)

Also, high-threshold Ca^{2+} currents and the persistent Na^+ current ($I_{Na(p)}$) could contribute to the depolarization of neurons during PDS, because these currents are activated at depolarized voltages. The intracellular recordings with BAPTA (an intracellular chelator of Ca^{2+}) containing pipettes confirmed the contribution of Ca^{2+} currents in the generation of

PDS (Timofeev et al., 2004), but also the contribution of K^+-currents such as I_h (Timofeev et al., 2002).

In conclusion, a PDS consists of (*a*) a summated EPSPs and IPSPs; and (*b*) an intrinsic current, $I_{Na(p)}$, as revealed by diminished depolarization in recordings with QX-314 in the recording micropipette. The hyperpolarization, related to the EEG depth-positive "wave," is a combination of K^+ currents (mainly $I_{K(Ca)}$ and I_{leak}) due to the strong depolarization during the PDS, which supports abundant entry of Na^+ and Ca^{2+} into neurons. And, the hyperpolarization-activated depolarizing sag, due to I_h, leads to a new paroxysmal cycle (for review see Timofeev and Steriade, 2004). The different synaptic and intrinsic currents activated by neocortical neurons during paroxysmal activity are summarized in Figure 4-6.
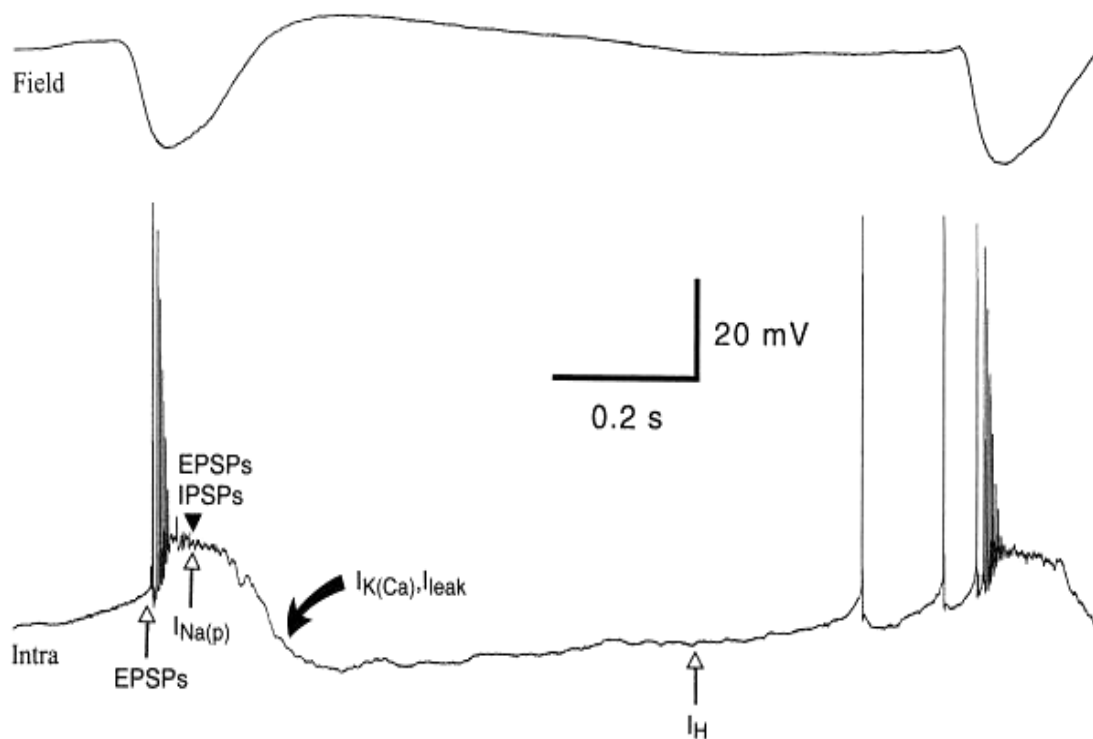
Figure 4-6. Tentative representation of different synaptic and intrinsic currents activated in neocortical neurons during paroxysmal activity. See text above for detailed discussion. (From: Timofeev and Steriade, 2004).

5 Ions in the brain

5.1 Neuronal activity alters ion distributions

As first demonstrated by Frankenhaeuser and Hodgkin, the transmembrane exchange of ions inherent in normal excitation can feed back and influence the cell whose activity caused the movement of ions in the first place (Frankenhaeuser and Hodgkin, 1956). Their indirect method consisted in measuring the hyperpolarizations that followed each spike during repetitive stimulation of a squid axon. Since it was known from earlier work that the hyperpolarizing afterpotential is generated by K^+ current, they concluded that K^+ ions released with the spikes accumulated in the restricted periaxonal space, and their buildup reduced the outward K^+ current and therefore repressed the hyperpolarization. The narrow gap between the axolemma of the squid giant axon and its Schwann cell sheath was thereafter referred to as "the Frankenhaeuser – Hodgkin space".

5.2 Extracellular potassium in the brain

The interstitial volume fraction in the CNS is about 13-30% of the total tissue volume in different regions and is generally close to 20% (McBain et al., 1990; Nicholson and Sykova, 1998). Therefore, it is expected that also in the cerebral interstitium of the mammalian brain, ion concentration would be altered by ion flow during neuron activation. The first evidence in this sense was offered by Kelly and Van Essen who showed depolarization of glial cells in the visual area of the cat cortex, presumably caused by an increased $[K^+]_o$, after adequate optical stimulation (Kelly and Van Essen, 1974).

More precise measurements were done thereafter with ion-selective microelectrodes that showed even spontaneous fluctuations of $[K^+]_o$ in the CNS, in the range of 0.2-0.5 mM, related to sleep spindles or burst firing (Somjen, 1979). Much more important changes were recorded when afferent pathways to the brain or spinal cord were stimulated by repetitive electrical pulses and during epileptiform seizures, which can drive $[K^+]_o$ to several times its normal level. For example, during prolonged maximal stimulation, $[K^+]_o$ rises to a more or less steady ceiling level between 6-12 mM, depending on the stimulated pathway (Moody et al., 1974; Heinemann and Lux, 1977).

5.3 Potassium regulation in the cortex

Potassium ions are released from the excited neurons with each action potential, each excitatory postsynaptic potential (EPSP) and each $GABA_B$ receptor-controlled inhibitory postsynaptic potential (IPSP). Loss of K^+ to the extracellular medium rises the $[K^+]_o/[K^+]_i$ ratio, and this can profoundly influence neuronal function. Since $[K^+]_o$ in the brain is between 2.9-3.4 mM, while $[K^+]_i$ is about 125 mM, and since the fractional volume of neurons is several times greater than that of the interstitial space, it is easy to see that neuronal activity will have a greater impact on the $[K^+]_o$ and on the $[K^+]_i$ (for review see Somjen, 2004). This increase in $[K^+]_o$ during neuronal activity could, if unrestrained, impair cerebral function. Therefore, by the cooperation of several mechanisms, the excess of K^+ in the brain can be restored to normal (Figure 5-1).

5.3.1 Recapture by the neurons

This function is achieved by neurons through the ATP-dependent membrane 3Na/2K pump (Cordingley and Somjen, 1978). During low-frequency firing the pump can almost keep up with outflow, most of the K^+ that has been released returns to the neurons following each spike and the $[K^+]_o$ is raised only slightly and transiently.

5.3.2 Diffusion

During high-frequency firing, but not necessarily synchronized, other processes must mediate the much higher $[K^+]_o$. If the high level of excitation is confined to a small focal area, then the excess K^+ that is not immediately recaptured can diffuse into the quieter region surrounding the active focus. This mechanism is able to dissipate the excess if and only if a sufficiently steep gradient of K^+ has been created, and will fail to work if $[K^+]_o$ is elevated in a large volume of tissue (Cordingley and Somjen, 1978). Once the high rate of firing has stopped, as the 3Na/2K pump pumps K^+ back into the neurons, a local low-$[K^+]_o$ region (sink), termed "postexcitation undershoot", is created and here the dispersed K^+ ions can return, determining the recovering of both $[K^+]_o$ and $[K^+]_i$ (Heinemann and Lux, 1975).
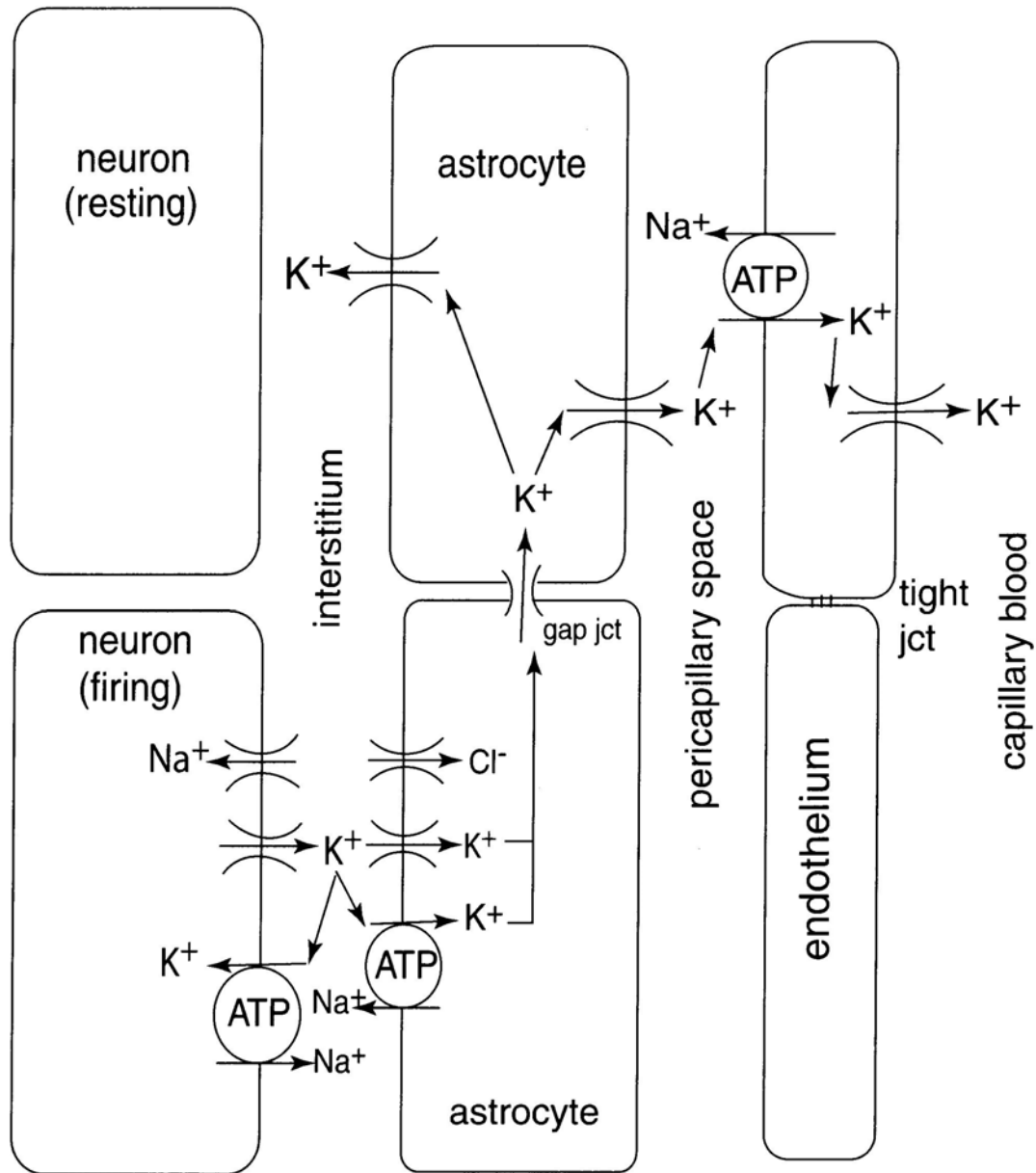
Figure 5-1. Diagram of the fate of K$^+$ released from neurons. K$^+$ is released through voltage-gated channels with action potentials and synaptic potentials. Normally, most of it is recaptured by the 3Na/2K ATPase ion pump. If retrieval does not keep pace with release, much of the excess is mopped up by glial cells, in part by the glial 3Na/2K ATPase, and in part driven by an electrochemical gradient through K$^+$ channels. Cl$^-$ is forced into the astrocyte with K$^+$ to maintain charge balance. Excess K$^+$ can move from one glial cell into its neighbors through gap junctions, causing K$^+$ release through K$^+$ channels at some distance into the interstitial (spatial buffering) or pericapillary spaces (siphoning). From the pericapillary spaces endothelial cells can take it up by the 3Na/2K ATPase ion pump and then discharge it into the capillary. (From: Somjen, 2004)

5.3.3 Glial regulation

When neuronal reuptake and extracellular diffusion fail to keep $[K^+]_o$ within normal limits, glial cells, mainly astrocytes, probably in cooperation with capillary endothelium, are believed to play an important part in the regulation of $[K^+]_o$ (for review see Somjen, 2004).

The uptake of K^+ from the interstitial fluid (ISF) into glial cells occurs through the cooperation of different biophysical processes:

1. Passive uptake of $[K^+]_o$, in combination with anions, mainly Cl^- (Ballanyi et al., 1987) (Figure 5-2).

2. Active uptake of $[K^+]_o$, in exchange against Na^+ by the glial Na^+/K^+ pump, stimulated by elevated $[K^+]_o$ (Ballanyi et al., 1987). Indeed, it was shown that in brain tissue slices, $[K^+]_i$ in glial cytoplasm increased whereas $[Na^+]_i$ decreased during neuronal activity (Ballanyi et al., 1987). Therefore, this mechanism can fulfill dual duty, for it not only buffers excess extracellular K^+ but it partially replenishes also a deficit of Na^+. Because the capacity of the astrocytes is larger than the interstitial space, a comparatively small increase in glial $[K^+]_i$ can buffer a much larger increase in $[K^+]_o$.

3. Spatial buffering of $[K^+]_o$, through the network of glial cells coupled by gap junctions (Orkand et al., 1966). The accumulated intraglial excess of K^+ would then initiate a current through intercellular gap junctions, this current conduction is very fast and that it why this mechanism is much more effective than diffusion over a distance, which is slow. Nevertheless, spatial buffering can work well only if clusters of excited neurons are surrounded by quiescent cells, but not when many neurons in a wide area are active (Kuffler, 1967). Besides, for such a spatial buffer to be effective, glial membranes must be highly permeable to K^+. This implies that altered glia, such as the ones found in many types of epileptic seizures and epilepsy, might have an impaired K^+ buffering which could facilitate extracellular accumulation of K^+ and thereby promote the generation of seizure activity (Heinemann et al., 2000).
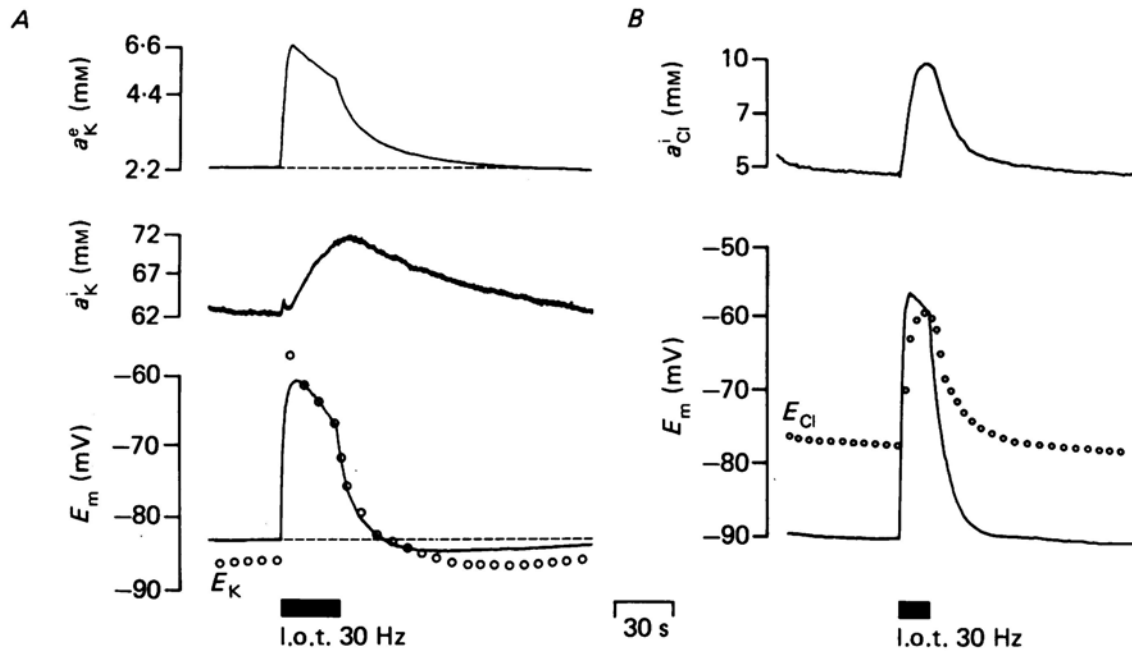
Figure 5-2. Uptake of K$^+$ and Cl$^-$ in glial cells. Recordings from glial cells in guinea pig hippocampal tissue slices. Responses are evoked by repetitive afferent stimulation of the lateral olfactory tract (l.o.t.). A. Measured values of intracellular (a^i_K) and extracellular (a^o_K) activities of K$^+$ in the glia were used to calculate the K$^+$ equilibrium potential (E_K; open circles) and to compare it with the membrane potential (Em). E_K is slightly more negative than Em during rest, briefly overshoots Em at the hught of the depolarization, and coincides closely with Em during repolarization. B. Rise of glial intracellular Cl$^-$ concentration in another glial cell. In this illustration, the Cl$^-$ equilibrium potential (E_{Cl}; open circles) before, during, and after repetitive stimulation of the l.o.t. was calculated using the measured value of the intracellular Cl$^-$ activity (a^i_{Cl}) and the Cl$^-$ activity in the bathing solution. (Modified from: Ballanyi et al., 1987)

5.4 Potassium and seizures

5.4.1 Potassium can induce seizures

It was first in 1957 when Feldberg and Sherwood demonstrated that injecting small amounts of KCl solution into the lateral ventricle of cats triggered epileptiform convulsions (Feldberg and Sherwood, 1957). But it was only in 1970 that Fertziger and Ranck formalized the "potassium hypothesis of seizure generation" (Fertziger and Ranck, 1970). The authors envisioned a loop in which overexcited neurons discharged excess K$^+$ into the interstitium, which then reinforced the excitation, rendering the seizure self-sustaining. According to their idea, seizures stopped when K$^+$-induced depolarization ultimately inactivated Na$^+$ currents, stopping the discharge and terminating the release of more K$^+$.

With the widespread use of isolated slices of brain tissue, it became possible to study more precisely the conditions for K^+-induced seizures. For example, modest elevation of K^+ in the bathing fluid facilitated the triggering of electrically induced paroxysmal activity (Somjen and Giacchino, 1985). But when $[K^+]_o$ rises up to 7-8 mM, spontaneous activity erupts (Rutecki et al., 1985; Korn et al., 1987; Traynelis and Dingledine, 1988), and it can take the form of brief interictal discharge or more prolonged ictal events. There are several effects of high $[K^+]_o$ that could engender epileptiform activity:

1. Elevated $[K^+]_o$ depolarizes the neurons, brings the membrane closer to the firing threshold, and stronger depolarization can induce firing (Kuffler, 1967; Forsythe and Redman, 1988).

2. High $[K^+]_o$ could also determine the depolarization of the presynaptic terminals that can trigger excessive firing of action potentials and activation of voltage-gated calcium channels, causing inappropriate release of transmitters (Singer and Lux, 1973; Hablitz and Lundervold, 1981).

3. Excess $[K^+]_o$ forces the uptake of Cl^-, mainly through the $N^+/K^+/Cl^-$ cotransporter (NKCC1) (Moore-Hoon and Turner, 1998), which renders GABA-mediated synaptic inhibition less effective (Korn et al., 1987; Chamberlin and Dingledine, 1988).

4. $[K^+]_o$ surplus also potentiates the persistent (slowly inactivating) Na^+ current ($I_{Na(P)}$) of neurons (Somjen and Muller, 2000), which is able to induce prolonged high-frequency spike bursts riding on large depolarizing waves (Alkadhi and Tian, 1996; Somjen, 2004).

5. Increased $[K^+]_o$ may transform the regular-spiking firing pattern of some neurons into intrinsically bursting patterns, which would increase the excitability of the network (Jensen et al., 1994).

6. The accumulation of $[K^+]_o$ could also result in neuronal swelling and electrotonic coupling between neurons (Traynelis and Dingledine, 1988; McNamara, 1994), leading to hyperexcitation (Perez Velazquez and Carlen, 2000).

5.4.2 Potassium accumulates in the interstitial space during seizures

For the potassium hypothesis of seizure generation to be accepted, it was necessary to demonstrate that excess K^+ accumulates in the ISF during seizures. Measurements of K^+ concentration at the surface of the brain found that excess K^+ was oozing from the surface during seizures (Fertziger and Ranck, 1970). Then, recordings of the depolarization of the so-called idle cells (correctly assumed to be glia) confirmed that $[K^+]_o$ increases during seizures (Sugaya et al., 1971).

The introduction of potassium-selective microelectrodes enabled the recording of impressive rise of $[K^+]_o$ during seizures (Vyskocil et al., 1972). During interictal discharges, brief modest increases were seen, but a much larger elevation was consistently recorded during many different types of ictal events (Prince et al., 1973; Lothman and Somjen, 1975, 1976), approaching the "ceiling" of 8-12 mM during sustained seizures (Heinemann and Lux, 1977). This steady ceiling level is established when a balance is reached between the outflow from and uptake into cells (neuron and glia).

5.4.3 Too much potassium can stop seizures

If the regulation of $[K^+]_o$ is overwhelmed and the ceiling level of 8-12 mM (Heinemann and Lux, 1977) is breached, the excessive depolarization of neurons suppresses excitation, as Na+ channels are inactivated and the cell becomes inexcitable (Fertziger and Ranck, 1970), and this may even lead to spreading depression (SD)-like phenomenon (Kager et al., 2000, 2002). Indeed, it has true shown that true tonico-clonic seizures may be followed by SD, and this has led to the suggestion that postictal depression may be due to SD-like depolarization (Van Harreveld and Stamm, 1954; Van Harreveld et al., 1956).

The most widely accepted mechanism of SD even today is Grafstein's K^+ hypothesis (Grafstein, 1956). According to his theory, K^+ release during intense neuronal firing accumulated in the restricted interstitial space of the brain and the excess of $[K^+]_o$ further depolarizes the very cells that released is, resulting in a vicious circle that leads first to increasing firing and eventually to inactivation of neuronal excitability. In the mean time, some of the accumulated K^+ diffuses through the interstitial space to neighboring cells, which then also depolarize, fire and go through the same cycle, thus producing the slowly

propagating wave of SD. The core of this hypothesis survives today but although we might indeed accept that the increase in $[K^+]_o$ is probably a key to the evolution of the SD process (Kager et al., 2000, 2002), it is not necessarily implicated in its propagation (Herreras and Somjen, 1993).

Other mechanisms by which an increased $[K^+]_o$ could stop seizures involve a differential inactivation of excitatory and inhibitory neurons (Aradi et al., 2004; Ziburkus et al., 2006), activation of Ih (Shin et al., 2008).

In conclusion, as already described, K^+ plays a key role in the regulation of neuronal activity both during normal and pathologic conditions and the regulation of its concentration is vitally important. In the CNS, K^+ can only be redistributed by dedicated neuronal and glial mechanisms that have different specific properties. While neurons are involved in the regulation of extracellular K^+ by virtue of their Na^+/K^+ pump and K^+ cotransporters, it is well established that macroglia, in particular astrocytes, have the predominant role in the proper homeostasis of extracellular K^+ by virtue of their Na^+/K^+ pump, K^+ contransporters, and membrane K^+ channels (Walz, 1987, 2000; Nedergaard et al., 2003). Nonetheless, the role of oligodendrocytes in extracellular K^+ homeostasis should not be neglected, since recent work has demonstrated that this type of macroglia is suitable for K^+ homeostasis as well (Chvatal et al., 1997; Chvatal et al., 1999), and may have reduced capability for ion homeostasis under certain pathological conditions (D'Ambrosio et al., 1999).

6   Reactive gliosis in epilepsy

Epilepsy is often accompanied by massive reactive gliosis (Kim, 2001; Blumcke et al., 2002; de Lanerolle and Lee, 2005), which is actually a common feature in virtually all insults to the CNS (D'Ambrosio, 2004). Glial reactivity, as defined by the occurrence of active cytological, immunological, morphological or functional response of glial cells to CNS insults, is thought to promote the functionally important processes of inflammation and tissue repair and participate in glial scar formation ("reactive gliosis"), which occurs after injury to the CNS (Penfield, 1929). However, the role of reactive glia, in particular

astrocytes, in the pathological sequelae of CNS insult remains controversial, especially with respect to its beneficial or detrimental influence on CNS recovery.

Although the significance of this alteration is poorly understood, recent findings suggest that modified astroglial functioning might have an important role in the generation and spread of seizure activity. Moreover, recent lines of evidence suggest that glial cells are potential novel targets for the treatment of CNS diseases. First, functional alterations of specific glial membrane channels, receptors and transporters have been discovered in several neurological disorders, including epilepsy (Heinemann et al., 2000; Steinhauser and Seifert, 2002; de Lanerolle and Lee, 2005; Seifert et al., 2006). Second, recent findings now link glial cells to modulation of synaptic transmission (Volterra and Steinhauser, 2004; Volterra and Meldolesi, 2005).

Alterations in astrocytic properties have been described both in human temporal lobe epilepsy (Heinemann et al., 2000) and in post-traumatic epilepsy (D'Ambrosio et al., 1999; Samuelsson et al., 2000). The most common pathology found in patients with medically-intractable temporal lobe epilepsy is hippocampal sclerosis, more generally termed mesial temporal sclerosis. Mesial temporal sclerosis is characterized by neuronal cell loss in specific hippocampal areas, gliosis, microvascular proliferation, and synaptic reorganization (Margerison and Corsellis, 1966; Mathern et al., 1997a; Blumcke et al., 1999). Early autopsy studies found mesial temporal sclerosis in 30-58% of temporal lobe epilepsy cases (Margerison and Corsellis, 1966); and similar proportions have been found in examination of specimens resected from patients undergoing surgery for medically intractable temporal lobe epilepsy (Mathern et al., 1997b; de Lanerolle et al., 2003). One striking hallmark of the sclerotic hippocampus is that while there is a specific pattern of neuronal loss, there is also reactive gliosis with hypertrophic glial cells exhibiting prominent GFAP staining and long, thick processes. Only a few studies have attempted to quantitate changes in astrocyte numbers and densities in epileptic tissue, and they all emphasize the important reactive gliosis (Krishnan et al., 1994; Mitchell et al., 1999; Briellmann et al., 2002).

Since both extracellular K^+ concentration and osmolarity have been shown to dramatically modulate neural excitability, it is plausible that changes in astrocytic K^+ or

water channels could contribute to hyperexcitability in epilepsy (Binder and Steinhauser, 2006). Indeed, recent studies have found changes in astroglial K^+-inward-rectifying channels (Kir) and aquaporin-4 (AQP4) water channels in temporal lobe epilepsy specimens (see below).

6.1 K^+ Channels

During neuronal hyperactivity, $[K^+]_o$ may increase from 3 mM to a ceiling of 10-12 mM; and K^+ released during high-frequency firing of neurons is thought to be primarily taken up by glial cells (Heinemann and Lux, 1977; Ballanyi et al., 1987; Somjen, 2004). Any impairment of glial K^+ uptake would be expected to be proconvulsant based on many previous studies. In the hippocampus, millimolar and even sub-millimolar increases in extracellular K^+ concentration powerfully enhance epileptiform activity (Rutecki et al., 1985; Traynelis and Dingledine, 1988; Feng and Durand, 2006). High-K^+ also reliably induces epileptiform activity in hippocampal slices from human patients with intractable temporal lobe epilepsy and hippocampal sclerosis (Gabriel et al., 2004).

A primary mechanism for K^+ reuptake and spatial K^+ buffering is thought to be via glial Kir channels (Orkand et al., 1966; Newman, 1986, 1993). There have been described multiple subfamilies of Kir channels (Kir1-Kir7) differing in tissue distribution and functional properties, but in brain astrocytes the expression of Kir4.1 has been investigated most thoroughly (Higashi et al., 2001; Hibino et al., 2004). It has been shown that pharmacological or genetic inactivation of Kir4.1 leads to impairment of extracellular K^+ regulation (Kofuji et al., 2000; Kofuji and Newman, 2004; Neusch et al., 2006). However, members of the strongly rectifying Kir2 family may also contribute to astroglial K^+ buffering (Neusch et al., 2003; Butt and Kalsi, 2006).

Down-regulation of astroglial Kir channels has been described in the CNS following various injury-induced reactive gliosis, such as in deafferented entorhinal cortex (Schroder et al., 1999), in freeze lesion-induced cortical dysplasia (Bordey et al., 2000; Bordey et al., 2001), and in traumatic (D'Ambrosio et al., 1999) and ischemic (Koller et al., 2000) brain injury. In addition, there have also been indications of downregulation of Kir currents in specimens from patients with temporal lobe epilepsy (Bordey and Sontheimer, 1998;

Hinterkeuser et al., 2000; Kivi et al., 2000; Schroder et al., 2000). For example, studies that used ion-sensitive microelectrodes and Ba^{2+}, a blocker of Kir channels, in hippocampal slices obtained from patients with or without mesial temporal sclerosis showed that Ba^{2+} augmented stimulus-evoked K^+ elevation in non-sclerotic but not in sclerotic specimens, suggesting an impairment in K^+ buffering in sclerotic tissue (Heinemann et al., 2000; Kivi et al., 2000). Also, direct evidence for down-regulation of Kir currents in the sclerotic CA1 region of hippocampus came from a comparative patch-clamp study in which a reduction in astroglial Kir currents was observed in sclerotic compared with non-sclerotic hippocampi (Hinterkeuser et al., 2000). These data indicate that dysfunction of astroglial Kir channels in the reactive astrocytes following various brain injuries could underlie impaired K^+ buffering (Steinhauser and Seifert, 2002).

6.2 Water Channels

The coordinated action of Kir4.1 and AQP4 channels in astroglial end-feet, which contact capillaries suggests that buffering of K^+ through Kir channels is dependent on concomitant transmembrane flux of water in the same cell (Seifert et al., 2006). Indeed, brain tissue excitability is exquisitely sensitive to osmolarity and the size of the extracellular space (ECS) (Schwartzkroin et al., 1998), hence, alterations in astroglial water regulation could also powerfully affect excitability. Decreasing ECS volume produces hyperexcitability and enhanced epileptiform activity (Dudek et al., 1990; Roper et al., 1992; Chebabo et al., 1995). Conversely, increasing ECS volume with hyperosmolar medium attenuates epileptiform activity (Dudek et al., 1990; Haglund and Hochman, 2005). These experimental data parallel extensive clinical experience indicating that hypoosmolar states lower seizure threshold while hyperosmolar states elevate seizure threshold (Andrew et al., 1989).

The aquaporins are a family of membrane proteins that function as water channels in many cell types and tissues in which fluid transport is crucial (Verkman, 2005). There is increasing evidence that water movement in the brain involves aquaporin channels (Amiry-Moghaddam and Ottersen, 2003c; Manley et al., 2004). A subtype of this family, AQP4 is expressed ubiquitously by glial cells, especially at specialized membrane domains including astroglial endfeet in contact with blood vessels and astrocyte membranes that

wrap glutamatergic synapses (Nielsen et al., 1997; Nagelhus et al., 2004). It has been demonstrated that the activity-induced radial water fluxes in neocortex could be caused by water movement via aquaporin channels in response to physiological activity (Holthoff and Witte, 2000; Niermann et al., 2001). Moreover, mice deficient in AQP4 have markedly decreased cerebral edema following water intoxication and focal cerebral ischemia (Manley et al., 2000) and impaired clearance of brain water in models of vasogenic edema (Papadopoulos et al., 2004), suggesting a functional role for AQP4 in brain water transport.

In sclerotic hippocampi obtained from patients with mesial temporal sclerosis there is an overall increase in AQP4 expression (Lee et al., 2004) and also a mislocalization of AQP4 with reduction in perivascular membrane expression (Eid et al., 2005). The authors hypothesized that the loss of perivascular AQP4 perturbs water flux, impairs K^+ buffering, and results in an increased propensity for seizures.

Several lines of evidence support the hypothesis that AQP4 and Kir4.1 may act jointly in K^+ and water regulation in the brain (Simard and Nedergaard, 2004):

1. K^+ reuptake into glial cells could be AQP4-dependent, as water influx coupled to K^+ influx is thought to underlie activity-induced glial cell swelling (Walz, 1987, 1992).

2. There is a subcellular co-localization of AQP4 and Kir4.1 (Connors et al., 2004; Nagelhus et al., 2004).

3. Kir4.1 knock-out mice, like AQP4 knock-out mice (Li and Verkman, 2001; Li and Prince, 2002), have impaired retinal and cochlear physiology presumably due to altered K^+ metabolism (Marcus et al., 2002; Rozengurt et al., 2003).

4. AQP4 knock-out mice have remarkably slowed K^+ reuptake in models of seizure and spreading depression *in vivo* (Padmawar et al., 2005; Binder and Steinhauser, 2006) associated with a near-threefold increase in seizure duration (Binder and Steinhauser, 2006).

5. Stimulation of hippocampal slices from alpha-syntrophin-deficient mice, which have a mislocalization of the AQP4 protein, demonstrates a deficit in extracellular K^+ clearance (Amiry-Moghaddam et al., 2003a).

7 Experimental post-traumatic epilepsy

As described previously, postlesional epilepsy is a common clinical problem which accounts for up to 12-13% of the epilepsies with a clearly defined cause (Annegers, 1994). Trauma that produces direct cortical injury, such as cortical contusion, hematoma, or penetrating cortical wounds, is associated with a high incidence of late-onset epilepsy, whereas less severe trauma resulting in cerebral concussion carries only a small risk of subsequent seizures (Annegers et al., 1998). Within 24 hours following head injury with penetrating wounds, up to 80 % of patients display clinical seizures (Kollevold, 1979; Dinner, 1993) and more than 50% of them develop late epilepsy (Salazar et al., 1985). Approximately one-third of patients with symptomatic localization-related epilepsy syndromes are refractory to available antiepileptic medications (Kwan and Brodie, 2000; Herman, 2002). Unfortunately, multiple clinical attempts to prevent posttraumatic epileptogenesis with simple prophylaxis using available antiepileptic drugs have been largely unsuccessful (Young et al., 1983; Temkin et al., 1999; Temkin, 2001). Improved treatments and prevention strategies await better delineation of the underlying mechanisms that are at present not well understood.

Epilepsy resulting from penetrating brain injury has been modeled, in experimental animals, by making chronic neocortical partial isolations ("undercuts" or "cortical islands") with virtually intact pial circulation and cortico-cortical connections (Sharpless and Halpern, 1962; Echlin and Battista, 1963). The undercut cortex becomes increasingly hyperexcitable and develops evoked prolonged ictal events and spontaneous interictal discharges both *in vitro* (Prince and Tseng, 1993; Jacobs et al., 2000; Li and Prince, 2002; Li et al., 2005; Jin et al., 2006) and *in vivo* (Topolnik et al., 2003a, b; Nita et al., 2006; Nita et al., 2007).

In our *in vivo* model (described in Topolnik et al., 2003a, b), 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 TC afferences. A standard knife (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. 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.

7.1 Acute traumatic injury

In acute *in vivo* experiments performed in cats under ketamine-xylazine, it was shown that the slow oscillation in the partially deafferented cortex decreased in amplitude immediately after the undercut (~5 minutes), and then, 2–3 hours later, recovered to almost its initial pattern (Topolnik et al., 2003b).

In the same study, partial cortical deafferentation led to development of paroxysmal-like activity, characterized by high-amplitude slow waves with morphological features of interictal spikes, or clear-cut electrographic seizures in ~40% of cases (Figure 7-1).

Moreover, the initiation of paroxysmal activity took place in the relatively intact, anterior part of the deafferented gyrus, with only weak or absence of such activity in the posterior part (Figure 7-2). Initiation of paroxysmal activity in the area adjacent to the undercut cortex was related to an increase in local synchrony. Field potentials and multi-unit activity in relatively intact areas occurred synchronously (with a time lag of ~2 ms) showing an increased local synchrony.

The time lag of propagation increased with transition to the more deafferented (posterior) areas, probably because of the relative hyperpolarization of deafferented neurons and their inability to reach firing threshold synchronously with more intact neurons. This may create conditions for a spatial localization of excitation and a permanent auto-excitation of neurons in more intact area through the recurrent network of excitatory

connections (Traub and Wong, 1982) which remained functional. In turn, increased positive feedback in an excitatory system could lead to the initiation of electrographic paroxysms (Topolnik et al., 2003b).
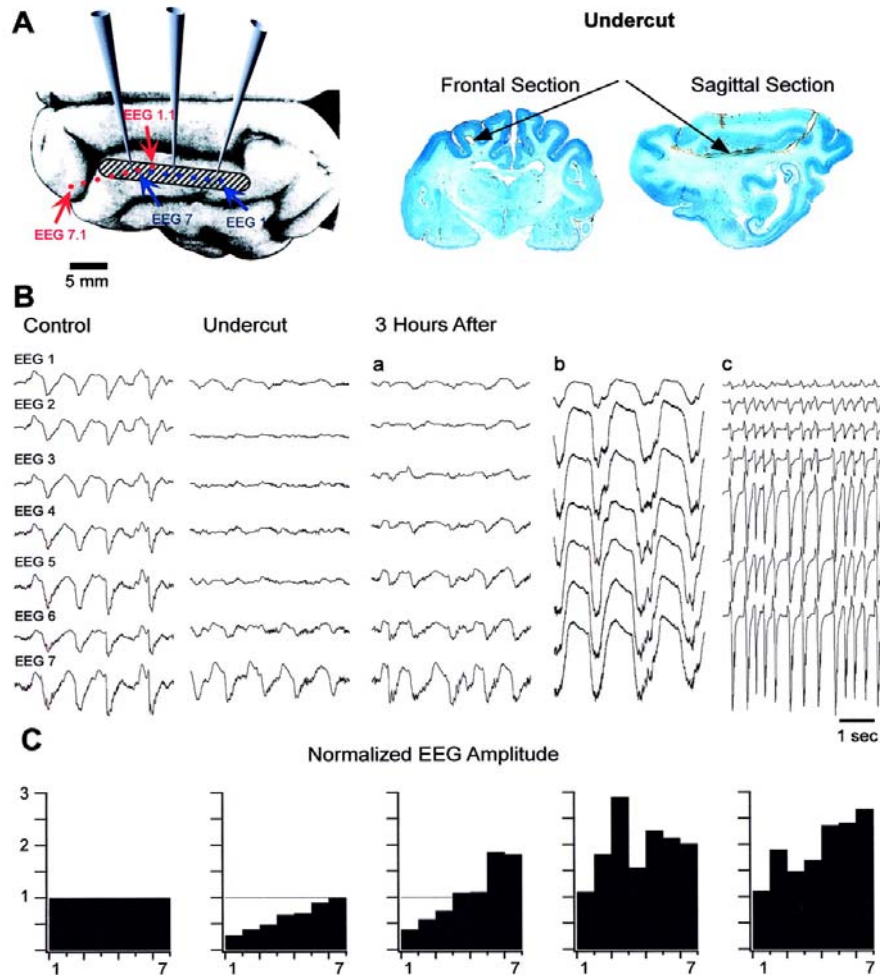
Figure 7-1. Slow oscillation in intact suprasylvian gyrus is modified by cortical undercut. A. Position of EEG arrays of electrodes and of intracellular pipettes, and histology of the undercut suprasylvian gyrus. Upper panel, dorsal view of the left hemisphere. The zone of undercut is tentatively indicated by shaded area. Blue points show the generally used position of EEG electrodes (EEG1–EEG7) along the suprasylvian gyrus. Red points show the second position of EEG electrodes (EEG1.1–EEG7.1) in the postcruciate gyrus and anterior part of suprasylvian gyrus. Position of pipettes is schematically indicated. Right panel shows frontal and parasagittal section of the left hemisphere from two different cats showing the extent of the undercut (indicated by arrows). B. Field potential recordings (EEG) from the intact cortex (left panel-CONTROL), undercut suprasylvian gyrus, immediately after the undercut (middle panel-UNDERCUT), and 3 h later (three right panels; a, b and c, three different animals). C. Normalized EEG amplitude (vertical axis) of the corresponding EEG from different sites (horizontal axis). Mean EEG amplitude for each electrode in control was taken as 1. EEG1-field potential recording from the posterior part of the suprasylvian gyrus, EEG7-recording from the anterior part of this gyrus. (From: Topolnik et al., 2003a).
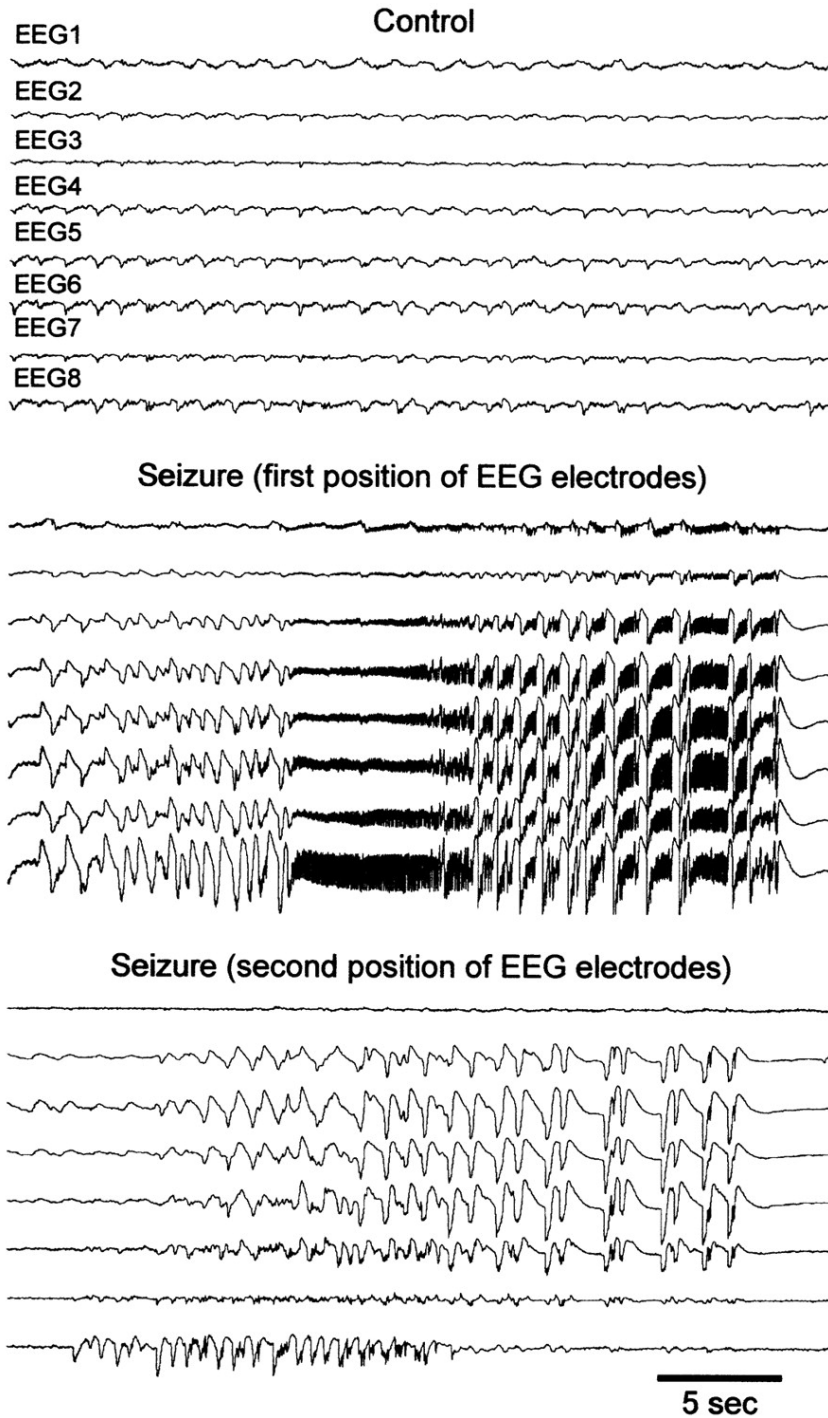
Figure 7-2. Spontaneous electrographic seizures in the suprasylvian gyrus were generated at the border between intact and undercut cortex. Field potential recordings (EEG) from the intact (upper panel) cortex, 2 h (middle panel) and 4 h (bottom panel) after the undercut. First position of EEG electrodes - electrodes located along the middle part of the undercut suprasylvian gyrus; second position of EEG electrodes - electrodes located in suprasylvian and postcruciate gyri (see Fig. 7-1.). Note initiation of electrographic seizures in the intact postcruciate gyrus (bottom panel). The amplitude of seizures was maximal in the anterior part of the undercut suprasylvian gyrus in any position of EEG electrodes. (From: Topolnik et al., 2003a.).

7.1.1 Mechanisms of acute trauma

Following acute trauma to the brain, the level of $[K^+]_o$ may increase due to neuronal and glial damage and, as described above, high $[K^+]_o$ leads to cellular depolarization, increased excitability and decreased inhibition promoting the development of seizures (Moody et al., 1974; Korn et al., 1987; Chamberlin and Dingledine, 1988; Traynelis and Dingledine, 1988).

Acute traumatic injury could also result in excessive accumulation of extracellular glutamate and in the raise of intracellular Ca^{2+} via transmitter- and voltage-gated channels (Faden et al., 1989; Lipton and Rosenberg, 1994; Sakowitz et al., 2002). Increased intracellular Ca^{2+} in turn enhances the response to glutamate (Yang and Benardo, 1997), favoring seizure activity. The increased Ca^{2+} influx (Choi, 1988) is a major activator of deleterious processes after neuronal injury, which might inhibit Na^+-K^+ pump activity engendering further accumulation of Ca^{2+} intracellularly (Fukuda and Prince, 1992) while the extracellular Ca^{2+} decreases (Morris et al., 1991; Wolf et al., 2001). However, previous studies show that only slight decrease in extracellular Ca^{2+} significantly increases the failure of synaptic transmission (Markram et al., 1998; Massimini and Amzica, 2001; Crochet et al., 2005; Seigneur and Timofeev, 2007), suggesting the possibility that mechanisms other than chemical synaptic transmission should be responsible for the neuronal synchronization during seizures. These alternative mechanisms could by either electrical coupling between different groups of neurons (Galarreta and Hestrin, 1999; Perez Velazquez and Carlen, 2000; Schmitz et al., 2001), between glial cells (Amzica et al., 2002), or via ephaptic interactions (Taylor and Dudek, 1982, 1984a, b; Grenier et al., 2003).

Another important factor engendering post-traumatic cortical over-excitation may be the I_h (Schwindt et al., 1988). It has previously been reported that, during seizures, there is an increase in the level of maximal hyperpolarization achieved by neurons between depolarizing events (Steriade et al., 1998), which would support an increase efficiency of I_h whose depolarizing sag may lead to activation of the low-threshold transient Ca^{2+} current (de la Peña and Geijo-Barrientos, 1996). An increased $[K^+]_o$ during acute seizures would

shift the reversal potential for all K^+-mediated currents and further involve I_h in the generation of seizures (Timofeev et al., 2002b).

The increases in intrinsic as well as synaptic neuronal excitability within the area adjacent to the undercut cortex are also able to engender seizures within a few hours after the undercut (Topolnik et al., 2003a). The increased number of IB neurons, acting as amplifiers (Timofeev et al., 2000) and a positive shift in the reversal potential for early IPSPs (Topolnik et al., 2003a) would both enhance the probability of seizures. Evoked potentials were dramatically increased in the relatively intact area of the undercut gyrus, but were comparable to before in the completely deafferented area, of the gyrus, suggesting an increased synaptic excitability of neurons from the relatively intact area, adjacent to undercut (Topolnik et al., 2003a).

In conclusion, acute undercut of the suprasylvian gyrus in cats, produces early post-traumatic increased in excitability, most likely generated by the alteration of neuronal milieu following brain injury. Nevertheless, chronic input deprivation of the cortex, due to deafferentation, triggers more complex structural changes of the brain. The possible differences between the pathogenetic processes underlying acute and chronic cortical hyperexcitability (Prince and Futamachi, 1970) and the frequent occurrence of epilepsy as a consequence of brain trauma, make it important to examine mechanisms of epileptogenesis after chronic cortical injury (Prince and Tseng, 1993).

7.2 Chronic traumatic injury

A previous study in anesthetized cats shows a progressively increase amplitudes of field potentials during both slow oscillation and SW/PSW seizures, as well as a gradual increased velocity of low-frequency activity propagation during both the slow oscillation and seizures (Nita et al., 2006) (Figure 7-3). Also data in non-anesthetized cats attest that the recurrent seizures with 4 Hz SW-complexes recorded following cortical penetrating wounds were modulated by the state of vigilance, occurring during the waking state, being enhanced during SWS, and absent during REM sleep (Nita et al., 2007) (Figure 7-4).

Injury can produce chronic hyperexcitability that triggers epileptogenesis by several different mechanisms, such as: (a) alterations in intrinsic membrane properties, (b) changes

in synaptic connectivity, (c) modifications of receptors and receptor subunits (d) variations in neuronal and glial density.

7.2.1 Mechanisms of chronic trauma

7.2.1.1 Alterations in intrinsic membrane properties

Axotomy of motoneurons has been known for some time to produce alterations in intrinsic membrane properties, such as increases in neuronal input resistance (IR) (Eccles et al., 1958). Similar observations have been made for corticospinal neurons axotomized at the level of the cervical spinal cord (Tseng and Prince, 1996).

In the epileptogenic undercut model, intracellular recordings from chronically undercut pyramidal neurons showed an increase in IR and membrane time constant compared with cells of control cortex *in vitro* (Prince and Tseng, 1993). These changes are likely attributable to an increase in membrane resistivity or capacitance, making the cell more electrotonically compact. Thus, for the same synaptic input, increases in IR and membrane time constant would result in larger amplitude and more prolonged postsynaptic events, hence afferent contacts would be more likely to evoke action potentials.

If the duration of excitatory activity is not rapidly truncated by inhibition, then activation of NMDA (N-methyl-D-aspartate) conductances via recurrent pyramidal collaterals can lead to late depolarizations and repetitive action potentials in pyramidal neurons, amplifying the enhanced excitatory input (Bush et al., 1999). An increase in postsynaptic NMDA conductance has been indeed demonstrated in some chronic models of temporal lobe epilepsy (Mody and Heinemann, 1987; Mody et al., 1992; Kohr et al., 1993; Lothman et al., 1995).

In addition to these changes in IR and membrane time constant, the relationship between applied current and adapted spike firing frequency (f-I slope) becomes steeper in both axotomized cells and cells in the undercut cortex *in vitro* (Prince and Tseng, 1993; Tseng and Prince, 1996).
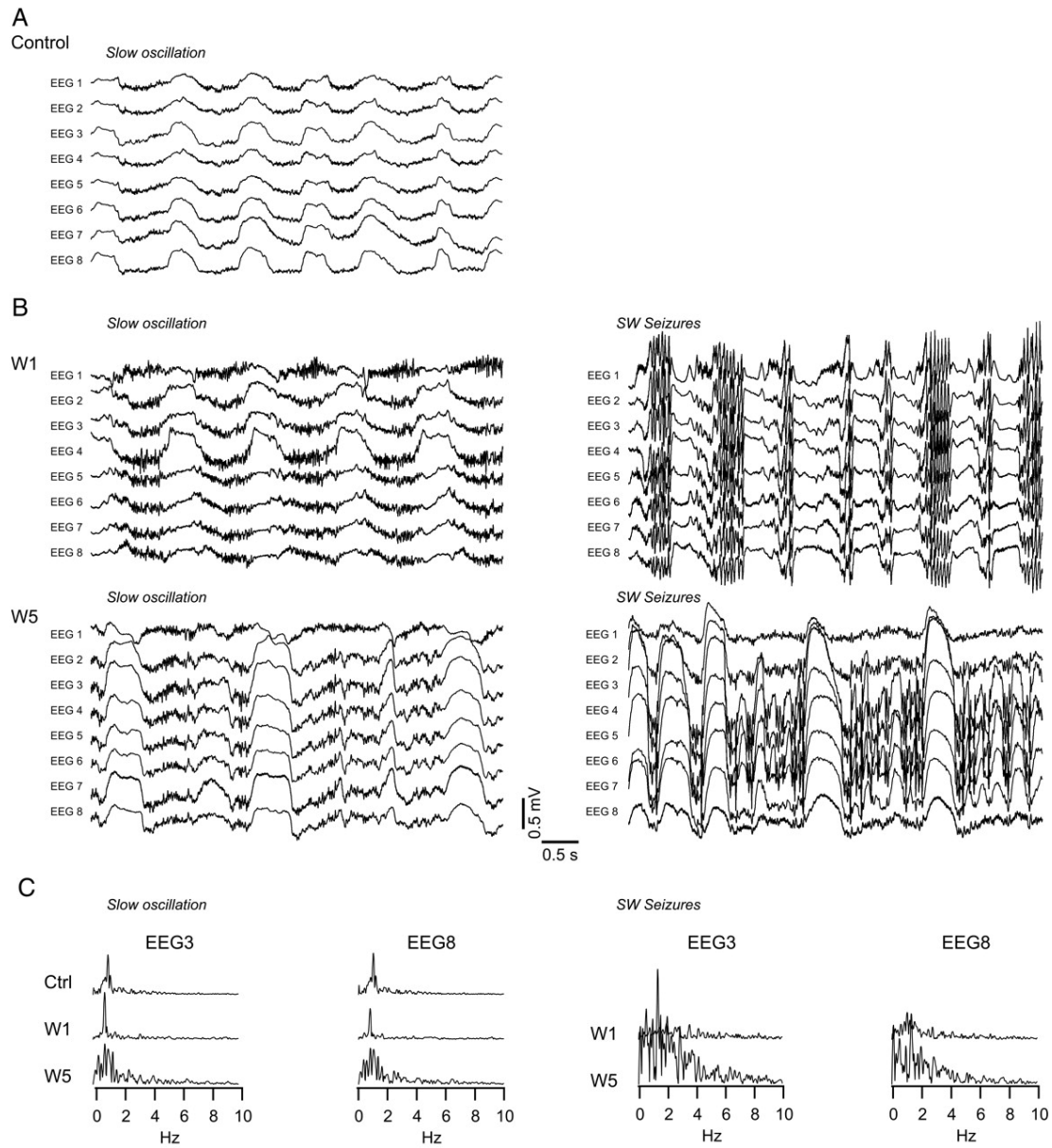
Figure 7-3. Patterns of EEG activity under ketamine–xylazine anesthesia. A: EEG field potentials showing slow oscillation in cat with intact suprasylvian gyrus. B: EEG field potentials during slow oscillation and SW/PSW seizures at 1 week (W1) and 5 weeks (W5) after cortical deafferentation. C: fast Fourier transform (FFT) of EEG activity shows an increased power of slow activities (<4 Hz) in anterior electrodes (EEG3) compared with posterior ones (EEG8), and in time from W1 to W5, both during slow oscillation and spike-wave (SW) seizures. Control data (Ctrl) are presented for slow oscillation epochs (From: Nita et al., 2006).
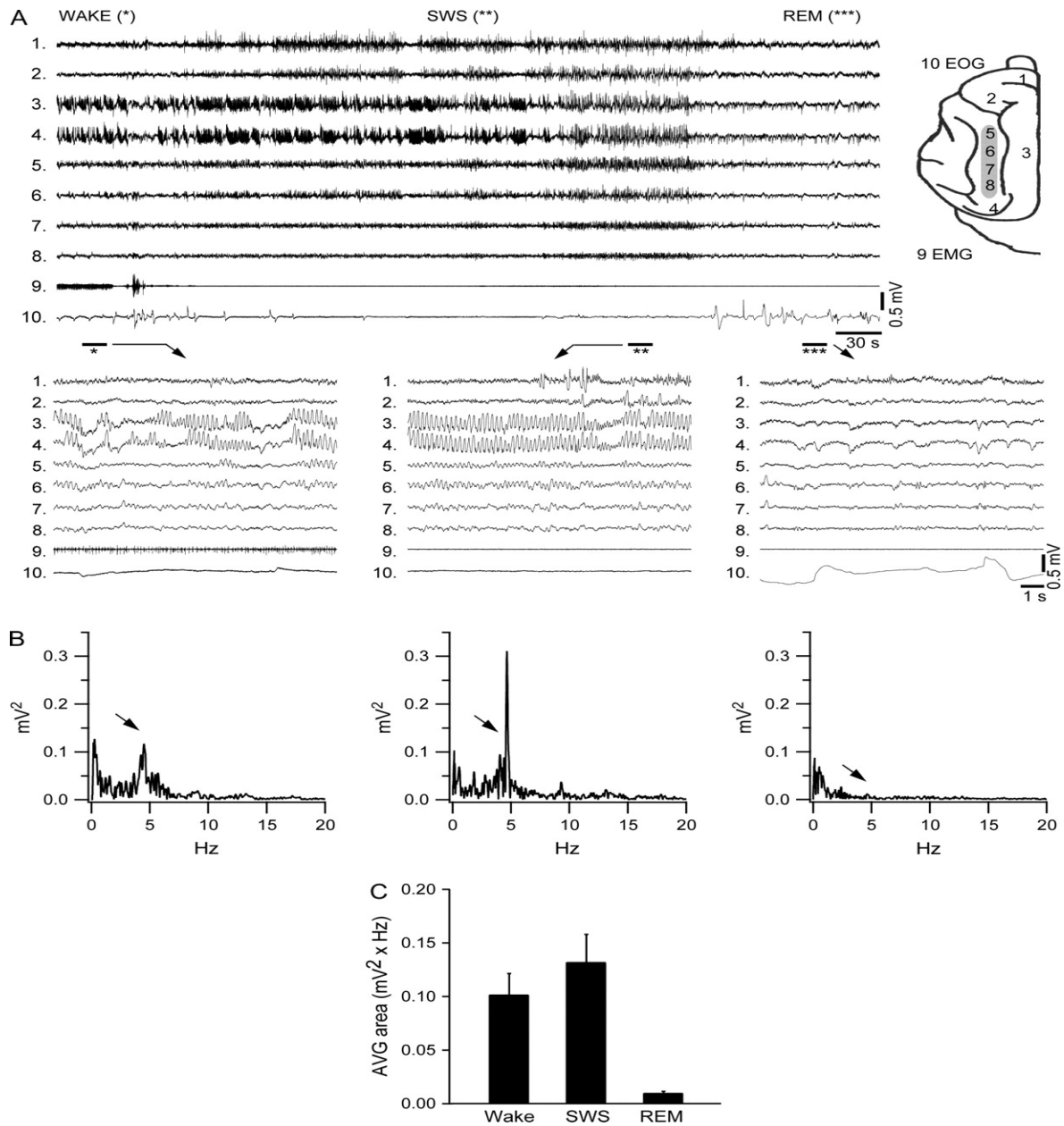
Figure 7-4 Sleep modulation of paroxysmal activities. (A) Top panel: polygraphic recording in a chronically implanted, naturally awake and sleeping cat, 10 days after the undercut. The placement of recording electrodes is depicted on right scheme, and the undercut is represented as a gray box. Bottom panels: occurrence of paroxysmal (3–4 Hz) activities was consistently found during wake, quasi-continuous during 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 with wake and disappears during REM sleep. (C) Pool data from 6 chronic cats of the FFT area in the 3- to 5-Hz domain, corresponding to electrode 3, from recordings performed between day 5 and day 15. (From: Nita et al., 2007).

This increases the slope of the input-output function of the affected neurons, so that for the same inward current neurons would generate more action potentials. Indeed, *in vivo* data in acutely deafferented cortex in cat show an enhancement between the amount of applied depolarization and the output firing rate in relatively intact neurons, while deafferented neurons demonstrated a weaker input-output relationship, with a greater level of frequency adaptation (Topolnik et al., 2003a). This effect may be attributable to a depression of the slow after-hyperpolarization and underlying Ca^{2+}-activated K^+ currents (Lancaster and Adams, 1986; Storm, 1990) that control repetitive firing (Franck and Schwartzkroin, 1985; Tseng and Prince, 1996).

7.2.1.2   Changes in synaptic connectivity

As already highlighted, epilepsy after penetrating brain injury, often becomes apparent only after a period. This latency to postlesional epileptogenesis in experimental models and humans may allow for anatomical and functional circuit reorganization. Sprouting and formation of new synaptic connections has previously been proposed as an epileptogenic mechanism (Purpura and Housepian, 1961; Sutula et al., 1989), although it is most likely not the only pathway to hyperexcitability, as blockade of sprouting does not prevent subsequent epileptogenesis in rats (Longo and Mello, 1997). Focal increases in the density of excitatory synapses determined with quantitative electron microscopy have been found in surgically resected tissue from patients with temporal lobe epilepsy (TLE) (Marco and DeFelipe, 1997). Axonal sprouting is also a common response to experimental central nervous system lesions (Schneider, 1973; Villablanca et al., 1988). Lesions of afferent cortical pathways have also been shown to induce sprouting of intrinsic cortical axons (Westenbroek et al., 1988; Darian-Smith and Gilbert, 1994).

Recurrent excitatory synapses formed by sprouting axons of pyramidal neurons and dentate gyrus granule cells (Perez et al., 1996; Sutula et al., 1998; Buckmaster et al., 2002; Smith and Dudek, 2002) are associated with enhanced functional excitation in hippocampal slices from epileptic humans and rat models of temporal lobe epilepsy (Molnar and Nadler, 1999; Lynch and Sutula, 2000; Scharfman et al., 2003). These injury-induced circuit reorganizations may contribute to epileptogenesis (Okazaki et al., 1999; Wuarin and Dudek, 2001).

An *in vitro* model specifically studied whether intracortical pyramidal neurons might sprout and form new connections, by examining the morphology of layer V neurons in the undercut cortex in rats (Salin et al., 1995). Total axonal length, number of axonal branches, and density of swellings or presumed boutons were all increased in undercut cortex relative to controls (Salin et al., 1995; Prince et al., 1997). The majority of the boutons on these layer V pyramidal cell axons occurred within layer V, where epileptiform activity is presumably initiated (Hoffman et al., 1994). But, is axonal sprouting of layer V pyramidal neurons in the partial neocortical isolation model of posttraumatic epileptogenesis associated with enhanced functional excitatory connectivity? Some results were offered by a study using scanning laser photostimulation and whole-cell recordings in undercut cortex *in vitro* indicating that layer V pyramidal cells in the injured cortex receive an excitatory synaptic input that is increased in spatial extent and intensity and is derived principally from presynaptic neurons in layer V (Jin et al., 2006).

7.2.1.3 Modifications of receptors and receptor subunits

Another potential way to promote hyperexcitability following brain trauma is a decrease in inhibitory receptors and/or neurons and/or an increase in glutamatergic excitatory receptors. However, autoradio-graphic receptor binding, positron emission tomography, and immunocytochemical investigations have not provided consistent results. Positron emission tomography studies of receptor binding suggest that benzodiazepine receptor levels, typically measured with $[^{11}C]$flumazenil, are reduced in hippocampus, thalamus, and temporal cortex in partial epilepsies, including cases of acquired lesions (McDonald et al., 1991; Juhasz et al., 1999). In contrast, benzodiazepine binding is increased in some types of developmental malformations (Duncan, 1999) and is not significantly altered in absence or generalized epilepsy (Savic et al., 1990; Prevett et al., 1995). Potential compensatory changes in benzodiazepine receptors and cases of unaltered levels have also been reported in patients with TLE (Savic et al., 1994; Duncan, 1999).

Another subject of debate is the $GABA_A$ receptor, which is notorious for being either hyperpolarizing or depolarizing, depending on age or on different pathological processes (De Groat, 1972; Mueller et al., 1983; Ben-Ari et al., 1989; Galanopoulou, 2007). The decision as to whether $GABA_A$ receptor activation will depolarize or hyperpolarize a

neuron is set by factors that control the gradients of anions, mainly Cl^- (Galanopoulou, 2007). Chloride homeostasis is controlled mostly by cation chloride cotransporters, from which, the principal Cl^- extruding cotransporters in neurons is K^+/Cl^- cotransporter (KCC2) (Payne et al., 1996), and the most studied importer of Cl^- is $Na^+/K^+/Cl^-$ cotransporter (NKCC1) (Moore-Hoon and Turner, 1998). Early in life, NKCC1 has the highest concentration and the developmental increase in KCC2, which eventually overcomes the influence of NKCC1, is required for the appearance of the mature, hyperpolarizing synaptic $GABA_A$ergic responses (Rivera et al., 1999; Hubner et al., 2001). Both *in vivo* and *in vitro* experiments have shown that a variety of insults in adult life, could lead to the reappearance of depolarizing GABA responses with ensuing calcium transients (for review see (Galanopoulou, 2007)). Such insults include brain trauma (Bonislawski et al., 2007), high temperature, hypotonic or hypertonic environment (van den Pol et al., 1996), nerve transections (van den Pol et al., 1996; Coull et al., 2003; Toyoda et al., 2003). The reversal of $GABA_A$ receptor function is often attributed to a decrease in KCC2 (Coull et al., 2003; Toyoda et al., 2003; Bonislawski et al., 2007), whereas NKCC1 does not necessarily change (Galeffi et al., 2004). Data from the subiculum of human epileptic tissue, show depolarizing GABA responses in the pyramidal neurons of the network that generates interictal-like activity (Cohen et al., 2002). Kindling of adult rodents has also been reported to alter the balance of cation chloride cotransporters, by the decreased KCC2 expression, and favor the appearance of depolarizing GABA responses (Rivera et al., 2002).

Functional receptor changes may also occur through alterations in subunit composition, engendering hyperexcitability and seizures. Tsunashima et al. investigated changes in $GABA_A$ receptor subunits in the well-known kainic acid model of TLE at several different time points (Tsunashima et al., 1997). Within the dentate gyrus, levels of messenger RNAs varied depending on the subunit as well as the time point examined. For instance, for the two most abundant α subunits, α-2 was initially decreased while α-4 was increased, and both returned to control levels after 7 days, and these changes in receptor subunit combinations could alter modulators of $GABA_A$ receptor currents (Tsunashima et al., 1997). Two such modifications in surgically resected tissue from patients with epilepsy have been reported: enhanced blockade of GABA currents by zinc (Shumate et al., 1998)

and reduction in the amplitude of GABA currents after high-frequency stimulation, possibly attributable to receptor desensitization (Isokawa, 1998).

Studies of immunocytochemical changes in glutamate receptor subunits in tissue from humans have also yielded inconsistent results. Generally, there is a decrease in glutamate receptors and receptor subunits that correlates with the amount of neuronal cell loss in TLE with hippocampal sclerosis (Bayer et al., 1995). However, a number of exceptions to this also exist. For instance, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionat (AMPA) receptor subunit, GluRl, appears to be increased specifically on proximal dendrites in the hippocampus in patients with TLE (Babb et al., 1996; Ying et al., 1998) as well as in some animal models (Babb et al., 1996; Mathern et al., 1997).

In the undercut model data show a strong down-regulation of immunocytochemically labeled GluRl and especially GluR2 subunits in deep layer V pyramidal neurons in all animals examined 3 days to 4 weeks after induction of the lesion (Jacobs et al., 2000). Counterstaining with thionin revealed that pyramidal cells lacking GluR2-immunoreactivity were present in the partially isolated cortex, suggesting that loss of GluR2 in tissue was not attributable solely to cell loss.

Nevertheless, a subpopulation of pyramidal neurons in superficial layer V remained strongly GluR2-immunoreactive. The location of these neurons, in superficial layer V, suggests that they correspond to the population of intrinsically bursting neurons that are selectively excited in slice preparations when low concentrations of bicuculline are added to the bathing medium (Chagnac-Amitai and Connors, 1989a).

A loss of the GluR2 subtype combined with sprouting may indicate that newly formed excitatory synapses have glutamate receptors that allow Ca^{2+} influx (Hume et al., 1991; Verdoorn et al., 1991), which may affect synaptic efficacy (Gu et al., 1996; Rozov et al., 1998) and result in prolonged depolarizations underlying repetitive high-frequency firing in epileptogenic neuronal aggregates (Matsumoto and Marsan, 1964).

7.2.1.4   Changes in neuronal density

7.2.1.4.1   Neuronal loss

Pathological studies of patients with a history of closed head trauma who later developed epilepsy (Hauser and Kurland, 1975) have revealed a spectrum of findings, ranging from cortical and white matter scars to isolated "Ammon's horn sclerosis," in which there is a selective loss of dentate hilar neurons and variable involvement of the pyramidal cell layers (Margerison and Corsellis, 1966). Surprisingly, adults with head trauma appear to have a more rapid progression of neuronal loss and processes that lead to epilepsy than children, as evidenced by a shorter latent period (Swartz et al., 2006).

In an animal model of post-traumatic epilepsy, brief impact applied to the surface of the cerebral cortex causes a selective, bilateral loss of hilar neurons and persistent changes in dentate granule cell excitability (Lowenstein et al., 1992). A direct relationship between the traumatic impact and hippocampal injury was supported by the observation that increasing forces of impact led to a greater amount of hilar neuron loss. The neuronal loss documented by cell counts was further substantiated by the identification of degenerating hilar neurons a few hours after impact and a decrease in cellular density in the hilus one week later (Lowenstein et al., 1992).

Although most of the studies agree that post-traumatic seizures both in experimental animals and in humans are associated with an overall neuronal loss, the data dealing with the preferential loss of GABAergic inhibitory neurons, again have provided inconsistent results. Several studies suggested that indeed GABA-ergic neurons might be selectively vulnerable to some forms of injury such as hypoxia (Sloper et al., 1980; Romijn et al., 1988), perinatal brain injury (Robinson et al., 2006), status epilepticus induced by convulsant agents (Franck and Schwartzkroin, 1985; Obenaus et al., 1993), excessive electrical stimulation (Sloviter, 1987, 1991), and neocortical isolations (Ribak et al., 1982; Ribak and Reiffenstein, 1982). Some other studies, however, found no change what so ever in the density of GABAergic neurons following brain injury (Franck et al., 1988; de Lanerolle et al., 1989; Davenport et al., 1990).

7.2.1.4.2 Neurogenesis

It is now well accepted that adult mammals including humans have stable populations of neural stem cells capable of replacing lost neurons following injury (Horner and Gage, 2000). A variety of insults, including ischemia (Liu et al., 1998; Gotts and Chesselet, 2005a, b), prolonged seizures (Parent et al., 2002), and mechanical injury (Ramaswamy et al., 2005), induce the proliferation of progenitors and the generation of immature neurons either in known neurogenic sites (Gould and Tanapat, 1997; Liu et al., 1998; Fallon et al., 2000; Magavi et al., 2000; Jin et al., 2001; Yoshimura et al., 2001) or in regions where neurogenesis normally does not occur (Johansson et al., 1999; Yamamoto et al., 2001b; Yamamoto et al., 2001a). Regrettably, the incorporation of new cells into the brain circuitry is not always beneficial. Parent et al. showed that prolonged seizures lead to generation of ectopic neurons or abnormal projections in the striatum and hippocampus. Although many of these ectopic neurons appear to have a very short life, this abnormal pattern of neurogenesis indicates that some of the brain's efforts toward repair may be misdirected and may accentuate rather than ameliorate the problem (Parent et al., 2002).

The data available about neurogenesis in the neocortex is very controversial. Some studies show there are no newly generated functional neurons in the neocortex even after injury, probably because of an unfavorable microenvironment for the local precursors (Arvidsson et al., 2002). On the contrary, some other studies demonstrate there is a region specific increase in number of proliferating cells following focal cerebral ischemia in rats (Zhang et al., 2001) and that a traumatic cortical injury resulted in the migration of cells from the subventricular zone to the injured cortex (Ramaswamy et al., 2005).

The existing evidence about the involvement of astrocytes in either neuronal death or neurogenesis appears also controversial. While astrocytes have been associated with creating a barrier against neurogenesis (Fitch et al., 1999) or even with facilitating neuronal loss in hippocampal sclerosis (Margerison and Corsellis, 1966), they have also been associated with the production of molecules that can be supportive to axonal regeneration and migration (Guth et al., 1985; Frisen et al., 1995; Mason et al., 2001; Song et al., 2002).

The absence of constitutive cortical neurogenesis reflects either an intrinsic limitation of the endogenous neural precursors' potential, or a lack of appropriate micro-environmental signals necessary for neuronal differentiation or survival. If we also take into consideration the fact that even when new neurons migrate from a neurogenic region they cannot survive for a long time in injured area (Arvidsson et al., 2002), the hypothesis of an hostile environment becomes more appealing. Indeed, multiple inhibitory cues have been identified after injury, including the formation of glia scars by astrocytes, reduced trophic factor support for neurons, and components of CNS myelin that inhibit regenerating axons. Some of the inhibitory myelin components are NogoA, MAG (Domeniconi et al., 2002; Liu et al., 2002), OMgp (Wang et al., 2002), and chondroitin sulfate proteoglycans.

7.2.1.5 Activity-related changes - homeostatic plasticity

Disturbances in the normal activity of the neuronal network, such as those produced by brain trauma, trigger a series of homeostatic mechanisms that would try to restore the activity within functional boundaries. These mechanisms will be discussed in the next section.

7.3 Homeostatic plasticity

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, 1989b), information transfer through the network (Sillito, 1975; Somers et al., 1995), and experience-dependent synaptic plasticity (Kirkwood and Bear, 1994; Hensch et al., 1998). Therefore, maintaining activity within functional boundaries is a crucial and in the same time a very challenging task for neurons in the CNS that receive thousands of synaptic inputs that need to be integrated in order to function accurately. 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 therefore composed of two contrasting but complementary forces: one that changes neuronal circuits gradually by creating selective variations between elements and another one that adjusts circuit properties in order to

stabilize the overall activity of the network. Thus, paradoxically, a stable biological system remains constant by being able to change and evolve.

In vitro studies demonstrate that a few days of pharmacological blockade of activity in cortical cell cultures determines an increased amplitude of mini excitatory postsynaptic currents (mEPSC) and EPSC in pyramidal cells (Turrigiano et al., 1998; Watt et al., 2000), as well as an increase in quantal release probabilities (Murthy et al., 2001). Conversely, prolonged enhanced activity levels reduced the size of mEPSCs (Lissin et al., 1998; Turrigiano et al., 1998; Leslie et al., 2001).

Not only synaptic, but also intrinsic excitability is regulated by activity. After chronic activity blockade, Na^+ currents increase and K^+ currents decrease, and this leads to an enhanced responsiveness of pyramidal cells to current injections (Desai et al., 1999). Recent data indicate that homeostatic plasticity also occurs *in vivo*, because sensory deprivation increased the average sizes of AMPA-mediated quantal currents by as much as 25% (Desai et al., 2002). These increases were mediated by global changes in the entire distribution of excitatory currents onto a given postsynaptic neuron and were well fitted by a multiplicative scaling model giving strong support to the idea that the synaptic scaling phenomenon, discovered and previously studied in culture, operates in the intact brain.

Does homeostatic plasticity play a role in trauma-induced epileptogenesis? Deafferentation occurs in humans after penetrating brain trauma and in animal undercut models, when a population of neurons is partially disconnected from the underneath structures and thus underexcited. This triggers homeostatic plasticity mechanisms that will try to restore normal activity by upregulating depolarizing influences and downregulating hyperpolarizing ones.

Recent *in silico* studies suggest that after a severe deafferentation of the cortical network, the homeostatic plasticity mechanisms have limitations, and will increase network excitability to the point where paroxysmal activity develops (Houweling et al., 2005; Frohlich et al., 2005). Thus, these data show that restoring a normal activity pattern with random non-synchronized firing of individual neurons may become impossible in the

severely deafferented network, and the same plasticity mechanisms may become maladaptive and lead to paroxysmal oscillations.

8 Rationale for the thesis

Animal models of the human condition are necessary to elucidate the mechanisms of epileptic seizures and to develop appropriate therapy that could control them. The study of PTE was very appealing to me particularly because the trigger of brain hyperexcitability is clearly identifiable in time, and this opens the possibilities of prevention therapy. Therefore, understanding the basic mechanisms of PTE is crucial if we aim at interfering with them immediately after cortical injury, to reduce the risk of developing epilepsy in some patients.

For the present work, we used the model of "partial cortical deafferentation" to mimic penetrating cortical wounds in humans, known to have a very high risk of PTE (Salazar et al., 1985; Weiss et al., 1986; Annegers et al., 1998). Although studies carried out in slices or cultures have been crucial in uncovering the role of different substances (Graber and Prince, 1999), ionic channels (Desai et al., 1999), receptors (Chen et al., 2007) in epilepsy, they cannot recreate the complexity of the whole brain in which different structures interact in order to maintain the fine balance between excitation and inhibition. To overcome these limitations we chose to carry out our experiments *in vivo*, at different time delays following deafferentation, to investigate the progressive nature of epileptogenesis. Moreover, parts of experiments explore the relationship between different states of vigilance, such as wake and natural sleep, and paroxysmal activity, as anesthetics could influence the background neuronal activity and contaminate our results.

The questions we tried to answer in the present work are: does the undercut modify neuronal and glial density? If this were true, do the excitatory and the inhibitory neurons have similar sensitivity to brain injury? Are the properties of glial cells similar in normal and in undercut cortex? Does chronic deafferentation induce changes in neuronal connectivity and intrinsic properties? Are these changes related to the progression of the disease? Is there a difference in excitability between neurons located in the partially

deafferented and in the relatively intact area of the suprasylvian gyrus, that would make the latter more likely to initiate paroxysmal activity? Are neuronal properties modulated by different states of vigilance, and how could this influence the susceptibility to seizure of these states of vigilance?

The guiding line of all the experiments was the hypothesis that chronic input deprivation of the undercut gyrus and the decreased network activity of the deafferented cortex activated homeostatic mechanisms, triggering major cortical reorganization, and probably a shift in the balance excitation-inhibition towards excitation, to compensate the reduced inputs to the undercut cortex.

9 Synaptic strength modulation following cortical trauma: a role in epileptogenesis

Sinziana Avramescu and Igor Timofeev

9.1 Résumé

Les traumatismes crâniens sont souvent suivis d'une hyperexcitabilité anormale menant à des crises convulsives et à l'épilepsie. Des études antérieures ont documenté la capacité des neurones néocorticaux de remodeler leurs connexions en réponse à de diverses lésions corticales et sous-corticales. Cependant, peu d'information est disponible au sujet des conséquences fonctionnelles des changements anatomiques qui suivent ce genre de traumatisme ou à propos de l'adaptation de la connectivité synaptique à la privation d'afférences neuronales produite par déafférentation chronique. Dans cette étude, nous avons enregistré des activités intracellulaires (IC) de neurones corticaux simultanément avec des activités extracellulaires (EC) et potentiels de champ des cellules avoisinantes dans le cortex du chat ayant subit une grande transection de la matière blanche sous le gyrus suprasylvien, en conditions aigues et chroniques (à 2, 4 et 6 semaines), chez des chats anesthésiés avec de la ketamine-xylazine. En utilisant des potentiels d'action EC pour calculer les variations moyennées du potentiel de membrane IC, nous avons trouvé une probabilité et une efficacité accrues de connexions entre les neurones après le traumatisme cortical. Les interactions inhibitrices n'ont montré aucun changement important dans le cortex traumatisé comparé au contrôle. L'efficacité synaptique renforcée a été accompagnée par une résistance membranaire augmentée, une hyperexcitabilité intrinsèque des neurones corticaux, ainsi que par une plus grande durée des périodes silencieuses dans le réseau. Nos données électrophysiologiques ont démontré les conséquences fonctionnelles des changements anatomiques du cortex traumatisé précédemment rapportés. Nous suggérons que la plasticité homéostatique synaptique compensant l'activité diminuée dans le cortex déafferenté mène à une hyperexcitabilité corticale incontrôlable et à des crises généralisées d'épilepsie.

9.2 Abstract

Traumatic brain injuries are often followed by abnormal hyperexcitability leading to acute seizures and epilepsy. Previous studies documented the rewiring capacity of neocortical neurons in response to various cortical and subcortical lesions. However, little information is available on the functional consequences of these anatomical changes following cortical trauma and the adaptation of synaptic connectivity to a decreased input produced by chronic deafferentation. In this study, we recorded intracellular (IC) activities of cortical neurons simultaneously with extracellular (EC) unit activities and field potentials of neighboring cells in cat cortex, following a large transection of the white matter underneath the suprasylvian gyrus, in acute and chronic conditions (at 2, 4 and 6 weeks) in ketamine-xylazine anesthetized cats. Using EC spikes to compute the spike-triggered averages of IC membrane potential, we found an increased connection probability and efficacy between cortical neurons weeks after cortical trauma. Inhibitory interactions showed no significant changes in the traumatized cortex compared to control. The increased synaptic efficacy was accompanied by enhanced input resistance and intrinsic excitability of cortical neurons, as well as by increased duration of silent network periods. Our electrophysiological data revealed functional consequences of previously reported anatomical changes in the injured cortex. We suggest that homeostatic synaptic plasticity compensating the decreased activity in the undercut cortex, leads to an uncontrollable cortical hyperexcitability and seizure generation.

9.3 Introduction

The central nervous system reveals a remarkable plasticity which enables the adaptation to changes in incoming volleys while still maintaining activity within functional boundaries (Turrigiano, 1999). Brain injury frequently generates aberrant excitability responsible for the increased frequency of acute seizures (Dinner, 1993; Topolnik et al., 2003b) and chronic epilepsy (Salazar et al., 1985).

Partially isolated neocortex is a well-established model of epileptogenesis following cortical trauma (Prince and Tseng, 1993; Hoffman et al., 1994; Jacobs et al., 2000; Topolnik et al., 2003b; Nita et al., 2007). The undercut cortex becomes increasingly hyperexcitable over a few weeks and progressively generates paroxysmal activity, both *in vivo* (Nita et al., 2006; Nita et al., 2007) and *in vitro* (Prince and Tseng, 1993; Salin et al., 1995). Several mechanisms may contribute to the development of hyperexcitability following cortical injury including changes in intrinsic neuronal properties (Esplin et al., 1994; Topolnik et al., 2003a), selective loss of inhibitory synapses (Ribak and Reiffenstein, 1982), increase of both inhibition and excitation (Bush et al., 1999), and axonal sprouting (Salin et al., 1995). The capacity of neocortical neurons to modify their axonal arborization has been documented following various cortical and subcortical lesions (Kuang and Kalil, 1990; McKinney et al., 1997). However, little is known on the functional consequences of these anatomical changes following cortical trauma and the adaptation of synaptic connectivity to a decreased input produced by chronic deafferentation.

Although studies carried out in slices or cultures have been crucial in uncovering the role of different substances (Graber and Prince, 1999), ionic channels (Desai et al., 1999) and receptors (Chen et al., 2007) in epilepsy, they cannot recreate the complexity of the whole brain in which different structures interact to maintain the fine balance between excitation and inhibition. To overcome these limitations we carried out the experiments *in vivo*, in order to investigate the gradual changes of synaptic efficacy and intrinsic excitability following cortical undercut. Most of previous studies assessing synaptic connectivity after brain trauma used only indirect methods to quantify the changes in synaptic connectivity (Li et al., 2005; Jin et al., 2006), since direct electrophysiological assessment of synaptic properties is difficult with currently available techniques (Li et al.,

2005); and was not considered feasible for quantitative analysis of functional connectivity (Jin et al., 2006).

In the present study, we quantified the efficacy of synaptic interactions *in vivo*, at different time delays following cortical deafferentation, to uncover the synaptic changes responsible for post-traumatic epilepsy. We found a boost in intrinsic excitability and synaptic connectivity several weeks after brain injury, accompanied by an increased efficacy of excitatory connections without significant changes of inhibitory connections, both in acute and chronic conditions. The decreased network activity in the deafferented cortex might have activated homeostatic synaptic plasticity (Turrigiano, 1999; Houweling et al., 2005; Trasande and Ramirez, 2007) leading to increased strength of excitatory synapses in order to compensate the reduced inputs to the undercut cortex.

A part of this work has been reported in an abstract form (Avramescu and Timofeev, 2006).

9.4 Materials and Methods

Animal preparation.

Experiments were performed in 37 ketamine-xylazine anesthetized cats (10–15 and 2–3 mg/kg, im, respectively) of both sexes, divided into three groups: control (n=13), acute undercut (n=12) and chronic undercut (n=12). The animals were paralyzed with gallamine triethiodide (20 mg/kg, iv) after the EEG showed typical signs of deep general anesthesia, essentially consisting of sequences of slow oscillation (0.5–1 Hz). Supplementary doses of anesthetics were administered at the slightest change towards an activated EEG pattern. Lidocaine (0.5%) infiltration was used for all pressure points or incision lines. General surgical procedures included: cephalic vein canulation for systemic liquid delivery (lactated Ringer's solution 5-10 ml/kg/h) and tracheotomy. The cats were ventilated artificially with the control of end-tidal CO_2 at 3.5–3.7% and heart rate (90–100 beats/min). The body temperature was maintained at 37–38°C with a heating pad. The stability of intracellular recordings was increased by the drainage of the cerebrospinal fluid from the cisterna magna, hip suspension, bilateral pneumothorax, and filling the hole made for recordings with a solution of 4% agar.

Cortical deafferentation.

The partial cortical deafferentation was performed by a large transection (13–15 mm postero-anteriorly, 3–4 mm medio-laterally and 3–4 mm deep) of the white matter under the suprasylvian gyrus. In order to produce the undercut, a custom designed "L" shape knife was inserted in the posterior part of the suprasylvian gyrus, rotated at a 90° angle and advanced rostrally, parallel to the surface of the gyrus. Therefore, the white matter below the posterior and middle part of the gyrus was transected whilst the anterior part was still connected to the underneath structures, hence creating conditions of partial cortical deafferentation.

At the end of experiments the animals received a lethal dose of sodium pentobarbital (50 mg/kg, i.v.). The brains were removed, sliced into 80-μm sections and stained with thionine in order to verify the extension and the position of the undercut (Fig. 9-1*A*). All experimental procedures were approved by the committee for animal care of

Laval University and were in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

Recordings.

Acute experiments were performed during the same day when the undercut was made, while chronic experiments were carried out at 2 weeks (n=4), 4 weeks (n=4) and 6 weeks (n=4) following cortical deafferentation, under ketamine-xylazine anesthesia (10–15 and 2–3 mg/kg, im). In control experiments, the recordings were performed in intact cortex.

The experimental approach was similar to the one described by (Matsumura et al., 1996). Simultaneous intracellular (IC) and extracellular (EC) activities of neighboring neurons were recorded in the partially deafferented area 7 of the left suprasylvian gyrus (Fig. 1*B,C*) with glass micropipettes (tip diameter <0.5 μm). The IC electrode was inserted in a vertical stereotaxic direction, while the EC electrode was inserted at an angle of 10-15° from vertical in the parasagittal plane, so that the tips of the electrodes were very close one to each other (less than 100 μm). The pipettes were filled with potassium acetate (2.5 M, in situ impedance 35-65 MΩ) for the IC recordings and with sodium chloride (1M, in situ impedance 5-10 MΩ) for local field potential and extracellular unit recordings. We carefully verified the morphology of each EC recorded spikes and we only included single unit recordings of the presynaptic neurons. However, it was possible that in some recordings the EC spikes corresponded to more than one presynaptic neuron. Each pair of neurons consisted of an EC recorded presynaptic spike and a postsynaptic IC recording.

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.

Twenty animals with deafferented suprasylvian gyrus displayed paroxysmal EEG activities (Fig. 9-1*E*). We chose not to include in our analysis this paroxysmal periods for several reasons. First, in the present study we were interested to quantify the changes in neuronal interactions that precede and favor the development of electrographic seizures. Second, during paroxysmal discharges the amplitude of the action potentials (29.1 ± 6.2

mV) decreases significantly (p<0.05, Student's T-test) and their variability (S.D.) increases compared to the slow oscillation (57.2 ± 2.5 mV) periods (Fig. 9-1*E*). This may bias the detection of EC spikes, which in this study was based on the morphology of spikes.

Data analysis.

All IC recorded neurons were classified, based on their pattern of response to depolarizing current pulses into one of four electrophysiological classes of neocortical neurons: regular-spiking (RS), intrinsically-bursting (IB), fast-rhythmic-bursting (FRB) or fast-spiking (FS) (Connors and Gutnick, 1990; Gray and McCormick, 1996; Steriade et al., 1998). The apparent input resistance of neurons was measured both during active and silent phase of the slow oscillation by averaging cellular responses to 5 trials of hyperpolarizing current pulses (0.5 nA, 0.1 s) during each phase. To evaluate the firing frequency of RS neurons we counted the number of spikes and we measured instantaneous firing rate as the reciprocal of the first inter-spike interval in response to depolarizing current (0.5 nA, 0.75 nA, 1.0 nA, 0.2 s) in control and in undercut cortex. We used the average of the values measured in 5 consecutive trials from ten RS neurons with similar membrane potential (-70 ± 5 mV) in each group.

To establish the possible synaptic connections between neurons, the EC recorded units were used to compute spike-triggered averages (STAs) of the membrane potential of IC recorded neurons (Fig. 9-1). We first averaged all segments ("AVG all") (Fig. 9-1*D1,2,4*) and subsequently, to reliably determine the response of the postsynaptic neuron to the presynaptic trigger, we removed manually all segments that revealed an action potential (AP) during 50 ms before or after the trigger. The average of the remaining segments was identified as "AVG no spikes" (Fig. 9-1*D3,4*). The minimum number of segments to detect a synaptic connection was 100, although most averages were obtained from 100 to 500 segments that remained after removing the APs.

Based on the polarity and the relative onset times of deflections in "AVG no spikes" we identified four basic types of interactions between the recorded cells. If the IC membrane potential revealed a depolarization event after the presynaptic AP, we identified it as an excitatory postsynaptic potential - "EPSP" (Fig. 9-2*A*) and if the onset of

depolarization occurred before the trigger, we classified it as a network excitation – "NE" (Fig. 9-2*B*). When a hyperpolarizing potential was recorded after the EC spike, we classified it as an inhibitory postsynaptic potential – "IPSP" (Fig. 9-2*C*) and if the onset of hyperpolarization occurred prior to the EC spike, we classified it as a network inhibition – "NI" (Fig. 9-2*D*). If the averaged amplitude of the IC membrane potential during 20 ms before or after the EC revealed no relation to the spike, we considered that the two cells were not interacting – "No response" (Fig. 9-2*E*).

Each averaged postsynaptic response was fitted with a sigmoid function and we estimated the beginning of the response at the point representing 10% of the slope. The same averaged responses were used to quantify the amplitude, latency, 0-100 % rise time and the duration at half amplitude for the four types of interactions (Fig. 9-2*F*).

For all direct synaptic connections (EPSPs and IPSPs) we analyzed each segment manually in order to distinguish between successful responses and failures. We established a jitter of 0.2 ms for the latency of the responses centered on a time window after the presynaptic trigger during which the responses were expected to occur. We then computed the first derivative of each individual segment included in the STAs and we considered a "response" those segments that displayed a positive peak (for excitatory connections) or a negative peak (for inhibitory connections) in the differentiated wave, during the established time window (Fig. 9-3*A*).

To clearly identify the response from the background synaptic noise, we measured from IC traces the amplitude of the synaptic potential in the time-window during which the responses were expected to occur, before (to measure the synaptic noise) and after the EC spike (to measure the amplitude of the response). Thereafter, we computed and superimposed the histograms of amplitude for both synaptic noise (grey bars) and for responses and failures (open bars) (Fig. 9-3*B,C*). In order to double-check the presence of responses, we plotted the amplitude of the responses and failures against the membrane potential and observed that for excitatory connections the responses were positive (open red circles) and the failures were centered on zero (open black circles), being either positive or negative depending on polarity of synaptic noise (Fig. 9-3*B*). For the inhibitory connections the amplitude of responses was negative at a depolarized membrane potential and positive

at a more hyperpolarized potential (open red circles), while the failures (open black circles) were also centered around zero line, and were either positive or negative (Fig. 9-3*C*). Since IPSPs occurring around reversal potential could be mistaken for failures, we calculated failure rates for IPSPs that occurred at least 5 mV above estimated reversal potential for IPSP. We also computed the skewness and kurtosis for the responses (excitatory and inhibitory) and for the noise.

It has been previously shown *in vitro*, in the undercut model, that GABA mediated postsynaptic potentials could become depolarizing during high activity periods, due to an impairment of Cl⁻ extrusion (Jin et al., 2005). In the present study, we estimated the reversal potential for all our recordings and it was always between -60 and -70 mV, while the potential for depolarizing GABA events could not be more depolarized than -50 mV. Moreover, neurons in the undercut cortex have a normal resting E_{Cl} and the impairment of Cl⁻ extrusion has been described to convert the hyperpolarizing GABAergic responses into depolarizing ones only during intense neuronal activity, such as during seizures (Jin et al., 2005), but here we only analyzed neuronal interactions during the seizure-free periods. Thus, depolarizing events after presynaptic spike recorded in the current study were EPSP and not depolarizing IPSPs.

For all direct excitatory connections, we calculated the failure rate as the percentage of all failures from the total number of segments of the STA and the coefficient of variation (c.v) was computed for successful responses only.

Statistical significance of comparative data was assessed by performing appropriate statistical tests: Student's t-test and Mann-Whitney test. Differences between means were considered significant at p<0.05. Numerical values are given as means ± standard error of mean (S.E.M.) accept where otherwise specified. In all statistical tests, the results obtained in acute conditions were compared to control and to chronic conditions.

9.5 Results

We recorded a total of 135 pairs of neurons in control and 263 in undercut cortex, from which 93 in control and 180 in deafferented cortex displayed an interaction: EPSP, IPSP, NE or NI), while the other EC-IC pairs the neurons were not connected.

In both control and undercut cortex (acute and chronic) the IC neurons revealed slow oscillatory pattern (Steriade et al., 1993; Contreras and Steriade, 1995). During depth-positivity the neurons were hyperpolarized and silent, and during depth-negativity they were depolarized (Fig. 9-1B). The incidence of paroxysmal activities increased following undercut as already reported in previous published *in vivo* data, both in acute and chronic conditions (Topolnik et al., 2003b; Nita et al., 2006). In acute conditions 8 out of 12 animals displayed paroxysmal EEG patterns consisting in paroxysmal discharges and/or clear-cut electrographic seizures (Fig. 9-1E), while in chronic conditions (2, 4 and 6 weeks after undercut) all animals (n=12) showed paroxysmal activity.

Synaptic interactions between cortical neurons

We identified four basic types of synaptic interactions between the triggering EC cell and the events in IC recorded neurons, based on the polarity and the relative onset times of deflections in the STAs. As we described in the method section, a pair of neurons was considered to be connected via a direct synaptic connection, either excitatory, if the STAs revealed an EPSP (Fig. 9-2A), or inhibitory, when the STAs revealed an IPSP (Fig. 9-2C). We also analyzed the histograms of noise and responses (Fig. 9-3B,C). The histograms of noise were always near normal distribution (skewness 0.22 ± 0.17, kurtosis 0.38 ± 0.08). The skewness for the EPSPs (1.29 ± 0.98) was always higher ($p<0.05$, Student's T-test) than that for the noise, while the skewness for the IPSPs (-0.74 ± 0.51) was smaller ($p<0.05$, Student's T-test). The kurtosis of the responses (-0.62 ± 0.29 for EPSPs and -1.1 ± 0.32 for IPSPs) was smaller ($p<0.05$, Student's T-test) than the kurtosis of the noise, suggesting a more flatten distribution of responses. Depolarizing potentials beginning before the presynaptic spike were termed NE (Fig. 9-2B) and the mechanisms that generated it could be a common excitatory synaptic input to both EC and IC cells from the same presynaptic cell(s). Similarly, hyperpolarizing potentials beginning before the

presynaptic spike, NI (Fig. 9-2D), were probably produced by a common excitatory synaptic input onto EC and a population of inhibitory interneurons which synapse on IC. There were also several cases in which an EPSP was superimposed on a NE type of response. All other mixed responses, such as an IPSP overlaying a NE, an EPSP over a NI, or an IPSP on top of a NI, were difficult to quantify and therefore were not included in the analysis.

Deafferentation induced changes in synaptic connectivity

The most common type of response in control and after undercut was NE followed by NI, while the direct synaptic connections (EPSP and IPSP) were the least frequent (Fig. 9-4A). The high frequency of NE could reflect the possibility that a spike has a larger chance to be preceded by network or neuronal depolarization. NI responses are less frequent as they could only be triggered when a local population of inhibitory neurons is able to send common synaptic input to both the EC and the IC cells, and it should also be taken into consideration that there are far less inhibitory neurons compared to excitatory ones.

We then measured the connection probability as the percentage of both EPSPs and IPSPs type of responses from the total number of interactions in a group. The probability of connection was similar in control (16.8 ± 2.8%) and in acute undercut (17.63 ±4.03%) groups and then progressively increased (p<0.05, Mann-Whitney) to 32.5 ± 7.5% at 2 weeks, 35.64 ± 3.89% at 4 weeks and 35.5 ± 6.98% at 6 weeks following deafferentation (Fig. 9-4B). There were no statistical significant changes when analyzing separately the direct excitatory and inhibitory connections.

The higher rate of paired recording in our study compared to connection rate from *in vitro* studies is probably due to the fact that we measured the connectivity *in vivo* with all the connections intact. In fact, *in vitro* studies support this assumption, showing that an increased slice thickness from only 400 to 500 μm resulted in more than a three fold increase in the probability of connection (from 1:15 to 7:32) (Thomson et al., 1996).

Since NE and NI s were the most frequent responses, we followed their dynamics at different time delays after cortical deafferentation. We observed no major changes in their

probability of occurrence in acute cortical trauma conditions ($77.33 \pm 5.19\%$ for NE and $10.01 \pm 3.73\%$ for NI) compared to control ($77.52 \pm 5.49\%$ for NE and $15.98 \pm 4.37\%$ for NI). Nevertheless, the incidence of NE and NI at 2 and 4 weeks after deafferentation revealed opposite changes, increasing with 10.9 % compared to acute conditions for NE and decreasing with 6.42 % for NI at 2 weeks ($p<0.05$, Mann-Whitney), while at 4 weeks the probability for NE decreased with 13.49% compared to control ($p<0.05$, Mann-Whitney), and increased for NI 15.78% ($p<0.05$, Mann-Whitney) (Fig. 9-4C,D). Therefore, the ratio between NE and NI, which was 4.8 in control, decreased dramatically at 4 weeks, reaching a 2.5 value and then boosted again to 7.2, almost double than the control conditions. These data provide support at cellular level for our previous observation, that the number of ictal events showed a plateau in the first 2 weeks, then decreased progressively, being almost absent after approximately 4 weeks and subsequently increased again, remaining constant for a long period of time (Nita et al., 2007).

In conclusion, the probability of connection, which includes both inhibitory and excitatory direct connections, is significantly larger in all the chronic stages of the undercut, whereas NE and NI display variations between the undercut groups. The high frequency of NE at 2 weeks is accompanied by a decrease incidence of NI; on the contrary, at 4 weeks the decreased expression of NE goes together with a higher frequency of NI, and at 6 weeks the NE and NI are balanced. This means that at 2 weeks after the penetrating injury there is a high incidence of excitation, at 4 weeks the inhibitory activity prevails and this might explain the decreased frequency of seizures at this time. Thereafter, the incidence of excitation increases again and the occurrence of seizures remains constant (Nita et al., 2007).

Modulation of synaptic efficacy following penetrating cortical trauma

We identified four types of neurons based on the electrophysiological properties of the intracellular recordings: RS, FRB, IB and FS. The general distribution expressed as means $\pm$ standard deviation was $73.22 \pm 9.85\%$ for RS, $11.17 \pm 4.03\%$ for FRB, $9.56 \pm 4.21\%$ for IB and $6.05 \pm 2.29\%$ for FS neurons. We observed a tendency towards a decreasing number of RS neurons at 4 and 6 weeks, together with an increased percentage of FRB and IB neurons, although it was not statistical significant. This heterogeneity of neurons could

have influenced the response of the postsynaptic cell. To test this possibility, we initially analyzed separately for each type of postsynaptic neuron (RS, FRB, IB and FS) the three parameters used to characterize the responses (amplitude, latency, rise time) in control conditions. Given that we found no significant differences between the groups, the synaptic efficacy was analyzed together for all recorded neurons.

The amplitude of excitatory connections, both EPSPs and NEs, increased at 2 and 6 weeks following deafferentation when compared to acute conditions ($p < 0.05$, Student's T-test), while at 4 weeks it was decreased ($p < 0.05$, Student's T-test) (Fig. 9-5A), showing therefore a similar trend with the incidence of NE. The amplitude of inhibitory connections (IPSP and NI) was not significantly modified following deafferentation (data not shown). However, there was a trend towards a reduction of inhibition at 2 weeks, while at 4 weeks after the undercut we observed a tendency towards enhanced inhibition (data not shown). In conclusion, the network excitability shifted towards excitation at 2 weeks after cortical undercut, diminished at 4 weeks and then increased again at 6 weeks, paralleling the alteration in the number of ictal events reported previously (Nita et al., 2007).

Both the duration and the rise time of EPSPs were significantly reduced 4 and 6 weeks following deafferentation. As expected, the latency was not modified in acute conditions after trauma, or after 2, 4 or 6 weeks, suggesting that axonal velocity and mediator release were similar in all experimental condition (Fig. 9-5A, bottom right). Similarly, the duration, rise time, and latency of NEs were reduced at 4 and 6 weeks after cortical deafferentation (Fig. 9-5C, bottom right). Instead, the duration, rise time, and latency were not significantly modified for IPSPs or NIs (data not shown).

The next step of our study was to identify the mechanism or the mechanisms responsible for the enhanced efficacy of excitatory connections following cortical trauma. The increased amplitude of averaged EPSPs could result either from (a) increased release probability, (b) larger number of release sites, (c) increased intrinsic excitability of postsynaptic neurons and (d) increase in postsynaptic receptor content. The later mechanism was not tested in the present study.

In order to test the strength of synaptic connections we computed the failure rate and the coefficient of variation (c.v.) for each monosynaptic excitatory connection. The failure rate of direct excitatory synaptic connections increased in acute undercut cortex to $85.76 \pm 0.78\%$ as compared to control $78.91 \pm 1.54\%$ ($p<0.05$, Student's T-test). Subsequently, it was significantly reduced 2 weeks after trauma ($82.32 \pm 1.18\%$) and increased at 4 weeks ($88.9 \pm 0.61\%$) when compared to acute conditions ($p<0.05$, Student's T-test) (Fig. 9-5B, left), explaining therefore the variation of amplitude which was higher at 2 weeks and the lowest at 4 weeks. However, the failure rate was always higher in the undercut cortex than in control; consequently, supplementary mechanisms should have been responsible for the increased amplitude of averaged excitatory responses in the undercut cortex.

The c.v. of excitatory postsynaptic responses reflects postsynaptic reliability of synaptic transmission (Markram et al., 1997). Similar to our previous observations (Crochet et al., 2005), the c.v. in control conditions was 1.44 ± 0.11 and it decreased to 1.04 ± 0.17 in acute undercut conditions, probably due to injury induced changes of neuronal interactions. The c.v. continued to decrease slowly even after the initial insult to the brain, being significantly different from acute ($p<0.05$, Student's T-test) at 4 weeks (0.52 ± 0.03) and appeared to be maintained at a similar value thereafter (Fig. 9-5B, middle). When investigating the relationship between the c.v. and the amplitude of responses, we found a correlation (Fig 9-5B, right) similar to previous studies (Markram et al., 1997). Moreover, a reduced c.v. is associated with the formation of new synapses and appears to be a sign of strong synaptic connection (Berninger et al., 1999).

The apparent discrepancy between the decreased release probability (increased failure rate) and the decreased c.v. likely occurs because the failure rate measures only the efficacy at the presynaptic site, while the c.v. of successful responses quantifies postsynaptic efficacy.

Neuronal hyperexcitability following trauma

In order to assess the intrinsic excitability of neurons in the injured cortex, we computed the neuronal input resistance as well as the firing frequency of neurons in response to depolarizing current pulses.

The input resistance of cortical neurons is higher during silent network states as compare to active states (Contreras et al., 1996; Steriade et al., 2001; Rudolph et al., 2007), except for layer 2-3 pyramidal neurons from rat somatosensory cortex (Waters and Helmchen, 2006). Following cortical trauma, the input resistance of neurons was similar in control (20.3 ± 1.85 MΩ during silent phase and 13.96 ± 1.31 MΩ during active state) and in acute conditions (21.43 ± 1.06 MΩ during silent phase and 14.59 ± 1.14 MΩ during active state), as reported before (Topolnik et al., 2003a). However, in chronically injured cortex the input resistance increased both during silent and active periods of the slow oscillation (p<0.05, Student's T-test), reaching a value of 30.52 ± 3.98 MΩ/ 20.83 ± 3.74 MΩ at 2 weeks, 40.49 ± 2.37 MΩ/ 31.44 ± 2.59 MΩ at 4 weeks and 39.45 ± 2.44 MΩ/ 26.88 ± 3.14 MΩ 6 weeks following cortical deafferentation (Fig. 9-6).

We further tested the relationship between the firing rate of RS neurons and the intensity of intracellularly applied current pulses in control and in undercut cortex. Our results revealed a significant decrease (p<0.05, Student's T-test) in both number of spikes and instantaneous firing rate after acute trauma to the brain compared to control conditions, for all intensities of current applied (0.5 nA, 0.75 nA, 1.0 nA). Nevertheless, the number of spikes and instantaneous firing rate increased thereafter at 2, 4, and 6 weeks when compared to acute (p<0.05, Student's T-test) (Fig. 9-7). When we compared the results obtained in chronically injured cortex to the control group, the increased number of spikes and instantaneous firing rate reached statistical significance only at 4 and 6 weeks after the undercut.

In agreement with previous study (Prince and Tseng, 1993), the firing threshold of neurons in chronic undercut cortex was not different from control (data not shown).

Increased duration of hyperpolarizing periods in the deafferented cortex

The occurrence of seizures during waking state has been related previously to the presence of periods of hyperpolarization during this state of vigilance, after cortical deafferentation (Nita et al., 2007). Therefore, we measured the duration of the hyperpolarizing events at mean membrane potential (Fig. 9-8) in control and in undercut cortex. To avoid disturbances caused by transient membrane potential fluctuations, we only took into account the hyperpolarizing periods lasting longer than 40 ms as in a previous study (Volgushev et al., 2006). The duration of hyperpolarizations was significantly increased in acute conditions compared to control ($p<0.05$, Student's T-test) and it continued to grow progressively at 2, 4 and 6 weeks after the undercut. When we compared the results obtained in chronically injured cortex to the acute group, they were statistically significant at 4 and 6 weeks ($p<0.05$, Student's T-test), nevertheless, when the comparison was made to the control group, then the difference was significant for all the three chronic groups (2, 4 and 6 weeks) (Fig. 9-8C).

The histograms of membrane potential computed for all IC recorded neurons after deafferentation revealed always a higher hyperpolarizing peak, in contrast to control neurons for which the depolarizing peak was higher, reflecting longer periods of hyperpolarization in the undercut vs. intact cortex. Furthermore, the histograms of neurons in the undercut cortex were constantly shifted towards a more hyperpolarized membrane potential (Fig. 9-8B). Similar results were presented formerly only for neurons recorded immediately after cortical trauma, in acute conditions (Topolnik et al., 2003a).

In order to test the assumption that the increased excitability in chronic undercut cortex is related to the decreased activity in the deafferented gyrus, we made correlations between the neuronal instantaneous firing rates in response to 1.0 nA current pulses and the duration of hyperpolarization in all tested groups. We found a significant correlation ($p<0.01$) at 4 and 6 weeks after the deafferentation, characterized at 4 weeks by the equation $y=0.1169x + 181.93$, an $R2=0.67$ and a global Pearson $r=0.82$, and at 6 weeks by the equation $y=0.1887x + 135.78$, an $R2=0.87$ and a global Pearson $r=0.86$ (Fig. 9-8D).

9.6 Discussion

In the present *in vivo* study, we demonstrated that following undercut, the cortical neuronal network undergoes reorganization leading to an overall increase in network excitability in chronic conditions. These changes are mediated by an increased connection probability, amplitude, rising phase of EPSPs, decreased c.v. and increased input resistance, as well as increased instantaneous firing rates. There were no significant changes in the efficacy of inhibitory synaptic relations.

The mechanisms accounting for seizure generation after an acute insult to the brain are probably different from those responsible for chronic hyperexcitability and epilepsy. We found an important boost of synaptic interactions, as well as an increased efficacy of excitatory connections starting with the second week after the brain injury, without any significant changes in acute conditions. These findings supports the fact that in humans, early administration of anticonvulsant medication decreases the percentage of early posttraumatic seizures but not that of chronic epilepsy (Temkin et al., 1990; Temkin et al., 1999; Chang and Lowenstein, 2003), since the mechanisms of the two are different. Moreover, our study evaluates the progressive transformation of synaptic interactions and intrinsic neuronal properties, therefore advancing the actual knowledge regarding the temporal window in which these synaptic changes occur and consequently the critical period during which antiepileptogenic prophylaxis may be attempted.

Early post-traumatic hyperexcitability (Topolnik et al., 2003b, a) is most likely generated by the dramatic alteration of neuronal milieu induced by the injury rather than by long-term anatomical changes of cortical networks. Previous studies suggest that the major activator of deleterious processes after neuronal injury is the increased Ca^{2+} influx (Choi, 1988) which might inhibit Na^{+}-K^{+} pump activity engendering further accumulation of Ca^{2+} intracellularly (Fukuda and Prince, 1992) while the extracellular Ca^{2+} decreases (Morris et al., 1991; Wolf et al., 2001) and this significantly increases the failure of synaptic transmission (Markram et al., 1998; Massimini and Amzica, 2001; Crochet et al., 2005; Seigneur and Timofeev, 2007). Accordingly, we found an increased failure rate of synaptic transmission in the acutely injured cortex compared to control.

Post-traumatic epilepsy arises probably from complex structural changes of neuronal networks (Lowenstein, 1996). The brain restores its activity after injury either by extensive reorganization of cortical connectivity such as axonal sprouting with formation of new synapses (Purpura and Housepian, 1961; Sutula et al., 1989; Salin et al., 1995; Dancause et al., 2005), by selective loss of inhibitory synapses (Ribak and Reiffenstein, 1982), or by increased synaptic and intrinsic neuronal responsiveness (Bush et al., 1999).

We found an increased efficacy of excitatory connections after cortical deafferentation characterized by an increased amplitude, decrease duration and rise time at 2 weeks and 6 weeks following trauma, but also an unexpected decreased amplitude after 4 weeks. This finding could be explained by the simultaneous massive increase in the failure rate of synaptic responses also seen 4 weeks after the injury and is also supported by the increased incidence of NI together with a decrease probability of NE at the same time. This matches very well with previous published data revealing a decreased number of ictal events from day 25 to day 40 after cortical undercut, followed by a subsequent increase of seizures to a stable rate (Nita et al., 2007). Our data follow the course of events also seen in humans, in which acute seizures are viewed as epiphenomena of the underlying brain disorder, with little independent impact on outcome from injury (Bladin et al., 2000), while late or remote seizures are thought to be the result of epileptogenesis following chronic changes of neural networks favoring excitation (Herman, 2002). In contrast to patients with early post-injury seizures, those with late-onset seizures are at greater risk of epilepsy (Bladin et al., 2000), probably because they express the already irreversible changes of neuronal network.

We then asked what could generate the increased amplitude of averaged responses at 2 and 6 weeks respectively, after cortical undercut. We envisaged several factors that could be incriminated individually or conjointly, such as modified reliability of synaptic transmission estimated as failure rate of postsynaptic responses, changes of intrinsic neuronal properties, reorganization of cortical connectivity, with formation of new synapses or revealing of otherwise unapparent excitatory connections. The failure rate was highest at 4 weeks, when the amplitude of responses was dramatically diminished and the number of failures decreased at 2 weeks, compared to acute conditions, which also was

consisted with increased amplitude of responses. We also observed that although the fluctuations of failure rate in the undercut cortex followed a similar trend to the amplitude of responses; the number of failures after the injury was much higher when compared to the intact cortex. Therefore, additional factors should have been responsible for the increased amplitude of averaged postsynaptic responses in the undercut cortex.

We found that neuronal input resistance in chronic conditions was increased, confirming previous findings obtained *in vitro* from chronically deafferented cortical neurons (Prince and Tseng, 1993; Tseng and Prince, 1996). In addition, we observed an increased number of spikes and instantaneous firing rate of neurons located in the traumatized cortex, supporting the hyperactivity of these neurons. Our results are also sustained by *in vitro* undercut models and by studies done in axotomized corticospinal neurons illustrating a steeper relationship between applied current and adapted spike firing frequency (f-I slope), therefore for the same inward current neurons would generate more action potentials (Prince and Tseng, 1993; Tseng and Prince, 1996). We assume that the increased neuronal firing in chronic conditions was a reflection of the increased input resistance which would make these neurons more responsive to excitatory events (Prince and Tseng, 1993; Jacobs et al., 2000), since we found no differences of action potential threshold, as also reported previously in the *in vitro* undercut model (Prince and Tseng, 1993). Interestingly, high values of input resistance have also been described in immature neocortical neurons, where they may be a factor that increases vulnerability to seizures (McCormick and Prince, 1987), and in surviving neurons from kainate-lesioned epileptogenetic hippocampi (Franck and Schwartzkroin, 1985; Franck et al., 1988).

The potential reorganization of axonal arbor of neocortical neurons has been demonstrated following a variety of injuries (Tsukahara et al., 1975; Villablanca et al., 1988; Kuang and Kalil, 1990). Cortical neuronal sprouting may also occur after direct trauma to the cortex (Maxwell et al., 1990; Fishman and Mattu, 1993) or in human pathological processes such as temporal lobe epilepsy (de Lanerolle et al., 1989; Sutula et al., 1989; Houser et al., 1990). In agreement with *in vitro* studies (Jin et al., 2006), we illustrate the increased efficacy of synaptic interactions following cortical injury, which could be the consequence of axonal sprouting. A progressively decreasing c.v. of

postsynaptic responses in injured cortex also correlated with a higher amplitude of individual responses, which appears to be the hallmark of strong synaptic connections (Berninger et al., 1999). The increased amplitude, together with the decreased duration and rise time of EPSPs following undercut could also be generated by increased presynaptic synchrony after cortical trauma, which boosts EPSPs and bi-synaptic IPSPs. Relatively small reduction in the efficacy of inhibition could lead to enlarged neuronal synchrony and to large expansion of activated cortical territory (Chagnac-Amitai and Connors, 1989; Prince and Tseng, 1993). For example, in the hippocampus, gradual blockade of GABAergic inhibition revealed excitatory connections between previously unconnected neurons (Miles and Wong, 1987).

Our results illustrate a progressive boost of connectivity and efficacy of excitatory connections following chronic cortical deafferentation, which is in agreement with a gradually increased propensity to seizures (Nita et al., 2006). Here, we show physiological consequences of axonal sprouting accompanying posttraumatic epileptogenesis (Salin et al., 1995).

The post-traumatic hyperexcitability of cortical neurons emerges probably from homeostatic synaptic plasticity, a mechanism that works to restore a stable pattern of activity whenever networks are perturbed (Turrigiano, 1999; Abbott and Nelson, 2000; Davis and Bezprozvanny, 2001). However, after prolonged periods of reduced activity, as it is the case of the deafferented suprasylvian gyrus, homeostatic synaptic plasticity may increase network excitability in an uncontrollable manner, leading to the development of paroxysmal activity (Houweling et al., 2005). Indeed, long-term activity blockade dramatically enhances cortical excitability and may lead to paroxysmal activity (Murthy et al., 2001; Burrone et al., 2002; Bausch et al., 2006). Here we demonstrate that longer periods of hyperpolarizing states correlate well with the increased firing rate starting one month after cortical deafferentation. Previous studies showed the occurrence of hyperpolarization periods during wake and REM sleep, when they don't normally appear, in chronic stages of the undercut (Nita et al., 2006). The same scenario is seen in clinical medical practice after severe head trauma that may produce cortical deafferentation, followed by seizures (Salazar et al., 1985). Early administration of antiepileptic drugs

known to enhance inhibition could not decrease the incidence of late epilepsy (Temkin, 2001) following cortical trauma and furthermore, antiepileptic drugs can exacerbate seizures (Perucca et al., 1998).

A better understanding of the role of homeostatic synaptic plasticity in generating post-traumatic epilepsy is needed, but if this hypothesis proves to be valid, then we can attempt to prevent seizure generation by augmenting cortical activity rather than decreasing it with antiepileptic medication.

9.7 Acknowledgements:

9.8 References

Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. NatNeurosci 3 Suppl:1178-1183.

Avramescu S, Timofeev I (2006) Synaptic synchrony modulation following cortical injury. Program No 23526/E6 2006 Neuroscience

Meeting Planner Atlanta, GA: Society for Neuroscience, 2006 Online.

Bausch SB, He S, Petrova Y, Wang XM, McNamara JO (2006) Plasticity of both excitatory and inhibitory synapses is associated with seizures induced by removal of chronic blockade of activity in cultured hippocampus. JNeurophysiol 96:2151-2167.

Berninger B, Schinder AF, Poo MM (1999) Synaptic reliability correlates with reduced susceptibility to synaptic potentiation by brain-derived neurotrophic factor. Learn Mem 6:232-242.

Bladin CF, Alexandrov AV, Bellavance A, Bornstein N, Chambers B, Cote R, Lebrun L, Pirisi A, Norris JW (2000) Seizures after stroke: a prospective multicenter study. Arch Neurol 57:1617-1622.

Burrone J, O'Byrne M, Murthy VN (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. Nature 420:414-418.

Bush PC, Prince DA, Miller KD (1999) Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. J Neurophysiol 82:1748-1758.

Chagnac-Amitai Y, Connors BW (1989) Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. JNeurophysiol 62:1149-1162.

Chang BS, Lowenstein DH (2003) Practice parameter: antiepileptic drug prophylaxis in severe traumatic brain injury: report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 60:10-16.

Chen Q, He S, Hu XL, Yu J, Zhou Y, Zheng J, Zhang S, Zhang C, Duan WH, Xiong ZQ (2007) Differential roles of NR2A- and NR2B-containing NMDA receptors in activity-dependent brain-derived neurotrophic factor gene regulation and limbic epileptogenesis. J Neurosci 27:542-552.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623-634.

Connors BW, Gutnick MJ (1990) Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci 13:99-104.

Contreras D, Steriade M (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. J Neurosci 15:604-622.

Contreras D, Timofeev I, Steriade M (1996) Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. J Physiol 494 (Pt 1):251-264.

Crochet S, Chauvette S, Boucetta S, Timofeev I (2005) Modulation of synaptic transmission in neocortex by network activities. Eur J Neurosci 21:1030-1044.

Dancause N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina EV, Stowe AM, Nudo RJ (2005) Extensive cortical rewiring after brain injury. J Neurosci 25:10167-10179.

Davis GW, Bezprozvanny I (2001) Maintaining the stability of neural function: a homeostatic hypothesis. AnnuRevPhysiol 63:847-869.

de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD (1989) Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res 495:387-395.

Desai NS, Rutherford LC, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. NatNeurosci 2:515-520.

Dinner DS (1993) Posttraumatic epilepsy. In: The treatment of epilepsy: principles (Wyllie E, ed), pp 654-658. Philadelphia: Lea & Fibinger.

Esplin MS, Abbott JR, Smart ML, Burroughs AF, Frandsen TC, Litzinger MJ (1994) Voltage-sensitive calcium channel development in epileptic DBA/2J mice suggests altered presynaptic function. Epilepsia 35:911-914.

Fishman PS, Mattu A (1993) Fate of severed cortical projection axons. J Neurotrauma 10:457-470.

Franck JE, Schwartzkroin PA (1985) Do kainate-lesioned hippocampi become epileptogenic? Brain Res 329:309-313.

Franck JE, Kunkel DD, Baskin DG, Schwartzkroin PA (1988) Inhibition in kainate-lesioned hyperexcitable hippocampi: physiologic, autoradiographic, and immunocytochemical observations. J Neurosci 8:1991-2002.

Fukuda A, Prince DA (1992) Excessive intracellular Ca^{2+} inhibits glutamate-induced $Na(^+)$-K^+ pump activation in rat hippocampal neurons. J Neurophysiol 68:28-35.

Graber KD, Prince DA (1999) Tetrodotoxin prevents posttraumatic epileptogenesis in rats. AnnNeurol 46:234-242.

Gray CM, McCormick DA (1996) Chattering cells: superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. Science 274:109-113.

Herman ST (2002) Epilepsy after brain insult: targeting epileptogenesis. Neurology 59:S21-26.

Hoffman SN, Salin PA, Prince DA (1994) Chronic neocortical epileptogenesis in vitro. JNeurophysiol 71:1762-1773.

Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV (1990) Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. J Neurosci 10:267-282.

Houweling AR, Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ (2005) Homeostatic synaptic plasticity can explain post-traumatic epileptogenesis in chronically isolated neocortex. CerebCortex 15:834-845.

Jacobs KM, Graber KD, Kharazia VN, Parada I, Prince DA (2000) Postlesional epilepsy: the ultimate brain plasticity. Epilepsia 41 Suppl 6:S153-S161.

Jin X, Huguenard JR, Prince DA (2005) Impaired Cl- extrusion in layer V pyramidal neurons of chronically injured epileptogenic neocortex. J Neurophysiol 93:2117-2126.

Jin X, Prince DA, Huguenard JR (2006) Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. J Neurosci 26:4891-4900.

Kuang RZ, Kalil K (1990) Specificity of corticospinal axon arbors sprouting into denervated contralateral spinal cord. J Comp Neurol 302:461-472.

Li H, Bandrowski AE, Prince DA (2005) Cortical injury affects short-term plasticity of evoked excitatory synaptic currents. J Neurophysiol 93:146-156.

Lowenstein DH (1996) Recent advances related to basic mechanisms of epileptogenesis. Epilepsy Res Suppl 11:45-60.

Markram H, Wang Y, Tsodyks M (1998) Differential signaling via the same axon of neocortical pyramidal neurons. Proc Natl Acad Sci U S A 95:5323-5328.

Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275:213-215.

Massimini M, Amzica F (2001) Extracellular calcium fluctuations and intracellular potentials in the cortex during the slow sleep oscillation. J Neurophysiol 85:1346-1350.

Matsumura M, Chen D, Sawaguchi T, Kubota K, Fetz EE (1996) Synaptic interactions between primate precentral cortex neurons revealed by spike-triggered averaging of intracellular membrane potentials *in vivo*. J Neurosci 16:7757-7767.

Maxwell WL, Follows R, Ashhurst DE, Berry M (1990) The response of the cerebral hemisphere of the rat to injury. I. The mature rat. Philos Trans R Soc Lond B Biol Sci 328:479-500.

McCormick DA, Prince DA (1987) Post-natal development of electrophysiological properties of rat cerebral cortical pyramidal neurones. J Physiol 393:743-762.

McKinney RA, Debanne D, Gahwiler BH, Thompson SM (1997) Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of posttraumatic epilepsy. Nat Med 3:990-996.

Miles R, Wong RK (1987) Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. J Physiol 388:611-629.

Morris ME, Leblond J, Agopyan N, Krnjevic K (1991) Temperature dependence of extracellular ionic changes evoked by anoxia in hippocampal slices. J Neurophysiol 65:157-167.

Murthy VN, Schikorski T, Stevens CF, Zhu Y (2001) Inactivity produces increases in neurotransmitter release and synapse size. Neuron 32:673-682.

Nita DA, Cisse Y, Timofeev I, Steriade M (2006) Increased propensity to seizures after chronic cortical deafferentation *in vivo*. JNeurophysiol 95:902-913.

Nita DA, Cisse Y, Timofeev I, Steriade M (2007) Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. Cereb Cortex 17:272-283.

Perucca E, Gram L, Avanzini G, Dulac O (1998) Antiepileptic drugs as a cause of worsening seizures. Epilepsia 39:5-17.

Prince DA, Tseng GF (1993) Epileptogenesis in chronically injured cortex: in vitro studies. JNeurophysiol 69:1276-1291.

Purpura DP, Housepian EM (1961) Morphological and physiological properties of chronically isolated immature neocortex. Exp Neurol 4:377-401.

Ribak CE, Reiffenstein RJ (1982) Selective inhibitory synapse loss in chronic cortical slabs: a morphological basis for epileptic susceptibility. Can J Physiol Pharmacol 60:864-870.

Rudolph M, Pospischil M, Timofeev I, Destexhe A (2007) Inhibition determines membrane potential dynamics and controls action potential generation in awake and sleeping cat cortex. J Neurosci 27:5280-5290.

Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, Dillon JD (1985) Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. Neurology 35:1406-1414.

Salin P, Tseng GF, Hoffman S, Parada I, Prince DA (1995) Axonal sprouting in layer V pyramidal neurons of chronically injured cerebral cortex. JNeurosci 15:8234-8245.

Seigneur J, Timofeev I (2007) Extracellular calcium depletion associated with low-threshold calcium spikes controls presynaptic mediator release in thalamus. Program No 8274/KK10 2007 Neuroscience Meeting Planner San Diego, CA: Society for Neuroscience, 2007 Online.

Steriade M, Nuñez A, Amzica F (1993) A novel slow (<1 Hz) oscillation of neocortical neurons *in vivo* : depolarizing and hyperpolarizing components. J Neurosci 13:3252-3265.

Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 85:1969-1985.

Steriade M, Timofeev I, Durmuller N, Grenier F (1998) Dynamic properties of corticothalamic neurons and local cortical interneurons generating fast rhythmic (30-40 Hz) spike bursts. J Neurophysiol 79:483-490.

Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L (1989) Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol 26:321-330.

Temkin NR (2001) Antiepileptogenesis and seizure prevention trials with antiepileptic drugs: meta-analysis of controlled trials. Epilepsia 42:515-524.

Temkin NR, Dikmen SS, Wilensky AJ, Keihm J, Chabal S, Winn HR (1990) A randomized, double-blind study of phenytoin for the prevention of post-traumatic seizures. N Engl J Med 323:497-502.

Temkin NR, Dikmen SS, Anderson GD, Wilensky AJ, Holmes MD, Cohen W, Newell DW, Nelson P, Awan A, Winn HR (1999) Valproate therapy for prevention of posttraumatic seizures: a randomized trial. J Neurosurg 91:593-600.

Thomson AM, West DC, Hahn J, Deuchars J (1996) Single axon IPSPs elicited in pyramidal cells by three classes of interneurones in slices of rat neocortex. J Physiol 496 (Pt 1):81-102.

Topolnik L, Steriade M, Timofeev I (2003a) Hyperexcitability of intact neurons underlies acute development of trauma-related electrographic seizures in cats in vivo. Eur J Neurosci 18:486-496.

Topolnik L, Steriade M, Timofeev I (2003b) Partial cortical deafferentation promotes development of paroxysmal activity. CerebCortex 13:883-893.

Trasande CA, Ramirez JM (2007) Activity deprivation leads to seizures in hippocampal slice cultures: is epilepsy the consequence of homeostatic plasticity? J Clin Neurophysiol 24:154-164.

Tseng GF, Prince DA (1996) Structural and functional alterations in rat corticospinal neurons after axotomy. J Neurophysiol 75:248-267.

Tsukahara N, Hultborn H, Murakami F, Fujito Y (1975) Electrophysiological study of formation of new synapses and collateral sprouting in red nucleus neurons after partial denervation. J Neurophysiol 38:1359-1372.

Turrigiano GG (1999) Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. Trends Neurosci 22:221-227.

Villablanca JR, Fomez-Pinilla F, Sonnier BJ, Hovda DA (1988) Bilateral pericruciate cortical innervation of the red nucleus in cats with adult or neonatal cerebral hemispherectomy. Brain Res 453:17-31.

Volgushev M, Chauvette S, Mukovski M, Timofeev I (2006) Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations [corrected]. J Neurosci 26:5665-5672.

Waters J, Helmchen F (2006) Background synaptic activity is sparse in neocortex. J Neurosci 26:8267-8277.

Wolf JA, Stys PK, Lusardi T, Meaney D, Smith DH (2001) Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. J Neurosci 21:1923-1930.

9.9 Figures
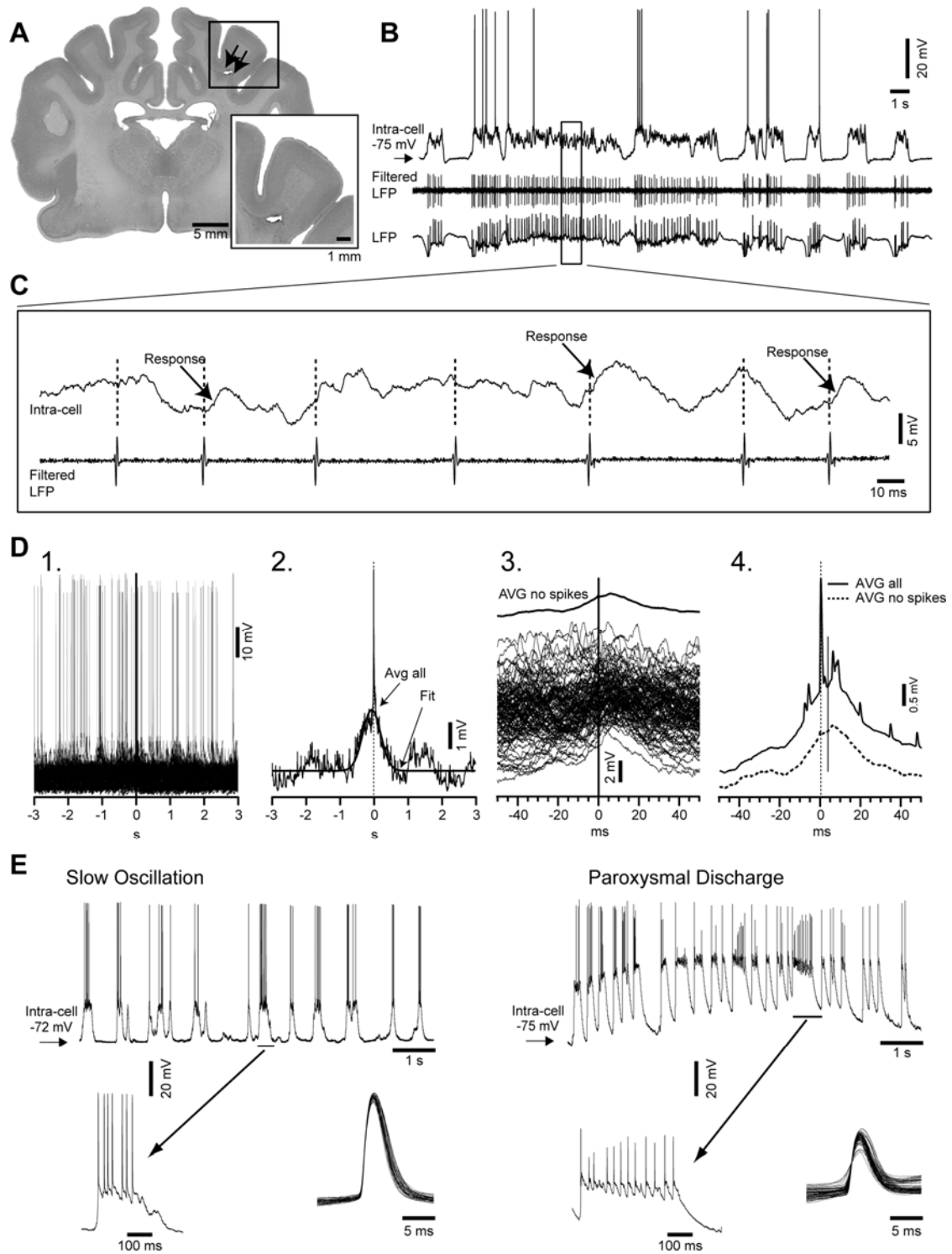
Figure 9-1. Experimental paradigm and methodology of analysis. A) A frontal section of cat brain (Nissl staining) depicting the large transection of the white matter under the suprasylvian gyrus (expanded in inset). Undercut is indicated by arrows. B) Simultaneous intracellular, local field potential (LFP) and unit activity, in cortical area 7 left, in a ketamine-xylazine anesthetized cat. Area designated with a rectangle is expanded in C. C) Intracellular recording (upper trace) and filtered local field potential (bottom trace). Different responses related with unit activity (filtered LFP) are indicated in the recorded neuron. D) 1. Superimposition of multiple segments (n=140) triggered by the spikes in the extracellularly recorded neuron. 2. Spike-triggered average (n=140) of intracellular segments (AVG all) fitted with a Gaussian function (Fit). 3. Panel illustrates all segments without action potentials triggered by the extracellular spikes (n=125), extracted from the intracellular recorded neuron, together with their average (AVG no spikes). 4. The average of all intracellular segments (AVG all) and the average of the segments without action potentials triggered by the presynaptic neuron (AVG no spikes). E) Intracellular recording of the same neuron during slow oscillation period (left panel) and during paroxysmal discharge (right panel). Periods indicated by horizontal bars are expanded below. Bottom right insets show superimposition of all action potentials detected during depicted periods of slow oscillation and paroxysmal discharge. Note the smaller amplitude and the higher variability of the action potentials during paroxysmal discharge periods as compared to the slow oscillation.
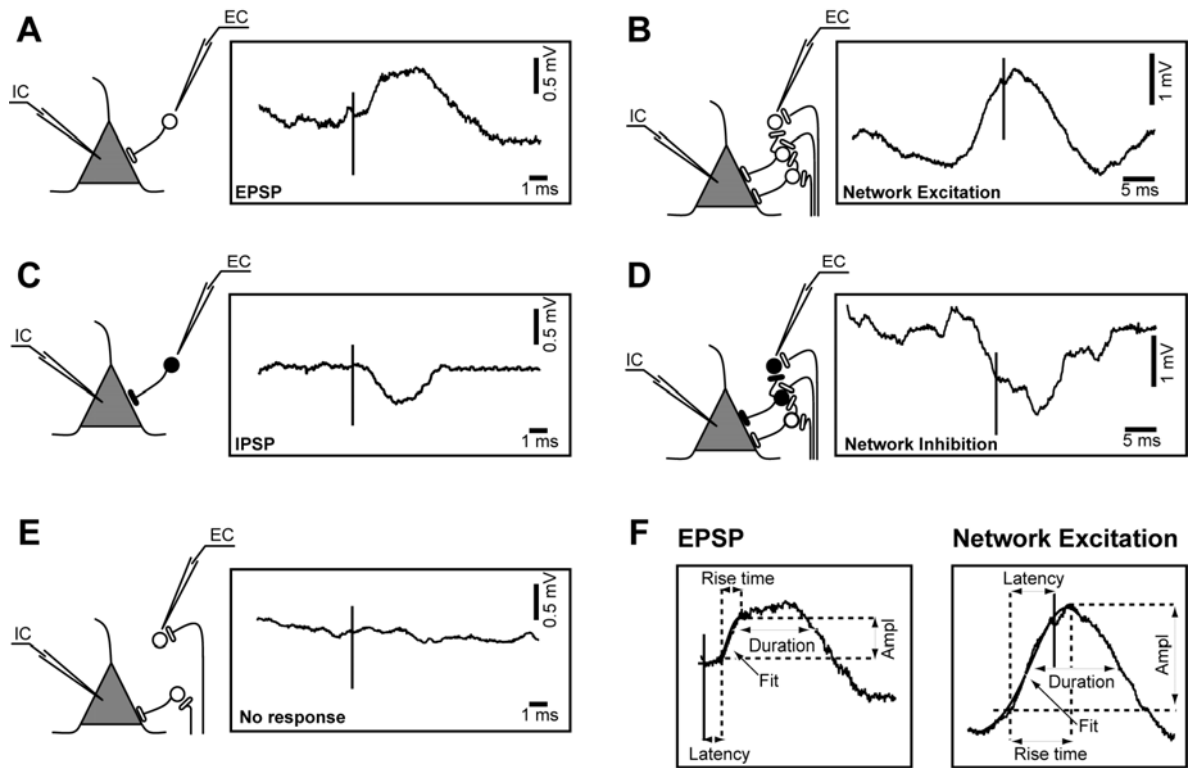
Figure 9-2. Different types of responses and possible designs of synaptic topology. Direct synaptic connections are depicted in panels A and C. Panels contain the scheme of a possible synaptic circuit triggering the average excitatory postsynaptic potential (EPSP, n=145), or inhibitory postsynaptic potential (IPSP, n=125). Pyramidal-shaped unit depicts the cell which was recorded intracellularly (IC); the round-shaped unit symbolizes the cell recorded extracellularly (EC). Open circle and buttons designate excitatory neurons respectively synapses, while the black ones the inhibitory neurons and synapses. Panels B and D illustrate synchronous network connections; network excitation (NE, n=178) and network inhibition (NI, n=131). Panel E depicts the non-responses (No response, n=155). Panel F schematize the methodology used for the quantification of responses. "Ampl" stands for amplitude, "Fit" stands for sigmoid fitting.
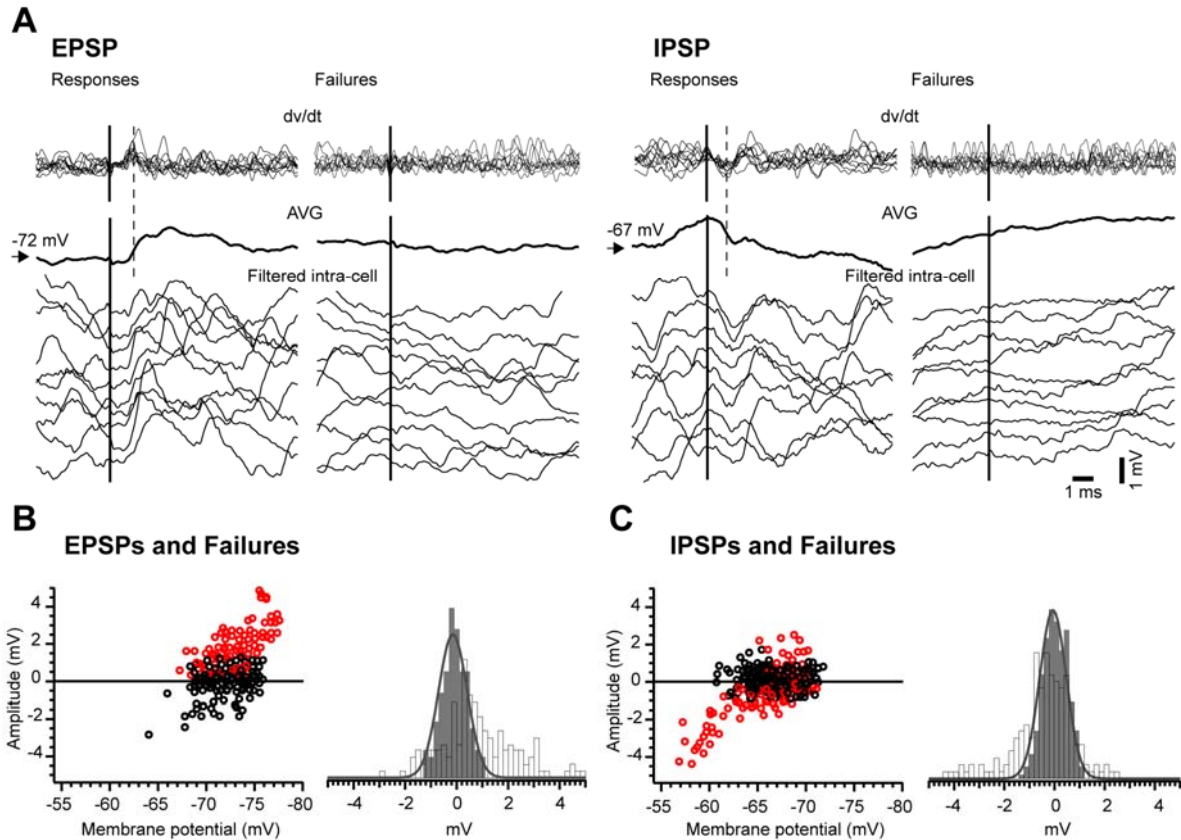
Figure 9-3. Examples of responses and failures in direct synaptic connections. A) Top panels contain the superimposition of the first derivative of the individual segments depicted as examples in the bottom panels. The middle panels depict the averages of all postsynaptic responses obtained from the pair of recorded neurons. Bottom panels show 10 individual examples of successful excitatory (left) and inhibitory (right) connection, together with examples of failures recorded in the same postsynaptic neuron. B) Amplitude of excitatory responses (open red circles) and failures (open black circles) plotted against the membrane potential and histograms of the amplitude of responses (open bars) and background noise (grey bars) in the excitatory connection topology. C) Amplitude of inhibitory responses (open red circles) and failures (open black circles) plotted against the membrane potential and histograms of the amplitude of responses (open bars) and network noise (gray bars) in the inhibitory connection topology. Noise histograms were fitted using a Gaussian function (plain grey line superimposed on grey histograms). Note the wider distribution to the right of the excitatory responses and of inhibitory responses to the left, compared with the network noise amplitude. The bin of the histogram is 0.2 mV.
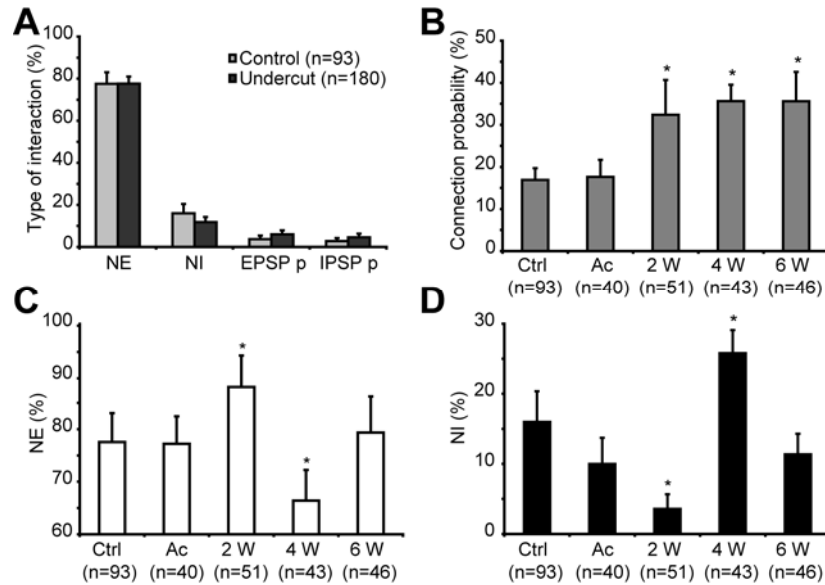
Figure 9-4. Changes of synaptic interactions following cortical deafferentation. A) Variation of the type of interactions: synchronous responses (NE and NI) and direct pure EPSP (EPSP p) and pure IPSP (IPSP p) connections in control (light grey bars) and undercut cortex (dark grey bars). Note that NE was the most frequent type of response both in control and in the undercut cortex. B) Variation of connection probability (only EPSPs and IPSPs were taken into account) at different time delays after cortical injury. Note the increased connectivity in chronic undercut cortex. C) The incidence of NE and D) NI in control and following cortical deafferentation. Ctrl – Control, Ac – Acute, 2W – 2 Weeks, 4W – 4 Weeks, 6W – 6 Weeks. "n" represents the total number of responses recorded in each group. Error bars indicate standard error of mean (s.e.m.). * $p < 0.05$, Mann-Whitney.

A EPSP
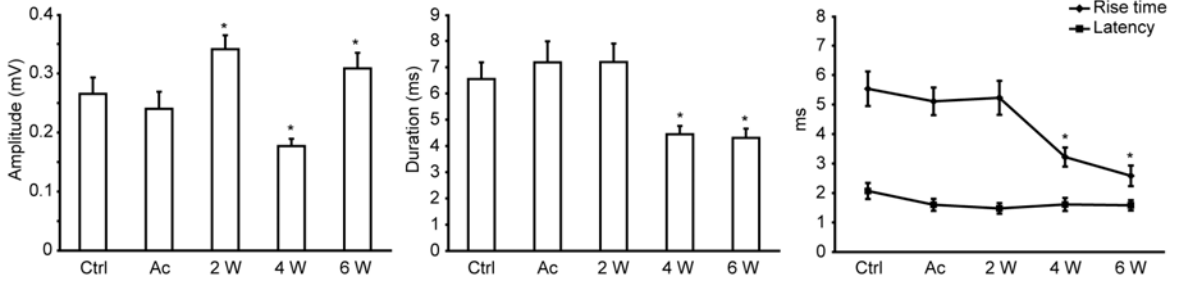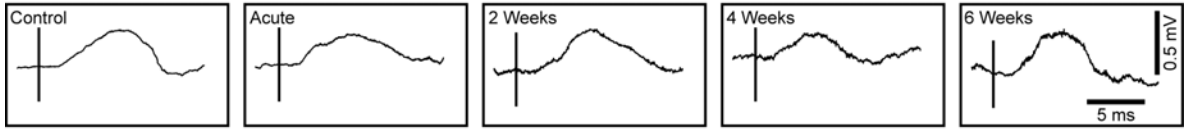
B
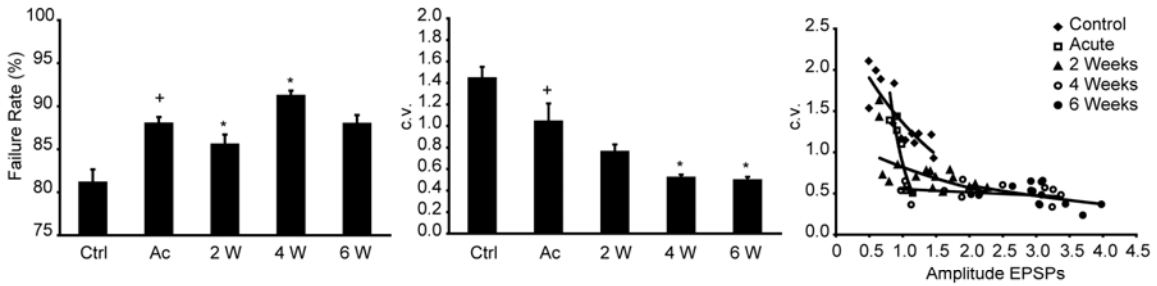
C NE
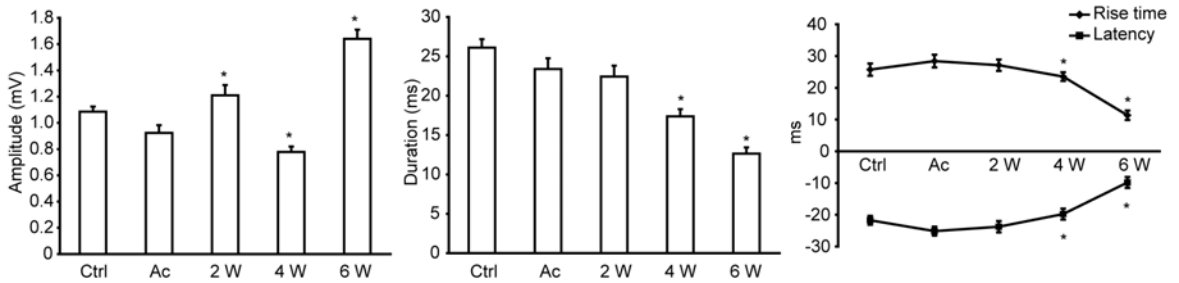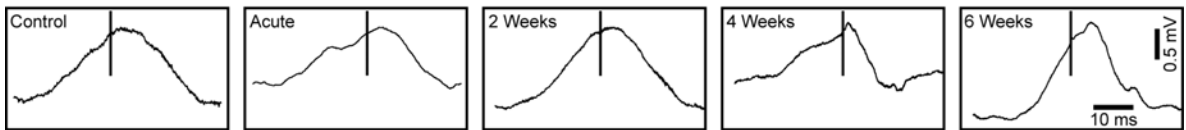
Figure 9-5. Modulation of synaptic interactions in acute and chronically deafferented cortex. A) Upper panel depicts examples of averaged postsynaptic responses for the direct synaptic connection (EPSP) in control and in undercut cortex. Lower panel shows the variation in amplitude, duration, rise time and latency of EPSPs in different conditions. B) Variation of the failure rate (left panel) and the dynamics of the coefficient of variation (c.v.) (middle panel) in control and following undercut. The right panel represents the plot of the coefficient of variation vs. EPSPs amplitude in control and in injured cortex. The values were fitted with an exponential function. Note the smallest values of c.v. at 4 and 6 weeks following undercut, supporting the increased synaptic strength. C) Upper panel depicts examples of averaged postsynaptic responses for the synchronous excitatory synaptic connections (NE) in control and in undercut cortex. Lower panel shows the variation in amplitude, duration, rise time and latency of NEs in different conditions. Ctrl – Control, Ac – Acute, 2W – 2 Weeks, 4W – 4 Weeks, 6W – 6 Weeks. Error bars indicate standard error of mean (s.e.m.). + p<0.05, Student's T-test (control vs. acute); * p<0.05, Student's T-test (acute vs. 2, 4 and 6 weeks respectively after undercut).
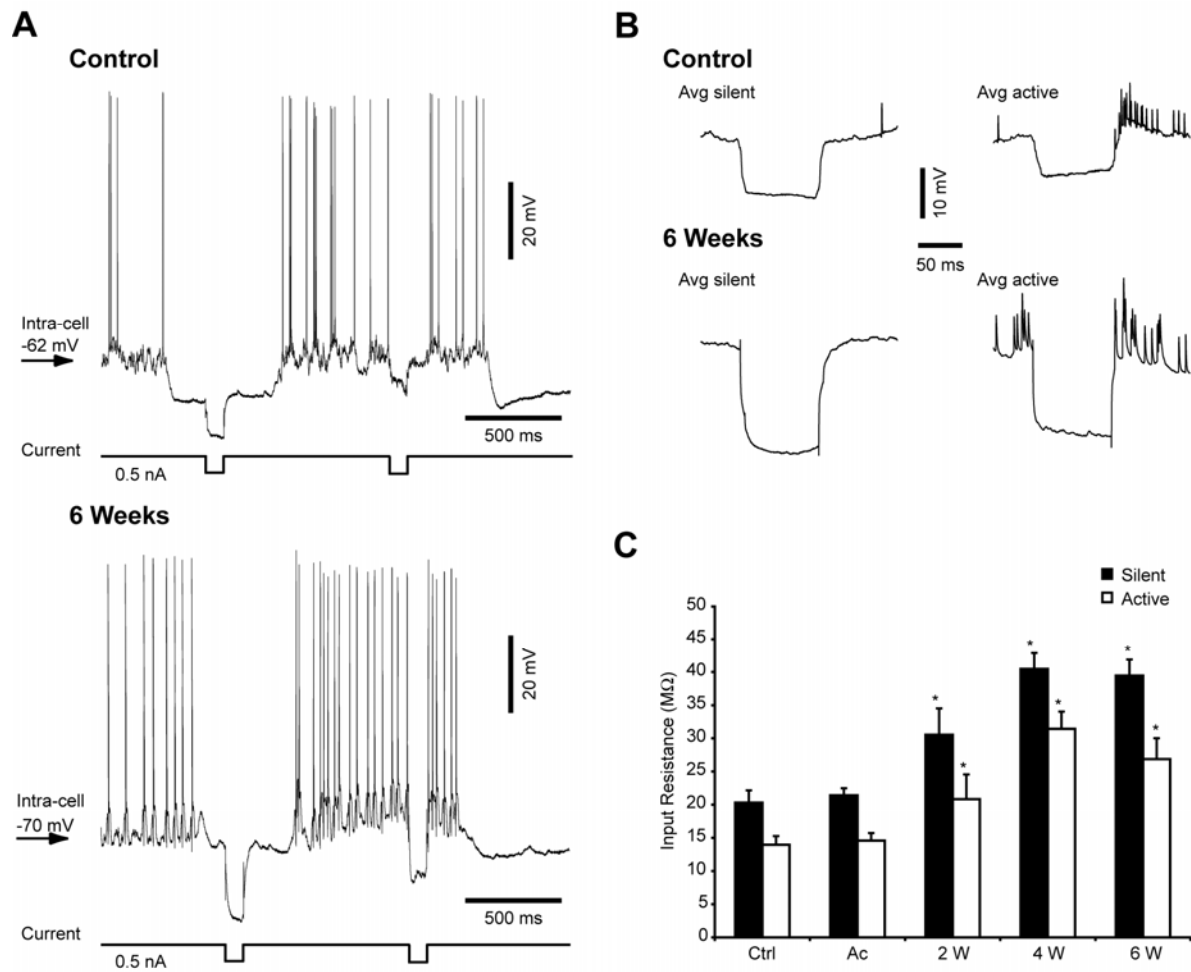
Figure 9-6. Increased neuronal input resistance in chronically undercut cortex. A) Examples of intracellular recordings of a RS neuron together with the corresponding EEG during intracellularly applied hyperpolarizing current pulses in control (upper panel) and 6 weeks (bottom panel) following cortical injury. B) Averages (Avg) of voltage responses to 5 trials of hyperpolarizing current (0.5 nA, 0.1 s) during each state of the network (silent and active). The averages correspond to the neurons depicted in A). C) Increased input resistance both during silent (black bars) and active (open bars) phase of the slow-oscillation in chronically injured cortex. Ctrl – Control, Ac – Acute, 2W – 2 Weeks, 4W – 4 Weeks, 6W – 6 Weeks. Bars represent the mean input resistance from 10 different neurons for each group. Error bars indicate standard error of mean (s.e.m.). * $p < 0.05$, Student's T-test.
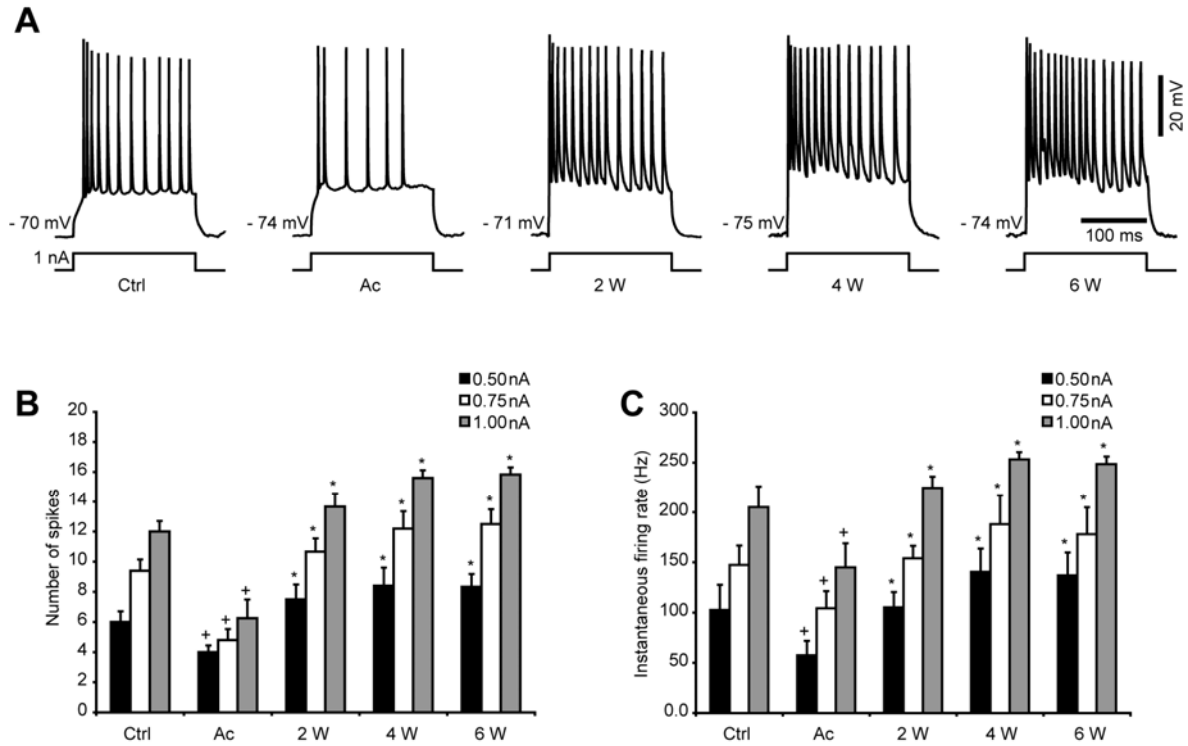
Figure 9-7. Progressive increase neuronal excitability following cortical undercut. A) Responses to depolarizing current pulses of RS neurons from control and undercut cortex. The membrane potential is indicated for each example. B) Number of spikes recorded in RS neurons in response to depolarizing current pulses of increasing intensity (0.5 nA in black, 0.75 nA in white, 1.0 nA in grey). C) Instantaneous firing rate (reciprocal of the first inter-spikes interval) in response to depolarizing current (0.5 nA in black, 0.75 nA in white, 1.0 nA in grey). Note the decline of both number of spikes and instantaneous firing rate immediately following injury, followed by the progressive increase in excitability at 2, 4 and 6 weeks after trauma. Ctrl – Control, Ac – Acute, 2W – 2 Weeks, 4W – 4 Weeks, 6W – 6 Weeks. Error bars indicate standard error of mean (s.e.m.). + $p < 0.05$, Student's T-test (control vs. acute); * $p < 0.05$, Student's T-test (acute vs. 2, 4 and 6 weeks respectively after undercut).
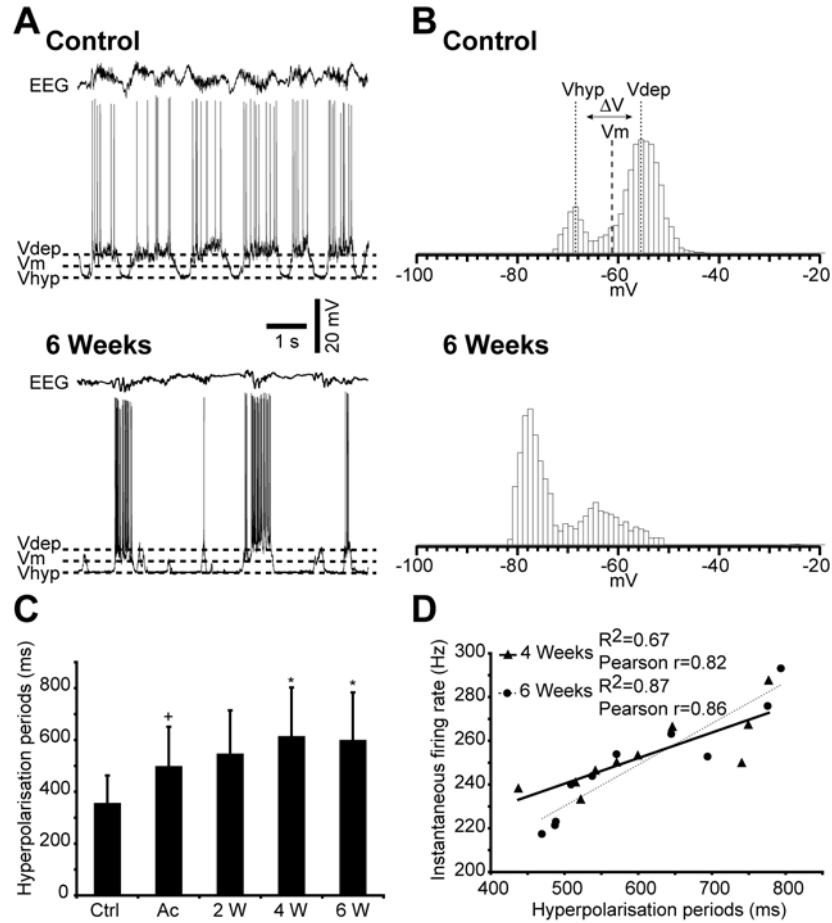
Figure 9-8. Increased duration of hyperpolarizing periods following cortical trauma. A) Intracellular recordings in control and 6 weeks after undercut, together with the corresponding EEG. Dashed lines indicate the membrane potential of the active (Vdep) and silent (Vhyp) phases of the slow-oscillation, as well as the mean membrane potential (Vm). B) Histograms of the cellular membrane potential corresponding to the intracellular recordings in A). The upper panel depicts also how the Vdep, Vhyp and Vm were calculated. The histograms were computed for 10 s duration, with a bin of 1mV. C) Mean duration of hyperpolarizing periods in control and following undercut (n=10 in each group), measured during 10 s for each neuron taken into account. Ctrl – Control, Ac – Acute, 2W – 2 Weeks, 4W – 4 Weeks, 6W – 6 Weeks. Error bars indicate standard error of mean (s.e.m.). + $p < 0.05$, Student's T-test (control vs. acute); * $p < 0.05$, Student's T-test (acute vs. 2, 4 and 6 weeks respectively after undercut). D) Correlations between the instantaneous firing rate (y axis) and the duration of hyperpolarization periods (x axis) at 4 weeks (triangles, correlation line solid) and at 6 weeks (circles, correlation line dotted) following cortical undercut. The coefficient of correlation R^2 and the global Pearson coefficient (r) are indicated on the graph.

10 Neocortical post-traumatic epileptogenesis is associated with loss of GABAergic neurons

Sinziana Avramescu, Dragos A. Nita and Igor Timofeev

10.1 Résumé

Les mécanismes subtils de l'épileptogenèse post-traumatique demeurent encore inconnus bien que l'incidence des épilepsies chroniques suite à des traumatismes corticaux pénétrants est très haute. Nous nous sommes demandé si les changements dans l'équilibre entre le nombre de neurones excitateurs et inhibiteurs pourraient expliquer la plus grande fréquence de crises épileptiques observées chez des chats ayant subit une déafférentation partielle du gyrus suprasylvien, pour un délai de moins de 6 semaines. Nous avons marqué tous les neurones corticaux avec des anticorps contre la protéine nucléaire neuronale spécifique (NeuN) et seulement les neurones inhibiteurs GABAérgiques avec soit des anticorps contre l'acide gamma-aminobutyrique (GABA) ou contre la décarboxylase de l'acide glutamique (GAD 65&67) sur des sections de cerveau obtenues à partir des animaux contrôles et épileptiques avec une déafférentation chronique du gyrus suprasylvien. Les quantifications des neurones marqués ont été effectués sur des tranches contrôles et sur des tranches provenant de cerveaux d'animaux sacrifiés à 2, 4 et 6 semaines après la déafférentation corticale, dans les gyri suprasylvien et marginal, ipsi- et contra-latéral au traumatisme. Chez tous les animaux épileptiques la perte neuronale a été limitée au niveau du gyrus suprasylvien. Les neurones inhibiteurs GABAérgiques ont été particulièrement plus sensibles à la déafférentation corticale que les neurones excitateurs, menant à une augmentation progressive du rapport entre l'excitation et l'inhibition, ce qui pourrait expliquer la fréquence augmentée des crises épileptiques dans le cortex chroniquement déafferenté.

10.2 Abstract

The subtle mechanisms of post-traumatic epileptogenesis remain unknown although the incidence of chronic epilepsy after penetrating cortical wounds is very high. Here, we investigated whether a change in the balance between the number of excitatory and inhibitory neurons could explain the increased frequency of seizures occurring within 6 weeks following partial deafferentation of the suprasylvian gyrus in cats. Immunohistochemical labeling of all neurons with neuronal-specific nuclear protein (NeuN) antibody, and of the GABAergic inhibitory neurons with either gamma-aminobutyric acid (GABA) or glutamic acid decarboxylase (GAD 65&67) antibodies, was performed on sections obtained from control and epileptic animals with chronically deafferented suprasylvian gyrus. Quantifications of labeled neurons were performed in control and at 2, 4 and 6 weeks following cortical deafferentation, in the suprasylvian and marginal gyri, both ipsi- and contra-lateral to the cortical trauma. In all epileptic animals the neuronal loss was circumscribed to the deafferented suprasylvian gyrus. Inhibitory GABAergic neurons were particularly more sensitive to cortical deafferentation than excitatory ones, leading to a progressively increasing ratio between excitation and inhibition towards excitation, which might explain the increased propensity to seizures in chronic undercut cortex.

10.3 Introduction

Traumatic head injuries are frequently followed by a progressive increase in the neuronal excitability, finally leading to seizures and epilepsy both in animal models (D'Ambrosio, et al., 2004, McKinney, et al., 1997, Nita, et al., 2006, Nita, et al., 2007, Topolnik, et al., 2003a) and in humans (Dinner, 1993, Salazar, et al., 1985). Normal brain function relies on a fine balance between excitation and inhibition, which could easily be disrupted following injury. Therefore, a reduced inhibition is thought to be particularly involved in the pathophysiology of epilepsy (Bernard, et al., 2000, Sloviter, 1987). A reduction of inhibition could result either from a loss of inhibitory synapses (Bloom and Iversen, 1971, Ribak, et al., 1982, Ribak and Reiffenstein, 1982), from alterations in GABA receptors (Bianchi, et al., 2002, Wallace, et al., 2001), or from a decreased number of GABAergic neurons (Buckmaster and Jongen-Relo, 1999, Dinocourt, et al., 2003, Hendry and Jones, 1986).

Several studies reported that GABAergic neurons might be selectively vulnerable to various injuries such as hypoxia (Romijn, et al., 1988, Sloper, et al., 1980), epilepsy induced by convulsive agents (Obenaus, et al., 1993, Ribak, et al., 1982), excessive electrical stimulation (Sloviter, 1987, Sloviter, 1992), and neocortical isolations (Ribak and Reiffenstein, 1982). On the other hand, some studies suggested that GABAergic neurons are selectively spared following some other insults (Mathern, et al., 1995, Tecoma and Choi, 1989). Nevertheless, the fact that epilepsy may be treated using drugs that enhance GABA receptor mediated inhibition (Fueta, et al., 2005, Yamauchi, et al., 2006) and that seizures can be triggered with GABA receptor blockers, such as penicillin and bicuculline (Karlsson, et al., 1992), suggests that altered inhibition might represent an important pathogenetic mechanism of chronic epileptogenesis.

Anatomical studies showed that the inhibitory GABA system is remarkably plastic and can be up- or down- regulated under conditions such as deafferentation or excessive stimulation (Hendry, et al., 1990, Hendry and Jones, 1988, Micheva and Beaulieu, 1995). This indicates that there also might be temporal variations of inhibition during the development of a chronic epileptogenic lesion that would give quite different results at two time points (Franck, et al., 1988, Franck and Schwartzkroin, 1985, Sloviter, 1992,

Whittington and Jefferys, 1994). Therefore, it is essential to study the ratio between excitation and inhibition at several different time delays following an injury that could promote cortical hyperexcitability and epilepsy.

In this study we used the model of partially isolated suprasylvian gyrus (Nita, et al., 2006, Nita, et al., 2007, Topolnik, et al., 2003a, Topolnik, et al., 2003b) to reveal anatomical changes that might explain the increased frequency of seizures observed in cats following cortical undercut. We hypothesized that chronic deafferentation triggers major cortical reorganization and possibly a shift in the balance excitation-inhibition towards excitation. This would promote the epileptogenetic mechanisms which might explain the high rate of epilepsy observed in patients with severe head trauma, and also the progressive nature of this process.

Some parts of the present data have been previously reported in an abstract form (Avramescu, et al., 2007).

10.4 Materials and methods

Animal preparation

Experiments were performed on 20 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 sodium thiopental anesthesia (30 mg/kg i.v.). The level of anesthesia was continuously monitored by EEG, heart rate, oxygen saturation of the arterial blood (aiming over 90%), and end-tidal CO_2 (~3.5%). General surgical procedures included: cephalic vein cannulation for systemic liquid delivery (lactated Ringer's solution 5-10 ml/kg/h) and lidocaine (0.5%) was used for infiltration of all pressure points or incision lines. Body temperature was maintained at 37–39°C using a heating pad. All the substances used in our experiments were provided by Sigma-Aldrich, Canada, unless otherwise specified.

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. A custom-designed knife was inserted in the posterior part of suprasylvian gyrus perpendicular to its surface for a depth of ~ 3 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 (see Fig. 10-1A). 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 received analgesic medication (anafen 2 mg/kg s.c.) for the next 72 hours.

Electrophysiological recordings

Field potential recordings in chronic conditions were obtained by means of concentric bipolar electrodes (Frederick Haer & Co, ME, USA) (see Fig. 10-1B), under ketamine & xylazine anesthesia (10-15 mg/kg and 2-3 mg/kg, respectively, i.m.), muscle paralysis with gallamine triethiodide (20mg/kg, i.v.), and artificial ventilation (20-30 cycles/min) seeking

an end-tidal CO_2 concentration around 3.5% (±0.4%). The craniotomy holes exposed the cerebral cortex and allowed the insertion of recording electrodes. Body temperature was maintained at 37–39°C and glucose (5% solution) was administered i.v. every 3–4 hours during experiments.

All experimental procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care, of NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Committee for Animal Care of Laval University. All efforts were made to minimize the number of animals used and their suffering.

Tissue preparation

The animals were sacrificed 2, 4 and 6 weeks respectively, after the initial trauma (W2 n = 5, W4 n = 5, W6 n = 5), while control animals had no cortical deafferentation. Briefly, animals were deeply anesthetized using a combination of anesthetics containing ketamine (60 mg/kg), xylazine (8.6 mg/ml) and acepromazine (0.9 mg/kg) and then perfused with normal saline followed by a fixative solution containing 4% paraformaldehyde, 0.05% glutaraldehyde in 0.1M PBS (pH 7.4). After perfusion, brains were removed and stored in 30% sucrose in PBS for cryoprotection. Coronal brain slices were sectioned with a freezing microtome (Jung Histoslide 2000, Leica, Germany) at 80 μm, through the middle of the suprasylvian gyri (7-10 mm posterior to the anterior limit of the gyrus). Sections obtained from control animals, 2W, 4W and 6W groups were then either processed for immunohistochemistry (see below) or stained with thionine (Nissl staining) to ascertain the extension of the undercut and to visualize the general cortical cytoarchitecture.

Immunohistochemistry

Free-floating sections were first incubated for 30 min in 1% H_2O_2 and 50% ethanol to inhibit endogenous peroxidase activity, than washed with PBS. Non-specific staining was minimized by incubating slices in 3% normal goat serum (NGS) (Vector Laboratories, Burlingame, CA, USA) for 1h at room temperature. Primary antibodies were diluted in 3% NGS and the sections were incubated with primary antibodies for 48 hours at 4°C. We assured a better penetration of the antibody by using 0.3% Triton both during pre-

incubation with the NGS and during incubation with the primary antibody. Anti-NeuN antibody was selected as a neuronal marker to reveal differences in the overall number of neurons among control and chronically deafferented cortical areas, while GABAergic neuronal populations were marked using anti-GAD65&67 or anti-GABA antiserum. After incubation with mouse Anti-NeuN antibody (dilution: 1:200, Chemicon, Temecula, CA, USA), sections were rinsed with PBS and incubated with their respective peroxidase conjugated secondary antibody (goat anti-mouse IgG; Chemicon, Temecula, CA, USA) for 2h, at a dilution of 1:200. Finally, sections were incubated in avidin-biotin complex (ABC, Vector Laboratories, Burlingame, CA, USA) for 2h and the reaction was revealed using 3–3'-diaminobenzidine (DAB) as a chromogen in the presence of nickel ammonium sulphate and cobalt chloride. Subsequently, sections marked with NeuN were double labeled with rabbit anti-GAD65&67 antiserum (dilution: 1:1500; Chemicon, Temecula, CA, USA) or rabbit anti-GABA antiserum (dilution 1:120; Chemicon, Temecula, CA, USA), after re-blocking against non-specific staining and peroxidase inactivation. We used both GAD/NeuN and GABA/NeuN labeling in all animal included in the study. Following the incubation with the biotinylated secondary antibody (biotinylated goat anti-rabbit IgG; dilution of 1:1500; Chemicon, Temecula, CA, USA) for 2h, sections were incubated in avidin-biotin complex (ABC, Vector Laboratories, Burlingame, CA, USA) for 2 h, and developed using DAB as a chromogen. Doubled-stained neurons were visualized as brown-colored cytoplasmic bodies (due to GAD65&67 or GABA reactivity) with black-blue nuclei (due to NeuN reactivity) (see Fig. 10-3B). In each experiment some sections were incubated without the primary antibody to determine staining specificity (negative controls). Several sections were labeled only with anti-GAD65&67, or anti-GABA, or NeuN antibodies (see Fig. 10-3A). Sections were mounted onto gelatin-coated slides, dehydrated in graded ethanol (50%, 70%, 90%, 95%, 100%), cleared in xylene and coverslipped for storage and analysis.

Image analysis

Sections were examined using a light microscope (Olympus BX61) equipped with the appropriate light sources (Olympus BX-UCB). Bright field images were acquired using a color CCD camera (Optronics, Goleta, CA, USA), a motorized stage (MAC5000, Ludl

Electronic Products, Exton, PA, USA), and a computer system equipped with Neurolucida software (V8.0, MicroBrightField, Williston, VT, USA) and Image-Pro Plus (V6.2, MediaCybernetics, Bethesda, MD, USA). Architectonic boundaries of the suprasylvian gyrus were determined using Nissl-stained sections anterior and posterior to the immunostained sections. The regions of interest were outlined, and double-labeled (NeuN/GAD, NeuN/GABA) or single-labed (NeuN) neurons were counted using Image-Pro Plus software. Staining was defined after thresholding for intensity, objects of interests were identified using predefined ranges of image analysis parameters from the Image-Pro Plus menu (such as mean density, area, and roundness); and parameters were equally applied to Z-stack images acquired at 5 μm through the thickness of the slices obtained from cortical layer V (Fig 10-6A). Parameters were adjusted to obtain optimal sensitivity and specificity, by testing them on single-labeled slices. In addition to the absolute densities of neurons we chose to illustrate neuronal densities as percentages relative to the values obtained from control for easier comparison, and to eliminate the possible underestimation of GABAergic neurons due to documented difficult penetration of GABA antibodies (Broman and Westman, 1988) or errors in the automatic detection. All double stained cells were considered inhibitory GABAergic neurons. We calculated the density of excitatory non-GABAergic neurons by subtracting the number of double-labeled inhibitory cells from the total number of neurons marked with NeuN.

Statistical analysis

The data were tested statistically using the Student's T-test and the analysis of variance (ANOVA) with the post hoc Tukey test adjusted for multiple comparisons. The test used is specified in the legend of each figure. Significant differences for all statistical testing were defined by a $p < 0.05$. Numerical data are represented as mean ± standard deviation (S.D.). All statistical tests were performed using statistical analysis software (SPSS 14.0, SPSS, Inc., Chicago, IL, USA).

10.5 Results

Development of seizures following cortical undercut

All animals with chronic partially deafferented cortex showed progressive paroxysmal patterns of the cortical slow oscillation (Fig. 10-1B left panels), similar to previous reports (Nita, et al., 2006). While in the early stages of the undercut (control and 2W groups) the EEG field recording showed regularly alternating patterns of the slow-oscillation, later stages were characterized by frequently occurring spikes and sharp transitions between the active and silent phases of the slow oscillation, giving rise to activities in the 3-4 Hz frequency range (Fig. 10-1C). The power spectra of the EEG signal also changed from a unimodal distribution at 2W with a peak at ~1 Hz corresponding to the slow oscillation frequency to a multimodal distribution at 6W with components at 3-4 Hz (Fig. 10-1C left panel).

SW/PSW seizures continuously developing from anesthesia-induced slow-oscillation were described in 30% experimental cats (Steriade and Contreras, 1995), as a result of the facilitating effect of ketamine & xylazine anesthesia (Steriade, et al., 1998). However, in the present experiments all anesthetized animals with chronic partial cortical deafferentation developed generalized seizures consisting in spike-wave (SW) complexes at ~3 Hz or SW and polyspike-wave (PSW) complexes at lower frequency (1.5-2.5 Hz) in the first weeks after deafferentation (2W-4W); intermingled later on with fast-runs ~10-20 Hz (6W) (Fig. 10-1B right panels), similar to the waveforms seen in humans with severe epileptic syndromes (Niedermeyer, 1969).

The increase in the power of slow oscillation from the early (2W) to the late stages of cortical undercut (4W and 6W) and the more severe patterns of seizures observed in chronic undercut at 6W suggest that epileptogenesis in the suprasylvian gyrus following trauma is a continuously evolving process and that in chronic stages the animals are more prone to develop severe seizures.

Cortical architecture in the injured cortex

Normal cortex has a specific cytoarchitecture, being horizontally organized into six laminae (Baillarger, 1840) and vertically into groups of synaptically linked cells, called neocortical minicolumns, that represent the basic processing units of the mature neocortex, and which are further grouped together by short-range horizontal connections into cortical columns (Mountcastle, 1957, Mountcastle, 1997).

Following chronic deafferentation of the suprasylvian gyrus by completely transecting the fibers in the white matter (Fig. 10-2A, right panel) we observed a gradual change in the normal distribution of neurons on columns and layers, particularly evident at 4W and 6W after the undercut was performed (Fig. 10-2B). The cortical cytoarchitecture was disorganized, lacking the expected arrangement of neurons on layers and columns typically seen in the normal hexalaminar neocortex (Fig. 10-2B, C).

Severe neuronal loss was observed in the deep layers of the suprasylvian gyrus (Fig. 10-2C) and the grey matter appeared to be shrunk (Fig. 10-2A, B, D). The few remaining neurons observed in the depth of the undercut cortex were abnormally oriented and showed cytological dystrophic properties, with small atypical and pycnotic nuclei (Fig. 10-2C).

The thickness of the cortical grey matter diminished progressively in the undercut suprasylvian gyrus (Fig. 10-2B, D) from 1.87 ± 0.17 mm in control animals to 1.72 ± 0.16 mm at 2 weeks, reaching a statistical significant difference ($p<0.01$, Student's T-test) at 4 weeks (1.5 ± 0.21 mm) and 6 weeks (1.45 ± 0.18 mm) (Fig. 10-2D). The linear progression of this alteration pleads for a biopathological process and correlates with the increased propensity to seizures reported after chronic cortical deafferentation.

Depth profile of neuronal death after penetrating cortical injuries

The Nissl stained sections were suggestive for an important reduction in neuronal number in the undercut cortex (Fig. 10-2B, C). Therefore, we specifically assessed the neuronal loss and the depth distribution of excitatory and inhibitory neurons following injury using immunohistochemical staining. All neuronal nuclei were labeled with the anti-NeuN (neuronal-specific nuclear protein) antibody, while the inhibitory GABAergic neurons were

labeled either with anti-GABA (gamma-aminobutyric acid) or anti-GAD 65&67 (glutamic acid decarboxylase) antibodies. The GAD, GABA and NeuN immunolabeling in control and undercut cortex showed a dramatically reduced number of neurons in the undercut suprasylvian gyrus compared to control animals (Fig. 10-3A).

Double immunohistochemical labeling (Fig. 10-3B) (both GAD/NeuN and GABA/NeuN) was used to build up depth profiles for the number of excitatory (labeled only with NeuN) and inhibitory GABAergic neurons (double-labeled), for each 100 microns of depth from the cortical surface on a total area of 0.6 mm^2, as it is seen in one particular example of double labeled neurons with GAD/NeuN (Fig. 10-4A) and GABA/NeuN (Fig. 10-4B) and in the averaged data from 5 different animals (Fig. 10-5).

The number of non-GABAergic, presumably excitatory neurons, decreased progressively after the initial trauma, reaching a first statistical significant value ($p<0.05$, Student's T-test) at 2 weeks at ~500 μm (Fig. 10-5A). The decreased density of excitatory neurons became more important at 4 and 6 weeks for all deep layer (1600-2000 μm) but also for some of the more superficial layers (Fig. 10-5A). The number of inhibitory GABAergic neurons was reduced significantly ($p<0.05$, Student's T-test) as early as 2 weeks after deafferentation for the deep layers and also for a few more superficial layers. The diminished density of inhibitory neurons was even more constant at 4 and 6 weeks for both the deep and the superficial layers (Fig. 10-5B). It is important to note that although the number of inhibitory neurons globally decreased; the excitatory neurons from the deep cortical layers were also affected, and the hexalaminar cortical structure was disrupted.

Preferential loss of inhibitory neurons after traumatic brain injuries

In order to quantify the degree of neuronal loss after cortical trauma, we measured the densities of neurons in the undercut suprasylvian gyrus, in the contralateral suprasylvian gyrus, as well as in the ipsi- and contra- lateral marginal gyrus in sham operated animal and after 2, 4 and 6 weeks following injury. For easier comparison, in addition to the absolute number of neurons per area of 0.07 mm^2 (Fig. 10-6B), we also present the relative neuronal densities (Fig. 10-6C). The total number of neurons stained with NeuN decreased with 5.35±3.6% from the control value at 2 weeks and became statistical significant ($p<0.05$,

ANOVA with post hoc Tukey test) at 4 weeks and 6 weeks in the undercut gyrus, while neuronal densities in the undamaged gyri were similar to control (Fig. 10-6B). Nevertheless, the inhibitory GABAergic neurons were much more affected by the traumatic injury, decreasing significantly ($p < 0.01$, ANOVA with post hoc Tukey test) as early as 2 weeks following undercut (Fig. 10-6B). The number of GABAergic neurons decreased even more ($p < 0.001$, ANOVA with post hoc Tukey test) at 4 and 6 weeks after the initial injury, whereas again they remained unchanged in the other gyri (Fig. 10-6B).

When analyzing globally the number of excitatory and inhibitory neurons only in the deafferented suprasylvian gyrus, we noticed once again that the density of excitatory neurons was significantly decreased ($p < 0.05$, ANOVA with post hoc Tukey test) at 4 weeks and 6 week following deafferentation (Fig. 10-6C). The inhibitory GABAergic neurons were far more affected by the penetrating cortical injury, declining significantly ($p < 0.01$, ANOVA with post hoc Tukey test) after only 2 weeks and even more dramatically ($p < 0.001$, ANOVA with post hoc Tukey test) after 4 weeks and 6 weeks (Fig. 10-6C).

To better illustrate the preferential loss of these inhibitory GABAergic neurons, we calculated the ratio between excitatory and inhibitory cells. Although the data obtained in stack images are very excellent source of globally estimating the relative loss of neurons in the undercut cortex compared to the intact one, they may however underestimate the absolute number of cells due to the well-known poor penetration of the GABA antibodies throughout the thickness of the sections. Therefore, we decided to compute the ratio between excitatory and inhibitory neurons using the manually quantified data. The ratio was 4.25 ± 0.63 in control, reflecting a density of about 20% for GABAergic neurons, as previously described (Gabbott and Somogyi, 1986). The preferential loss of these inhibitory neurons was reflected by a progressively increasing ratio between the excitatory and the inhibitory neurons from control to 2 weeks ($p < 0.05$, ANOVA with post hoc Tukey test), and then to 4 and 6 weeks ($p < 0.01$, ANOVA with post hoc Tukey test) (Fig. 10-6D).

10.6 Discussion

In this study we illustrate the progressive anatomical changes that might explain the increased frequency of seizures observed after penetrating cortical wounds. We used the undercut cortex as a paradigm for post-traumatic seizures and we found an important disorganization of the cortical layers following injury and a progressive loss of neurons (NeuN-positive cells) in the deafferented suprasylvian gyrus. The inhibitory GABAergic neurons (GABA/GAD 65&67-positive) were particularly more sensitive to cortical trauma than the excitatory ones, leading to a relative raise in the number of excitatory neurons that might explain the increased propensity to seizures following penetrating cortical injury.

A higher frequency of seizures following head trauma has been described both in humans (Dinner, 1993, Marcikic, et al., 1998, Salazar, et al., 1985) and in animal models (Nita, et al., 2006, Prince and Tseng, 1993, Topolnik, et al., 2003a, Topolnik, et al., 2003b). The mechanisms responsible for this outcome include changes in intrinsic properties of pyramidal neurons (Esplin, et al., 1994, Topolnik, et al., 2003b), enhanced excitatory synaptic conductances without altered inhibition (Bush, et al., 1999, Houweling, et al., 2005), but also disorganization of normal cerebral cytoarchitecture either as microgyria and cortical sclerosis (Jacobs, et al., 1999a, Jacobs, et al., 1999b, Marin-Padilla, 1999, Marin-Padilla, et al., 2002, Swartz, et al., 2006), or neuronal death (Lowenstein, et al., 1992, Marin-Padilla, 1999, Marin-Padilla, et al., 2002, Swartz, et al., 2006).

Neuronal loss following cortical deafferentation

A prominent characteristic observed in many types of epilepsy is the massive and widespread neuronal degeneration occurring in different brain areas (Ebert, et al., 2002, Garrido Sanabria, et al., 2006), produced either as a consequence of the acute excitotoxic damage (Gorter, et al., 2003), or following recurrent chronic seizures, both in humans and animals (reviewed in Cendes, 2005). However, our data demonstrate cortical atrophy and substantial neuronal loss mainly due to the massive disappearance of GAD 65&67 and GABA-positive neurons in the suprasylvian gyrus. There were no statistical differences in cortical neuronal densities between control and 2W-4W-6W animals in the other analyzed gyri. The finding that neuronal loss in cats with chronically deafferented suprasylvian gyrus

is strictly limited to the undercut suggests that the cause of neuronal loss was the deafferentation and not the subsequent seizures present in these animals (Nita, et al., 2007).

Our results are further supported by studies done both in animals and in humans, in which the deafferentation was generated in a non-traumatic manner, by sensorial deprivation, such as limb amputation, ischemic nerve obstruction or visual deprivation (Chen, et al., 2002, Chen, et al., 1998, Hendry and Jones, 1986, Ziemann, et al., 1998). It was shown that deafferentation of the somatosensory (Welker, et al., 1989) or visual cortex (Hendry and Jones, 1986) in animals led to a reduction in the number of neurons containing GABA or its synthesizing enzyme, glutamic acid decarboxylase (GAD). Similarly, studies using transcranial magnetic stimulation in humans clearly demonstrate a reduction of GABAergic cortical inhibition and an enhancement of excitation following amputation of the upper limb (Schwenkreis, et al., 2000), lower limb (Chen, et al., 1998) and after nerve ischemia (Ziemann, et al., 1998).

Post-traumatic cortical architecture disorganization

We found a preferential loss of GABA-ergic neurons, similar to what has been described both in human (de Lanerolle, et al., 1989, Robbins, et al., 1991) and animal temporal lobe epilepsy (Sloviter, 1987), but also a progressive disruption of the cortical hexalaminar structure analogous to morphological studies done in epileptic patients resistant to antiepileptic drugs showing a complete disorganization of the cortical lamination accompanied by abnormalities in the morphology and distribution of inhibitory neurons (Spreafico, et al., 1998a, Spreafico, et al., 1998b). Recent data in humans show an important reduction of neocortical thickness and complexity in patients with mesial temporal lobe epilepsy (Lin, et al., 2007). Moreover, Marín-Padilla et al. showed that in children with brain trauma, the neurons directly damaged by the initial insult or incapable of reestablishing functional connections will die, while neurons capable of reestablishing post-injury functional connections will survive and will start a slow process of reorganization resulting in progressive cortical dysplasia (Marin-Padilla, et al., 2002). They also illustrated that in post-traumatic epileptic children (as in shaken infant syndrome) the amount of residual gray matter displays areas of complete destruction alternating with areas of dysplastic tissue without lamination, similar to what we describe in the chronically

deafferented suprasylvian gyrus. In the light of these data, the increased resistance to antiepileptic therapy of the patients with posttraumatic epilepsy may rely on an abnormal laminar cortical structure and neuronal death. Given that most of the new antiepileptic drugs exert their anticonvulsant action through enhancement of inhibitory-mediated neurotransmission (White, 1999), they possibly fail to control some posttraumatic epilepsy because of the low number of inhibitory neurons in the damaged cortex.

To sum up, we report that penetrating brain injuries initiate a morphological cortical disruption (loss of neurons, laminar disorganization) capable of shifting the balance between excitation and inhibition towards excitation in the deafferented cortex, increasing the susceptibility to seizures of chronically injured brains.

10.7 Acknowledgements

10.8 References

Avramescu, S., Nita, D. A., and Timofeev, I., 2007. Loss of excitatory and inhibitory neurons accompanies cortical post-traumatic epilepsy. Program No. 164.15/Z17 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2007. Online.

Baillarger, J., 1840. Recherches sur la structure de la couche corticale des circonvolutions du cerveau. Mémoires de l'Académie royale de médecine, Paris 8**,** 149 -183.

Bernard, C., Cossart, R., Hirsch, J. C., Esclapez, M., and Ben-Ari, Y., 2000. What is GABAergic inhibition? How is it modified in epilepsy? Epilepsia 41 Suppl 6**,** S90-95.

Bianchi, M. T., Song, L., Zhang, H., and Macdonald, R. L., 2002. Two different mechanisms of disinhibition produced by GABAA receptor mutations linked to epilepsy in humans. J Neurosci 22**,** 5321-5327.

Bloom, F. E., and Iversen, L. L., 1971. Localizing 3H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. Nature 229**,** 628-630.

Broman, J., and Westman, J., 1988. GABA-immunoreactive neurons and terminals in the lateral cervical nucleus of the cat. J. Comp. Neurol. 274**,** 467-482.

Buckmaster, P. S., and Jongen-Relo, A. L., 1999. Highly specific neuron loss preserves lateral inhibitory circuits in the dentate gyrus of kainate-induced epileptic rats. J Neurosci 19**,** 9519-9529.

Bush, P. C., Prince, D. A., and Miller, K. D., 1999. Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. J Neurophysiol 82**,** 1748-1758.

Cendes, F., 2005. Progressive hippocampal and extrahippocampal atrophy in drug resistant epilepsy. Curr. Opin. Neurol. 18**,** 173-177.

Chen, R., Cohen, L. G., and Hallett, M., 2002. Nervous system reorganization following injury. Neuroscience 111, 761-773.

Chen, R., Corwell, B., Yaseen, Z., Hallett, M., and Cohen, L. G., 1998. Mechanisms of cortical reorganization in lower-limb amputees. J. Neurosci. 18, 3443-3450.

D'Ambrosio, R., Fairbanks, J. P., Fender, J. S., Born, D. E., Doyle, D. L., and Miller, J. W., 2004. Post-traumatic epilepsy following fluid percussion injury in the rat. Brain 127, 304-314.

de Lanerolle, N. C., Kim, J. H., Robbins, R. J., and Spencer, D. D., 1989. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res 495, 387-395.

Dinner, D. S., 1993. Posttraumatic epilepsy. In: Wyllie, E., (Ed., The treatment of epilepsy: principles. Lea & Fibinger, Philadelphia, pp. 654-658.

Dinocourt, C., Petanjek, Z., Freund, T. F., Ben-Ari, Y., and Esclapez, M., 2003. Loss of interneurons innervating pyramidal cell dendrites and axon initial segments in the CA1 region of the hippocampus following pilocarpine-induced seizures. J Comp Neurol 459, 407-425.

Ebert, U., Brandt, C., and Loscher, W., 2002. Delayed sclerosis, neuroprotection, and limbic epileptogenesis after status epilepticus in the rat. Epilepsia 43 Suppl 5, 86-95.

Esplin, M. S., Abbott, J. R., Smart, M. L., Burroughs, A. F., Frandsen, T. C., and Litzinger, M. J., 1994. Voltage-sensitive calcium channel development in epileptic DBA/2J mice suggests altered presynaptic function. Epilepsia 35, 911-914.

Franck, J. E., Kunkel, D. D., Baskin, D. G., and Schwartzkroin, P. A., 1988. Inhibition in kainate-lesioned hyperexcitable hippocampi: physiologic, autoradiographic, and immunocytochemical observations. J Neurosci 8, 1991-2002.

Franck, J. E., and Schwartzkroin, P. A., 1985. Do kainate-lesioned hippocampi become epileptogenic? Brain Res 329, 309-313.

Fueta, Y., Kunugita, N., and Schwarz, W., 2005. Antiepileptic action induced by a combination of vigabatrin and tiagabine. Neuroscience 132**,** 335-345.

Gabbott, P. L., and Somogyi, P., 1986. Quantitative distribution of GABA-immunoreactive neurons in the visual cortex (area 17) of the cat. Exp. Brain Res. 61**,** 323-331.

Garrido Sanabria, E. R., Castaneda, M. T., Banuelos, C., Perez-Cordova, M. G., Hernandez, S., and Colom, L. V., 2006. Septal GABAergic neurons are selectively vulnerable to pilocarpine-induced status epilepticus and chronic spontaneous seizures. Neuroscience 142**,** 871-883.

Gorter, J. A., Goncalves Pereira, P. M., van Vliet, E. A., Aronica, E., Lopes da Silva, F. H., and Lucassen, P. J., 2003. Neuronal cell death in a rat model for mesial temporal lobe epilepsy is induced by the initial status epilepticus and not by later repeated spontaneous seizures. Epilepsia 44**,** 647-658.

Hendry, S. H., Fuchs, J., deBlas, A. L., and Jones, E. G., 1990. Distribution and plasticity of immunocytochemically localized GABAA receptors in adult monkey visual cortex. J Neurosci 10**,** 2438-2450.

Hendry, S. H., and Jones, E. G., 1986. Reduction in number of immunostained GABAergic neurones in deprived-eye dominance columns of monkey area 17. Nature 320**,** 750-753.

Hendry, S. H., and Jones, E. G., 1988. Activity-dependent regulation of GABA expression in the visual cortex of adult monkeys. Neuron 1**,** 701-712.

Houweling, A. R., Bazhenov, M., Timofeev, I., Steriade, M., and Sejnowski, T. J., 2005. Homeostatic synaptic plasticity can explain post-traumatic epileptogenesis in chronically isolated neocortex. Cereb.Cortex 15**,** 834-845.

Jacobs, K. M., Hwang, B. J., and Prince, D. A., 1999a. Focal epileptogenesis in a rat model of polymicrogyria. J Neurophysiol 81**,** 159-173.

Jacobs, K. M., Kharazia, V. N., and Prince, D. A., 1999b. Mechanisms underlying epileptogenesis in cortical malformations. Epilepsy Res 36**,** 165-188.

Karlsson, G., Kolb, C., Hausdorf, A., Portet, C., Schmutz, M., and Olpe, H. R., 1992. GABA_B receptors in various in vitro and in vivo models of epilepsy: a study with the GABA_B receptor blocker CGP 35348. Neuroscience 47, 63-68.

Lin, J. J., Salamon, N., Lee, A. D., Dutton, R. A., Geaga, J. A., Hayashi, K. M., Luders, E., Toga, A. W., Engel, J., Jr., and Thompson, P. M., 2007. Reduced neocortical thickness and complexity mapped in mesial temporal lobe epilepsy with hippocampal sclerosis. Cereb. Cortex 17**,** 2007-2018.

Lowenstein, D. H., Thomas, M. J., Smith, D. H., and McIntosh, T. K., 1992. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. J Neurosci 12**,** 4846-4853.

Marcikic, M., Melada, A., and Kovacevic, R., 1998. Management of war penetrating craniocerebral injuries during the war in Croatia. Injury 29**,** 613-618.

Marin-Padilla, M., 1999. Developmental neuropathology and impact of perinatal brain damage. III: gray matter lesions of the neocortex. J Neuropathol Exp Neurol 58**,** 407-429.

Marin-Padilla, M., Parisi, J. E., Armstrong, D. L., Sargent, S. K., and Kaplan, J. A., 2002. Shaken infant syndrome: developmental neuropathology, progressive cortical dysplasia, and epilepsy. Acta Neuropathol. (Berl.) 103**,** 321-332.

Mathern, G. W., Babb, T. L., Vickrey, B. G., Melendez, M., and Pretorius, J. K., 1995. The clinical-pathogenic mechanisms of hippocampal neuron loss and surgical outcomes in temporal lobe epilepsy. Brain 118 (Pt 1)**,** 105-118.

McKinney, R. A., Debanne, D., Gahwiler, B. H., and Thompson, S. M., 1997. Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of posttraumatic epilepsy. Nat Med 3**,** 990-996.

Micheva, K. D., and Beaulieu, C., 1995. Neonatal sensory deprivation induces selective changes in the quantitative distribution of GABA-immunoreactive neurons in the rat barrel field cortex. J Comp Neurol 361**,** 574-584.

Mountcastle, V. B., 1957. Modality and topographic properties of single neurons of cat's somatic sensory cortex. J Neurophysiol 20**,** 408-434.

Mountcastle, V. B., 1997. The columnar organization of the neocortex. Brain 120 (Pt 4)**,** 701-722.

Niedermeyer, E., 1969. The Lennox-Gastaut syndrome: a severe type of childhood epilepsy. Dtsch. Z. Nervenheilkd. 195**,** 263-282.

Nita, D. A., Cisse, Y., Timofeev, I., and Steriade, M., 2006. Increased propensity to seizures after chronic cortical deafferentation in vivo. J. Neurophysiol. 95**,** 902-913.

Nita, D. A., Cisse, Y., Timofeev, I., and Steriade, M., 2007. Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. Cereb. Cortex 17**,** 272-283.

Obenaus, A., Esclapez, M., and Houser, C. R., 1993. Loss of glutamate decarboxylase mRNA-containing neurons in the rat dentate gyrus following pilocarpine-induced seizures. J Neurosci 13**,** 4470-4485.

Prince, D. A., and Tseng, G. F., 1993. Epileptogenesis in chronically injured cortex: in vitro studies. J.Neurophysiol. 69**,** 1276-1291.

Ribak, C. E., Bradburne, R. M., and Harris, A. B., 1982. A preferential loss of GABAergic, symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex. J Neurosci 2**,** 1725-1735.

Ribak, C. E., and Reiffenstein, R. J., 1982. Selective inhibitory synapse loss in chronic cortical slabs: a morphological basis for epileptic susceptibility. Can. J. Physiol. Pharmacol 60**,** 864-870.

Robbins, R. J., Brines, M. L., Kim, J. H., Adrian, T., de Lanerolle, N., Welsh, S., and Spencer, D. D., 1991. A selective loss of somatostatin in the hippocampus of patients with temporal lobe epilepsy. Ann Neurol 29**,** 325-332.

Romijn, H. J., Ruijter, J. M., and Wolters, P. S., 1988. Hypoxia preferentially destroys GABAergic neurons in developing rat neocortex explants in culture. Exp. Neurol. 100**,** 332-340.

Salazar, A. M., Jabbari, B., Vance, S. C., Grafman, J., Amin, D., and Dillon, J. D., 1985. Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. Neurology 35**,** 1406-1414.

Schwenkreis, P., Witscher, K., Janssen, F., Dertwinkel, R., Zenz, M., Malin, J. P., and Tegenthoff, M., 2000. Changes of cortical excitability in patients with upper limb amputation. Neurosci. Lett. 293**,** 143-146.

Sloper, J. J., Johnson, P., and Powell, T. P., 1980. Selective degeneration of interneurons in the motor cortex of infant monkeys following controlled hypoxia: a possible cause of epilepsy. Brain Res. 198**,** 204-209.

Sloviter, R. S., 1987. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 235**,** 73-76.

Sloviter, R. S., 1992. Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. Neurosci Lett 137**,** 91-96.

Spreafico, R., Battaglia, G., Arcelli, P., Andermann, F., Dubeau, F., Palmini, A., Olivier, A., Villemure, J. G., Tampieri, D., Avanzini, G., and Avoli, M., 1998a. Cortical dysplasia: an immunocytochemical study of three patients. Neurology 50**,** 27-36.

Spreafico, R., Pasquier, B., Minotti, L., Garbelli, R., Kahane, P., Grand, S., Benabid, A. L., Tassi, L., Avanzini, G., Battaglia, G., and Munari, C., 1998b. Immunocytochemical investigation on dysplastic human tissue from epileptic patients. Epilepsy Res. 32**,** 34-48.

Steriade, M., Amzica, F., Neckelmann, D., and Timofeev, I., 1998. Spike-wave complexes and fast components of cortically generated seizures. II. Extra- and intracellular patterns. J. Neurophysiol. 80**,** 1456-1479.

Steriade, M., and Contreras, D., 1995. Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. J. Neurosci. 15**,** 623-642.

Swartz, B. E., Houser, C. R., Tomiyasu, U., Walsh, G. O., DeSalles, A., Rich, J. R., and Delgado-Escueta, A., 2006. Hippocampal cell loss in posttraumatic human epilepsy. Epilepsia 47**,** 1373-1382.

Tecoma, E. S., and Choi, D. W., 1989. GABAergic neocortical neurons are resistant to NMDA receptor-mediated injury. Neurology 39**,** 676-682.

Topolnik, L., Steriade, M., and Timofeev, I., 2003a. Hyperexcitability of intact neurons underlies acute development of trauma-related electrographic seizures in cats in vivo. Eur J Neurosci 18**,** 486-496.

Topolnik, L., Steriade, M., and Timofeev, I., 2003b. Partial cortical deafferentation promotes development of paroxysmal activity. Cereb.Cortex 13**,** 883-893.

Wallace, R. H., Marini, C., Petrou, S., Harkin, L. A., Bowser, D. N., Panchal, R. G., Williams, D. A., Sutherland, G. R., Mulley, J. C., Scheffer, I. E., and Berkovic, S. F., 2001. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28**,** 49-52.

Welker, E., Soriano, E., and Van der Loos, H., 1989. Plasticity in the barrel cortex of the adult mouse: effects of peripheral deprivation on GAD-immunoreactivity. Exp. Brain Res. 74**,** 441-452.

White, H. S., 1999. Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. Epilepsia 40 Suppl 5**,** S2-10.

Whittington, M. A., and Jefferys, J. G., 1994. Epileptic activity outlasts disinhibition after intrahippocampal tetanus toxin in the rat. J Physiol 481 (Pt 3)**,** 593-604.

Yamauchi, T., Kaneko, S., Yagi, K., and Sase, S., 2006. Treatment of partial seizures with gabapentin: double-blind, placebo-controlled, parallel-group study. Psychiatry Clin. Neurosci. 60**,** 507-515.

Ziemann, U., Hallett, M., and Cohen, L. G., 1998. Mechanisms of deafferentation-induced plasticity in human motor cortex. J. Neurosci. 18**,** 7000-7007.

10.9 Figures
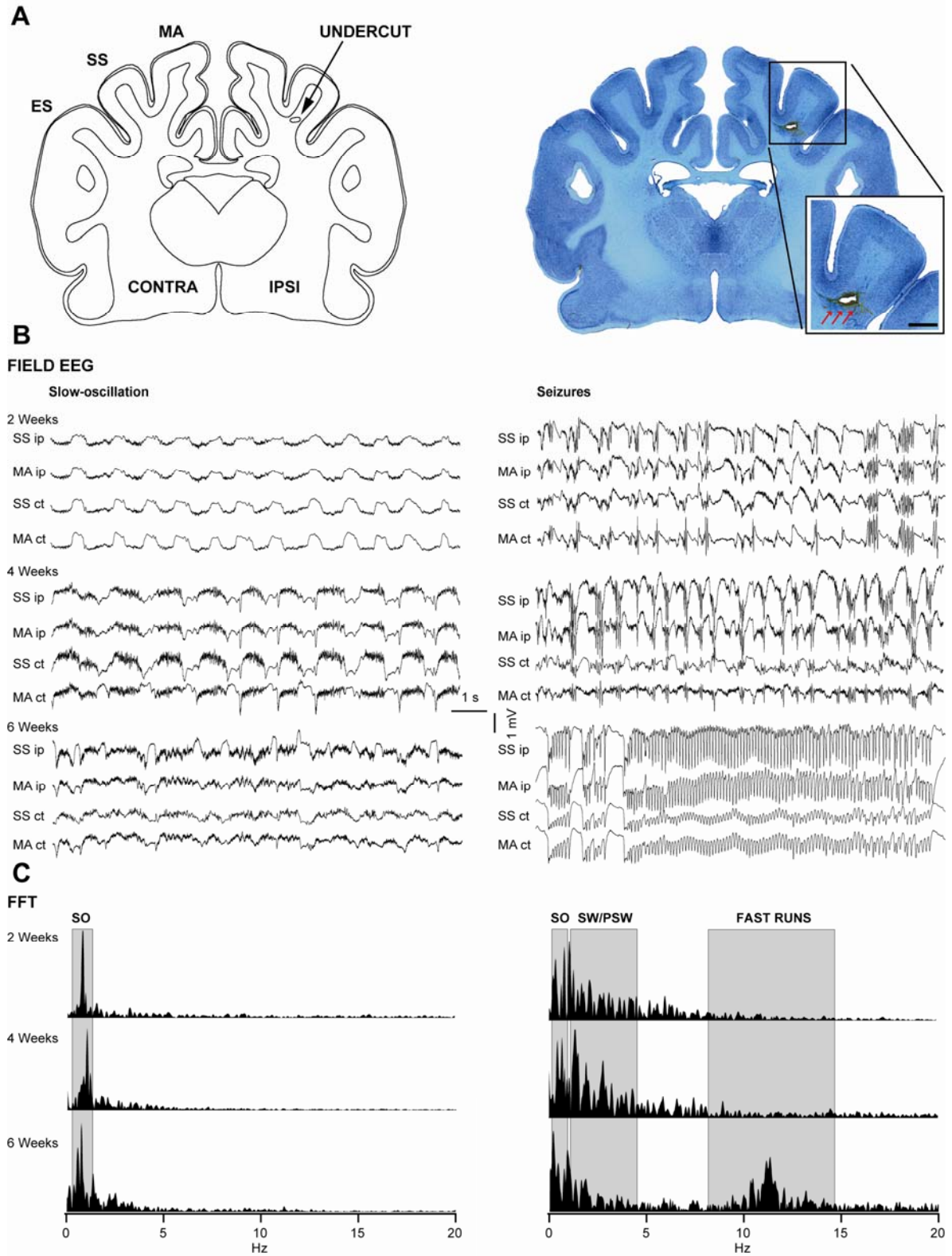
Figure 10-1. Increased propensity to seizures after chronic cortical deafferentation. Frontal section (schema in the left panel, Nissl staining in the right panel) of cat brain. The extent of the damage caused by the knife is expanded in the inset. The scale bar in the bottom right corner of the panel represents 4 mm for the complete brain section and 2 mm for the inset (A). EEG field potentials during slow oscillation (left) and seizures (right) at 2 weeks (2W), 4 weeks (4W) and 6 weeks (6W) respectively, after brain injury. The EEG was recorded in the undercut gyrus (SS ip), in the marginal gyrus ipsi- (MA ip) and contra-lateral (MA ct) to the undercut and in the contra-lateral suprasylvian gyrus (SS ct) (B). Fast Fourier transformation (FFT) of EEG activity during cortical slow-oscillation (left panels) and seizures (right panels) at different time intervals following cortical trauma (C). With progression in time, 3-4 Hz spike wave (SW) and poli-spike-wave (PSW) activities (PSW) emerged from the slow-oscillation and seizures progressively developed from SW complexes to SW/PSW activities intermingled with fast-runs (10-20 Hz) as observed in patients with Lennox-Gastaut syndrome.
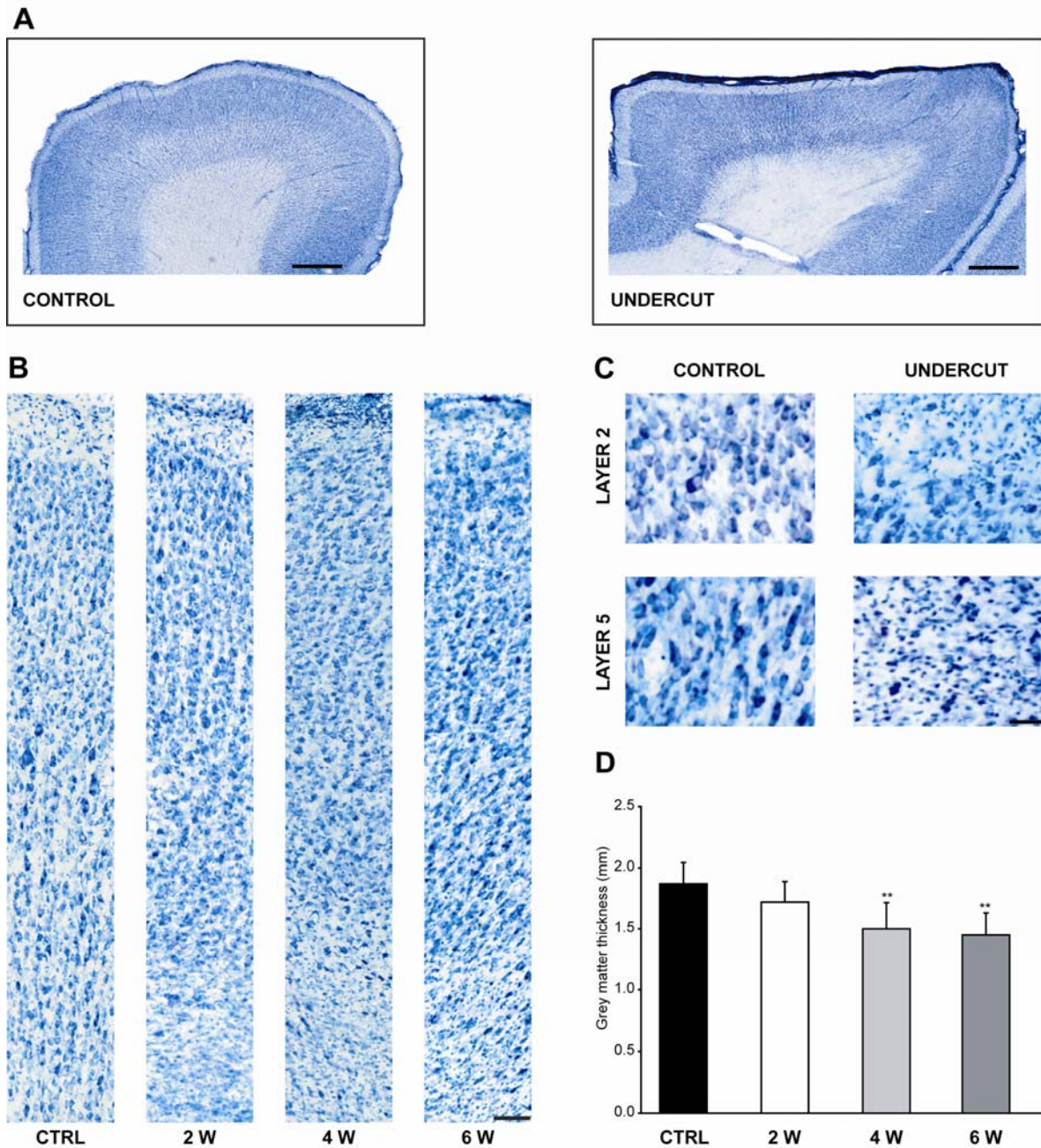
Figure 10-2. Penetrating brain wounds cause a reduction of grey matter's thickness and the disorganization of cortical architecture. Nissl staining of the suprasylvian gyrus in control (left panel) and 6 weeks following cortical undercut (right panel) (A). Cortical depth profile of the suprasylvian gyrus with Nissl staining, showing the normal cellular distribution in control (CTRL) and the progressive disorganization and shrinkage of the grey matter at 2 weeks (2W), 4 weeks (4W) and 6 weeks (6W) respectively after deafferentation (B). Illustration of neuronal morphology in the cortical layer 2 (upper panel) and layer 5 (lower panel) in control (left) and undercut cortex (right) (C). Quantification of cortical width in control (CTRL) and at 2 weeks (2W), 4 weeks (4W) and 6 weeks (6W) after undercut (average, n=10) (D). ** $p<0.01$, Student's T-test. Scale bars are represented at the bottom right corner of each panel and represent 1 mm in (A), 100 μm in (B) and 50 μm in (C).

A GAD   CONTROL   UNDERCUT

GABA

NeuN

B GAD / NeuN   GABA / NeuN
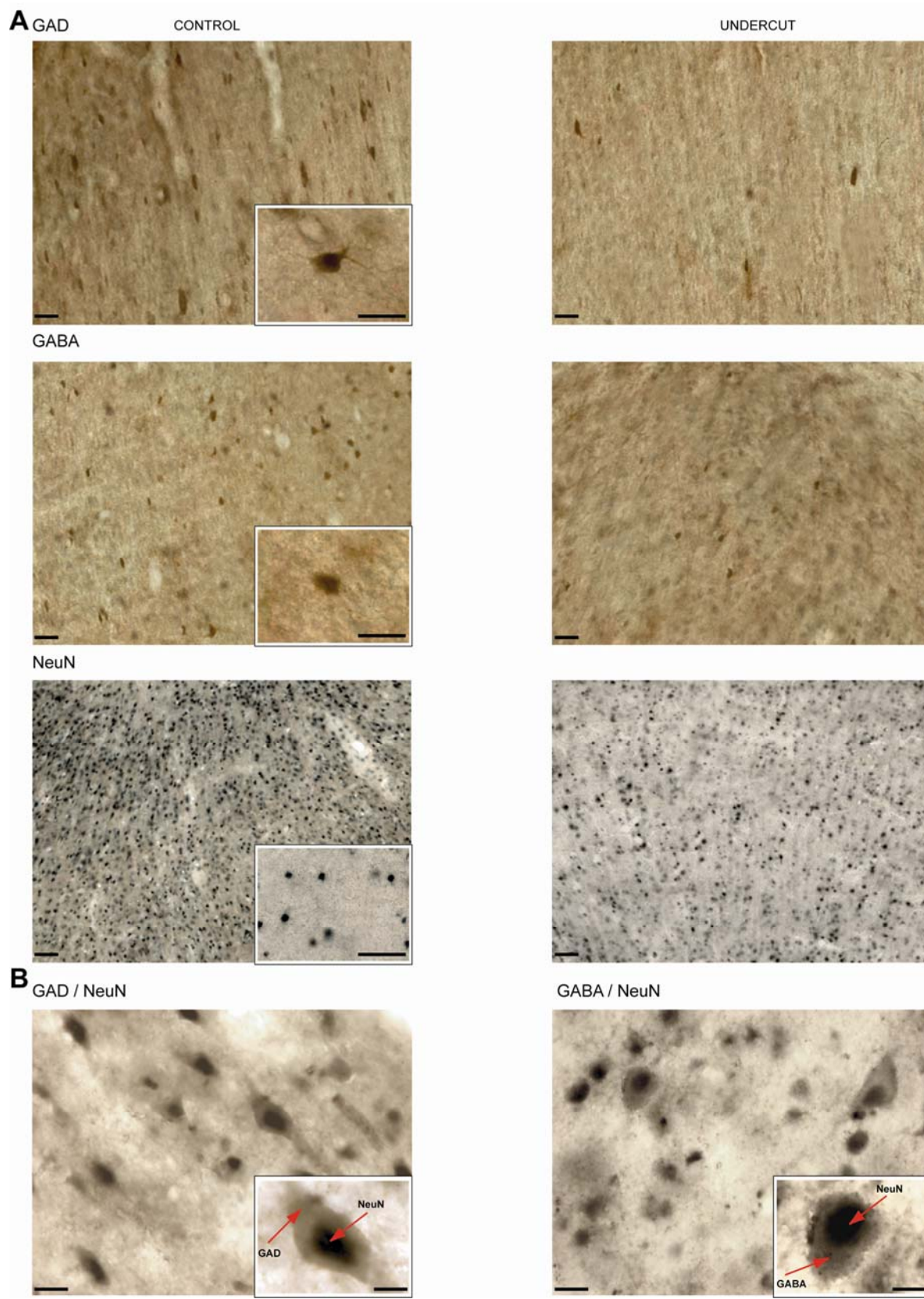
Figure 10-3. Immunohistochemical labeling of cortical neurons. Staining for glutamic acid decarboxylase (GAD 65/67) (upper panel), gamma-aminobutyric acid (GABA) (middle panel) and neuronal nuclear protein (NeuN) (lower panel) in control (left) and undercut cortex (right). The insets show the simple GAD, GABA and NeuN labeling of one neuron (A). Double staining GAD & NeuN (left panel) and GABA & NeuN (right panel). Insets depict the double labeling of inhibitory neurons. Note the nucleus labeled in grey-black (DAB - Ni, Cr enhancement) and the cytoplasm in brown (DAB) (B). Scale bars in (A) stand for 100 μm (50 μm in the insets). Scale bars in (B) represent 20 μm (10 μm in the insets).

A GAD/NeuN

CTRL   2W   4W   6W

B GABA/NeuN

CTRL   2W   4W   6W
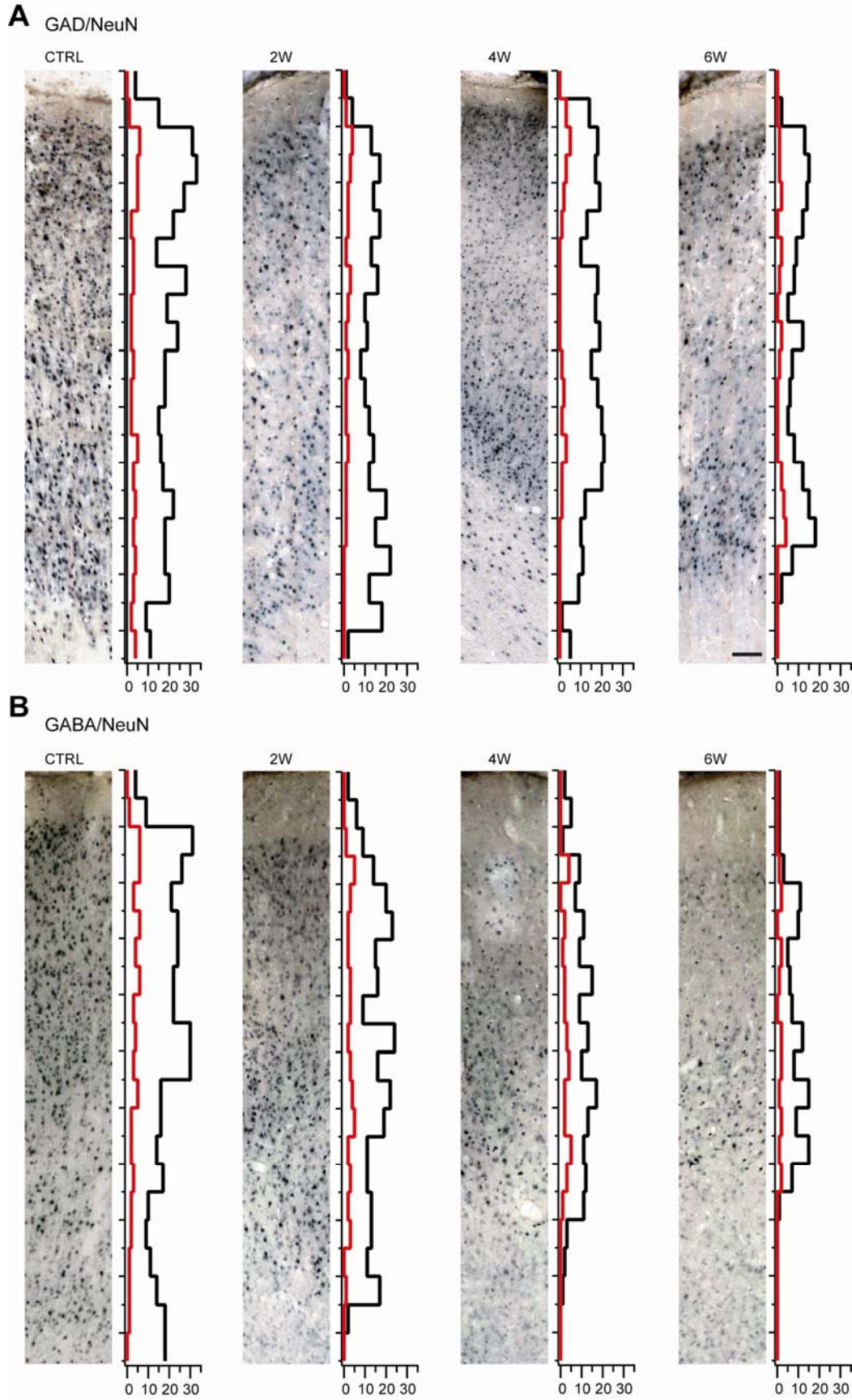
Figure 10-4. Examples of cortical depth profiles showing the distribution of excitatory and inhibitory neurons in control and undercut cortex. Individual examples of neuronal profiles double stained with GAD and NeuN in control (CTRL) and after deafferentation at 2 weeks (2W), 4 weeks (4W) and 6 weeks (6W) (A). Similar examples as the ones presented in A for neurons double stained with GABA and NeuN (B). Graphs on the right side of the profiles depict the number of labeled cells (x axis) on 0.03 mm^2 areas at the corresponding depth (y axis). Scale bar represents 100 μm.

A Excitatory (NeuN-GAD)   Excitatory (NeuN-GABA)

B Inhibitory (GAD)   Inhibitory (GABA)

CTRL ●
2W ■
4W ▶
6W ◆
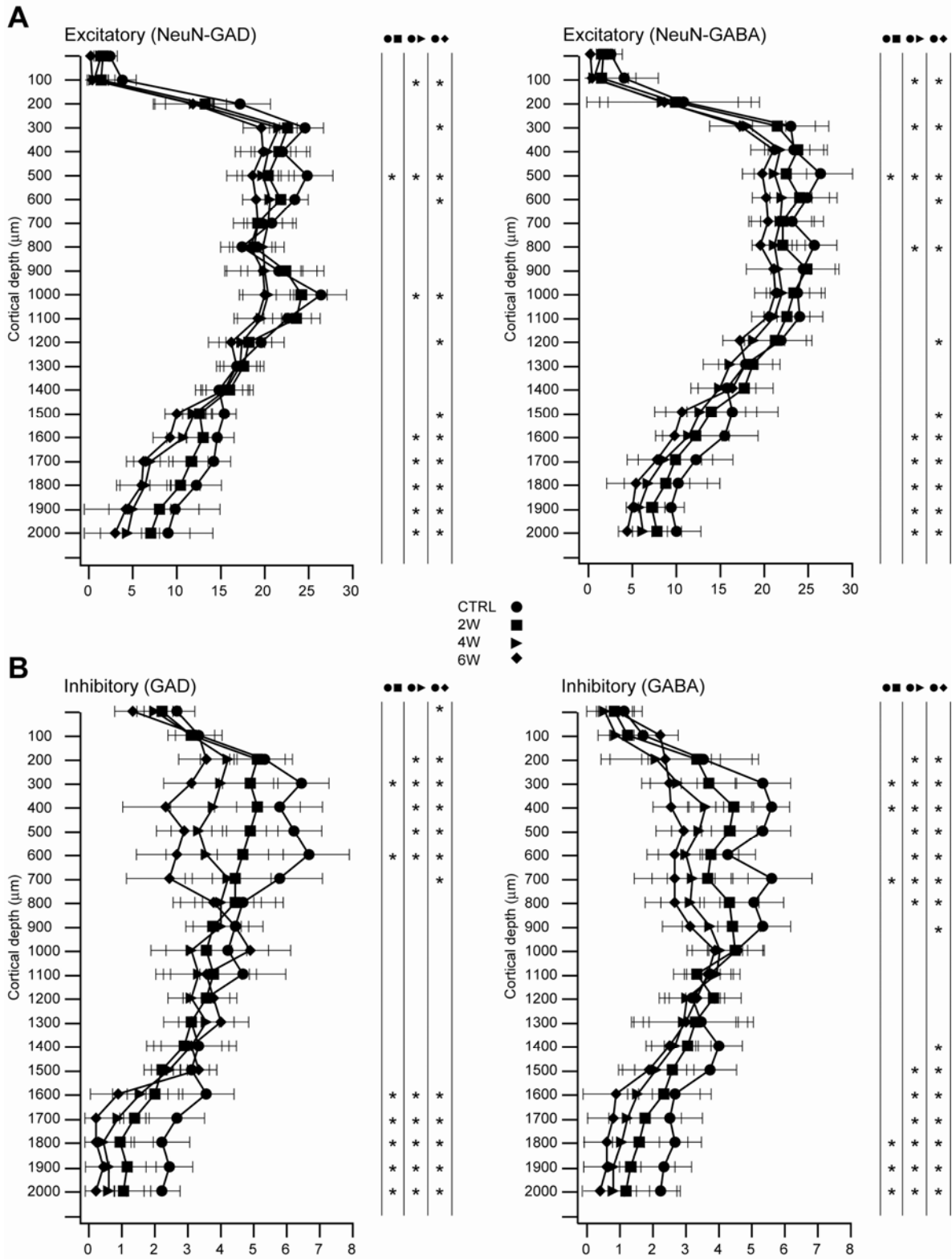
Figure 10-5. Depth profile distribution of neuronal densities in control and after cortical trauma. Average distribution of excitatory neurons (NeuN minus GAD – left panel and NeuN minus GABA – right panel) on 0.03 mm^2 areas (x axis) at each 100 μm of cortical depth (y axis) from 5 different animals in control (black circles) and at 2 weeks (black squares), 4 weeks (black triangles) and 6 weeks (black rhombi) (A). Average distribution of inhibitory neurons (GAD - left panel and GABA – right panel) on 0.03 mm^2 areas (x axis) at each 100 μm of cortical depth (y axis) from 5 different animals in control (black circle) and at 2 weeks (black square), 4 weeks (black triangle) and 6 weeks (black rhombus) (B). Note the progressive loss of excitatory neurons mostly in the deep layers and the loss of inhibitory neurons both in the deep layers and in the more superficial ones. The diagram on the right side of each graph represents the statistical significant difference (control - 2weeks left, control - 4weeks middle, control - 6weeks right) in neuronal densities for every 100 μm of cortical depth * p<0.05, Student's T-test.

A STACK 40x — ALL NEURONS — GAD/GABA NEURONS

B NeuN distribution — GAD distribution — GABA distribution

C

D Excitatory / Inhibitory
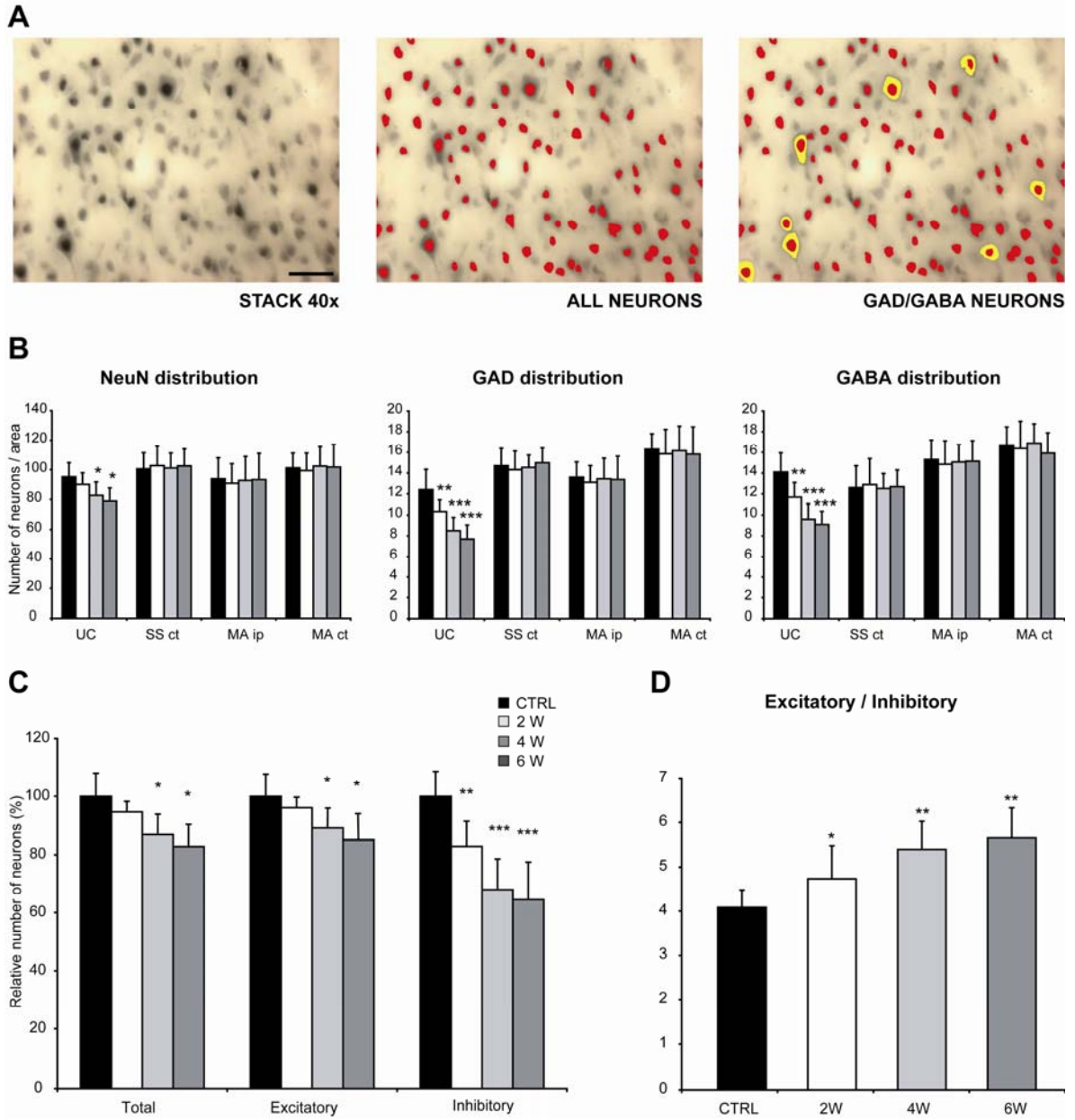
Figure 10-6. Changes in the balance between excitation and inhibition towards excitation in chronically deafferented cortex. Stack images acquired with a 40x objective (left panel) were used for automatic quantification using Image-Pro software. Images depict the selection process of all neurons (labeled with NeuN) and of specifically labeled inhibitory neurons (double-labeled with either GAD or GABA) based on predefined ranges of image analysis parameters equally applied to all acquired z-stack average images (A). Distribution of the total number of neurons (NeuN distribution) and of inhibitory neurons (GAD and GABA distribution) in control (black bars), and after undercut at 2 weeks (white bars), 4 weeks (light grey bars) and 6 weeks (dark grey bars). Neuronal densities are expressed as number of neurons per each analyzed area of 0.07 mm^2 (B). Percentage of total neuronal loss in the undercut gyrus, together with progressive loss of excitatory neurons and inhibitory neurons. Note the more significant reduction of inhibitory neurons (C). The ratio of excitatory/inhibitory neurons in control (CTRL) and at 2 weeks (2W), 4 weeks (4W) and 6 weeks (6W) after deafferentation. Note the increased shift in the excitation-inhibition balance toward excitation following cortical trauma which might account for the increased propensity to seizures (D). Scale bar represents 40 µm. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ANOVA with post hoc Tukey test.

11 Laminar distribution of spontaneous cortical activity following cortical deafferentation

Sinziana Avramescu, Dragos A. Nita and Igor Timofeev

11.1 Résumé

Suite aux traumatismes crâniens pénétrants, le cerveau devient progressivement hyperexcitable et génère des activités paroxystiques spontanées. Des études antérieures ont montré que la déafférentation corticale partielle favorise le développement de crises électrographiques, initiées dans le tissu cérébral relativement intact. Néanmoins, les conséquences de la privation chronique d'afférences neuronales par déafférentation sur l'aspect de la décharge neuronale ou sur la génération de l'oscillation corticale lente demeurent peu connues. Dans la présente étude, nous avons enregistré l'activité corticale spontanée à plusieurs profondeurs dans le cortex à différents moments après la déafférentation partielle du gyrus suprasylvien, in vivo, pendant différents états de vigilance. Nous avons trouvé que la fréquence neuronale de décharge augmente progressivement pendant le sommeil à ondes lentes comparé à l'état de veille, particulièrement dans la partie relativement intacte du gyrus déafferenté. L'analyse laminaire de l'activité multiunitaire a indiqué qu'après la déafférentation la plus grande fréquence neuronale de décharge apparaît initialement dans les couches moyennes et profondes du cortex, autant dans la partie antérieure que postérieure du gyrus suprasylvien. Plusieurs semaines après la déafférentation, dans le cortex complètement déafferenté, la fréquence neuronale maximale de décharge a été enregistrée dans les couches corticales plus superficielles, alors que les neurones situés dans les couches profondes avaient un très basniveau de décharge. Toutefois, le profil de profondeur de décharges dans la partie antérieure, partiellement intacte, du gyrus est resté semblable en tout temps. L'analyse de la densité des sources de courant (CSD) a prouvé que l'oscillation lente corticale spontanée commençait également dans les couches corticales plus profondes, tôt après la déafférentation, mais que dans les étapes ultérieures, dans le gyrus complètement déafferenté, elle était déclenchée dans les couches plus superficielles et ensuite se propageait vers les couches inférieures. À la lumière de ces données, nous envisageons que suite à une déafférentation corticaleles neurones profonds vont dégénérer et ceci causera une fréquence de décharge diminuée dans les couches profondes et une relocalisation parallèle de l'origine des activités corticales spontanées.

11.2 Abstract

After penetrating cortical wounds, the brain becomes progressively hyperexcitable and generates spontaneous paroxysmal activity. Previous studies documented that partial cortical deafferentation promotes the development of electrographic seizures, starting in the relatively intact cerebral tissue. However, little is known regarding the consequences of chronic input deprivation on the neuronal firing pattern or on the generation of slow cortical oscillation. In the present study, we recorded the spontaneous cortical activity through the depth of the cortex at different time delays following partial deafferentation of the suprasylvian gyrus, *in vivo*, during different states of vigilance. We found a progressively increased neuronal discharge frequency during slow-wave sleep compared to waking state, particularly in the relatively intact part of the deafferented gyrus. Laminar analysis of multiunit activity revealed the highest firing rate in the middle and deep layers of the cortex, in early stages of the undercut, both in the anterior and in the posterior parts of the suprasylvian gyrus. Several weeks after deafferentation, in the undercut cortex, the maximal firing rate shifted towards the more superficial cortical layers, while the fewer neurons from deep layers had a very low total discharge rate. Nevertheless, the depth profile of firing frequency in the anterior, partially intact, part of the gyrus was similar in early and in late stages of the undercut. Current-source-density analysis showed that the spontaneous cortical slow oscillation originated also in the deeper cortical layers early after undercut, but in later stages, in the completely deafferented gyrus, it was initiated in the more superficial layers and then spread towards the lower ones. In the light of these data, we envisage that following cortical undercut, deeply laying neurons will degenerate and this will cause a decreased firing rate in the deep layers and a parallel relocation in the origin of the spontaneous cortical activity.

11.3 Introduction

The incidence of epilepsy following brain trauma depends on the type and severity of the underlying injury, and is highest after penetrating cortical wounds (Annegers et al. 1980; Salazar et al. 1985). Approximately one-third of patients with symptomatic localization-related epilepsy syndromes are refractory to available antiepileptic medication (Kwan and Brodie 2000). Although there were several clinical attempts to prevent posttraumatic epileptogenesis by simple prophylaxis with antiepileptic drugs, they have been largely unsuccessful (Temkin 2001). Therefore, prevention strategies and even improved treatments would benefit from better understanding the underlying mechanisms of posttraumatic seizures.

The use of partial neocortical isolation is a well-studied model of posttraumatic epilepsy both *in vivo* (Nita et al. 2006; 2007; Topolnik et al. 2003a; 2003b) and *in vitro* (Jacobs et al. 2000; Jin et al. 2006; Li et al. 2005; Li and Prince 2002; Prince and Tseng 1993). Previous studies illustrate a profound reorganization of the neuronal network in the undercut cortex, such as axonal sprouting (Salin et al. 1995) and increased connectivity (Avramescu and Timofeev 2008), changes in intrinsic neuronal properties (Avramescu and Timofeev 2008; Esplin et al. 1994; Topolnik et al. 2003a), selective degeneration of inhibitory synapses (Ribak and Reiffenstein 1982), and neuronal loss mainly in the deep cortical layers (Avramescu et al. 2007). It has also been shown that the epileptiform activity is initiated in the intact cortex surrounding the undercut (Nita et al. 2007; Topolnik et al. 2003a; 2003b) and that chronic cortical deafferentation is associated with loss of neurons, particularly in the deep cortical layers (Avramescu et al. 2007). Data on the differences between intact and undercut cortex is nevertheless scarce, and the effect of chronic deafferentation on the initiation and propagation of the cortical slow oscillation remains unknown.

The cerebral cortex is able to spontaneously generate a highly synchronized slow oscillatory rhythm (0.1–0.5 Hz), alternating between periods of activity and silence of the thalamocortical networks (Contreras and Steriade 1995; Steriade et al. 1993; Steriade et al. 2001; Timofeev et al. 2001; Volgushev et al. 2006). This slow rhythmic activity occurs during natural slow-wave-sleep (SWS), but also under anesthesia and even in isolated

cortical preparations (Antognini and Carstens 2002; Mahon et al. 2001; Sanchez-Vives and McCormick 2000; Timofeev et al. 2000). Blocking GABA$_A$ receptors in the neocortex has also been described as very effective in generating synchronous activity in isolated slices (Connors 1984; Gutnick et al. 1982) and *in vivo* (Castro-Alamancos and Borrell 1995; Gloor et al. 1977; Steriade and Contreras 1998). Recordings of spontaneous cortical activities show that they originate typically in the deep cortical layers and then propagate toward the more superficial ones, both *in vitro* (Sanchez-Vives and McCormick 2000) and *in vivo* (Chauvette et al. 2008; Volgushev et al. 2006). Cortical undercut generates an important loss of neurons, particularly inhibitory, in the deep cortical layers, and this could have profound influence on the generation and propagation of the slow oscillation.

In the present study, we tested the effects of chronic cortical deafferentation on the activity pattern of neurons deprived of subcortical inputs and how this might influence the generation and propagation of the cortical slow oscillation. Slicing the brain for *in vitro* preparation and anesthesia *in vivo* alter the normal patterns of neuronal activity. To overcome these limitations we chose to carry our experiments *in vivo*, in non-anesthetized cats, to follow the gradual changes of cortical activity during wake and natural sleep, from early to late stages of cortical deafferentation. We found a shift in the origin of the cortical slow oscillation from the deep cortical layers, in control and in early stages of the undercut, to the more superficial ones, in later stages, which was accompanied by a decreased total discharge rate of deeply laying neurons in chronic undercut cortex.

11.4 Materials and methods

Surgery and chronic electrodes implantation

Experiments were performed on 7 chronically implanted male cats. All 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 the electroencephalogram (EEG) and cardiac frequency (aiming 90-110 beats/min) and supplemental doses of anesthetic were given at the slightest tendency toward an activated EEG pattern. Oxygen saturation of the arterial blood and end-tidal CO_2 were also monitored. General procedures also included cephalic vein canulation for systemic liquid delivery (lactated Ringer's solution 5-10 ml/kg/h), lidocaine (0.5%) infiltration of all pressure points or incision lines, and maintenance of body temperature between 37–38°C with a heating pad.

The cerebral cortex was exposed by craniotomy and a large undercut (13–15 mm postero-anteriorly, 3–4 mm medio-laterally and 3–4 mm deep) was performed in the white matter below the suprasylvian gyrus, while keeping intact the blood supply. The detailed surgical procedure is described elsewhere (Topolnik et al., 2003; Nita et al., 2007; Avramescu et al., 2008).

Two recording chambers were implanted over the intact dura above the suprasylvian gyrus, one in the anterior part, relatively intact, and the other one in the posterior, partially deafferented, part of the gyrus, for subsequent juxtacellular recordings with tungsten microelectrodes and for depth profile recordings with single-shank 16-channels Michigan Probes.

Chronically implanted tungsten electrodes for local field potential recordings (with the tip in the cortical depth at about 1 mm from the surface) were placed bilaterally over frontal, temporal and occipital areas. Pairs of Ag/AgCl electrodes were placed around the orbit through the frontal sinus to record the electrooculogram (EOG), into the neck muscles for the electromyogram (EMG), and were used to monitor the states of vigilance. Ag/AgCl references were placed on the nasium and bilaterally under the skin in the vicinity of the

ears. The skull was reconstituted using acrylic dental cement, all electrode wires were soldered to a connector socket, and 4 bolts were placed in the cement to allow non-painful fixation of the cat's head in a stereotactic 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 short recovery period (2–3 days), cats were trained to stay in the stereotactic frame and few days later they were able to sleep and displayed clearly identifiable states of vigilance (wake (W), slow wave sleep (SWS), and rapid eye movement (REM) sleep.

Recordings started 4–5 days after surgery and lasted up to 5 months. Two to three recording sessions, each lasting 3-4 h, were performed daily in a dark and silent environment under video surveillance, and usually contained periods of W, SWS and REM sleep. Body movements and position of the eyelids were noted. Cats were not deprived of sleep between recording sessions, and during recordings they could move their limbs and make postural adjustments.

Behavioral seizures were considered an "end-limit point" of any experiments, as consented with the local ethic committee. At the end of the experiments or at the first sign of clinically manifest seizures cats were given a lethal dose of intravenous sodium pentobarbital (50 mg/kg). After all experiments the extension of the undercut was verified on Nissl stained (thionine) sections. 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.

Electrophysiological recordings

Recordings of local field potentials (LFP) across the cortical depth were performed with 16-channel silicon probes (University of Michigan Center for Neural Communication Technology, Ann Arbor, MI). Probe recording sites were separated by 150 μm and had impedances of 8.6–10.2 MΩ at 1 kHz. The probe was lowered into the brain under visual

guidance through a small hole performed in the dura, oriented normal to the cortical surface, until the most superficial recording site was aligned with the surface. EEG was obtained from the LFP signals by filtering between 0.1 Hz and 100 Hz, and multi-unit activity was evidenced by filtering the LFP signal between 0.5-10 kHz.

Extracellular unit recordings were also obtained using tungsten electrodes with an impedance of 1.5 MΩ at 1 kHz (FHC Inc., Bowdoin, ME). Signals were amplified and filtered between 0.5-10 kHz.

Spike detection procedure

The extracellular recordings (sampling rate 20 kHz) were filtered (0.5-10 kHz) to remove the slow frequency signal component. For each filtered signal we chose a negative threshold of minus five standard deviations of the extracellular trace, to extract spikes. Each time the threshold was crossed, a corresponding point was assigned as time for action potential, and was then used to calculate the total firing rate. We computed the sequential histogram of firing rate (0.1s bin) on segments of one minute from the recordings performed with tungsten electrodes, and on 20 seconds segments from the multiunit activity recorded by means of Michigan probes. Multiunit activity was analyzed; no attempt was made to extract single units. We computed the total firing rate by summating the discharge frequency of all extracellularly recorded neurons.

Current source density analysis

LFPs from the 16-channel probes were used to calculate the current source density (CSD) of the spontaneous cortical activity, according to the original description (Mitzdorf 1985; Mitzdorf and Singer 1978). Briefly, the one-dimensional CSD was derived from the second spatial derivative of the LFP data using the following formula:

$$(\delta^2\Phi/\delta z^2)=[\Phi(z+n\Delta z) - 2\Phi(z) + \Phi(z-n\Delta z)]/(n\Delta z)^2$$

where Φ is the LFP at the vertical depth of the probe z, Δz is the inter-recording site distance (150 μm in the present study), and nΔz represents the differentiation grid (in our case n=1).

The CSD analyses were performed on periods of 10 seconds recorded during SWS. In CSD traces, current sinks are indicated by downward deflections and sources by upward deflections. To facilitate visualization of CSD profiles, we generated color image plots using linear interpolation along the depth axis.

Statistical analysis

Statistical significance of comparative data was assessed by performing Student's T-test. Differences between means were considered significant at $p<0.05$. Numerical data are represented as mean ± standard deviation (S. D.). All statistical tests were performed using statistical analysis software (SPSS 14.0, SPSS, Inc., Chicago, IL, USA).

11.5 Results

Progressively increased firing rate following undercut

We recorded depth EEG activity during wake and SWS at day 7 and day 56 following cortical deafferentation, in the relatively intact area 5 of cat cortex located in the anterior part of the suprasylvian gyrus and in the partially deafferented area 21, located in the posterior portion of the gyrus. Starting with day 7 we observed sparse ictal events in 6 out of the 7 cats, consisting in patches of spike-waves (SW) and poly-spike-waves (PSW) complexes, with a frequency of 3-4 Hz (Fig. 11-1A). These ictal events occurred intermittently during wake and SWS and were absent during REM sleep, as previously reported (Nita et al. 2007). Additionally, we observed that most of the ictal events in early stages of the undercut, were initiated in the anterior part of the suprasylvian gyrus, and were not always transmitted to the middle and posterior part of the gyrus (Fig. 11-1A, right panel, see rectangle), in agreement with previous data (Nita et al., 2007). At day 56 after deafferentation, the SW/PSW complexes occurred in all animals, were more numerous compared to early stages and spread to the whole suprasylvian gyrus (Fig. 11-1C).

Previous studies showed an increased frequency of ictal events during SWS compared to the other states of vigilance (Nita et al. 2007). Therefore, we first investigated whether the increased seizure activity during SWS was related to an increased total firing rate of neurons compared to waking state. We measured the spontaneous total firing rate of the extracellular multiunit activity during wake and SWS in the anterior and the posterior part of the suprasylvian gyrus. At 7 days after undercutting the brain, the spontaneous total firing frequency was similar during SWS and wake, although a trend towards increasing frequency during SWS was observed both in the anterior and the posterior parts of the suprasylvian gyrus (Fig. 11-1B), as also described in intact brain (Steriade et al. 2001). Equally, the number of spikes per minute was comparable between wake and SWS at day 7 (Fig. 11-1E). Conversely, in the late stages of the undercut, at day 56, the firing frequency and the number of spikes/minute increased significantly ($p < 0.05$, Student's T-test) during SWS compared to wake in both anterior and posterior suprasylvian gyrus (Fig. 11-1D, E). We also noted a reduction of the firing rate in the partially deafferented area 21 compared to the relatively intact area 5 during all states of vigilance, which became significant

(p<0.05, Student's T-test) at day 56 during waking state and was even more pronounced during SWS (p<0.01, Student's T-test) (Fig. 11-1).

In a previous study we observed a decreased density of deeply laying cortical neurons several weeks after deafferentation (Avramescu et al. 2007). Since neuronal network reorganization could have profound influence on the total firing rate of neurons located in different layer, we next analyzed the distribution of neuronal firing throughout the depth of the cortex.

Depth profile of neuronal firing

We used 16-channels Michigan probes, to record multiunit activity in the anterior and the posterior parts of the suprasylvian gyrus during wake and SWS, at 7, 28, 42 and 56 days respectively, following cortical deafferentation. In all recordings done at 7 days after undercut or earlier, the sequential histogram of summated discharge frequencies showed a relatively regular pattern of neuronal discharge during waking state, while during SWS the histograms revealed brief periods of high-frequency firing interrupted by silent periods corresponding to the hyperpolarizing phases of the slow oscillation. In early stages of the undercut (days 7 and 28), the mean total firing rate had the tendency to be higher during SWS compared to waking state, in the anterior, as well as in the posterior part of the undercut gyrus (Fig. 11-2, Fig. 11-4) and reached a statistical significant difference in late stages - day 42 and 56 (p<0.05, Student's T-test) (Fig. 11-3, Fig. 11-4). Note the higher variability (pointed up by the S.D. and the histograms of the summated firing rates) of the firing frequency during wake, in late stages of the undercut compared to early stages. This illustrates that the regular discharge pattern recorded during waking at day 7 was interrupted by periods of increased firing frequency at day 56, although the pattern remained more regular than the one characterizing SWS (Fig. 11-2, Fig. 11-3).

The laminar analysis of the firing rates computed at day 7 revealed the highest total firing rates in the deep cortical layers (positions 10-14) (Fig. 11-2A, Fig. 11-3A, Fig. 11-4A). This pattern of firing was maintained in the relatively intact anterior part of the suprasylvian gyrus even 56 days after cortical undercut (Fig. 11-2B). However, in the partially deafferentated part of the gyrus, during late stages of the undercut (days 42 and

56), the firing rate was diminished in the deep cortical layers (positions 11-16), while it was significantly increased (p<0.05, Student's T-test) more superficially in the cortex (positions 4-10) compared to the corresponding layers both from the anterior and the posterior parts of the gyrus in early stages (Fig. 11-3B, Fig. 11-4A). This finding is supported by our previous data showing an important neuronal degeneration in the deep cortical layers six weeks following deafferentation (Avramescu et al. 2007).

Similar to previous data in acute undercut cortex, in anesthetized cats (Topolnik et al. 2003a), the firing frequency was significantly diminished (p<0.05, Student's T-test) in the partially deafferented part of the gyrus compared to the relatively intact part in all states of vigilance, starting from day 7 after undercut. The differences were even more important (p<0.01, Student's T-test) at day 56 after deafferentation.

Remarkably, the spontaneous activity in the posterior part of the undercut cortex undergoes systematic changes: the spiking activity is high at 7 days after deafferentation, then the activity decreases at day 28 (p<0.05, Student's T-test) during both W and SWS and then increases again at day 42 and 56 to values much higher than in early stages (p<0.01, Student's T-test) and reaches a plateau (Fig. 11-4B). However, in the anterior part of the undercut the cortical firing activity at day 28 is similar to the one recorded at day 7, suggesting different morphological and/or electrophysiological changes in the two sites (Fig. 11-4B). This finding is congruent to our previous results, showing a decrease in seizure occurrence at 20-30 days after undercut, followed by an increase in the number of ictal events and reaching a plateau at about 50 days after isolation (Nita et al., 2007).

The increased total firing rate during SWS in chronically injured cortex could be produced either by a higher firing or bursting activity after undercut, or because previously silent neurons started to discharge.

Increased bursting during SWS in chronically injured cortex

The local field potentials recorded by Michigan probes in the posterior part of the undercut suprasylvian gyrus, in area 21, during SWS, were filtered between 0.5-10 kHz to identify multiunit activity and between 0-100 Hz to highlight the slow cortical activity. We then

matched the extracellular multiunit activity with the corresponding cortical oscillation for all the 16 recording sites of the probe. As expected, during SWS the neurons were firing during active states of the cortical slow oscillation and they were quiet throughout the silent periods. At day 56 after deafferentation, however, the instantaneous firing rate was increased at the beginning of the active periods during SWS, compared to the pattern of activity displayed during SWS at day 7 (Fig. 11-5B). This higher neuronal discharge during SWS was characterized by a higher number of spikes and a smaller interspike interval at day 56 than similar active periods recorded during SWS at day 7 (Fig. 11-5). The bursting activity could explain the increased firing rate recorded through the cortical depth in late stages of the undercut, during SWS (Fig. 11-2B, Fig. 11-3B, Fig. 11-4).

Laminar origin of the slow-oscillation in the undercut cortex

The cortical reorganization described anatomically in chronically deafferented cortex seamed to profoundly influence the cortical activity. Total neuronal firing in the deep cortical layers decreased, being instead augmented in the more superficial layers. The shift in firing distribution to the upper layers could be associated with a similar shift in the generation of spontaneous cortical activities, known to originate in the deep layers, in normal cortex (Sanchez-Vives and McCormick 2000; Volgushev et al. 2006).

The data recorded by Michigan probes during SWS was used to perform current-source-density (CSD) analysis in order to test whether cortical deafferentation could also influence the initiation of spontaneous cortical activity. The 16 site linear array silicone probes record voltages through the depth of the neocortex and allow calculating CSDs that are displayed as color contour plots. We compared the data from the anterior and the posterior parts of the suprasylvian gyrus at day 7, with the recordings from day 56 after undercut (Fig. 11-6A, Fig. 11-7A).

The CSD analysis performed at day 7 after undercut, both in the anterior and in the posterior parts of the suprasylvian gyrus, revealed strong current sinks originating in the lower cortical layers (usually positions 12-14) with corresponding current sources around them, which propagated upward to the more superficial layers (positions 5-7) (Fig. 11-6B, Fig. 11-7B, left panels). At day 56, the cortical slow oscillation appeared to be initiated

also, in the deep layers where current sinks and sources first originated and then spread to the upper layers (Fig. 11-6B, right panel).

Conversely, in the chronic stages of the undercut, CSD analysis of the data recorded in the posterior, partially deafferented part, of the suprasylvian gyrus shows a more superficial origin of the cortical slow oscillation (Fig. 11-7B, right panel). We identified very strong sinks in the more superficial cortical layers (positions 3-5) during active states, which then propagated downward, towards lower layers (Fig. 11-7B, right panel). We also noticed a decreased amplitude of the cortical activity recorded in the deeply inserted channels (positions 13-16) at day 56 compared to day 7 in the partially deafferented part of the gyrus (Fig. 11-7A, B, right panel).

SW/PSW ictal events at ~3Hz occurred in most of the recordings at day 7 (5 out of 7 cats) and in all of the recordings from day 56, both in the anterior and in the posterior parts of the undercut suprasylvian gyrus. At day 56, the ictal events occurred incessantly during SWS and the CSD analysis of the spontaneous cortical activity revealed that their origin was in the deep cortical layers of the anterior part, relatively intact, of the suprasylvian gyrus (Fig. 11-8A, B, left panel). However, the ictal events recorded in the posterior, partially deafferented, part of the gyrus at day 56 originated in the upper cortical layers, as demonstrated by CSD analysis, and then spread to deeper layers, matching the shift of increased neuronal discharge toward the superficial layers (Fig. 11-8A, B, right panel).

11.6 Discussion

In the present study we recorded spontaneous extracellular neuronal activity *in vivo*, during wake and natural sleep, in cats with partial deafferentation of the suprasylvian gyrus, to quantify the effects of chronic input deprivation on neuronal discharge pattern and on the initiation of cortical slow oscillation. We show a higher firing frequency during SWS than during wake in chronically undercut gyrus, which is apparent through the entire cortical depth in the anterior, relatively intact, part of the gyrus, while in the posterior, partially deafferented cortex, it only occurs in the superficial layers. The cortical firing activity in the posterior part of the undercut decreases 4 weeks after deafferentation compared to the first week and than increases again, reaching a plateau after 6 weeks. Additionally, we show that early after undercut, the spontaneous active states of the slow oscillation originate in deep cortical layers, similar to intact cortex, while in chronically deafferented posterior part of the gyrus, the spontaneous activity is initiated more superficialy and then it propagates to the deep layers.

Previous intracellular recordings from normal cortex during different states of vigilance showed that the relatively regular pattern of discharge during wake changed during SWS into an alternating pattern of brisk neuronal firing and silent state, but the mean firing frequency remained similar between wake and SWS (Steriade et al. 2001). In early stages of the undercut, we recorded a similar pattern of activity, regular discharge during wake and alternating active and silent phases during SWS, with an overall equal firing rate between the two behavioral states. However, in later stages of the undercut the multiunit extracellular recordings displayed a much higher total firing rate during SWS than during waking state, but also higher compared to the corresponding states of vigilance recorded several weeks earlier. Chronic input deprivation or activity blockade, as in the case of the deafferented suprasylvian gyrus, have been shown to trigger homeostatic plasticity mechanisms that dramatically enhance cortical excitability (Bausch et al. 2006; Burrone et al. 2002; Houweling et al. 2005; Murthy et al. 2001) in the attempt of restoring network activity to previous level (Davis and Bezprozvanny 2001; Turrigiano 1999). The increased excitability of the undercut cortex has various origins, from an increased efficacy of excitatory connections (Avramescu and Timofeev 2008; Jin et al. 2006) and axonal

sprouting (Salin et al. 1995), to a selective loss of inhibitory synapses (Ribak and Reiffenstein 1982), inhibitory neurons (Avramescu et al. 2007) and increased intrinsic neuronal responsiveness (Avramescu and Timofeev 2008; Bush et al. 1999; Prince and Tseng 1993).

The involvement of homeostatic plasticity mechanisms in triggering the increased posttraumatic cortical hyperexcitability is further sustained by the fact that we recorded a much higher total firing rate during SWS in chronically injured cortex, suggesting that the periods of disfacilitation characterizing SWS contributed to the up-regulation of neuronal excitability. Indeed, the extracellular multiunit activity revealed increased discharge frequency at precisely at the beginning of active states of the slow oscillation in the chronically deafferented gyrus, compared to early stages of the undercut when the silent phases were followed by tonic neuronal firing with lower frequency, as revealed by the longer inter-spike-interval. Our previous data from intracellular recordings in anesthetized animals also showed that longer hyperpolarizing periods correlated with the increased firing rate, starting one month after cortical deafferentation (Avramescu and Timofeev 2008). Furthermore, in chronic cortical undercut, hyperpolarization periods were described during wake and REM sleep, (Nita et al. 2007), when they don't normally appear (Timofeev et al. 2001).

The increase in total firing rate could be produced by (a) an increased in bursting activity, by (b) an increased tonic discharge frequency, and/or by (c) initiation of firing activity in previously silent neurons, following cortical deafferentation. (a) Previous data show that during natural states of vigilance, in intact cortex, the incidence of IB cells is only 4 % (Steriade et al. 2001). Conversely, there is an increased occurrence of bursting neurons in deafferented preparations (Timofeev et al. 2000) as well as a higher frequency of bursts in the undercut cortex (Nita et al. 2007). (b) Another possible factor that could contribute to the higher total firing rate after deafferentation is the increased tonic discharge frequency due to higher input resistance which increases the instantaneous firing rate of individual neurons in the undercut (Avramescu and Timofeev 2008). (c) In addition, silent neurons could start to discharge and thus, increase the total firing rate after injury. Upper layer cells rarely fire action potentials (Waters and Helmchen 2006, Chauvette et al. 2008).

However, we show here that neurons in the superficial layers already fire at 7 days following deafferentation and at day 56 there is a major increase in the total firing rate, supporting the assumption that indeed previous silent state could start to fire, in the attempt to restore neuronal activity to normal levels.

Our multiunit recordings revealed a reduced mean total firing rate in the partially deafferented cortex compared to the firing rate of the neurons in the relatively intact area 5, during the same states of vigilance. In addition, the depth profile of neuronal firing showed a significant reduction in the level of spontaneous firing particularly in the deep cortical layers in area 21 of the chronically deafferented gyrus compared to early stages of the undercut and to the neurons from area 5, less affected by the cortical undercut. Similarly, simultaneous recordings from the anterior and the posterior parts of the suprasylvian gyrus performed several hours after undercutting the brain showed a low level of spontaneous synaptic activity in area 21, while more efficient synaptic events were described in the relatively intact area 5 (Topolnik et al. 2003a). However, we previously demonstrate a progressively increased intrinsic excitability of neurons located in the deafferented cortex corresponding to the aggravation of paroxysmal activity (Avramescu and Timofeev 2008). In conclusion, we propose that indeed only relatively intact neurons in area 5, which display an increased spontaneous firing rate, may actually initiate ictal events following cortical deafferentation, but the increased intrinsic excitability and responsiveness of neurons located in the posterior part of the gyrus are essential for the rapid spread of seizure activity over the whole cortical surface.

We also show here a reduced spontaneous activity at day 28 following deafferentation, restricted to the posterior part of the undercut cortex. Thereafter, the activity increases again to much higher values than in early stages of brain trauma and reaches a plateau, confirming the progressive nature of epileptogenesis and suggesting different morphological and/or electrophysiological changes of neurons located in the two sites (anterior and posterior) of the undercut. These data are supported also by recordings in chronic neocortical slabs showing an increased spontaneous activity a few days after isolation (1.2-1.4 Hz), which decreased after about 10 days to 0.8-1 Hz and then increased again and became stable in chronic stages (Timofeev, unpublished data). In addition, in a

previous study we also found an increased network inhibition at 4 weeks after undercut (Avramescu and Timofeev, 2008), which coud explain the decreased activity as well as the transient decreased frequency of seizures described at about 3 weeks after isolation (Nita et al., 2007).

In a histological study of the undercut cortex we showed formerly a progressive degeneration of neurons, particularly inhibitory GABAergic, located in the deep cortical layers, which paralleled the gradual increased paroxysmal activity. The decreased density of the deeply laying neurons explains therefore, the changes in laminar distribution of neuronal firing recorded in the completely deafferented suprasylvian gyrus. This observation raises further questions regarding the initiation of the slow oscillation exclusively by neurons from deep cortical layers (Sanchez-Vives and McCormick 2000).

Our data from CSD analysis of the spontaneous slow oscillation during SWS, support the observation that slow oscillation usually originates in the deep cortical layers and then spreads toward the more superficial ones, when recordings were done in the anterior part of the deafferented suprasylvian gyrus. The slow oscillation recorded in the posterior, partially deafferented, part of the gyrus, was also initiated in the deep cortical layers only at early stages of the undercut. However, in more chronic stages, characterized by degeneration of the deeply laying neurons, the origin of the slow oscillation shifted towards more superficial layers, where the remaining neurons formed a more intact network. This observation has major theoretical consequences: the initiation of slow waves requires a large number of interconnected elements (Timofeev et al. 2000) and is not primarily based on intrinsic neuronal currents (Sanchez-Vives and McCormick 2000).

Previous studies showed that the epileptiform discharges produced by application of various pro-convulsant drugs *in vitro* and *in vivo*, as well as the SW activity in a genetic model of absence seizures, have a preferential laminar susceptibility of epileptiform activity, particularly at a depth corresponding to layer V (Chatt and Ebersole 1982; Ebersole and Chatt 1985; Pockberger et al. 1984; Polack et al. 2007). Several factors have been incriminated, such as the presence of burst-generating neurons within middle cortical layers that serve to synchronize large populations of cortical cells in layers V and II-III (Connors 1984; Gutnick et al. 1982), or the existence of large pyramidal neurons with

specific firing pattern such as post-hyperpolarization excitation (Spain et al. 1991), or an increased synaptic excitability mediated by NMDA receptors (D'Antuono et al. 2006). Also, a higher intrinsic neuronal excitability (Polack et al. 2007) possibly due to altered hyperpolarization-activated cation current (I_h) which could promote bursting activity (Zhang et al. 2003), or a decrease of synaptic inhibition with increased excitation (Yang et al. 2007) could be responsible for the higher propensity of layer V neurons to initiate seizure activity. However, in all these studies the cortical input was presumably intact or, the recordings were performed in acute conditions after trauma. Therefore, the effects of long term input deprivation and/or that of neuronal degeneration was overlooked. Here, we show that indeed the ictal events recorded in the relatively intact gyrus originated indeed in the deep layers, both in early and more chronic stages of the undercut. However, after chronic undercut, the seizure activity recorded in the partially deafferented cortex originated in the superficial cortical layers.

In conclusion, following penetrating cortical wounds, the firing rate of deeply laying neurons is decreased, while it increases in the more superficial layers. As a consequence, the laminar initiation of spontaneous activity is altered and the cortical slow oscillation is generated in the upper cortical layers.

11.7 Acknowledgements

11.8 References

Annegers JF, Grabow JD, Groover RV, Laws ER, Jr., Elveback LR, and Kurland LT. Seizures after head trauma: a population study. *Neurology* 30: 683-689, 1980.

Antognini JF, and Carstens E. In vivo characterization of clinical anaesthesia and its components. *British journal of anaesthesia* 89: 156-166, 2002.

Avramescu S, Nita DA, and Timofeev I. Loss of excitatory and inhibitory neurons accompanies cortical post-traumatic epilepsy. *Program No 16415/Z17 2007 Neuroscience Meeting Planner San Diego, CA: Society for Neuroscience, 2007 Online* 2007.

Avramescu S, and Timofeev I. Synaptic strength modulation following cortical trauma: a role in epileptogenesis. *J Neurosci (in press)* 2008.

Bausch SB, He S, Petrova Y, Wang XM, and McNamara JO. Plasticity of both excitatory and inhibitory synapses is associated with seizures induced by removal of chronic blockade of activity in cultured hippocampus. *JNeurophysiol* 96: 2151-2167, 2006.

Burrone J, O'Byrne M, and Murthy VN. Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* 420: 414-418, 2002.

Bush PC, Prince DA, and Miller KD. Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. *JNeurophysiol* 82: 1748-1758, 1999.

Castro-Alamancos MA, and Borrell J. Contribution of NMDA and nonNMDA glutamate receptors to synchronized excitation and cortical output in the primary motor cortex of the rat. *Brain research bulletin* 37: 539-543, 1995.

Chatt AB, and Ebersole JS. The laminar sensitivity of cat striate cortex to penicillin induced epileptogenesis. *Brain research* 241: 382-387, 1982.

Chauvette S, Volgushev M, and Timofeev I. Origin of active states in local neocortical networks during slow sleep oscillation. *Cereb Cortex (in revision)* 2008.

Connors BW. Initiation of synchronized neuronal bursting in neocortex. *Nature* 310: 685-687, 1984.

Contreras D, and Steriade M. Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci* 15: 604-622, 1995.

D'Antuono M, Inaba Y, Biagini G, D'Arcangelo G, Tancredi V, and Avoli M. Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures. *Genes, brain, and behavior* 5: 73-84, 2006.

Davis GW, and Bezprozvanny I. Maintaining the stability of neural function: a homeostatic hypothesis. *AnnuRevPhysiol* 63: 847-869, 2001.

Ebersole JS, and Chatt AB. Differences between strychnine and penicillin epileptogenesis suggest a laminar organization of neocortical inhibition. *Brain research* 340: 390-396, 1985.

Esplin MS, Abbott JR, Smart ML, Burroughs AF, Frandsen TC, and Litzinger MJ. Voltage-sensitive calcium channel development in epileptic DBA/2J mice suggests altered presynaptic function. *Epilepsia* 35: 911-914, 1994.

Gloor P, Quesney LF, and Zumstein H. Pathophysiology of generalized penicillin epilepsy in the cat: the role of cortical and subcortical structures. II. Topical application of penicillin to the cerebral cortex and to subcortical structures. *Electroencephalography and clinical neurophysiology* 43: 79-94, 1977.

Gutnick MJ, Connors BW, and Prince DA. Mechanisms of neocortical epileptogenesis in vitro. *JNeurophysiol* 48: 1321-1335, 1982.

Houweling AR, Bazhenov M, Timofeev I, Steriade M, and Sejnowski TJ. Homeostatic synaptic plasticity can explain post-traumatic epileptogenesis in chronically isolated neocortex. *CerebCortex* 15: 834-845, 2005.

Jacobs KM, Graber KD, Kharazia VN, Parada I, and Prince DA. Postlesional epilepsy: the ultimate brain plasticity. *Epilepsia* 41 Suppl 6: S153-S161, 2000.

Jin X, Prince DA, and Huguenard JR. Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. *J Neurosci* 26: 4891-4900, 2006.

Kwan P, and Brodie MJ. Early identification of refractory epilepsy. *The New England journal of medicine* 342: 314-319, 2000.

Li H, Bandrowski AE, and Prince DA. Cortical injury affects short-term plasticity of evoked excitatory synaptic currents. *JNeurophysiol* 93: 146-156, 2005.

Li H, and Prince DA. Synaptic activity in chronically injured, epileptogenic sensory-motor neocortex. *JNeurophysiol* 88: 2-12, 2002.

Mahon S, Deniau JM, and Charpier S. Relationship between EEG potentials and intracellular activity of striatal and cortico-striatal neurons: an in vivo study under different anesthetics. *Cereb Cortex* 11: 360-373, 2001.

Mitzdorf U. Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. *Physiological reviews* 65: 37-100, 1985.

Mitzdorf U, and Singer W. Basic patterns of synaptic activity in visual cortex of normal and monocularly deprived cats: a current source density analysis of electrically evoked potentials [proceedings]. *J Physiol* 284: 120P, 1978.

Murthy VN, Schikorski T, Stevens CF, and Zhu Y. Inactivity produces increases in neurotransmitter release and synapse size. *Neuron* 32: 673-682, 2001.

Nita DA, Cisse Y, Timofeev I, and Steriade M. Increased propensity to seizures after chronic cortical deafferentation in vivo. *JNeurophysiol* 95: 902-913, 2006.

Nita DA, Cisse Y, Timofeev I, and Steriade M. Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. *Cereb Cortex* 17: 272-283, 2007.

Pockberger H, Rappelsberger P, and Petsche H. Penicillin-induced epileptic phenomena in the rabbit's neocortex II. Laminar specific generation of interictal spikes after the application of penicillin to different cortical depths. *Brain research* 309: 261-269, 1984.

Polack PO, Guillemain I, Hu E, Deransart C, Depaulis A, and Charpier S. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J Neurosci* 27: 6590-6599, 2007.

Prince DA, and Tseng GF. Epileptogenesis in chronically injured cortex: in vitro studies. *JNeurophysiol* 69: 1276-1291, 1993.

Ribak CE, and Reiffenstein RJ. Selective inhibitory synapse loss in chronic cortical slabs: a morphological basis for epileptic susceptibility. *Can J Physiol Pharmacol* 60: 864-870, 1982.

Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, and Dillon JD. Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. *Neurology* 35: 1406-1414, 1985.

Salin P, Tseng GF, Hoffman S, Parada I, and Prince DA. Axonal sprouting in layer V pyramidal neurons of chronically injured cerebral cortex. *J Neurosci* 15: 8234-8245, 1995.

Sanchez-Vives MV, and McCormick DA. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nature neuroscience* 3: 1027-1034, 2000.

Spain WJ, Schwindt PC, and Crill WE. Post-inhibitory excitation and inhibition in layer V pyramidal neurones from cat sensorimotor cortex. *J Physiol* 434: 609-626, 1991.

Steriade M, and Contreras D. Spike-wave complexes and fast components of cortically generated seizures. I. Role of neocortex and thalamus. *JNeurophysiol* 80: 1439-1455, 1998.

Steriade M, Nunez A, and Amzica F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci* 13: 3252-3265, 1993.

Steriade M, Timofeev I, and Grenier F. Natural waking and sleep states: a view from inside neocortical neurons. *JNeurophysiol* 85: 1969-1985, 2001.

Temkin NR. Antiepileptogenesis and seizure prevention trials with antiepileptic drugs: meta-analysis of controlled trials. *Epilepsia* 42: 515-524, 2001.

Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, and Steriade M. Origin of slow cortical oscillations in deafferented cortical slabs. *CerebCortex* 10: 1185-1199, 2000.

Timofeev I, Grenier F, and Steriade M. Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. *Proc Natl Acad Sci U S A* 98: 1924-1929, 2001.

Topolnik L, Steriade M, and Timofeev I. Hyperexcitability of intact neurons underlies acute development of trauma-related electrographic seizures in cats in vivo. *Eur J Neurosci* 18: 486-496, 2003a.

Topolnik L, Steriade M, and Timofeev I. Partial cortical deafferentation promotes development of paroxysmal activity. *CerebCortex* 13: 883-893, 2003b.

Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci* 22: 221-227, 1999.

Volgushev M, Chauvette S, Mukovski M, and Timofeev I. Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations [corrected]. *J Neurosci* 26: 5665-5672, 2006.

Waters J, and Helmchen F. Background synaptic activity is sparse in neocortex. J Neurosci 26: 8267-8277, 2006.

Yang L, Benardo LS, Valsamis H, and Ling DS. Acute injury to superficial cortex leads to a decrease in synaptic inhibition and increase in excitation in neocortical layer V pyramidal cells. *JNeurophysiol* 97: 178-187, 2007.

Zhang Y, Oliva R, Gisselmann G, Hatt H, Guckenheimer J, and Harris-Warrick RM. Overexpression of a hyperpolarization-activated cation current (Ih) channel gene modifies the firing activity of identified motor neurons in a small neural network. *J Neurosci* 23: 9059-9067, 2003.

11.9 Figures

A

Day 7 Wake

Undercut Anterior

Undercut Posterior

EMG

Day 7 SWS

0.5 mV

1 s

B

Day 7 Wake Anterior

Day 7 SWS Anterior

Day 7 Wake Posterior

Day 7 SWS Posterior

C

Day 56 Wake

Undercut Anterior

Undercut Posterior

EMG

Day 56 SWS

0.5 mV

1 s

D

Day 56 Wake Anterior

Day 56 SWS Anterior

Day 56 Wake Posterior

Day 56 SWS Posterior

E

Undercut Anterior

Undercut Posterior

Wake
SWS

Day 7   Day 56

Day 7   Day 56

Figure 11-1. Modulation of ictal events by the state of vigilance in early and chronic undercut. Panels A) and C) depict the LFP in the anterior and posterior parts of the undercut, during wake (left panels) and SWS (right panels), at 7 and 56 days following the cortical deafferentation. Rectangle in panel A (right), indicates that in early stages of the undercut not all epileptiform discharges initiated in the relatively intact part of the suprasylvian gyrus are transmitted to the partially deafferented part. Panels B) and D) contain filtered LFP (0.5-10 kHz) and the sequential histograms (0.1 s bin) of firing rate (ordinate in Hz), corresponding to the spontaneous cortical activity depicted in A) and C). E) Global quantifications of the mean firing rate, calculated as the number of spikes/minute, in the anterior and posterior parts of the deafferented cortex during wake and SWS. Note an increased firing rate in anterior (intact) compared to posterior undercut (more deafferented) further enhanced by the SWS. +, $p<0.05$, ++, $p<0.01$ (comparison between day 7 and day 56); **, $p<0.01$ (comparison between Wake and SWS).
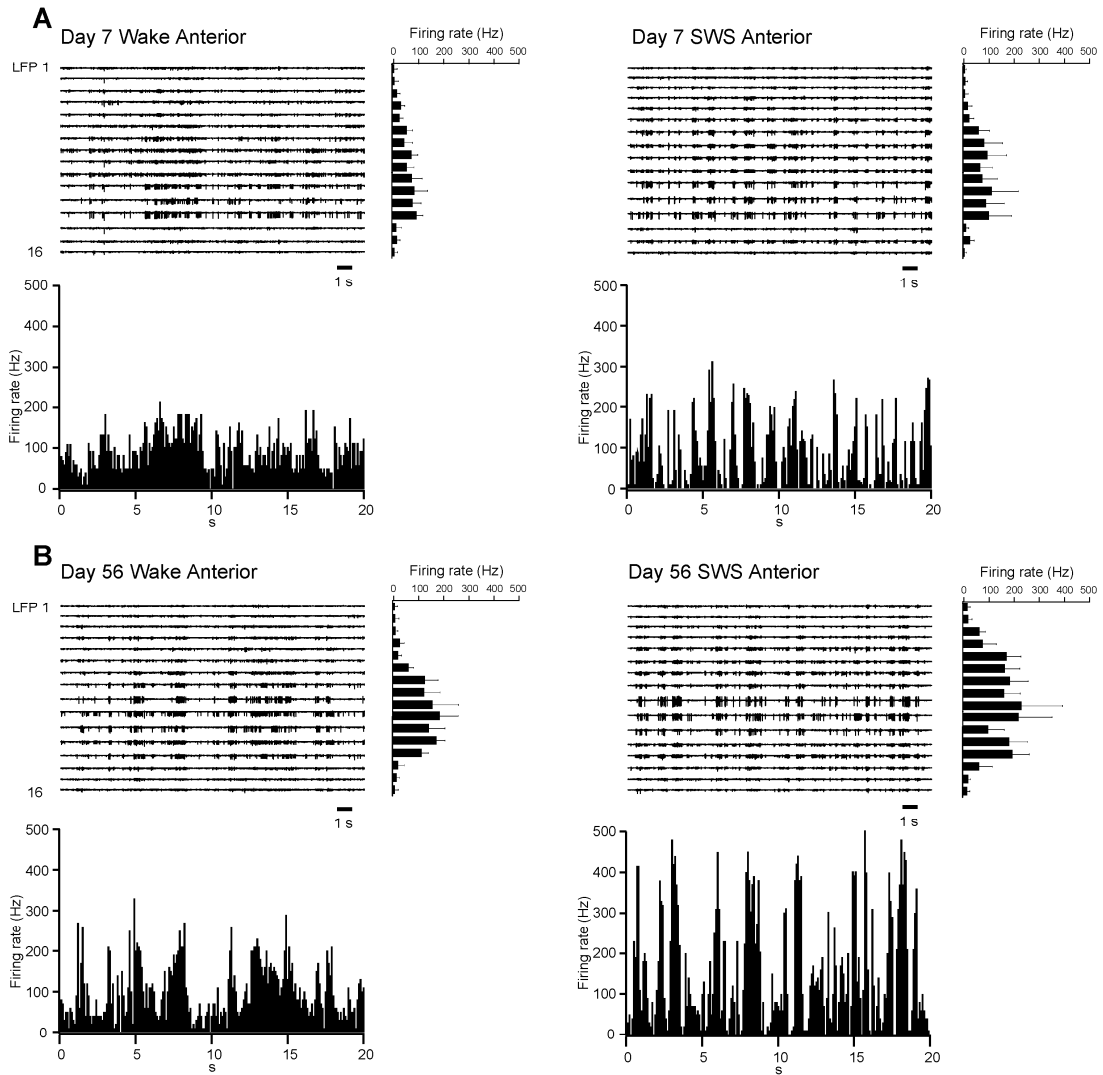
Figure 11-2. Laminar profile of multi-unit activities in the anterior part of the undercut in A) early and B) chronic stages. Panel A) contains filtered LFP (0.5-10 kHz) during wake and SWS at 7 days following the undercut together with the mean rate of unit activities. Panel B) contains filtered LFP (0.5-10 kHz) during wake and SWS at 56 days following the cortical deafferentation. The corresponding histograms of summated firing rate (ordinate in Hz) are depicted in the bottom panels (0.1 s bin) and the laminar distribution of the mean firing rate is shown on the right of each panel containing LFP recordings. Bars indicate S.D.
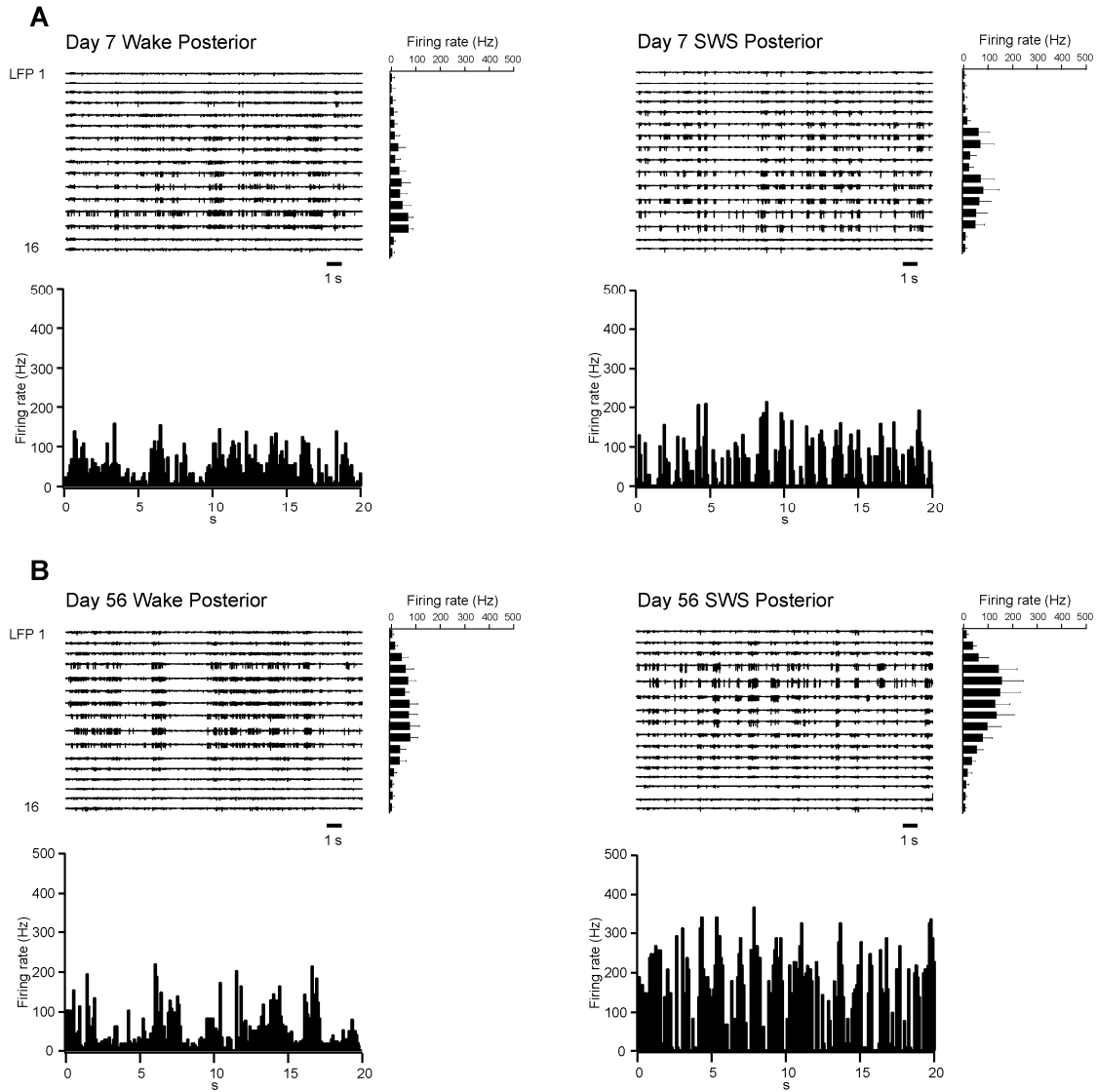
Figure 11-3. Laminar profile of multi-unit activities in the posterior part of the undercut in A) early and B) chronic stages. Panel A) contains filtered LFP (0.5-10 kHz) during wake and SWS at 7 days following the undercut together with the mean rate of unit activities. Panel B) contains filtered LFP (0.5-10 kHz) during wake and SWS at 56 days following the cortical deafferentation. The corresponding histograms of summated firing rate (ordinate in Hz) are depicted in the bottom panels (0.1 s bin) and the laminar distribution of the mean firing rate is shown on the right of each panel containing LFP recordings. Note the higher frequency of discharge in the superficial layers of the chronically deafferented gyrus. Bars indicate S.D.
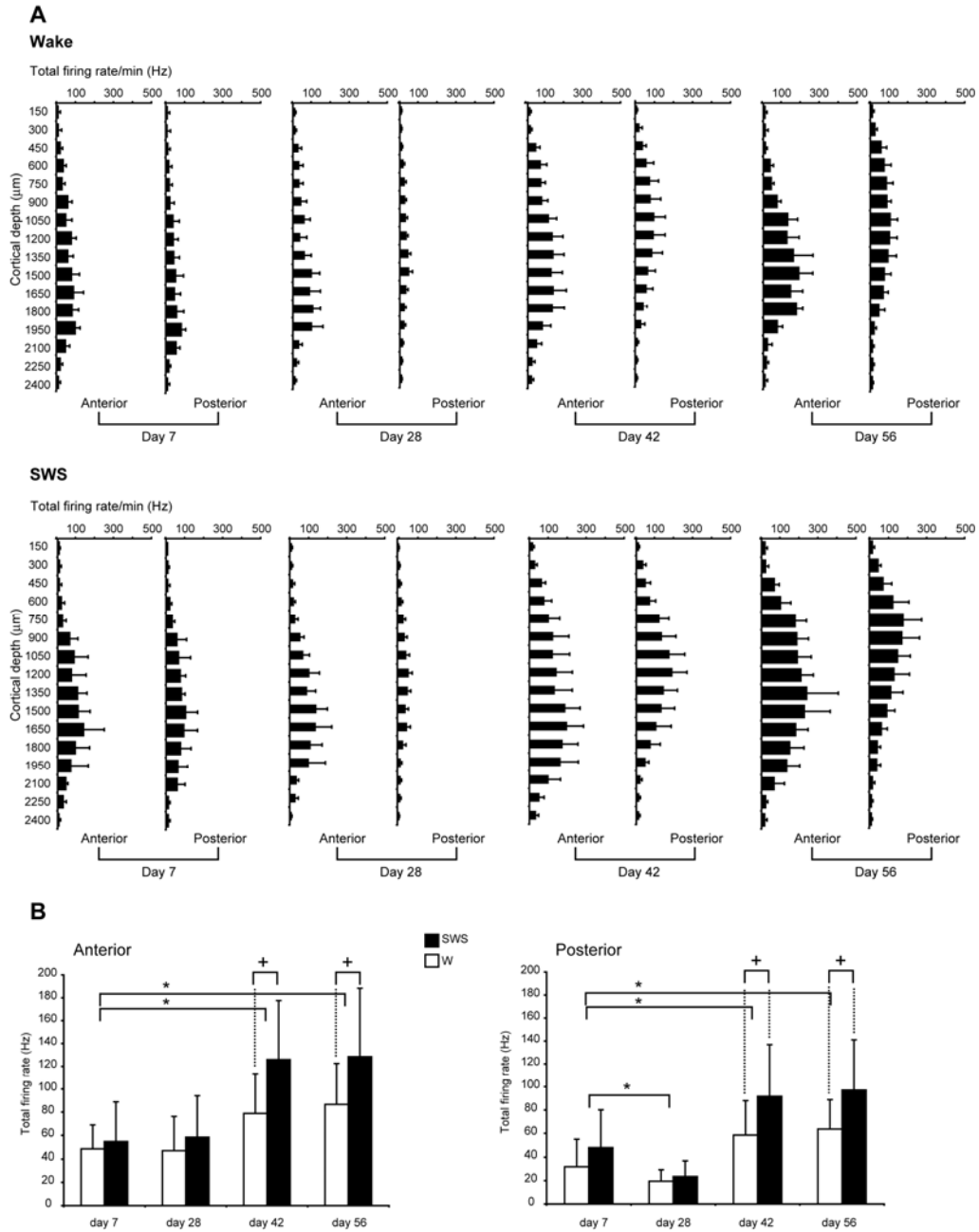
Figure 11-4. The dynamics of the total neuronal firing rate following cortical deafferentation during wake and SWS. A) The histograms of total firing rate (ordinate in Hz) depict the laminar distribution of firing rate during wake (upper panels) and during SWS (lower panels), at different time delays after undercut (days 7, 28, 42, 56). Note the decreased firing rate at day 28 and the shift of neuronal discharge towards the superficial layers at days 42 and 56 in the posterior part of the deafferented gyrus. B) The total firing rate following cortical deafferentation has a different distribution in the anterior (left panel) and in the posterior (righr panel) parts of the undercut, both during wake (open bars) and during SWS (black bars). Note the higher firing rate during SWS compared to wake in all groups.*, p<0.05 (days 28, 42 and 56 compared to day 7); +, p<0.05 (SWS compared to Wake). Bars indicate S.D.
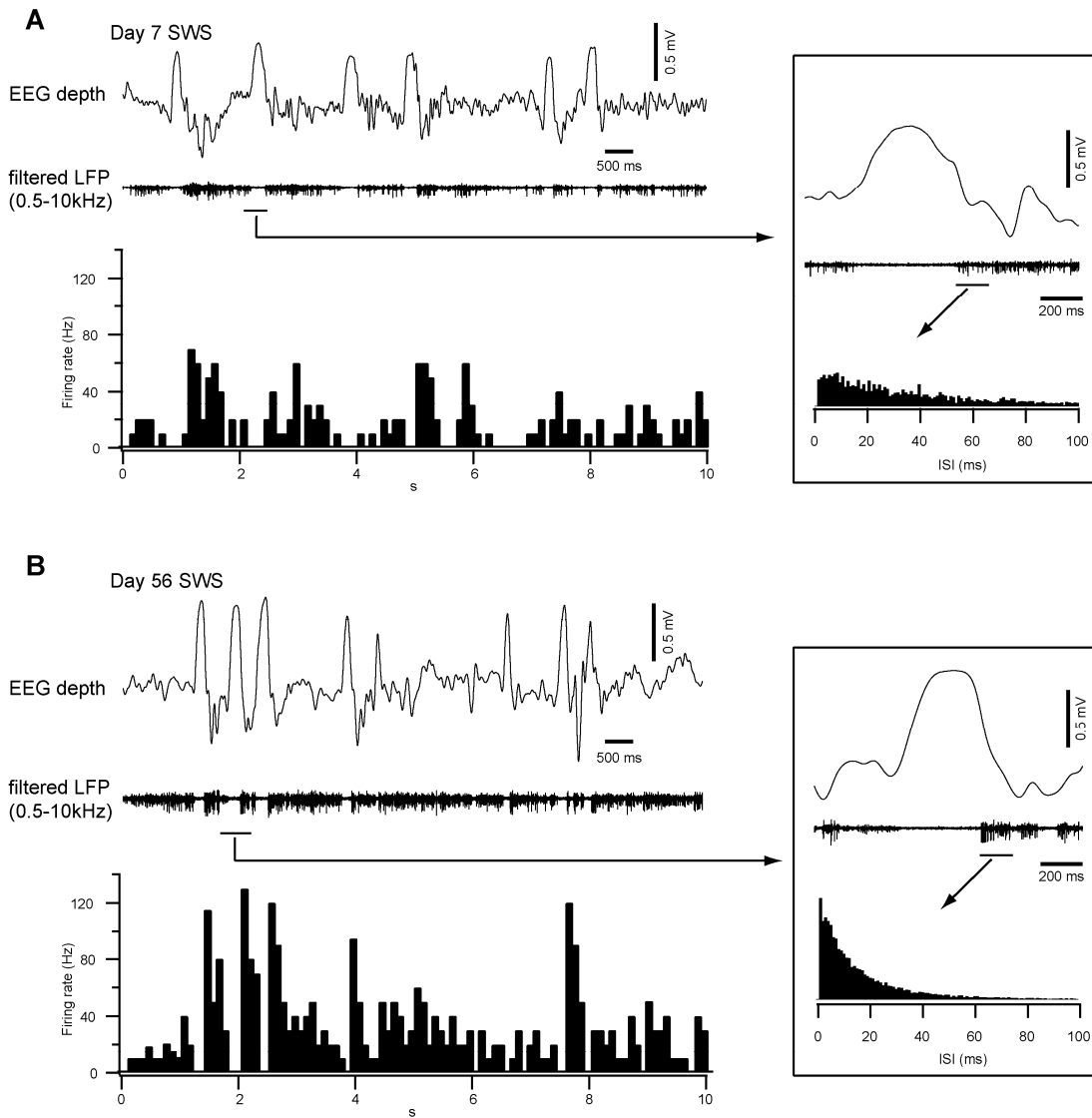
Figure 11-5. Higher discharge rate at the beginning of the active phase of the slow oscillation, in chronic undercut. Panel A) depicts the early stages of the undercut (7 days) during SWS. The filtered LFP (0.5-10 kHz) demonstrates the occurrence of unit discharges during the depth-negative phase of the slow-oscillation. In the inset at right inter-spike interval calculation (ISI) shows a tonic discharge during the active phase of the cortical slow-oscillation. Panel B) contains data from the chronic stages of the undercut (day 56) during SWS. An increased firing rate is observed together with the occurrence of clusters of action potentials suggesting bursting activity (peak of ISI between 2.5-5 ms) following the periods of disfacilitation characterizing the silent phase of the slow-oscillation, as depicted in the inset on the right.
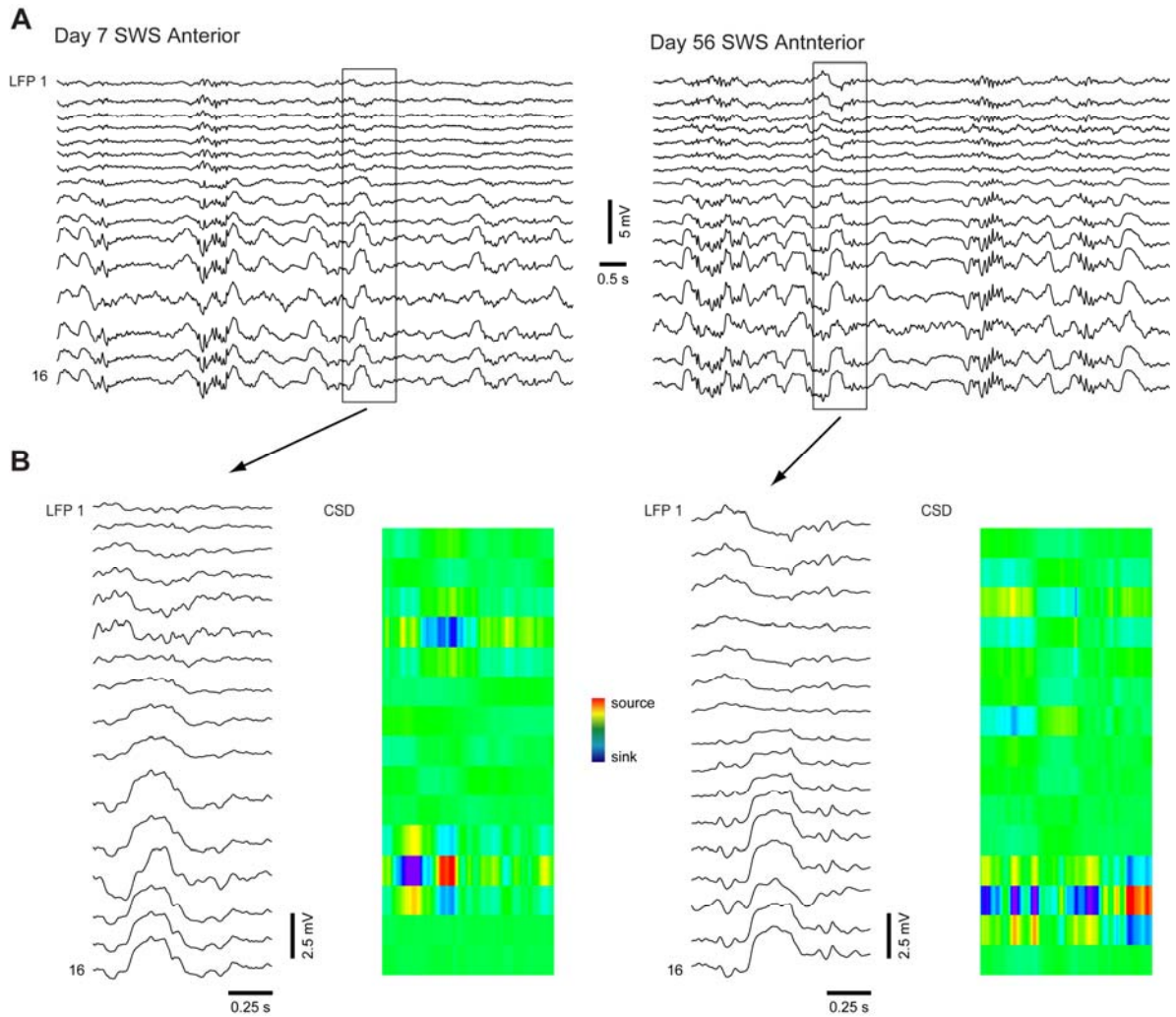
Figure 11-6. Local field-potentials (LFP) and current-source density (CSD) analysis of the slow oscillation in the anterior undercut in early and chronic stages. Panel A) contains the LFP recordings at 7 (left panel) and 56 days (right panel) following cortical deafferentation. The segment indicated by the rectangle is expanded in B) and the CSD analysis of the same segment is depicted on the right. Note that the spontaneous cortical activity is initiated in the deep cortical layers and then spreads to the upper ones.
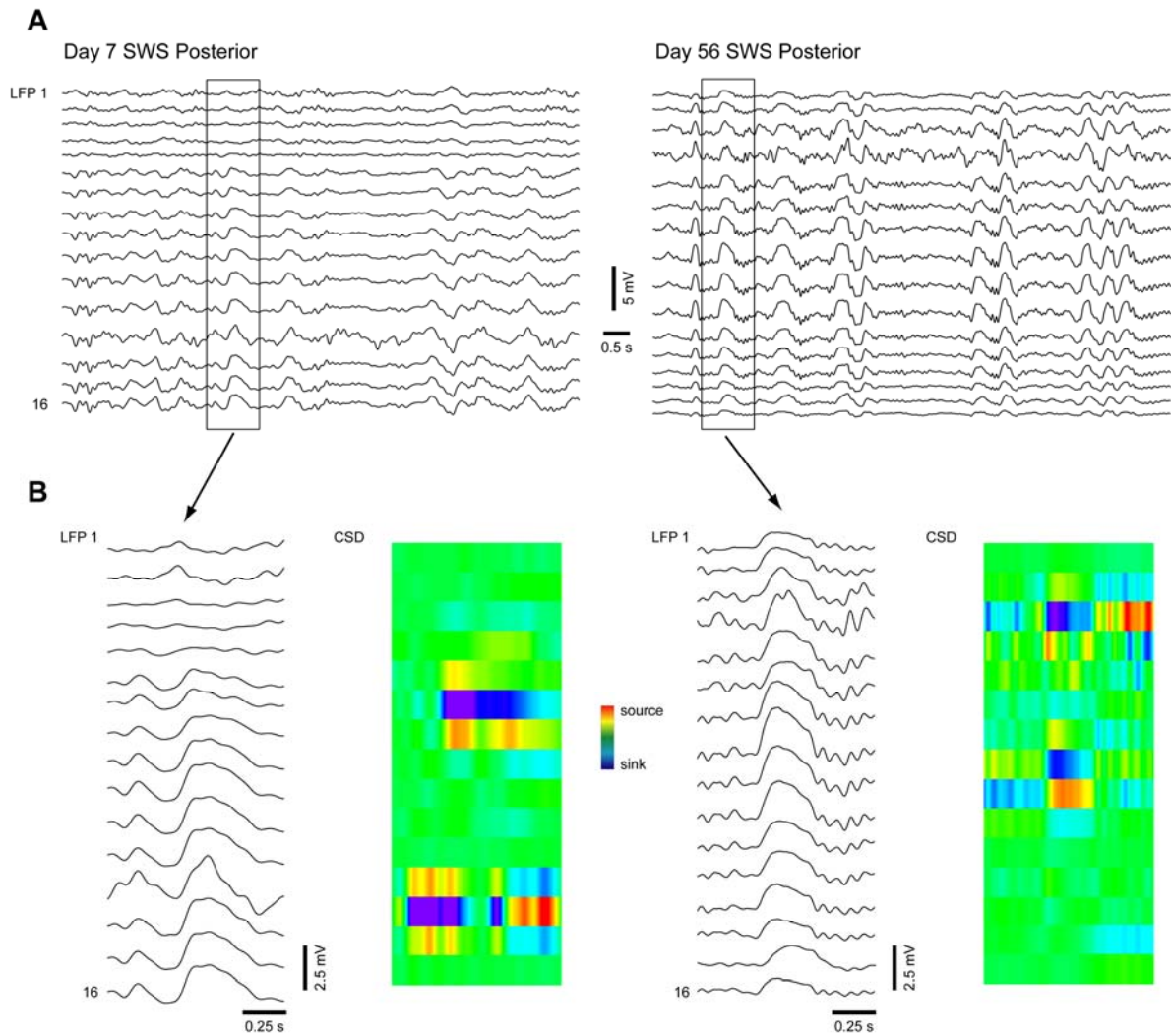
Figure 11-7. Local field-potentials (LFP) and current-source density (CSD) analysis of the slow oscillation in the posterior undercut in early and chronic stages. Panel A) contains the LFP recordings at 7 (left panel) and 56 days (right panel) following cortical deafferentation. The segment indicated by the rectangle is expanded in B) and the CSD analysis of the same segment is depicted on the right. Note that the spontaneous cortical activity is initiated in the deep cortical layers only at day 7, while at day 56 it is shifted towards the more superficial layers.
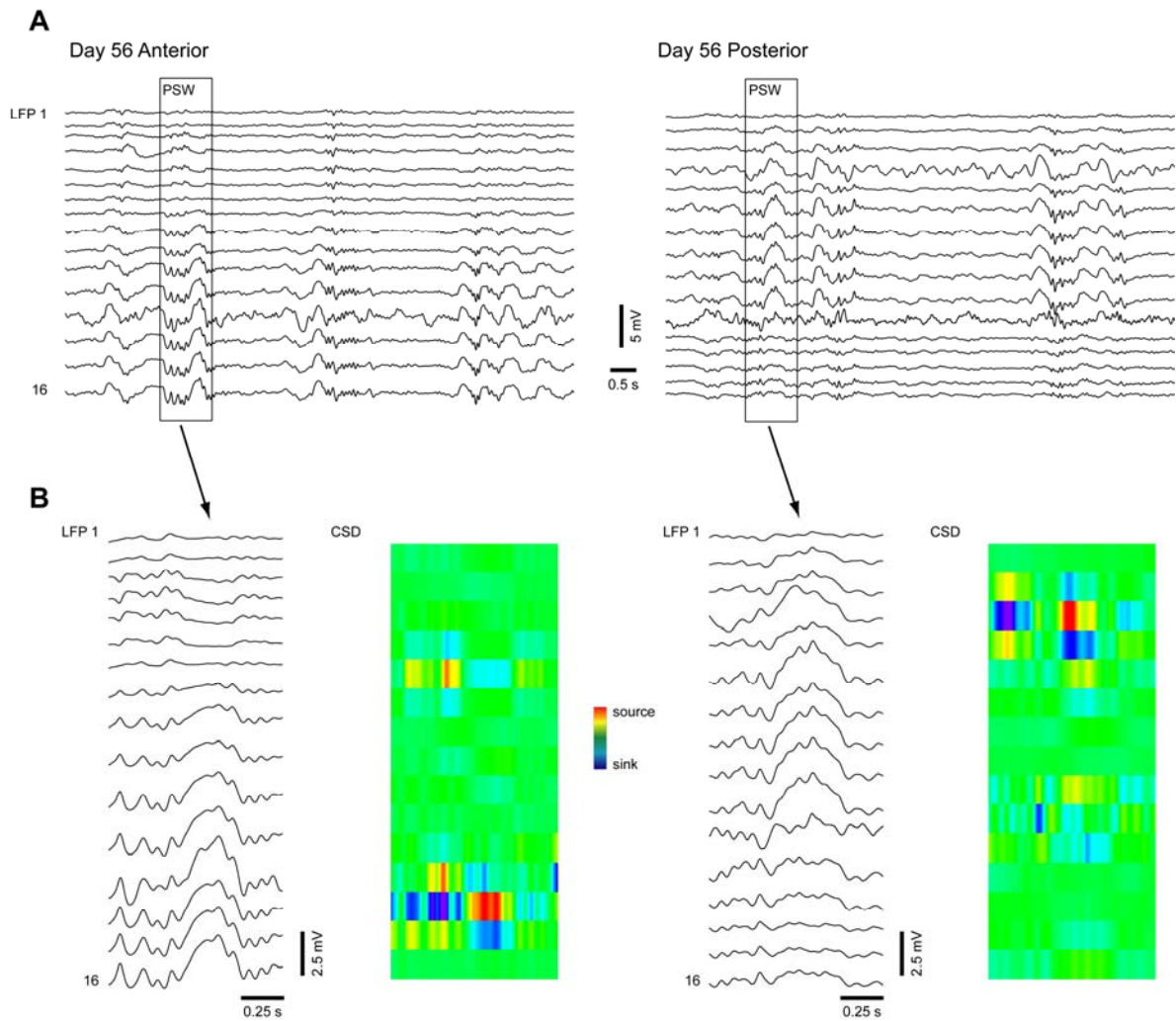
Figure 11-8. Local field-potentials (LFP) and current-source density (CSD) analysis of the paroxysmal activity in the anterior and posterior undercut. Panel A) contains the LFP recordings at day 56 from the anterior (left panel) and the posterior (right panel) parts of the deafferented gyrus. The segment indicated by the rectangle, representing an example of PSW, is expanded in B) and the CSD analysis of the same segment is depicted on the right. Note that the PSW activity is initiated in the deep cortical layers in the relatively intact area of the undercut gyrus, while it originates in the more superficial layers, in the partially deafferented part of the gyrus.

12 Posttraumatic reactive gliosis is associated with paroxysmal activity and impaired potassium clearance

Sinziana Avramescu, Dragos A. Nita and Igor Timofeev

12.1 Résumé

La fonction des astrocytes réactifs est altérée suite à divers traumatismes crâniens, ce qui mène à une dérégulation du potassium (K^+) et à une excitabilité neuronale accrue. Nous avons entrepris cette étude afin d'examiner si les traumatismes corticaux de nature pénétrante sont associés à une dérégulation de la concentration extracellulaire du potassium ($[K^+]_o$) lié à l'altération des propriétés des cellules gliales réactives pendant le sommeil naturel et lors de crises épileptiques. Nos expériences ont été effectuées chez des chats non-anesthésiés avec isolation partielle du gyrus suprasylvien, un modèle d'épilepsie post-traumatique bien établi. Des enregistrements polygraphiques (EEG, EOG, EMG) ainsi qu'une détection de la $[K^+]_o$ par des microélectrodes spécifiques ont été exécutés pendant différents états de vigilance. Tous les animaux ayant subit une déafférentation chronique partielle corticale ont développés des activités EEG paroxystiques dans un délai d'un mois à compter du moment de l'isolation corticale. L'incidence des crises épileptiques était maximale pendant le sommeil à ondes lentes (SWS), a diminué pendant l'état de veille et était minimale ou absente pendant le sommeil à mouvements oculaires rapides (REM). Pendant le SWS, la $[K^+]_o$ était diminuée lors des états silencieux et augmentée durant les phases actives de l'oscillation corticale lente, autant dans le cortex déafferenté que dans le cortex intact, et a également augmenté pendant les crises spontanées d'épilepsie de type pointe-onde. En plus, lors de la transition entre le sommeil SWS et REM, nous avons observé une concentration augmentée et une période d'enlèvement du K^+ extracellulaire rallongée dans le gyrus déafferenté comparé au cortex contra-latéral intact. Nos données démontrent un enlèvement du K^+ globalement altéré dans le cortex partiellement isolé, en particulier pendant les transitions entre SWS et sommeil REM, ce qui pourrait être le résultat de l'activité gliale altérée suite à des traumatismes corticaux.

12.2 Abstract

Previous studies have documented an impaired function of the reactive astrocytes following various brain injuries, leading to dysfunctional clearance of potassium (K^+) and increased excitability. We undertook this study in order to test whether penetrating cortical wounds are associated with abnormal regulation of extracellular K^+ concentration ($[K^+]_o$) due to altered properties of reactive glial cells, during natural sleep and seizures. Experiments were performed in non-anesthetized cats with partially isolated suprasylvian gyrus, a well-know model of posttraumatic epilepsy. Polygraphic recordings (EEG, EOG, EMG) together with a detection of $[K^+]_o$ by K^+-sensitive microelectrodes were performed during different states of vigilance. All animals with chronic partially deafferented cortex developed paroxysmal EEG activities within one month after cortical undercut. The incidence of seizures was maximal during slow wave sleep (SWS), decreased during wake (W) and was minimal or absent during rapid eye movement (REM) sleep. Throughout SWS $[K^+]_o$ decreased during silent states and increased during active phases of the cortical slow oscillation, both in control and intact cortex, and was also raised during spontaneous spike-wave seizures. Additionally, during the transition between SWS and REM sleep, we found an increased concentration and clearance time of extracellular K^+ in the undercut gyrus compared to the intact contralateral cortex. Our data show an overall impaired clearance of K^+ in the undercut cortex, particularly during the transition between SWS to REM sleep, which could originate in the impaired glial activity described in the injured brain.

12.3 Introduction

Posttraumatic epilepsy has an incidence of 53% after penetrating brain injuries (Salazar et al., 1985) and accounts for up to 12-13% of the epilepsies with a clearly defined cause (Annegers, 1994). Epilepsy is often accompanied by massive reactive gliosis (Kim, 2001; Blumcke *et al.*, 2002; de Lanerolle & Lee, 2005), which is actually a common feature in virtually all insults to the central nervous system (D'Ambrosio, 2004). Alterations in astrocytic properties have been described both in human temporal lobe epilepsy (Heinemann et al., 2000) and in post-traumatic epilepsy (D'Ambrosio et al., 1999; Samuelsson et al., 2000). Although the significance of this alteration is poorly understood, recent findings suggest that modified astroglial functioning might have an important role in the generation and spread of seizure activity (D'Ambrosio et al., 1999; Schroder et al., 1999; Kivi et al., 2000; Bordey et al., 2001). Previous studies have emphasized the essential role of glial cells in regulating extracellular potassium concentration ($[K^+]_o$) (Orkand et al., 1966; Somjen, 1979), as well as the fact that an increased $[K^+]_o$ can augment neuronal excitability and thereby contribute to the initiation and spread of seizure activity (Rutecki *et al.*, 1985; Korn *et al.*, 1987; Traynelis & Dingledine, 1988). It is therefore, counterintuitive that disturbances of glial properties could facilitate extracellular accumulation of K^+ and perturb normal function of neuronal network. However, no information is available on the relationship between $[K^+]_o$, different states of vigilance and seizure activity in the neocortex. So far, most of the data on K^+ variations in sleep and epilepsy were obtained in non-physiological conditions, such as using electrical stimulation and/or different anesthetics or pro-convulsive drugs.

Partially isolated neocortex is a well-known model of posttraumatic epilepsy (Prince & Tseng, 1993; Hoffman *et al.*, 1994; Jacobs *et al.*, 2000; Topolnik *et al.*, 2003a; Nita *et al.*, 2007). The undercut cortex becomes increasingly hyperexcitable over a few weeks and progressively generates paroxysmal activity both *in vitro* (Prince & Tseng, 1993; Jacobs *et al.*, 2000; Li & Prince, 2002; Li *et al.*, 2005; Jin *et al.*, 2006) and *in vivo* (Topolnik *et al.*, 2003b, a; Nita *et al.*, 2006; Nita *et al.*, 2007). The incidence of ictal events following cortical deafferentation depends on the state of vigilance, occurring during the waking state, being enhanced during SWS, and absent during REM sleep (Nita et al., 2007). However,

there is no data on the exact frequency and duration of spike-wave (SW) seizures during each state of vigilance, nor on the possible influence of seizure activity on sleep architecture, in cortical posttraumatic epilepsy. In humans, both frontal and temporal lobe epilepsy are associated with a decrease of sleep efficiency index, a decrease in the percentage of REM sleep and an increase percentage of the stage 2 SWS (Touchon et al., 1991; Crespel et al., 1998; Crespel et al., 2000).

Earlier *in vivo* studies showed an enhancement of $[K^+]_o$ at the transition between SWS and REM sleep in the reticular formation (Satoh et al., 1979), the lateral geniculate nucleus (Satoh et al., 1982) and the hippocampus of cats (Satoh et al., 1991). Therefore, it is important to investigate whether this increased $[K^+]_o$ at the transition SWS-REM could also be recorded in the cerebral cortex and whether it is changed in the undercut cortex during epileptogenesis.

We undertook this study to test whether dysfunctional reactive astrocytes following cortical undercut are associated with an impaired K^+ clearance and whether this disturbance of $[K^+]_o$ could alter the different states of vigilance, in behaving epileptic cats.

12.4 Materials and methods

Ethical approval

All experimental procedures were performed in accordance with the guidelines published by National Institutes of Health for the Care and Use of Laboratory Animals, and all experimental procedures 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.

Surgery and electrode implantation

Experiments were performed on 5 male cats in chronic conditions. All 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 the electroencephalogram (EEG) and cardiac frequency (aiming 90-110 beats/min). Oxygen saturation of the arterial blood and end-tidal CO_2 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 37–38°C with a heating pad.

The cerebral cortex was exposed by craniotomy and a large undercut was performed in the white matter below the suprasylvian gyrus in order to produce a partial cortical deafferentation by transecting the thalamo-cortical projections, while keeping intact the blood supply. The detailed surgical procedure is described elsewhere (Topolnik *et al.*, 2003b, a; Nita *et al.*, 2007; Avramescu & Timofeev, 2008). Coaxial bipolar electrodes (with the tip in the cortical depth at about 0.8-1 mm and the ring placed at the cortical surface) were placed bilaterally over frontal, temporal and occipital cortical areas. Recording chambers were implanted over the intact dura, above the suprasylvian gyri both ipsi- and contra-lateral to the undercut, for subsequent intracellular and ion recordings with glass micropipettes. Ag/AgCl references were placed on the nasium and bilaterally under the skin in the vicinity of the ears.

Pairs of Ag/AgCl electrodes were placed around the orbit through the frontal sinus to record the electro-oculogram (EOG); coated stainless steel electrodes were inserted into the neck muscles for the electromyogram (EMG), in order to monitor the states of vigilance. The skull was reconstituted using acrylic dental cement, all electrode wires were soldered to a connector socket, and 4 bolts were placed in the cement to allow non-painful fixation of the cat's head in a stereotactic 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 short recovery period (2–3 days), cats were trained to stay in the stereotactic frame and few days later they were able to sleep and displayed clearly identifiable states of vigilance (wake (W), slow wave sleep (SWS), and rapid eye movement (REM) sleep).

Polysomnographic recordings and sleep staging

Polysomnographic recordings started 5–7 days after surgery and lasted up to 4 months. Two to three recording sessions, each lasting for 3-4 h, were performed daily in a dark and silent environment under video surveillance, and usually contained periods of W, SWS and REM sleep. Body movements and position of the eyelids were noted. Cats were not deprived of sleep between recording sessions, and during recordings they could move their limbs and make postural adjustments.

Recordings were scored on 30 seconds epochs in four sleep stages: waking state (W), light slow-waves sleep (LSWS), deep slow-waves sleep (DSWS) and REM, based on adapted criteria from previously described sleep staging method in cats (Hess et al., 1953; Ursin, 1968, 1970), and each epoch was assigned to the sleep stage that had the longest duration during that respective period of time. Briefly, classic criteria include low voltage fast waves (>16 Hz) during W; high voltage sleep spindles (12-14 Hz) more prominent in frontal leads and high voltage delta waves (1-4 Hz) present in less than 50% of epoch's duration on a background of low voltage activity during LSWS; persistent high voltage delta waves (1-4 Hz) over more than 50% of the epoch's duration during DSWS; and low voltage fast waves combined with rapid eyes movements and muscular atonia during REM sleep (Ursin, 1968).

Following cortical undercut cats gradually developed 4 Hz spike wave (SW) and poly-spike-waves (PSW) seizures occurring incessantly during wake, SWS (Nita et al., 2007), overlapping on the background EEG activity. Therefore, to differentiate between W, LSWS and DSWS while 4 Hz SW/PSW seizures were present we took into consideration the relative quantity of delta activities per epoch in the 0.1-3 Hz frequency domain instead of the 1-4 Hz delta band considered in previously described criteria. Well sustained neck muscular tonus was present during W, LSWS, DSWS and it was absent during REM sleep.

The number and the duration of the ictal events build on 4 Hz SW/PSW were counted for each 30 seconds epoch. The time onset was considered at 10% of the amplitude of the sigmoid fitting for the first wave in the PSW complex, and the offset at 10% of the amplitude of the sigmoid fitting of the last wave. Similarly, 10% of the amplitude of the sigmoid fitting was used to determine the onset of the silent phase of the cortical slow oscillation.

Intracellular recordings and K^+-sensitive-microelectrodes

Intracellular recordings from presumed glial cells were obtained with glass micropipettes (tip diameter <0.5 μm) filled with potassium acetate (3 M, *in situ* impedance 35-50 MΩ) 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. The intracellular signals were passed through a high-impedance amplifier with an active bridge circuitry (bandwidth DC to 9 kHz). Intraglial recordings were considered for analysis only if the membrane potential (Vm) was more negative than −70 mV upon impalement, the recordings were stable without current injection, and with no action potentials fired at impalement, exit or by imposing Vm more positive than -55 mV.

Extracellular K^+ concentration was measured with K^+-sensitive microelectrodes (KSM) built from double-barrel glass capillaries in which one barrel was used for ion detection and the other as reference. K^+-sensitive barrel was pretreated with Dimethylchlorosilane, dried at 120°C for 2 hours, and the tip filled with the K^+ ionophore I-cocktail A (Fluka). The rest of the K^+-sensitive pipette was filled with KCl (0.2 M), while the reference capillary was filled with NaCl (2 M).

The K^+ microelectrode was calibrated prior and after each recording in artificial CSF solutions (NaCl 126 mM, KCl 2.3 mM, $NaHCO_3$ 26 mM, $MgSO_4$ 1.3 mM, $CaCl_2$ 2.4 mM, KH_2PO_4 1.2 mM, glucose 15 mM, HEPES 5 mM, thiourea 0.4 mM and 3% dextran 70000 with a final pH of 7.35 at 37°C) in which the K^+ concentration was adjusted between 1 and 25 mM by substituting NaCl with KCl. The relationship between concentration and voltage was derived in accordance with the Nicolsky–Eisenmann equation (Ammann, 1986). We used only K^+-sensitive electrodes with sensitivity within the prevailing range (1–6 mM) better than 10 mV/mM. All electrical signals were digitized (20 kHz sampling rate) and stored for off-line analysis.

End of experiments

Behavioral seizures were considered an "end-limit point" of any experiments, as consented with the local ethic committee. At the end of the experiments or at the first sign of clinically manifest seizures cats were given a lethal dose of intravenous sodium pentobarbital (50 mg/kg). After all experiments the extension of the undercut was verified on Nissl stained (thionine) sections.

GFAP Immunocytochemistry

At the end of the experiment animals were anesthetized with sodium pentobarbital overdose and transcardially perfused with 1000 ml of 0.9% cold saline (4°C), followed by 2000 ml of 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.35) over a 45 minutes period. Brains were removed and post-fixed in the paraformaldehyde solution overnight, then cryoprotected by allowing to sink (24-36 hr) in a 30% sucrose in 0.1 M phosphate buffer solution. Tissue sectioning (50 μm) was performed on a freezing microtome (Jung Histoslide 2000, Leica, Germany), and only slices from the central part of the undercut were included in the study (~5-7 mm rostraly from the entrance of the knife used for undercutting the cortex).

For GFAP immunocytochemistry, free-floating sections were incubated in a solution of 3% normal goat serum (NGS, Vector Laboratories, Burlingame, CA, USA), 0.1% Triton X-100 (TX), and 1.0% bovine serum albumin (BSA, Vector Laboratories,

Burlingame, CA, USA) in 0.1 M Tris-buffered saline (TBS; pH 7.4) for 2 hours to block nonspecific staining. Sections were then transferred to the primary antiserum containing 1:800 dilution of antibody against GFAP (Chemicon, Temecula, CA, USA) in 0.1 M TBS with 3% NGS, 0.1% TX, and 1.0% BSA. After incubation for 24 hours, the tissue was rinsed in 0.1 M TBS, pH 7.4, five times for 10 min. Secondary antibody treatment included a 3 hours incubation at room temperature, in a 1:300 solution of biotinylated goat anti-rabbit IgG (Chemicon, Temecula, CA, USA) in 0.1 M TBS with 3% NGS, 0.1% TX, and 1% BSA. A rinse cycle followed (5 times, 10 minutes each), then a 1 hour incubation in a 1:500 Elite avidin-biotin horseradish peroxidase complex (ABC, Vector Laboratories, Burlingame, CA, USA) in 3% NGS, 0.1% TX, and 1% BSA in 0.1 M TBS. After three rinses in 0.1 M TBS and three rinses in 0.1 M Tris-buffer (TB), pH 7.6, the sections were immersed in 0.05% 3,3-diaminobenzenidine (DAB) in 0.1 M TB solution, pH 7.6, for 5 minutes. Sections were mounted onto gelatin-coated slides, dehydrated in graded ethanol (50%, 70%, 90%, 95%, 100%), cleared in xylene and coverslipped for storage and analysis.

Image analysis

Sections were examined using a light microscope (Olympus BX61) equipped with the appropriate light sources (Olympus BX-UCB). Bright field images were acquired using a color CCD camera (Optronics, Goleta, CA, USA), a motorized stage (MAC5000, Ludl Electronic Products, Exton, PA, USA), and a computer system equipped with Neurolucida software (V8.0, MicroBrightField, Williston, VT, USA). In order to assess the density of GFAP+ cells we averaged the numbers obtained from 50 random fields inspected with a 40x objective, both in the grey and in the white matter of the ipsi- and contra-lateral suprasylvian gyri, on each slide. We included in the study 30 sections from 5 different animals in control and 6 weeks after cortical deafferentation. In order to eliminate the possible underestimation due to difficult penetration of the antibody or lack of sensibility, we chose to illustrate the densities of GFAP^{+} cells as percentages relative to the values obtained from control and not as absolute numbers.

Statistical analysis

The data were tested statistically using the Student's T-test and the analysis of variance (ANOVA) with the post hoc Tukey test adjusted for multiple comparisons. Significant differences for all statistical testing were defined by a $p<0.05$. Numerical data are represented as mean $\pm$ standard deviation (S.D.). Statistical tests were performed using statistical analysis software (SPSS 14.0, SPSS, Inc., Chicago, IL, USA).

12.5 Results

The normal cortical slow oscillation (<1 Hz) comprises two different activity levels: an active state in which cortical neurons are depolarized and tonically fire action potentials, and a silent state in which cortical neurons are hyperpolarized (Steriade et al., 1993; Contreras et al., 1996). In agreement with previous reports (Nita et al., 2006; Nita et al., 2007) following chronic deafferentation of the suprasylvian gyrus all animals displayed sharp transitions between active and silent states during cortical slow oscillation, and 7-14 days following the undercut they developed seizures consisting in spike-wave (SW) and polyspike-wave (PSW) complexes at 4 Hz. Polysomnographic recordings during the sleep-wake cycle showed that paroxysmal activities are expressed incessantly during wake, they appear enhanced during slow-wave sleep, and are abolished during REM sleep (Fig. 12-2).

Reactive gliosis following penetrating cortical trauma

Immunocytochemical studies on the GFAP$^+$ cell population of the cat cortex were performed to characterize the histological nature of astrocytic changes after cortical deafferentation. The quantification of the degree of reactive astrogliosis following brain trauma was done by comparing the astrocytic density in the undercut suprasylvian gyrus with the data obtained from control brain slices. As the two principal classes of astrocytes – protoplasmic astrocytes found in the grey matter and fibrous astrocytes in the white matter (Peters et al., 1976), have different biochemical properties (Raff et al., 1983) and different susceptibility to injury (Shannon et al., 2007), we decided to quantify separately the density of astrocytes in the white and grey matter.

The number of astrocytes was significantly higher 6 weeks following cortical deafferentation compared to control both in the white and in the grey matter. Nevertheless, the density of astrocytes in the white matter increased by only 42.23 % compared to control (p<0.05, ANOVA with post hoc Tukey test), whereas it increased by as much as 134.67 % in the grey matter (p<0.01, ANOVA with post hoc Tukey test) (Fig. 12-1).

Epilepsy during wake and sleep

Since it is well-known that the various types of seizure are affected differently by the circadian sleep-wake cycle (Steriade, 1974; Kellaway, 1985), but also by the ultradian rapid-eye-movement/slow-wave-sleep (REM/SWS) cycle (Langdon-Down and Brain, 1929; Patry, 1931), we quantified the frequency of ictal events during each of the different stages of sleep and during waking (Fig. 12-2, 12-3). Also, because stage 2 of SWS in humans has been reported to particularly facilitate generalized epilepsies (Touchon *et al.*, 1991; Bazil & Walczak, 1997) we decided to divide SWS into two stages – LSWS and DSWS (see materials and methods for details), as previously used for electrophysiological recordings in cats (Ursin, 1968).

When analyzing the sequential organization of sleep stage episodes we observed that the head restrained animals spent, during afternoon time, 31.06±5.42% in LSWS and only 25.53±7.21% in DSWS and 6.8±3.7% in REM sleep (Fig. 12-3*B*). Also, REM sleep was almost always (81.39%) proceeded by DSWS (Fig. 12-3*A*), as previously reported (Ursin, 1968, 1970).

The highest number of ictal events, as well as the longest ictal periods, were recorded during LSWS, while being completely absent or dramatically decreased during REM sleep (Fig. 12-2, 12-3). Therefore, more than half (50.48±8.45%) of ictal events occurred during LSWS, occupying 14.45±1.53% of this sleep stage, while during REM sleep only 0.55±0.43% of seizures emerge, taking up just 0.7±0.21% of this period. The percentage of seizures during W and DSWS was only 25.52±10.2% and 23.45±16.73% respectively, and occupied 6.1±1.95% of W and 8.61±3.71% of DSWS time.

Phasic variation of $[K^+]_o$ during SWS and SW activity

As it has been shown that posttraumatic reactive astrocytes have altered electrophysiological properties, leading to impaired K^+ homeostasis (D'Ambrosio et al., 1999), we first investigated the phasic $[K^+]_o$ variations during SWS and SW activity.

During stable periods of quite wakefulness, the average resting level of $[K^+]_o$ was 3.1±0.63 mM in the suprasylvian gyrus contralateral to the undercut, and in the undercut

cortex the baseline value raised slightly to 3.3±0.87 mM, without reaching statistical significance. The slow-oscillation EEG pattern recorded during DSWS was associated with a matching variation of $[K^+]_o$, which decreased during silent states and increased during active states (Fig. 12-4A). The amplitude of $[K^+]_o$ variations was similar in intact cortex (0.2±0.03 mM) and in the undercut cortex (0.21±0.05 mM), but lower than the values recorded in ketamine-xylazine anesthetized cats (Amzica et al., 2002).

Occasionally, the continuous pattern of slow-oscillation was interrupted by spontaneous paroxysmal activity of ~3Hz (Fig. 12-2, Fig. 12-4*B*). Following such periods, the KSM recorded an increased $[K^+]_o$ reaching on average a value of 0.27±0.1 mM (Fig. 12-4*B*), suggesting either an enhanced neuronal firing or/and an impaired K^+ clearance.

$[K^+]_o$ during activated brain states

We expected the abnormalities of brain function due to traumatic brain injuries to be most apparent during the transitions from a slow oscillating pattern, as seen in SWS, to an activated EEG pattern as displayed during REM sleep and waking state.

Indeed, the transition between SWS and REM sleep was always marked by a temporary increased of $[K^+]_o$, both ipsi- and contra- lateral to the undercut cortex, which decreased again to baseline levels after several seconds (Fig. 12-5*A*). Nevertheless, the increased $[K^+]_o$ reached higher values and lasted longer in the undercut suprasylvian gyrus compared to intact cortex (Fig. 12-5*A*). On average, the variation of $[K^+]_o$ at the transition between SWS and REM sleep was 0.3±0.05 mM in the intact cortex and returned to the baseline in 103.83±23.9 s. For the same transition SWS-REM the variation of $[K^+]_o$ in the undercut cortex was much higher than in the intact cortex (p<0.05, Student's T test) reaching a value of 0.51±0.22 mM and returning to the baseline in 128.17±26.6 s (Fig. 12-6).

Conversely, the transition between SWS and W was matched by a smaller and more transient increase of $[K^+]_o$, of similar amplitude in the undercut and intact cortex (Fig. 12-5*B*). On average, the $[K^+]_o$ varied with 0.1±0.02 mM in the intact cortex and with

0.13±0.05 mM in the undercut, and returned to baseline in 55.5±15.57 s in the intact cortex and in 60.33±20.22 s in the undercut.

Impaired function of glial cells following brain injury

One of the possible causes of the increased $[K^+]_o$ could be the impaired glial function after penetrating cortical wounds. Therefore, we performed intracellular recordings of glial cells both in the ipsi- and contra-lateral to the undercut suprasylvian gyri. We included in the study 39 glial cells recorded in the undercut cortex, from which 22 underwent a transition between two different states of vigilance (either SWS-REM or SWS-W), and 42 glial cells recorded in the intact cortex, 25 of which underwent a transition.

During SWS, in the intact gyrus, all intraglial recordings displayed slow oscillation consisting in low-amplitude alternations of Vm above its resting level, with an averaged amplitude of 1.8±0.41 mV (Fig. 12-7*A*), similar to previous reports (Amzica et al., 2002). Also, the majority of glial cells recorded in the intact cortex (80%) displayed a hyperpolarized membrane potential during the transitions from SWS to either REM sleep or W, while 22% were depolarized, as previously reported (Seigneur et al., 2006). Conversely, in the undercut gyrus, the vast majority (90.9%) of intraglial recordings during SWS showed no variation of the membrane potential neither during SWS, nor during transitions from SWS to REM or/and W. The rest of 9.09% (2 out of 22) of glial cells recorded in the injured cortex had a variation of the membrane potential of 1.1±0.35 mV during SWS and displayed a long-lasting depolarization of the membrane potential during the transitions between SWS and REM and SWS and W. These findings sustain the possible impairment of glial function, which could explain deficient K^+ clearance from the extracellular space.

12.6 Discussion

In the present study we demonstrate *in vivo*, in non-anesthetized cats that reactive gliosis following penetrating cortical wounds is associated with increased paroxysmal activity, particularly during LSWS, and with impaired K^+ clearance in the injured gyrus. We also show here, for the first time, the variations of $[K^+]_o$ in the neocortex, corresponding to the active and silent phases of the cortical slow oscillation during natural SWS, as well as corresponding to the transitions from SWS to more activated brain states, such as REM sleep and W. Moreover, following cortical deafferentation we observed an increased $[K^+]_o$ during SW activity and a higher $[K^+]_o$ accompanied by longer $[K^+]_o$ clearance in the undercut gyrus compared to the intact contralateral cortex. We observed additionally an increased immunostaining for $GFAP^+$ cells in the injured gyrus, together with impaired electrophysiological properties of glial cells in the same gyrus. Therefore, we suggest that these dysfunctional reactive astrocytes are responsible for the increased $[K^+]_o$ following brain injury, leading to an impaired neuronal function.

The close relationship between sleep and epilepsy is well documented both in experimental animals (Steriade, 1974; Nita et al., 2007; Nita et al., 2008) and in humans (Bazil & Walczak, 1997; Herman *et al.*, 2001). We also show here that the incidence of ictal events is modulated by the state of vigilance, occurring during the waking state, being enhanced during SWS, particularly during LSWS, and almost absent during REM sleep. The activating properties of SWS on epileptiform discharges could probably be ascribed to the highly synchronous oscillations in the corticothalamic systems, which is the characteristic of this sleep stage (Steriade & Contreras, 1995). Similarly, in humans, SWS and mostly stage 2 has a synchronizing effect shown by increase in frequency and spreading of seizures, while REM sleep prevents generalized discharges of generalized epilepsies (Bazil & Walczak, 1997; Herman *et al.*, 2001). A surprising finding of the present study was the fact that the duration of LSWS appeared highly increased in the detriment of REM sleep, which was decreased compared to data reported formerly in uninjured cats, although the time spent in W and DSWS was similar to these previous reports (Sterman et al., 1965; Ursin, 1968) This might be explained by the fact that seizure activity is known to disrupt sleep pattern in humans, reducing REM, as well as deep stages

of sleep, while increasing stage 1 (Bazil et al., 2000), so it probably has a similar effect in animals. Another possible explanation is that the animals in this study were head restrained.

As impaired K^+ homeostasis has been previously linked to reactive astrocytosis following various brain injuries (D'Ambrosio et al., 1999; Schroder et al., 1999; Kivi et al., 2000; Schroder et al., 2000; Bordey et al., 2001), we decided to investigate the variations of $[K^+]_o$ during natural sleep and SW seizures. Throughout stable periods of DSWS, the KSM showed an increased $[K^+]_o$ during the active phase and a decreased $[K^+]_o$ during the silent state of the slow oscillation. This relationship between $[K^+]_o$ and the cortical slow oscillation has been demonstrated previously using ketamine-xylazine anesthesia (Amzica et al., 2002). Nevertheless, the K^+ variations reported in that study were higher (0.78 ± 0.13 mM) than in our experiments (0.2 ± 0.03 mM), probably due to the effect of anesthesia, since both ketamine and xylazine influence neuronal activity, promoting oscillatory behavior in the thalamocortical system (Buzsaki et al., 1991).

Similarly, we observed a transient increase in $[K^+]_o$ following SW seizures (0.27 ± 0.1 mM). Previous studies show brief modest increases of $[K^+]_o$ during interictal discharges, and a much larger elevation during different types of ictal events (Prince et al., 1973; Lothman and Somjen, 1975, 1976). Also, data recorded during anesthesia and using pro-convulsive pharmacological agents (Moody *et al.*, 1974; Amzica & Steriade, 2000) or electrical stimulation (Heinemann and Lux, 1977) showed an increase of $[K^+]_o$ typically in the range of 2-3 mM, reaching occasionally values of 6 mM (Moody et al., 1974), or even approaching the "ceiling" of 8-12 mM during sustained seizures (Heinemann and Lux, 1977). At variance with these studies, we show here a much lower increase in $[K^+]_o$ (0.27 ± 0.1 mM), occurring immediately after but not during seizure. These discrepancies arise probably because we recorded unprovoked occurring SW and PSW activities in behaving animals, following cortical undercut. Our data are therefore, not contaminated by the effect of anesthesia or of sudden pharmacological/electrical excitatory stimulation of a large neuronal population, both of which are not a physiological phenomenon. Our recordings are performed following a traumatic brain injury that increases the duration and number of silent periods of brain oscillation (Avramescu and Timofeev, 2008; Nita et al., 2007). This global decrease in neuronal activity could thus be responsibe for the lower

$[K^+]_o$ and the delayed response we recorded in the undercut cortex. In addition, we show here an impaired K^+ clearance in the deafferented gyrus which could further explain the persistence of high $[K^+]_o$ after SW complexes, because of a delayed K^+ reuptake. To our knowledge, this is the first *in vivo* study reporting the phasic modulation of $[K^+]_o$ during active and silent phases of the cortical slow oscillation during natural SWS, as well as the variations of $[K^+]_o$ during spontaneous SW seizures.

We then explored the modulation of $[K^+]_o$ at the transitions between SWS and REM sleep, as well as between SWS and W, both ipsi- and contra- lateral to the undercut suprasylvian gyrus. Earlier *in vivo* studies have shown an enhancement of $[K^+]_o$ at the transition between SWS and REM sleep in the reticular formation (Satoh et al., 1979), the lateral geniculate nucleus (Satoh et al., 1982) and the hippocampus of cats (Satoh et al., 1991). In the intact suprasylvian gyrus we obtained similar $[K^+]_o$ increases of 0.3±0.05 mM, returning to baseline in 103.83±23.9 s. However, in the undercut cortex, the variation of $[K^+]_o$, for the same transition SWS-REM, was much higher, increasing by 0.51±0.22 mM, and the clearance time was longer, 128.17±26.6 s. When examining the transition from SWS to W, we also observed a small increase of $[K^+]_o$, but there were no statistical significant differences between the values and clearance time recorded in the undercut and the intact cortex. All of the above data suggests that the clearance of the small increases of $[K^+]_o$ accumulated during the active phase of the slow oscillation, during single SW seizures or/and during the transitions from SWS to W was similar in the undercut and in the intact cortex. The defective mechanisms of K^+ reuptake became apparent only when high amounts of K^+ accumulated in the injured cortex, such as at the transition between SWS and REM sleep. Then, the $[K^+]_o$ increased more and the washing out took longer time in the undercut gyrus compared to the intact cortex.

Previous studies depict K^+ as a very important modulator of synaptic transmission and excitability, showing that mild elevation of $[K^+]_o$ from the resting level of 3.5 mM to 5 mM increased neuronal excitability (Balestrino et al., 1986; Rausche et al., 1990), further raising $[K^+]_o$ to 7-8 mM promoted spontaneous and even epileptiform activity (Rutecki *et al.*, 1985; Korn *et al.*, 1987; Traynelis & Dingledine, 1988), but at higher levels, $[K^+]_o$ suppressed excitability and induced spreading depression (Kager et al., 2000, 2002). The

levels of $[K^+]_o$ we recorded at the transition between SWS and REM sleep are therefore, in the excitatory range. Indeed, it has been documented in kindled cats that SWS-REM transitions and SWS are the sleep periods most vulnerable to secondary generalized seizures (Shouse, 1986). Similarly, in humans with temporal lobe epilepsy, in which secondary generalized seizures tend to be state-dependent, seizures have been described to occur more frequently during transitional periods into REM sleep and during SWS (Cadhilac, 1982). Therefore, the transient increased $[K^+]_o$ that marks the transition to REM sleep could be responsible for the increased propensity to seizures of this transitional sleep stage. The higher level of $[K^+]_o$ recorded in the undercut suprasylvian gyrus during the transition between SWS and REM sleep could be generated either by an increased neuronal firing following deafferentation or/and by an impaired K^+ clearance.

A primary mechanism for K^+ reuptake and spatial K^+ buffering is considered to be to be via glial inwardly rectifying K^+ channels (K_{ir} channels) in glial cells (Orkand et al., 1966; Newman, 1986, 1993). Downregulation of astroglial K_{ir} channels has been described in the CNS following various injury-induced reactive gliosis, such as in deafferented entorhinal cortex (Schroder et al., 1999), in freeze lesion-induced cortical dysplasia (Bordey et al., 2001), and in traumatic (D'Ambrosio et al., 1999) and ischemic (Koller et al., 2000) brain injury. In addition, there have also been indications of downregulation of Kir currents in specimens from patients with temporal lobe epilepsy (Bordey & Sontheimer, 1998; Hinterkeuser *et al.*, 2000; Kivi *et al.*, 2000; Schroder *et al.*, 2000). These data indicate that dysfunction of astroglial K_{ir} channels in the reactive astrocytes following various brain injuries could underlie impaired K^+ buffering (Steinhauser & Seifert, 2002). Similarly, we found an increased density of GFAP$^+$ cells, suggestive of reactive astrogliosis, associated with an increased $[K^+]_o$ restricted to the cortical undercut gyrus.

In intact brain, *in vivo*, glial cells follow closely the active and silent states of the slow oscillation and respond to cortical activation, in most of the cases, by a hyperpolarized membrane potential due to a direct effect of acetylcholine mainly on muscarinic glial receptors (Amzica & Steriade, 2000; Amzica *et al.*, 2002; Seigneur *et al.*, 2006). However, less frequently, glial membrane potential may be depolarized during cortical activation by an indirect effect, namely through the activation of neurons which further could released K^+

and glutamate in the extracellular space, both of which are able to depolarize glial membrane (Kettenmann & Schachner, 1985; Amzica & Steriade, 2000; Amzica *et al.*, 2002; Seigneur *et al.*, 2006). In our experiments, all glial cells recorded in the intact suprasylvian gyrus had a slow oscillatory pattern of the membrane potential during SWS, whereas during the transitions to either REM sleep or W most of the glia hyperpolarized and only a small percentage were depolarized. Conversely, in the undercut suprasylvian gyrus most of glial cells had no variations of the membrane potential neither during SWS, nor during transitions from SWS to REM or/and W, supporting previous studies which demonstrated the decreased capacity of posttraumatic reactive glia to uptake extracellular K^+ (D'Ambrosio et al., 1999; D'Ambrosio, 2004). Moreover, no glial cell recorded in the undercut cortex displayed a hyperpolarization during activated brain activity, suggesting that these glia could not respond to the expected acetylcholinergic stimulation. Our finding is supported by a previous study documenting almost a complete loss of cholinergic staining following deafferentation of the suprasylvian gyrus in cat (Krnjevic & Silver, 1965). This means that the undercut deprived the suprasylvian gyrus of the cholinergic activation from the subcortical structures and therefore, the glial cells from the deafferented gyrus could not be hyperpolarized.

In conclusion, we show here that reactive gliosis following cortical deafferentation in cats, is associated with an increased paroxysmal activity particularly during LSWS and with an impaired potassium clearance, probably due to astrocytic dysfunction.

12.7 Acknowledgements

12.8 References

Ammann D. (1986). *Ion sensitive microelectrodes*. Springer, Berlin.

Amzica F, Massimini M & Manfridi A. (2002). Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. *J Neurosci* **22,** 1042-1053.

Amzica F & Steriade M. (2000). Neuronal and glial membrane potentials during sleep and paroxysmal oscillations in the neocortex. *J Neurosci* **20,** 6648-6665.

Annegers JF. (1994). The natural course of epilepsy: an epidemiologic perspective. In *The surgical management of epilepsy*, ed. Wyler A.R. HBP, pp. 3-7. Butterworth-Heinemann, Boston.

Avramescu S & Timofeev I. (2008). Synaptic strength modulation following cortical trauma: a role in epileptogenesis. *JNeurosci (in press)*.

Balestrino M, Aitken PG & Somjen GG. (1986). The effects of moderate changes of extracellular K+ and Ca2+ on synaptic and neural function in the CA1 region of the hippocampal slice. *Brain research* **377,** 229-239.

Bazil CW, Castro LH & Walczak TS. (2000). Reduction of rapid eye movement sleep by diurnal and nocturnal seizures in temporal lobe epilepsy. *Archives of neurology* **57,** 363-368.

Bazil CW & Walczak TS. (1997). Effects of sleep and sleep stage on epileptic and nonepileptic seizures. *Epilepsia* **38,** 56-62.

Blumcke I, Thom M & Wiestler OD. (2002). Ammon's horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. *Brain pathology (Zurich, Switzerland)* **12,** 199-211.

Bordey A, Hablitz JJ & Sontheimer H. (2000). Reactive astrocytes show enhanced inwardly rectifying K$^+$ currents in situ. *Neuroreport* **11,** 3151-3155.

Bordey A, Lyons SA, Hablitz JJ & Sontheimer H. (2001). Electrophysiological characteristics of reactive astrocytes in experimental cortical dysplasia. *Journal of neurophysiology* **85,** 1719-1731.

Bordey A & Sontheimer H. (1998). Properties of human glial cells associated with epileptic seizure foci. *Epilepsy Res* **32,** 286-303.

Buzsaki G, Kennedy B, Solt VB & Ziegler M. (1991). Noradrenergic Control of Thalamic Oscillation: the Role of alpha-2 Receptors. *The European journal of neuroscience* **3,** 222-229.

Cadhilac J. (1982). Complex partial seizures and REM sleep. In *Sleep and Epilepsy*, ed. Sterman M.B. SMN, Passouan P., pp. 315-324. Academic Press, New York.

Contreras D, Timofeev I & Steriade M. (1996). Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J Physiol* **494 (Pt 1),** 251-264.

Crespel A, Baldy-Moulinier M & Coubes P. (1998). The relationship between sleep and epilepsy in frontal and temporal lobe epilepsies: practical and physiopathologic considerations. *Epilepsia* **39,** 150-157.

Crespel A, Coubes P & Baldy-Moulinier M. (2000). Sleep influence on seizures and epilepsy effects on sleep in partial frontal and temporal lobe epilepsies. *Clin Neurophysiol* **111 Suppl 2,** S54-59.

D'Ambrosio R. (2004). The role of glial membrane ion channels in seizures and epileptogenesis. *Pharmacology & therapeutics* **103,** 95-108.

D'Ambrosio R, Maris DO, Grady MS, Winn HR & Janigro D. (1999). Impaired K$^+$ homeostasis and altered electrophysiological properties of post-traumatic hippocampal glia. *J Neurosci* **19,** 8152-8162.

de Lanerolle NC & Lee TS. (2005). New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. *Epilepsy Behav* **7,** 190-203.

Heinemann U, Gabriel S, Jauch R, Schulze K, Kivi A, Eilers A, Kovacs R & Lehmann TN. (2000). Alterations of glial cell function in temporal lobe epilepsy. *Epilepsia* **41 Suppl 6,** S185-189.

Herman ST, Walczak TS & Bazil CW. (2001). Distribution of partial seizures during the sleep--wake cycle: differences by seizure onset site. *Neurology* **56,** 1453-1459.

Hess R, Jr., Koella WP & Akert K. (1953). Cortical and subcortical recordings in natural and artificially induced sleep in cats. *Electroencephalography and clinical neurophysiology* **5,** 75-90.

Hinterkeuser S, Schroder W, Hager G, Seifert G, Blumcke I, Elger CE, Schramm J & Steinhauser C. (2000). Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. *The European journal of neuroscience* **12,** 2087-2096.

Hoffman SN, Salin PA & Prince DA. (1994). Chronic neocortical epileptogenesis in vitro. *JNeurophysiol* **71,** 1762-1773.

Jacobs KM, Graber KD, Kharazia VN, Parada I & Prince DA. (2000). Postlesional epilepsy: the ultimate brain plasticity. *Epilepsia* **41 Suppl 6,** S153-S161.

Jin X, Prince DA & Huguenard JR. (2006). Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. *J Neurosci* **26,** 4891-4900.

Kager H, Wadman WJ & Somjen GG. (2000). Simulated seizures and spreading depression in a neuron model incorporating interstitial space and ion concentrations. *Journal of neurophysiology* **84,** 495-512.

Kager H, Wadman WJ & Somjen GG. (2002). Conditions for the triggering of spreading depression studied with computer simulations. *Journal of neurophysiology* **88,** 2700-2712.

Kellaway P. (1985). Sleep and epilepsy. *Epilepsia* **26 Suppl 1,** S15-30.

Kettenmann H & Schachner M. (1985). Pharmacological properties of gamma-aminobutyric acid-, glutamate-, and aspartate-induced depolarizations in cultured astrocytes. *J Neurosci* **5,** 3295-3301.

Kim JH. (2001). Pathology of epilepsy. *Experimental and molecular pathology* **70,** 345-367.

Kivi A, Lehmann TN, Kovacs R, Eilers A, Jauch R, Meencke HJ, von Deimling A, Heinemann U & Gabriel S. (2000). Effects of barium on stimulus-induced rises of $[K^+]_o$ in human epileptic non-sclerotic and sclerotic hippocampal area CA1. *The European journal of neuroscience* **12,** 2039-2048.

Koller H, Schroeter M, Jander S, Stoll G & Siebler M. (2000). Time course of inwardly rectifying K^+ current reduction in glial cells surrounding ischemic brain lesions. *Brain research* **872,** 194-198.

Korn SJ, Giacchino JL, Chamberlin NL & Dingledine R. (1987). Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *Journal of neurophysiology* **57,** 325-340.

Krnjevic K & Silver A. (1965). A histochemical study of cholinergic fibres in the cerebral cortex. *Journal of anatomy* **99,** 711-759.

Langdon-Down M & Brain WR. (1929). *Time of day in relation to convulsions in epilepsy.*, vol. 2. Lancet.

Li H, Bandrowski AE & Prince DA. (2005). Cortical injury affects short-term plasticity of evoked excitatory synaptic currents. *Journal of neurophysiology* **93,** 146-156.

Li H & Prince DA. (2002). Synaptic activity in chronically injured, epileptogenic sensory-motor neocortex. *JNeurophysiol* **88,** 2-12.

Moody WJ, Futamachi KJ & Prince DA. (1974). Extracellular potassium activity during epileptogenesis. *Exp Neurol* **42,** 248-263.

Newman EA. (1986). High potassium conductance in astrocyte endfeet. *Science (New York, NY* **233,** 453-454.

Newman EA. (1993). Inward-rectifying potassium channels in retinal glial (Muller) cells. *J Neurosci* **13,** 3333-3345.

Nita DA, Cisse Y & Timofeev I. (2008). State-dependent slow outlasting activities following neocortical kindlling in cats. *Exp Neurol.* DOI: 10.1016-j.Exp Neurol.2008.02.010.

Nita DA, Cisse Y, Timofeev I & Steriade M. (2006). Increased propensity to seizures after chronic cortical deafferentation in vivo. *JNeurophysiol* **95,** 902-913.

Nita DA, Cisse Y, Timofeev I & Steriade M. (2007). Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. *Cereb Cortex* **17,** 272-283.

Orkand RK, Nicholls JG & Kuffler SW. (1966). Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *Journal of neurophysiology* **29,** 788-806.

Patry FL. (1931). The relation of time of day, sleep and other factors to the incidence of epileptic seizures. *Amer J Psychiat* **10,** 789–813.

Peters A, Palay SL & Webster HdF. (1976). *The Fine Structure of the Nervous System: the Neurons and Supporting Cells*. Saunders, Philadelphia.

Prince DA & Tseng GF. (1993). Epileptogenesis in chronically injured cortex: in vitro studies. *JNeurophysiol* **69,** 1276-1291.

Raff MC, Abney ER, Cohen J, Lindsay R & Noble M. (1983). Two types of astrocytes in cultures of developing rat white matter: differences in morphology, surface gangliosides, and growth characteristics. *J Neurosci* **3,** 1289-1300.

Rausche G, Igelmund P & Heinemann U. (1990). Effects of changes in extracellular potassium, magnesium and calcium concentration on synaptic transmission in area CA1 and the dentate gyrus of rat hippocampal slices. *Pflugers Arch* **415,** 588-593.

Rutecki PA, Lebeda FJ & Johnston D. (1985). Epileptiform activity induced by changes in extracellular potassium in hippocampus. *Journal of neurophysiology* **54,** 1363-1374.

Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D & Dillon JD. (1985). Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. *Neurology* **35,** 1406-1414.

Samuelsson C, Kumlien E, Flink R, Lindholm D & Ronne-Engstrom E. (2000). Decreased cortical levels of astrocytic glutamate transport protein GLT-1 in a rat model of posttraumatic epilepsy. *Neuroscience letters* **289,** 185-188.

Satoh T, Sakagucci M & Eguchi K. (1982). Extracellular potassium ion activity during PGO spike in cat lateral geniculate nucleus. *Sleep* **5,** 213-217.

Satoh T, Watabe K & Eguchi K. (1979). Enhancement during REM sleep of extracellular potassium ion activity in the reticular formation. *Brain research* **174,** 180-183.

Satoh T, Yokota T & Kitayama S. (1991). Enhancement of potassium ion activity in cat hippocampus during REM sleep. *Sleep* **14,** 2-4.

Schroder W, Hager G, Kouprijanova E, Weber M, Schmitt AB, Seifert G & Steinhauser C. (1999). Lesion-induced changes of electrophysiological properties in astrocytes of the rat dentate gyrus. *Glia* **28,** 166-174.

Schroder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP & Steinhauser C. (2000). Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. *Epilepsia* **41 Suppl 6,** S181-184.

Seigneur J, Kroeger D, Nita DA & Amzica F. (2006). Cholinergic action on cortical glial cells in vivo. *Cereb Cortex* **16,** 655-668.

Shannon C, Salter M & Fern R. (2007). GFP imaging of live astrocytes: regional differences in the effects of ischaemia upon astrocytes. *Journal of anatomy* **210,** 684-692.

Shouse MN. (1986). State disorders and state-dependent seizures in amygdala-kindled cats. *Exp Neurol* **92,** 601-608.

Somjen GG. (1979). Extracellular potassium in the mammalian central nervous system. *Annual review of physiology* **41,** 159-177.

Steinhauser C & Seifert G. (2002). Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *European journal of pharmacology* **447,** 227-237.

Steriade M. (1974). Interneuronal epileptic discharges related to spike-and-wave cortical seizures in behaving monkeys. *Electroencephalography and clinical neurophysiology* **37,** 247-263.

Steriade M & Contreras D. (1995). Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. *J Neurosci* **15,** 623-642.

Steriade M, Nunez A & Amzica F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci* **13,** 3252-3265.

Sterman MB, Knauss T, Lehmann D & Clemente CD. (1965). Circadian sleep and waking patterns in the laboratory cat. *Electroencephalography and clinical neurophysiology* **19,** 509-517.

Topolnik L, Steriade M & Timofeev I. (2003a). Hyperexcitability of intact neurons underlies acute development of trauma-related electrographic seizures in cats in vivo. *The European journal of neuroscience* **18,** 486-496.

Topolnik L, Steriade M & Timofeev I. (2003b). Partial cortical deafferentation promotes development of paroxysmal activity. *CerebCortex* **13,** 883-893.

Touchon J, Baldy-Moulinier M, Billiard M, Besset A & Cadilhac J. (1991). Sleep organization and epilepsy. *Epilepsy research* **2,** 73-81.

Traynelis SF & Dingledine R. (1988). Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *Journal of neurophysiology* **59,** 259-276.

Ursin R. (1968). The two stages of slow wave sleep in the cat and their relation to REM sleep. *Brain research* **11,** 347-356.

Ursin R. (1970). Sleep stage relations within the sleep cycles of the cat. *Brain research* **20,** 91-97.

12.9 Figures
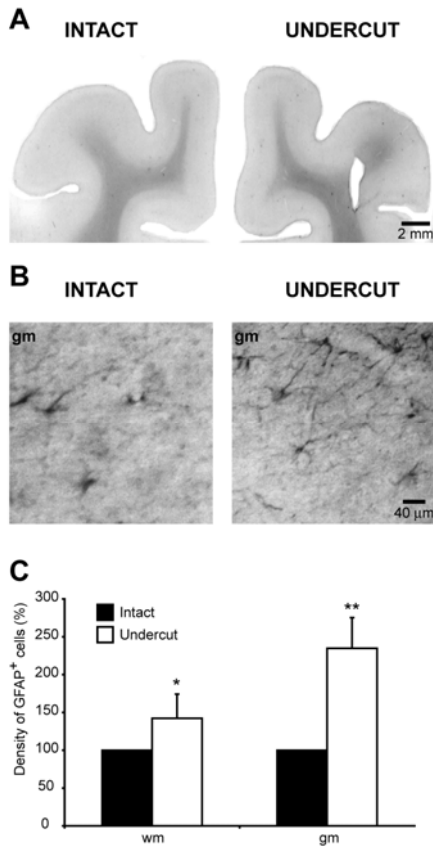
Figure 12-1. Immunocytochemical labeling of GFAP⁺ cells. A) Frontal sections of cat brain immunohistochemically stained with GFAP, showing the intact (left) and the undercut (right).Note the increased staining in the grey matter above the undercut following chronic deafferentation. B) Details of staining in the grey matter (gm) from the intact (left) and undercut (right) cortex. C) Quantification of the relative number of labeled astrocytes in the white matter (wm) and grey matter (gm) in the intact and undercut cortex. Note the higher increase in the density of GFAP⁺ cells in the grey matter compared to the white matter. *, $p<0.05$; **, $p<0.01$.
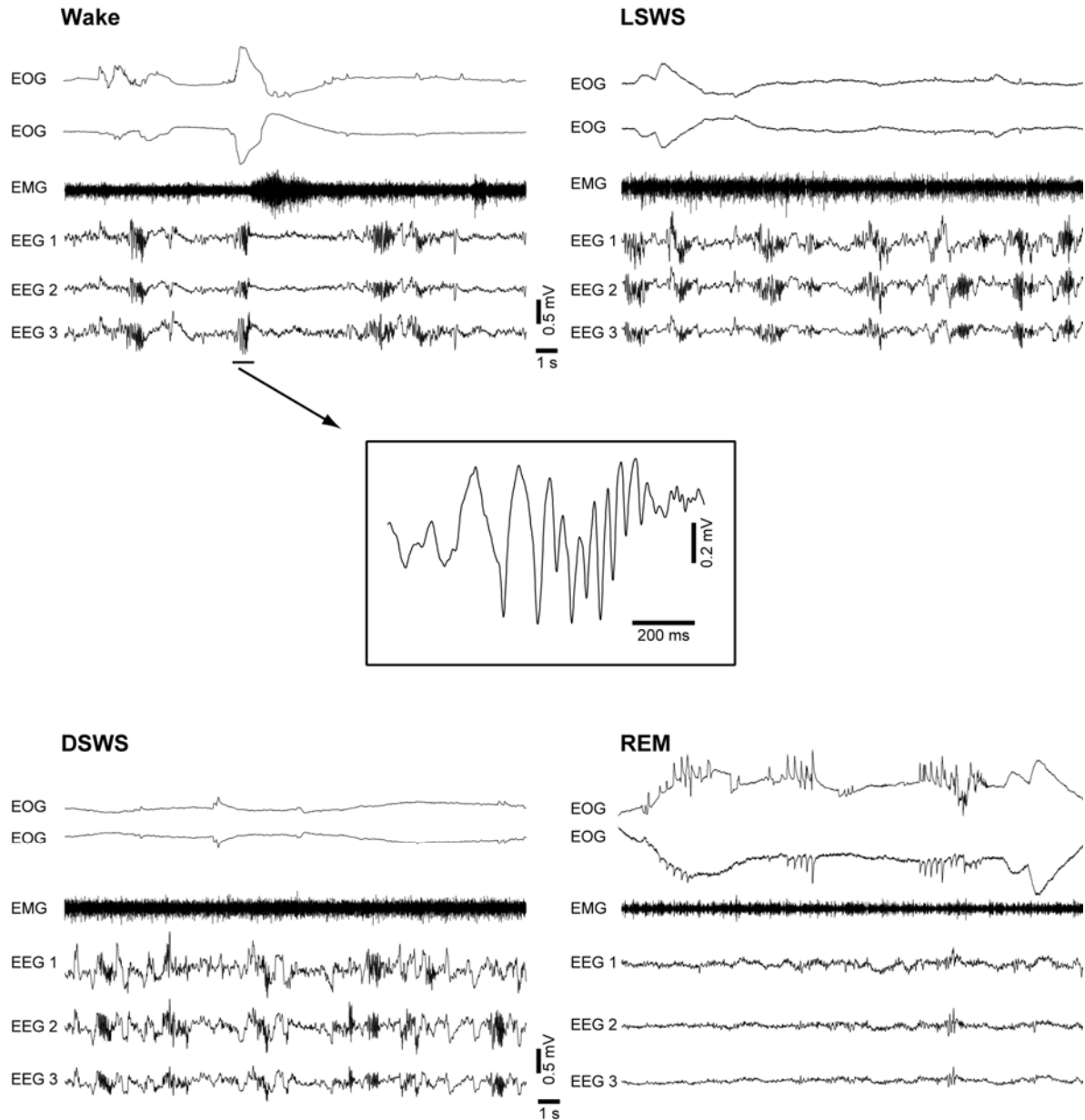
Figure 12-2. Relationship of ictal (4 Hz) activities with different states of vigilance. Polysomnographic recordings in a cat with a chronic deafferentation of the suprasylvian gyrus during waking state, light slow-wave sleep (LSWS), deep slow-wave sleep (DSWS), and rapid-eye movements sleep (REM). The underlined ictal event (~ 4 Hz) is expanded in the inset. Paroxysmal activities are expressed incessantly during wake, they appear enhanced during slow-wave sleep (especially LSWS), and are abolished during REM sleep. EOG = electro-oculogram, EMG = electro-miogram, EEG = electroencephalogram in (1) - frontal, (2) - temporal, and (3) - occipital areas of the cerebral hemisphere ipsi-lateral to the undercut.
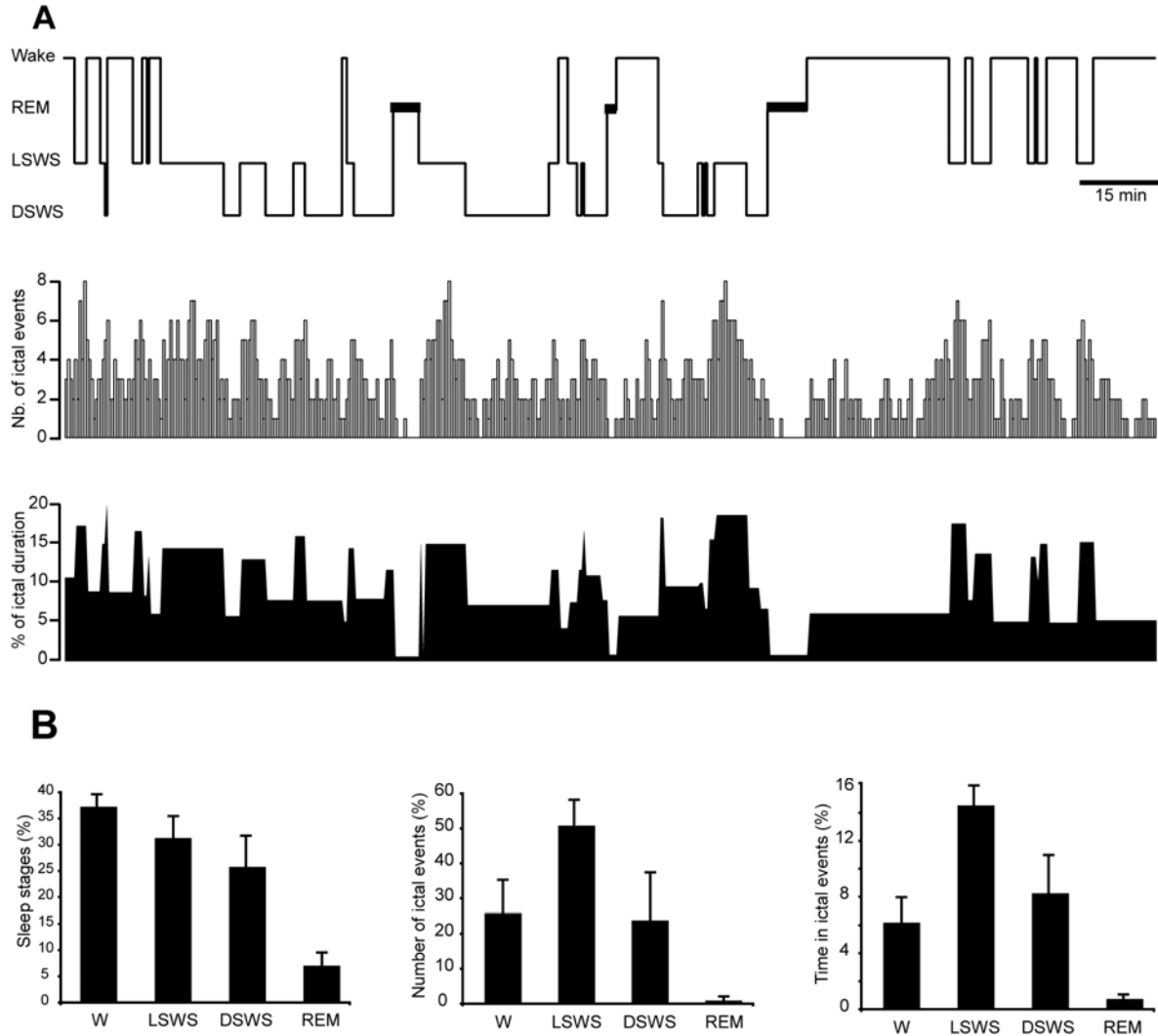
Figure 12-3. Incidence of ictal events during different phases of the sleep-wake cycle. A) Pattern of sleep of an experimental epileptic animal (upper panel) together with the total number of ictal events (middle panel) and the relative duration of ictal events on each 30 seconds epoch used for sleep staging (bottom panel). B) Global quantification of the contribution of each sleep phase to the architecture of sleep-wake cycle in chronically epileptic animals (left panel), percentage of the total number of ictal events (middle panel) per each state of vigilance, and of the time spent in ictal events during wake and sleep (right panel) for all recorded animals. Note the increased ictal activity during LSWS. W = wake, LSWS = light slow wave sleep, DSWS = deep slow wave sleep, REM = rapid eye movement sleep.
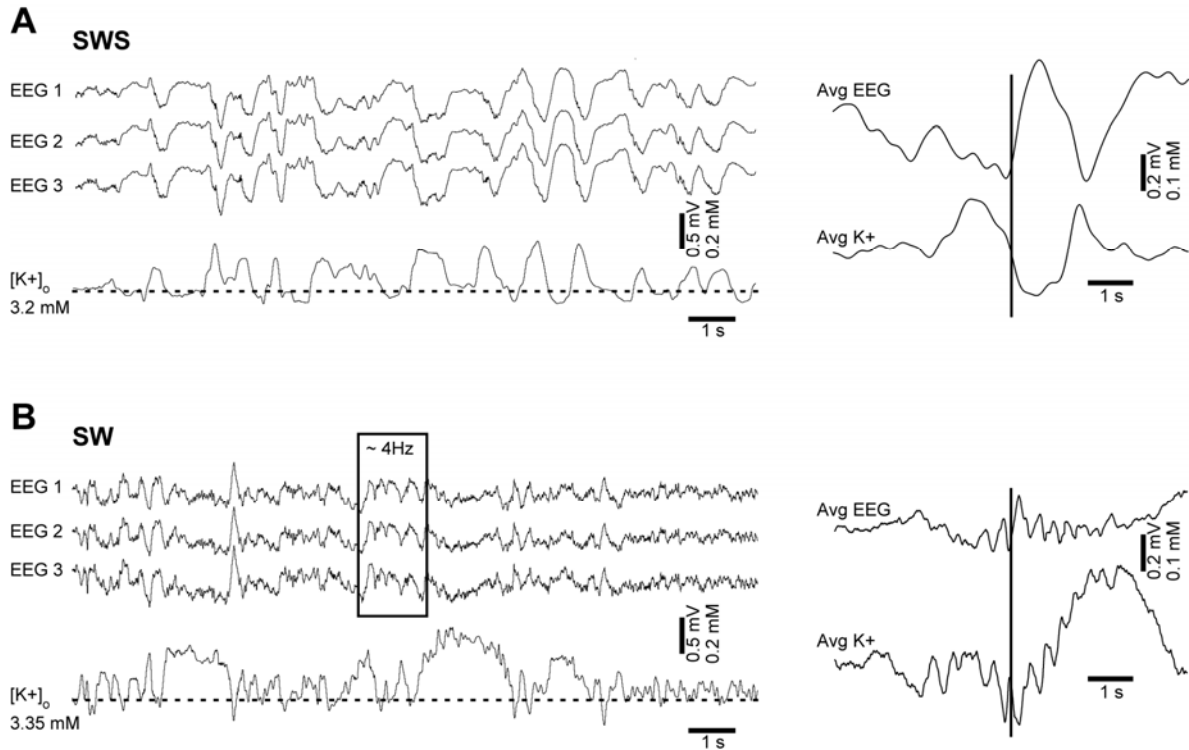
Figure 12-4. Phasic variation of the extracellular potassium concentration during sleep and ictal events. Electroencephalographic recordings from the frontal area (EEG 1), temporal area (EEG2) and occipital area (EEG 3) of the (A) cerebral hemisphere contra-lateral to the undercut during slow-wave sleep (SWS), and of the (B) cerebral hemisphere ipsi-lateral to the undercut during 4 Hz spike-wave (SW) ictal events, in cats with chronic deafferentation of the suprasylvian gyrus. Right panels contain averaged responses of both EEG recordings and extracellular potassium (n = 50 for each Avg).
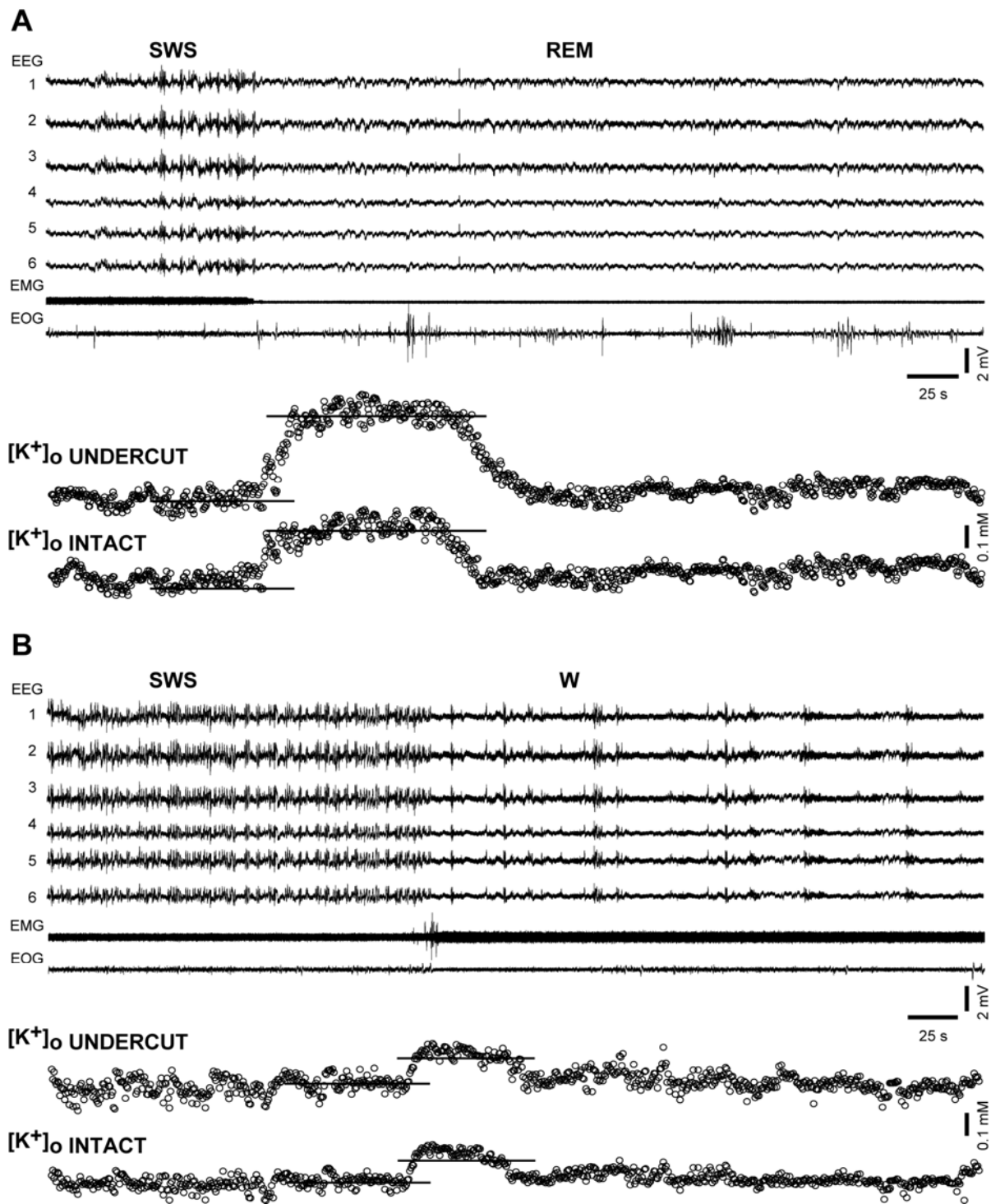
Figure 12-5. Steady increase of the extracellular potassium concentration ($[K^+]_o$) following cortical activation. A) Variation of $[K^+]_o$ in the undercut and contra-lateral intact cortex at the transition between slow-wave sleep (SWS) and rapid-eye movements sleep (REM). B) Variation of $[K^+]_o$ in the undercut and contra-lateral intact cortex at the transition between slow-wave sleep (SWS) and wake (W). The increase of $[K^+]_o$ is more ample and lasts longer in chronically deafferented cortex compared to intact cortex. EEG = electroencephalogram in (1) - frontal, (2) - temporal, and (3) - occipital areas of the cerebral hemisphere contra-lateral to the undercut; EEG (4) - frontal, (5) - temporal, and (6) - occipital areas of the cerebral hemisphere ipsi-lateral to the undercut; EOG = electro-oculogram; EMG = electro-miogram.
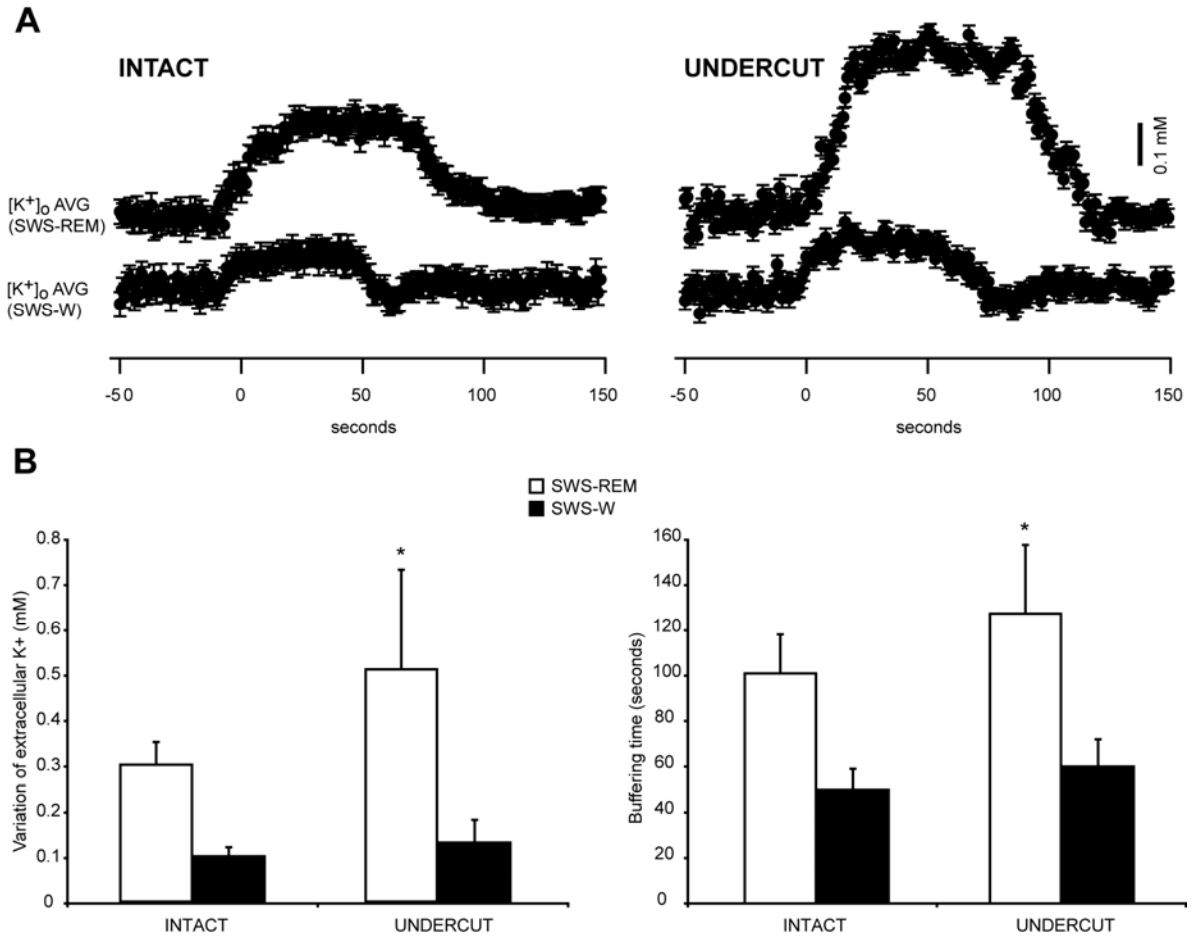
Figure 12-6. Average increase of the extracellular potassium concentration ($[K^+]_o$) following cortical activation. A) Variation of the recorded $[K^+]_o$ in the intact cortex (left panel) and chronically deafferented cortex (right panel) during the transitions from slow-wave sleep (SWS) to rapid-eye movements (REM) sleep and from SWS to wake (W). B) Quantification of the amplitude and duration of $[K^+]_o$ increase following cortical activation in all recorded animals. Note the higher increase of $[K^+]_o$ at the transition SWS-REM compared to SWS-W, particularly in the deafferented cortex. *, $p<0.05$
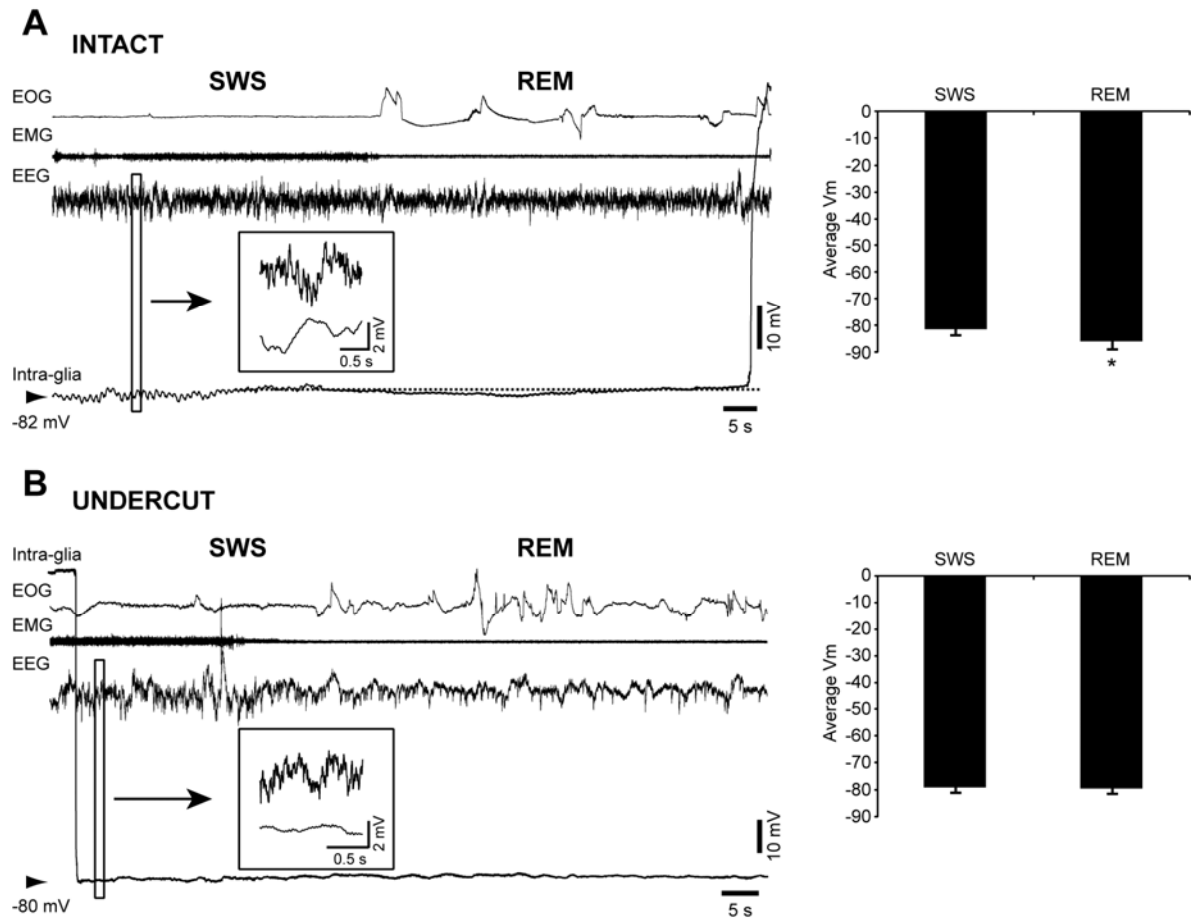
Figure 12-7. Intracellular recordings of presumed glial cells during cortical activation from slow-wave sleep (SWS) to REM sleep in intact (A) and chronically deafferented (B) cortex. Insets depict the relationship of the local field potential (EEG) to the intracellular recordings. Right panels contain the averaged variation of glial membrane potential (Vm) computed on 30 seconds windows before and after the activation. Note the hyperpolarization of the glial Vm in the intact cortex and the lack of variation in the undercut cortex. EOG = electro-oculogram; EMG = electro-miogram. *, $p<0.05$.

13 Conclusions

In the present work, the cellular and homeostatic network mechanisms of posttraumatic seizures have been studies, in an *in vivo* model. Our results shed new light on the cellular mechanisms of posttraumatic epilepsy, both from a histological and from a physiological point of view. We also explored the relationship between these anatomical and electrophysiological changes and different states of vigilance. The results were presented and discussed in detail in the preceding sections. This section will present a general discussion of the findings and an integrated model of them.

The study dealt mainly with four different topics, all of them tightly interconnected and related to the changes induced by the cortical deafferentation:

1. The modulation of synaptic and intrinsic neuronal properties following undercut,

2. The progressive loss of neurons, particularly inhibitory GABAergic, correlated with a gradual increase propensity to seizures after deafferentation,

3. Laminar redistribution of neuronal discharge and initiation of spontaneous cortical activities,

4. Posttraumatic reactive astrocytosis and dysfunctional glia associated with an impaired K^+ clearance.

As shown previously, a higher frequency of seizures following head trauma has been described both in humans (Salazar et al., 1985; Weiss et al., 1986; Annegers et al., 1998) and in animal models (Prince and Tseng, 1993; Topolnik et al., 2003a, b; D'Ambrosio et al., 2004; Nita et al., 2006). The mechanisms responsible for this outcome include changes in intrinsic properties of pyramidal neurons (Esplin et al., 1994; Topolnik et al., 2003b), enhanced excitatory synaptic conductances without altered inhibition (Bush et al., 1999; Houweling et al., 2005), but also disorganization of normal cerebral cytoarchitecture either as microgyria and cortical sclerosis (Jacobs et al., 1999a; Jacobs et

al., 1999b; Marin-Padilla, 1999; Marin-Padilla et al., 2002), or neuronal death (Lowenstein et al., 1992; Marin-Padilla, 1999; Marin-Padilla et al., 2002; Swartz et al., 2006).

Epileptogenesis following cortical deafferentation is probably prompted by long-term complex structural changes of neuronal networks (Lowenstein, 1996), triggered by the chronic input deprivation of the cortex superjacent to the undercut. The progressive nature of posttraumatic epilepsy described in humans (Annegers, 1994; Herman, 2002) as well as in animals (Nita et al., 2006) imposed three directions of our experiments: first, to investigate possible anatomical changes related both to neurons and glia that could contribute to the evolution of PTS following undercut; second, to explore the electrophysiological outcome of these anatomical modifications; third, to compare the results at different time delays following trauma, so we could understand and describe the gradual changes and maybe identify the time-limit to which prophylactic therapy could be efficient in preventing late epileptogenesis.

13.1 Posttraumatic neuronal loss and cortical disorganization

Studies in children with brain trauma showed that the neurons directly damaged by the initial insult or incapable of reconnecting with other neurons died, while neurons capable of reestablishing post-injury functional connections survived and started a process of reorganization (Marin-Padilla et al., 2002). Similarly, we found an important neuronal loss particularly in the deep cortical layers, close to the undercut, mainly due to the massive disappearance of GABAergic neurons and restricted to the injured gyrus (Fig. 10-5).

The higher sensitivity of GABAergic neurons changed the balance between excitation and inhibition towards progressive increased excitation, which matched the aggravation of the paroxysmal activity displayed by the animals. Our results are supported by studies in which the deafferentation was produced in a non-traumatic manner, such as somatosensory (Welker et al., 1989) or visual (Hendry and Jones, 1986) deafferentation in animals, which lead also to a reduction in the number of neurons containing GABA or its synthesizing enzyme, GAD. Similarly, studies in humans demonstrate as well, a reduction of GABAergic cortical inhibition and an enhancement of excitation following limb

amputation (Chen et al., 1998; Schwenkreis et al., 2000) or nerve ischemia (Ziemann et al., 1998).

We also found a progressive disruption of the cortical hexalaminar structure analogous to morphological studies done in epileptic patients resistant to antiepileptic drugs showing a complete disorganization of the cortical lamination accompanied by abnormalities in the morphology and distribution of inhibitory neurons (Spreafico et al., 1998b; Spreafico et al., 1998a). Moreover, in post-traumatic epileptic children the amount of residual gray matter displays areas of complete destruction alternating with areas of dysplastic tissue without lamination, similar to the cortical disorganization and decreased thickness of the gray matter that we observed in our experiments (Fig. 10-2). Reduced neocortical thickness and cortical complexity were also reported recently in humans with mesial temporal lobe epilepsy and hippocampal sclerosis (Lin et al., 2007).

The increased propensity to seizures following cortical undercut, despite neuronal loss and chronic input deprivation, suggests extensive reorganization of cortical connectivity and intrinsic cellular properties, which would make the neurons more excitable, generating epileptiform activity.

13.2 Homeostatic plasticity following chronic cortical deafferentation

The post-traumatic hyperexcitability of chronically injured cortical neurons emerges probably from homeostatic plasticity, a mechanism that works to restore a stable pattern of activity whenever networks are perturbed (Turrigiano, 1999; Abbott and Nelson, 2000; Davis and Bezprozvanny, 2001), mechanism triggered probably by the decreased activity in the undercut gyrus. This type of hypersensitivity by denervation is apparent in almost all investigated tissues, from smooth and skeletal muscles to glands and neurons (Cannon and Rosenblueth, 1949). However, after prolonged periods of reduced activity, as it is the case of the deafferented suprasylvian gyrus, homeostatic synaptic plasticity may increase network excitability in an uncontrollable manner, leading to the development of paroxysmal activity (Houweling et al., 2005). Indeed, long-term activity blockade

dramatically enhances cortical excitability and may lead to paroxysmal activity (Murthy et al., 2001; Burrone et al., 2002; Bausch et al., 2006).

In acute cortical deafferentation however, the increased excitability leading to the development of electrographic seizures (Topolnik et al., 2003b), is caused mainly by alterations of neuronal milieu induced by the injury, such as high $[K^+]_o$ which promotes cellular depolarization (Traynelis and Dingledine, 1988), accumulation of extracellular glutamate (Lipton and Rosenberg, 1994) and increased Ca^{2+} influx (Choi, 1988). The raise of intracellular Ca^{2+} concentration triggers a decrease in the concentration of extracellular Ca^{2+} (Wolf et al., 2001) and this significantly increases the failure of synaptic transmission (Markram et al., 1998; Massimini and Amzica, 2001; Crochet et al., 2005; Seigneur and Timofeev, 2007). Accordingly, we found an increased failure rate of synaptic transmission in the acutely injured cortex compared to control (Fig. 9-5).

In chronic undercut cortex, on the other hand, the decreased activity due to long-lasting input deprivation and the loss of neurons triggered indeed homeostatic mechanism. We demonstrated with intracellular recordings that longer periods of hyperpolarization correlate well with the increased firing rate of the same neuron, starting one month after cortical deafferentation (Fig. 9-8). Also, previous studies showed the occurrence of hyperpolarization periods during wake and REM sleep, when they don't normally appear, in chronic stages of the undercut (Nita et al., 2006). Additionally, we found in late stages of the undercut, an increased neuronal connectivity and a higher efficacy of excitatory connections (Fig. 9-4 and Fig. 9-5), suggestive of important structural reorganization such as axonal sprouting and formation of new synapses (Salin et al., 1995; Prince et al., 1997; Dancause et al., 2005). The intrinsic properties of the neurons were also modified towards increased excitability manifested by a progressively increased input resistance, number of spikes and instantaneous firing rate of neurons located in the traumatized cortex (Fig. 9-6 and Fig. 9-7), as previously described in the *in vitro* model of cortical undercut (Prince and Tseng, 1993; Tseng and Prince, 1996).

Our data obtained after both acute and chronic brain injuries follow the course of events also seen in humans, in which acute seizures are viewed as epiphenomena of the underlying brain disorder, with little independent impact on outcome from injury (Bladin et

al., 2000), while late or remote seizures are thought to be the result of epileptogenesis following chronic changes of neural networks favoring excitation (Herman, 2002). In contrast to patients with early post-injury seizures, those with late-onset seizures are at greater risk of epilepsy (Bladin et al., 2000), probably because they express the already irreversible changes of neuronal network.

13.3 Laminar redistribution of spontaneous cortical activity

The involvement of homeostatic plasticity mechanisms in triggering the increased posttraumatic cortical hyperexcitability is further sustained by the fact that we recorded a dramatic increase of total firing rate during natural SWS in chronically injured cortex (Fig. 11-1, 11-2, 11-3), although previous studies *in vivo* showed a similar firing frequency during wake and SWS in normal cortex (Steriade et al., 2001). This finding suggests that the periods of disfacilitation characterizing SWS contributed to the up-regulation of neuronal excitability and as shown formerly, neurons were probably more likely to display bursts at the end of hyperpolarizing periods (Nita et al., 2006).

The loss of neurons particularly from deep cortical layers of the undercut gyrus, as well as the information that the seizure activity is more likely to start from the anterior, relatively intact, part of the deafferented gyrus (Topolnik et al., 2003b), prompted us to explore the potential changes of neuronal discharge pattern and initiation of spontaneous cortical activity after undercut. Indeed, the depth profile of neuronal firing showed a significant reduction in the level of spontaneous firing particularly in the deep cortical layers of area 21 from chronically deafferented gyrus compared to early stages of the undercut and compared to the neurons from area 5, less affected by the cortical undercut (Fig. 11-3).

This decreased total discharge rate of deeply laying neurons, restricted to the posterior, partially deafferented part of the chronically undercut gyrus, raises further questions regarding the laminar origin of the spontaneous cortical activity in different parts of the gyrus, following deafferentation. *In vitro* recordings demonstrate that the slow oscillation of the neocortex is generated through a recurrent network of excitatory connections that generate a self-sustained period of depolarization, initiated by layer 5

pyramidal neurons, but propagates to deeper and more superficial layers (Sanchez-Vives and McCormick, 2000). Using CSD analysis of the spontaneous slow oscillation during SWS, we show that it originates indeed in the deep cortical layers and then spreads toward the more superficial ones, when recordings were done in the anterior part of the deafferented suprasylvian gyrus. The slow oscillation recorded in the posterior, completely deafferented, part of the gyrus, was initiated in the deep cortical layers only at early stages of the undercut. Nevertheless, in more chronic stages, characterized by degeneration of the deeply laying neurons, the origin of the slow oscillation and of the SW/PSW activity shifted to the more superficial layers and propagated from there through the cortical depth (Fig. 11-7 and Fig. 11-8).

13.4 Glial dysfunction and K^+ clearance impairment

Experimental data substantiating the hypothesis of a dialogue between neurons and glia in epilepsy have been obtained in studies of hippocampus and neocortex since the 1960s (Grossman and Hampton, 1968; Sypert and Ward, 1971; Dichter et al., 1972). Reactive gliosis occurs after virtually all injuries to the CNS (Penfield, 1929; D'Ambrosio, 2004) and it has been shown that glia found in areas of reduced neuronal density have an impaired capacity for spatial K^+ buffering (Heinemann et al., 2000). Similar alterations in astrocytic properties have been described in human temporal lobe epilepsy (Kivi et al., 2000; Gabriel et al., 2004) and in animal models of post-traumatic epilepsy (D'Ambrosio et al., 1999; Samuelsson et al., 2000).

We found an increased density of $GFAP^+$ cells and dysfunctional membrane properties of presumed glia, associated with an impaired K^+ clearance during the transition from SWS to REM sleep, in the undercut suprasylvian gyrus compared to the contra-lateral, intact gyrus (Fig. 12-5 and Fig. 11-6). Although a small increase of $[K^+]_o$ was also observed at the transition between SWS and wake, there were no statistical significant differences between the undercut and the intact cortex, suggesting that the defective mechanisms of K^+ reuptake became apparent only when high amounts of K^+ accumulated in the injured cortex. Downregulation of astroglial Kir channels has been described in the CNS following various injury-induced reactive gliosis, such as in deafferented entorhinal cortex (Schroder et al., 1999), in freeze lesion-induced cortical dysplasia (Bordey et al., 2000; Bordey et al.,

2001) and in traumatic (D'Ambrosio et al., 1999) and ischemic (Koller et al., 2000) brain injury. In addition, there have also been indications of downregulation of Kir currents in specimens from patients with temporal lobe epilepsy (Bordey and Sontheimer, 1998; Hinterkeuser et al., 2000; Kivi et al., 2000; Schroder et al., 2000). These data indicate that dysfunction of astroglial Kir channels in the reactive astrocytes following various brain injuries could underlie impaired K^+ buffering (Steinhauser and Seifert, 2002).

Studies in kindled cats showed that SWS-REM transitions and SWS are the sleep periods most vulnerable to secondary generalized seizures (Shouse, 1986). Similarly, in humans with temporal lobe epilepsy, in which secondary generalized seizures tend to be state-dependent, seizures have been described to occur more frequently during transitional periods into REM sleep and during SWS (Cadhilac, 1982). Therefore, the transient increased $[K^+]_o$ that marks the transition to REM sleep could be responsible for the increased propensity to seizures of this transitional sleep stage.

In conclusion, reactive gliosis following cortical deafferentation in cats is associated with an increased paroxysmal activity particularly during LSWS and with an impaired potassium clearance, probably due to astrocytic dysfunction.

13.5 Final remarks

In the present work we propose, as illustrated in Fig. 13-1, that following cortical deafferentation of the suprasylvian gyrus in cats, the death of deeply laying neurons and the chronic input deprivation lead first, to a redistribution of discharge rate in the upper layers of the deafferented part of the gyrus; secondly, they trigger homeostatic mechanisms that would subsequently modify intrinsic neuronal properties towards hyperexcitability by increased input resistance and firing rate, would increase neuronal connectivity and the efficacy of excitatory connections. Also, cortical trauma induces reactive gliosis and dysfunctional glial cells, responsible for the impaired K^+ clearance in the injured cortex, which additionally increases the excitability of neurons, generating finally paroxysmal activity.
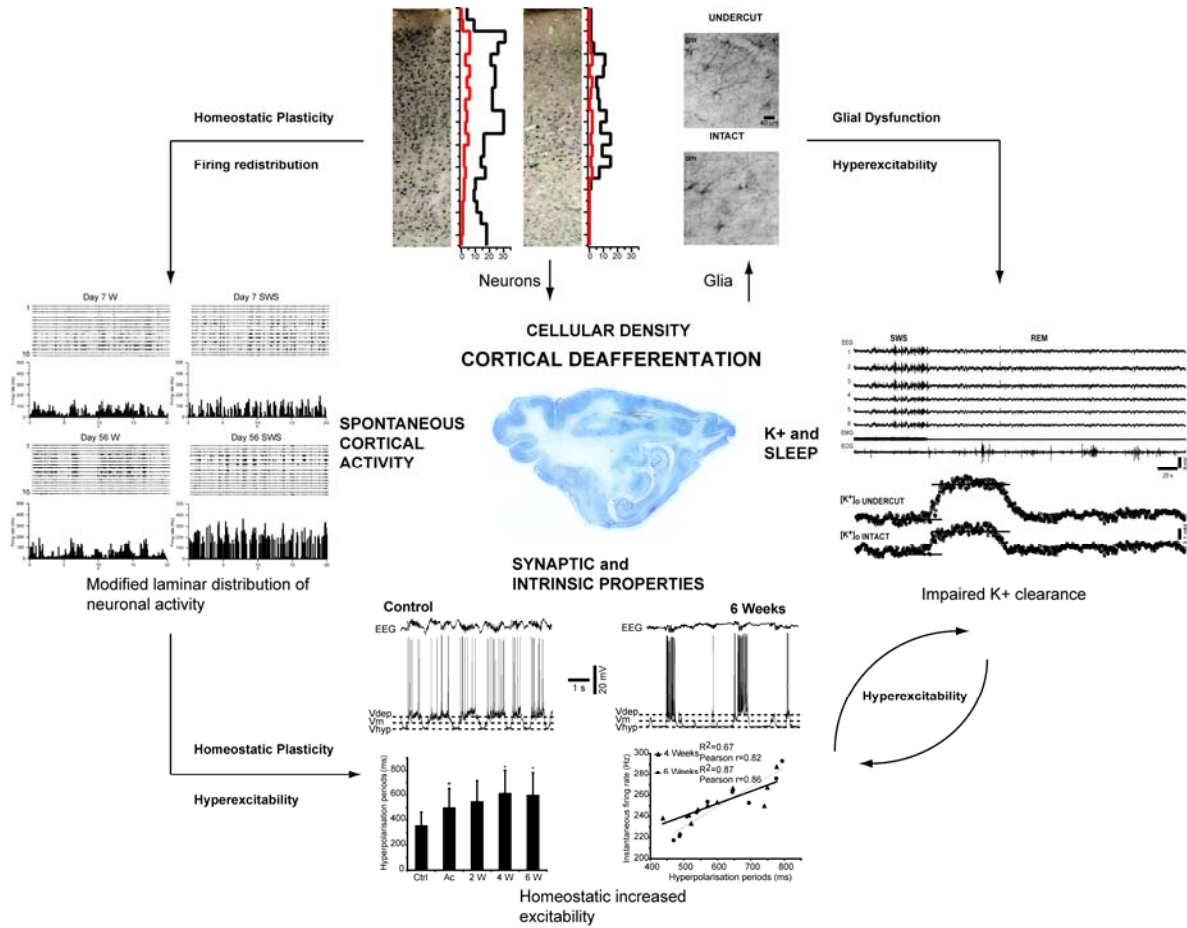
Figure 13-1. Integrated mechanisms of epileptogenesis following chronic cortical deafferentation.

I hope the data presented here sheds new light on the basic mechanisms of posttraumatic epilepsy, and more than that, I hope my work will stimulate other researchers in the field of epileptology and will goad them into approaching many of the still unsolved issues of epilepsy.

References

Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. NatNeurosci 3 Suppl:1178-1183.

Agathonikou A, Panayiotopoulos CP, Giannakodimos S, Koutroumanidis M (1998) Typical absence status in adults: diagnostic and syndromic considerations. Epilepsia 39:1265-1276.

Aicardi J, Levy Gomes A (1992) Clinical and electroencephalographic symptomatology of the 'genuine' Lennox-Gastaut syndrome and its differentiation from other forms of epilepsy of early childhood. Epilepsy Res Suppl 6:185-193.

Ajmone-Marsan C, Ralston B (1956) Thalamic control of certain normal and abnormal cortical rhythms. Electroencephalogr Clin Neurophysiol 8:559-582.

Alkadhi KA, Tian LM (1996) Veratridine-enhanced persistent sodium current induces bursting in CA1 pyramidal neurons. Neuroscience 71:625-632.

Amiry-Moghaddam M, Ottersen OP (2003c) The molecular basis of water transport in the brain. Nat Rev Neurosci 4:991-1001.

Amiry-Moghaddam M, Williamson A, Palomba M, Eid T, de Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP (2003b) Delayed K+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. Proc Natl Acad Sci U S A 100:13615-13620.

Amiry-Moghaddam M, Otsuka T, Hurn PD, Traystman RJ, Haug FM, Froehner SC, Adams ME, Neely JD, Agre P, Ottersen OP, Bhardwaj A (2003a) An alpha-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. Proc Natl Acad Sci U S A 100:2106-2111.

Amzica F, Massimini M, Manfridi A (2002) Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. J Neurosci 22:1042-1053.

Andermann F, Robb JP (1972) Absence status. A reappraisal following review of thirty-eight patients. Epilepsia 13:177-187.

Andrew RD, Fagan M, Ballyk BA, Rosen AS (1989) Seizure susceptibility and the osmotic state. Brain Res 498:175-180.

Annegers JF (1994) The natural course of epilepsy: an epidemiologic perspective. In: The surgical management of epilepsy (Wyler A.R. HBP, ed), pp 3-7. Boston: Butterworth-Heinemann.

Annegers JF, Hauser WA, Coan SP, Rocca WA (1998) A population-based study of seizures after traumatic brain injuries. N Engl J Med 338:20-24.

Annegers JF, Grabow JD, Groover RV, Laws ER, Jr., Elveback LR, Kurland LT (1980) Seizures after head trauma: a population study. Neurology 30:683-689.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 8:963-970.

Babb TL, Mathern GW, Leite JP, Pretorius JK, Yeoman KM, Kuhlman PA (1996) Glutamate AMPA receptors in the fascia dentata of human and kainate rat hippocampal epilepsy. Epilepsy Res 26:193-205.

Bal T, von Krosigk M, McCormick DA (1995) Synaptic and membrane mechanisms underlying synchronized oscillations in the ferret lateral geniculate nucleus in vitro. J Physiol 483 (Pt 3):641-663.

Baldy-Moulinier M (1986) Inter-relationships between sleep and epilepsy. In: Recent advances in epilepsy (Pedley TAaM, B.S., ed), pp 37–55. Edinburgh: Churchill Livingstone.

Ballanyi K, Grafe P, ten Bruggencate G (1987) Ion activities and potassium uptake mechanisms of glial cells in guinea-pig olfactory cortex slices. J Physiol 382:159-174.

Bausch SB, He S, Petrova Y, Wang XM, McNamara JO (2006) Plasticity of both excitatory and inhibitory synapses is associated with seizures induced by removal of chronic blockade of activity in cultured hippocampus. JNeurophysiol 96:2151-2167.

Bayer TA, Wiestler OD, Wolf HK (1995) Hippocampal loss of N-methyl-D-aspartate receptor subunit 1 mRNA in chronic temporal lobe epilepsy. Acta Neuropathol 89:446-450.

Bazil CW, Walczak TS (1997) Effects of sleep and sleep stage on epileptic and nonepileptic seizures. Epilepsia 38:56-62.

Bazil CW, Castro LH, Walczak TS (2000) Reduction of rapid eye movement sleep by diurnal and nocturnal seizures in temporal lobe epilepsy. Arch Neurol 57:363-368.

Beghi E (2003) Overview of studies to prevent posttraumatic epilepsy. Epilepsia 44 Suppl 10:21-26.

Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. J Physiol 416:303-325.

Berger H (1929) Uber des EEG des menschen. Arch Psych Nervkrankh:87: 527.

Binder DK, Steinhauser C (2006) Functional changes in astroglial cells in epilepsy. Glia 54:358-368.

Bladin CF, Alexandrov AV, Bellavance A, Bornstein N, Chambers B, Cote R, Lebrun L, Pirisi A, Norris JW (2000) Seizures after stroke: a prospective multicenter study. Arch Neurol 57:1617-1622.

Blumcke I, Thom M, Wiestler OD (2002) Ammon's horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. Brain Pathol 12:199-211.

Bonislawski DP, Schwarzbach EP, Cohen AS (2007) Brain injury impairs dentate gyrus inhibitory efficacy. Neurobiol Dis 25:163-169.

Bordey A, Sontheimer H (1998) Properties of human glial cells associated with epileptic seizure foci. Epilepsy Res 32:286-303.

Bordey A, Hablitz JJ, Sontheimer H (2000) Reactive astrocytes show enhanced inwardly rectifying K+ currents in situ. Neuroreport 11:3151-3155.

Bordey A, Lyons SA, Hablitz JJ, Sontheimer H (2001) Electrophysiological characteristics of reactive astrocytes in experimental cortical dysplasia. J Neurophysiol 85:1719-1731.

Boucetta S, Chauvette S, Bazhenov M, Timofeev I (2008) Focal generation of paroxysmal fast runs during electrographic seizures. Epilepsia (DOI: 10.1111/j.1528-1167.2008.01707.x).

Briellmann RS, Kalnins RM, Berkovic SF, Jackson GD (2002) Hippocampal pathology in refractory temporal lobe epilepsy: T2-weighted signal change reflects dentate gliosis. Neurology 58:265-271.

Bruns J, Jr., Hauser WA (2003) The epidemiology of traumatic brain injury: a review. Epilepsia 44 Suppl 10:2-10.

Buckmaster PS, Zhang GF, Yamawaki R (2002) Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. J Neurosci 22:6650-6658.

Burrone J, O'Byrne M, Murthy VN (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. Nature 420:414-418.

Bush PC, Prince DA, Miller KD (1999) Increased pyramidal excitability and NMDA conductance can explain posttraumatic epileptogenesis without disinhibition: a model. J Neurophysiol 82:1748-1758.

Butt AM, Kalsi A (2006) Inwardly rectifying potassium channels (Kir) in central nervous system glia: a special role for Kir4.1 in glial functions. J Cell Mol Med 10:33-44.

Cadhilac J (1982) Complex partial seizures and REM sleep. In: Sleep and Epilepsy (Sterman M.B. SMN, Passouan P., ed), pp 315-324. New York: Academic Press.

Cannon WB, Rosenblueth A (1949) The supersensitivity of denervated structures: a law of denervation. New York: Macmillan.

Castro-Alamancos MA (1999) Neocortical synchronized oscillations induced by thalamic disinhibition in vivo. J Neurosci 19:RC27.

Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L (1991) Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. Epilepsia 32:778-782.

Caveness WF, Meirowsky AM, Rish BL, Mohr JP, Kistler JP, Dillon JD, Weiss GH (1979) The nature of posttraumatic epilepsy. J Neurosurg 50:545-553.

Chagnac-Amitai Y, Connors BW (1989a) Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. JNeurophysiol 62:1149-1162.

Chagnac-Amitai Y, Connors BW (1989b) Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. J Neurophysiol 61:747-758.

Chamberlin NL, Dingledine R (1988) GABAergic inhibition and the induction of spontaneous epileptiform activity by low chloride and high potassium in the hippocampal slice. Brain Res 445:12-18.

Charpier S, Leresche N, Deniau JM, Mahon S, Hughes SW, Crunelli V (1999) On the putative contribution of GABA(B) receptors to the electrical events occurring during spontaneous spike and wave discharges. Neuropharmacology 38:1699-1706.

Chebabo SR, Hester MA, Aitken PG, Somjen GG (1995) Hypotonic exposure enhances synaptic transmission and triggers spreading depression in rat hippocampal tissue slices. Brain Res 695:203-216.

Chen Q, He S, Hu XL, Yu J, Zhou Y, Zheng J, Zhang S, Zhang C, Duan WH, Xiong ZQ (2007) Differential roles of NR2A- and NR2B-containing NMDA receptors in activity-dependent brain-derived neurotrophic factor gene regulation and limbic epileptogenesis. J Neurosci 27:542-552.

Chen R, Corwell B, Yaseen Z, Hallett M, Cohen LG (1998) Mechanisms of cortical reorganization in lower-limb amputees. J Neurosci 18:3443-3450.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623-634.

Chvatal A, Anderova M, Ziak D, Sykova E (1999) Glial depolarization evokes a larger potassium accumulation around oligodendrocytes than around astrocytes in gray matter of rat spinal cord slices. J Neurosci Res 56:493-505.

Chvatal A, Berger T, Vorisek I, Orkand RK, Kettenmann H, Sykova E (1997) Changes in glial K+ currents with decreased extracellular volume in developing rat white matter. J Neurosci Res 49:98-106.

Clark S, Wilson WA (1999) Mechanisms of epileptogenesis. Adv Neurol 79:607-630.

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R (2002) On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 298:1418-1421.

Cole AJ (2000) Is epilepsy a progressive disease? The neurobiological consequences of epilepsy. Epilepsia 41 Suppl 2:S13-22.

Commission on Epidemiology and Prognosis ILAE (1981) Guidelines for studies on epilepsy.

Connors NC, Adams ME, Froehner SC, Kofuji P (2004) The potassium channel Kir4.1 associates with the dystrophin-glycoprotein complex via alpha-syntrophin in glia. J Biol Chem 279:28387-28392.

Cordingley GE, Somjen GG (1978) The clearing of excess potassium from extracellular space in spinal cord and cerebral cortex. Brain Res 151:291-306.

Coull JA, Boudreau D, Bachand K, Prescott SA, Nault F, Sik A, De Koninck P, De Koninck Y (2003) Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature 424:938-942.

Crespel A, Baldy-Moulinier M, Coubes P (1998) The relationship between sleep and epilepsy in frontal and temporal lobe epilepsies: practical and physiopathologic considerations. Epilepsia 39:150-157.

Crespel A, Coubes P, Baldy-Moulinier M (2000) Sleep influence on seizures and epilepsy effects on sleep in partial frontal and temporal lobe epilepsies. Clin Neurophysiol 111 Suppl 2:S54-59.

Crochet S, Chauvette S, Boucetta S, Timofeev I (2005) Modulation of synaptic transmission in neocortex by network activities. Eur J Neurosci 21:1030-1044.

D'Ambrosio R (2004) The role of glial membrane ion channels in seizures and epileptogenesis. Pharmacol Ther 103:95-108.

D'Ambrosio R, Maris DO, Grady MS, Winn HR, Janigro D (1999) Impaired K(+) homeostasis and altered electrophysiological properties of post-traumatic hippocampal glia. J Neurosci 19:8152-8162.

D'Ambrosio R, Fairbanks JP, Fender JS, Born DE, Doyle DL, Miller JW (2004) Post-traumatic epilepsy following fluid percussion injury in the rat. Brain 127:304-314.

Dancause N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina EV, Stowe AM, Nudo RJ (2005) Extensive cortical rewiring after brain injury. J Neurosci 25:10167-10179.

Darian-Smith C, Gilbert CD (1994) Axonal sprouting accompanies functional reorganization in adult cat striate cortex. Nature 368:737-740.

Davenport CJ, Brown WJ, Babb TL (1990) GABAergic neurons are spared after intrahippocampal kainate in the rat. Epilepsy Res 5:28-42.

Davis GW, Bezprozvanny I (2001) Maintaining the stability of neural function: a homeostatic hypothesis. AnnuRevPhysiol 63:847-869.

De Groat WC (1972) GABA-depolarization of a sensory ganglion: antagonism by picrotoxin and bicuculline. Brain Res 38:429-432.

de la Pena E, Geijo-Barrientos E (1996) Laminar localization, morphology, and physiological properties of pyramidal neurons that have the low-threshold calcium current in the guinea-pig medial frontal cortex. J Neurosci 16:5301-5311.

de Lanerolle NC, Kim JH, Williamson A, Spencer SS, Zaveri HP, Eid T, Spencer DD (2003) A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: evidence for distinctive patient subcategories. Epilepsia 44:677-687.

de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD (1989) Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res 495:387-395.

de Lanerolle NC, Lee TS (2005) New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. Epilepsy Behav 7:190-203.

Desai BT, Whitman S, Coonley-Hoganson R, Coleman TE, Gabriel G, Dell J (1983) Seizures and civilian head injuries. Epilepsia 24:289-296.

Desai NS, Rutherford LC, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. NatNeurosci 2:515-520.

Desai NS, Cudmore RH, Nelson SB, Turrigiano GG (2002) Critical periods for experience-dependent synaptic scaling in visual cortex. NatNeurosci 5:783-789.

Destexhe A (1998) Spike-and-wave oscillations based on the properties of GABAB receptors. J Neurosci 18:9099-9111.

Dichter MA, Ayala GF (1987) Cellular mechanisms of epilepsy: a status report. Science 237:157-164.

Dichter MA, Herman CJ, Selzer M (1972) Silent cells during interictal discharges and seizures in hippocampal penicillin foci. Evidence for the role of extracellular K+ in the transition from the interictal state to seizures. Brain Res 48:173-183.

Dinner DS (1993) Posttraumatic epilepsy. In: The treatment of epilepsy: principles (Wyllie E, ed), pp 654-658. Philadelphia: Lea & Fibinger.

Domeniconi M, Cao Z, Spencer T, Sivasankaran R, Wang K, Nikulina E, Kimura N, Cai H, Deng K, Gao Y, He Z, Filbin M (2002) Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. Neuron 35:283-290.

Dudek FE, Obenaus A, Tasker JG (1990) Osmolality-induced changes in extracellular volume alter epileptiform bursts independent of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. Neurosci Lett 120:267-270.

Dulac O (2003) Atypical absence. Commission of Classification and Terminology of the International League Against Epilepsy

Dulac O, N'Guyen T (1993) The Lennox-Gastaut syndrome. Epilepsia 34 Suppl 7:S7-17.

Dulac O, Engel J, Jr. (2003) Lennox-Gastaut Syndrome. Commission of Classification and Terminology of the International League Against Epilepsy

Duncan JS (1999) Positron emission tomography receptor studies. Adv Neurol 79:893-899.

Eccles JC, Libet B, Young RR (1958) The behaviour of chromatolysed motoneurones studied by intracellular recording. J Physiol 143:11-40.

Echlin FA, Battista A (1963) Epileptiform Seizures from Chronic Isolated Cortex. Arch Neurol 9:154-170.

Eid T, Lee TS, Thomas MJ, Amiry-Moghaddam M, Bjornsen LP, Spencer DD, Agre P, Ottersen OP, de Lanerolle NC (2005) Loss of perivascular aquaporin 4 may underlie deficient water and K+ homeostasis in the human epileptogenic hippocampus. Proc Natl Acad Sci U S A 102:1193-1198.

Elwes RD, Johnson AL, Shorvon SD, Reynolds EH (1984) The prognosis for seizure control in newly diagnosed epilepsy. N Engl J Med 311:944-947.

Epilepsy CoEaPotILA (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 30:389-399.

Esclapez M, Hirsch JC, Khazipov R, Ben-Ari Y, Bernard C (1997) Operative GABAergic inhibition in hippocampal CA1 pyramidal neurons in experimental epilepsy. Proc Natl Acad Sci U S A 94:12151-12156.

Esplin MS, Abbott JR, Smart ML, Burroughs AF, Frandsen TC, Litzinger MJ (1994) Voltage-sensitive calcium channel development in epileptic DBA/2J mice suggests altered presynaptic function. Epilepsia 35:911-914.

Faden AI, Demediuk P, Panter SS, Vink R (1989) The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science 244:798-800.

Fallon J, Reid S, Kinyamu R, Opole I, Opole R, Baratta J, Korc M, Endo TL, Duong A, Nguyen G, Karkehabadhi M, Twardzik D, Patel S, Loughlin S (2000) In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. Proc Natl Acad Sci U S A 97:14686-14691.

Feldberg W, Sherwood SL (1957) Effects of calcium and potassium injected into the cerebral ventricles of the cat. J Physiol 139:408-416.

Feng Z, Durand DM (2006) Effects of potassium concentration on firing patterns of low-calcium epileptiform activity in anesthetized rat hippocampus: inducing of persistent spike activity. Epilepsia 47:727-736.

Fertziger AP, Ranck JB, Jr. (1970) Potassium accumulation in interstitial space during epileptiform seizures. Exp Neurol 26:571-585.

Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 46:470-472.

Fitch MT, Doller C, Combs CK, Landreth GE, Silver J (1999) Cellular and molecular mechanisms of glial scarring and progressive cavitation: in vivo and in vitro analysis of inflammation-induced secondary injury after CNS trauma. J Neurosci 19:8182-8198.

Forsythe ID, Redman SJ (1988) The dependence of motoneurone membrane potential on extracellular ion concentrations studied in isolated rat spinal cord. J Physiol 404:83-99.

Franck JE, Schwartzkroin PA (1985) Do kainate-lesioned hippocampi become epileptogenic? Brain Res 329:309-313.

Franck JE, Kunkel DD, Baskin DG, Schwartzkroin PA (1988) Inhibition in kainate-lesioned hyperexcitable hippocampi: physiologic, autoradiographic, and immunocytochemical observations. J Neurosci 8:1991-2002.

Frankenhaeuser B, Hodgkin AL (1956) The after-effects of impulses in the giant nerve fibres of Loligo. J Physiol 131:341-376.

French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD (1993) Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol 34:774-780.

Frey LC (2003) Epidemiology of posttraumatic epilepsy: a critical review. Epilepsia 44 Suppl 10:11-17.

Frisen J, Haegerstrand A, Risling M, Fried K, Johansson CB, Hammarberg H, Elde R, Hokfelt T, Cullheim S (1995) Spinal axons in central nervous system scar tissue are closely related to laminin-immunoreactive astrocytes. Neuroscience 65:293-304.

Frohlich F, Bazhenov M, Timofeev I, Sejnowski TJ (2005) Maintenance and termination of neocortical oscillations by dynamic modulation of intrinsic and synaptic excitability. Thalamus & Related Systems 3:147-156.

Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M (2003) Excitatory GABA input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells. Neuroscience 119:265-275.

Fukuda A, Prince DA (1992) Excessive intracellular Ca2+ inhibits glutamate-induced Na(+)-K+ pump activation in rat hippocampal neurons. J Neurophysiol 68:28-35.

Fusco L, Vigevano F (1993) Ictal clinical electroencephalographic findings of spasms in West syndrome. Epilepsia 34:671-678.

Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ER, Eilers A, Kivi A, Zeller M, Meencke HJ, Cavalheiro EA, Heinemann U, Lehmann TN (2004) Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. J Neurosci 24:10416-10430.

Galanopoulou AS (2007) Developmental patterns in the regulation of chloride homeostasis and GABA(A) receptor signaling by seizures. Epilepsia 48 Suppl 5:14-18.

Galarreta M, Hestrin S (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402:72-75.

Galeffi F, Sah R, Pond BB, George A, Schwartz-Bloom RD (2004) Changes in intracellular chloride after oxygen-glucose deprivation of the adult hippocampal slice: effect of diazepam. J Neurosci 24:4478-4488.

Gastaut H, Roger J, Soulayrol R, Saint-Jean M, Tassinari CA, Regis H, Bernard R, Pinsard N, Dravet C (1966) Childhood epileptic encephalopathy with diffuse slow spike-waves (otherwise known as "Petit mal variant") or Lennox syndrome. Epilepsia 7:139-179.

Giaretta D, Avoli M, Gloor P (1987) Intracellular recordings in pericruciate neurons during spike and wave discharges of feline generalized penicillin epilepsy. Brain Res 405:68-79.

Gibbs EL, Gibbs FA (1947) Diagnostic and localizing value of electroencephalographic studies in sleep. Res Pbl Assoc Res Nerv Ment Dis 26:366–376.

Gibbs FA, Gibbs EL (1952) Atlas of Electroencephalography, 2nd edn. Cambridge, MA: Addison-Wesley.

Gibbs FA, Gibbs EL, Lennox WG (1939) The influence of blood sugar level on the wave and spike formation in petit mal epilepsy. Archives of Neurology and Psychiatry (Chicago) 47:1111-1116.

Gloor P (1979) Generalized epilepsy with spike-and-wave discharge: a reinterpretation of its electrographic and clinical manifestations. The 1977 William G. Lennox Lecture, American Epilepsy Society. Epilepsia 20:571-588.

Goldstein M (1990) Traumatic brain injury: a silent epidemic. Ann Neurol 27:327.

Gotts JE, Chesselet MF (2005a) Mechanisms of subventricular zone expansion after focal cortical ischemic injury. J Comp Neurol 488:201-214.

Gotts JE, Chesselet MF (2005b) Migration and fate of newly born cells after focal cortical ischemia in adult rats. J Neurosci Res 80:160-171.

Gould E, Tanapat P (1997) Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience 80:427-436.

Gowers WR (1885) Epilepsy and Other Chronic Convulsive Diseases. London: Williams Wood.

Graber KD, Prince DA (1999) Tetrodotoxin prevents posttraumatic epileptogenesis in rats. AnnNeurol 46:234-242.

Grafstein B (1956) Mechanism of spreading cortical depression. J Neurophysiol 19:154-171.

Grenier F, Timofeev I, Crochet S, Steriade M (2003) Spontaneous field potentials influence the activity of neocortical neurons during paroxysmal activities in vivo. Neuroscience 119:277-291.

Grossman RG, Hampton T (1968) Depolarization of cortical glial cells during electrocortical activity. Brain Res 11:316-324.

Gu JG, Albuquerque C, Lee CJ, MacDermott AB (1996) Synaptic strengthening through activation of Ca2+-permeable AMPA receptors. Nature 381:793-796.

Guberman A, Cantu-Reyna G, Stuss D, Broughton R (1986) Nonconvulsive generalized status epilepticus: clinical features, neuropsychological testing, and long-term follow-up. Neurology 36:1284-1291.

Guth L, Barrett CP, Donati EJ, Anderson FD, Smith MV, Lifson M (1985) Essentiality of a specific cellular terrain for growth of axons into a spinal cord lesion. Exp Neurol 88:1-12.

Hablitz JJ, Lundervold A (1981) Hippocampal excitability and changes in extracellular potassium. Exp Neurol 71:410-420.

Haglund MM, Hochman DW (2005) Furosemide and mannitol suppression of epileptic activity in the human brain. J Neurophysiol 94:907-918.

Hahn YS, Fuchs S, Flannery AM, Barthel MJ, McLone DG (1988) Factors influencing posttraumatic seizures in children. Neurosurgery 22:864-867.

Halasz P (1991) Runs of rapid spikes in sleep: a characteristic EEG expression of generalized malignant epileptic encephalopathies. A conceptual review with new pharmacological data. Epilepsy Res Suppl 2:49-71.

Hauser WA, Annegers JF, Rocca WA (1996) Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. Mayo Clin Proc 71:576-586.

Hauser WA, Kurland LT (1975) The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. Epilepsia 16:1-66.

Hauser WA, Tabaddor K, Factor PR, Finer C (1984) Seizures and head injury in an urban community. Neurology 34:746-751.

Heinemann U, Lux HD (1975) Undershoots following stimulus-induced rises of extracellular potassium concentration in cerebral cortex of cat. Brain Res 93:63-76.

Heinemann U, Lux HD (1977) Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. Brain Res 120:231-249.

Heinemann U, Gabriel S, Jauch R, Schulze K, Kivi A, Eilers A, Kovacs R, Lehmann TN (2000) Alterations of glial cell function in temporal lobe epilepsy. Epilepsia 41 Suppl 6:S185-189.

Hendry SH, Jones EG (1986) Reduction in number of immunostained GABAergic neurones in deprived-eye dominance columns of monkey area 17. Nature 320:750-753.

Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 282:1504-1508.

Herman ST (2002) Epilepsy after brain insult: targeting epileptogenesis. Neurology 59:S21-26.

Herman ST, Walczak TS, Bazil CW (2001) Distribution of partial seizures during the sleep--wake cycle: differences by seizure onset site. Neurology 56:1453-1459.

Herreras O, Somjen GG (1993) Analysis of potential shifts associated with recurrent spreading depression and prolonged unstable spreading depression induced by microdialysis of elevated K+ in hippocampus of anesthetized rats. Brain Res 610:283-294.

Hibino H, Fujita A, Iwai K, Yamada M, Kurachi Y (2004) Differential assembly of inwardly rectifying K+ channel subunits, Kir4.1 and Kir5.1, in brain astrocytes. J Biol Chem 279:44065-44073.

Higashi K, Fujita A, Inanobe A, Tanemoto M, Doi K, Kubo T, Kurachi Y (2001) An inwardly rectifying K(+) channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. Am J Physiol Cell Physiol 281:C922-931.

Hinterkeuser S, Schroder W, Hager G, Seifert G, Blumcke I, Elger CE, Schramm J, Steinhauser C (2000) Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. Eur J Neurosci 12:2087-2096.

Hoffman SN, Salin PA, Prince DA (1994) Chronic neocortical epileptogenesis in vitro. JNeurophysiol 71:1762-1773.

Holthoff K, Witte OW (2000) Directed spatial potassium redistribution in rat neocortex. Glia 29:288-292.

Horner PJ, Gage FH (2000) Regenerating the damaged central nervous system. Nature 407:963-970.

Houweling AR, Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ (2005) Homeostatic synaptic plasticity can explain post-traumatic epileptogenesis in chronically isolated neocortex. CerebCortex 15:834-845.

Hrachovy RA, Frost JD, Jr. (1989) Infantile spasms. Pediatr Clin North Am 36:311-329.

Hubner CA, Stein V, Hermans-Borgmeyer I, Meyer T, Ballanyi K, Jentsch TJ (2001) Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. Neuron 30:515-524.

Hume RI, Dingledine R, Heinemann SF (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253:1028-1031.

Isokawa M (1998) Modulation of GABAA receptor-mediated inhibition by postsynaptic calcium in epileptic hippocampal neurons. Brain Res 810:241-250.

Isokawa M, Mello LE (1991) NMDA receptor-mediated excitability in dendritically deformed dentate granule cells in pilocarpine-treated rats. Neurosci Lett 129:69-73.

Jackson J (1870) A study of convulsions, Transactions St. Andrews Medical Graduates Association, volume iii. Reprintred in: J Taylor, G Holmes, F M R Walshe (eds), Selected Writings of John Hughlings Jackson 1:8-36 (1958).

Jacobs KM, Graber KD, Kharazia VN, Parada I, Prince DA (2000) Postlesional epilepsy: the ultimate brain plasticity. Epilepsia 41 Suppl 6:S153-S161.

Jacobs KM, Kharazia VN, Prince DA (1999a) Mechanisms underlying epileptogenesis in cortical malformations. Epilepsy Res 36:165-188.

Jacobs KM, Mogensen M, Warren E, Prince DA (1999b) Experimental microgyri disrupt the barrel field pattern in rat somatosensory cortex. Cereb Cortex 9:733-744.

Jallo JI, Narayan RK (2000) Craniocerebral trauma. In: Bradley WG, Daroff RB, Fenichel GM et al , eds Neurology in clinical practice Boston: Butterworth-Heinemann:1055– 1087.

Janz D (1953) Matutinal epilepsies; comparison with nocturnal and sleep epilepsies. Arch Psychiatr Nervenkr Z Gesamte Neurol Psychiatr 191:73-98.

Janz D (1962) The grand mal epilepsies and the sleeping-waking cycle. Epilepsia 3:69-109.

Jasper HH, Kershman J (1941) Electroencephalographic classification of the epilepsies. Archives of Neurology and Psychiatry 45:903-943.

Jayakar PB, Seshia SS (1991) Electrical status epilepticus during slow-wave sleep: a review. J Clin Neurophysiol 8:299-311.

Jennett B (1975) Epilepsy after non-missile head injury. England: William Heinemann Medical Books.

Jensen MS, Azouz R, Yaari Y (1994) Variant firing patterns in rat hippocampal pyramidal cells modulated by extracellular potassium. J Neurophysiol 71:831-839.

Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci U S A 98:4710-4715.

Jin X, Prince DA, Huguenard JR (2006) Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. J Neurosci 26:4891-4900.

Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J (1999) Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96:25-34.

Johnston D, Brown TH (1981) Giant synaptic potential hypothesis for epileptiform activity. Science 211:294-297.

Johnston D, Brown TH (1984) The synaptic nature of the paroxysmal depolarizing shift in hippocampal neurons. Ann Neurol 16 Suppl:S65-71.

Juhasz C, Nagy F, Muzik O, Watson C, Shah J, Chugani HT (1999) [11C]Flumazenil PET in patients with epilepsy with dual pathology. Epilepsia 40:566-574.

Kager H, Wadman WJ, Somjen GG (2000) Simulated seizures and spreading depression in a neuron model incorporating interstitial space and ion concentrations. J Neurophysiol 84:495-512.

Kager H, Wadman WJ, Somjen GG (2002) Conditions for the triggering of spreading depression studied with computer simulations. J Neurophysiol 88:2700-2712.

Kellaway P (1985) Sleep and epilepsy. Epilepsia 26 Suppl 1:S15-30.

Kellaway P, Hrachovy RA, Frost JD, Jr., Zion T (1979) Precise characterization and quantification of infantile spasms. Ann Neurol 6:214-218.

Kelly JP, Van Essen DC (1974) Cell structure and function in the visual cortex of the cat. J Physiol 238:515-547.

Kim JH (2001) Pathology of epilepsy. Exp Mol Pathol 70:345-367.

Kirkwood A, Bear MF (1994) Hebbian synapses in visual cortex. JNeurosci 14:1634-1645.

Kivi A, Lehmann TN, Kovacs R, Eilers A, Jauch R, Meencke HJ, von Deimling A, Heinemann U, Gabriel S (2000) Effects of barium on stimulus-induced rises of [K+]o in human epileptic non-sclerotic and sclerotic hippocampal area CA1. Eur J Neurosci 12:2039-2048.

Kobayashi K, Nishibayashi N, Ohtsuka Y, Oka E, Ohtahara S (1994) Epilepsy with electrical status epilepticus during slow sleep and secondary bilateral synchrony. Epilepsia 35:1097-1103.

Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. Neuroscience 129:1045-1056.

Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA (2000) Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. J Neurosci 20:5733-5740.

Kohr G, De Koninck Y, Mody I (1993) Properties of NMDA receptor channels in neurons acutely isolated from epileptic (kindled) rats. J Neurosci 13:3612-3627.

Koller H, Schroeter M, Jander S, Stoll G, Siebler M (2000) Time course of inwardly rectifying K(+) current reduction in glial cells surrounding ischemic brain lesions. Brain Res 872:194-198.

Kollevold T (1979) Immediate and early cerebral seizures after head injuries. Part IV. J Oslo City Hosp 29:35-47.

Korn SJ, Giacchino JL, Chamberlin NL, Dingledine R (1987) Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. J Neurophysiol 57:325-340.

Kostopoulos G, Gloor P, Pellegrini A, Gotman J (1981) A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: microphysiological features. Exp Neurol 73:55-77.

Kotagal P (1995) Multifocal independent Spike syndrome: relationship to hypsarrhythmia and the slow spike-wave (Lennox-Gastaut) syndrome. Clin Electroencephalogr 26:23-29.

Kriegstein AR, Suppes T, Prince DA (1987) Cellular and synaptic physiology and epileptogenesis of developing rat neocortical neurons in vitro. Brain Res 431:161-171.

Krishnan B, Armstrong DL, Grossman RG, Zhu ZQ, Rutecki PA, Mizrahi EM (1994) Glial cell nuclear hypertrophy in complex partial seizures. J Neuropathol Exp Neurol 53:502-507.

Kuffler SW (1967) Neuroglial cells: physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. Proc R Soc Lond B Biol Sci 168:1-21.

Kwan P, Brodie MJ (2000) Early identification of refractory epilepsy. N Engl J Med 342:314-319.

Lancaster B, Adams PR (1986) Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons. J Neurophysiol 55:1268-1282.

Langdon-Down M, Brain WR (1929) Time of day in relation to convulsions in epilepsy.: Lancet.

Lee TS, Eid T, Mane S, Kim JH, Spencer DD, Ottersen OP, de Lanerolle NC (2004) Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. Acta Neuropathol 108:493-502.

Leite JP, Babb TL, Pretorius JK, Kuhlman PA, Yeoman KM, Mathern GW (1996) Neuron loss, mossy fiber sprouting, and interictal spikes after intrahippocampal kainate in developing rats. Epilepsy Res 26:219-231.

Lemieux JF, Blume WT (1986) Topographical evolution of spike-wave complexes. Brain Res 373:275-287.

Lennox WG, Davis JP (1950) Clinical correlates of the fast and the slow spike-wave electroencephalogram. Pediatrics 5:626-644.

Leslie KR, Nelson SB, Turrigiano GG (2001) Postsynaptic depolarization scales quantal amplitude in cortical pyramidal neurons. J Neurosci 21:RC170.

Li H, Prince DA (2002) Synaptic activity in chronically injured, epileptogenic sensory-motor neocortex. JNeurophysiol 88:2-12.

Li H, Bandrowski AE, Prince DA (2005) Cortical injury affects short-term plasticity of evoked excitatory synaptic currents. J Neurophysiol 93:146-156.

Li J, Verkman AS (2001) Impaired hearing in mice lacking aquaporin-4 water channels. J Biol Chem 276:31233-31237.

Lin JJ, Salamon N, Lee AD, Dutton RA, Geaga JA, Hayashi KM, Luders E, Toga AW, Engel J, Jr., Thompson PM (2007) Reduced neocortical thickness and complexity mapped in mesial temporal lobe epilepsy with hippocampal sclerosis. Cereb Cortex 17:2007-2018.

Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330:613-622.

Lissin DV, Gomperts SN, Carroll RC, Christine CW, Kalman D, Kitamura M, Hardy S, Nicoll RA, Malenka RC, von ZM (1998) Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. ProcNatlAcadSciUSA 95:7097-7102.

Liu BP, Fournier A, GrandPre T, Strittmatter SM (2002) Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. Science 297:1190-1193.

Liu J, Solway K, Messing RO, Sharp FR (1998) Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. J Neurosci 18:7768-7778.

Loiseau P, Duche B, Pedespan JM (1995) Absence epilepsies. Epilepsia 36:1182-1186.

Longo BM, Mello LE (1997) Blockade of pilocarpine- or kainate-induced mossy fiber sprouting by cycloheximide does not prevent subsequent epileptogenesis in rats. Neurosci Lett 226:163-166.

Lothman EW, Somjen GG (1975) Extracellular potassium activity, intracellular and extracellular potential responses in the spinal cord. J Physiol 252:115-136.

Lothman EW, Somjen GG (1976) Functions of primary afferents and responses of extracellular K+ during spinal epileptiform seizures. Electroencephalogr Clin Neurophysiol 41:253-267.

Lothman EW, Rempe DA, Mangan PS (1995) Changes in excitatory neurotransmission in the CA1 region and dentate gyrus in a chronic model of temporal lobe epilepsy. J Neurophysiol 74:841-848.

Lowenstein DH (1996) Recent advances related to basic mechanisms of epileptogenesis. Epilepsy Res Suppl 11:45-60.

Lowenstein DH, Thomas MJ, Smith DH, McIntosh TK (1992) Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. J Neurosci 12:4846-4853.

Lynch M, Sutula T (2000) Recurrent excitatory connectivity in the dentate gyrus of kindled and kainic acid-treated rats. J Neurophysiol 83:693-704.

Magavi SS, Leavitt BR, Macklis JD (2000) Induction of neurogenesis in the neocortex of adult mice. Nature 405:951-955.

Malow BA (2007) The interaction between sleep and epilepsy. Epilepsia 48 Suppl 9:36-38.

Malow BA, Aldrich MS (2000) Localizing value of rapid eye movement sleep in temporal lobe epilepsy. Sleep Med 1:57-60.

Malow BA, Selwa LM, Ross D, Aldrich MS (1999) Lateralizing value of interictal spikes on overnight sleep-EEG studies in temporal lobe epilepsy. Epilepsia 40:1587-1592.

Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, Chan P, Verkman AS (2000) Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med 6:159-163.

Marco P, DeFelipe J (1997) Altered synaptic circuitry in the human temporal neocortex removed from epileptic patients. Exp Brain Res 114:1-10.

Marcus DC, Wu T, Wangemann P, Kofuji P (2002) KCNJ10 (Kir4.1) potassium channel knockout abolishes endocochlear potential. Am J Physiol Cell Physiol 282:C403-407.

Margerison JH, Corsellis JA (1966) Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. Brain 89:499-530.

Marin-Padilla M (1999) Developmental neuropathology and impact of perinatal brain damage. III: gray matter lesions of the neocortex. J Neuropathol Exp Neurol 58:407-429.

Marin-Padilla M, Parisi JE, Armstrong DL, Sargent SK, Kaplan JA (2002) Shaken infant syndrome: developmental neuropathology, progressive cortical dysplasia, and epilepsy. Acta Neuropathol (Berl) 103:321-332.

Markand ON (1977) Slow spike-wave activity in EEG and associated clinical features: often called 'Lennox' or "Lennox-Gastaut' syndrome. Neurology 27:746-757.

Markram H, Wang Y, Tsodyks M (1998) Differential signaling via the same axon of neocortical pyramidal neurons. Proc Natl Acad Sci U S A 95:5323-5328.

Marks DA, Kim J, Spencer DD, Spencer SS (1992) Characteristics of intractable seizures following meningitis and encephalitis. Neurology 42:1513-1518.

Mason HA, Ito S, Corfas G (2001) Extracellular signals that regulate the tangential migration of olfactory bulb neuronal precursors: inducers, inhibitors, and repellents. J Neurosci 21:7654-7663.

Massimini M, Amzica F (2001) Extracellular calcium fluctuations and intracellular potentials in the cortex during the slow sleep oscillation. J Neurophysiol 85:1346-1350.

Mathern GW, Babb TL, Armstrong DL (1997a) Hippocampal sclerosis. In: Epilepsy: A comprehensive textbook (Engel J PT, ed), pp 133-155. Philadelphia: Lippincott.

Mathern GW, Bertram EH, 3rd, Babb TL, Pretorius JK, Kuhlman PA, Spradlin S, Mendoza D (1997b) In contrast to kindled seizures, the frequency of spontaneous epilepsy in the limbic status model correlates with greater aberrant fascia dentata excitatory and inhibitory axon sprouting, and increased staining for N-methyl-D-aspartate, AMPA and GABA(A) receptors. Neuroscience 77:1003-1019.

Mathern GW, Babb TL, Leite JP, Pretorius K, Yeoman KM, Kuhlman PA (1996) The pathogenic and progressive features of chronic human hippocampal epilepsy. Epilepsy Res 26:151-161.

Mathern GW, Bertram EH, 3rd, Babb TL, Pretorius JK, Kuhlman PA, Spradlin S, Mendoza D (1997) In contrast to kindled seizures, the frequency of spontaneous epilepsy in the limbic status model correlates with greater aberrant fascia dentata excitatory and

inhibitory axon sprouting, and increased staining for N-methyl-D-aspartate, AMPA and GABA(A) receptors. Neuroscience 77:1003-1019.

Matsumoto H, Marsan CA (1964) Cortical Cellular Phenomena in Experimental Epilepsy: Interictal Manifestations. Exp Neurol 9:286-304.

Matsumoto H, Ayala GF, Gumnit RJ (1969) Effects of intracellularly injected currents on the PDS and the hyperpolarizing after-potential in neurons within an epileptic focus. Electroencephalogr Clin Neurophysiol 26:120.

McDonald JW, Garofalo EA, Hood T, Sackellares JC, Gilman S, McKeever PE, Troncoso JC, Johnston MV (1991) Altered excitatory and inhibitory amino acid receptor binding in hippocampus of patients with temporal lobe epilepsy. Ann Neurol 29:529-541.

McBain CJ, Traynelis SF, Dingledine R (1990) Regional variation of extracellular space in the hippocampus. Science 249:674-677.

McNamara JO (1994) Cellular and molecular basis of epilepsy. J Neurosci 14:3413-3425.

Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM (1993) Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. Epilepsia 34:985-995.

Mitchell LA, Jackson GD, Kalnins RM, Saling MM, Fitt GJ, Ashpole RD, Berkovic SF (1999) Anterior temporal abnormality in temporal lobe epilepsy: a quantitative MRI and histopathologic study. Neurology 52:327-336.

Mody I, Heinemann U (1987) NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. Nature 326:701-704.

Mody I, Heinemann U, MacDonald JF, Salter MW (1992) Recruitment of NMDA receptors into synaptic transmission after kindling-induced epilepsy and its possible mechanism. Epilepsy Res Suppl 8:307-310; discussion 310-301.

Molnar P, Nadler JV (1999) Mossy fiber-granule cell synapses in the normal and epileptic rat dentate gyrus studied with minimal laser photostimulation. J Neurophysiol 82:1883-1894.

Moody WJ, Futamachi KJ, Prince DA (1974) Extracellular potassium activity during epileptogenesis. Exp Neurol 42:248-263.

Moore-Hoon ML, Turner RJ (1998) Molecular and topological characterization of the rat parotid Na+-K+-2Cl- cotransporter1. Biochim Biophys Acta 1373:261-269.

Morris ME, Leblond J, Agopyan N, Krnjevic K (1991) Temperature dependence of extracellular ionic changes evoked by anoxia in hippocampal slices. J Neurophysiol 65:157-167.

Mueller AL, Chesnut RM, Schwartzkroin PA (1983) Actions of GABA in developing rabbit hippocampus: an in vitro study. Neurosci Lett 39:193-198.

Murthy VN, Schikorski T, Stevens CF, Zhu Y (2001) Inactivity produces increases in neurotransmitter release and synapse size. Neuron 32:673-682.

Nagelhus EA, Mathiisen TM, Ottersen OP (2004) Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience 129:905-913.

Neckelmann D, Amzica F, Steriade M (1998) Spike-wave complexes and fast components of cortically generated seizures. III. Synchronizing mechanisms. J Neurophysiol 80:1480-1494.

Neckelmann D, Amzica F, Steriade M (2000) Changes in neuronal conductance during different components of cortically generated spike-wave seizures. Neuroscience 96:475-485.

Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26:523-530.

Neusch C, Weishaupt JH, Bahr M (2003) Kir channels in the CNS: emerging new roles and implications for neurological diseases. Cell Tissue Res 311:131-138.

Neusch C, Papadopoulos N, Muller M, Maletzki I, Winter SM, Hirrlinger J, Handschuh M, Bahr M, Richter DW, Kirchhoff F, Hulsmann S (2006) Lack of the Kir4.1 channel subunit abolishes K+ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K+ regulation. J Neurophysiol 95:1843-1852.

Newman EA (1986) High potassium conductance in astrocyte endfeet. Science 233:453-454.

Newman EA (1993) Inward-rectifying potassium channels in retinal glial (Muller) cells. J Neurosci 13:3333-3345.

Ng SK, Hauser WA, Brust JC, Susser M (1988) Alcohol consumption and withdrawal in new-onset seizures. N Engl J Med 319:666-673.

Nicholson C, Sykova E (1998) Extracellular space structure revealed by diffusion analysis. Trends Neurosci 21:207-215.

Niedermeyer E (1969) The Lennox-Gastaut syndrome: a severe type of childhood epilepsy. Dtsch Z Nervenheilkd 195:263-282.

Niedermeyer E (1999) Abnormal EEG patterns (epileptic and paroxysmal). In: Electroencephalography: Basic Principles, Clinical Applications, and Related Fields, ed E Niedermeyer and F Lopes da Silva, Baltimore: Williams & Wilkins:235-260.

Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 17:171-180.

Niermann H, Amiry-Moghaddam M, Holthoff K, Witte OW, Ottersen OP (2001) A novel role of vasopressin in the brain: modulation of activity-dependent water flux in the neocortex. J Neurosci 21:3045-3051.

Nita DA, Cisse Y, Timofeev I, Steriade M (2006) Increased propensity to seizures after chronic cortical deafferentation in vivo. JNeurophysiol 95:902-913.

Nita DA, Cisse Y, Timofeev I, Steriade M (2007) Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. Cereb Cortex 17:272-283.

O'Brien JL, Goldensohn ES, Hoefer PF (1959) Electroencephalographic abnormalities in addition to bilaterally synchronous 3 per second spike and wave activity in petit mal. Electroencephalogr Clin Neurophysiol 11:747-761.

Obenaus A, Esclapez M, Houser CR (1993) Loss of glutamate decarboxylase mRNA-containing neurons in the rat dentate gyrus following pilocarpine-induced seizures. J Neurosci 13:4470-4485.

Okazaki MM, Molnar P, Nadler JV (1999) Recurrent mossy fiber pathway in rat dentate gyrus: synaptic currents evoked in presence and absence of seizure-induced growth. J Neurophysiol 81:1645-1660.

Olafsson E, Gudmundsson G, Hauser WA (2000) Risk of epilepsy in long-term survivors of surgery for aneurysmal subarachnoid hemorrhage: a population-based study in Iceland. Epilepsia 41:1201-1205.

Olsen TS (2001) Post-stroke epilepsy. Curr Atheroscler Rep 3:340-344.

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J Neurophysiol 29:788-806.

Padmawar P, Yao X, Bloch O, Manley GT, Verkman AS (2005) K+ waves in brain cortex visualized using a long-wavelength K+-sensing fluorescent indicator. Nat Methods 2:825-827.

Panayiotopoulos CP (1997) Absence epilepsies. In: Epilepsy: a comprehensive textbook (Engel JJ, Pedley TA, eds), pp 2327–2346. Philadelphia: Lippincott-Raven.

Panayiotopoulos CP (2002a) Autonomic seizures and autonomic status epilepticus specific to childhood. Arch Pediatr Adolesc Med 156:945.

Panayiotopoulos CP (2002b) Absence status epilepticus. In: International League Against Epilepsy, Classification and Terminology.

Panayiotopoulos CP (2002c) A clinical guide to epileptic syndromes and their treatment. Chipping Norton, UK: Bladon Medical Publishing.

Papadopoulos MC, Manley GT, Krishna S, Verkman AS (2004) Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. Faseb J 18:1291-1293.

Parent JM, Valentin VV, Lowenstein DH (2002) Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. J Neurosci 22:3174-3188.

Park SA, Lee BI, Park SC, Lee SJ, Kim WJ, Lee JH, Kim JY (1998) Clinical courses of pure sleep epilepsies. Seizure 7:369-377.

Passouant P, Latour H, Cadilhac J (1951) Morpheic epilepsy. Ann Med Psychol (Paris) 109:526-540.

Patry FL (1931) The relation of time of day, sleep and other factors to the incidence of epileptic seizures. Amer J Psychiat 10:789–813.

Patry G, Lyagoubi S, Tassinari CA (1971) Subclinical "electrical status epilepticus" induced by sleep in children. A clinical and electroencephalographic study of six cases. Arch Neurol 24:242-252.

Payne JA, Stevenson TJ, Donaldson LF (1996) Molecular characterization of a putative K-Cl cotransporter in rat brain. A neuronal-specific isoform. J Biol Chem 271:16245-16252.

Penfield W (1929) The mechanisms of cicatricial contraction in the brain. Brain 50:499–517.

Perez Velazquez JL, Carlen PL (2000) Gap junctions, synchrony and seizures. Trends Neurosci 23:68-74.

Perez Y, Morin F, Beaulieu C, Lacaille JC (1996) Axonal sprouting of CA1 pyramidal cells in hyperexcitable hippocampal slices of kainate-treated rats. Eur J Neurosci 8:736-748.

Pinault D, Leresche N, Charpier S, Deniau JM, Marescaux C, Vergnes M, Crunelli V (1998) Intracellular recordings in thalamic neurones during spontaneous spike and wave discharges in rats with absence epilepsy. J Physiol 509 (Pt 2):449-456.

Pollen DA (1964) Intracellular Studies of Cortical Neurons During Thalamic Induced Wave and Spike. Electroencephalogr Clin Neurophysiol 17:398-404.

Prevett MC, Lammertsma AA, Brooks DJ, Cunningham VJ, Fish DR, Duncan JS (1995) Benzodiazepine-GABAA receptor binding during absence seizures. Epilepsia 36:592-599.

Prince DA, Futamachi KJ (1970) Intracellular recordings from chronic epileptogenic foci in the monkey. Electroencephalogr Clin Neurophysiol 29:496-510.

Prince DA, Lux HD, Neher E (1973) Measurement of extracellular potassium activity in cat cortex. Brain Res 50:489-495.

Prince DA, Tseng GF (1993) Epileptogenesis in chronically injured cortex: in vitro studies. JNeurophysiol 69:1276-1291.

Prince DA, Jacobs K (1998) Inhibitory function in two models of chronic epileptogenesis. Epilepsy Res 32:83-92.

Prince DA, Salin P, Tseng GF, Hoffman S, Parada I (1997) Axonal sprouting and epileptogenesis. Adv Neurol 72:1-8.

Purpura DP, Housepian EM (1961) Morphological and physiological properties of chronically isolated immature neocortex. Exp Neurol 4:377-401.

Ramaswamy S, Goings GE, Soderstrom KE, Szele FG, Kozlowski DA (2005) Cellular proliferation and migration following a controlled cortical impact in the mouse. Brain Res 1053:38-53.

Ribak CE, Reiffenstein RJ (1982) Selective inhibitory synapse loss in chronic cortical slabs: a morphological basis for epileptic susceptibility. Can J Physiol Pharmacol 60:864-870.

Ribak CE, Bradburne RM, Harris AB (1982) A preferential loss of GABAergic, symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex. J Neurosci 2:1725-1735.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251-255.

Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M (2002) BDNF-induced TrkB activation down-regulates the K+-Cl- cotransporter KCC2 and impairs neuronal Cl-extrusion. J Cell Biol 159:747-752.

Robinson S, Li Q, Dechant A, Cohen ML (2006) Neonatal loss of gamma-aminobutyric acid pathway expression after human perinatal brain injury. J Neurosurg 104:396-408.

Rodin EA (1972) Medical and social prognosis in epilepsy. Epilepsia 13:121-131.

Roger J, Dravet C, Bureau M (1989) The Lennox-Gastaut syndrome. Cleve Clin J Med 56 Suppl Pt 2:S172-180.

Romijn HJ, Ruijter JM, Wolters PS (1988) Hypoxia preferentially destroys GABAergic neurons in developing rat neocortex explants in culture. Exp Neurol 100:332-340.

Roper SN, Obenaus A, Dudek FE (1992) Osmolality and nonsynaptic epileptiform bursts in rat CA1 and dentate gyrus. Ann Neurol 31:81-85.

Rozengurt N, Lopez I, Chiu CS, Kofuji P, Lester HA, Neusch C (2003) Time course of inner ear degeneration and deafness in mice lacking the Kir4.1 potassium channel subunit. Hear Res 177:71-80.

Rozov A, Zilberter Y, Wollmuth LP, Burnashev N (1998) Facilitation of currents through rat Ca2+-permeable AMPA receptor channels by activity-dependent relief from polyamine block. J Physiol 511 (Pt 2):361-377.

Rutecki PA, Lebeda FJ, Johnston D (1985) Epileptiform activity induced by changes in extracellular potassium in hippocampus. J Neurophysiol 54:1363-1374.

Sakowitz OW, Unterberg AW, Stover JF (2002) Neuronal activity determined by quantitative EEG and cortical microdialysis is increased following controlled cortical impact injury in rats. Acta Neurochir Suppl 81:221-223.

Salazar AM, Jabbari B, Vance SC, Grafman J, Amin D, Dillon JD (1985) Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study. Neurology 35:1406-1414.

Salin P, Tseng GF, Hoffman S, Parada I, Prince DA (1995) Axonal sprouting in layer V pyramidal neurons of chronically injured cerebral cortex. JNeurosci 15:8234-8245.

Samuelsson C, Kumlien E, Flink R, Lindholm D, Ronne-Engstrom E (2000) Decreased cortical levels of astrocytic glutamate transport protein GLT-1 in a rat model of posttraumatic epilepsy. Neurosci Lett 289:185-188.

Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 3:1027-1034.

Sato S, Dreifuss FE, Penry JK (1973a) The effect of sleep on spike-wave discharges in absence seizures. Neurology 23:1335-1345.

Sato T, Yamamoto M, Nakahama H (1973b) Influence of synchronized sleep upon spontaneous and induced discharges of single units in visual system. Exp Brain Res 16:533-541.

Savic I, Pauli S, Thorell JO, Blomqvist G (1994) In vivo demonstration of altered benzodiazepine receptor density in patients with generalised epilepsy. J Neurol Neurosurg Psychiatry 57:797-804.

Savic I, Widen L, Thorell JO, Blomqvist G, Ericson K, Roland P (1990) Cortical benzodiazepine receptor binding in patients with generalized and partial epilepsy. Epilepsia 31:724-730.

Scharfman HE, Sollas AL, Berger RE, Goodman JH (2003) Electrophysiological evidence of monosynaptic excitatory transmission between granule cells after seizure-induced mossy fiber sprouting. J Neurophysiol 90:2536-2547.

Schmitz D, Schuchmann S, Fisahn A, Draguhn A, Buhl EH, Petrasch-Parwez E, Dermietzel R, Heinemann U, Traub RD (2001) Axo-axonal coupling. a novel mechanism for ultrafast neuronal communication. Neuron 31:831-840.

Schneider GE (1973) Early lesions of superior colliculus: factors affecting the formation of abnormal retinal projections. Brain Behav Evol 8:73-109.

Schroder W, Hager G, Kouprijanova E, Weber M, Schmitt AB, Seifert G, Steinhauser C (1999) Lesion-induced changes of electrophysiological properties in astrocytes of the rat dentate gyrus. Glia 28:166-174.

Schroder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP, Steinhauser C (2000) Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. Epilepsia 41 Suppl 6:S181-184.

Schwartzkroin PA, Baraban SC, Hochman DW (1998) Osmolarity, ionic flux, and changes in brain excitability. Epilepsy Res 32:275-285.

Schwenkreis P, Witscher K, Janssen F, Dertwinkel R, Zenz M, Malin JP, Tegenthoff M (2000) Changes of cortical excitability in patients with upper limb amputation. Neurosci Lett 293:143-146.

Schwindt PC, Spain WJ, Foehring RC, Stafstrom CE, Chubb MC, Crill WE (1988) Multiple potassium conductances and their functions in neurons from cat sensorimotor cortex in vitro. J Neurophysiol 59:424-449.

Seifert G, Schilling K, Steinhauser C (2006) Astrocyte dysfunction in neurological disorders: a molecular perspective. Nat Rev Neurosci 7:194-206.

Seigneur J, Timofeev I (2007) Extracellular calcium depletion associated with low-threshold calcium spikes controls presynaptic mediator release in thalamus. Program No 8274/KK10 2007 Neuroscience Meeting Planner San Diego, CA: Society for Neuroscience, 2007 Online.

Sharpless SK, Halpern LM (1962) The electrical excitability of chronically isolated cortex studied by means of permanently implanted electrodes. ElectroencephalogrClinNeurophysiol 14:244-255.

Shouse MN (1986) State disorders and state-dependent seizures in amygdala-kindled cats. Exp Neurol 92:601-608.

Shouse MN, Farber PR, Staba RJ (2000) Physiological basis: how NREM sleep components can promote and REM sleep components can suppress seizure discharge propagation. Clin Neurophysiol 111 Suppl 2:S9-S18.

Shumate MD, Lin DD, Gibbs JW, 3rd, Holloway KL, Coulter DA (1998) GABA(A) receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. Epilepsy Res 32:114-128.

Sillito AM (1975) The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat. JPhysiol 250:305-329.

Simard M, Nedergaard M (2004) The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 129:877-896.

Singer W, Lux HD (1973) Presynaptic depolarization and extracellular potassium in the cat lateral geniculate nucleus. Brain Res 64:17-33.

Sloper JJ, Johnson P, Powell TP (1980) Selective degeneration of interneurons in the motor cortex of infant monkeys following controlled hypoxia: a possible cause of epilepsy. Brain Res 198:204-209.

Sloviter RS (1987) Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 235:73-76.

Sloviter RS (1991) Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. Hippocampus 1:41-66.

Smith BN, Dudek FE (2002) Network interactions mediated by new excitatory connections between CA1 pyramidal cells in rats with kainate-induced epilepsy. J Neurophysiol 87:1655-1658.

Somers DC, Nelson SB, Sur M (1995) An emergent model of orientation selectivity in cat visual cortical simple cells. JNeurosci 15:5448-5465.

Somjen GG (1979) Extracellular potassium in the mammalian central nervous system. Annu Rev Physiol 41:159-177.

Somjen GG (2004) Ions in the brain: Normal function, Seizures, and Stroke: Oxford University Press, Inc.

Somjen GG, Giacchino JL (1985) Potassium and calcium concentrations in interstitial fluid of hippocampal formation during paroxysmal responses. J Neurophysiol 53:1098-1108.

Somjen GG, Muller M (2000) Potassium-induced enhancement of persistent inward current in hippocampal neurons in isolation and in tissue slices. Brain Res 885:102-110.

Song H, Stevens CF, Gage FH (2002) Astroglia induce neurogenesis from adult neural stem cells. Nature 417:39-44.

Spreafico R, Battaglia G, Arcelli P, Andermann F, Dubeau F, Palmini A, Olivier A, Villemure JG, Tampieri D, Avanzini G, Avoli M (1998a) Cortical dysplasia: an immunocytochemical study of three patients. Neurology 50:27-36.

Spreafico R, Pasquier B, Minotti L, Garbelli R, Kahane P, Grand S, Benabid AL, Tassi L, Avanzini G, Battaglia G, Munari C (1998b) Immunocytochemical investigation on dysplastic human tissue from epileptic patients. Epilepsy Res 32:34-48.

Steinhauser C, Seifert G (2002) Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. Eur J Pharmacol 447:227-237.

Steriade M (1974) Interneuronal epileptic discharges related to spike-and-wave cortical seizures in behaving monkeys. Electroencephalogr Clin Neurophysiol 37:247-263.

Steriade M (2003) Neuronal substrates of sleep and epilepsy. Cambridge, UK: Cambridge University Press.

Steriade M, Contreras D (1995) Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. J Neurosci 15:623-642.

Steriade M, Contreras D (1998) Spike-wave complexes and fast components of cortically generated seizures. I. Role of neocortex and thalamus. J Neurophysiol 80:1439-1455.

Steriade M, Amzica F (1999) Intracellular study of excitability in the seizure-prone neocortex in vivo. J Neurophysiol 82:3108-3122.

Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 85:1969-1985.

Steriade M, Amzica F, Neckelmann D, Timofeev I (1998) Spike-wave complexes and fast components of cortically generated seizures. II. Extra- and intracellular patterns. J Neurophysiol 80:1456-1479.

Storm JF (1990) Potassium currents in hippocampal pyramidal cells. Prog Brain Res 83:161-187.

Sugaya E, Karahashi Y, Sugaya A, Haruki F (1971) Intra- and extra-cellular potentials from "idle" cells in cerebral cortex of cat. Jpn J Physiol 21:149-157.

Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L (1989) Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol 26:321-330.

Sutula T, Zhang P, Lynch M, Sayin U, Golarai G, Rod R (1998) Synaptic and axonal remodeling of mossy fibers in the hilus and supragranular region of the dentate gyrus in kainate-treated rats. J Comp Neurol 390:578-594.

Swartz BE, Houser CR, Tomiyasu U, Walsh GO, DeSalles A, Rich JR, Delgado-Escueta A (2006) Hippocampal cell loss in posttraumatic human epilepsy. Epilepsia 47:1373-1382.

Sypert GW, Ward AA, Jr. (1971) Unidentified neuroglia potentials during propagated seizures in neocortex. Exp Neurol 33:239-255.

Tassinari CA, Volpi L, Michelucci R (1999) Electrical status epilepticus during slow sleep. Commission of Classification and Terminology of the International League Against Epilepsy

Tassinari CA, Bureau M, Dravet C, Dalla Bernardina B, Roger J (1985) Epilepsy with continuous spikes and waves during slow sleep. In: Roger J, Dravet C, Bureau M, Dreifuss FE, Wolf P, editors Epileptic syndromes in infancy, childhood and adolescence London: John Libbey:194-204.

Taylor CP, Dudek FE (1982) Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. Science 218:810-812.

Taylor CP, Dudek FE (1984a) Excitation of hippocampal pyramidal cells by an electrical field effect. J Neurophysiol 52:126-142.

Taylor CP, Dudek FE (1984b) Synchronization without active chemical synapses during hippocampal afterdischarges. J Neurophysiol 52:143-155.

Temkin NR (2001) Antiepileptogenesis and seizure prevention trials with antiepileptic drugs: meta-analysis of controlled trials. Epilepsia 42:515-524.

Temkin NR, Dikmen SS, Anderson GD, Wilensky AJ, Holmes MD, Cohen W, Newell DW, Nelson P, Awan A, Winn HR (1999) Valproate therapy for prevention of posttraumatic seizures: a randomized trial. J Neurosurg 91:593-600.

Timofeev I, Steriade M (2004) Neocortical seizures: initiation, development and cessation. Neuroscience 123:299-336.

Timofeev I, Bazhenov M (2005) Mechanisms of cortical trauma induced epileptogenesis and seizures. Recent Res Devel Physiol: 99-139.

Timofeev I, Grenier F, Steriade M (1998) Spike-wave complexes and fast components of cortically generated seizures. IV. Paroxysmal fast runs in cortical and thalamic neurons. J Neurophysiol 80:1495-1513.

Timofeev I, Grenier F, Steriade M (2002a) The role of chloride-dependent inhibition and the activity of fast-spiking neurons during cortical spike-wave electrographic seizures. Neuroscience 114:1115-1132.

Timofeev I, Grenier F, Steriade M (2004) Contribution of intrinsic neuronal factors in the generation of cortically driven electrographic seizures. J Neurophysiol 92:1133-1143.

Timofeev I, Bazhenov M, Sejnowski T, Steriade M (2002b) Cortical hyperpolarization-activated depolarizing current takes part in the generation of focal paroxysmal activities. Proc Natl Acad Sci U S A 99:9533-9537.

Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, Steriade M (2000) Origin of slow cortical oscillations in deafferented cortical slabs. CerebCortex 10:1185-1199.

Topolnik L, Steriade M, Timofeev I (2003a) Hyperexcitability of intact neurons underlies acute development of trauma-related electrographic seizures in cats in vivo. Eur J Neurosci 18:486-496.

Topolnik L, Steriade M, Timofeev I (2003b) Partial cortical deafferentation promotes development of paroxysmal activity. CerebCortex 13:883-893.

Touchon J, Baldy-Moulinier M, Billiard M, Besset A, Cadilhac J (1991) Sleep organization and epilepsy. Epilepsy Res Suppl 2:73-81.

Touchon J, Baldy-Moulinier M, Billiard M, Besset A, Valmier J, Cadilhac J (1987) Organization of sleep in recent temporal lobe epilepsy before and after treatment with carbamazepine. Rev Neurol (Paris) 143:462-467.

Toyoda H, Ohno K, Yamada J, Ikeda M, Okabe A, Sato K, Hashimoto K, Fukuda A (2003) Induction of NMDA and GABAA receptor-mediated Ca2+ oscillations with KCC2 mRNA downregulation in injured facial motoneurons. J Neurophysiol 89:1353-1362.

Traub RD, Wong RK (1982) Cellular mechanism of neuronal synchronization in epilepsy. Science 216:745-747.

Traub RD, Borck C, Colling SB, Jefferys JG (1996) On the structure of ictal events in vitro. Epilepsia 37:879-891.

Traynelis SF, Dingledine R (1988) Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. J Neurophysiol 59:259-276.

Tseng GF, Prince DA (1996) Structural and functional alterations in rat corticospinal
　　　　neurons after axotomy. J Neurophysiol 75:248-267.

Tsunashima K, Schwarzer C, Kirchmair E, Sieghart W, Sperk G (1997) GABA(A) receptor
　　　　subunits in the rat hippocampus III: altered messenger RNA expression in kainic
　　　　acid-induced epilepsy. Neuroscience 80:1019-1032.

Turrigiano GG (1999) Homeostatic plasticity in neuronal networks: the more things
　　　　change, the more they stay the same. Trends Neurosci 22:221-227.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-
　　　　dependent scaling of quantal amplitude in neocortical neurons. Nature 391:892-896.

van den Pol AN, Obrietan K, Chen G (1996) Excitatory actions of GABA after neuronal
　　　　trauma. J Neurosci 16:4283-4292.

Van Harreveld A, Stamm JS (1954) Consequences of cortical convulsive activity in rabbit.
　　　　J Neurophysiol 17:505-520.

Van Harreveld A, Stamm JS, Christensen E (1956) Spreading depression in rabbit, cat and
　　　　monkey. Am J Physiol 184:312-320.

Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B (1991) Structural
　　　　determinants of ion flow through recombinant glutamate receptor channels. Science
　　　　252:1715-1718.

Verkman AS (2005) More than just water channels: unexpected cellular roles of
　　　　aquaporins. J Cell Sci 118:3225-3232.

Villablanca JR, Fomez-Pinilla F, Sonnier BJ, Hovda DA (1988) Bilateral pericruciate
　　　　cortical innervation of the red nucleus in cats with adult or neonatal cerebral
　　　　hemispherectomy. Brain Res 453:17-31.

Volterra A, Steinhauser C (2004) Glial modulation of synaptic transmission in the
　　　　hippocampus. Glia 47:249-257.

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the
　　　　revolution continues. Nat Rev Neurosci 6:626-640.

Vyskocil F, Kritz N, Bures J (1972) Potassium-selective microelectrodes used for
　　　　measuring the extracellular brain potassium during spreading depression and anoxic
　　　　depolarization in rats. Brain Res 39:255-259.

Walz W (1987) Swelling and potassium uptake in cultured astrocytes. Can J Physiol
　　　　Pharmacol 65:1051-1057.

Walz W (1992) Mechanism of rapid K(+)-induced swelling of mouse astrocytes. Neurosci
　　　　Lett 135:243-246.

Walz W (2000) Role of astrocytes in the clearance of excess extracellular potassium.
　　　　Neurochem Int 36:291-300.

Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, Neve RL, He Z (2002)
　　　　Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite
　　　　outgrowth. Nature 417:941-944.

Watanabe K, Negoro T, Aso K, Matsumoto A (1993) Reappraisal of interictal
　　　　electroencephalograms in infantile spasms. Epilepsia 34:679-685.

Watt AJ, van Rossum MC, MacLeod KM, Nelson SB, Turrigiano GG (2000) Activity
　　　　coregulates quantal AMPA and NMDA currents at neocortical synapses. Neuron
　　　　26:659-670.

Weiss GH, Salazar AM, Vance SC, Grafman JH, Jabbari B (1986) Predicting posttraumatic
　　　　epilepsy in penetrating head injury. Arch Neurol 43:771-773.

Welker E, Soriano E, Van der Loos H (1989) Plasticity in the barrel cortex of the adult mouse: effects of peripheral deprivation on GAD-immunoreactivity. Exp Brain Res 74:441-452.

Westenbroek RE, Westrum LE, Hendrickson AE, Wu JY (1988) Ultrastructure of synaptic remodeling in piriform cortex of adult rats after neonatal olfactory bulb removal: an immunocytochemical study. J Comp Neurol 274:334-346.

Williamson PD, French JA, Thadani VM, Kim JH, Novelly RA, Spencer SS, Spencer DD, Mattson RH (1993) Characteristics of medial temporal lobe epilepsy: II. Interictal and ictal scalp electroencephalography, neuropsychological testing, neuroimaging, surgical results, and pathology. Ann Neurol 34:781-787.

Wolf JA, Stys PK, Lusardi T, Meaney D, Smith DH (2001) Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. J Neurosci 21:1923-1930.

Wong RK, Prince DA (1978) Participation of calcium spikes during intrinsic burst firing in hippocampal neurons. Brain Res 159:385-390.

Wuarin JP, Dudek FE (2001) Excitatory synaptic input to granule cells increases with time after kainate treatment. J Neurophysiol 85:1067-1077.

Yang L, Benardo LS (1997) Epileptogenesis following neocortical trauma from two sources of disinhibition. J Neurophysiol 78:2804-2810.

Yaqub BA (1993) Electroclinical seizures in Lennox-Gastaut syndrome. Epilepsia 34:120-127.

Yaqub BA, Waheed G, Kabiraj MM (1997) Nocturnal epilepsies in adults. Seizure 6:145-149.

Yamamoto S, Yamamoto N, Kitamura T, Nakamura K, Nakafuku M (2001a) Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. Exp Neurol 172:115-127.

Yamamoto S, Nagao M, Sugimori M, Kosako H, Nakatomi H, Yamamoto N, Takebayashi H, Nabeshima Y, Kitamura T, Weinmaster G, Nakamura K, Nakafuku M (2001b) Transcription factor expression and Notch-dependent regulation of neural progenitors in the adult rat spinal cord. J Neurosci 21:9814-9823.

Ying Z, Babb TL, Comair YG, Bushey M, Touhalisky K (1998) Increased densities of AMPA GluR1 subunit proteins and presynaptic mossy fiber sprouting in the fascia dentata of human hippocampal epilepsy. Brain Res 798:239-246.

Yoshimura S, Takagi Y, Harada J, Teramoto T, Thomas SS, Waeber C, Bakowska JC, Breakefield XO, Moskowitz MA (2001) FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. Proc Natl Acad Sci U S A 98:5874-5879.

Young B, Rapp RP, Norton JA, Haack D, Walsh JW (1983) Failure of prophylactically administered phenytoin to prevent post-traumatic seizures in children. Childs Brain 10:185-192.

Zhang RL, Zhang ZG, Zhang L, Chopp M (2001) Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 105:33-41.

Ziemann U, Hallett M, Cohen LG (1998) Mechanisms of deafferentation-induced plasticity in human motor cortex. J Neurosci 18:7000-7007.