Neuronal Substrates of Sleep and Epilepsy

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Chapter 5 Neuronal mechanisms of seizures

This chapter is about the intrinsic neuronal properties and network operations that underlie different forms of seizures. The antagonism between concepts emphasizing the "epileptic neuron" or "epileptic networks" is obsolete as both voltage-gated properties of single neurons and synaptic articulations within different forebrain structures (neocortex, thalamus, corticothalamic loops, archicortex, and related systems) are crucial for the generation and spread of electrical paroxysms.

The knowledge of intrinsic cell properties has continuously evolved due to *in vitro* work conducted in the neocortex, thalamus, and hippocampus [1]. A series of studies pointed to various ionic currents that are implicated in potentiating the susceptibility to seizures.

The discovery of voltage-dependent Na^+ and Ca^{2+} channels in dendrites changed the model of dendrites with only passive properties and demonstrated that different intrinsic currents can amplify synaptic signals [2], which may eventually lead to abnormal cellular excitation and paroxysmal discharges [3].

The intrinsic propensity of some neocortical and hippocampal neurons to bursting is also a factor that predisposes to seizures. In fact, there is a continuum of variation in burstiness of cortical neurons. *In vivo* experiments, using intracellular recordings in acutely prepared and chronically implanted animals, have shown different incidences of intrinsically bursting (IB) neurons in different experimental conditions, depending on the degree of background firing, as well as the transformation of IB into regular-spiking (RS) neurons with enhanced synaptic activity (see ***2.1.2*** and ***2.1.3*** in Chapter 2) [4]. *In vitro*, the continuum extends from non-bursters to bursters that are induced by extrinsic depolarization as well as to spontaneously bursting neurons [5]. In CA1 hippocampal slices, this development in neuronal properties is modulated by the extracellular concentration of K^+, $[K^+]_o$. The effects of increasing $[K^+]_o$ on firing characteristics is not due to depolarization per se because depolarizing current injection does not convert RS into IB cells; rather, the increase in $[K^+]_o$ reduces the driving force of outward K^+ currents, thereby

[1] Reviewed in Llinás (1988); Gutnick and Mody (1995); Crill (1996); Huguenard (1996); Hoffman et al. (1997); Magee et al. (1998).

[2] For example, the current transmitted from dendrites to soma was measured by glutamate iontophoresis onto apical dendrites of neocortical pyramidal neurons in layer V while the soma was voltage-clamped with a second electrode (Schwindt and Crill, 1995). The results of this study showed that the persistent Na^+ current ($I_{Na(p)}$) in the dendrites can amplify synaptic signals mediated by NMDA receptors.

[3] Ward and Schmidt (1961) anticipated the recent findings on dendritic neuronal properties and their possible role in generating paroxysms by postulating that stretching dendrites may endow their arbor with epileptic properties.

[4] Much less often, neocortical neurons with regular-spiking patterns during natural slow-wave sleep develop their discharges into intrinsically bursting patterns at slightly depolarized levels during wakefulness (see Fig. 2.14 in Chapter 2).

[5] Jensen et al. (1994); Jensen and Yaari (1997).

increasing depolarizing afterpotentials (DAPs) and triggering additional spikes [5]. This transformation is probably one of the factors that explain the generation of recurring hippocampal seizures by high-K^+ solutions [6]. Perfusion with high $[K^+]_o$ has also been used in neocortical slices to increase the incidence and synchronization of bursting neurons [7]. Burst generation allows neurons to amplify signals. This led to the hypothesis that bursting neurons are pacemakers of epileptiform discharges [8]. A new class of voltage-gated K^+ channels, of the Kv3 subfamily, enables fast repolarization of action potentials, without compromising spike amplitude, and allows neurons to fire at very fast frequencies [9], which may also be a factor behind the transformation of normal into paroxysmal discharges. The frequency of spike generation may determine the extent of invasion of action potentials through the fine axonal arbors [10] in the cortex and other structures that are critical for epileptogenesis.

In a certain proportion of neocortical neurons, the hyperpolarization-activated cation current, I_H, is implicated in the repolarization of the membrane potential from the hyperpolarization associated with the "wave" component of cortically generated spike-wave complexes [11], and in the production of subsequent paroxysmal depolarizing shifts (see also section **5.6**).

Thus, a mosaic of intrinsic cell properties, changes in extracellular ion concentrations, as well as intracellular acidification [12], may play a role in the induction and/or duration of seizures.

However, the decisive role in the spread of focal or widespread synchronization of cellular discharges is played by neuronal circuitry. This view, which arises from *in vivo* investigations on brain-intact animals, was also expressed by some *in vitro* investigators who regarded epileptic properties as requiring activities in intact neuronal circuits. Thus the "need to refocus our attention on circuitry" was emphasized, and it was stated that the results from *in vitro* slices "do not argue that there are no epileptic cells per se, but do indicate that expression of epileptic properties require a minimum circuitry and/or environment" [13]. In the same vein, other investigators studying neocortical slices assumed that, although small regions of cortex may sustain synchronous activity, such limited circuits are not adequate to support spontaneous oscillations for prolonged periods of time [14].

This chapter will mainly focus on network operations underlying various forms of paroxysmal activities but will also provide data on intrinsic neuronal properties implicated in seizures. The available evidence points to the progressive build-up of seizures through synaptic operations within the cerebral cortex, the role of

[6] Zuckermann and Glaser (1968). This study was performed *in vivo*. In hippocampal slices too, perfusion with elevated K^+ is a common procedure for eliciting paroxysmal activity (Traynelis and Dingledine, 1988; Leschinger et al., 1993), similar to that occurring in tonico-clonic seizures.

[7] Connors (1984).

[8] Schwartzkroin and Prince (1978); Gutnick et al. (1982). Bursting neurons are located prevalently, but not exclusively (Chen et al., 1996; Nishimura et al., 2001), in layer V (see Connors and Amitai, 1995). Layer V neurons that have been partially deafferented, an experimental condition that increases the propensity to seizures (see section **5.9**), have higher input resistance and longer time constants than controls (Prince and Tseng, 1993). In such neurons, the decrease in the slow Ca^{2+}-activated K^+ current ($I_{K(Ca)}$) that underlies the spike afterhyperpolarization may lead to an increased input-output function of neurons and thus contribute to the generation of seizures. Similar changes in intrinsic neuronal properties of CA1 hippocampal pyramidal cells have been reported after kainic acid lesions (Franck and Schwartzkroin, 1985) that are known as an effective tool for triggering seizures.

[9] Rudy and McBain (2001) were "unaware of any neuronal type capable of sustained or repetitive high-frequency firing that does not express at least one of the Kv3.1-Kv3.4 genes" (p. 520).

[10] Westerfield et al. (1978); Manor et al. (1991).

[11] Timofeev et al. (2002a).

[12] Xiong et al. (2000). In this study, intracellular pH (pH_i) was investigated in hippocampal slices from the dentate gyrus. During seizures induced by low-Ca^{2+}, high-K^+ perfusion, manipulation of pH_i changed the duration of large-amplitude population "spikes", with acidification resulting in the early termination of paroxysmal activity.

[13] This conclusion belongs to a chapter by Schwartzkroin (1983) in a symposium volume on neuronal hyperexcitability (p. 99–100).

[14] Chagnac-Amitai and Connors (1989).

[15] Pedley (1987).

[16] Kellaway et al. (1960).

[17] Thomas and Klass (1968); Hughes (1980).

[18] Reiher et al. (1977); White et al. (1977).

[19] Lipman and Hughes (1968).

synaptic interactions between inhibitory and projection neurons within the thalamus, the control of thalamic neurons by corticofugal projections during different types of paroxysmal activity, the short- and long-range projections linking hippocampus with related systems in seizures, and the role of cholinergic and other generalized modulatory systems in altering the susceptibility to paroxysms. All these data, presented below in different sections, justify experimental designs in intact-brain animals.

5.1. Patterns of different epileptic seizures in humans and animals

Before a classification of epileptic seizures and a description of their phenomenology, some patterns that mimic paroxysmal discharges [15] should be briefly mentioned.

Some of the EEG entities that may seem, but are not, epileptogenic include:

(a) 14- and 6-Hz positive EEG "spikes" associated with paroxysmal abdominal pain and autonomic disturbances [16].

(b) "Phantom spike-wave bursts" at ~6 Hz, with a very short duration, which do not necessarily indicate epileptic seizures but, if their amplitudes exceed ~50 μV, may predict the occurrence of seizures in more than half of subjects investigated [17].

(c) Small sharp EEG "spikes", with exceedingly short duration (<70 ms), also called benign epileptiform transients of sleep, that have little or no relation to epilepsy [18].

(d) Rhythmic mid-temporal wave-bursts at a frequency of about 5–7 Hz, which mainly occur during drowsiness and have little or no relation to psychomotor seizures within the same frequency band [19].

Despite the great diversity in the EEG patterns and clinical manifestations of epileptic syndromes, common neuronal mechanisms may underlie a series of electrical paroxysms. These mechanisms are best studied by using multi-site extra- and intracellular recordings *in vivo* and *in vitro*. Because our experimental data that aimed to reveal the intracellular mechanisms underlying brain paroxysms, which is the aim of this chapter, have mainly addressed seizures with electrographic patterns resembling those observed in absence epilepsy and Lennox–Gastaut syndrome, I shall focus in more detail on these entities (sections **5.5** and **5.6**).

Firstly (section **5.2**), I discuss the development of seizures, often without discontinuity, from various types of normal brain oscillations, particularly those that define drowsiness, somnolence or slow-wave sleep (SWS) – behavioral states during which many types

[20] Niedermeyer (1999a).

[21] Steriade and Amzica (1999).

[22] Gotman and Marciani (1985).

of seizures preferentially occur. Although it is commonly assumed that SWS rhythms are only within the relatively low frequency range (<15 Hz), fast (20–60 Hz) and very fast (80–200 Hz) rhythms also occur over the depolarizing phase of the cortically generated slow sleep oscillation (0.5–1 Hz) (see section *3.2.3.2* in Chapter 3), and both low-frequency and very fast-frequency rhythms may evolve under particular conditions into electrical paroxysms that mimic various EEG patterns of clinical seizures. As will be shown in section **5.2**, the appearance of peculiar oscillations with increased amplitudes may reliably predict the occurrence of seizures and, thus, the analysis of their underlying mechanisms may open new therapeutic avenues.

Next (section **5.3**), I deal with afterdischarges (ADs) that follow brain electrical stimulation or repetitive sensory (photic and auditory) signals. As is the case with normal oscillations developing into paroxysmal ones (section **5.2**), the patterns of ADs may faithfully reproduce, often with higher amplitudes and changing features, the shape and frequency of responses elicited by repetitive stimuli. Although epileptic seizures are defined by their "spontaneous", repetitive recurrence, the analysis of electrically and sensory evoked ADs at the intracellular level provides insights into the mechanisms of reflex epilepsy. Indeed, cerebral scars, synchronous stimuli applied to central pathways, and repetitive sensory volleys, such as stroboscopic flash-lights and repeated sounds, all acting on a hyperexcitable cortex, can trigger epileptic seizures in susceptible animals and humans.

The characteristics of EEG interictal "spikes" or "polyspikes", their difference from full-blown seizures in cortical and thalamic neurons, and the role played by intrinsic membrane properties and excitatory/inhibitory synaptic coupling in their generation are analyzed in section **5.4**. These paroxysmal events, with short duration (20–70 ms) on conventional EEG paper recordings [20] but up to ~200 ms with intracellular recordings from neocortex [21], may be isolated, interictal, postictal, and their frequency may increase after a seizure [22]. Although such unitary events are basic elements of paroxysmal activity and their rhythmic recurrence may reliably announce the onset of a fully developed seizure (see section **5.6**), not all of them reflect an epileptic condition and some are physiological waves that should be distinguished from pathological ones on the basis of morphology and especially age, as EEG "spikes" of old age do not have the same significance as in a newborn [20].

In section **5.5** I discuss the mechanisms of seizures consisting of typical spike-wave (SW) or polyspike-wave (PSW) complexes at ~3 Hz, lasting more than 3–5 s and less than 30 s, as in absence

(petit-mal) epilepsy. Although 3 Hz is the classical frequency of SW/PSW complexes, they may be faster at the onset of the seizure and slow down to 3 Hz or 2.5 Hz at the end of the paroxysmal episode [20]. The relatively faster (4–5 Hz) SW complexes with shorter duration seem to occur preferentially in subjects older than 15 years [23]. Clinically, these seizures are defined as a paroxysmal loss of consciousness only, with abrupt and sudden onset and offset, without aura or postictal state, accompanied by tonic deviation of gaze. The sudden impairment of responsiveness may also be associated with different degrees of other motor (clonic, tonic or atonic) components [24]. Absence seizures are primarily a disease of childhood and adolescence, with a peak at about age 6–7 years, but typical absence seizures, with SW complexes at 3 Hz and rolling up of the eyeballs, have been observed as early as 6 months [25]. The term *absence* is valid only for those seizures that occur during wakefulness because during natural sleep, when there is an increased incidence of such paroxysms (see section **5.5**), or in experimental animals under anesthesia, the subjects prone to these seizures are already quite absent. I would refrain from commenting on the habit of some investigators who are working on isolated brain slices and still use the term *absence seizures*. Besides, any loss of consciousness is an *absence*, making this term virtually useless when applied to generalized SW seizures [26]. The electrical patterns of these seizures will be analyzed extensively in section **5.5** because of the large numbers of experimental studies using intracellular recordings in neocortex and thalamus, both *in vivo* and *in vitro*, to elucidate the mechanisms underlying the EEG "spike" and "wave" components, as well as the origin of these seizures, usually defined as suddenly generalized and bilaterally synchronous. This definition originated with the concept of a deeply located "centrencephalic" system [27] and the fact that so-called "absence" seizures were induced in the cortex by electrical stimulation of midline thalamic nuclei at 3 Hz, at a critical level of barbiturate anesthesia [28]. In that study, only SW-like *responses* were evoked in the cortex, but no self-sustained activity. As to the existence of a "centrencephalic" system that would produce bilaterally synchronous SW complexes, it should be mentioned that there are no bilaterally projecting thalamic neurons. Brainstem core neurons with generalized projections disrupt, rather than produce, SW seizures [29]. A series of experimental studies, to be reported in section **5.5**, point to the progressive build-up of SW/PSW seizures at ~3 Hz that obey the rule of synaptic circuits, sequentially distributed through short- and long-range circuits in corticocortical and corticothalamic synaptic networks. Earlier and more recent EEG studies and toposcopic analyses

[23] Gastaut (1968).

[24] Dreifuss (1997). The classification of epileptic seizures in the chapter by this author was dictated by several factors, among them the need for an appropriate therapy. However, the author recognized, in the line of an idea expressed by John Hughlings Jackson, a pioneer of clinical neurology in the United Kingdom (see Chapter 1), that a utilitarian classification may be different from a scientific one based on morphological and physiological criteria. Jackson made the first distinction between generalized absence seizures and partial epileptic fits. For the first clinical description and origin of the term *absence*, dating back to the early 18th century, and the origin of the term *pyknolepsy* used to describe frequent daily attack, see Dreifuss (1997) and another chapter in the same textbook (Stefan and Snead, 1997).

[25] Cavazzuti et al. (1989).

[26] Porter (1993). Nonetheless, behavioral changes associated with SW seizures in the feline generalized epilepsy model indicate that, even in those animals, using instrumental conditioning procedures, the ability to respond to sensory stimuli was deeply altered during SW complexes, whereas the responsiveness between SW bursts remained unimpaired (Taylor-Courval and Gloor, 1984).

[27] Jasper and Kershman (1941); Penfield and Jasper (1954). Facing the repeated evidence that the "centrencephalic system" that is "found in the diencephalon and mesencephalon" (Penfield and Rasmussen, 1950, p. 19) cannot account for consciousness or for the generation of generalized seizures, Jasper (1990, p. 3–4) quoted a passage of Penfield in which he wrote: "It would be absurd to suppose that this central integration could take place without implication of cortical areas. . . . To suppose that centrencephalic integration is possible without utilization of the cortex would be to return to the thinking of Descartes and to enthrone again a spiritual homunculus in . . . such area as the nearby pineal gland". Also, concerning the origin of generalized seizures, Jasper mentioned the findings of Naquet and colleagues on photomyoclonic epilepsy, who failed to confirm the genesis of such seizures in the "centrencephalic system", the view of Gastaut who proposed instead the "holencephalic" hypothesis as a probable mechanism of generalized seizures, and Gloor's hypothesis on a corticoreticular system, rather than the

centrencephalic one. Gloor's view is probably the closest to the reality (see our data on seizures generated in corticothalamic systems, in the main text of section **5.5**).

[28] Jasper and Droogleever-Fortuyn (1949).

[29] Danober et al. (1995).

[30] Jasper and Hawkes (1938); Petsche (1962).

[31] Lemieux and Blume (1986). In another study, the investigation of apparently bilateral synchronous SW complexes in children with sleep-activated seizures similarly showed that the interhemispheric time differences during SW activity were 12–26 ms (Kobayashi et al., 1994).

[32] Gibbs and Gibbs (1952). Hypsarrhythmia derives form the Greek word *hypselos*, which means high and indicates the high amplitude of paroxysmal EEG waves in this epileptic syndrome.

[33] Gastaut et al. (1966); Niedermeyer (1969).

[34] Niedermeyer (1999b, p. 507).

[35] Gibbs et al. (1939).

[36] West (1841).

[37] Kellaway et al. (1979) distinguished three major groups of infantile spasms: extensor, flexor, and mixed flexor-extensor.

in humans and animals have also indicated that some SW seizures are locally generated and result from multiple, independent cortical foci [30] and topographical analyses of SW complexes in humans showed that the EEG "spike" component propagates from one hemisphere to another with time-lags as short as 15 ms [31], which cannot be estimated by visual inspection. This explains why absence seizures are less detrimental than grand-mal epilepsy, which implicates more widespread neuronal manifestations. Indeed, one of the characteristics of SW seizures is that there is little or no disruption of cognitive abilities after an ictal event. The origin of SW seizures within the thalamus or cortex was and continues to be hotly debated. However, the corticothalamic system is a reciprocally connected loop and, although some studies placed exclusive emphasis on one or another component of this unified entity, a congruent conclusion was ultimately reached; namely, that neocortical excitability represents the leading factor in controlling thalamic events during this type of seizures (see section **5.5**).

Thereafter (section **5.6**) I present data on the cellular bases of electrographic patterns in the Lennox–Gastaut syndrome, a clinical entity related to what is called *infantile spasms*, corresponding to the EEG notion of *hypsarrhythmia* [32]. The major distinction between the infantile spasms and Lennox–Gastaut syndrome [33] resides in age: the infantile spasms occurring between 4 and 30 months, whereas the Lennox–Gastaut encephalopathy usually starts between 1 and 10 years [20]. Otherwise, "no separating line is drawn between the attacks occurring in infantile spasms and Lennox–Gastaut syndrome" [34]. The Lennox–Gastaut syndrome was also called petit-mal *variant* [35] to differentiate its relatively slow (1.5–2.5 Hz) SW complexes from the classical SW complexes at 3–4 Hz occurring in absence epilepsy. The electrographic pattern of the Lennox–Gastaut syndrome is characterized by SW/PSW complexes, often associated with fast runs at 10–20 Hz, but up to 30 Hz (Fig. 5.1), and the background activity may be completely disorganized and chaotic. Clinically, infantile spasms were first described in the mid 19th century by a British physician [36] who gave his name to this epileptic condition, the West syndrome. The triad consists of different forms of spasms, such as myoclonic jerks and flexion spasms [37], EEG hypsarrhythmia, and mental retardation. This is a severe epileptic disorder and, generally, children with abnormal computed tomography do not become normal in follow-up. Although generally placed in the category of generalized seizures, focal clinical seizures are not uncommon and, indeed, our intracellular recordings of an electrographic pattern in cats, resembling that observed in clinical Lennox–Gastaut syndrome (see Fig. 5.1),

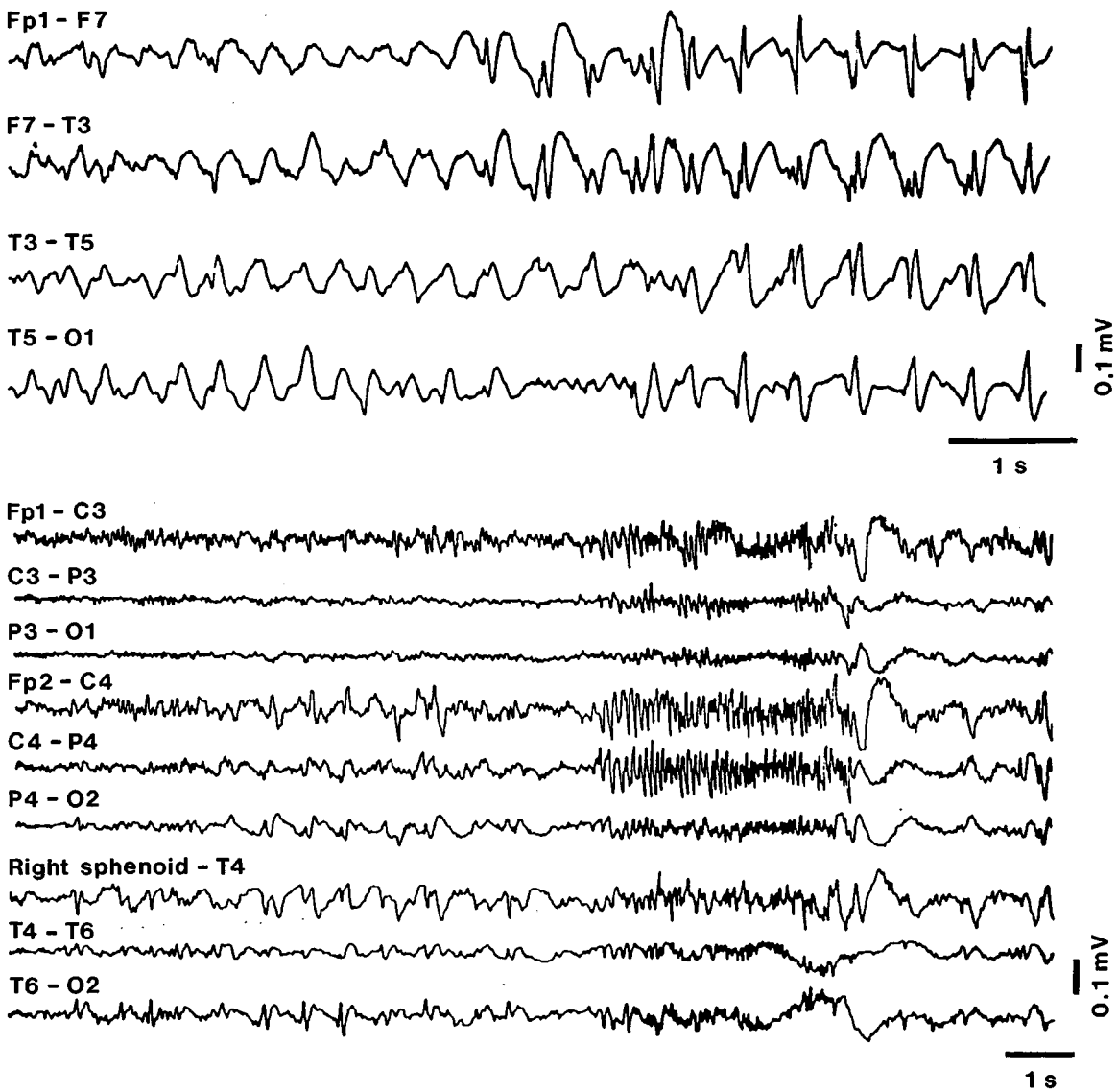


Fig. 5.1 EEG patterns in Lennox–Gastaut syndrome. *Top*, child with severe epileptic seizure disorder and generalized spike-wave (SW) complexes, mostly around 2 Hz. *Bottom*, rapid run in a 19-year-old epileptic patient, with maximum of fast waves in anterior leads. Also note a few slow SW complexes in the right temporo-occipital region. Modified from Niedermeyer (1999 a-b).

[38] This term was introduced by Gowers (1885).

[39] Zifkin and Dravet (1997).

[40] This term was introduced by Jasper et al. (1951).

[41] Gibbs et al. (1938); Lennox (1951).

[42] Feindel and Penfield (1954).

[43] Gloor et al . (1982); Gloor (1992).

[44] Gloor (1997).

demonstrate that the onset of many such spontaneously occurring seizures occurs focally in the neocortex, only to spread subsequently to thalamus (see section **5.6**).

Section **5.7** deals with grand-mal [38], tonico-clonic seizures. As such paroxysms with rich motor activity [34, 39] cannot easily be explored intracellularly in behaving animals that display convulsions, since intracellular recordings require perfect stability, I do not have personal experimental data with such seizures. In that section, I will only briefly describe the cellular and molecular processes that have been tentatively implicated in these seizures.

Mesial structures in the temporal lobe, including hippocampus and related systems (among them, parahippocampal gyrus and amygdala nuclear complex), play an important role in seizures that are included in the category of "temporal lobe epilepsy" [40]. The cellular mechanisms of seizures occurring in the hippocampus and amygdala are discussed in section **5.8**. Although some use the term temporal lobe epilepsy (TLE) as a synonym for psychic or psychomotor (complex partial) seizures [41], many patients with TLE have grand-mal seizures and some psychic seizures may arise from the fronto-orbital region [34]. Clinically, many patients with psychic seizures display déjà vu manifestations, first described by Hughlings Jackson [24], and have an emotional aura. A hallucinatory, dreaming-like experience and/or a feeling of strangeness often announce these seizures. These experiential phenomena have been intensively investigated since Jackson [24] and, more recently, by Penfield and his collaborators [27, 42] and Gloor [43, 44]. The hallucinations are mainly visual or auditory. Experiential phenomena can be affective, perceptual, and mnemonic, and they include elements from the individual's past. The most common affect is fear, from mild anxiety to terror, and arises suddenly. Fear can be elicited by stimulation of the amygdala [43]. It is known that wild monkeys are transformed into docile animals if the amygdala is lesioned and patients with bilateral lesions of the amygdala nuclear complex are unable to recognize facial expressions of fear (see ***2.2.3*** in Chapter 2). Positive emotions are less often seen in TLE and they may consist of exhilaration and erotic feelings. The mnemonic-like phenomena occurring in TLE are déjà vu illusions, which can also be elicited by electrical stimulation of the temporal lobe, and memory recall. In Gloor's data, the incidence of memory recall was higher by stimulating the amygdala than the hippocampus [44]. The mnemonic events evoked by seizures or electrical stimulation are explained by a series of parallel-distributed neuronal networks, including the direct projections from the amygdala, as well as amygdala projections mediated by perirhinal cortices, to

association neocortical areas implicated in polymodal sensory integration [45]. The onset of experiential phenomena in TLE is most often the amygdala, followed by the hippocampus [46], while scalp recordings generally fail to display significant changes in EEG. Indeed, psychomotor or complex partial seizures are more likely to occur when abnormal EEG signs are confined to the temporal lobe [47].

Brainstem seizures occur using auditory stimulation in rats with massive ablation of forebrain structures [48] and stimulating the brainstem reticular core of cats and rabbits with precollicular transection [49]. In contrast to forebrain seizures, brainstem-induced paroxysms are mainly tonic, with running/bouncing convulsions [50]. As yet, there is no intracellular analysis of brainstem-evoked seizures. There are controversial results concerning the requirement of an intact brainstem for the expression of forebrain-induced seizures [51] or the possibility of eliciting seizures in animals with precollicular transection [52]. It seems that the brainstem is not required for seizures induced by convulsant agents in a highly discrete epileptogenic site within the deep prepiriform cortex, area tempestas [53].

In section **5.9** I discuss the cellular aspects of seizures occurring after injury and deafferentation of cortical and thalamic tissues. Postlesional epilepsy accounts for more than 10% of the epilepsies with a defined cause [54]. Cortical lesions result in the development of chronic epileptogenesis [55]. The question of whether the cerebral cortex displays autonomous activity [56] or exhibits activity only when driven from other sources [57] has been the subject of hot debate. The absence of activity in earlier experiments on isolated cortical tissue [57] could have been due to cortical hypoxia, as more recent studies demonstrate that, although quite dissimilar to the activity of intact cortex, small isolated neocortical slabs display periods of spiking activity interspersed with long epochs during which miniature postsynaptic potentials (PSPs) are observed [58]. Cortical slabs isolated from human cortex at the time of surgery display paroxysmal, high-voltage activity [59]. Such epileptiform events may occur as a result of hypersensitivity by denervation, which is apparent in almost all investigated tissues, from the smooth and skeletal muscles to glands and neurons [60]. The value of this hypothesis was recently demonstrated by the higher incidence of spontaneous and electrically induced seizures in cortical slabs or undercut cortex *in vivo* [61]. One of the mechanisms underlying the appearance of stroke-induced epileptic foci could be the hyperexcitability in hypoperfused peri-ischemic areas [62]. Experimentally, pronounced hyperexcitability also occurs

[45] Amaral and Price (1984); Amaral et al. (1992).

[46] Fish et al. (1993).

[47] Gabor and Ajmone-Marsan (1968).

[48] Garcia-Cairasco et al. (1993).

[49] Kreindler et al. (1958). The results of this study, conducted in cats, were confirmed in rabbits by Bergmann et al. (1963) who circumscribed in the midbrain reticular core a low-threshold convulsive area.

[50] Browning and Nelson (1986).

[51] Velasco and Velasco (1990).

[52] Magistris et al. (1988).

[53] Browning et al. (1993).

[54] Annegers (1994).

[55] For a review of techniques used in chronically isolated cortical tissue, see Halpern (1972).

[56] Bremer (1958a).

[57] Burns (1951, 1958).

[58] Timofeev et al. (2000a).

[59] Echlin et al. (1952).

[60] Cannon and Rosenblueth (1949). The hypersensitivity by denervation may explain some unexpected results, such as the increased duration of wakefulness after chronic excitotoxic lesions of the midbrain reticular neurons. Such lesions firstly produce a loss of the waking state, but the time spent in wakefulness is increased 7–8 days after the lesion (Kitsikis and Steriade, 1981), probably due to an increased excitability of denervated structures (thalamus and basal forebrain) that are targets of midbrain reticular neurons and are also implicated in cortical activation.

[61] Topolnik et al. (2001, 2003).

[62] Pohlmann-Eden et al. (1996).

in remote brain areas after focal ischemia, as demonstrated by multi-unit recordings [63]. The mechanisms accounting for epileptogenesis in the injured and isolated brain tissue have been investigated using intracellular recordings *in vitro* and *in vivo* (see section **5.9**).

Experimental data substantiating the hypothesis of a dialogue between neurons and glia in epilepsy [64] have been obtained in studies of hippocampus and neocortex since the 1960s [65], in more recent *in vitro* studies [66], and by using dual intracellular recordings of these two cellular types *in vivo* [67]. These data are discussed in section **5.10**. The potential importance of glial cells in the synchronization and propagation of paroxysmal activity is due to the presence of receptors for different neurotransmitters (such as glutamate and GABA) on membranes of glial cells [68], the release of some transmitters by glial cells [69], and the display of intrinsic Ca^{2+} oscillations [70]. Thus, glial cells cannot be simply thought of as passively reflecting neuronal activity.

Finally, the effects of epileptic seizures on sleep cerebral states are discussed in section **5.11**.

5.2. Sleep and epilepsy: normal oscillations during non-REM sleep developing into seizures

The adage "sleep and epilepsy are common bedfellows" is supported by much clinical and experimental evidence showing that epileptic seizures of different types preferentially occur during slow-wave sleep (SWS or non-REM), whereas REM sleep is a relatively non-epileptic state [71] (see details in sections **5.5** and **5.6**).

The prevalence of paroxysmal activity during SWS is observed not only with full-blown seizures but also with interictal EEG "spiking". Most patients show maximal EEG "spiking" in SWS stages 3 and 4; because of less frequent interictal "spikes" in waking and REM sleep, recordings during these states of vigilance provide better localization of these signs for the presurgical assessment of temporal lobe epilepsy [72].

5.2.1. From low-frequency (7–15 Hz) sleep rhythms or augmenting responses to seizures

Although the clinical loss of consciousness in absence epilepsy with ~3-Hz SW complexes is only evident when such seizures occur during the waking state, the electrical correlates of these seizures preferentially appear during SWS, more often in early stages 1–2, and less often in stages 3–4, toward the end of SWS, when the

[63] Witte and Freund (1999).

[64] Orkand (1969).

[65] Grossman and Hampton (1968); Sypert and Ward (1971); Dichter et al. (1972). The density of neurons and glia in resected tissue from patients with temporal lobe epilepsy was analyzed and the authors concluded that glial density influenced the transition from interictal to ictal states, while neuronal density influenced the propagation of seizures (Spencer et al., 1999).

[66] In slices from the sclerotic CA1 area of chronic epileptic humans and pilocarpine-induced seizures in rats, Heinemann et al. (2000) found that, in areas of reduced neuronal density, there is an impairment of glial capacity for spatial K^+ buffering.

[67] Amzica and Steriade (1998b, 2000); Amzica and Neckelmann (1999); Amzica et al. (2002).

[68] Bormann and Kettenmann (1988); MacVicar et al. (1989); Steinhäuser and Gallo (1996).

[69] Levi and Gallo (1995); Araque et al. (1999).

[70] Parri et al. (2001) reported that recurrent astrocytic Ca^{2+} transients take place every 5–6 min, propagate through the glial syncytium, and trigger glutamate-dependent activity in neurons.

[71] Mahowald and Schenck (1997).

[72] Sammaritano et al. (1991).

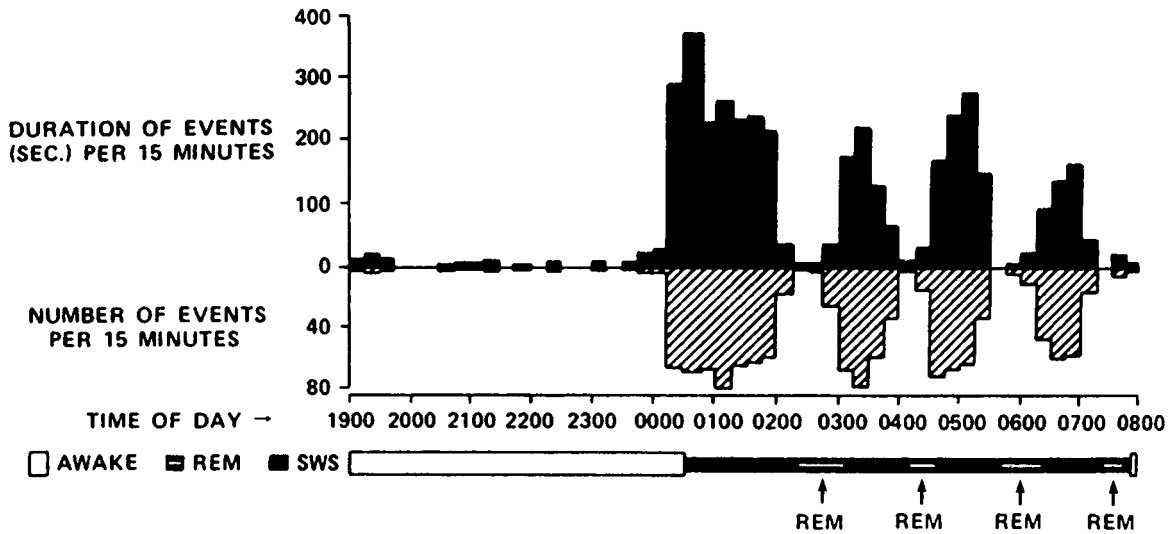


Fig. 5.2 The duration and number of SW complexes during waking, SWS, and REM sleep during the night in humans. Note the greatest incidence of SW complexes at the onset of SWS and obliteration during REM sleep. Abscissa shows the time of day and night. Modified from Kellaway and Frost (1983).

[73] Ross et al. (1966); Sato et al. (1973).

[74] Kellaway (1985). Shouse et al. (1996) discussed the possible relation between K-complexes, a major electrographic sign of light sleep, and childhood absence epilepsy. Basically similar relations between the K-complex in intracellular activities of cortical neurons and field potentials have been found during slow sleep oscillation and the paroxysmal events during complex SW seizures, the latter being just an exaggeration in amplitude and increase in frequency of the former (Steriade et al., 1998a; see sections **5.6**).

[75] Steriade (1974).

rhythmicity of SW complexes declines [73]. The appearance of seizures with pure SW and/or SW/PSW complexes at about 3 Hz during the early SWS stages is usually explained by their close relationship with sleep spindles at 7–15 Hz [74], which are a hallmark of stage 2, although experimental data show the presence of neocortical SW seizures in the absence of the thalamus and spindles (see section ***5.5.3***). Figure 5.2 shows the much higher incidence of 3-Hz SW complexes at the onset of SWS in humans, their decrement during successive SWS cycles, and their virtual absence in REM sleep.

The relation between sleep spindles and SW seizures is supported by animal experiments in which augmenting responses, the model of spindles (see section **4.2** in Chapter 4), lead progressively to self-sustained, epileptiform activity. Several forms of such transformations, from normal to paroxysmal events, have been reported. In chronically implanted, naturally drowsy or sleeping macaques, stimulation at 10 Hz (within the frequency range of spindles) applied to the motor thalamus elicits augmenting responses in precentral motor cortex, followed by typical SW complexes at ~3 Hz in the cortical depth, sometimes without reflection at the cortical surface (Fig. 5.3). The fact that the SW electrical seizure activity was only seen in the cortical depth suggested that intracortical, focal potentials generate this type of paroxysmal activity [75], which only

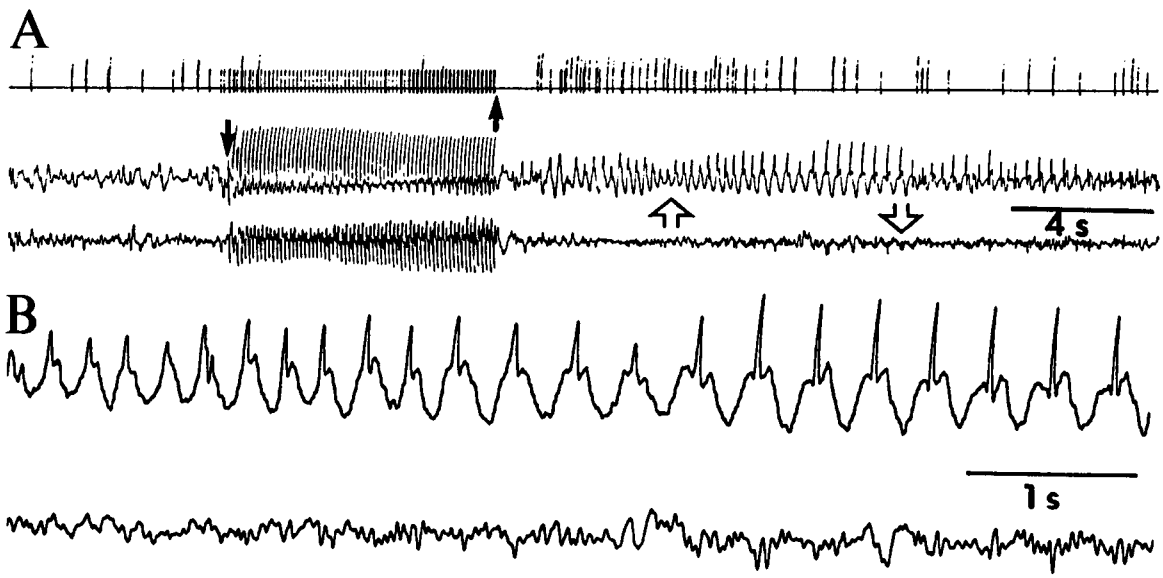


Fig. 5.3 Thalamic stimulation at 10 Hz elicits augmenting cortical responses, followed by self-sustained SW complexes at ~3 Hz. Chronically implanted monkey (*Macaca mulatta*) during a state of drowsiness. *A*, three ink-written traces show (from top to bottom): unit spikes (used to deflect the pen of the EEG machine; each deflection exceeding the lowest level represents a group of high-frequency discharges), focal slow waves (recorded by the same microelectrode as used for unit recording), and EEG surface waves. *B*, expanded portion of EEG activities depicted between arrows during self-sustained seizure in *A*. In depth- and surface-EEG recordings, positivity downwards. Modified from Steriade (1974).

[76] Golshani et al. (2001).

[77] Steriade and Contreras (1995).

subsequently spreads to other cortical areas and thalamus. Supporting evidence for the cortical generation of SW seizures is fully reported in section **5.5**.

Cortical augmenting responses are reflected in the thalamus. In particular, thalamic reticular (RE) GABAergic neurons are driven by corticofugal volleys more efficiently than thalamocortical (TC) neurons, because the numbers of some glutamate receptor subunits are much higher at corticothalamic synapses in RE neurons, compared to TC neurons [76] (see section ***2.4.3*** in Chapter 2). This accounts for the RE-mediated inhibition of the majority of TC neurons during cortically generated SW seizures (see section **5.5**). During the self-sustained seizure that follows thalamic stimulation eliciting augmenting responses at 10 Hz or during seizures developing "spontaneously" from the slow sleep oscillation, each paroxysmal depolarizing shift (PDS) in cortical neurons is faithfully followed by target RE neurons [77]. This is illustrated in Fig. 5.4, depicting a cortical seizure triggered by a pulse-train applied to the thalamic ventrolateral (VL) nucleus at a frequency (10 Hz) that is generally used to evoke augmenting responses. With a 10-Hz stimulation lasting 2 s, a 20-s electrical seizure was induced in the cortex and, without exception, every

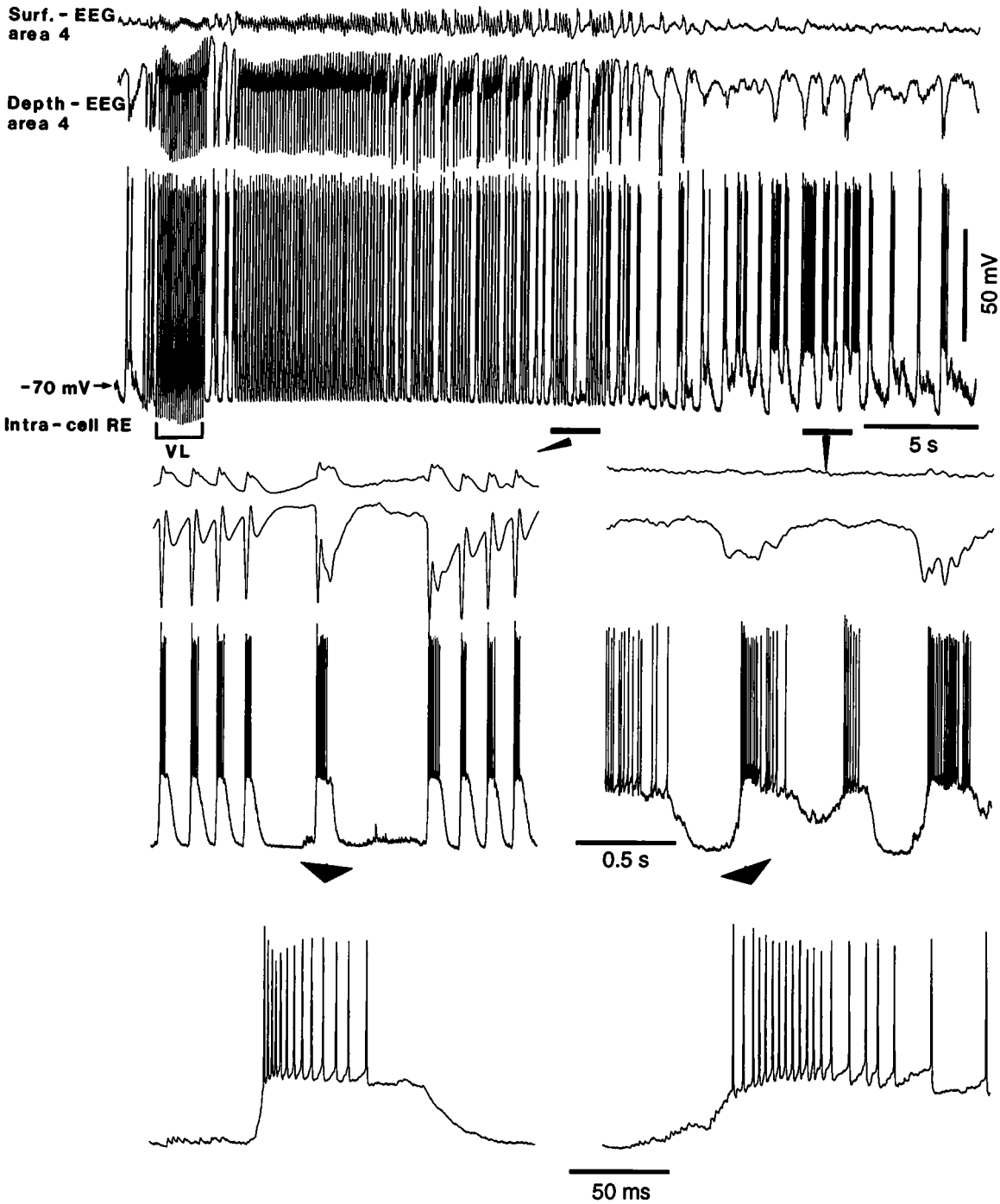
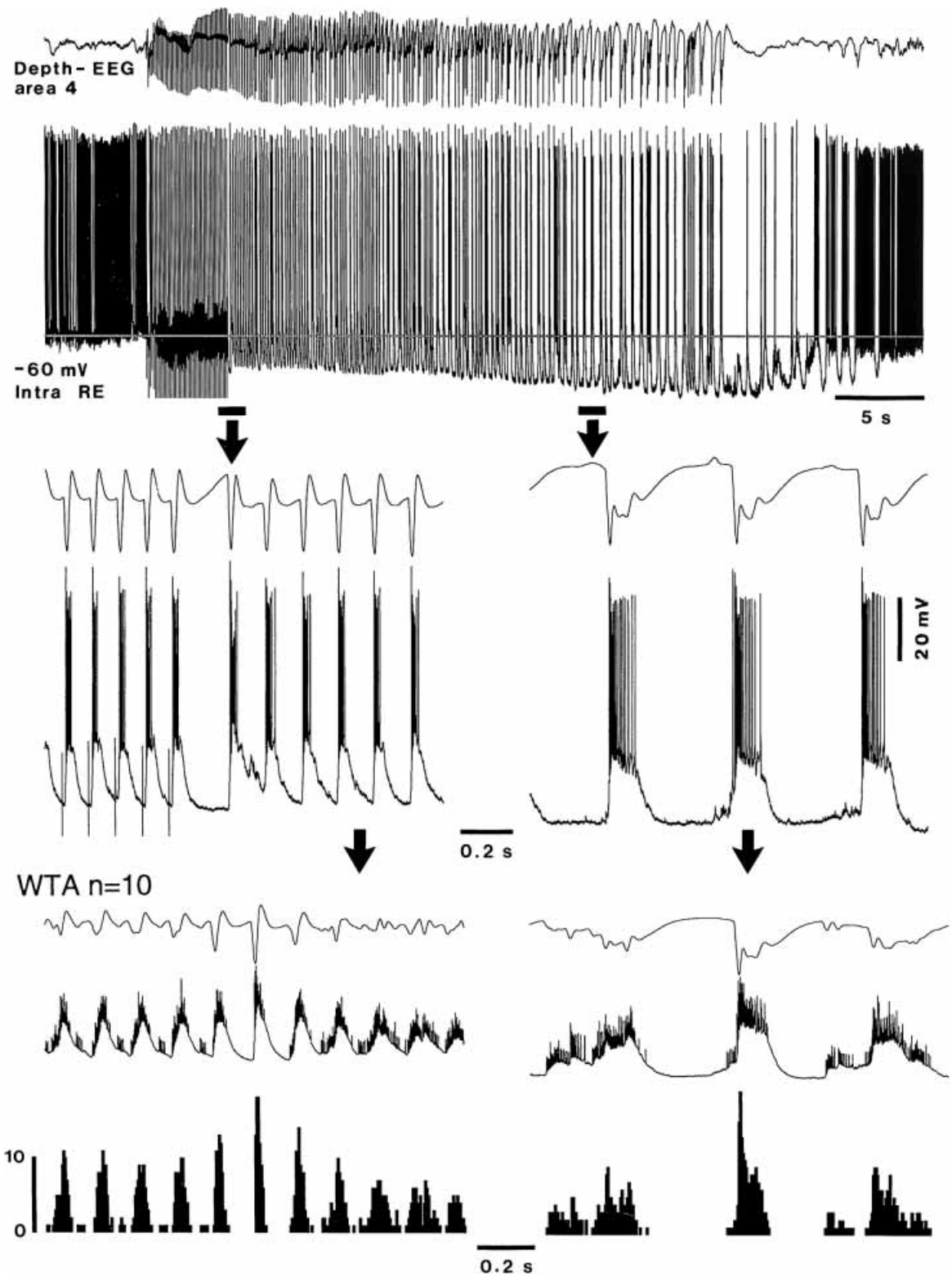


Fig. 5.4 Burst discharges in thalamic RE neurons follow normal and paroxysmal synchronous activities in neocortex. Cat under ketamine-xylazine anesthesia. Intracellular recording of RE neuron (rostral pole), together with surface- and depth-EEG from motor cortex (area 4). Stimulation at 10 Hz, lasting 2 s, was applied to thalamic VL nucleus (indicated by bar). Seizure lasted for ~20 s. Two parts marked by horizontal bars are expanded below (arrowheads). Each cortical paroxysmal depolarizing shift (PDS) was followed by a spike-burst in the RE neuron (middle panel, left). After seizure cessation, an episode of slow oscillation is seen and each depth-negative (excitatory) component in the cortical EEG is similarly followed by a spike-burst in the RE neuron (middle panel, right). One burst from each period (seizure and slow oscillation) is further expanded below. Note at bottom right, the accelerando-decelerando pattern, typical of RE neurons during sleep oscillations. From Steriade and Timofeev (2001).


Depth- EEG
area 4
-60 mV
Intra RE
5 s
20 mV
0.2 s
WTA n=10
10
0
0.2 s

[78] Steriade et al. (1993f). Earlier data also showed that augmenting responses can be elicited in primary somatosensory cortex by 10-Hz stimulation of white matter, in animals with complete lesion of the thalamic ventrobasal complex, and that augmenting responses develop into self-sustained seizures (Steriade and Yossif, 1974).

PDS in the EEG recorded from the cortical depth was associated with a high-frequency spike-burst in the intracellularly recorded RE neuron (middle and bottom left panels). Outside the seizure epoch, when the neuron resumed its slowly rhythmic pattern, the depth-negative field potential of the slow oscillation (reflecting summated depolarizations and firing in a pool of cortical neurons) invariably triggered spike-bursts with accelerando-decelerando patterns (middle and bottom right panels), typical of RE neurons during SWS.

Stimulation of dorsal thalamic nuclei at 10 Hz elicited self-sustained activity that was initiated at the same frequency, and displayed the same pattern, as responses evoked by electrical stimulation. Thus, the cortical seizure started with runs at ~9–10 Hz and then shifted to SW and PSW complexes at ~2 Hz. The increased number of action potentials in RE cell, from single spikes or spike-doublets to high-frequency spike-bursts, probably occurred through progressive hyperpolarization of the RE cell (Fig. 5.5). The progressive hyperpolarization was associated with an increasing number of spikes per burst. Averaged activities triggered by the depth-negative cortical waves during both the initial runs at ~9 Hz and SW/PSW complexes at ~2 Hz showed the synchronous cortico-RE discharges in both components of the seizure (bottom panels in Fig. 5.5).

Other data on the progressive transformation of augmenting responses into cortical SW seizures are reported in section ***4.3.3*** of Chapter 4 (see Fig. 4.45 in intact corticothalamic systems and Fig. 4.49 in isolated neocortical slab).

The fact that cortical networks alone, in the absence of the thalamus, are able to develop both augmenting responses and self-sustained seizures was demonstrated in athalamic animals [78]. Figure 5.6 shows that repeated pulse-trains at 10 Hz, eliciting augmenting responses in callosal pathways, are associated with depolarization (7 mV) and an increased number of action potentials in spike-bursts, eventually followed by a self-sustained seizure consisting of two components: (*a*) relatively short (~50 ms) spike-bursts,

Fig. 5.5 (opposite) Self-sustained seizure in the neocortex and an RE neuron, elicited by dorsal thalamic stimulation at 10 Hz. Cat under ketamine-xylazine anesthesia. Intracellular recording of a rostral RE neuron together with depth-EEG from area 4. Stimulation consisted of pulse-trains (10 Hz, 5 s) applied to the ventrolateral (VL) nucleus. Parts indicated by horizontal bars below the intracellular trace are expanded below (arrows). The seizure started with runs at ~9 Hz, close to the frequency used during the stimulation period, and then shifted to SW and PSW complexes at ~2 Hz. Bottom panels depict wave-triggered averages (WTA) from the two epochs of fast runs (left) and SW/PSW complexes (right). Reference time was the depth-negative field potentials from area 4. Note that the RE neuron was progressively hyperpolarized during the seizure induced by electrical stimulation of the dorsal thalamus. From Timofeev et al. (1998).

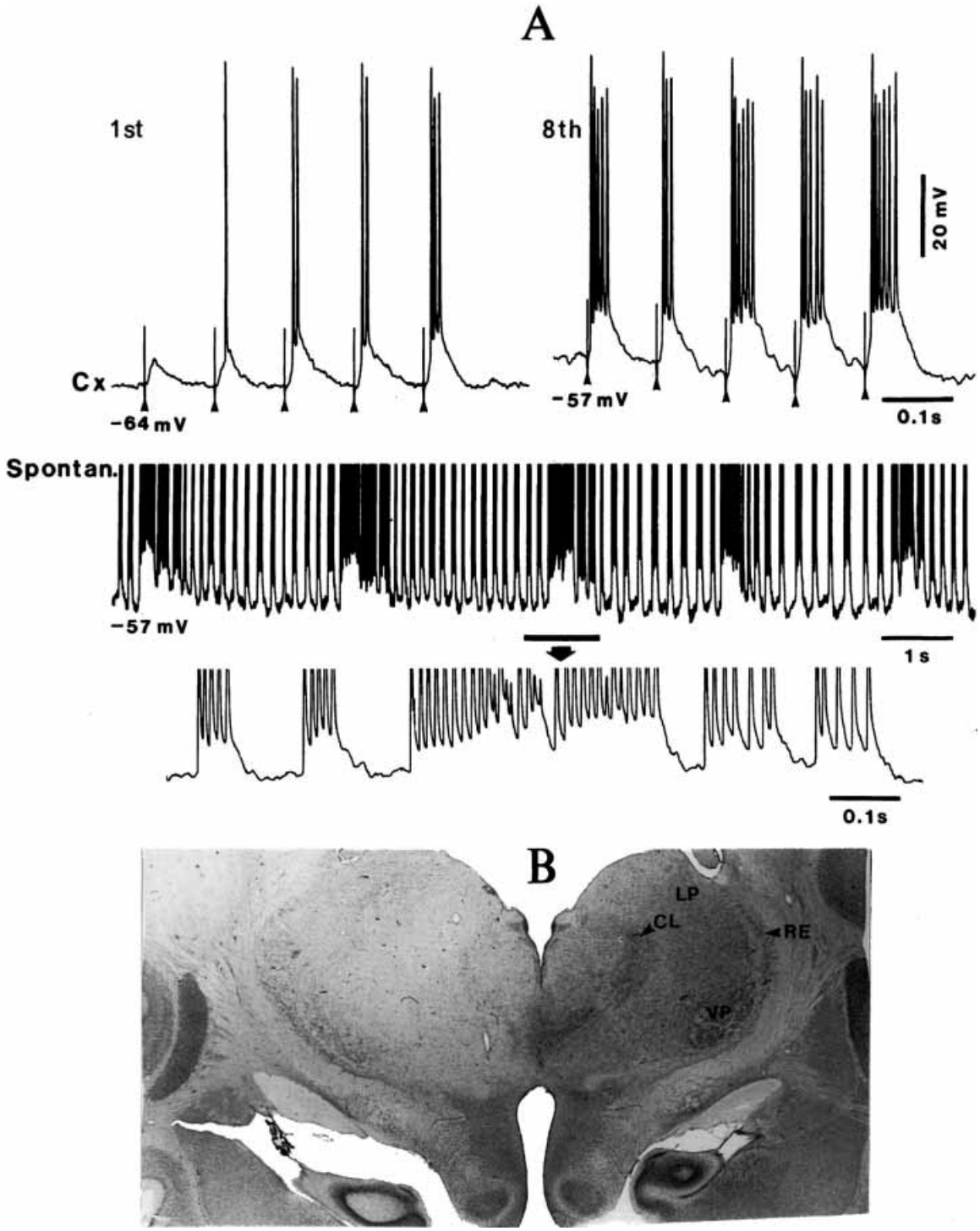


Fig. 5.6 Self-sustained seizure in a neocortical neuron after augmenting responses evoked by rhythmic callosal stimulation of the homotopic point in the contralateral hemisphere. Thalamically lesioned cat by means of kainic acid (see *B*). Urethane anesthesia. Intracellular recording of neuronal activity at a depth of 1.5 mm in area 7. *A*, responses to pulse-trains (each consisting of five stimuli at 10 Hz), repeated every 3 s, applied to contralateral area 7. The intracortical augmenting responses to the first and eighth pulse-trains are illustrated. Note depolarization by about 7 mV and increased number of action potentials within bursts after repetitive stimulation. Below, self-sustained paroxysmal discharges after augmenting responses (spikes truncated). Period marked by horizontal bar is expanded below (arrow). *B*, thalamic lesion. CL, LP and RE: central lateral, lateral posterior and reticular thalamic nuclei. Modified after Steriade et al. (1993f) and Steriade (1998).

[79] Steriade et al. (1996a-b).

[80] Grenier et al. (2001).

[81] Allen et al. (1992).

[82] Traub et al. (2001).

[83] Bragin et al. (1999a, 2000).

[84] Grenier et al. (2003).

occurring rhythmically within a frequency range close to that used in the preceding period of stimulation; and (*b*) prolonged (500–600 ms) spike-bursts, recurring within the frequency range of the slow oscillation (0.3–0.4 Hz) that dominated the background activity [78].

Thus, spontaneously occurring low-frequency oscillations (such as sleep spindles) or their experimental model (augmenting responses) may lead to self-sustained neocortical seizures, which are initiated at approximately the same frequency as that of spontaneous or evoked waves and develop into SW/PSW seizures at 2–3 Hz.

5.2.2. From very fast (80-200 Hz) rhythms during the slow sleep oscillation to seizures

During SWS, the depolarizing phase of the slow neocortical oscillation includes fast (20–60 Hz) rhythms [79] and very fast (80–200 Hz) rhythms, called ripples [80]. These activities were recorded under anesthesia as well as in chronically implanted, naturally sleeping cats (see section *3.2.3.2* and Figs. 3.40–3.42 in Chapter 3). There are several types of neocortical and hippocampal seizures with fast and very fast oscillatory components, 50–80 Hz [81], 80–130 Hz [82], and 200–500 Hz [83]. The mechanism(s) responsible for the initiation of neocortical seizures from very fast oscillations, in particular from ripples (80–200 Hz), is an important topic as it may lead to therapeutic avenues against seizures.

That ripples recorded during the slow sleep oscillation could play a role in initiating seizures is suggested by the strong correlation between neuronal excitation and the intensity of ripples [80]. To be considered as involved in seizure initiation, neocortical ripples have to be present at the transition between normal and paroxysmal activity, to show significantly increased amplitudes at the very onset of seizure, compared to epochs prior to it, and there should be fewer or no seizures in conditions that diminish neocortical ripples. Recent extra- and intracellular multi-site recordings from different neocortical areas show that, indeed, ripples are particularly strong at the onset of seizures and they decline afterwards, halothane antagonizes the occurrence of both ripples and seizures, and stimulation mimicking the pattern of ripples is a most efficient paradigm to induce seizures associated with ripple activity at the level of field potentials and individual neurons [84]. Some of these data are discussed in section **5.6**, dealing with seizures of the Lennox–Gastaut type. Here, I focus on data using electrical stimulation within the frequency range of ripples (~100 Hz).

The 100-Hz stimulation paradigm faithfully mimics the ripples crowning EEG "spikes" that occur at the onset of seizures. This

is illustrated in Fig. 5.7 by comparing spontaneous and evoked seizures during the same experiment. The 100-Hz stimulation also evokes focal seizures, within the region close to the stimulation, in non-anesthetized, chronically implanted animals [84]. When seizures are elicited using this type of stimulation, ripples appear in the EEG and/or intracellular recordings between trains of stimuli, prior to the occurrence of full-blown seizures, and neuronal activities within ripple frequencies outlast stimulation at 100 Hz, in the form of repetitive action potentials (Fig. 5.8) or subthreshold membrane potential fluctuations. The evoked ripples in paroxysmal neuronal activities are only seen in neocortical neurons, and not in simultaneously recorded thalamocortical neurons (Fig. 5.8).

The relation between ripples and seizures is more than just a temporal correlation, as data suggest that ripples are implicated in the generation of the first EEG "spike" of seizures. During repetitive seizures ripples do not appear with the same intensity *after* the first EEG "spike" is expressed as they should if they were conditional on the occurrence of the seizure. Rather, the first EEG "spike" slowly builds up with ripples present from the beginning of seizure; thereafter, successive EEG "spikes" are associated with less intense ripples (Fig. 5.9). In contrast, foci that follow the primary site display more abruptly rising successive EEG "spikes", with ripples on top of them [84]. This suggests that ripples could be dependent on neuronal depolarization during EEG "spikes" in secondary sites, but that they are involved in generating the first EEG "spike" in the primary site. Seizures in secondary sites are probably triggered by projections from the primary site.

When seizures evolve without discontinuity from the slow oscillation, strong ripples occur just before the transition between normal and paroxysmal activity. The comparison between non-paroxysmal (Fig. 5.9*A*) and the first paroxysmal (Fig. 5.9*B*) EEG-depth negativities (that reflect summated depolarizations in a pool of neurons) shows that they were similar at their onset; however, before the paroxysmal negativity reached the maximal value displayed by the non-paroxysmal one, ripples appeared over it and were present until the negativity reached its full paroxysmal peak. Thus, ripples of strong amplitude are present from the very onset of the transition between normal and paroxysmal activities.

5.3. Electrically and sensory-induced afterdischarges

The afterdischarges (ADs) elicited by electrical stimulation of various brain structures are related to seizures induced by

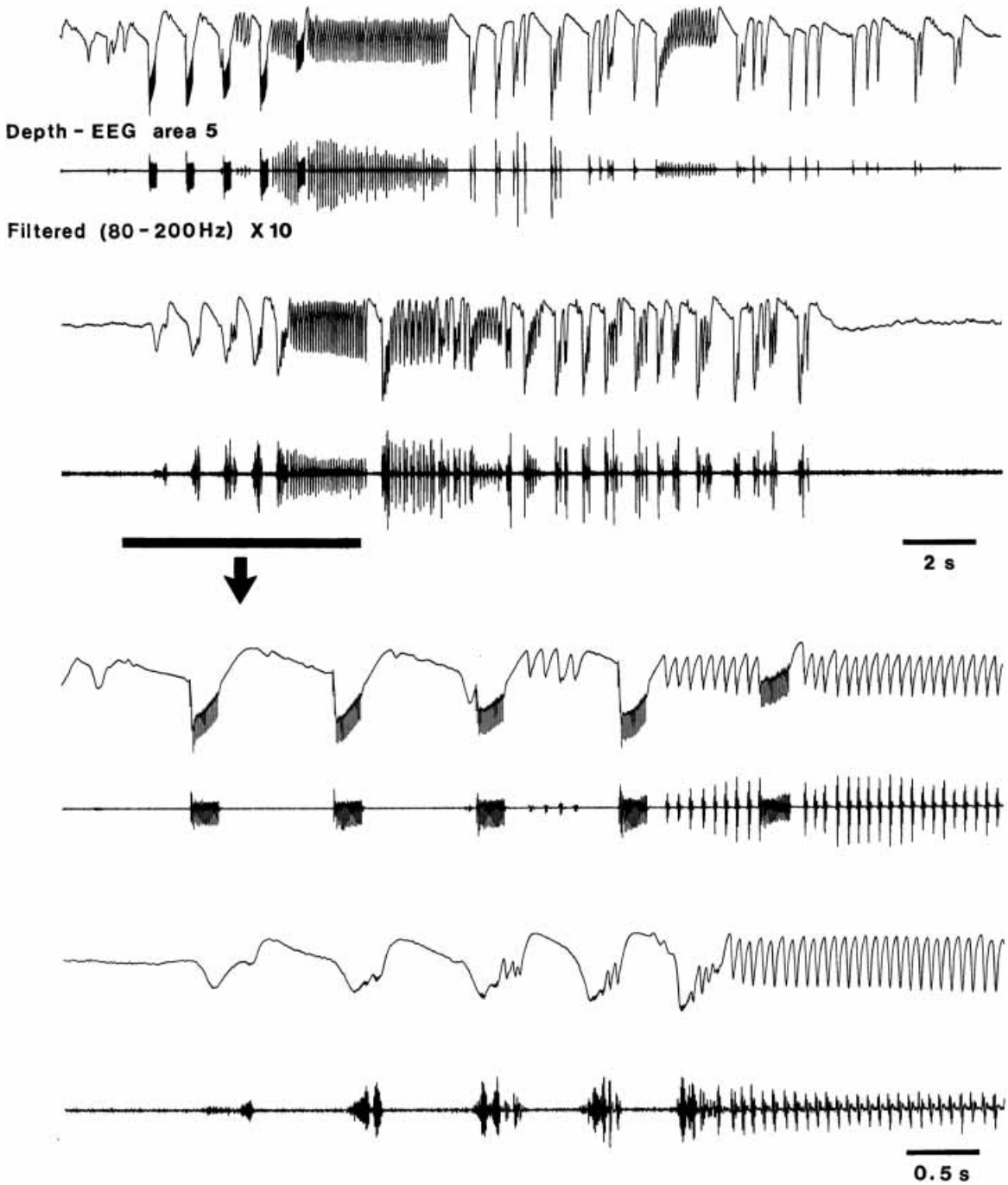


Fig. 5.7 Pulse-trains at 100 Hz mimic the onset of spontaneously occurring seizures. Cat under ketamine-xylazine anesthesia. Field potential recordings from area 7 along with the filtered trace between 80 and 200 Hz (amplified × 10). Top panels display one evoked (above) and one spontaneous (below) seizure from the same experiment. The onset of both seizures is expanded in the bottom panels to show the similarity between the stimulation paradigm (20 shocks at 100 Hz repeated every second) and the EEG "spikes" with ripples at the onset of the spontaneous seizure. From Grenier et al. (2003).

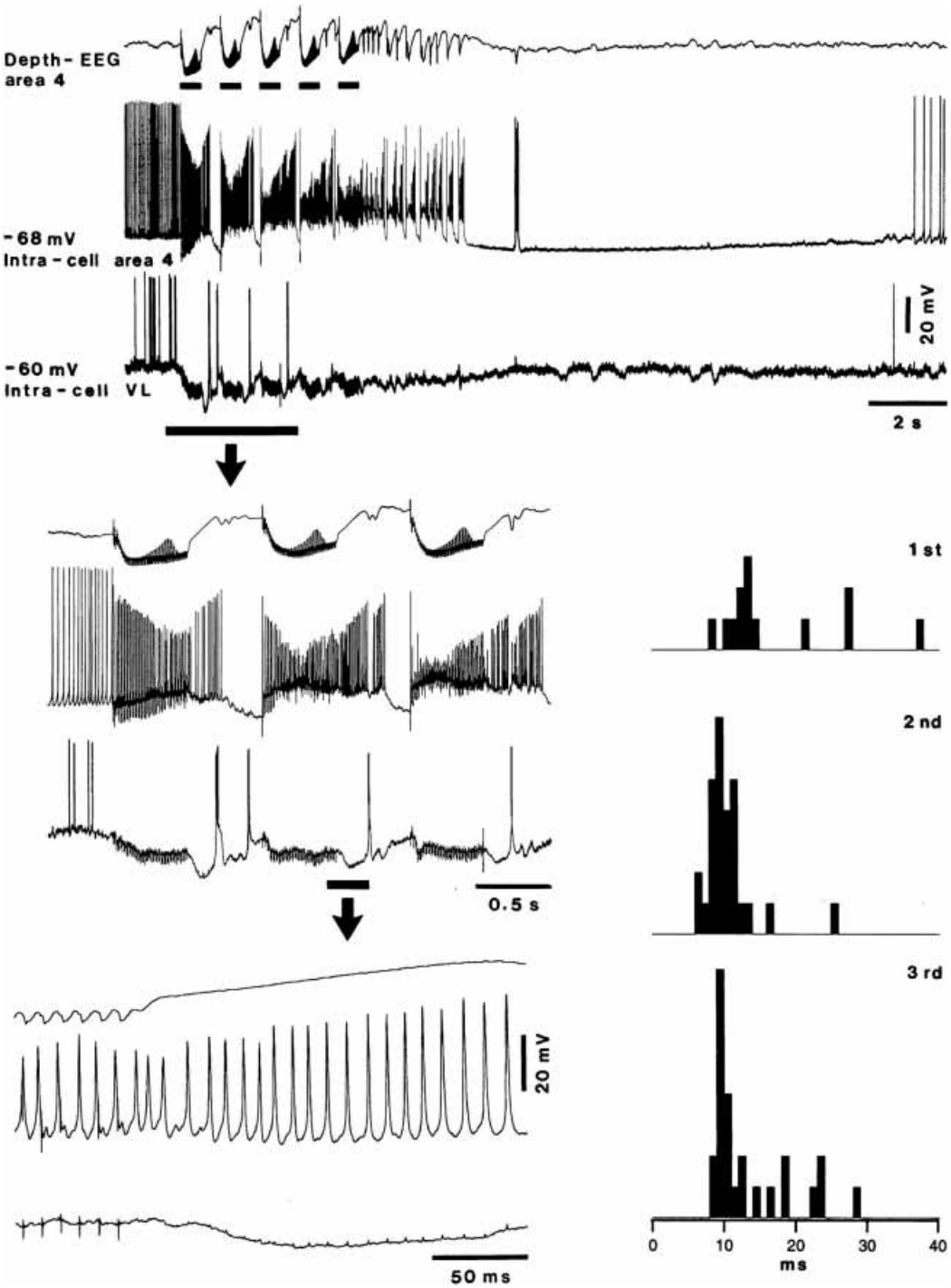


Fig. 5.8 Ripples outlast electrical stimulation at 100 Hz that leads to a self-sustained seizure in neuronal firing. Cat under ketamine-xylazine anesthesia. Dual intracellular recordings from a thalamocortical (TC) cell in the ventrolateral (VL) nucleus and an area 4 cortical cell as well as a field potential recording from area 4. Electrical stimulation of area 4 (five trains of 50 stimuli at 100 Hz, marked by horizontal bars below EEG) led to a brief seizure. The top panel displays the period of stimulation and the ensuing seizure. The first three pulse-trains of stimulation are expanded on the left, in the middle panel. The second pulse-train is further expanded at bottom left. Note the continuation of spikes in the cortical cell after the stimulation has ceased. Note also the absence of 100-Hz activity in the TC cell. On the right interspike histograms (ISIHs) are shown for the periods following the first three trains of stimulation. Note that most ISIs are clustered around values (8–11 ms) corresponding to ripple frequencies between 80 and 120 Hz. From Grenier et al. (2003).