Absence-like seizures in adult rats following pilocarpine-induced status epilepticus early in life

Abstract

Administration of pilocarpine causes epilepsy in rats if status epilepticus (SE) is induced at an early age. To determine in detail the electrophysiological patterns of the epileptogenic activity in these animals, 46 Wistar rats, 7-17 days old, were subjected to SE induced by pilocarpine and electro-oscillograms from the cortex, hippocampus, amygdala, thalamus and hypothalamus, as well as head, rostrum and vibrissa, eye, ear and forelimb movements, were recorded 120 days later. Six control animals of the same age range did not show any signs of epilepsy. In all the rats subjected to SE, iterative spike-wave complexes (8.1 ± 0.5 Hz in frequency, 18.9 ± 9.1 s in duration) were recorded from the frontal cortex during absence fits. However, similar spike-wave discharges were always found also in the hippocampus and, less frequently, in the amygdala and in thalamic nuclei. Repetitive or single spikes were also detected in these same central structures. Clonic movements and single jerks were recorded from all the rats, either concomitantly with or independently of the spike-wave complexes and spikes. We conclude that rats made epileptic with pilocarpine develop absence seizures also occurring during paradoxical sleep, showing the characteristic spike-wave bursts in neocortical areas and also in the hippocampus. This is in contrast to the well-accepted statement that one of the main characteristics of absence-like fits in the rat is that spike-wave discharges are never recorded from the hippocampal fields.

Introduction

Epidemiological studies indicate that human epileptic disorders frequently have their onset early in life. The highest incidence of epilepsy is found in childhood and adolescence (1,2). From 1 to 8% of all epileptic patients present at least one episode of status epilepticus (SE) and a considerable number of these patients die as a consequence of SE (2-5). Mortality due to SE ranges from 2.5 to 20% in adults (5) and may reach moderately higher values of up to 25% in children (2-8). These data suggest that SE is a prominent factor in epilepsy, as also demonstrated experimentally in rats and mice (9-14). In humans, late epileptic seizures may occur years after SE (4) and it should be pointed out that...
in children and adults who die of SE, lesions have been found in several areas of the brain (2,4,8).

Although epilepsy may become manifest at any age, the onset of its causing lesion does often occur shortly after birth, when the nervous system is still immature and highly susceptible to metabolic, traumatic and inherited damaging factors (3). The phases of neural maturation during which such factors exert their influence seem to be a key factor in determining the threshold for the development of any kind of epileptic seizure, either convulsive or non-convulsive (15-18).

Many manifestations of epilepsy can be studied more closely in laboratory animals than in humans, providing valuable information about this disease. Among such manifestations, SE and epileptic discharges in several areas of the brain are the most convenient to be studied in animal models, also for testing antiepileptic drugs. The pilocarpine model of epilepsy in rats is well suited to the study of several kinds of epilepsy that mimic human epileptic fits. In fact, systemic administration of pilocarpine, a cholinergic muscarinic agonist, to rats 7 to 10 days after birth causes mild behavioral manifestations, such as motor hyper- or hypoactivity, head and body tremor and scratching. When injected into 11- to 17-day-old rats, however, pilocarpine usually induces far more severe effects, such as mastication, salivation, eye blinking, jaw and limb clonic movements, rearing, and generalized convulsions. In both cases, however, the rats eventually progress to SE (18-20). The long-term consequences of SE, such as those observed in adult rats and mice (10,11), are spontaneous seizures secondarily generalized and accompanied by structural damage to the brain. Chronic epilepsy thus provoked is similar in many aspects to human temporal lobe epilepsy and as such is a good model for this human disease.

Even brief episodes of SE may cause injury to neurons in several parts of the brain. Particularly vulnerable to SE caused by pilocarpine are the hippocampal interneurons (21) but granule cells in the dentate gyrus also are lesioned, exhibiting, like the interneurons, morphological features of apoptosis and necrosis (22,23). It is interesting to note that reinduction of SE does not provoke greater damage to these neurons (24).

Since recording the electrical activity (electro-oscillograms) of a large number of cortical and subcortical sites in the brain is of the utmost relevance for the study of epileptic seizures, the present study was carried out to record the electro-oscillograms from several brain areas and to assess the body movements of adult rats subjected to systemic pilocarpine administration at the age of 7 to 17 days. Some unexpected manifestations of this type of epilepsy were detected by the wide span analysis of the electrical activity of the brain in these animals.

**Material and Methods**

Forty-six Wistar rats of either sex (16 females and 30 males), permanently kept in a temperature-controlled environment under a 12-h light-dark cycle, were injected ip with a single dose of pilocarpine (320 mg/kg) between 7 and 17 days after birth (10,18). Six age-matched control animals were injected with 0.9% saline. The animals were subjected to implantation of electrodes 120 days after SE (10,20) for recording the electro-oscillograms and body movements. Preliminary studies showed that after this interval following pilocarpine administration the changes in the electrophysiological manifestations of epilepsy were stabilized.

Parallel nichrome electrodes 150 µm in diameter, bent at 90° at the distal 0.5 mm and isolated except at the end cut section (to detect potentials from a small area), were stereotaxically implanted under 50 mg/kg ip cyclo-hexylamine (Ketalar®) anesthesia. The electrodes were implanted over cortical areas 3 and 10 according to skull references...
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(25,26) that make it possible to implant the electrodes over specific cytoarchitectonic and functional (27) areas through 1-mm holes in the bone.

Similar but straight electrodes parallel and isolated except at the end cut cross-section were stereotaxically implanted into the CA1 and CA3 hippocampal fields according to the Paxinos and Watson atlas (28). In 10 rats electrodes were also implanted in the amygdala and in the ventral posterolateral thalamic and ventromedial hypothalamic nuclei. Nichrome electrodes were implanted in the trapezius muscles, in each external canthus of both eyes, in the muscle pad that moves the snout and vibrissae and in the biceps brachialis, to record head, eye, rostrum and vibrissa and forelimb movements, respectively. Not all the electro-oscillograms or all the movements were recorded in every experiment.

The electrodes were connected to the pins of a 26-pin socket, which in turn was anchored to the bone by two brass screws 1.4 mm in diameter and thereafter cemented to the skull with a rapidly polymerizing methacrylate. After electrode implantation the animals were kept in individual cages, with free access to food and water. The procedures for implanting the electrodes were performed with extreme care to avoid edema that usually results in unreliable recordings before one week post-surgery.

For the electro-oscillographic recording, which was performed with a 21-channel Nihon-Kohden electroencephalograph, the rat was placed in a small cubic Faraday metal cage (0.40 x 0.40 x 0.40 m) for electrical shielding, and the socket fixed to the bone was connected to the recorder input panel. Recordings, which began 2-3 days after electrode implantation, were continuously monitored for 6-8 h and repeated every three days for four weeks. Since it is well known that spike-wave discharges (SWD) and absence seizures are specifically blocked by certain drugs, in 6 rats ethosuximide was injected (200 mg/kg, ip) to find out if the spike-wave complexes would be blocked.

At the end of the recording series each rat was injected with an overdose of cyclohexylamine and a small, marking electrolytic lesion was provoked at the distal tip of every subcortical electrode, intended to check its correct placement. The head was subsequently perfused with 250 ml of 0.9% saline and with a similar amount of 10% formalin thereafter. The brain was removed and kept in formalin for seven days at 10°C, after which 20-µm frozen sections were Nissl-stained, only for the purpose of identifying the sites where the electrodes had been implanted.

Results

Neocortical spike-wave bursts

SWD were recorded from cortical areas 10, 3 and 17 of all experimental rats. The epileptic patterns were very similar for all 46 animals. The spike-wave complexes were bilateral and synchronous, although seldom with a similar voltage on both sides; occasionally, in the same animal, SWD started 0.1 to 1 s earlier on one side or the other. The SWD frequency ranged from 7.2 to 9.9 Hz (mean 8.1 ± 0.5 Hz) and the bursts, that lasted on average 18.9 ± 9.1 s, occurred irregularly at a frequency of about 50 to 150 per hour. Within the time elapsed from the first to the last recording session no change in periodicity of the SWD was detected. Quite often such SWD were followed by a low frequency pattern (2 to 4.8 Hz) of discharges. While SWD occurred the animal remained immobilized but sometimes, and unexpectedly, clonic movements also accompanied the electrophysiological seizure.

Figure 1 shows three typical consecutive cortical SWD seizures similar in morphology and frequency (9.5 Hz) in area 10 of both brain hemispheres. Due to their high functional significance and relevance, these po-
tentials will be described here in detail. In area 3 SWD oscillated at the same frequency on both sides but were different in morphology, although the electrodes were placed precisely over the contralateral symmetric site and their resistance was closely similar, thus excluding the possibility of the difference being due to an artifact. Following the first and second SWD bursts, delta waves (3.1 Hz) occurred, coupled to a spike in the second burst, a pattern that was also a very common finding. As usual, the SWD complexes started as two or three slow waves not followed by spikes; then, the spikes started, closely linked to the delta waves, increased in voltage and disappeared before the slow waves, as is illustrated in Figure 1 (see inset). The inset shows with a higher definition that the earliest and the last potentials of a burst are only slow waves and that the slow wave and the spike component of the SWD burst are closely related (a and b). As will be discussed later, this is precisely the pattern for human absence seizure of the petit mal type.

The SWD were readily blocked by ethosuximide (200 mg/kg), an efficient antiepileptic drug. In 4 rats with recurrent SWD bursts in which we injected ethosuximide, the epileptic bursts were consistently and entirely blocked for 1 h.

Most SWD neocortical bursts were accompanied by concomitant potentials in the hippocampus and other subcortical sites. In the same animal the cortical bursts may differ in voltage from the subcortical ones at different epochs. Figure 2 illustrates such correlations. In Figure 2a the low voltage cortical burst (in A10, A3 and A17) is concomitant with a high voltage SWD burst in the hippocampal fields CA1 and CA3 as well as in the basolateral nucleus of the amygdala and in the ventromedial nucleus of the hypothalamus. The events in Figure 2c and d were equivalent in all leads. At the same time clonic movements occurred in several parts of the body, which were visually detectable during the seizure. In Figure 2b repetitive spikes occur concomitantly in the subcortical structures, a pattern also observed for the seizures in Figure 2c and d.

In Figure 3 the electro-oscillograms of an SWD seizure are reproduced from a com-

![Figure 1](image1.png)

![Figure 2](image2.png)
puter-generated recording to increase the resolution of the potentials. In the right and left frontal areas 10 the spike-wave complexes are very clear but similar and synchronic complexes are present also in area A3 and in the hippocampus, mainly in the right CA1. The delta waves in the right left area 10, each followed by a spike, are in fact high voltage, low frequency SWD (with a saturated negative component). Even during an interval of 49 to 49.5 s, when the SWD are absent from areas 10, they are quite clear in the right CA1.

In two rats, SWD were recorded during periods of paradoxical sleep. Figure 4a illustrates a normal episode of paradoxical sleep with rostrum and eye movements in one of the animals, characterized by desynchronization in area 10 and theta waves in area 3, in the hippocampal CA1 field and in the basolateral nucleus of the amygdala. The burst of SWD in Figure 4b appears suddenly in neocortical areas 10, 3 and 17 and in CA1 and is accompanied by phasic eye and ear movements. In Figure 4c, paradoxical sleep is again entirely normal.

In Figure 5 a short period of SWD with light clonic movements breaks through an episode of paradoxical sleep but, differing from the previous example, it is followed by wakefulness, during which desynchronization is present in area 3, whereas in all the remaining leads theta waves occur. Concomitantly with the wakefulness electro-oscillogram, intense movements of the head, rostrum, eyes, ears and forelimb do appear with a normal aspect.

Discussion

The most significant findings of the present study are: i) permanent absence-like epilepsy develops in adult rats in which SE has been provoked by pilocarpine administration 7 to 17 days after birth; ii) iterative SWD occur bilaterally in the neocortex (8.1 ± 0.5 Hz in frequency, 18.9 ± 9.1 s in duration); iii)
during SWD the rats are generally immobilized, as in human absence seizures, but clonic movements and single jerks may occur concomitantly or dissociated from the SWD; iv) the neocortical SWD are similar to those found in all genetically epileptic rats; v) the SWD bursts can be entirely suppressed by ethosuximide; vi) similar SWD also always occur in the hippocampus and frequently appear in the basolateral nucleus of the amygdala, thalamic ventrolateral nucleus and hypothalamic ventromedial nucleus; vii) SWD may occur during paradoxical sleep.

Spike and wave complexes and immobilization are characteristic of absence fits in humans. Such seizures in humans may also be accompanied by jerks and clonic movements. Since our rats also showed such characteristics it is probable that their epilepsy is at least partially equivalent to that occurring in humans.

The pathognomonic electrophysiological signs of absence, or petit mal, epilepsy were studied in depth by several authors as soon as electroencephalography became available to neurologists. In humans, absence seizures are electroencephalographically characterized by spike-wave complexes oscillating at nearly 3 Hz in both hemispheres, mainly in the frontal lobe. Recordings of potentials from both cortex and thalamic nuclei of six children with frequent absence fits (29) showed that the spike-wave complexes start in thalamic nuclei as delta waves that are transmitted to the cortex with increasing voltage as a function of time. Williams (29) advanced the hypothesis that the spike originates in deep cortical layers and propagates to the thalamic nuclei, with the consequent occurrence of a wave-spike pattern. After attaining a peak voltage, the SWD decreased in the opposite sequence, i.e., the spike diminished in voltage and eventually ceased while the delta wave was also progressively reduced in voltage and finally subsided. This pattern is similar to the cortical recruiting due to stimulation of midline and intralaminar nuclei (26), in agreement with the currently accepted hypothesis of a thalamic origin for SWD (30). It thus seems that the slow wave originates and progresses in the thalamus as a recruiting response and ascends to the cortex, where it also recruits the spike (29). Probably, the thalamically originated delta wave triggers the cortical spike, which in turn is projected back to thalamic nuclei; a resonant loop might thus arise and be responsible for the epileptic fit, which fails to occur when the spike is not recruited (29).

The SWD we found in pilocarpine-treated rats evolved just as the human SWD in petit mal. Recent microelectrode studies have shown that the spikes of spike-wave complexes are associated with impulses leaving the cortex, thus supporting the hypothesis of the cortical origin of these potentials (29).

It should be pointed out, however, that the spike-wave complex may be triggered by penicillin (31,32) in intact cats and by metrazol in the isolated cortex, which is suggestive of a specific cortical circuitry that is set to produce complex spike-wave complexes if adequately activated by subcortical
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Bilateral synchronous cortical spike-wave complexes have also been provoked by local application of kainic acid to the cingulate gyrus of cats (33), which introduces a complicating factor in the hypothesis of a thalamic origin of SWD.

The involvement of the hippocampus in absence seizures is of the utmost relevance for the understanding of its pathophysiology. However, this is still a controversial issue. In rodents, models of genetic absence seizures have been described (34,35) in which spike-wave potentials were shown in cortical areas but not in the hippocampus. In the present study, in which epilepsy was provoked by treatment with pilocarpine, we have detected such discharges also in the hippocampus, as well as in the amygdala, thalamus and hypothalamus. This discrepancy may be attributed to the lesions provoked by the drug in different central structures, among which the hippocampus, amygdala and thalamus (21-24). It should be stressed, however, that in our rats the SWD in the hippocampus, amygdala and thalamus were not independent of the cortical ones but were concomitant, indicating that they are being driven by the same SWD pacemaker.

The question of SWD in the hippocampus has long been a matter of discussion. For instance, in stargazer mutant mice the study of the expression of neuropeptide Y in dentate gyrus granule cells (36) revealed that it is concentrated in mossy fibers following SWD seizures, a fact considered to reflect the gene induction triggered by synchronous bursting. The results of the present study clearly demonstrate that SWD are present in the hippocampus, which has been denied (33) due to the study of single/multi-unit recordings from the sensorimotor cortex, the ventrolateral thalamus and the hippocampal CA1 and CA3 fields. Whereas these recordings demonstrated a tight correlation between the unit recordings and the spike component of the SWD in the cortex and thalamus, no correlation was detected in the hippocampus. Another demonstration of the lack of hippocampal activation during absence seizures induced by GABA is the expression of α1 and α4 subunit mRNA in thalamic relay nuclei but not in the hippocampus (37). Patterns of identifiable spike-wave components were recorded from the human hippocampus (38) during temporal lobe epilepsy but such discharges were taken as the effect of rhythmic patterns generated within the hippocampus but without any relation to SWD epilepsy (39).

In kainate-treated rats, a protocol that induces epilepsy, CA1 pyramidal cells and putative inhibitory neurons in the stratum oriens were reduced within eight days after kainate administration and axon branching of CA1 pyramidal cells was increased several weeks after kainate treatment (24). In rats submitted to pilocarpine administration interneurons in CA1, amygdala and piriform cortex have been shown to be primarily lesioned; repeated seizures seem to increase the number of inhibitory interneurons that are destroyed, thus facilitating the pathological excitation that results in epileptic seizures, and pilocarpine also damages cortical neurons one day after being injected in rats (24). It is very interesting and relevant that when domoic acid is injected in normal rats, it causes immediate SE, which may last up to 24 h, but if injected in spontaneously epileptic rats SE is either short-lasting or even absent (40).

In the laboratory of one of us, a strain of rats (FMUSP-rats) with spontaneous absence-like seizures was found (41,42), in which SWD are profuse in the hippocampus and other subcortical nuclei. In these rats the absence epilepsy is spontaneous (probably genetic) and thus does not involve a drug-induced lesion of the hippocampus. The occurrence of SWD in the FMUSP-rats is not compatible with the statement that in the hippocampus such discharges never occur in spontaneously epileptic rats. It is interesting that, despite the lack of electrophysiological
epileptogenic potentials in the hippocampus of another genetically epileptic absence strain of rats, a marked increase in local glucose utilization in the dorsal hippocampus (+36%), in the ventral hippocampus (+37%) and in the dentate gyrus has been consistently observed, in addition to increased metabolism in several other central structures (43).

The data cited above reveal that there is a serious discrepancy among several studies regarding the presence of SWD in the hippocampus. Several researchers adhered to the dogmatic conclusion that the hippocampus is not involved in the so-called absence models, whereas a few investigators disagree with this position. Unless some experiments have not been correctly performed, which is unlikely, it should be accepted that in some species or strains the hippocampus is involved in the production of SWD and absence seizures, whereas in others it is not.

Lesioning induced by pilocarpine is only partially understood. It does probably involve many regions of the central nervous system that have not yet been investigated, a fact that limits reasoning about which central areas are in fact affected and how some functional epileptic manifestations can be explained. For instance, pilocarpine-induced SE in early life significantly changes the predominance of movements exhibited during paradoxical sleep (20). Eye movements, which in normal rats occur only during 13% of the time, last 42.8% of the time in pilocarpine-induced epilepsy, whereas rostrum movements, which occur during 48% of the time in normal rats (Valle AC and Timo- Iaria C, unpublished results), decrease to only 8.1% in the epileptic animals (20). Since paradoxical sleep depends on pontine structures, impairment of such structures is likely to occur in epileptic rats.

Jerks do occur in about 30% of children with absence attacks (2), which in some patients arise from a diffuse epileptic encephalopathy, such as Lennox-Gastaut syndrome. Thus, it is likely that the seizures provoked by pilocarpine mimic this syndrome, since absence attacks and jerks and clonic movements did occur consistently in the rats studied here.

Absence seizures have been considered to be generated in thalamic circuits, possibly arising from aberrant thalamocortical circuits (30), a hypothesis that is consistent with the centrencephalic model proposed by Penfield and Jasper (44) to explain the loss of consciousness during epileptic seizures. It is remarkable that, although their morphol- ogy is very similar, the frequency of oscillation of the spike-wave complexes in humans (about 3 Hz) and rodents (about 8 Hz) is quite different. If the hypothesis that generalized absence seizures are a perturbation of neocortical rhythmicity in favor of the rhythmic burst firing mode is correct, this difference may be attributed to the specificity of frequency patterns in the nervous system of organisms occupying different phylogenetic positions. A good example of such specificity is that whereas desynchronization is characteristic of arousal and desynchronized (paradoxical) sleep in most cortical areas in man and other primates, in rats and other rodents it is present almost exclusively in the frontal cortex, while theta waves characterize both states of the wakefulness-sleep cycle (45). It is certainly not a coincidence that SWD occur within the frequency domain of theta waves (7 to 10 Hz). Probably, the oscillatory frequency of theta waves is related to SWD frequency because some circuits that give origin to both phenomena are the same or are influenced by circuits in which theta waves are present.

The prompt and enduring effect of ethosuximide administration in blocking the SWD in pilocarpine-induced seizures points to a high similarity between such fits in our rats and in absence seizures of genetic origin.

A relevant issue in the present study is that in two rats SWD occurred during episodes of paradoxical sleep. Epileptic absence is clearly related to interruption of the
conscious process, and dreaming activity, which is a conscious activity, occurs during paradoxical sleep. This is a very interesting and relevant phenomenon that remains to be studied in depth because it may bring information about consciousness itself. Petit mal seizures are well characterized by the sudden block of consciousness and total arrest of responses to sensory stimulation. If such a drastic effect is concomitant with a real absence of mental activity is not known. It may be only an interruption of the connection between the sensory channels and the conscious processing of information, or the absence of memory consolidation, which does not allow the patient to recall anything that occurred during the seizure. If this is the case, the occurrence of dreaming activity in rats during the epileptic seizure, as we found here, is not surprising. Anyhow, this interesting problem requires further advanced experimental investigation.

References


40. Alves RSC (1999). Status epilepticus in rats induced by domoic acid. Doctoral thesis. School of Medicine, University of São Paulo, São Paulo, SP, Brazil.


