Developmental Brain Research, 37 (1987) 43–58
Elsevier

BRD 50633

The susceptibility of rats to pilocarpine-induced seizures is age-dependent

Esper A. Cavalheiro[1], Delrio F. Silva[1], Waldemar A. Turski[2], Lineu S. Calderazzo-Filho[1], Zuner A. Bortolotto[1] and Lechoslaw Turski[2,*]

[1]*Laboratorio de Neurologia Experimental, Departamento de Neurologia e Neurocirurgia, Escola Paulista de Medicina, Sao Paulo (Brazil) and* [2]*Department of Pharmacology, Medical School, Lublin (Poland)*

(Accepted 28 April 1987)

Key words: Pilocarpine; Maturation; Seizure; Brain damage; Development; Rat

Behavioral, electroencephalographic and morphological changes induced by systemic administration of pilocarpine hydrochloride were studied in 3–90-day-old rats. Pilocarpine, 100, 200 and 380 mg/kg, presented a characteristic array of behavioral patterns in developing rats. Hyper- or hypoactivity, tremor, loss of postural control, scratching, head bobbing and myoclonic movements of the limbs dominated the behavior in 3–9-day-old rats. No overt motor seizures were observed in this age group. More intense behavioral signs evolving in some animals to limbic seizures and status epilepticus occurred when pilocarpine was administered in 12-day-old-rats. The electrographic activity in these animals progressed from low voltage spiking registered concurrently in the hippocampus and cortex during the first week of life into localized epileptic activity in the hippocampus, which spread to cortical recordings during the second week of life. No morphological alterations were detected in the brains of 3–12-day-old rats subjected to the action of pilocarpine, 100–380 mg/kg. The adult pattern of behavioral and electroencephalographic sequelae after pilocarpine was encountered in 15–21-day-old rats. Akinesia, tremor and head bobbing progressed in 15–21-day-old rats given pilocarpine, 100–380 mg/kg, to motor limbic seizures and status epilepticus. The lethal toxicity of pilocarpine reached 50% during the third week of life. This increased susceptibility to the convulsant action of pilocarpine was characterized by a shortened latency for behavioral and electrographic signs, and an increased severity of seizures relative to older and younger rats. In 15–21-day-old rats subjected to pilocarpine-induced convulsions high voltage fast activity superposed over hippocampal θ-rhythm, progressed into high voltage spiking and spread to cortical records. The electrographic activity became well synchronized and then developed into seizures and status epilepticus. Morphological analysis of frontal forebrain sections in 15–21-day-old rats which underwent status epilepticus after pilocarpine revealed no damage or an attenuated pattern of damage. In 15–21-day-old rats which presented epilepsy-related brain damage, morphological breakdown was seen in the hippocampus, amygdala, olfactory cortex, neocortex and certain thalamic nuclei. No damage was detected in the substantia nigra and lateral thalamic nucleus. An adult pattern of the damage to the brain, in terms of extent and topography, was present in 4–5-week-old rats. The increased susceptibility to the convulsant action of pilocarpine observed during the third week of life gradually decreased with age and reached the mature level in 35–60-day-old rats. The different sensitivity of developing rats to the convulsant action of pilocarpine may be related to the immaturity of neuronal networks in the brain engaged in the generation and spread of seizure activity.

INTRODUCTION

'Psychomotor' or temporal lobe epilepsy is the most common form of epilepsy in adult man[19,30,44]. This form of epilepsy is seen in children only after the age of 3 years[19]. The clinical experience has until recently left some uncertainty as to whether spread of seizures, and extent and topography of the seizure-related cell death in immature human brain resembles that commonly seen in adults suffering from complex partial seizures[19,30].

The pilocarpine model of epilepsy offers an attractive feature in that intractable seizures and status epilepticus can be induced rapidly following pilocarpine treatment. In addition, spontaneous motor seizures continue to be observed several weeks after treat-

* Present address: Department of Neuropsychopharmacology, Schering AG, P.O. Box 650133, Sellerstr. 6-8, D-1000 Berlin 65, F.R.G.

Correspondence: E.A. Cavalheiro, Laboratorio de Neurologia Experimental, Departamento de Neurologia e Neurocirurgia, Escola Paulista de Medicina, R. Botucatu 862, CEP 04023 Sao Paulo, SP, Brazil.

ment[57,59–61]. This model of seizures resembles the functional impact of human temporal lobe epilepsy in that rats and mice undergoing treatment with pilocarpine display morphological alterations in the brain comparable in many aspects to those known from human pathology[30,57,60,61]. This model of seizures has been successfully utilized to predict anticonvulsant action of drugs in mature rodents[55,56,59] and to select drugs active against complex partial seizures[59].

It is not firmly established whether seizures and seizure-related brain damage induced by pilocarpine in adult rats differ from those produced by the drug during the development. The present study was therefore undertaken to complement previous investigations in adult rats by comparing behavioral, electroencephalographic (EEG) and neuropathological sequelae of seizures produced by pilocarpine in developing rats.

Some of these data have been communicated to the 5th Meeting of the International Society for Developmental Neuroscience in Chieti (Italy)[10] and to the 16th Annual Meeting of the Society for Neuroscience in Washington DC (U.S.A.)[49].

MATERIALS AND METHODS

Animals

Subjects were male and female Wistar rats aged 3–90 days, housed under environmentally controlled conditions (7.00–19.00 h lights on (light/dark cycle); 22–24 °C) and with food and water available ad libitum. The rats were bred in our laboratories. The pups were housed together with their mother in individual cages until the age chosen for study or until weaning at day 24 (the day of birth was considered as day 0). For assessment of seizure susceptibility subjects were selected randomly from a litter of the appropriate age. No rats were used for more than one injection. To avoid interlitter variations in susceptibility to seizures, different treatment groups were represented in each litter. The determination of time of behavioral and electrographic testing for different age and treatment groups was performed by means of a randomized method. During the observation session the rats were separated from their mothers, and were returned to the litter after completion of monitoring of the behavior and EEG. Behavioral and electrographic assessments took place between 8.00 and 18.00 h.

Drugs

Pilocarpine hydrochloride and methylscopolamine nitrate were purchased from Sigma, St. Louis, MO, U.S.A., freshly dissolved in saline and administered i.p. or s.c. in a volume of 0.1 ml/10 g b. wt. (immature rats) or 0.5 ml/100 g b. wt. (adult rats). Pilocarpine was administered i.p. in doses of 100, 200 or 380 mg/kg. Methylscopolamine, 1 mg/kg, was administered s.c. 30 min prior to the injection of either dose of pilocarpine.

Surgery and electrophysiological procedures

Animals less than 30 days of age were lightly anesthetized with ether and placed in a stereotaxic frame. The head of the animal was fixed between two concave acrylic pads, which were previously molded on rats of the same age to fit exactly the lateral shape of the skull. The neck and the jaw rested on a flat subaxic guidance, which could be moved to ensure the desired hight and inclination of the head. The fixation of the skull was completed by a horizontal bar resting on the nasal bones. These modifications of the stereotaxic instrument ensured adequate positioning of the skull for electrode implantation. Bipolar and monopolar electrodes (tip diameter 100 μm) were chronically implanted under stereotaxic guidance[48] in the right and left sensorimotor cortex and in the hippocampus by means of a technique which allows recording of the electrical activity in freely moving animals[11].

One or 2 h after recovery from anesthesia, baseline EEG recordings were made for 30 min and the animals were given i.p. injections of pilocarpine in doses of 100, 200 and 380 mg/kg. EEG recordings were made continuously and behavior noted for periods ranging from 4 to 6 h following the i.p. injection of pilocarpine. In some animals additional recordings were made between 10.00 and 18.00 h.

Animals at the age of 30–90 days were anesthetized with a chloral hydrate/pentobarbital mixture (0.4 ml/100 g b. wt.) and placed in a conventional stereotaxic apparatus. The chloral hydrate/pentobarbital mixture contained chloral hydrate (4.25 g; Merck, Darmstadt, F.R.G.), pentobarbital (0.97 g; Nembutal; Ceva, Neuilly-sur-Seine, France), magnesium

sulphate (2.13 g; Merck, Darmstadt, F.R.G.), propylene glycol (42.8 ml; Merck, Darmstadt, F.R.G.) and ethanol 95% (11.5 ml; Merck, Darmstadt, F.R.G.), and was made up to 100 ml with distilled water. For EEG recordings, bipolar twisted electrodes (tip diameter 100 μm, interelectrode distance 500 μm) were positioned in the dorsal hippocampus (AP 3.0–4.0; L +1.5–2.0; V +2.3–2.6)[24,48]. Surface recordings were led from screws positioned bilaterally over the occipital cortex. Three to 5 days after surgery, animals were subjected to the same methodological procedure and drug treatment as described for younger animals. In all rats, the correct location of the implanted deep electrodes was histologically confirmed using Cresyl violet-stained serial sections.

Morphological analysis

The brains were processed for morphological examination by light microscopy 1–3 (developing and adult rats) and 5–15 (adult rats) days after the administration of pilocarpine. The animals were anesthetized with an overdose of pentobarbital and perfused by the fixative containing 10% acetic acid, 10% formaldehyde and 80% methanol. The brains were allowed to fix in situ at 4 °C for 24 h, then removed and processed for paraffin embedding. Subsequently, serial sections of the entire brain were cut coronally at 10 μm, with every tenth section being mounted on a glass slide and stained with Cresyl violet or according to Fink and Heimer's technique.

RESULTS

Behavior

Pilocarpine, when administered systemically in adult rats in doses exceeding 350 mg/kg, elicits an array of persistently recurring behavioral alterations[60,61]. The akinesia, ataxic lurching, gustatory automatisms and head tremor dominate the animals' behavior 5–10 min following the injection. After 10–15 min this behavior progresses to motor limbic seizures with rearing, forelimb clonus, salivation, intense masticatory movements and falling. Motor limbic seizures commence after 20–30 min, recur every 2–5 min, and lead to status epilepticus after a delay of 50–60 min. The lethal toxicity of pilocarpine, 380 mg/kg, in adult rats is less than 30%[60,61].

Pilocarpine, 100 and 200 mg/kg, renders animals akinetic and cataleptic immediately following the injection[60,61]. Mild tremor of the head, myoclonic

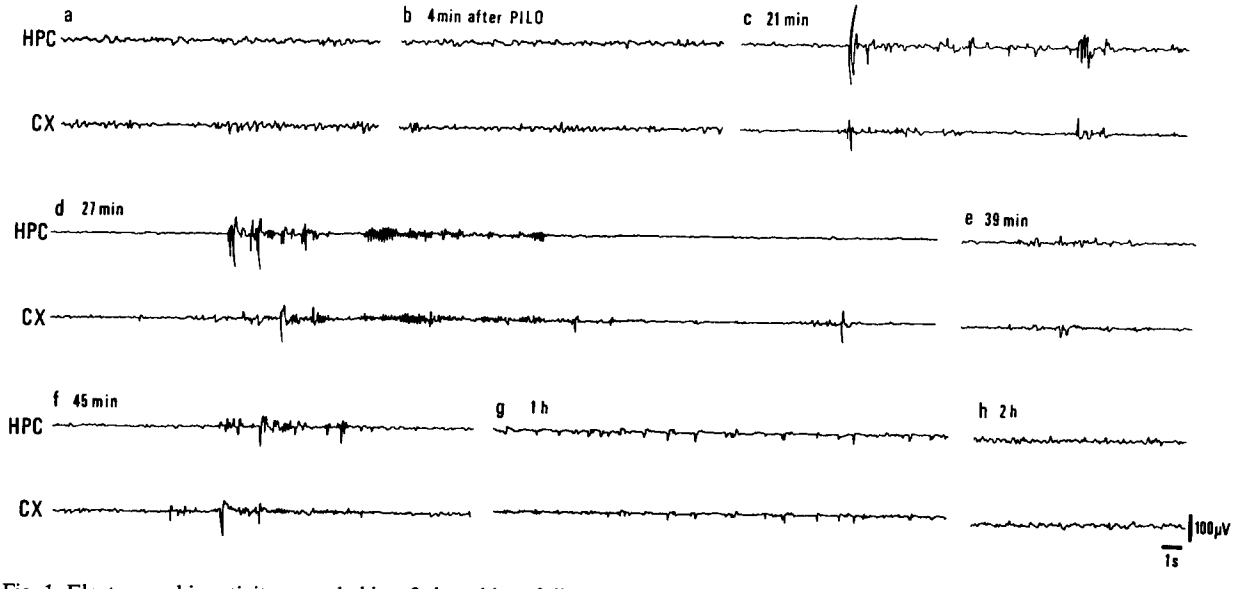


Fig. 1. Electrographic activity recorded in a 3-day-old rat following systemic administration of pilocarpine (PILO), 380 mg/kg. a: pre-drug control recordings. b: unchanged EEG patterns 4 min after the injection of PILO. c–g: continuous recordings to illustrate electrographic events 21 min–1 h after injection of PILO. Isolated spikes or polyspikes are infrequently recorded in the hippocampus and cortex; h: 2 h after the injection of PILO, the EEG in the hippocampus and cortex returns to the pre-drug pattern (a). HPC, hippocampus; CX, cortex.

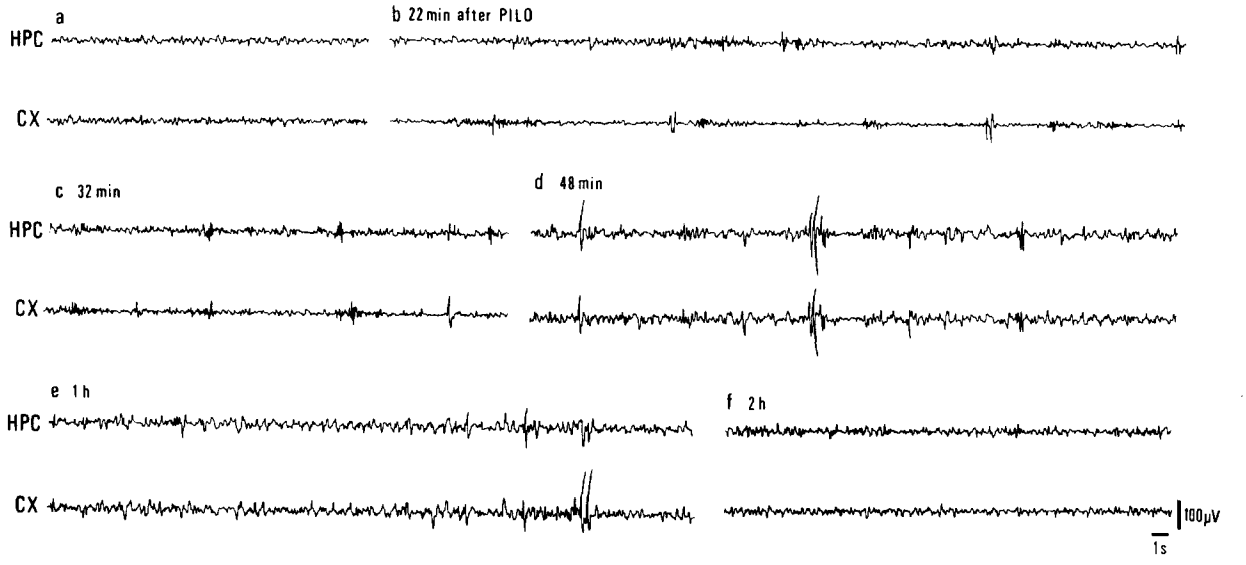


Fig. 2. Electrographic activity registered after systemic administration of pilocarpine (PILO), 380 mg/kg, in a 6-day-old rat. a: pre-drug control recordings. b–e: continuous recordings to illustrate electrographic changes 22 min–1 h after injection of PILO. Isolated spikes and groups of polyspikes are registered in hippocampus and cortex. The background activity is more complex (d,e). f: 2 h after the injection of PILO, the EEG in the hippocampus and cortex returns to the pre-drug pattern (a). HPC, hippocampus; CX, cortex.

movements of the forelimbs, head bobbing, scratching and teeth chattering comprise the behavior for up to 1–2 h postinjection. This activity subsides within the following 30–45 min and behavior is indistinguishable from that seen in saline-treated control rats. The rats treated with pilocarpine, 100 and 200 mg/kg, develop neither generalized seizures nor status epilepticus[60,61].

In 3–6-day-old rats, pilocarpine, 100 (n = 15), 200 (n = 16) and 380 mg/kg (n = 15), induced behavioral

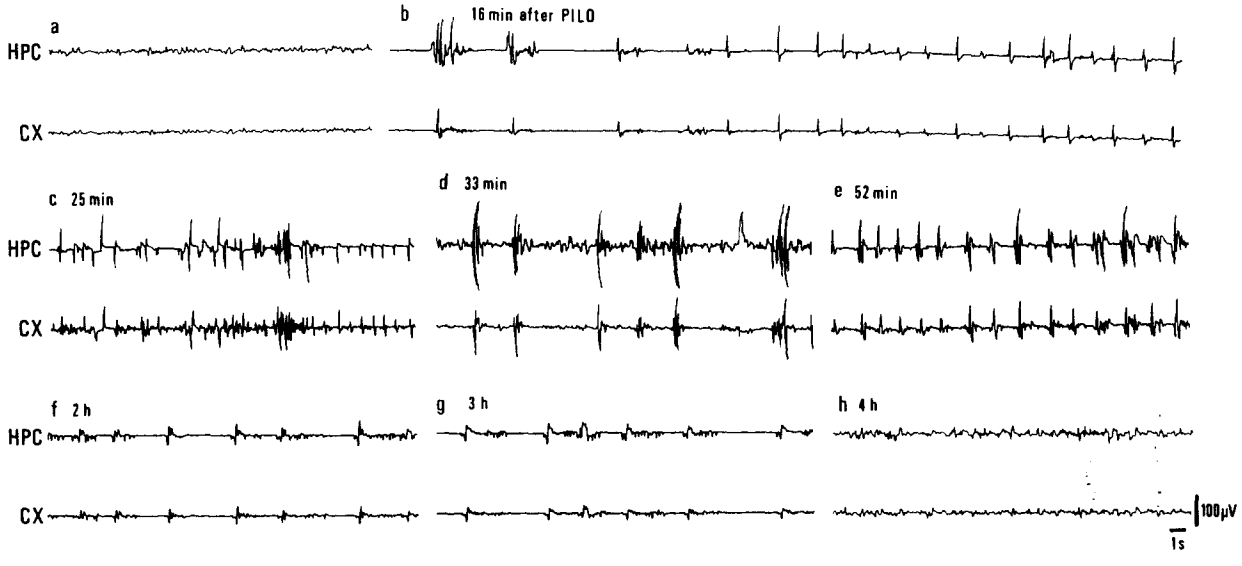


Fig. 3. Recordings to illustrate the effect of systemic pilocarpine (PILO), 380 mg/kg in a 10-day-old rat. a: pre-drug control recordings. b–g: electrographic correlates 16 min–3 h after injection of PILO. Note a concurrent activation of the hippocampus and cortex with spikes and polyspikes. h: 4 h after the injection of PILO, the EEG in the hippocampus and cortex returns to the pre-drug pattern (a). HPC, hippocampus; CX, cortex.

TABLE I

The incidence of motor limbic seizures and status epilepticus, the lethal toxicity, and the latency for the first electrographic (EEG) changes recorded from the hippocampus or cortex following the administration of pilocarpine, 100–380 mg/kg, in developing rats

Animals in the EEG group are also included in the analysis of behavioral effects, e.g. row 1 0/90 equals 90 animals observed behaviorally 45 of which were also monitored electrographically.

Age (days)	*Motor limbic seizures*	*Status epilepticus*	*Lethal toxicity*	*First EEG changes (min)*
3–6	0/90	0/90	0/90	25.3 ± 6.9 (n = 45)
7–12	24/94	7/94	0/94	14.0 ± 5.7 (n = 44)
15–21	87/121	76/121	58/121	4.2 ± 2.2 (n = 41)
24–60	38/50	23/50	12/50	12.4 ± 4.8 (n = 16)
90	10/24	10/24	2/24	11.0 ± 5.3 (n = 10)

changes which included hyperactivity followed by hypoactivity, tremor of the head or whole body tremor, loss of righting reflex and scratching movements. These behaviors appeared immediately following the injection of pilocarpine and lasted for up to 90 min. No clear-cut relationship between the dose of pilocarpine and behavioral changes was detected in 3–6-day-old rats.

The pattern of behavioral alterations induced by pilocarpine in 7–12-day-old rats consisted of more complex behavioral alterations and followed a typical dose–response relationship. The animals subjected to pilocarpine, 100 mg/kg (n = 12), presented continuous scratching, body tremor and masticatory automatisms lasting for 1–2 h. The dose of 200 mg/kg (n = 18) induced additionally clonic movements of

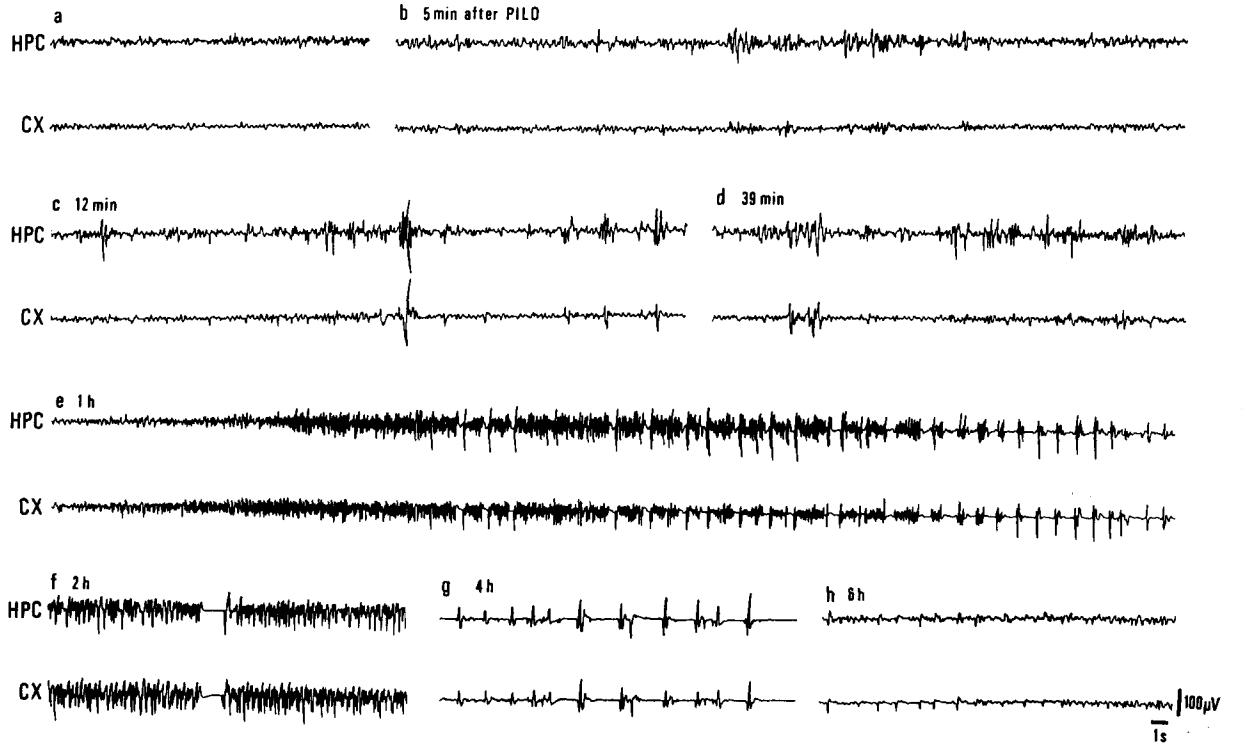


Fig. 4. Recordings to demonstrate the effect of systemic pilocarpine (PILO), 380 mg/kg, in a 12-day-old rat. a: pre-drug control recordings. b: electrographic correlates 5 min after PILO administration characterized by significant background activity in the hippocampus and isolated spikes. c,d: spiking activity spreads to cortical recordings 10–30 min following PILO. e: first electrographic seizure registered in both recordings 1 h after injection of PILO. The build up of electrical activity is highly synchronized in both recordings. f: electrographic activity registered 2 h after the injection of PILO during status epilepticus. g: progressive normalization of the electrographic activity observed 4 h after the injection of PILO. h: isolated spikes and normal background activity are registered 6 h post-injection. HPC, hippocampus; CX, cortex.

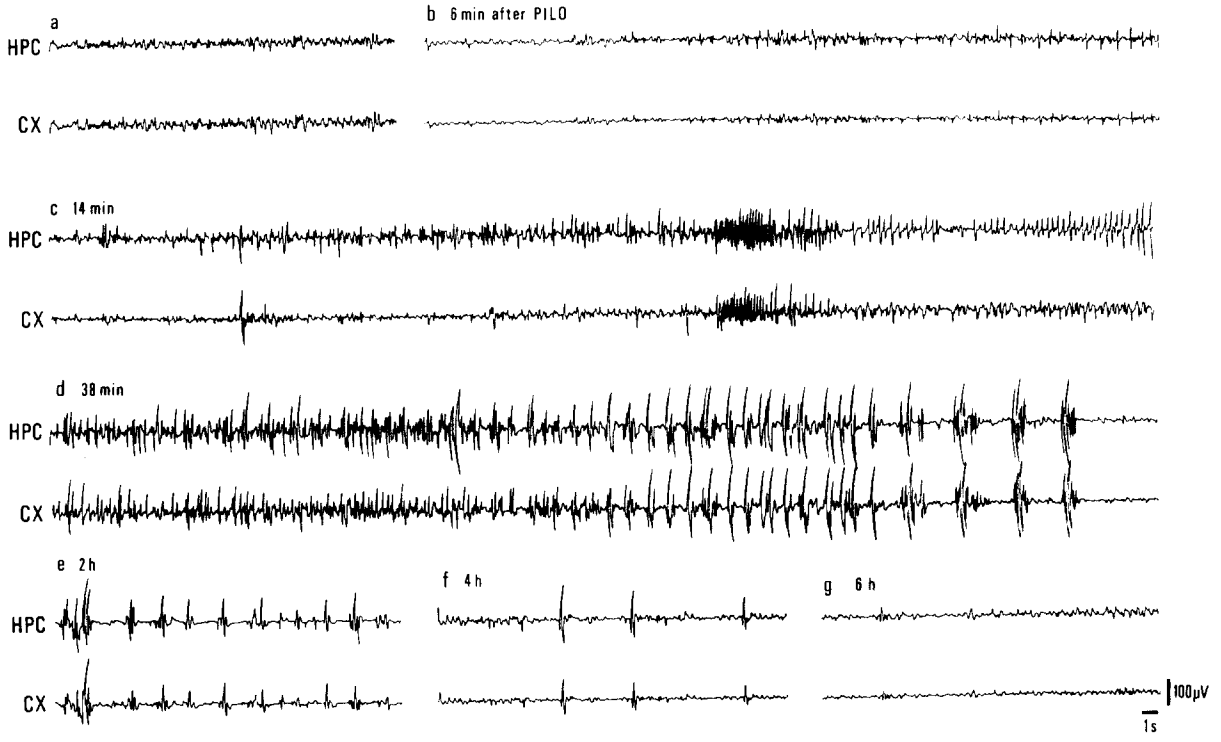


Fig. 5. Electrographic recordings demonstrating the effect of systemic administration of pilocarpine (PILO), 100 mg/kg, in a 15-day-old rat. a: pre-drug control recordings. b: electrographic correlates 6 min after injection of PILO. Isolated spikes initially observed in the hippocampal recording rapidly spread to cortical lead. c: first electrographic seizure registered 14 min after injection of PILO. The seizure activity is initially recorded in the hippocampus. d: the electrographic seizure registered 38 min following the administration of PILO. The seizure activity is highly synchronized in hippocampal and cortical recordings. e,f: progressive normalization of the electrographic activity observed 2–4 h after the injection of PILO. g: 6 h after the injection of PILO, the EEG in the hippocampus and cortex returns to the pre-drug pattern (a). HPC, hippocampus; CX, cortex.

the forelimbs, head bobbing and loss of postural control. In animals receiving pilocarpine, 380 mg/kg (n = 20), these signs were more pronounced and frequently interrupted by periods of immobility. Motor seizures were observed in 11 out of 16 animals at the age of 11–12 days. The seizures lasted up to 60 s and were characterized by orofacial automatisms, salivation, clonic movements of the paws, rearing and falling. Four rats (4/16) evolved to status epilepticus.

In 15–21-day-old rats, the initial phase of motor alterations commenced 3–5 min following the administration of pilocarpine regardless of the dose. This period was characterized by scratching movements, body tremor and masticatory automatisms. This pattern of behavioral alterations lasted for up to 2 h and was seen in 60% of rats subjected to pilocarpine, 100 mg/kg (18/30), and in 30% of rats receiving 200 mg/kg (7/24). The remaining animals from both dosage groups and rats receiving 380 mg/kg (n = 26) of pilocarpine presented, with a delay of 10–15 min wet shakes, clonic movements of the forepaws, rearing and running which culminated in generalized seizures. These seizures, which may be considered similar to those observed in adult animals, recurred every 3–5 min and evolved into typical status epilepticus (48/55). Thirty-eight out of 48 rats which developed status epilepticus following pilocarpine, 100–380 mg/kg, died in the course of seizures (38/48). This finding suggests, that the rats at the age of 15–21 days were more sensitive to the convulsant action of pilocarpine relative to adult and younger animals (3–12 days old). The latency for the occurrence of initial motor signs was shorter and lower doses of pilocarpine, 100 and 200 mg/kg, could induce severe seizures or status epilepticus.

In 24–60-day-old rats (n = 34), pilocarpine, 100–380 mg/kg, induced behavioral changes similar to those observed in adult rats (n = 14)[60,61].

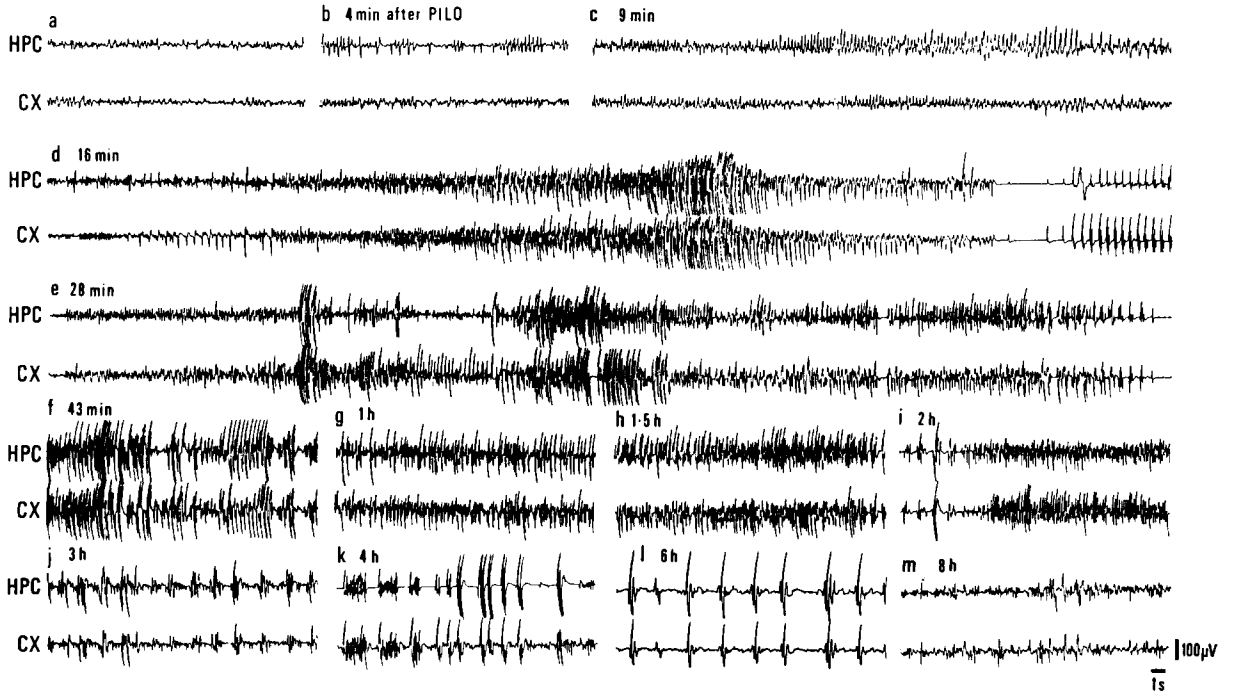


Fig. 6. Recordings to illustrate the effect of systemic administration of pilocarpine (PILO), 200 mg/kg, in a 18-day-old rat. a: pre-drug control recordings. b,c: electrographic correlates 4–9 min after injection of PILO. Hippocampal background activity is replaced by high-voltage spiking which sometimes spreads to cortical recordings (c). d: the first electrographic seizure registered in both recordings 16 min after injection of PILO. The build up of electrical activity is highly synchronized in both recordings. e: the ictal periods progressively grow in duration and complexity between 20 and 40 min post-injection and finally result in status epilepticus (f). f–i: electrographic activity registered 43 min–2 h after the injection of PILO during status epilepticus. j–l: progressive normalization of the electrographic activity observed 3–6 h after the injection of PILO. m: isolated spikes highly synchronized in both recordings are registered up to 8 h post-injection. HPC, hippocampus; CX, cortex.

Electroencephalography

The pattern of electrographic changes induced by pilocarpine, 100 (n = 17), 200 (n = 12) and 380 mg/kg (n = 15), in 3–6-day-old rats included flattening of the background activity in the hippocampus and cortex. Spikes of low amplitude and low frequency were registered concurrently in both recordings (Fig. 1c–g and Fig. 2b–e). The spikes appeared sometimes in sequences of 5–15 s, which correlated well with behavioral arrest. This type of electrographic activity started after a long delay (25.3 ± 6.9 min; Table I) following pilocarpine and lasted up to 1 h. The electrographic activity progressively normalized in hippocampal and cortical recordings after 1–2 h (Fig. 1h and Fig. 2f).

In 7–10-day-old rats (n = 22), the electrographic changes induced by pilocarpine were similar to those registered in animals during the first week of life. However, they had a more complex morphology (Fig. 3b–g) and a clear-cut dose–response relationship was now evident. The epileptic activity, which appeared simultaneously in hippocampus and cortex, comprised of spikes or polyspikes. Infrequently, spike and wave-like complexes were registered (Fig. 3e). These changes started 17.4 ± 5.2 min after the administration of pilocarpine, recurred in bursts of 10–30 s, and lasted up to 3 h. In 11–12-day-old rats, pilocarpine, 100 and 200 mg/kg (n = 14), induced initially electrographic changes in the hippocampus (latency of 8.2 ± 3.4 min) consisting of spikes and polyspikes, which sometimes spread to cortical recordings (Fig. 4b–d). In 9 of 14 rats receiving these doses of pilocarpine (9/14), the electrographic changes lasted up to 1–2 h and were followed by a progressive normalization of the activity in the EEG. In the remaining 5 animals treated with 100 and 200 mg/kg (5/14) and in those receiving 380 mg/kg of pilocarpine (8/8), the electrographic activity evolved, for the first

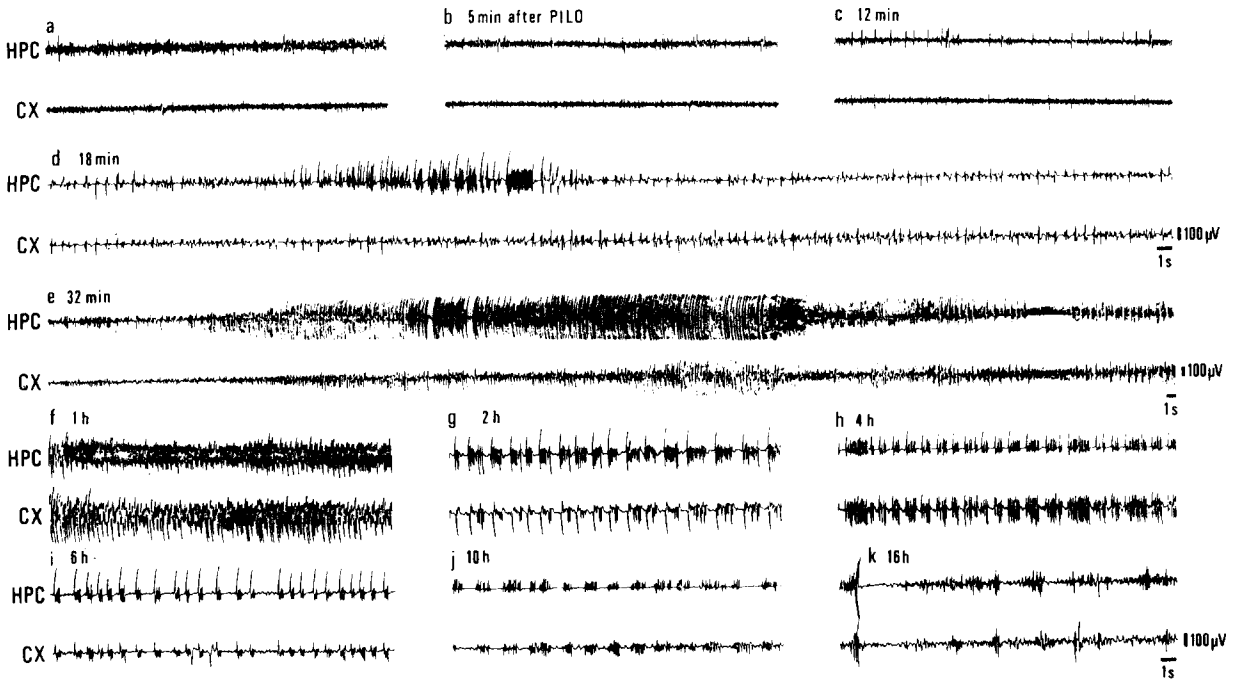


Fig. 7. The build up of seizure activity produced by pilocarpine (PILO), 380 mg/kg, in a 30-day-old rat. a: pre-drug control recordings. b: significant θ-rhythm in the hippocampus and fast activity in cortex registered 5 min after injection of PILO. c,d: high-voltage spiking activity superposes over hippocampal θ-rhythm 12 min after injection of PILO and progresses into polyspikes with cortical involvement (d) 6 min later. e: electrographic seizure registered 32 min after injection of PILO. High-voltage fast activity and prominent spiking precede the evolution of the seizure in the hippocampus. Cortical recordings display less pronounced changes. f–h: electrographic activity during status epilepticus 1-4 h after PILO. i,j: progressive normalization of the electrographic activity within 6–10 h post-injection. k: by 16 h, the EEG progressively returns to the pre-drug pattern (a), although short lasting bursts of polyspikes are registered in both recordings. HPC, hippocampus; CX, cortex.

time during the postnatal period, to a well-organized pattern of electrographic seizures (Fig. 4e). The seizure activity in these animals consisted of fast, high-voltage spiking activity followed by polyspike, wave or spike and wave complexes, and lasted for up to 60–70 s. The duration and frequency of electrographic seizures progressively increased with time and finally resulted in status epilepticus in 3 rats (3/13) (Fig. 4f).

In 15–21-day-old rats, seizure activity usually started in the hippocampus after a short delay (4.2 ± 2.2 min; Table I) following the injection of pilocarpine and remained restricted to this structure in 5 out of 15 animals receiving pilocarpine, 100 mg/kg (5/15) and in one out of 11 rats treated with 200 mg/kg (1/11) (Figs. 5b and 6b,c). In the remaining animals receiving 100 or 200 mg/kg and in rats subjected to 380 mg/kg (n = 15) of pilocarpine, the epileptic activity rapidly spread to cortical recordings (Fig. 5c) and evolved into well-developed electrographic seizures (Figs. 5d and 6d,e). The status epilepticus (28/35) was reached more rapidly in 15–21-day-old rats (38.7 ± 5.7 min) relative to adult animals (68.1 ± 12.4 min, n = 18). The EEG displayed also different electrographic elements consisting of bursts of polyspikes (Fig. 6f–i), polyspike and wave (Fig. 6k) or spike and wave complexes (Figs. 5e,f and 6l) during the status epilepticus in these animals. In the rats, which survived status epilepticus (5/15), the electrographic activity progressively returned to a pre-drug pattern, although isolated spikes were observed for up to 8 h after the injection of pilocarpine.

In 24–60-day-old rats (n = 16), the electrographic patterns induced by different doses of pilocarpine were similar to those observed in adult animals (Figs. 7 and 8, Table I), although the latency for status epilepticus was shorter relative to adult rats.

Morphological studies

Examination of frontal forebrain sections with light microscopy after application of pilocarpine, 100, 200 or 380 mg/kg, in 3–6-day-old rats (n = 12)

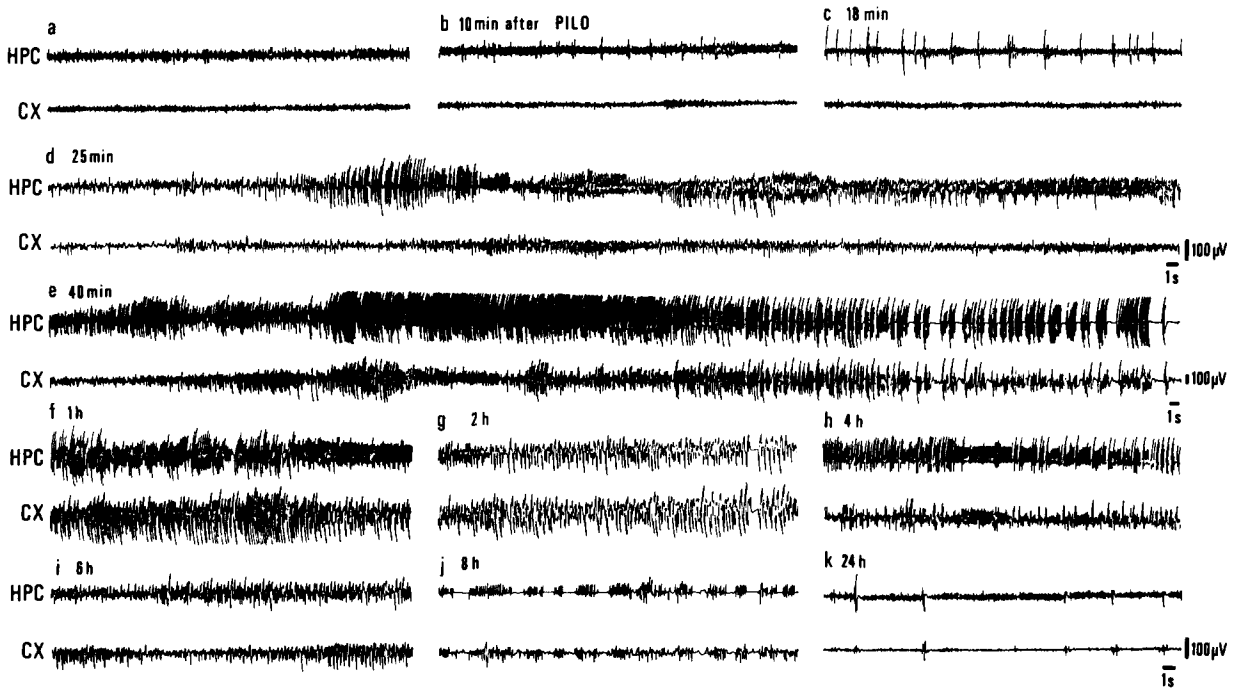


Fig. 8. Electrographic recordings illustrating the evolution of electrographic activity elicited by systemic pilocarpine (PILO), 380 mg/kg, in a 60-day-old rat. a: pre-drug control recordings. b,c: θ-rhythm and fast activity prevail in the hippocampal recordings and cortex 10–18 min after the injection of PILO. Spiking activity alternates with and superposes over hippocampal θ-rhythm. d: the first electrographic seizure registered 25 min after injection of PILO. High-voltage fast activity and prominent spiking occurs almost exclusively in hippocampal recordings. e: continuous recording illustrating electrographic seizure registered 40 min after injection of PILO. High-voltage spiking and bursts of polyspiking build up rapidly in an electrographic seizure. The waxing of the seizure is faster in the hippocampal recording. f–h: electrographic activity during status epilepticus 1–4 h after PILO. i,j: progressive normalization of the electrographic activity within 6–8 h post-injection. k: by 24 h, the EEG progressively returns to the pre-drug pattern (a), although isolated spikes are registered in both recordings. HPC, hippocampus; CX, cortex.

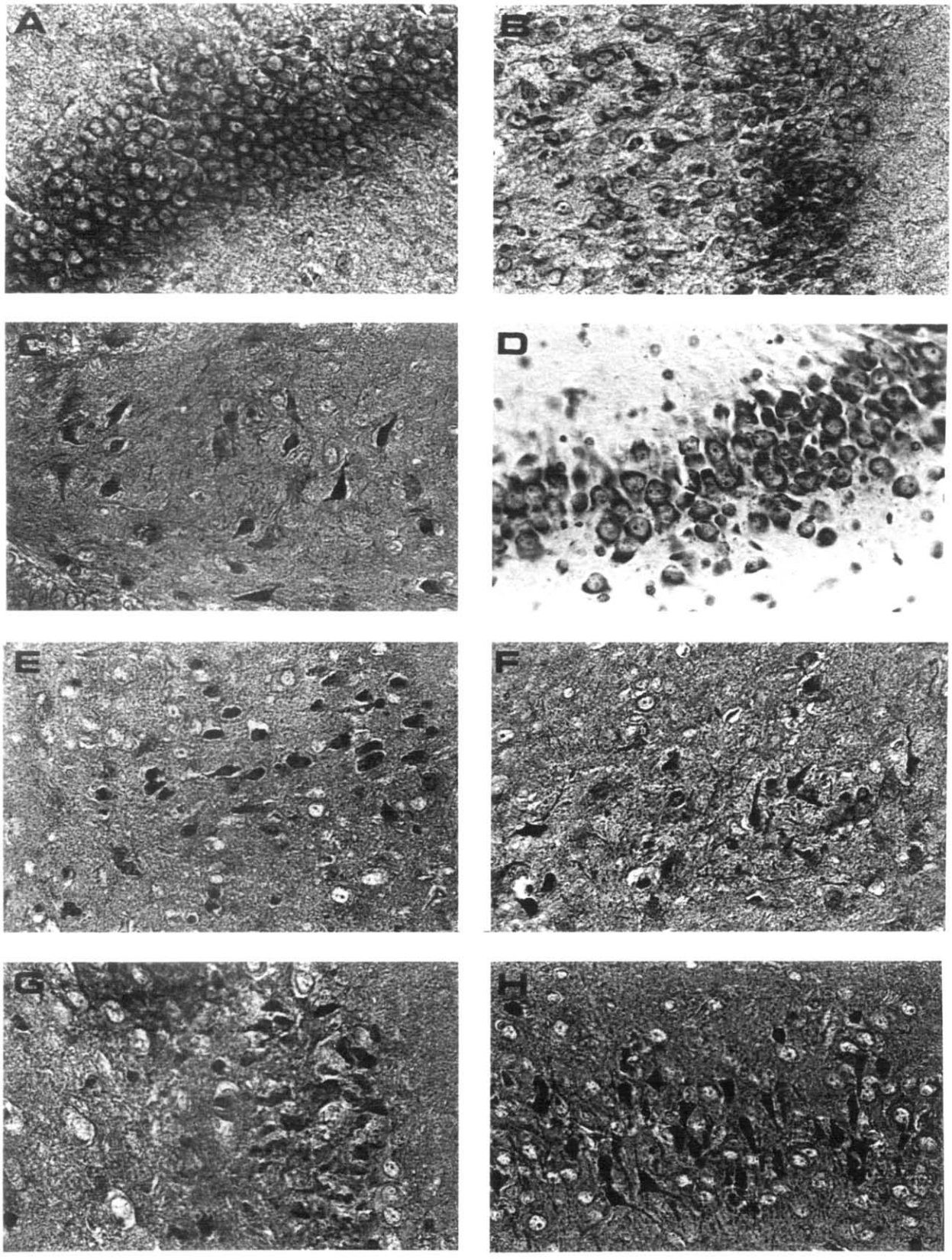
revealed no morphological alterations throughout the entire brain (Fig. 9A,B).

In 7–10-day-old rats subjected to the convulsant action of pilocarpine, 100–380 mg/kg (n = 8), there were also no apparent morphological alterations in the brain.

Seizures produced by pilocarpine, 100–380 mg/kg, may result in the epilepsy-related brain damage in 11–21-day-old rats (5/14); however, the presence of the damage is not an invariant consequence of convulsions at this age. In 11–21-day-old rats, which developed brain damage following seizures induced by pilocarpine, the extent of the damage was less and its topography different relative to that observed in adult rats[60,61].

In the 11–21-day-old group, 14 out of 52 rats developed and survived status epilepticus following pilocarpine (100–380 mg/kg); 5 of these rats showed evidence of brain damage. This damage was seen as neuronal loss in the hippocampus (Fig. 6C,D), thalamus (Fig. 9E,F), olfactory cortex (Fig. 9G,H), septum (Fig. 9I), hypothalamus (Fig. 9J), amygdala (Fig. 9K) and neocortex. The topography of the damage in this age group deserves particular attention with emphasis placed on the distribution of morphological alterations in the hippocampus, thalamus and substantia nigra. In rats at the age of 11–12 and 15 days, the damage to the dorsal hippocampus extended into subfields CA_4 (Fig. 9C) and CA_1. No morphological alterations were encountered in the ventral hippocampus. The damage to the ventral hippocampus was detected after 18–21 days of age. No morphological alterations were seen in the dentate gyrus, while the lateral septum was severely damaged.

Within the thalamus, damage occurred in the dorsomedial (Fig. 9E), reuniens (Fig. 9F), rhomboideus, paratenial, paraventricular, anterior and posterior


A
B
C
D
E
F
G
H

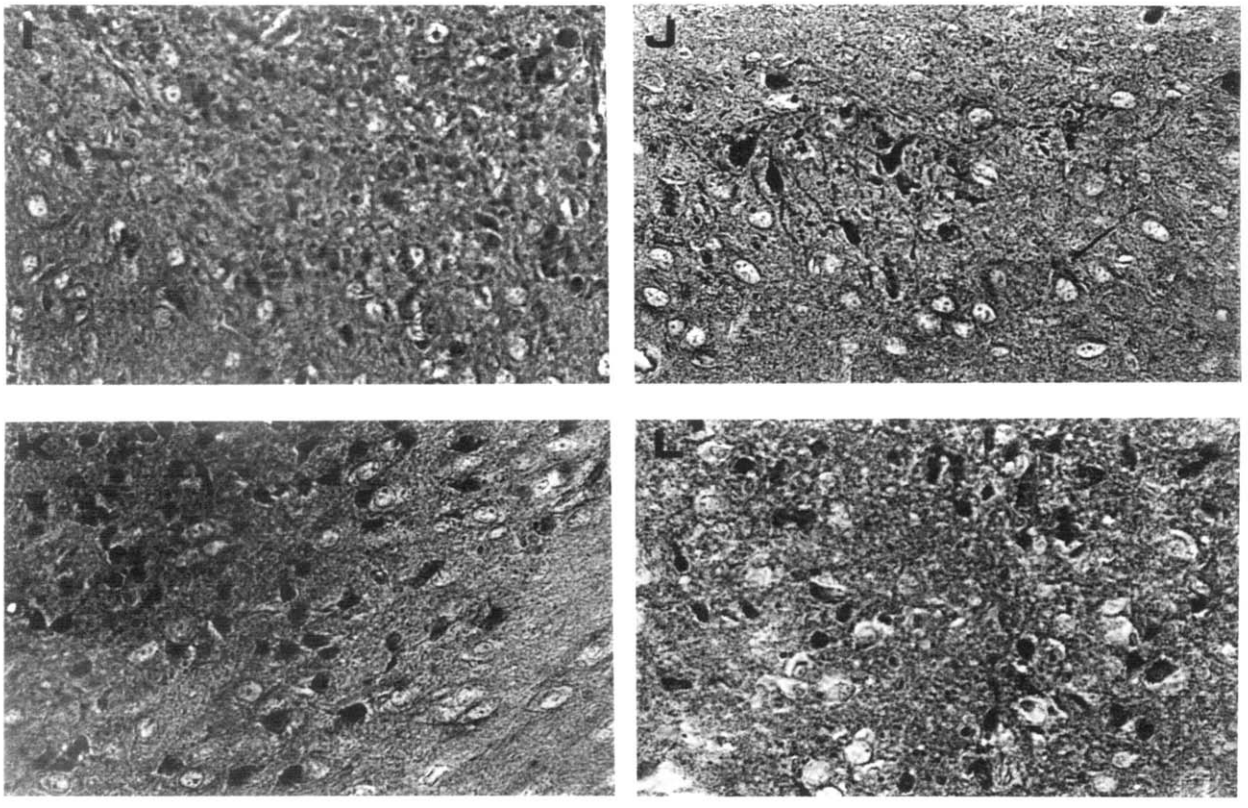


Fig. 9. Neuropathological sequelae of seizures produced by pilocarpine in developing rats. A: photomicrograph demonstrating normal cytoarchitecture of the CA_1 subfield of the dorsal hippocampus in a 3-day-old rat subjected to the action of pilocarpine, 380 mg/kg. Survival time: 48 h. Fink–Heimer stain. B: no morphological changes are discernible throughout the entire pyriform cortex in a 4-day-old rat after the treatment with pilocarpine, 380 mg/kg. Survival time: 48 h. Fink–Heimer stain. C: shrunken and darkened neurons in the CA_4 subfield of the dorsal hippocampus in a 16-day-old rat subjected to the convulsant action of pilocarpine, 200 mg/kg. No morphological alterations were seen in the dentate gyrus. Survival time: 72 h. Fink–Heimer stain. D: pathological alterations in the hippocampal CA_3 subfield after systemic administration of pilocarpine, 200 mg/kg, in an 18-day-old rat. Note cell loss, narrowings and darkening of pyramidal neurons. Survival time: 72 h. Cresyl violet stain. E: severely shrunken cells in the mediodorsal thalamic nucleus in a 21-day-old rat after seizures produced by pilocarpine, 200 mg/kg. Survival time: 72 h. Fink–Heimer stain. F: morphological structure of the reuniens thalamic nucleus after seizures induced by pilocarpine, 200 mg/kg, in a 20-day-old rat. The tissue became disrupted and most neurons are shrunken and darkened. Survival time: 72 h. Fink–Heimer stain. G: disruption of the cytoarchitecture of the pyriform cortex in a 20-day-old rat treated with pilocarpine, 200 mg/kg. Severely shrunken and darkened cells are visible throughout the entire region. Survival time: 72 h. Fink–Heimer stain. H: shrunken, argyrophylic neurons in the infralimbic cortex in a 25-day-old rat following seizures induced by pilocarpine, 380 mg/kg. Survival time: 4 days. Fink–Heimer stain. I: extensive morphological breakdown in the lateral septum in a 24-day-old rat after treatment with pilocarpine, 200 mg/kg. Survival time: 72 h. Fink–Heimer stain. J: neuronal degeneration in the hypothalamus detected in a 21-day-old rat after seizures induced by pilocarpine, 200 mg/kg. Survival time: 72 h. Fink–Heimer stain. K: breakdown of morphological structure of the cortical amygdaloid nucleus in a 16-day-old rat following seizures elicited by pilocarpine, 200 mg/kg. Survival time: 72 h. Fink–Heimer stain. L: disruption of the cytoarchitecture and neuronal loss in the cortical amygdaloid nucleus after seizures induced by pilocarpine, 380 mg/kg, in a 28-day-old rat. The majority of neurons became shrunken and darkened, and the neuropil appears swollen and edematous. Survival time: 5 days. Fink–Heimer stain. A–L: ×196.

nuclei. No neuronal degeneration was found in the lateral thalamic nuclei.

No morphological alterations were seen in the substantia nigra.

The amygdala and olfactory system underwent breakdown of the morphological structure. Neuronal degeneration in the amygdala principally affected cortical (Fig. 9K), lateral and the basal nuclear complex. Severely shrunken and darkened neurons were found in the pyriform cortex (Fig. 9G) and entorhinal cortex. Within the neocortex the damage was limited to areas located dorsally to the rhinal sulcus. Little damage was detected in the hypothalamus (Fig. 9J).

In rats 24–60 days of age ($n = 8$) the pattern of the

brain damage following seizures induced by pilocarpine became progressively similar to that detected in adult animals (Fig. 9L)[60]. The substantia nigra and lateral thalamic nucleus became sensitive to the damaging action of pilocarpine between the 4th and 5th week after birth.

The damaged regions of the brain showed a total destruction of morphological structures with extensive neuronal loss resembling the adult pattern after 28–42 days of age (Fig. 9L). The extent of the damage for up to 30 days of age was limited to severe shrinkage and darkening of neurons. The neuronal tissue in the brains taken from younger animals remained relatively resistant to swelling, vacuolization and edema (Fig. 9A–K).

DISCUSSION

The present study provides evidence that in rats neuronal networks necessary for the convulsant action of pilocarpine, a cholinergic muscarinic agonist, do not become functional until the third week of life. These findings most likely reflect immaturity of the cholinergic system in the rat brain, since biochemical and morphological analyses of the developing brain indicate that cholinergic neurons do not attain functional maturity until 18–20 days after birth[6,13,14,27,29,36,45,50].

These results are consistent with the observations of akinesia and catalepsy in developing rats in response to pilocarpine reported by Baez et al.[2] and Campbell et al.[8]. Pilocarpine has been found to induce neither akinesia nor catalepsy in rats before 15–20 day of age[2]. Similarly, more recent observations have reported locomotor stimulatory effects of scopolamine, a cholinergic muscarinic antagonist, only in animals aged at least 20 days[8].

The resistance of rats to the convulsant action of pilocarpine during the first two weeks of life may be a consequence of the immaturity of cholinergic neuronal circuits and not of an immature blood–brain barrier[7]. This suggestion is supported by experiments involving the co-administration of pilocarpine and methylscopolamine, a quaternary muscarinic antagonist which does not readily enter the brain and was used to prevent peripheral side-effects. In these experiments animals 3–12 days old receiving high doses of pilocarpine showed locomotor hyperactivity, tail rigidity and tremor.

Similarly, one can conclude that the relative insensitivity of 3–12-day-old rats to convulsant action of pilocarpine as revealed by behavioral monitoring, electroencephalography and morphology is a consequence of immaturity of the cholinergic neuronal networks and not an inability of these rats to seizure[9,15,20,26,29,31,33,39,51–53,62,63,65].

The susceptibility of rats to the convulsant action of pilocarpine and lethal toxicity of the drug is dramatically increased during the third week of life. The physiological significance of this finding is unclear. However, strikingly similar results have been reported by Baez et al.[2]. These authors, while studying the cataleptogenic action of pilocarpine in developing rats, found that 15-day-old animals may develop convulsions following pilocarpine in doses which are non-convulsant in rats aged 10 or 20 days[2].

While previous clinical and experimental studies have not explicitly focused on the complexity of age-dependence of sensitivity to seizures, there is a precedence for the idea that there is a critical period of development during which seizure susceptibility is enhanced[34,37,46,62,64]. Newborn infants are resistant to febrile convulsions, children between 6 months and 5 years show a high incidence of these seizures, after which the frequency of febrile seizures decreases with age[37]. Interestingly, an afterdischarge threshold necessary for the development of seizures in kindled rats is also related to the age[20,34], being highest in 15-day-old rats, lowest in 35- and intermediate in 60–84-day-old rats[34]. Similar profiles of seizure susceptibility were observed in developing rats treated with tungstic acid[64]. Newborn rats are relatively insensitive to tungstic acid, show high sensitivity to tungstic acid-induced convulsions during the third week of life, while during further development the seizure susceptibility normalizes and gradually reaches the adult level[64].

Our findings also form an interesting parallel to developmental studies with kainic acid-induced seizures in rats[1,3,9,12]. The rat pups aged 15–18 days presented the lowest threshold for kainic acid-induced convulsions and the highest mortality, while they did not develop epilepsy-related brain damage following status epilepticus[1]. Electrographically these animals showed a sequential pattern of seizure evolution with

primary activation of the hippocampus[12]. In 33–37-day-old rats the severity of seizures induced by kainic acid was much less and mortality lower[1]. In this age group status epilepticus led to the damage of the forebrain resembling the adult pattern[1,5]. In 1–14-day-old rats, kainic acid induced neither motor limbic seizures nor status epilepticus and the brains from these rats presented no epilepsy-related damage[1,3]. In the electroencephalogram registered in these rats no changes or primary activation of the cortex were detected during the first two weeks of life[12]. An autoradiographic analysis with the use of the 2-deoxyglucose technique demonstrated a prominent metabolic activation of the substantia nigra in the course of kainic acid-induced seizures in 33–37-day- and older animals but not in 15–18-day-old pups[1,5].

The remarkable similarity between our finding on critical period of susceptibility to seizures induced by pilocarpine in rats and findings of others in the kindling model[34], tungstic acid-[64] and kainic acid-induced convulsions[1,3,12] lends credence to the inference that a global immaturity may be considered important in determining seizure susceptibility of the brain at this stage of development.

Such a conclusion could also be applicable to in vitro studies on hippocampal slices from immature rabbit, kitten and rat[46,47,54]. Schwarzkroin and Kunkel[46,47] showed that at a critical period of development (8–12 postnatal days) hippocampal tissue can generate long-lasting depolarizations spontaneously or in response to stimulation. A spontaneous seizure-like activity was also seen in hippocampal tissue from immature rabbit at an apparently critical age: the hippocampal tissue from rabbits at the age of less than 1 week or from healthy adults did not generate spontaneous depolarization events[46]. Swann and Brady[54] detected a developmental time window between postnatal days 9 and 19, during which the rat hippocampal neurons generated prolonged afterdischarges in response to penicillin. This capacity was reduced after 24–25 days of age and during the first postnatal week.

Morphological analysis of brains from developing rats subjected to high doses of pilocarpine showed a remarkable age dependence in the susceptibility of the brain to epilepsy-related cell death. No damage is seen in the brains of 3–12-day-old rats following pilocarpine treatment. The third week of postnatal life is critical for the occurrence of the epilepsy-related damage in the brain. This period of development is characterized by a rapid progress in the morphology and complexity of the electroencephalogram, and a dramatic increase in the susceptibility to seizure in response to pilocarpine. The lethal toxicity of pilocarpine during the third week after birth is also particularly high. The incidence of the damage to the brain in rats subjected to the treatment with pilocarpine is not an invariant consequence of seizures and status epilepticus during the second and third week of life. Thus, although 12–21-day-old rats usually develop status epilepticus, they typically do not present epilepsy-related damage in their brains.

Morphological analysis of brains from 12–21-day-old animals, which survived status epilepticus and developed seizure-related brain damage following the treatment with pilocarpine, presents a characteristic pattern and distribution of the damage. The topography of the damage in these rats resembles well that usually encountered in adult rats[60,62] with the exception of the lateral thalamic nucleus and substantia nigra. The damage to the lateral thalamic nucleus and the substantia nigra is not seen until the 4th–5th week of postnatal life. The extent of the damage in developing rats is considerably less relative to that observed in the brains of adult rats subjected to the convulsant action of pilocarpine. The topography and extent of the damage resemble the adult pattern after 28–42 days of age.

The finding on unexpected resistance of the lateral thalamic nucleus and substantia nigra to the brain-damaging action of pilocarpine awaits discussion. The lateral thalamic nucleus is typically destroyed following the seizures induced by pilocarpine in adult rats and mice[57,60,61]. This brain area remains intact following seizures and status epilepticus induced by systemic administration of kainic acid[5] or by focal microinjections of convulsants (bicuculline, picrotoxin, morphine, cholinomimetics, kainic acid, folic acid[4,23,41,42,58,61]) into the amygdala. The physiological relevance of these discrepancies remains unclear.

The substantia nigra undergoes extensive morphological damage in the course of intractable seizures induced in adult rats by systemic administration of pilocarpine and fluorothyl[38,61]. Microinjections of cholinomimetics, morphine and γ-aminobutyrate (GABA) antagonists into the rat amygdala may also

lead to a destruction of the substantia nigra[23,41,58,61]. The observation on the resistance of the substantia nigra to epilepsy-related damage in immature rats subjected to a high-dose treatment with pilocarpine is valid in terms of the role ascribed to this region in governing the seizure generation and spread in the mature brain[22,28,55,56]. Focal application of GABA agonists, γ-vinyl-GABA or excitatory amino acid antagonists into the substantia nigra prevents the development of seizures produced by pentylenetetrazol, bicuculline, maximal electroshock, amygdala kindling, ethanol withdrawal, fluorothyl and pilocarpine in adult rats[16,21,25,28,32,55,56].

Surprisingly, intranigral injections of the GABA agonist, muscimol, in 16–17-day-old rats enhanced their susceptibility to fluorothyl-induced seizures[32,35,40]. This apparent functional discrepancy in the control of seizure spread between the adult and immature substantia nigra reflects well the differences in the sensitivity of the substantia nigra to epilepsy-related brain damage. It is tempting to relate this difference to seemingly different stages of maturity. The major developmental shift in terms of morphological maturation occurs in the substantia nigra during the 3rd week of life[43]. The electrophysiological analysis of the functional state of the striatonigral pathway during the development shows excitatory and inhibitory responses to caudate stimulation at the age of less than 40 days and a pure inhibitory response profile thereafter[18]. The pathophysiological significance of these observations remains uncertain.

The age-related differences in the susceptibility of developing rats to seizures produced by pilocarpine reflect the complexity of seizure generation and spread in the immature brain and provide evidence for an apparent distinction between the mechanisms of epileptogenesis in the mature and developing nervous system.

ACKNOWLEDGEMENTS

This research was supported by grants from FAPESP, CNPq, FINEP and Polish Academy of Sciences.

REFERENCES

1 Albala, B.J., Moshe, S.L. and Okada, R., Kainic acid-induced seizures: a developmental study, *Dev. Brain Res.*, 13 (1984) 139–148.
2 Baez, L.A., Eskridge, N.K. and Schein, R., Postnatal development of dopaminergic and cholinergic catalepsy in the rat, *Eur. J. Pharmacol.*, 36 (1976) 155–162.
3 Ben-Ari, Y., Tremblay, E., Berger, M. and Nitecka, L., Kainic acid seizure syndrome and binding sites in developing rats, *Dev. Brain Res.*, 14 (1984) 284–288.
4 Ben-Ari, Y., Tremblay, E. and Ottersen, O.P., Injections of kainic acid into the amygdaloid complex of the rat: an electrographic, clinical and histological study in relation to the pathology of epilepsy, *Neuroscience*, 5 (1980) 515–528.
5 Ben-Ari, Y., Tremblay, E., Riche, D., Ghilini, G. and Naquet, R., Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline or pentetrazole: metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy, *Neuroscience*, 6 (1981) 1361–1391.
6 Ben-Arak, J. and Dudai, Y., Cholinergic binding sites in rat hippocampal formation: properties and ontogenesis, *Brain Res.*, 166 (1979) 245–257.
7 Braun, L.D., Cornford, E.M. and Oldendorf, W.H., Newborn rabbit blood-brain barrier is selectively permeable and differs substantially from the adult, *J. Neurochem.*, 34 (1980) 147–152.
8 Campbell, B.A., Lytle, L. and Fibiger, H., Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat, *Science*, 166 (1969) 637–639.
9 Cavalheiro, E.A., de Feo, M.R. and Ricci, G.F., Intracortical and intrahippocampal injections of kainic acid in developing rats: an electrographic study, *Electroencephalogr. Clin. Neurophysiol.*, 56 (1983) 480–486.
10 Cavalheiro, E.A., Silva, D.F., Turski, L. and Lima, J.G.C., Behavioural and EEG changes induced by systemic pilocarpine in developing rats, *Int. J. Dev. Neurosci.*, 2 (1984) S62.
11 Cherubini, E., de Feo, M.R., Mecarelli, O. and Ricci, G.F., A simple method for implanting electrodes in freely moving neonatal rats, *J. Neurosci. Meth.*, 6 (1982) 175–177.
12 Cherubini, E., de Feo, M.R., Mecarelli, O. and Ricci, G.F., Behavioral and electrographic patterns induced by systemic administration of kainic acid in developing rats, *Dev. Brain Res.*, 9 (1983) 69–77.
13 Coyle, J.T. and Yamamura, H.I., Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain, *Brain Res.*, 118 (1976) 429–440.
14 Crain, B., Cotman, C.W., Taylor, D. and Lynch, G.S., A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat, *Brain Res.*, 63 (1973) 195–204.
15 De Feo, M.R., Mecarelli, O. and Ricci, G.F., Bicuculline- and allylglycine-induced epilepsy in developing rats, *Exp. Neurol.*, 90 (1985) 411–421.
16 DeSarro, G., Meldrum, B.S. and Reavill, C., Anticonvulsant action of 2-amino-7-phosphonoheptanoic acid in the substantia nigra, *Eur. J. Pharmacol.*, 106 (1985) 175–179.
17 Fink, R.P. and Heimer, L., Two methods for selective impregnations of degenerating axons and their synaptic end-

ings in the central nervous system, *Brain Res.*, 4 (1967) 369–374.
18 Fisher, R.S., Levine, M.S., Hull, C.D. and Buchwald, N.A., Postnatal ontogeny of evoked neuronal responses in the substantia nigra of the cat, *Dev. Brain Res.*, 3 (1982) 443–462.
19 Gastaut, H. and Broughton, R., *Epileptic Seizures. Clinical and Electrographic Features, Diagnosis and Treatment,* Thomas, Springfield, IL, 1972.
20 Gilbert, M.E. and Cain, D.P., A developmental study of kindling in the rat, *Dev. Brain Res.*, 2 (1982) 321–328.
21 Gonzales, L.P. and Hettinger, M.K., Intranigral muscimol suppresses ethanol withdrawal seizures, *Brain Res.*, 298 (1984) 163–166.
22 Iadarola, M.J. and Gale, K., Substantia nigra: site of anticonvulsant activity mediated by γ-aminobutyric acid, *Science,* 218 (1982) 1237–1240.
23 Ikonomidou-Turski, C., Cavalheiro, E.A., Turski, W.A., Bortolotto, Z.A. and Turski, L., Convulsant action of morphine, (D-Ala^2,D-Leu^5]-enkephalin and naloxone in the rat amygdala: electroencephalographic, morphological and behavioural sequelae, *Neuroscience,* 20 (1987) 669–684.
24 Kònig, J.F.R. and Klippel, R.A., *The Rat Brain. A Stereotaxic atlas of the Forebrain and Lower Parts of the Brain Stem,* Williams and Wilkins, Baltimore, 1963.
25 La Gal La Salle, G., Kaijima, M. and Feldblum, S., Abortive amygdaloid kindled seizures following microinjection of γ-vinyl-GABA in the vicinity of substantia nigra in rats, *Neurosci. Lett.*, 36 (1982) 69–74.
26 Mares, P., Maresova, D., Trojan, S. and Fischer, J., Ontogenetic development of rhythmic thalamo-cortical phenomena in the rat, *Brain Res. Bull.*, 8 (1982) 765–769.
27 Matthews, D.A., Nadler, J.V., Lynch, G.S. and Cotman, C.W., Development of cholinergic innervation in the hippocampal formation of the rat, *Dev. Biol.*, 36 (1974) 130–141.
28 McNamara, J.O., Galloway, M.T., Rigsbee, L.C. and Shin, C., Evidence implicating substantia nigra in regulation of kindled seizure threshold, *J. Neurosci.*, 4 (1984) 2410–2417.
29 Milner, T.A., Loy, R. and Amaral, D.G., An anatomical study of the development of the septo-hippocampal projection in the rat, *Dev. Brain Res.*, 8 (1983) 343–371.
30 Meldrum, B.S. and Corsellis, J.A.N., Epilepsy. In J. Hume Adams, J.A.N. Corsellis and L.W. Duchen (Eds.), *Greenfield's Neuropathology,* Arnold, London, 1985, pp. 921–950.
31 Moshe, S.L. and Albala, B.J., Maturational changes in postictal refractoriness and seizure susceptibility in developing rats, *Ann. Neurol.*, 13 (1983) 552–557.
32 Moshe, S.L. and Albala, B.J., Nigral muscimol infusions facilitate the development of seizures in immature rats, *Dev. Brain Res.*, 13 (1984) 305–308.
33 Moshe, S.L., Albala, B.J., Ackermann, R.F. and Engel, J., Increased seizure susceptibility of the immature brain, *Dev. Brain Res.*, 7 (1983) 81–85.
34 Moshe, S.L., Sharpless, N.S. and Kaplan, J., Kindling in developing rats: variability of afterdischarge thresholds with age, *Brain Res.*, 211 (1981) 190–195.
35 Moshe, S.L., Sperber, E.F., Wurpel, J.N.D. and Sharpless, N.S., Age-related changes in striatal dopamine activity following nigral muscimol infusions, *Dev. Brain Res.*, 31 (1987) 129–132.
36 Nadler, J.V., Matthews, D.A., Cotman, C.W. and Lynch, G.S., Development of cholinergic innervation in the hippocampal formation of the rat, *Dev. Biol.*, 36 (1974) 142–154.
37 Nelson, K.B. and Ellenberg, J.H., *Febrile Seizures,* Raven, New York, 1981.
38 Nevander, G., Ingvar, M., Auer, R. and Siesjö, B.K., Status epilepticus in well-oxygenated rats causes neuronal necrosis, *Ann. Neurol.*, 18 (1985) 281–290.
39 Okada, R., Moshe, S.L. and Albala, B.J., Infantile status epilepticus and future seizure susceptibility in the rat, *Dev. Brain Res.*, 15 (1984) 177–183.
40 Okada, R., Moshe, S.L., Wong, B.Y., Sperber, E.F. and Zhao, D., Age-related substantia nigra-mediated seizure facilitation, *Exp. Neurol.*, 93 (1986) 180–187.
41 Olney, J.W., deGubareff, T. and Labruyere, J., Seizure related brain damage induced by cholinergic agents, *Nature (London),* 301 (1983) 520–522.
42 Olney, J.W., Fuller, T.A. and deGubareff, T., Kainate-like toxicity of folates, *Nature (London),* 292 (1981) 165–167.
43 Phelps, P.E. and Adinolfi, A.M., The postnatal development of the substantia nigra: a light and electron microscopy study, *J. Comp. Neurol.*, 209 (1982) 123–138.
44 Porter, R.J., Antiepileptic drugs: efficacy and inadequacy. In B.S. Meldrum and R.J. Porter (Eds.), *Current Problems in Epilepsy, Vol. 4, New Antiepileptic Drugs,* Libbey, London, 1986, pp. 3–15.
45 Rotter, A., Field, P.M. and Raisman, G., Muscarinic receptors in the central nervous system of the rat. III. Postnatal development of binding of [^{3}H]propylbenzilylcholine mustard, *Brain Res. Rev.*, 1 (1979) 185–205.
46 Schwartzkroin, P.A., Epileptogenesis in the immature central nervous system. In P.A. Schwartzkroin and H.V. Wheal (Eds.), *Electrophysiology of Epilepsy,* Academic, London, 1984, pp. 389–412.
47 Schwartzkroin, P.A. and Kunkel, D.D., Electrophysiology and morphology of the developing hippocampus of fetal rabbits, *J. Neurosci.*, 2 (1982) 448–462.
48 Sherwood, N.M. and Timiras, P.S., *A Stereotaxic Atlas of the Developing Rat Brain,* Univ. California Press, Berkeley, 1970.
49 Silva, D.F., Calderazzo-Filho, L.S., Turski, L. and Cavalheiro, E.A., Susceptibility to seizures induced by pilocarpine in developing rats, *Soc. Neurosci. Abstr.*, 12 (1986) 1375.
50 Singh, V.K. and McGeer, E.G., Choline acetyltransferase in developing rat brain and spinal cord, *Brain Res.*, 127 (1977) 159–163.
51 Snead, O.C. and Stephens, H.I., Ontogeny of cortical and subcortical electroencephalographic events in unrestrained neonatal and infant rats, *Exp. Neurol.*, 82 (1983) 249–269.
52 Snead, O.C. and Stephens, H.I., The ontogeny of seizures induced by leucine-enkephalin and β-endorphin, *Ann. Neurol.*, 15 (1984) 594–598.
53 Staudacherova, D., Mares, P., Kozakova, H. and Camutaliova, M., Ontogenetic development of acetylcholine and atropine epileptogenic cortical foci in rats, *Neuroscience,* 3 (1978) 749–753.
54 Swann, J.W. and Brady, R.J., Penicillin-induced epileptogenesis in immature rat CA_3 hippocampal pyramidal cells, *Dev. Brain Res.*, 12 (1984) 243–254.
55 Turski, L., Cavalheiro, E.A., Schwarz, M., Turski, W.A., Moraes Mello, L.E.A., Bortolotto, Z.A., Klockgether, T. and Sontag, K.H., Susceptibility of seizures produced by pilocarpine in rats after microinjection of isoniazid and γ-vi-

nyl-GABA into the substantia nigra, *Brain Res.*, 370 (1986) 294–309.

56 Turski, L., Cavalheiro, E.A., Turski, W.A. and Meldrum, B.S., Excitatory neurotransmission within substantia nigra pars reticulata regulates threshold for seizures produced by pilocarpine in rats: effects of intranigral 2-amino-7-phosphonoheptanoate and N-methyl-D-aspartate, *Neuroscience*, 18 (1986) 61–77.

57 Turski, W.A., Cavalheiro, E.A., Bortolotto, Z.A., Moraes Mello, L.E.A., Schwarz, M. and Turski, L., Seizures produced by pilocarpine in mice: a behavioral, electroencephalographic and morphological analysis, *Brain Res.*, 321 (1984) 237–253.

58 Turski, W.A., Cavalheiro, E.A., Calderazzo-Filho, L.S., Kleinrok, Z., Czuczwar, S.J. and Turski, L., Injections of picrotoxin and bicuculline into the amygdaloid complex of the rat: an electroencephalographic, behavioural and morphological analysis, *Neuroscience*, 14 (1985) 37–53.

59 Turski, W.A., Cavalheiro, E.A., Coimbra, C., Berzaghi, M.P., Ikonomidou-Turski, C. and Turski, L., Only certain antiepileptic drugs prevent seizures induced by pilocarpine, *Brain Res. Rev.*, 12 (1987) 281–305.

60 Turski, W.A., Cavalheiro, E.A., Schwarz, M., Czuczwar, S.J., Kleinrok, Z. and Turski, L., Limbic seizures produced by pilocarpine in rats: a behavioural, electroencephalographic and neuropathological study, *Behav. Brain Res.*, 9 (1983) 315–336.

61 Turski, W.A., Czuczwar, S.J., Kleinrok, Z. and Turski, L., Cholinomimetics produce seizures and brain damage in rats, *Experientia*, 39 (1983) 1408–1411.

62 Vernadakis, A. and Woodbury, D.M., The developing animal as a model, *Epilepsia*, 10 (1969) 163–178.

63 Wolf, G. and Keilhoff, G., Kainate and glutamate neurotoxicity in dependence on the postnatal deveopment with special reference to hippocampal neurons, *Dev. Brain Res.*, 14 (1984) 15–21.

64 Yamauchi, T., Hirabayashi, Y., Mohri, Y. and Kataoka, N., Ontogenetic studies of seizure patterns and seizure activities induced by cortical focus, *Folia Psych. Neurol. Jap.*, 30 (1976) 241–252.

65 Zouhar, A., Mares, P. and Brozek, G., Electrocorticographic activity elicited by metrazol during ontogenesis in rats, *Arch. Int. Pharmacodyn. Ther.*, 248 (1980) 280–288.