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Disturbance of Motivated Behavior in Rats by Epileptic Afterdischarges

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Summary

Nearly all epileptic seizures in patients are characterized by deranged consciousness. We started to study changes in motivated behavior (drinking in thirsty rats) as a possible analogue of compromised consciousness during and after epileptic seizures. Epileptic afterdischarges (ADs) were elicited by stimulation of the dorsal hippocampus and/or thalamus. Rats with implanted electrodes (deprived of water for 24 hours) were trained to lick water from a narrow tube. After pretraining ADs were elicited eight times in each animal and access to water was allowed during different phases of the AD. Stimulation did not affect licking if no AD was induced. If stimulation was successful, licking was stopped in nearly 70 % of stimulations and modified (biting the tube) in 30 %. Hippocampal ADs (characterized by serrated waves in the EEG and by an arrest of behavior with subsequent automatisms) completely blocked licking, signs of recovery appeared during the interval between the AD and recurrent AD and it progressed during recurrent ADs. Thalamic ADs abolished licking in 82% of cases and immediately after ADs normal licking reappeared in 49 % of these observations. Our results suggest that changes in motivated behavior might serve as an analogue of compromised human consciousness.

Key words

Epileptic seizures • Rat • Hippocampus • Thalamus • Behavior

Introduction

Approximately 20% of human epilepsies are resistant to contemporaneous pharmacotherapy. The majority of these cases exhibits complex partial (temporal) seizures without generalization (nonconvulsive seizures characterized by disturbance of consciousness and epileptic automatisms – Wieser 1987) or with secondary generalization to convulsions. Therefore adequate models of this type of seizure are necessary to study the mechanisms of their generation, spread and arrest. There are two possibilities of how to model these seizures: to induce them pharmacologically or by electrical stimulation. Among pharmacological models, kainic acid-induced seizures are most common. Originally suggested as a model of temporal lobe seizures by Nadler (1981) and Ben Ari (1985) the data about this model were reviewed by Babb *et al.* (1995). Another model, introduced by Turskis and Cavalheiro (Turski *et al.* 1983, Cavalheiro 1995) are pilocarpine-induced seizures. A modification of this model is represented by lithium-pilocarpine seizures allowing a ten times lower dose of pilocarpine to be used, which thereby reduces possible peripheral cholinomimetic effects (Honchar *et al.* 1983). Both these models have in common a severe convulsive status epilepticus at the beginning with marked nerve cell death and a subsequent development of spontaneous seizures. They are excellent for studies of the generation of seizure activity, its generalization and chronic epileptogenesis as well as brain damage and they are models of epilepsy as a process. Because of their severity and chronicity it is practically impossible to use

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them for studying the mechanisms of seizure arrest and the changes accompanying this event. For these studies, electrically induced models are more convenient. Epileptic afterdischarges (ADs) elicited by stimulation of limbic structures might be used either in isolation to model a single epileptic seizure (Dyer *et al.* 1979, Swartzwelder *et al.* 1979) or in a chronic experiment like kindling, to model progressive epileptogenesis (Goddard *et al.* 1969, McNamara and Wada 1998).

We have used low doses of kainic acid (Mikulecká *et al.* 1999, 2000a) or of pilocarpine (in a lithium-pilocarpine modification – Mikulecká *et al.* 2000b, Kršek *et al.* 2001) to study behavioral changes during and after nonconvulsive seizures. These models were adequate for studying behavioral changes during nonconvulsive seizures but not so good for studies of early postseizure behavior because it is impossible to exactly establish the end of seizures. In the present work, we try to overcome this inconvenience with a model of electrically elicited ADs where the epileptogenic agent (rhythmic electrical stimulation) is active for an exactly timed period and then the duration of the self-sustained epileptic ADs can be precisely measured. We decided to use stimulation of the dorsal hippocampus because of its detailed description in the literature (Dyer *et al.* 1979, Swartzwelder *et al.* 1979) and of our previous experience with this model (Mareš *et al.* 1985, Velíšek *et al.* 1989). For comparison with another type of epileptic AD, thalamically-induced ADs characterized by spike-and-wave rhythm and clonic seizures (Kolínová *et al.* 1979) were used. As a model of motivated behavior, licking water from a narrow tube was exploited. This method was transferred from the Bureš' laboratory (Bureš *et al.*1976).

Methods

The first experimental group was formed by 12 adult albino rats of the Wistar strain. The animals were implanted with stimulation electrodes (two twisted Teflon-coated wires with a diameter of 0.2 mm; coordinates AP=3.5, L=3.5, H=4 mm in relation to bregma – Fifková and Maršala 1960) into the right dorsal hippocampus. The recording electrode (Teflon-coated wire) was stereotaxically introduced into the left hippocampus at the same coordinates as the stimulation electrodes. The second group consisted of 8 rats with stimulation electrodes localized in the right thalamus (AP=2.5, L=2, H=4.5 mm) and one recording electrode at the same coordinates in the left thalamus. Animals of both groups were implanted with epidural cortical recording electrodes (silver balls) over the sensorimotor (AP=0, L=2.5 mm) areas of both hemispheres as well as with a silver indifferent electrode inserted into the nasal bone. Two stainless steel screws were put into the bone to anchor the assembly. All electrodes were connected to an eletrical plug and the whole assembly was fixed to the scull by means of fast curing dental acrylic. The animals were allowed to recover for one week after the surgery.

A box with a bottle of water ending in a narrow tube hidden behind a sliding door was used for experiments. At first, the animals were trained to lick water from the tube when the door was open. The tongue of the rat interrupted a light beam in the tube so that licking could be registered. To increase motivation, rats were deprived of water for 24 hours before the exposure. All the animals were able to immediately find the source of water after two or three training sessions (repeated with at least a 5-day interval). Some of the rats tried to open the sliding door. In the experimental session an epileptic AD was elicited by rhythmic electrical stimulation of the dorsal hippocampus and/or thalamic nuclei (15-s series of 2-4-V pulses lasting 1 ms at a frequency of 9 Hz). A 10-s access to water was allowed 2-3 s after the beginning of stimulation, immediately after the end of stimulation, and 10 s after the end of the AD. If recurrent AD was present, the door was open immediately after the first sharp waves appeared and remained open again for 10 s. Each animal was stimulated 8 times, the interval between an AD and subsequent stimulation was 20 min. EEG activity was continuously recorded, one channel was used for registration of licking. The presence and type of epileptic ADs including the presence of the recurrent ADs as well as the presence or absence of licking were evaluated.

Histological control demonstrated in all 12 animals included in the first experimental group a localization of both stimulation and recording electrodes in the CA3 area of the dorsal hippocampus. Tips of the thalamic stimulation electrodes in the second group were localized either in the lateral anterior nucleus or at the border between lateral anterior and mediodorsal nuclei.

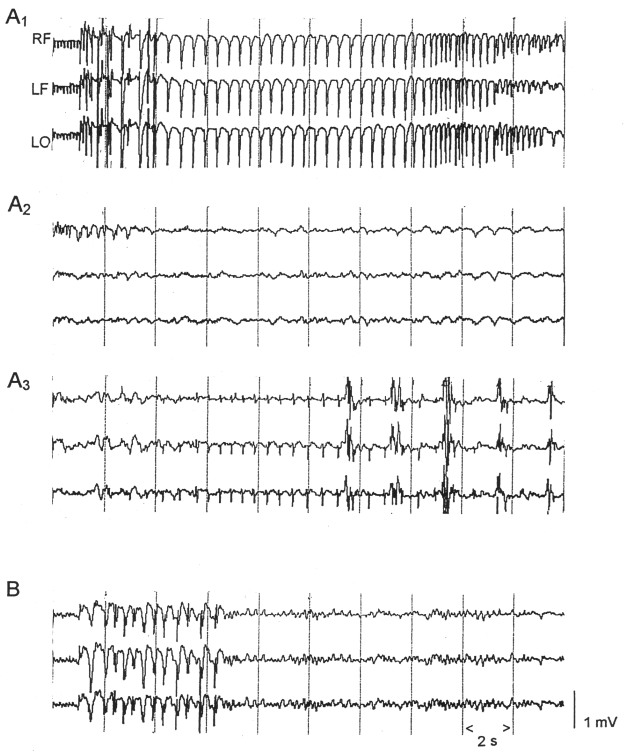

Fig. 1. *EEG recording of two types of afterdischarges A_1 - Upper part of the recording demonstrates the end of stimulation and an AD, A_2 - middle part represents a continuation of the first part with the end of ADs, A_3 - lower part demonstrates a recurrent afterdischarge Between the middle and lower part of the recording 20 s are omitted B – Spike-and-wave AD Individual leads from top to bottom: RF – right frontal cortical electrode, LF – left frontal cortical electrode, LO – left occipital cortical electrode, always in a reference connection Time mark = 2 s, amplitude calibration = 0 5 mV*

Results

Hippocampal stimulation: Epileptic ADs were elicited in 75 out of 96 stimulations. They were formed by huge delta waves with superimposed fast activity of low-amplitude spikes (serrated waves - Fig.1). The average duration of the ADs was 34±7 (mean ± S.E.M.). They were accompanied by immobilization at the beginning, then chewing and sniffing was present and towards the end of ADs wet dog shakes (WDS) appeared as the most conspicuous automatism. Nearly all ADs (N=61) were followed by a recurrent AD. The interval between the first and recurrent ADs lasted 67±12 s on the average. The EEG pattern of the recurrent ADs was more simple than that of the first ADs, they were characterized by sharp delta waves of high amplitude. The recurrent ADs were accompanied by WDS (seen also as huge artifacts in the EEG), other automatisms did not appear. The duration of recurrent ADs was 25±6 s on the average.

Licking was not compromised during stimulations that did not elicit an AD (N=21), nor it was compromised during the corresponding poststimulation periods (data not shown). In contrast, normal licking appeared only for a short period at the beginning of the stimulations that resulted in ADs. It was quickly replaced by a sequence of modified behavior – animals tried to bite the tube – and a complete absence of licking. During the ADs the animals did not exhibit an interest in the tube. In 61 cases when recurrent ADs were registered, the interval between the two phases of ADs was characterized by an absence of licking and/or (in 41% of cases) by modified behavior. Recovery of normal licking was seen during the recurrent ADs when more than half of the ADs was accompanied by regular licking whereas modified licking or absence of this behavior was observed in a minority of cases (Fig.2).

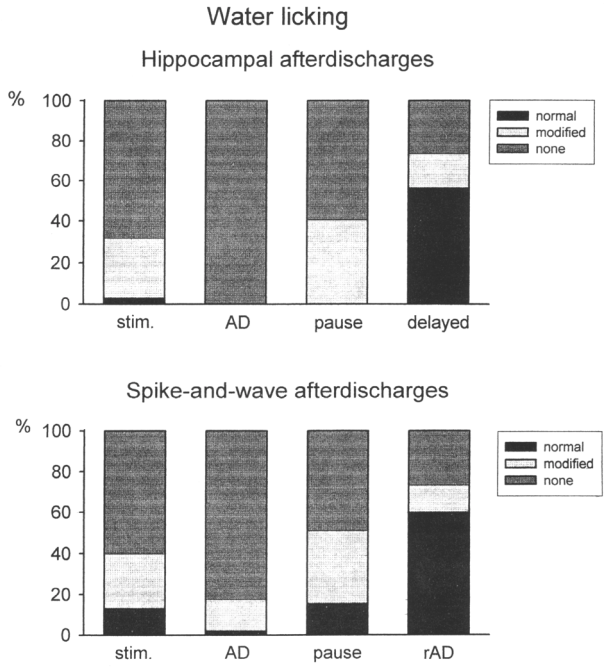

Fig. 2. *Representation of normal, modified and absent water licking during different phases of observation A – hippocampal type of ADs, B – spike-and-wave ADs Abscissa from left to right: stimulation (stim.), afterdischarge (AD), interval between the first and the recurrent afterdischarges (pause), recurrent after-discharge (rAD), respectively the corresponding time period in B Ordinate: percentage of the three types of behavior during individual phases*

Thalamic stimulation: Forty-five out of 64 stimulations elicited an AD characterized by rhythmic spike-and-wave activity (usually with a more pronounced spike component) accompanied by clonic seizures of the head and forelimb muscles. These ADs (with average duration of 13±2 s) were not followed by recurrent AD. The remaining stimulations either did not elicit ADs at all (N=13) or induced an AD of the "hippocampal" type (N=6) usually as a response to one of the last stimulations. These six stimulations that elicited serrated wave ADs were excluded from evaluation.

The thirteen stimulations without ADs did not modify water licking (data not shown). Stimulations eliciting ADs compromised licking in the same way as in the hippocampal experimental group. Spike-and-wave ADs did not abolish licking in all cases – normal and/or modified licking was present in 14% of ADs. Licking started to recover immediately after the end of ADs so that it was observed in 41% of cases (normal licking in 6%, modified in 35%). At the longest interval the percentage of the three different behaviors was the same as during the recurrent ADs in the hippocampal group, i.e. at this time the recovery of water licking was similar after both types of Ads.

Discussion

The two types of epileptic ADs represent models of different types of human epileptic seizures. The same type of ADs as that elicited by hippocampal stimulation in our animals (also with recurrent ADs) was described repeatedly in amygdala kindling experiments (Engel et al. 1978). The correlation is clear: characteristic EEG pattern, arrest of behavior and/or automatisms appearing mostly towards the end of the AD correspond to human complex partial seizures without secondary generalization (Wieser 1987). On the other hand, ADs induced by thalamic stimulation might be taken as a model of human myoclonic seizures – they are also characterized by a presence of a spike-and-wave rhythm in the EEG and by marked motor seizures (Janz et al. 1998) – but the correlation is not so equivocal as in the case of hippocampal ADs.

There were some similarities as well as differences between the two types of ADs. The influence of stimulation on water licking was identical in both models – stimulations that did not elicit epileptic ADs did not compromise this behavior whereas the stimulations that led to ADs stopped licking. There are two possible explanations: either ADs start during the stimulation and this obscured the AD-related cessation of licking, or there was also a threshold for the behavioral disturbance. The first possibility appears less probable: spike-and-wave ADs elicited by thalamic (Chocholová et al. 1977) or cortical stimulation (Kubová et al. 1999) never start during stimulation and changes in thalamocortical evoked potentials during the stimulation series appear only towards the end of stimulation (Mareš et al. 1983) whereas licking is stopped at the very beginning of stimulation. Therefore we are in favor of the second possibility but to prove it further experiments have to be performed.

A difference was found in the behavior during and immediately after ADs: hippocampal ADs abolished licking during the ADs and the recovery was slow whereas spike-and-wave ADs were unable to completely block this behavior and the presence of licking (mostly in a modified form) was substantially more frequent during the immediate postictal phase. The different licking behavior during the ADs is likely connected with the different mechanisms of the two types of ADs. The serrated wave type of ADs elicited by hippocampal stimulation is generated in limbic structures – the same type of ADs may be induced not only by stimulation of the hippocampus (Dyer et al. 1979, Swartzwelder et al. 1979) but also by stimulation of the amygdala (Goddard et al. 1969), entorhinal (Sutula et al. 1986) or piriform cortex (Löscher and Ebert 1996). Unfortunately, the generator of spike-and-wave AD accompanied by clonic seizures is not fully known. The EEG pattern (spike-and-wave rhythm) is of thalamocortical origin (Avanzini et al. 1992) and the motor pattern of clonic, "minimal" seizures is generated in the basal forebrain (Browning and Nelson 1986). The connection between the two generators and thus the complete list of structures involved (and those left undisturbed) is not known. The spike-and-wave rhythm elicited by low doses of pentylenetetrazol (not accompanied by clonic seizures) was found to block the licking behavior (Schickerová et al. 1989) but the distribution of epileptic activity in the brain must be different in this case as indicated by the absence of obvious motor seizures. Therefore these data could not be directly compared with our present findings. The difference in the postictal period may be ascribed to a different duration of postictal depression which is longer after hippocampal ADs than after thalamically-induced spike-and-wave ADs (Mareš and Marešová 1989). The later recovery of normal motivated behavior is the same in both models.

A marked difference was found between the first phase of ADs and the recurrent ADs in the hippocampus. This finding speaks in favor of different mechanisms of the two phases of the epileptic ADs. The recurrent AD was described in detail in kindling experiments but there are no further data on its generation (Racine et al. 1981).

Our experiments addressed a general question concerning experimental models of epileptic seizures – what might be taken for an analogue of disturbed consciousness as one of the most conspicuous symptoms of human epileptic seizures? We tried to demonstrate that a highly motivated behavior – drinking in thirsty rats – may be used as a measure. We have shown that it is compromised in three different types of epileptic seizures modeling human seizures with deranged consciousness: rhythmic metrazol activity (a model of human absence seizures – Schickerová et al. 1989), hippocampal ADs (a model of human complex partial seizures – present results) and ADs induced by thalamic stimulation (a possible model of human myoclonic seizures – present results). It remains to demonstrate that a model of simple partial seizures (neocortical epileptic foci), i.e. the only type of human epileptic seizures with preserved consciousness, does not interfere with the motivated behavior studied in our experiments.

Appendix

I entered the laboratory of Jan Bureš as a Ph.D. student when my boss decided that I have to change topics of my disertation from clinical to experimental neurophysiology. Jan Bureš started to train me in the basic electrophysiological methods and durnig my one-year stage he gave me a solid background in the field. Only much later I realized how good this background was. A few years later I became a scientist in the Department of Epilepsy of the same institute where Jan Bureš chaired Department of Neurophysiology of Memory. It was a good luck for me to have a possibility to discuss my projects with Jan and it saved me from at least few dead ends in my research plans. He was and is an excellent teacher and a very good friend ready to give advices not only to students. I am happy that I met him and I am proud to be his friend.

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