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A STUDY OF SPONTANEOUS AND EVOKED ACTIVITY IN THE RAT HIPPOCAMPUS UNDER HELIUM-OXYGEN HIGH PRESSURE

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Several findings in man and animals indicate that high pressure induces neurological disturbances in the spinal cord (Hugon et al. 1981; Fagni et al. 1982) as well as in the brain (high pressure nervous syndrome (HPNS), Rostain and Naquet 1974). Nevertheless, the pressure effects on the CNS are still not understood, possibly because of the relative complexity of the integrated systems usually studied. In the present study we examined pressure effects on the dorsal hippocampus of the rat, a structure which provides a relatively simple anatomy and a well understood physiology.

The most characteristic hippocampal EEG activity is a rhythmic slow activity (RSA, 6–8 Hz) which occurs, in the rat, predominantly only if animals are in an aroused state, during 'voluntary' movements, or during paradoxical sleep (Vanderwolf 1969; Winson 1974). RSA seems to be generated in the pyramidal layer of the CA1 region and in the molecular layer of the dentate gyrus (Winson 1974; Bland and Whishaw 1976). Our first index of the effect of pressure on the CNS physiology was to compare the RSA recorded in the CA1 field, under normal conditions and at high pressure, in the same rats.

The laminar organization of the hippocampus is of substantial advantage in the study of synaptic transmission (Andersen et al. 1971). For instance, a monosynaptic pathway can be activated by electrical stimulation of the commissural fibres which evokes a population EPSP of the pyramidal cells in the contralateral CA1 region. By increasing the stimulus intensity, a superimposed CA1 population spike of these cells can be evoked (Andersen 1960; Lynch and Schubert 1980). We used these potentials, evoked by two patterns of stimulation (single pulse or paired-pulse), as a second index of the effect of pressure on the excitability of central neurones.

Under normal conditions repetitive electrical stimulation of the hippocampus, with an appropriate intensity, is well known to induce afterdischarges, a model of epilepsy (Burchfield 1981; Goddard 1981; Racine et al. 1981; Gutnick et al. 1982). We examined the effect of pressure on the electrical threshold for evoking such hippocampal afterdischarges (AD threshold). The AD threshold was taken as an index of the epileptogenic property of the hyperbaric environment.

Our results show that the spontaneous activity of the hippocampus, the commissural evoked potentials and the AD threshold are reversibly modified by pressure.

Methods

(1) Surgery

Twenty-nine male Sprague-Dawley rats, weighing 300–350 g, were studied for spontaneous EEG and evoked responses through chronic implants. Surgery was performed under pentobarbital anaesthesia (40 mg/kg, i.p.). Bipolar stimulation electrodes were made of twisted platinum wires (diameter 0.090 mm) and were implanted into the left dorsal hippocampus (3.5 mm posterior to

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bregma; 2.5 mm lateral to the midline) to stimulate the axons of the CA3 pyramidal cells. Similar electrodes were positioned in the right dorsal hippocampus (P = 3.5 mm; L = 2.0 mm) for monopolar recording (against a ground electrode) of the corresponding commissural evoked potential and of spontaneous activity in the CA1 field. The final positions of the electrodes were optimized with regard to the shape, the size and the stability of the commissural evoked potentials. A silver wire fixed under the skin of the neck served as reference and ground electrode.

At the end of the experiments the animals were deeply anaesthetized with sodium pentobarbital, then perfused with physiological saline and 10% formalin and their brains removed. Coronal frozen sections (0.040 mm) were cut and mounted for histological determination of the sites of electrode tips.

(2) Environmental control

The animals were placed, individually or in pairs, in a hyperbaric chamber of 164 litres. Linear compressions were performed under a helium oxygen atmosphere at a rate of 0.5 or 1 bar/min, until a pressure of 91 bars was reached. The animals remained at this pressure for 2–4 h and then were decompressed at a linear rate of 0.07 bar/min.

The presence of soda lime in the chamber ensured a partial pressure of carbon dioxide lower than 0.005 bar. The partial pressure of oxygen was maintained between 0.21 and 0.25 bar during the compression and at the maximum pressure, and then increased to 0.5 bar during the decompression. The ambient temperature was regulated at $29 \pm 0.5°C$ at 2 bars and progressively raised 1°C every 30 bars, in order to maintain a constant comfortable temperature.

(3) Experimental procedures

Seven to 10 days after surgery recordings were performed: 1 h before compression under a normobaric normoxic atmosphere of helium-oxygen (control), then at different pressures during compression, at the maximal pressure and after the end of the decompression. Continuous visual observation of the animals allowed us to relate behavioural and electrophysiological data. Only records from awake (active or immobile) animals were analysed.

Spectral analysis of hippocampal spontaneous activity was performed off line on a PDP 11 (Digital) computer (BW = 0–50 Hz). Each power spectrum was calculated on 15–20 samples of 8 sec each, devoid of artifact. The initial slope of the population EPSP and the peak-to-peak amplitude of the population spike were measured on averages of 8 responses (by means of an Apple II computer) evoked at a rate of 0.1 Hz (pulse duration = 0.1 msec). Input-output curves and paired-pulse facilitation curves of these responses were then analysed.

The AD threshold was studied separately on 7 rats under normal conditions, and on 4 other rats under high pressure (91 bars). The threshold was established according to the following procedure: a train (60 Hz; 1 sec duration; 1 msec pulse duration; constant current monophasic pulses) was applied every 15 min. The selected pulse polarity was the one that, with a single pulse, evoked the highest commissural dendritic response. The pulse intensity of each train was 0.005 mA higher than the pulse intensity of the preceding train. The required intensity for evoking the first AD was taken as threshold value.

Results

(1) Motor disturbances and changes in spontaneous hippocampal activity

Fig. 1A shows an example of hippocampal spontaneous activity recorded in an awake immobile rat under normobaric conditions. The spectral analysis of this EEG type displayed two main bands: 2–4 Hz and 5–8 Hz.

During the 0.5 bar/min compressions no change was noticed in the electrographic records, or in the spectral analysis of the spontaneous hippocampal activity. On the other hand, the 14 rats compressed at this rate displayed the characteristic motor disturbances of the HPNS: tremor, jerks and, in some cases, seizures.

The tremor appeared between 40 and 60 bars. Clinically, it resembled the one that we previously described in the cat (Fagni et al. 1982). It can be

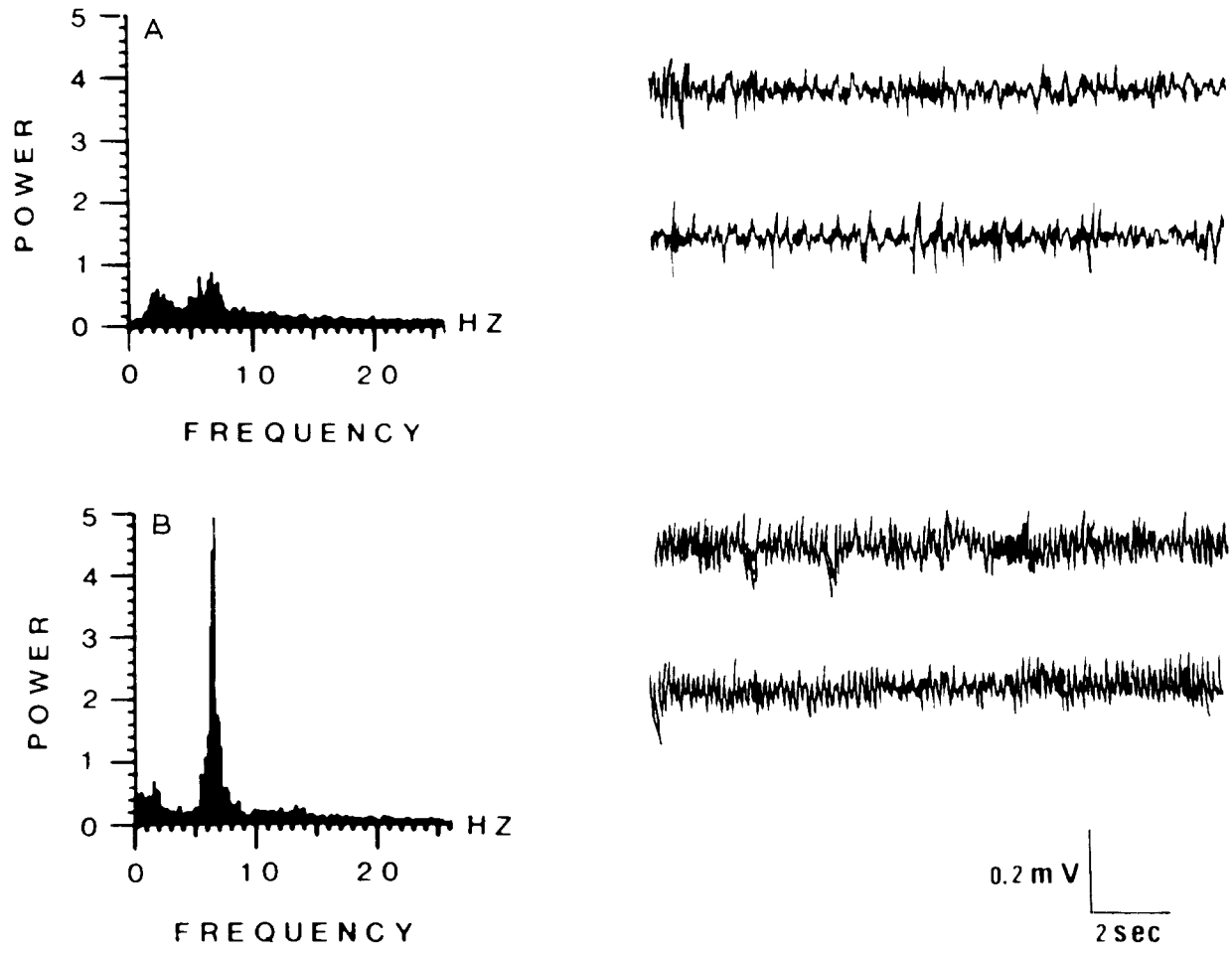

Fig. 1. Electrographic records (on the right) and power spectra (on the left) of spontaneous activity in the CA1 region of the rat hippocampus. A: at 1 bar (0 m); B: at 91 bars (900 m) after 1 bar/min compression. Note in B the sustained regular 5–8 Hz activity and, in the corresponding spectrum, the increase in the density of the 5–8 Hz band.

defined as a postural tremor, more pronounced in the head and the limbs, and enhanced during kinetic activities. Its amplitude increased with pressure. Spontaneous intermittent jerks appeared between 60 and 80 bars. They were generalized to the whole body; occurring at first at intervals of several minutes, they became more frequent (every 30–60 sec) and more intense as the pressure increased.

All motor disturbances vanished during the constant pressure period at 91 bars, while spectral analysis of the hippocampal EEG showed, in 3 rats only, a 100% increase in the power of the 5–8 Hz band without any apparent change in the electrographic records. No significant modification was found in the 2–4 Hz band. At this maximal pressure 3 other rats displayed tonic-clonic convulsions (1–2 min duration), followed by a long period of atony and akinesia. It is worth noting that only 1 rat displayed hippocampal seizures (Fig. 2) during the motor convulsions.

With faster compressions (1 bar/min; 8 rats) the tremor and a regular 5–8 Hz hippocampal activity appeared, in all animals, between 30 and 40 bars. Jerks appeared between 30 and 50 bars. All these disturbances intensified as pressure

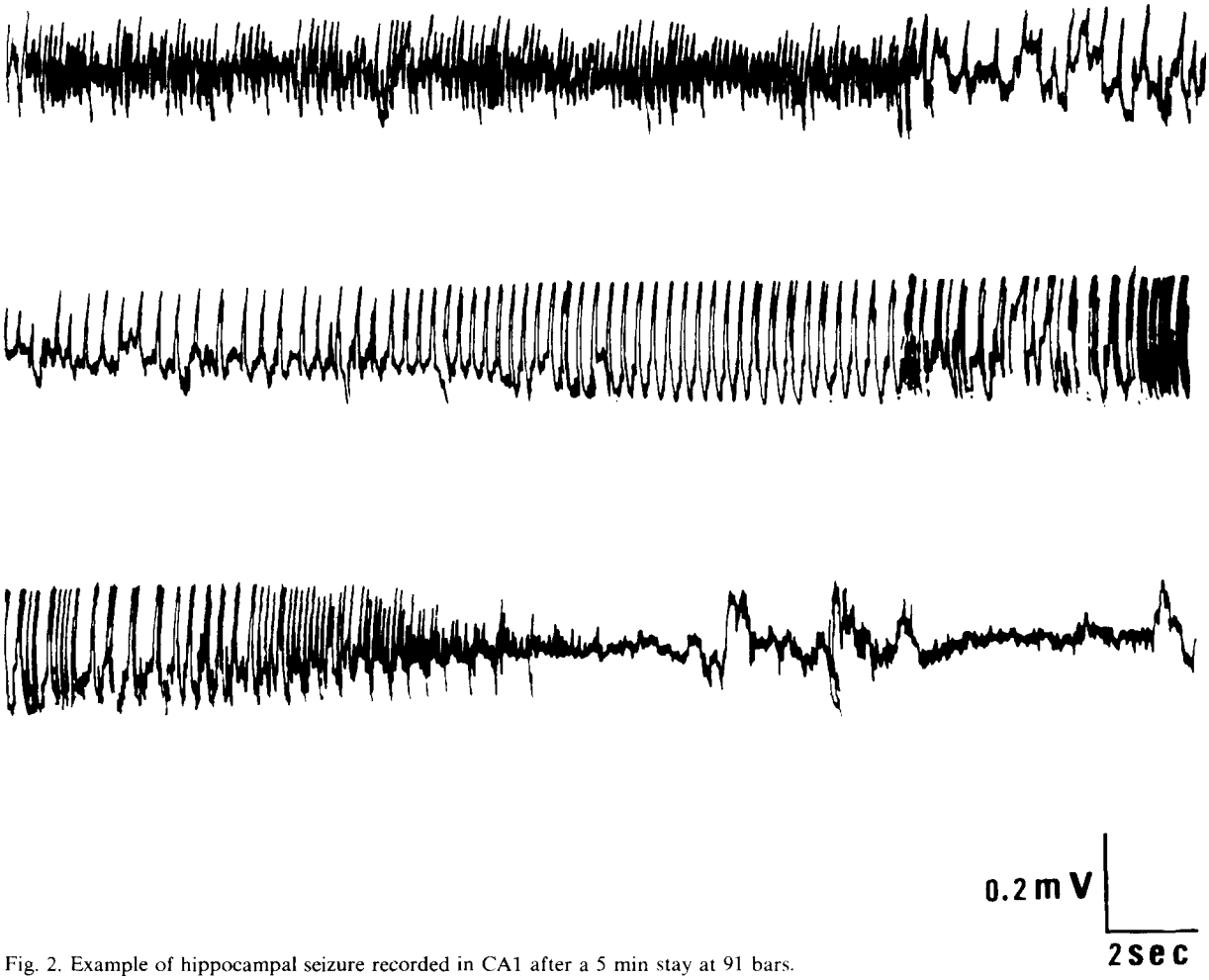

Fig. 2. Example of hippocampal seizure recorded in CA1 after a 5 min stay at 91 bars.

increased. At 91 bars, the 5–8 Hz hippocampal rhythm became abnormally sustained (Fig. 1B), while the tremor and the jerks were more intense.

In 5 of the 8 rats, motor disturbances slowly lessened during the constant pressure period at 91 bars, but the 5–8 Hz hippocampal rhythm could persist until the beginning of decompression. The sustained 5–8 Hz hippocampal rhythm was not necessarily associated with classical 'voluntary movements,' such as walking, rearing, grooming or jumping, which are classically related to normal RSA. Spectral analysis of the spontaneous hippocampal activity showed a 300–600% increase in the power of the 5–8 Hz band without any significant change in its peak frequency. At the same pressure, the 2–4 Hz band tended to shift to a 0–2 Hz band with no significant modification in its power (Fig. 1B).

In the 3 other rats compressed at 1 bar/min, tremor, then jerks, were followed, at 91 bars, by tonic-clonic convulsions similar to those observed with 0.5 bar/min compressions. Only 2 of them displayed hippocampal seizures during these motor convulsions.

(2) Changes in hippocampal evoked potentials

Histological verification of electrode positions showed that stimulation was in the CA1 pyramidal layer-stratum radiatum region, and recording from the stratum oriens-CA1 pyramidal layer zone.

(a) Input-output curves. Stimulation from 0.085 to 0.100 mA evoked a population EPSP (Fig. 3A), and stimulation above 0.100 mA evoked a superimposed population spike (Fig. 3B). The same figure shows input-output curves of these responses recorded at 1 bar, 91 bars and after the end of the decompression. Comparing these curves, one can see that both population EPSP and population spike were significantly smaller at 91 bars. However, the change in the population EPSP was not apparent when the population EPSP was maximal. The input-output curve obtained after the end of the decompression shows that this change was reversible. Within the limits of our investigations this depression did not seem to depend on the compression rate.

(b) Paired-pulse facilitation curves. Fig. 4 shows paired-pulse facilitation curves of population EPSPs evoked by homosynaptic stimulation of the commissural fibres. Stimuli were equal and adjusted so as to evoke submaximal population EPSPs. Under normal conditions these paired-pulse curves showed a marked facilitation of the test response for 20–40 msec interpulse intervals. This paired-pulse facilitation was greatly depressed at 91 bars. The same figure shows that this change was partially reversible after the end of the decompression.

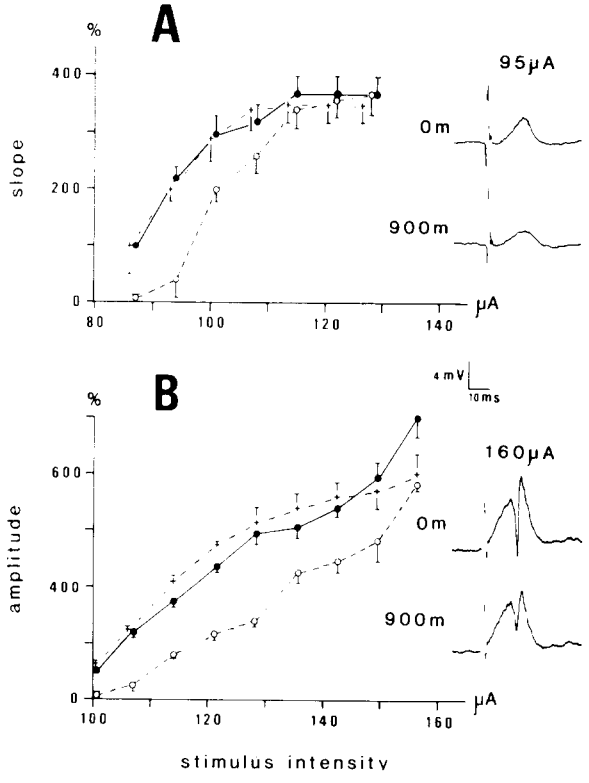

Fig. 3. Input-output curves of commissural responses in the CA1 region of the rat hippocampus. A: on the right: population EPSPs evoked by 0.095 mA stimuli at 1 bar (0 m) and at 91 bars (900 m); on the left: input-output curves for the values of the initial slope of the population EPSP at 1 bar (filled circles), at 91 bars (open circles) and after the end of the decompression (crosses). Each point (mean ± S.E.M.; n = 18 animals) of the curves is expressed as percentage of the value obtained at 1 bar for a 0.085 mA stimulus. B: on the right: population spikes evoked by 0.160 mA stimuli at 1 bar (0 m) and at 91 bars (900 m); on the left: input-output curves of the amplitude (peak to peak) of the population spike (same legend as A, n = 10 animals).

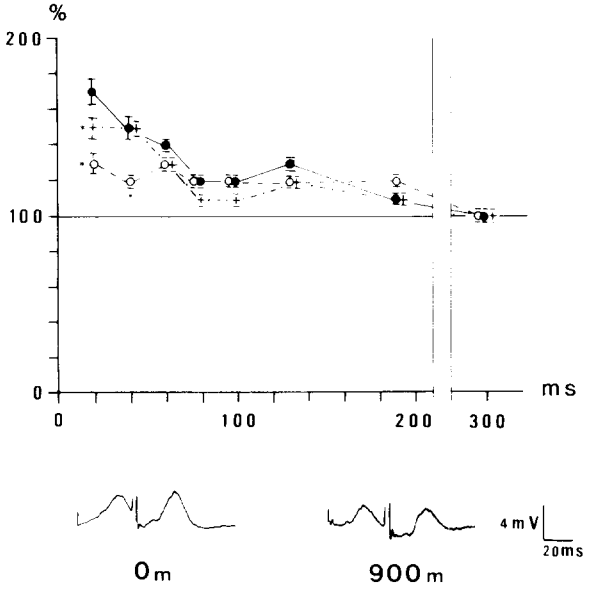

Fig. 4. Upper part: paired-pulse facilitation curves of the population EPSP in the CA1 region of the rat hippocampus at 1 bar (0 m, filled circles), 91 bars (900 m, open circles) and at 1 bar after the end of the decompression (crosses). Identical stimulus intensities were used at 1 bar (before and after decompression) and at 91 bars. Each point represents the initial slope (m ± S.E.M; n = 18 animals) of the second response expressed as percentage of the initial slope of the first response. The points indicated by asterisks are significantly different from those of the control curve (filled circles; Student's t test, $P < 0.025$). Lower part: population EPSPs evoked by paired-pulse stimulation (0.100 mA, 40 msec pulse interval) at 1 bar (0 m) and at 91 bars (900 m).

(3) Change in the afterdischarge threshold

Fig. 5 shows examples of recorded afterdischarges evoked by seizure threshold stimulation at normal pressure and at high pressure. Under normobaric conditions the AD threshold was 0.062 ± 0.006 mA (m ± S.E.M.; n = 7). At 91 bars, after a 0.5 bar/min compression, the AD threshold was 0.032 ± 0.005 mA (m ± S.E.M.; n = 4). These 2 values are significantly different ($P < 0.03$; Mann and Whitney non-parametric test). Two weeks after the end of the decompression, the group of rats tested under pressure was studied again in normal environmental conditions: the AD threshold was 0.057 ± 0.007 mA (m ± S.E.M.; n = 4). This value

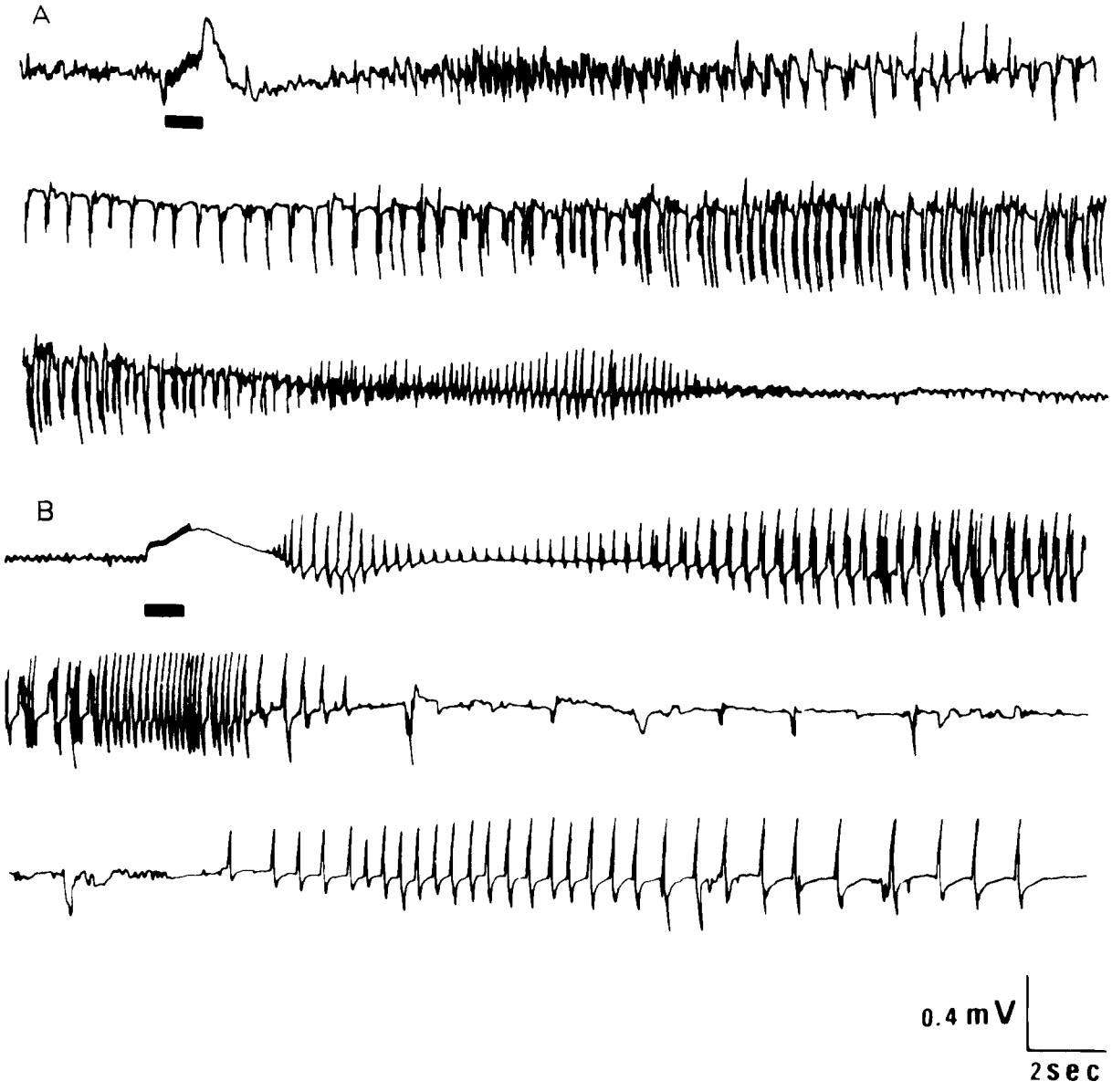

Fig. 5. Examples of afterdischarges evoked in the CA1 region of the rat hippocampus by seizure threshold stimulation (solid bars). A: 1 bar (0.040 mA stimulation); B: 91 bars (0.030 mA stimulation). Note the generation of secondary afterdischarges at 91 bars in B.

is significantly different from the value obtained in these animals under pressure and not significantly different from the value obtained in the group of rats tested under normal conditions.

Discussion

The present results show that the HPNS is accompanied, in the rat, by changes in hippocampal bioelectrical activities. These changes include: (1) the appearance of a sustained 5–8 Hz regular spontaneous activity, the spectral density of which increases with pressure and, in some cases, is followed by seizures; (2) a marked decrease in the responses of CA1 pyramidal cells to stimulation of their commissural afferents; (3) a reduction in paired-pulse facilitation; and (4) a 50% decrease in the AD threshold. These changes are roughly reversible after decompression. It is worth emphasizing that hyperbaric motor seizures can occur without any apparent seizure activity in the dorsal hippocampus. This finding strongly suggests that the hippocampus is not necessarily involved in the HPNS motor seizures.

The sustained large amplitude regular 5–8 Hz hippocampal rhythm that we observed under pressure might reflect a continued state of arousal. Nevertheless, this theta-like activity was not correlated with classical characteristic behaviours associated, in normal conditions, with RSA (Lopes da Silva and Arnolds 1978; Robinson 1980). Therefore, it seems more likely that this sustained rhythm would result from hippocampal dysfunctioning rather than being a normal RSA. In this case the enhancement of this theta-like rhythm might result from a pressure effect on the septal area, a structure which is well known to generate hippocampal RSA, or on the hippocampus itself.

So far it seems precarious to relate this hippocampal dysfunction to the pressure-induced motor disturbances since we did not see any clear correlation between the observable abnormal motor events (tremor, jerks and convulsions) and hippocampal EEG changes: (1) the tremor and the jerks disappeared during the constant pressure period at 91 bars, while the abnormal RSA-like hippocampal rhythm persisted until the beginning of the decompression; and (2) the pressure-induced motor disturbances were not systematically accompanied by hippocampal seizures. These observations support the hypothesis of different sites of action of pressure (Fagni et al. 1982).

The increase in the spectral density of the regular sustained 5–8 Hz hippocampal activity observed at high pressure was concomitant with a decrease in the size of commissural evoked potentials. This is apparently in contrast to the results of various authors (Buzsaki et al. 1981; Jeantet and Jaffard 1981) who described a facilitation of hippocampal commissural responses during periods of RSA in rodents, in normal physiological and environmental conditions. This apparent discrepancy suggests that, as discussed above, the 5–8 Hz regular hippocampal rhythm appearing under pressure is probably an abnormal activity rather than a normal RSA.

Both population EPSP and population spike were reduced under pressure. These changes indicate a decrease in the excitability of the commissural afferents-CA1 pyramidal cell system. So far, we shall tentatively propose the hypothesis of a general alteration of membrane bioelectrical processes involved in EPSP and action potential generation, which suffices to explain the changes observed in the evoked commissural responses. At the moment, hyperbaric experiments at cellular level are required for further speculation on the mechanisms of this apparent pressure-induced hippocampal hypoexcitability.

The paired-pulse test was performed only on population EPSPs in order not to evoke propagated responses to the first stimulus. This experimental situation may explain the absence of inhibition of the second response which might have been induced by the activation of recurrent inhibitory processes if a population spike had been evoked by a first high intensity stimulus. The paired-pulse facilitation of the population EPSP, under normal conditions, would be due to the release of a larger amount of neurotransmitter by the excited terminals (Creager et al. 1980). Therefore, the reduction in the paired-pulse facilitation observed under pressure supports the hypothesis of an alteration of synaptic transmission. This hypothesis does not exclude the possibility, men-

tioned before, of a general (pre- and postsynaptic) alteration of some membrane properties resulting in hypoexcitability of the tested system.

The phenomenon of electrically induced ADs is generally considered as a model of epilepsy. So the decrease in AD threshold that we observed under pressure strongly suggests that hyperbaric environments are favourable to the development of epileptic-like states. This observation is in agreement with the findings of Kauffman et al. (1977), Brauer et al. (1979) and Rostain (Thesis 1980) who described neocortical 'epileptic' activities in mammals under high pressures of inert gases. Possible relations between the decrease in AD threshold and the decrease in the size of commissural responses observed under pressure need further study. Nevertheless, it seems unlikely that this facilitation would result from either: (1) hyperexcitability of commissural afferents, or (2) hyperexcitability of CA1 pyramidal cells, since all these changes would give rise to facilitated population EPSPs and population spikes.

The present findings show that high pressures have a synchronizing effect on the hippocampal EEG and also that they can be considered as an epileptogenic agent on this part of the brain. In that respect, the hippocampus appears to react to pressure in a similar manner to various other parts of the brain studied so far (Halsey 1982). On the other hand, the pressure-induced hypoexcitability that we found in this structure contrasts with the pressure-induced hyperexcitability that has been reported in other neurophysiological systems such as the muscle-spinal cord system in the study of the Hoffmann reflex or peripheral nerve-somatosensory cortex system in the study of somatic evoked potentials (Hugon et al. 1981). Hence it seems that high pressures exert protean effects on nervous structures and do not act uniformly on the CNS.

Summary

High pressures affect the physiology of the central nervous system. For a better understanding of this effect, we examined the hippocampal activity in the rat under high pressures (91 bars) of helium-oxygen.

Effects of high pressure on hippocampal physiology are: (1) an abnormally sustained 5–8 Hz pattern of spontaneous activity, followed, in some cases, by seizures; (2) a marked decrease in the responses of CA1 pyramidal cells to stimulation of their commissural afferents; and (3) a 50% decrease in the afterdischarge threshold.

On the basis of the relatively well understood hippocampal physiology in normobaric conditions, our observations suggest that high pressures induce hypoexcitability of afferents and/or target cells.

Résumé

Une activité spontanée et évoquée hippocampique chez le rat sous haute pression d'hélium-oxygène

Les hautes pressions induisent une altération fonctionnelle du système nerveux central. A la recherche d'une meilleure compréhension de cet effet de la pression, nous avons étudié l'activité hippocampique chez le rat sous hautes pressions (91 bars) d'hélium-oxygène.

Nous avons mis en évidence des modifications physiologiques de l'hippocampe qui se traduisent par: (1) une activité spontanée périodique à 5–8 Hz ample et anormalement soutenue, éventuellement suivie de crises électrographiques; (2) une nette dépression des réponses des cellules pyramidales de la région CA1 évoquées par la stimulation de leurs afférences commissurales; et (3) un abaissement de 50% du seuil de déclenchement des post-décharges.

Sur la base des données physiologiques normobares relatives à l'hippocampe, nos observations suggèrent que les hautes pressions induiraient une hypoexcitabilité des afférences et/ou de leurs cellules cibles.

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