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Kindling of the mesencephalic reticular formation in rats
(ラットの中脳網様体のキンドリング)

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Introduction

Experimental evidence suggests that the brainstem reticular formation is involved in the manifestation of generalized tonic seizures (Kreindler et al., 1958 ; Bergmann et al., 1963 ; Browning et al., 1981 ; Burnham et al., 1981 ; Browning, 1987). In the present study, we investigated the kindling of the mesencephalic reticular formation (MRF) and its influence on subsequent amygdala (AMY) kindling in rats.

Methods

Experiment 1 : MRF kindling

Twelve male Sprague-Dawley rats weighing 300-400 g were used. Under pentobarbital anesthesia, bipolar electrodes made of twisted stainless steel wire, 200 μ A in diameter, were stereotaxically (Paxinos and Watson, 1986) inserted into the unilateral MRF (P 5.8, L 1.7, V 6.6 mm ; the deep mesencephalic nucleus) and the ipsilateral AMY (P 2.8, L 4.8, V 9.5 mm ; the anterior basolateral amygdaloid nucleus). Two extradural cortical electrodes (stailess steel screws) were inserted into the skull over the ipsilateral motor cortex and cerebellar cortex (the latter was used as the reference electrode).

Two weeks after the surgical procedure, the afterdischarge (AD) threshold of the MRF was determined by 1-sec stimulations at 100 μ A (60 Hz biphasic square pulses). Every 24 hours, stimulation was increased by 100 μ A until ADs were induced. Thereafter, the MRF was stimulated once per day at the AD threshold until 3 consecutive generalized tonic-clonic seizures of at least 50 sec in duration were induced (the MRF group, n = 6).

Experiment 2 : AMY kindling

Twenty-four hours after the final MRF stimulation, 3 of the 6 MRF group rats underwent daily AMY kindling. Kindling of the AMY at the AD threshold (100 μ A)

was continued until 3 consecutive stage 5 seizures (described below) were induced. The AMY kindled seizures were classified by a modification of Racine (1972), as follows : stage 1, rhythmic mouth and facial movements ; stage 2, rhythmic head nodding ; stage 3, unilateral forelimb clonus ; stage 4, bilateral forelimb clonus and rearing ; and stage 5, falling. Rats that did not undergo MRF kindling were used as controls (n = 6).

Experiment 3 : MRF restimulation

MRF restimulation at the AD threshold was performed 24 hours and 30 days after AMY kindling in 2 rats from the MRF group, respectively.

On completion of the experiments, all animals were deeply anesthetized and their brains were perfused, serially sectioned (40 μ m), and stained by hematoxylin and eosin. Histological examination showed that all the electrode tips were located in the intended structures.

Results

Experiment 1

The mean AD threshold at the MRF was 317 μ A (range 200-500 μ A) and the mean number of ADs required to complete MRF kindling was 12.8 (range 5 – 22). Kindling caused a progressive increase in AD duration and complexity in all the MRF stimulation group rats ; the initial and final mean AD duration were 17.8 sec (range 8 – 27 sec) and 66.8 sec (range 50 – 88 sec), respectively ($p < 0.05$ by Wilcoxon one-sample test). The initial stimulation elicited only a brief generalized tonic seizure (Fig. 1A). However, the final stimulation produced a prolonged generalized tonic-clonic seizure (Fig. 1B). Although AD appeared only in the subcortical structures (MRF and AMY) during the initial seizure, AD also appeared in the motor cortex during the final seizure.

The mean number of stimulations required to reach stage 5 was 9.2 (range 6 – 12) in the control group and 4.0 (range 3 – 5) in the MRF group. This difference was statistically significant ($p < 0.05$ by Mann-Whitney U test). The MRF group rats showed a significantly higher incidence of a generalized tonic seizure with a prolonged loss of postural control (> 4 sec, range 5 – 18 sec) than the controls during stage 5 seizures (3/3 and 0/6, respectively ; $p < 0.05$ by chi-square test). Fig. 3 is an example of a stage 5 seizure in the MRF group.

Both at 24 hours and at 30 days after AMY kindling, MRF restimulation induced a generalized tonic-clonic seizure that was similar to the final generalized seizure of the MRF kindling session.

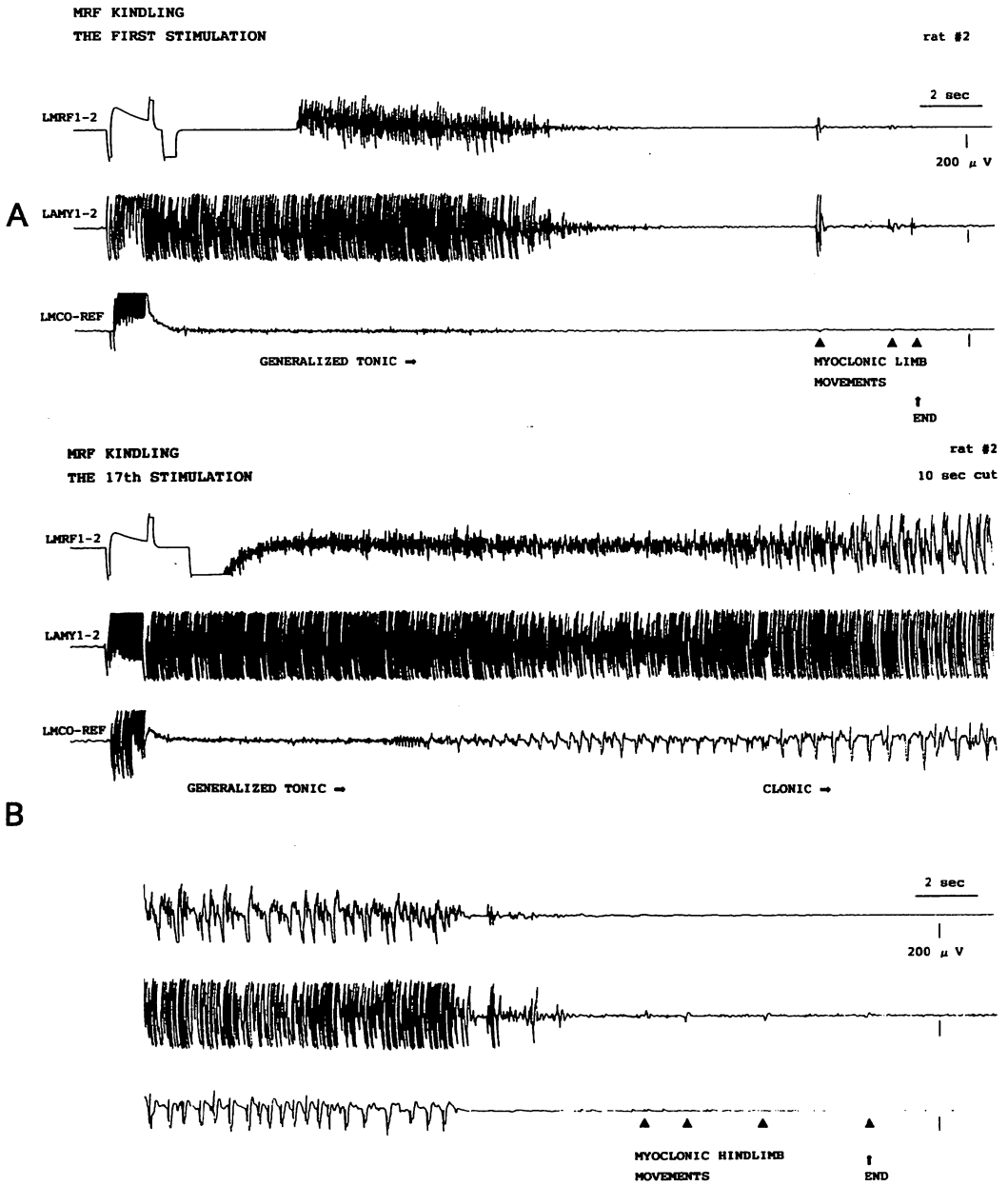

Fig. 1. A typical seizure development induced by repeated mesencephalic reticular formation (MRF)-stimulation. The rat displayed afterdischarges (AD) 17 times. A : First stimulation : AD threshold (ADT) 500 μ A/1 sec, a brief generalized tonic seizure, AD = 25 sec. B : Seventeenth stimulation : ADT 500 μ A/1 sec, a prolonged generalized tonic-clonic seizure, AD = 55 sec. LMRF, left MRF ; LAMY, left amygdala ; LMCO, left motor cortex ; REF, reference electrode.

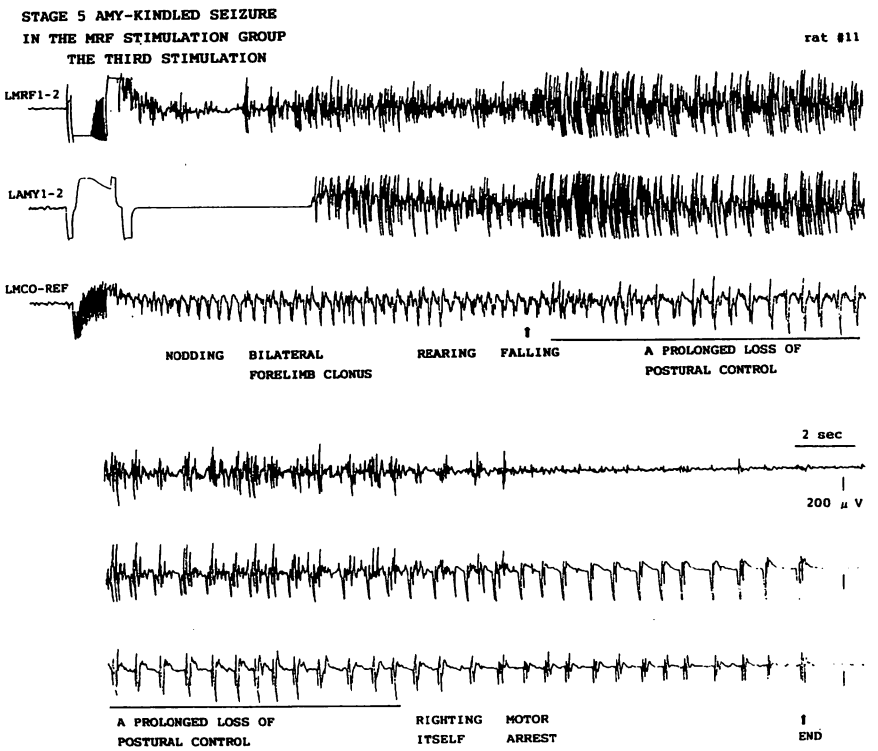

Fig. 2. Stage 5 amygdala (AMY) - kindled seizure in mesencephalic reticular formation (MRF) - kindled rat. At the third stimulation of left AMY, the rat developed a stage 5 seizure with a prolonged loss of postural control (18 sec) and generalized tonic seizure. LMRF, left MRF ; LAMY, left AMY ; LMCO, left motor cortex ; REF, reference electrode.

Discussion

MRF-kindled rats showed a progressive change in the behavioral seizure pattern and progressive AD growth involving the motor cortex. Furthermore, the MRF restimulation experiment indicated that the increased seizure susceptibility established by MRF kindling was persistent. These findings indicate that the MRF can be kindled effectively.

In the AMY kindling experiment, MRF-kindled rats reached stage 5 significantly faster than the control group rats. This finding supports the hypothesis that the vertical (limbic-brainstem) rather than horizontal pathways (interlimbic pathway through the forebrain commissures) are crucial for the development of the AMY kindling (Wada and Osawa,1974 ; Wada and Sato, 1974, 1975 ; Cain, 1985 ; Racine et al., 1986). The MRF -kindled group, on the other hand, had a significantly higher incidence of a tonic seizure

associated with a protracted loss of postural control (> 4 sec, range 5 – 18 sec) as compared with the control group during stage 5 seizures, suggesting that AMY kindling utilizes the increased seizure susceptibility of the brainstem induced by the previous MRF kindling.

We conclude that the MRF can be kindled effectively, and that MRF kindling has a facilitory influence on subsequent AMY kindling.

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Summary

We examined the effect of repeated mesencephalic reticular formation (MRF) -stimulation and its influence on subsequent amygdala (AMY) kindling in rats (n = 6). Five to 22 daily MRF stimulations at an afterdischarge (AD) -inducing threshold (200 – 500 μ A, 60 Hz biphasic square pulses, 1 sec) produced a progressive increase in AD duration and recruitment of behavioral seizures. Although the initial stimulation elicited only a brief tonic seizure, the final stimulation produced a prolonged, generalized tonic-clonic seizure (GTCS). Subsequent AMY kindling (3 of 6 rats) resulted not only in more rapid kindling, but also in tonic seizure associated with a prolonged loss of postural control (5 – 18 sec) not observed in animals undergoing AMY kindling without previous MRF kindling (n = 6). Furthermore, MRF restimulation (2 of the 3 MRF group rats), both at 24 hours and 30 days after AMY kindling, induced GTCS that was similar to the final GTCS of the MRF kindling session. These findings indicate that the MRF can be kindled effectively and that subsequent AMY kindling utilizes the proconvulsant neuroplastic changes that have been already established by MRF kindling.