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Amygdala Kindling in Rats with Brainstem Bisection
(脳幹両側切断ラットの扁桃体Kindling)

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Amygdala Kindling in Rats with Brainstem Bisection

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〈Introduction〉

A facilitory effect of amygdala (AM) kindling at the primary site (PS) on the AM kindling development at the contralateral secondary site (SS) has been called positive transfer effect (PTE)[10, 21]. In rats, total forebrain bisection (bisection of the corpus callosum, anterior and posterior commissures, massa intermedia, habenular and hippocampal commissures) prior to AM kindling had no effect on PTE[13]. It has been therefore assumed that the interhemispheric pathway achieving PTE in rat AM kindling is located in the brainstem[13]. Recent study suggested that the structural integrity of the ventral prepontine area might be crucial for PTE[11]. In the latter study, however, the small number of rats invites other studies to confirm these findings. In this paper, we reported the effect of the selective midsaggital brainstem bisection including the prepontine area on SS kindling as well as PS kindling in rats.

〈Methods〉

Twenty-six male hooded rats of the Royal Victoria Hospital strain (weighing 300–330g) were used. The animals were divided into three groups; the ventral bisection (n = 11), dorsal bisection (n = 9), and control group (n = 5).

In the two bisection groups, under pentobarbital anesthesia, the brainstem bisection and electrodes implantation were carried out stereotaxically[15]. The bisection was made by using a flexible arc-shaped blade (stainless steel wire : 32 G in diameter) which was inside the straight guide cannula (22 G in diameter). The method of ventral bisection is as follows : 1) at 5.0㎜ posterior from the bregma (the midline), the guide cannula was vertically inserted to point A (5.0㎜ from the skull), 2) the blade was pushed out

of the tip of the cannula to the caudal direction (3.5㎜), 3) the tip of the cannula was lowered to point B (8.5㎜ from the skull) and subsequently elevated to point A, and 4) the blade was completely pulled in and the cannula was vertically removed from the brain. In the dorsal group, point A and B was 1.0㎜ and 7.5㎜ from the skull, respectively. The control animals received the cannula insertion (7.5㎜ from the skull) without bisection. In all the three groups, bipolar electrodes made of twisted nichrome steel wire, 127 ㎛ in diameter, were implanted into AM bilaterally (2.3㎜ posterior, 4.8㎜ lateral, and 9.5㎜ ventral from the bregma).

Two weeks after the operation, the left AM (PS) was stimulated sequentially with a 1 sec train of constant current 60Hz sine wave beginning at 50 ㎂ with a 25 ㎂ step increase at 10min interstimulus intervals until localized afterdischarges (AD) was induced. The first current intensity to induce AD was designed as the AD threshold (ADT). AM kindling commenced with once a day stimulation at the ADT. The kindled seizures were classified by a modification of Racine[16], as follows : stage 1, rhythmic mouth and facial movement ; stage 2, rhythmic head nodding ; stage 3, unilateral forelimb clonus ; stage 4, bilateral forelimb clonus and rearing ; stage 5, falling. Kindling at PS was continued until four consecutive stage 5 seizures were produced. When a rat failed to reach stage 5 in spite of the appearance of seven consecutive stage 4 seizures, PS kindling ended. After a week's rest interval, the right AM (SS) kindling began in the same manner of PS kindling.

Upon completion of the kindling experiments, all animals were deeply anesthetised and their brains were perfused, serially sectioned (40 ㎛), and stained by cresyl violet.

〈Results〉

The animals tolerated the ventral or dorsal bisection well and no behavioral complications were found. There was no significant difference in body weight among the three groups at the end of the experiment (range ; 400 − 550g). The electrode placements in the amygdalae were verified by microscopic examination of the sections.

Table 1 provides a profile of the AM kindling. It was noteworthy that in the dorsal group five of nine animals failed to reach stage 5 at PS and SS, whereas all the rats of the control and ventral bisection group reached stage 5 at both sites.

There was no significant group difference in the ADT, number of stimulations for

stages 4/5, and the AD duration of the first stages 4/5 not only in PS but also in SS kindling (multiple comparison by Tukey's method). The ventral and dorsal bisection group, as well as the control group, showed a statistically significant acceleration of SS kindling in stages 4/5 and no significant difference was found in the saving rate of the number of stimulations to reach stages 4/5 among the three groups (multiple comparison by Tukey's method). Thus, the present bisections did not affect PTE.

Fig. 1 depicts the bisected areas of these groups. The areas of the two types of bisections ranged from the midbrain to the pons (5.0 − 8.5㎜ posterior from the bregma), overlapping one another. The five animals of the dorsal group, which did not reach stage 5, had the largest bisection area (Fig. 1B, solid lines).

		ADT (μA)	Number of stimulations to produce stages 4 and 5		AD duration (sec) at first stages 4 and 5	
			4	5	4	5
Ventral bisection						
(N = 11)	PS	56.8± 3.3	8.9± 0.8	10.5± 0.9	61.1± 6.7	57.2± 7.4
	SS	52.3± 2.2	3.0± 0.4* (S; 67%)	3.8± 0.4* (S; 64%)	69.5± 5.6	66.8± 4.6
Dorsal bisection						
(N = 9)	PS	55.6± 3.5	10.2± 0.6	10.8± 0.9a	60.7± 6.6	60.0± 3.7a
	SS	50.0± 0.0	2.6± 0.3* (S; 74%)	3.8± 0.4*a (S; 65%)	72.0± 8.0	71.3± 9.0a
Control						
(N = 5)	PS	60.0± 5.5	11.2± 1.7	12.0± 1.6	55.6± 8.6	57.8± 5.3
	SS	50.0± 0.0	3.4± 0.4* (S; 70%)	4.2± 0.5* (S; 65%)	56.6± 4.8	61.8± 4.8

Table 1 Kindling profile of the ventral bisection, dorsal bisection, and control group
Values are means ± SEM. AD, afterdischarges; ADT, afterdischarges threshold; PS, primary site; SS, secondary site; S, saving rate [A − B/A × 100 (%) where A is the mean number of stimulations at PS and B is the mean number of stimulations at SS]; *, $p < 0.01$ as compared with PS of each group (Wilcoxon one sample test); a, data obtained from four rats with stage 5 of the dorsal group.

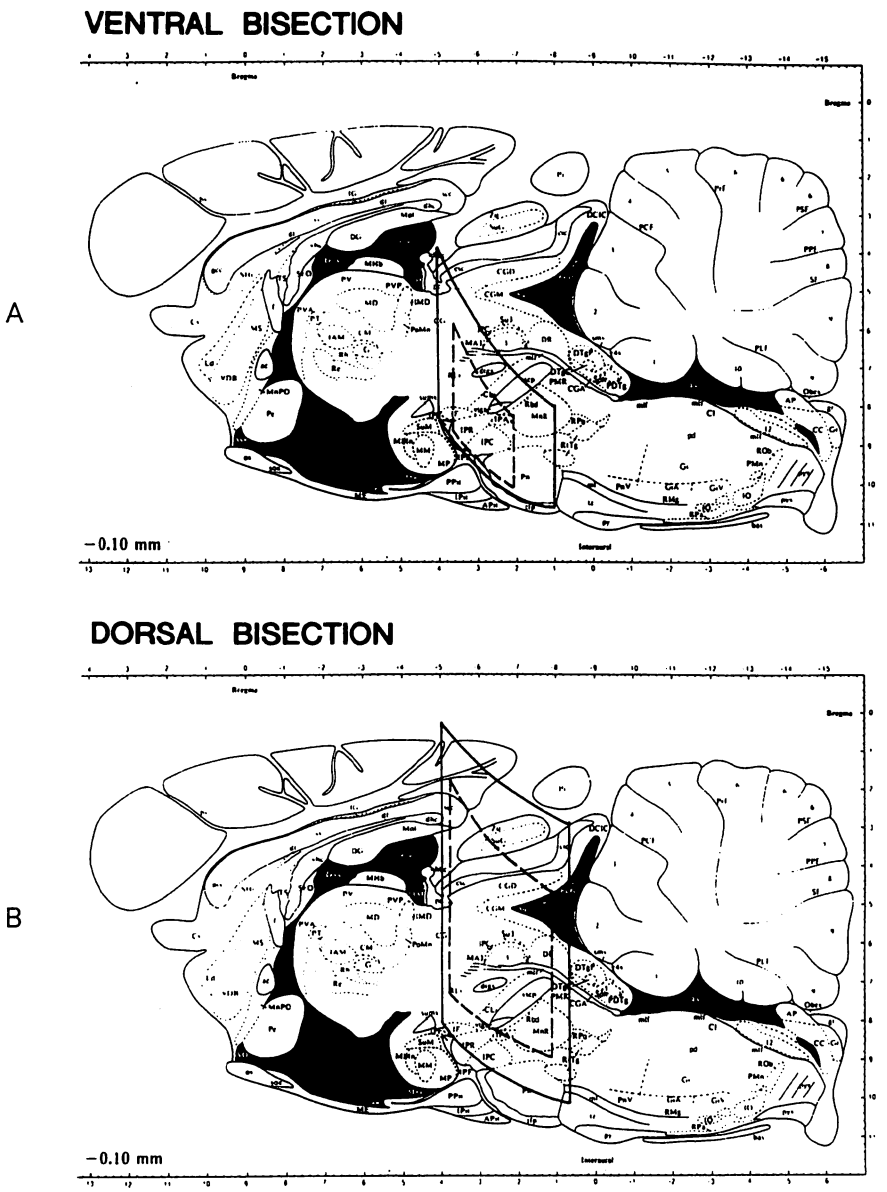

Fig. 1 Schematic diagram (taken from Paxinos and Watson[15]) showing the bisected area of the ventral (A) and dorsal (B) group. In each group, solid lines represent the area in the rat with the largest bisection, and broken lines that in the rat with smallest bisection. Five of 9 rats of the dorsal group, which did not develop a stage 5 seizure at PS and SS, had the largest bisection area.

〈Discussion〉

In this study, the brainstem bisection did not have an influence on the rate of AM kindling development (stages 4/5) at PS and SS. The present bisectioned structures of the brainstem, therefore, does not seem to be associated with AM kindling development and the mechanism of PTE in rats. In recent preliminary study in our laboratory, a) the midsagittal brainstem bisection did not affect AM kindling development at PS, while b) some rats with the bisection of the brainstem including the ventral prepontine area did not show PTE[11]. We could not confirm the latter finding b), although the ventral prepontine area was bisectioned in the present study.

We have recently found out that the midline electrolytic lesion of the midbrain retarded AM kindling at PS and inhibited PTE at SS in rats[8], suggesting that the midbrain structures (nuclei and/or tracts) not only regulate AM kindling development but also participate in the mechanism of PTE. This lesion study substantiates McIntyre's view[13] that the brainstem mechanism is important in producing PTE.

In the present study, several rats with almost complete bisection from the midbrain to the pons failed to develop a stage 5 seizure both at PS and at SS. These findings suggest that this bisection has an inhibitory influence on the seizure mechanism of a stage 5 which is characterized by falling as a result of the tonic extension of the trunk and hindlimbs. Tonic seizures are considered to depend on neural substrates in the brainstem but not require the integrity of the forebrain[1-7, 9, 12], although the specific substrates within the brainstem responsible for producing tonic seizures have not been ellucidated. The reticular formation (the midbrain to the pons) is likely to be involved in the manifestation of tonic convulsions, because electrical stimulation applied to the reticular formation triggers tonic convulsions[1, 7, 12], whereas lesions within this area can attenuate tonic convulsions[2-4, 6]. In feline AM kindling, an emergence of afterdischarges in the ipsilateral and then contralateral midbrain reticular formation during stage 3 (head nodding) ushered in a new phase of afterdischarges development that culminated in a pattern of total synchronization in the forebrain structures and the reticular formation to reach stage 6 (a generalized convulsive seizure with falling)[18]. In addition, unilateral lesions (ipsilateral to stimulated AM) in the midbrain reticular formation elevated the generalized seizure-triggering threshold and larger lesions in the midbrain reticular formation precluded the production of a stage 6 seizure in cats[20], while bisection of

the forebrain did not prevent inducing a stage 6 convulsion[19]. Considering these findings and anatomical evidence that there are complicated horizontal (i. e., traversing the midline) as well as vertical neuronal connections within the brainstem (the midbrain to the pons)[14, 15], it is possible that the extensive midline bisection in the brainstem may produce a function alteration in the reticular formation, so that the manifestation of a convulsion with falling is precluded. The bisection in the present study may result in lesioning of the dorsal and median raphe nuclei. These lesions, however, do not seem to participate in preventing a stage 5 seizure since midbrain raphe lesions were reported to facilitate the development of AM kindling in rats[17].

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Summary

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This study examined the effect of selective midsaggital bisection of the brainstem (the midbrain to the pons) on amygdala kindling at the primary site (PS) and the secondary site (SS). The development of SS kindling as well as that of PS kindling was unaffected by the bisection. Rats bisectioned extensively, however, failed to reach a final stage 5 seizure (i. e., a convulsion with falling) in both amygdala kindling. These findings suggest that in rat amygdala kindling the present bisectioned structures participate in the seizure mechanism of a convulsion with falling, while the specific region which is responsible for positive transfer effect is not located in the structures.