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Kindling of the Interpeduncular Nucleus in Rats
（ラットの脚間核のKindling）

Chiba Shigeru, Wada Juhn A.

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Shigeru Chiba[1] and Juhn A. Wada[2]

[1] Department of Psychiatry and Neurology, Asahikawa Medical College, Asahikawa 078, Japan

[2] Neurosciences and Neurology, Health Sciences Centre Hospital, University of British Columbia, Vancouver, B.C. V6T 2A1, Canada

〈Introduction〉

The role of the interpeduncular nucleus (IPN) in seizure mechanism remains unknown. In rats, lesions of IPN retarded the development of amygdala (AM) kindling in rats (Ackermann and Engel, 1978). Glucose utilization in IPN increased during Soman (potent acetylcholinesterase inhibitor) induced seizures (McDonough et al., 1983) or kainic acid induced seizures (Chastain et al., 1987). During a carbachol (acetylcholine agonist) kindled seizure, on the other hand, glucose utilization in IPN and brainstem tectum was reported to decrease (Wasterlain et al., 1981). In the postictal phase of AM kindling, the neural networks linking IPN was reported to play an active role in the mechanism of termination of the seizure (Namba et al., 1989). In this study, to ellucidate whether IPN is capable of evoking propagated seizure activity, or whether or how it plays a role in limbic seizures, we initially applied repeated electrical stimulation at an afterdischarges (AD)-inducing intensity to IPN in rats. As the stimulation resulted in progressive development of AD duration and behavioral seizures, we subsequently examined the effect of repeated IPN stimulation on AM kindling development in these animals.

〈Methods〉

Experiment 1 : IPN stimulation

Fourteen male hooded adult rats of Royal Victoria Hospital strain (weighing 300 – 350g) were used (IPN stimulation group, N = 9 ; IPN non-stimulation group, N = 5). Under pentobarbital anesthesia, bipolar electrodes made of twisted wire, 127 μm in diameter, were stereotaxically implanted into IPN (5.8mm posterior from the bregma, 9.0mm from the skull) and the left and right AM (2.8mm posterior and 4.8mm lateral from the bregma,

and 9.5㎜ from the skull) in all the rats of the non-stimulation and 7/9 rats of the stimulation group. In the remaining 2 rats of the stimulation group, surface electrodes (stainless steel screws) were implanted into the skull over the bilateral sensorimotor areas, and the bipolar electrodes described above were inserted into the IPN and left AM.

Two weeks after the operation, AD threshold was determined at IPN in the stimulation group. IPN was stimulated initially for 1sec at 200 μA (60Hz biphasic sine wave). If AD was not induced, the current was increased by 200 μA steps at > 10㎖in intervals up to 800 μA. Once AD was induced, the stimulation intensity was repeatedly delivered until 5 consecutive generalized tonic-clonic seizures or 15 times of AD were produced. If the intensity could not produce AD during the process, the intensity was raised in the following order ; 1000 μA/1sec, 600 μA/2sec, 800 μA/2sec, 1000 μA/2sec, 800 μA /3sec, and 1000 μA/3sec.

Experiment 2 : amygdala kindling

On the day following the last stimulation of IPN, animals received daily stimulation to the left AM (the primary site, PS) at AD threshold which was determined by beginning at 50 μA with a 25 μA step increase at 10㎖in interstimulus intervals. Stimulation to AM was repeated until five consecutive stage 5 seizures (described later) were observed. After a rest interval of 24 hours, the right AM (the secondary site, SS) kindling began in a manner identical to that of PS kindling. Finally, 24 hours after the completion of SS kindling, PS kindling was retested. Rats which received the same handling as the stimulation group received, without IPN stimulation, were used as the IPN non-stimulation group. The 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 ; stage 5, falling.

Upon completion of Experiment 2, the animals were deeply anesthetized and their brain were perfused, serially sectioned (40 μ㎜), and stained by cresyl violet.

〈Results〉

Experiment 1

The initial AD threshold at IPN was 400 − 800 μA (mean 600 μA) / 1sec, and the last

threshold was 600 – 1000 (mean 889 μA) / 1 – 3sec (mean 1.7sec). Thus AD threshold at IPN tended to increase slightly (no significance ; Wilcoxon one sample test).

The mean number of AD induced by IPN stimulation was 13.3 (11 – 15 times). The AD duration at IPN was markedly prolonged as the number of AD increased ; the initial and the last mean AD duration were 5.9sec (3 – 14sec) and 34.7sec (14 – 90sec), respectively ($p < 0.01$ by Wilcoxon one sample test).

Seven of 9 animals showed the developement of behavioral seizures, as well as the prolongation of AD duration, in the following order ; class I, motor arrest with tonic extension of neck and bilateral forelimbs ; class II, generalized bisynchronous tonic seizures consisting of tonic flexion of neck and trunk, tonic extension of forelimbs, and partial or complete extention of hindlimbs [in two rats the tonic seizure were followed by limbic kindling-like seizures (LKS) that were equivalent to stages 1 – 2 of AM kindling seizures] ; class III, class II followed by bisynchronous clonic seizures [in the two rats mensioned above the tonic-clonic seizures were followed by LKS which evolved to secondarily generalized seizures equivalent to stage 5 of AM kindling seizures)]. The remaining 2/9 rats stayed at class I but showed a prolongation of AD duration. Fig. 1 dipicts the typical development of seizures induced by IPN stimulation. AD propagated more extensively as the seizure class progressed, namely ; class I, AD appeared mainly in IPN ; II, AD in IPN and AM ; and III, AD in IPN, AM and sensorimotor areas (the latter data obtained from two rats with surface electrodes of sensorimotor areas).

Experiment 2

Table 1 shows a profile of AM kindling in the IPN stimulation and non-stimulation group. The AD threshold at PS, SS, and PS retested (PSR) between the two groups was not statistically significant. The stimulation group reached stage 5 significantly faster than the non-stimulation group in PS kindling, while there was no significant difference in the number of stimulations to reach stage 5 in SS and PSR kindling between these groups. No significant group difference was found in AD duration at first stage 5 of PS, SS, and PSR kindling. The stimulation group, on the other hand, had a significantly higher incidence of rats which exhibited a long loss of postural control with generalized tonic seizures (> 4sec, range 5 – 20sec) as compared with the non-stimulation group at stage 5 of all the kindling.

Histological examination showed that the electrode tips were in the intended structures.

〈Discussion〉

This study demonstrated that repeated IPN stimulation produced the progressive change of motor seizures and propagation of AD to previously non-involved structures (AM /sensorimotor cortices). In our preliminary study, the behavioral and electrographical changes elicited by the IPN stimulation persisted 14 days after the last stimulation, namely ; rats with consecutive class III seizures showed the same seizure after a rest interval of 14 days (Chiba and Wada, unpublished data). These findings suggest that IPN is capable of not only evoking propagated seizure activity but also kindling.

Neuroanatomical studies revealed that in the rat the major projections to IPN originated

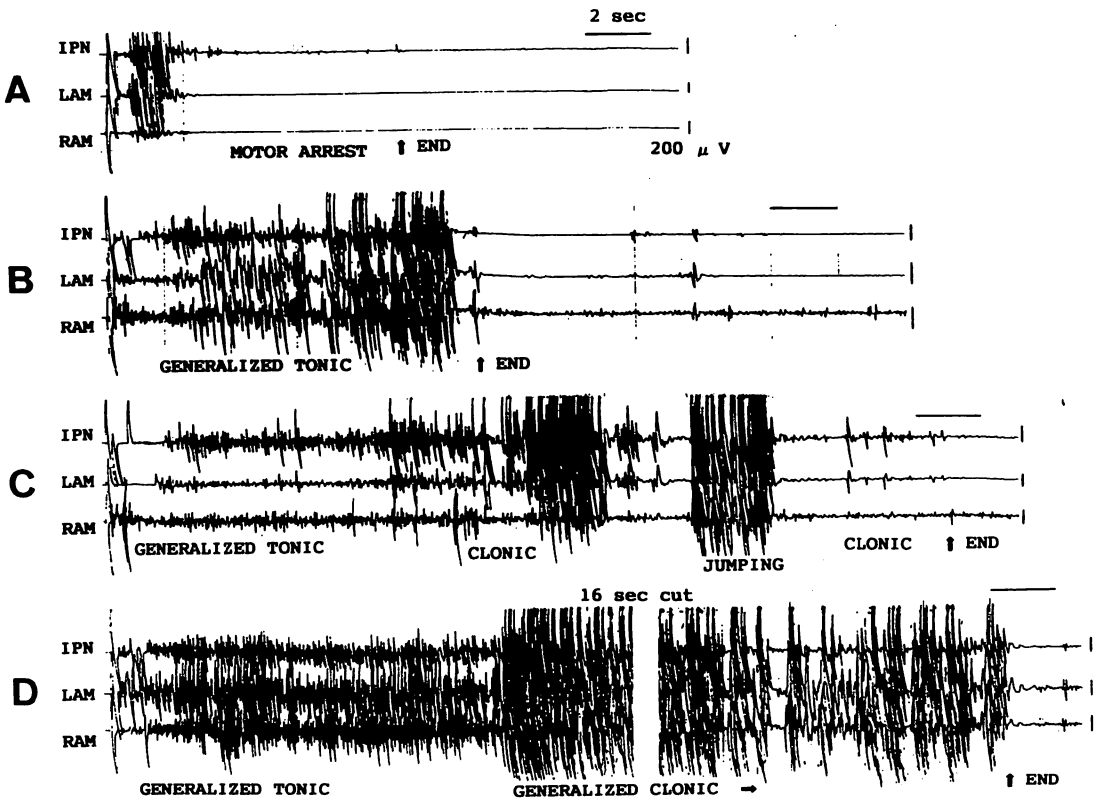

Fig. 1 A typical seizure development induced by the interpeduncular nucleus stimulation. The rat had a total of 11 times of afterdischarges (AD). A (1st, 600 μA /1sec), motor arrest, AD = 8sec ; B (3rd, 600 μA/1sec), a generalized tonic seizure, AD = 10sec ; C (7th, 1000 μA/1sec), a generalized tonic-clonic seizure, AD = 22sec ; D (11th, 1000 μA), a generalized tonic-clonic seizure, AD = 42sec. IPN, interpeduncular nucleus ; LAM, left amygdala ; RAM, right amygdala.

from the raphe nuclei, dorsal tegmental nucleus, medial habenular nucleus, and nucleus of the diagonal band of Broca (Marchand et al., 1980 ; Constabile and Flumerfelt, 1981 ; Sutherland, 1982 ; Hamill et al., 1984 ; Woolf and Butcher, 1985 ; Shibata et al., 1986 ; Nieuwenhuys et al., 1991), while IPN projected to the raphe, adjacent reticular formation, dorsal tegmental nucleus, metencephalic central gray, hippocampus, and septum (Sutherland, 1982 ; Shibata and Suzuki, 1984 ; Groenewegen et al. 1986 ; Nieuwenhuys et al., 1991). From these facts it may be deduced that some of these structures connected with IPN should be involved in the development of IPN kindled seizures.

Rats with IPN stimulation showed generalized convulsions consisting of tonic flexion of neck and trunk, tonic extension of forelimbs, and partial or complete tonic extension of hindlimbs. This configuration of the generalized convulsions is remniscent of that of generalized seizures triggered electrically from the mesencephalic reticular formation (Kreindler et al., 1958 ; Bergmann et al., 1981 ; Burnham et al., 1981 ; Maton et al., 1992). Furthermore, in rat dorsal tegmentum (the mesencephalic reticular formation) kindling in which electrical stimulations were applied daily for 40 days to the reticular formation of the mesencephalon at the threshold intensity necessary to elicit an EEG and tonic seizure response, the initial tonic seizures evolved into tonic-clonic fits with bilateral myoclonias following the tonic phase ; in some animals, the tonic seizures were followed

	ADT (μ A)	Number of stimulations to reach stage 5		AD duration (sec) at first stage 5	Number of rats with a long loss of postural control[a]	
P S						
Stimulation N = 9	50.0	3.2 (1-6)	*	56.3 (22-78)	8	**
Non-stimulation N = 5	55.0 (50-75)	11.0 (8-15)		54.4 (25-94)	0	
S S						
Stimulation N = 7	50.0	4.4 (3-6)		81.4 (39-132)	6	**
Non-stimulation N = 5	55.0 (50-75)	4.6 (2-8)		74.4 (40-100)	0	
P S R						
Stimulation N = 7	50.0	1.0		83.4 (22-128)	6	**
Non-stimulation N = 5	55.0 (50-75)	1.0		69.8 (40-106)	0	

Table 1 Kindling profile of the interpeduncular nucleus stimulation and non-stimulation groups. Values are means (numbers in parentheses ; range). a, > 4sec (range ; 5 – 20sec). *, $p < 0.01$ by Mann-Whitney U test ;**, $p < 0.01$ by χ^2-test. AD, afterdischarges ; ADT, AD threshold ; PS, primary site ; SS, secondary site ; PSR, primary site retested.

by occasional mastication, and clonic jerks of the face and the forelimbs (Maton et al., 1992). A similar profile of the seizure development was observed in IPN kindled rats. On the basis of these findings, it is assumed that IPN kindling involves the mesencephalic reticular formation in direct or indirect way, and that these two kindling from the mesencephalic reticular formation and IPN have a common neuronal substrate in the seizure development. However, there are some discrepancies between them, which are probably related to different brainstem structures involved in the initiation of the seizures: 1) the initial behavioral symptom was tonic seizures in the reticular formation kindling while it was motor arrest with tonic extension of neck and bilateral forelimbs in IPN kindling; and 2) high voltage epileptiform discharges in AM were not detected during the tonic seizures triggered from the mesencephalic reticular formation (Burnham et al., 1981) whereas the discharges in AM were observed during the tonic seizures elicited by IPN stimulation.

In our study, IPN kindled rats reached stage 5 significantly faster than the non-stimulation group in PS kindling. The IPN kindled group also had a significantly higher incidence of rats which exhibited a long loss of postural control with tonic seizures (> 4sec) as compared with the non-stimulation group in stage 5 seizures of any kindling. These findings suggest that AM kindling makes use of the proconvulsant neuroplastic changes in the brainstem substrate that have been already established by IPN kindling.

This positive transfer effect from IPN to AM supports the view that mainly vertical rather than horizontal pathways of the propagation of epileptiform discharges are crucial for the development of kindling (Wada and Sato, 1974, 1975; Wada and Osawa, 1976; Cain, 1985; Racine et al., 1986). How IPN plays a role in limbic seizures, however, remains unknown. Considering the fact that lesions of IPN retarded development of AM kindled seizures but did not prevent a stage 5 seizure (Ackermann et al., 1978), IPN does not appear to be part of a crucial seizure-conducting pathway. Instead, IPN is likely to participate in the seizure-regulating system.

In conclusion, we observed that repeated electrical stimulation of IPN resulted in the progressive change of motor seizures and propagation of AD to previously non-involved structures, suggesting that IPN was capable of kindling. IPN kindling played a facilitory role in subsequent amygdala kindling.

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Summary

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Shigeru Chiba[1] and Juhn A. Wada[2]

[1] Department of Psychiatry and Neurology, Asahikawa Medical College, Asahikawa 078, Japan

[2] Neurosciences and Neurology, Health Sciences Centre Hospital, University of British Columbia, Vancouver, B.C. V6T 2A1, Canada

To ellucidate the role of the interpeduncular nucleus (IPN) in the seizure mechanism, we examined the effect of repeated electrical stimulation of IPN on the behavioral and electrographical seizures in rats (N = 9). IPN was stimulated daily at an afterdischarges-inducing threshold (400 – 1000 μA, 1 – 3sec). The repeated appearance of AD (11 – 15 times) resulted in the development of the AD duration (9/9 rats) and behavioral seizures (7/9 rats) of which final form was generalized tonic-clonic seizures or those followed by limbic kindling-like seizures. On the other hand, rats with IPN stimulation showed a significantly more easily induced amygdala kindling and had a significantly higher incidence of rats which exhibited a long loss of postural control with tonic seizures (> 4 sec) as compared with rats without IPN stimulation (N = 5). These findings suggest that IPN is capable of evoking seizure activity and kindling (IPN kindling), and that AM kindling makes use of the proconvulsant neuroplastic changes in the brainstem substrate that have been already established by IPN kindling.