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Positive Transfer Effect of Amygdaloid Kindling in Developing Rats

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To study the hypothesis that seizure susceptibility in the young rat brain is higher than that in the adult brain, positive transfer effect (PTE) in amygdaloid kindling in rats was investigated at varing ages: 15 days, 18 days, 28 days, 40 days and 70 days. Although PTE was observed regardless of age, it was more pronounced in weaning rats than in adult rats.

Key words: amygdaloid kindling; development; rat; seizure susceptibility; transfer effect

Kindling is the progression of behavioral and electrographical seizure induced by repeated activation of neural pathways with initial subclinical stimulations (Goddard et al., 1969). Once kindling is established, the kindling effect is sustained for a long period without electrical stimulations. In addition, after the establishment of kindling at one site in the brain (primary site, PS), a significantly lower number of stimulations can induce kindling at another site (secondary site, SS), called the "positive transfer effect" (PTE) (Goddard et al., 1969; Racine, 1972; Cain, 1985). These data suggest that the separate secondary epileptic foci can be created with minor stimuli in comparison to the original stimuli required for the induction of the 1st focus.

We previously reported that young rats developed amygdaloid kindling with a smaller number of stimulations and had a higher susceptibility than adult rats (Matsuda et al., 1993, 1995). Difference of the PTE between young and adult brains may add the deeper understanding on the age dependency of kindling induction in rat brains, but only reports on 16-day-old rats (Kawawaki et al., 1987) and 20-day-old rats (Chiba et al., 1994) are available to date. No reports are available on the PTE in the younger rats. To shed a light on a working hypothesis that the young rat brain is more susceptible to kindling, we investigated the PTE in rats at various ages, including those before weaning.

Materials and Methods

Animals

Male Wistar rats aged 70- [n = 6] postnatal days (referred to as adult rats) and rats of either sex aged 15- [n = 5], 18- [n = 6], 28- [n = 5] and 40- [n = 5] postnatal days (referred to as young rats) were used in these studies. The animals were kept in an environmentally controlled room (12/12 h light/dark cycle, lights on at 0700, temperature 20–22˚C, humidity 30–40%) in the Animal Care Center at Tottori University, with food and water available ad libitum except during the experimental procedure. All animal experiments were performed

Abbreviations: AD, after-discharge; ADD, AD duration; ADT, AD threshold; PS, primary site; PTE, positive transfer effect; SS, secondary site

in accordance with the Guidelines set out by the Tottori University Committee for Animal Experimentation.

Kindling preparation

Bipolar stainless-steel electrodes were implanted 3 days prior to the kindling stimulations in the 15-, 18- and 28-day-old groups, and 7 days prior in the 40- and 70-day-old groups. The rats were anesthetized with pentobarbital (Nembutal, Abbot, Chicago, IL; 25 mg/kg intraperitoneally for young rats and 50 mg/kg intraperitoneally for adult rats) and then placed in a streotaxic apparatus with the skull set level. Bipolar stainless-steel electrodes (0.1 mm in diameter for young rats and 0.2 mm in diameter for adult rats) were implanted bilaterally into the amygdala according to the streotaxic atlas of Sherwood and Timiras (1970) for young rats and König and Klippel (1979) for adult rats: the site of implantation in the both amygdala to the bregma was 1.8 mm posterior, 3.5 mm lateral and 7.2 mm inferior in 15- and 18-day-old groups; 2.0 mm posterior, 3.8 mm lateral and 7.4 mm inferior in the 28-day-old group; 2.5 mm posterior, 3.8 mm lateral and 7.8 mm inferior in the 40-day-old group; and 3.0 mm posterior, 4.8 mm lateral and 8.8 mm inferior in the 70-day-old group. A reference electrode was placed in the frontal sinus in the young rats, and in the frontal bone in the adult rats. All electrodes were secured to the skull with dental cement.

Kindling procedure

Kindling procedure followed according to the method described by Yoshioka et al. (2000). Briefly, the after-discharge (AD) threshold (ADT) was determined by 60 Hz electrical stimulation for 1 s beginning at 50 μA and increasing its intensity by 50 μA every 10 min until AD was elicited. After determining the ADT, the rats were subjected to stimulation at an ADT for 1 s every 30 min in the 15- and 18-day-old groups; for 3 s every 30 min in the 28-day-old group; and for 3 s every 60 min in the 40- and 70-day-old groups until Stage-5 seizures appeared 5 times. The 5 appearances of Stage-5 seizures were regarded as a completion of kindling. In the present study, the inter-stimulation intervals and durations of stimulation were different among the rats at respective ages because the PS (right) kindling and SS (left) kindling must be completed within a single day. After one day following completion of the PS kindling in 15-, 18-, 28-, 40- and 70-day-old groups, SS kindling was performed until Stage-5 seizures appeared.

It is known that the behavioral manifestations of amygdaloid kindling seizures in young rats were different from those in adult rats (Moshé, 1981). Therefore, we chose 2 different classification scales of kindling seizures for young and adult rats. The behavioral seizure stages were assessed with the classification proposed by Moshé (1981) for 15-, 18- and 28-day old groups: Stage 0, no manifestations; Stage 1, facial movements; Stage 2, head turning to stimulation site or head nodding; Stage 3, alternating independent forelimb clonus or unilateral forelimb clonus; Stage 4, bilateral clonus or rotatory movements of tonically extended forelimbs and Stage 5, loss of balance; and by Racine (1972) for 40- and 70-day-old groups: Stage 0, no manifestations; Stage 1, mouth and facial movements; Stage 2, head nodding; Stage 3, contralateral forelimb clonus; Stage 4, bilateral forelimb clonus with rearing and Stage 5, rearing and falling. Electrographic recording in the amygdala was also performed before and during kindling stimulation, and the AD durations (ADDs) elicited by each stimuli were measured. ADDs elicited by the 1st ADT stimulation at PS kindling and SS kindling (1st response ADDs) and at the 1st Stage-5 seizure (1st Stage-5 ADDs) were measured. Locations of the electrodes tips were confirmed histologically.

Data analysis

All data were expressed as mean ± SEM. Statistical analysis of the data was performed using Wilcoxon's rank sign sum test, Student's paired *t*-test, Kruskall-Wallis' test and one-way analysis of variance followed by post hoc analysis of Fisher's protected least significant difference.

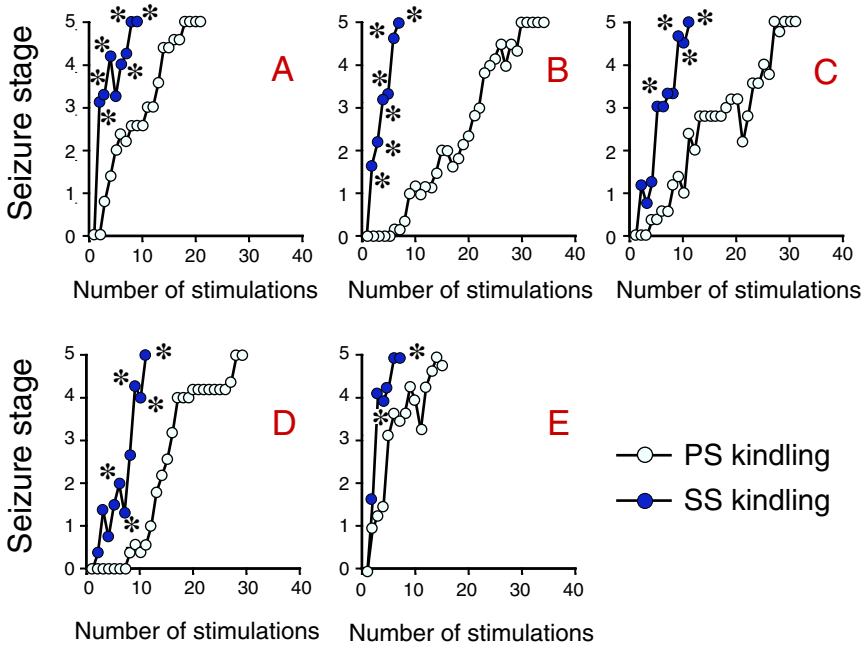

Fig. 1. Amygdaloid kindling development stimulated at the primary site (PS) and the secondary site (SS) in young and adult rats aged 15 (**A**), 18 (**B**), 28 (**C**), 40 (**D**) and 70 days (**E**). The group mean seizure stages are plotted over the number of stimulations in rats. Significance of differences in the seizure stages at equivalent number of stimulations compared with PS of each group are indicated by asterisks (*$P < 0.05$) as obtained from Wilcoxon's rank sum singed test.

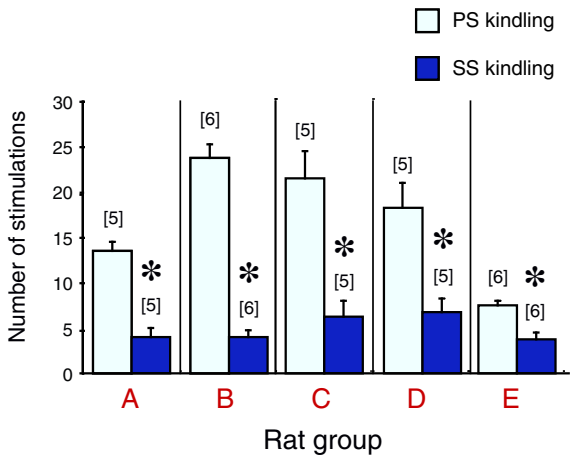

Fig. 2. Comparison of the number of stimulations required to the 1st Stage-5 seizure between the primary site (PS) and the secondary site (SS) kindling in rats at different ages of 15 (**A**), 18 (**B**), 28 (**C**), 40 (**D**) and 70-days (**E**). At every age, the number of stimulations required to the 1st Stage-5 seizure is significantly smaller at SS than PS. Values are mean ± SEM. Brackets indicate the number of animals. Significance of differences in the number of stimulations until the 1st Stage-5 appearance compared with PS kindling are indicated by asterisks (*$P < 0.05$) as obtained from Wilcoxon's rank sum singed test.

Results

Figure 1 shows kindling development stimulated at the PS and SS in 5 groups of 15-, 18-, 28-, 40- and 70-day-old rats. Kindling progressed more rapidly at the SS than at the PS in all groups.

The number of stimulations required to reach the 1st Stage-5 seizure (kindling rate) was compared in PS and SS kindling at each age: the rate was significantly lower in SS kindling ($P < 0.05$) than in PS kindling in all groups (Fig. 2). The kindling rate during PS kindling of the 5 groups in the age-increasing order was 13.4 ± 1.1, 23.5 ± 1.5, 21.2 ± 3.0, 18.2 ± 2.7 and 7.3 ± 1.4, respectively. The 70-day-old group was significantly faster in kindling than 15- ($P < 0.05$), 18- ($P < 0.0001$), 28- ($P < 0.0001$) and 40-day old groups ($P < 0.001$). Among younger groups, the 15-day-old group was significantly faster in kindling than 18- ($P < 0.01$) and 28-day-old groups ($P < 0.05$).

Table 1. After-discharge durations (ADDs) at 1st after-discharge threshold stimulation and 1st Stage-5 appearance at primary site (PS) and secondary site (SS) kindling in rats at different ages

Rats	Kindling	First response ADDs (s)	First Stage-5 ADDs (s)
15-day-old [5]	PS	16.4 ± 1.4 *	82.4 ± 10.3
	SS	39.0 ± 5.8 *	118.0 ± 27.6
18-day-old [6]	PS	12.3 ± 1.1	74.2 ± 12.5
	SS	23.0 ± 14.1	74.8 ± 12.5
28-day-old [5]	PS	12.2 ± 1.5 *	60.2 ± 13.2
	SS	35.6 ± 9.1 *	70.4 ± 14.3
40-day-old [5]	PS	15.0 ± 3.9	74.8 ± 15.1
	SS	20.0 ± 5.3	65.2 ± 16.4
70-day-old [6]	PS	21.8 ± 5.0	106.0 ± 17.8
	SS	41.5 ± 18.3	91.5 ± 19.9

Values are mean ± SEM.
[ ], the number of animals.
* $P < 0.05$ by Studen's paired *t*-test.

The ratio of the kindling rate in SS kindling to that in PS kindling (SS/PS) was analyzed: the 5 groups showed, in the age-increasing order, 0.30 ± 0.10, 0.17 ± 0.03, 0.35 ± 0.12, 0.36 ± 0.09 and 0.51 ± 0.03, respectively. Analysis of variance indicated significant differences in SS/PS ratio between young and adult groups [$F(4, 22) = 3.033$; $P < 0.05$]. The SS/PS level of the 18-day-old group was significantly lower ($P < 0.01$) by post hoc analysis than that of the 70-day-old group.

Table 1 compares ADDs at the time of 1st stimuli and 1st Stage-5 development between PS and SS kindling: differences in 1st-response ADDs between PS and SS kindling were significant in the 15- ($P < 0.05$) and 28-day-old groups ($P < 0.05$), but not in the other groups. In addition, ADDs at the 1st Stage-5 appearance did not differ between PS and SS kindling among all groups.

Discussion

Most studies on PTE have been of adult animals. In the present study, we investigated whether PTE may occur age-dependently in developing rats. The kindling rate in 70-day-old adult rats was significantly faster in SS kindling than in PS kindling. Furthermore, the rate in younger rat groups was significantly faster in SS kindling than in PS kindling. These results indicate that PTE in amygdaloid kindling occurred in developing rats at various ages as well as adult rats.

Moshé et al. (1995) reported that postnatal age-related changes in seizure susceptibility were not linear. The seizure susceptibility to amygdaloid kindling in rats was decreased in the 1st postnatal week, but increased in the 2nd and 3rd weeks. In addition, 30- to 35-day-old rats were resistant to the development of amygdaloid kindling (Moshé, 1981; Moshé et al., 1981) and kainic acid-induced seizures (Albala et al., 1984). In the present study, the 70-day-old group reached Stage 5 more rapidly as compared with other groups. Further, the 15-day-old group also reached more rapidly as compared with 18- and 40-day-old groups. Hence, it seems that adult rats have an increased seizure susceptibility as compared with young rats. However, Chiba et al. (1992) reported no differences in kindling rate among 20-, 30- and 60-day-old rats when hourly electrical stimulation at a same suprathreshold current level for 1 s was applied in all groups 10 times daily. Moshé (1981) also reported that when the rats received the same electrical stimulation at a 400-μA current for 1 s hourly, the kinding rates were similar among 15-, 17-, 18-, 48- and 72-day-old rats, whereas more stimulations were necessary for the 34-day-old rats. The reasons for the different rates of progression

between the literature (Moshé, 1981; Chiba et al., 1992) and our results can be attributed not only to characteristics related to differences in age but also to differences in experimental conditions, such as inter-stimulation intervals, durations of stimulation, strength of stimuli or implantation sites of electrodes.

In order to examine the seizure susceptibility at a precise age, we performed kindling with the stimulation conditions set to the extent possible so that the kindling stimulations at PS and SS kindling would be completed within 1 day for each of the age. However, the experimental conditions could not be made entirely consistent. Firstly, the inter-stimulation intervals were different among groups. Moshé et al. (1983) reported that stimulations at 60-min intervals induced kindling of the amygdala in both adult and sucking rats. However, stimulations at 15-min intervals disrupted kindling in adult rats, but induced kindling in suckling pup rats. These results suggest that the pup rats appear to have relatively short seizure refractory periods. Therefore, the 40- and 70-day-old rats received electrical stimulation of longer inter-stimulus intervals as compared with the 15-, 18- and 28-day-old rats. Secondly, the durations of stimulation were also different among groups. Holmes and Thompson (1987) have found that in rapid kindling model, 29-day-old rats kindled when the duration of stimulation was 10 s. However, the animals stimulated with 1-s stimulation did not. They suggested that the key factor in rapid kindling was the duration of stimulation. Thus, we chose the longer duration of stimulation for kindling procedure for the 28-, 40- and 70-day-old groups than for the 15- and 18-day-old groups. Thirdly, Moshé et al. (1981) also reported that the ADTs varied with age, being highest in the suckling rats, lowest in the 35-day-old rats and intermediate in the 60- and 85-day-old rats. However, previous studies (Goddard et al., 1969; Racine, 1972) have demonstrated that the kindling development did not significantly differ at the current intensities used for their stimulus between the ADT level and the suprathreshold level. In the present experiments, we chose the ADT levels as the strength of stimuli in all the groups. However, the possibility that the differences in the durations of stimulation and strength of stimuli among the groups influence the kindling development needs to be taken into account. Finally, it is well known that the rate at which the animals developed kindled seizures showed some differences according to the regional distribution of the electrode site (Goddard et al., 1969). In the present experiments, the electrode tips were located in the amygdala in all groups. The coordinates of implantation of electrodes to the bregma were somewhat different among all groups; therefore, the possibility cannot be excluded that the present age-related changes in the kindling rats were due to the differences in the sites of implantation.

Increased seizure susceptibility in the young brain has been obtained in experiments including studies on amygdala kindling (Moshé, 1981; Moshé et al., 1983), hippocampal kindling (Haas et al., 1990), or kainic acid- (Albara et al., 1984) or pentylentetrazole- (Weller and Mostofsky, 1995) induced seizure. In addition, Haas et al. (1990) have found that young rats receiving alternating stimulations in the amygdala and hippocampus develop progressively more severe seizures than adult rats, indicating that the young brain might be less able to suppress the generalization of seizures than the adult brain. Our observations on PTE revealed that SS/PS ratios were significantly smaller in the 18-day-old rats than in the adult rats when the kindling rates in PS and SS kindling were compared for calculating SS/PS ratios. However, there were no significant differences in SS/PS ratios between the 15-, 28- or 40-day-old rats and 70-day-old ones. These results indicate that the savings in the number of stimulations required to produce the 1st Stage-5 seizure during the SS kindling were larger only in weaning rats than in adult rats. Chiba et al. (1994) analyzed SS/PS ratios until the appearance of generalized convulsions and the prolongation of ADDs at each stage in SS kindling: based on the findings, they reported that PTE was more strongly manifested in 20-day-old rats than in mature rats (Chiba et al., 1994): among our data, those of the 18-day-old group

alone were consistent with theirs. Le Gal La Salle (1981) analyzed the relationship between the initial ADDs and the kindling rate, and observed that rats which initially responded to the amygdaloid stimulation by long AD tended to kindle faster than those which responded by short AD. In the present study, the kindling rates in SS kindling were shorter than in PS kindling, but ADDs at 1st ADT stimulation in SS kindling were not always longer than in PS kindling. In addition, comparisons of ADDs showed no difference in excitability. These results were in disagreement with those reported by Chiba et al. (1994). Absence of increased ADDs at the 1st Stage-5 seizure during SS kindling suggests that increased ADDs are not a necessary correlate of enhanced kindling susceptibility.

What are the factors that may be responsible for the enhanced seizure susceptibility of the young brain? In young rats, the level of brain norepinephrine is lower than in mature rats (Milby et al., 1979; Morris et al., 1980), and the γ-aminobutyric acid inhibition system in the substantia nigra in the former is less well developed (Wurpel et al., 1988). Recently, we have found that α-2 agonist clonidine facilitates kindling development in 14-day-old rats, whereas it retards in adult rats (Yoshioka et al., 2000). The increased susceptibility to kindling development in young rats might be due to these ontogenetic changes in the neurochemical system.

Some reports have suggested involvement of the brain stem in generalized convulsions and PTE in mature rat amygdaloid kindling (McIntyre, 1975; Cain, 1985; Chiba and Wada, 1997). Chiba and Wada (1997) reported that electrolytic lesioning of the midbrain prior to amygdala kindling resulted in not only retardation at PS kindling but also abolishment of PTE. These results suggest that the brain stem participates not only in the development of PS kindling but also in the mechanism of transhemispheric PTE. In the present study, it was found that the PTE was observed in rats regardless of ages. These findings suggest that the epileptiform activity at the PS developed into the contralateral hemisphere via the brain stem in rats at various ages. Thus, a similar study of the brain-stem lesioning reported by Chiba and Wada (1997) may give some answers on the role of the brain stem responsible for the PTE formation in young rats.

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