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Neonatal damage to neocortex abolishes the anxiolytic action of diazepam in adult rats

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Abstract

A neonatal cerebral cortical lesion was made in rats and the effects of diazepam on ultrasonic isolation calls in pups and footshock-elicited ultrasonic distress calls in young adult rats were assessed. There was no indication that the cortical lesion influenced the production of the ultrasonic distress calls in either pups or adults. Diazepam attenuated the ultrasonic isolation calls in all the pups with and without cortical lesion, and the distress calls in normal adult rats. However, diazepam failed to exert the effect in rats which received a neonatal cortical lesion. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), another anxiolytic, was effective to diminish the distress calls even in the adult rats which had had the neonatal damage to the cortex. These findings indicate that the intact cerebral cortex is not always required for production of ultrasonic distress calls; however, the development of the neuronal mechanism involving benzodiazepine receptors to inhibit the ultrasonic expression of anxiety or fear in adult rats is dependent on the integrity of the cerebral cortex.

Keywords: Ultrasonic distress call; Cerebral cortex; Lesion; Anxiolytic mechanism; Diazepam; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide)

1. Introduction

It is well known that rat pups and adult rats emit ultrasonic signals when they need to deliver conspecific messages (Sales and Pye, 1974; Brown, 1976; Nyby and Whitney, 1978; Miczek et al., 1991). The ultrasonic vocalizations which are most likely to form a means of purposeful communication are distress calls. Rat pups produce a train of pulses of 100–300 ms duration with 30–50 kHz sound frequencies when they are isolated from the mother or nest (Noirot, 1968; Sewell, 1970; Allin and Banks, 1972). The ultrasonic calls are potent stimuli for maternal retrieval (Smotherman et al., 1974; Bell, 1979). Such ultrasonic calls decline sharply in the third week after birth (Noirot, 1968; Okon, 1972; Naito and Tonoue, 1987) and never appear in the adult. The adult rat emits ultrasounds with a frequency of 20–30 kHz and duration up to 1.5 s frequently when they take a posture of submission to attacking opponents (Takahashi et al., 1983; Takeuchi and Kawashima, 1986; Miczek et al., 1991), anticipate pain (Tonoue et al., 1986; Sales et al., 1986; Van der Poel et al., 1989) or become startled at acoustic stimuli (Kaltwasser, 1990).

It has been demonstrated that the ultrasonic vocalization in the rat pup is markedly attenuated by treatments with various anxiolytic drugs (Gardner, 1985a,b; Insel et al., 1986; Gardner and Budhrum, 1987; Mos and Oliver, 1989; Carden and Hofer, 1990; Winslow and Insel, 1991a). Also, the ultrasonic distress calls in adult rats were found to be diminished following treatments with benzodiazepines, opiates and 5-HT receptor agonists which are representative anxiolytic substances (Tonoue et al., 1986, 1987; Sales et al., 1986; Cuomo et al, 1988; Van de Poel et al., 1989; Vivian and Miczek, 1990). Therefore, ultrasonic vocalizations of both infant and adult rats have been thought to be a promising animal model for the study of the anxiogenic or anxiolytic brain mechanism, and as a screening method for anxiolytics (Kaltwasser, 1991; Winslow and Insel, 1991b).

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However, there exists a striking difference in ultrasonic vocalization between adult rats and pups. Whereas rat pups under 16 days of age unexceptionally emit the ultrasound when they are isolated and exposed to low ambient temperature, an extensive variation among individual adult rats exists in the production of ultrasonic distress calls when they are confronted with a provocative situation (Tonoue et al., 1986; Sales et al., 1986). The generation of ultrasonic expression of the distress state in adult rats thus seems to be more dependent on the individual life history of the animals. Although our attempts to identify relevant causes to differentiate the ultrasonic vocalization responses in adult rats have so far been unsuccessful, it seems reasonable to assume that some individuality in expressing distress is ontogenetically forged.

The situation described above led us to assess the role of the cerebral cortex in the ultrasonic distress calls in the rat. As far as we know no information is available on the control by the cerebral cortex of the ultrasonic distress calls in the adult rat. We report here that neonatal lesion of the cerebral cortex does not impair the production of the ultrasonic distress calls in either pups or adult rats, but does nullify the attenuating effect of diazepam, a benzodiazepine anxiolytic, on the ultrasonic distress calls in the adult rat.

2. Materials and methods

2.1. Subjects

Male and female Wistar-Imamichi strain rats from 14 litters produced in this laboratory were used. They were maintained in a temperature (21 ± 1°C)- and light (0700–2100 h)-controlled room with free access to food and water. On the day after parturition (day 1), each litter was culled to 8 pups (balancing the sex ratio as closely as possible). The pups were then randomly assigned to five treatment groups with the constraint that no littermate of the same sex was assigned to the same group.

The groups were (1) intact; (2) sham-operated; (3) lesion of the right cortex; (4) lesion of the left cortex; and (5) bilateral lesion of the cortex. The animals were raised in family groups until weaning on day 22. Thereafter, they were raised in groups of 4–5 rats of the same sex.

2.2. Surgical procedure

The lesion was made by removing the cortical tissue on day 2. Anesthesia was induced by cooling the pup's whole body in a freezing box (−5°C). The pup was placed on a cooling pad (−5°C) during surgery. This procedure induced hypothermia in the pup (10–15°C) and no reaction or response to nociceptive stimuli was evoked whatsoever. For making bilateral lesions of the cerebral cortex, incisions were made bilaterally at 2 mm lateral to the midline at the cranial bone between the bregma and the lambda. Aspiration of the cortical tissue was done with a polyethylene tube (o.d. 2 mm, i.d. 1.5 mm) with a diagonally cut opening. For making bilateral lesions an attempt was made to remove as much of the cortical tissue under the incision as possible and no attention was paid to avoiding damage to the hippocampus.

For making a relatively small unilateral lesion in the cortex, a small incision was made at the right or left cranial bone and care was taken to remove only the cerebral cortical tissue under the incision.

Sham-operated animals were anesthetized by cooling as described for the lesioned pups and received bilateral incisions in the cranial bone. After receiving treatment for the wounds, the animals were warmed in water at 36°C and returned to maternal care. There was no subject mortality following surgery.

2.3. Induction and measurement of ultrasonic vocalization

2.3.1. Isolation calls of pups

Measurement of ultrasound emission of isolated pups was made on day 6 or day 9. Since the sex difference in the ultrasonic vocalization activity in pups is not great (Naito and Tonoue, 1987), pups of both sexes were used in this study. The procedure for measurement was the same as previously described (Naito and Tonoue, 1987). Briefly, the pup was removed in isolation from littermates to a 1000 ml glass beaker with as bedding a 1 cm thick paper towel. The beaker was then placed 10 cm below a microphone in a sound-attenuated and cooled (10 ± 2°C) chamber. Ultrasound measurement started at 1 min after the door of the chamber was closed.

2.3.2. Ultrasonic distress calls of adult rats

Animals 60–70 days old were put in an unescapable cage (17 × 21 × 18 cm) with a grid floor. The cage was placed 10 cm below a microphone. The adult rat then received a series of electric footshocks (1 s, 2 mA) at intervals of 1 min (10 shocks at most). When the rat squeaked or emitted ultrasonic distress calls for longer than 1 min during the delivery of footshocks, delivery was interrupted in order to minimize the averse stimuli and to enhance the acquisition of ultrasonic vocalization for avoiding the stimuli. When the rat did not emit ultrasounds, another series of stimulations was delivered next day. Only those rats which came within 4 series of footshock delivery, to show an ultrasonic vocalization response for longer than 3 min after being put in the stimulation cage or receiving the first single

footshock were used in assessing the effect of anxiolytic drugs. The measurement of ultrasound emitted by adult rats started 1 min after the door of the chamber was closed and when the first shock was delivered.

Ultrasonic waves transduced by a condenser microphone (Kunitachi Acoustic Lab, ACM-20F) were amplified and fed to an electronic device (Diamedical System, DMP-350) which measures the average frequency and average amplitude of sine waves in 5-ms time bins and successively outputs them to a personal computer in realtime through an A/D converter. Sound production with frequencies higher than 20 kHz was continuously monitored on a computer screen. For the waves with frequencies higher than 20 kHz, the average amplitude in a 5-ms time bin was accumulated for 3 min after starting measurement. The data are therefore combined products of the intensity and duration of ultrasonic vocalization. The data were expressed in arbitrary units.

2.4. Assessment of effect of drugs

Ultrasonic isolation calls of pups were measured for 3 min in the morning of day 6 and day 9. The pup was returned at once to maternal care. Then, 2 h later, diazepam (Yamanouchi Seiyaku, Japan) was subcutaneously injected at the nape of the neck at a dose of 1.0 mg/kg (0.5 mg/ml suspension in 0.9% saline with 1% Tween 80). The isolation calls were measured for 3 min 10, 30, 50, and 120 min after diazepam injection.

The shock-elicited ultrasonic distress calls in adult rats 70–90 days old were measured for 3 min in the morning of the day following the day when the vocalization response was obtained. Two hours later diazepam prepared as described above or 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, RBI, USA) was subcutaneously injected. 8-OH-DPAT was dissolved in 0.9% saline and injected at a dose of 0.01 or 0.5 mg/kg. The volume injected was 2 ml/kg. Ultrasound measurement was repeated 10, 30, 60 and 120 min after the injection.

The effect of drugs on the ultrasonic vocalizations was expressed quantitatively as the vocalization activity after injection as a percentage of that before injection on the same day in each rat. Statistical analysis was made by means of a Mann-Whitney non-parametric two-tailed test.

2.5. Morphology

After completion of the measurements, the rats were given a lethal dose of sodium pentobarbital and were perfused with 10% formol-saline solution. The brain was removed and fixed in 10% formalin. The lesion of the brain cortical surface was macroscopically examined and recorded. The brain was then cut coronally at the center of the lesion and the depth of lesion was examined.

3. Results

The location and extent of the cortical lesion varied among rats. In the bilateral lesion group, the lesion was found at the caudal third of area 1 and area 2 of the frontal cortex, at most of the hindlimb area and agranular retrosplenial cortex. In some rats the lesion was found also at the area 2 mediomedial part of the occipital cortex (according to the cortical map by Zilles, 1985). In these rats damage of various extent to the hippocampus was also observed (Fig. 1). In the unilateral lesion groups, on the other hand, the lesion was found at the hindlimb area in most rats. In some rats the lesion extended to the frontal edge of the occipital

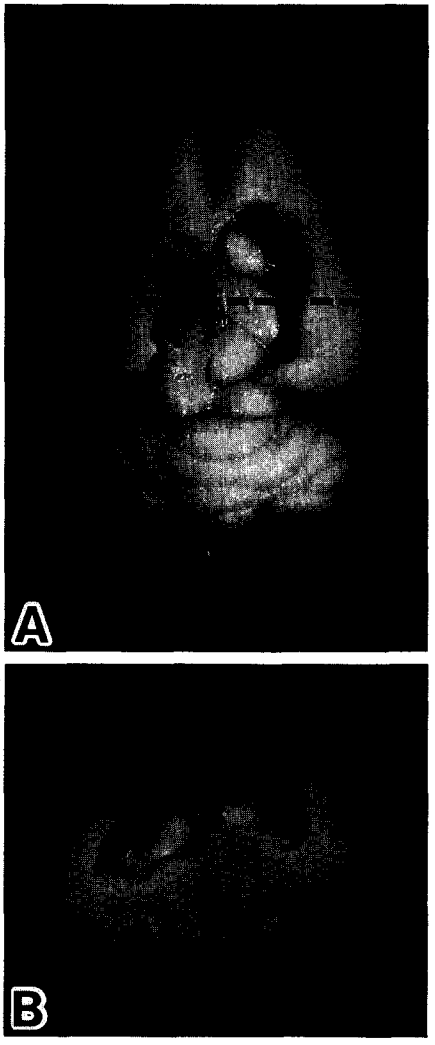

Fig. 1. Representative bilateral lesion of the cerebral cortex. A: Dorsal view. B: Sagittal view of the cut surface indicated by broken line on the dorsal view.

Table 1
Ultrasonic (US) isolation calls in rat pups with and without cortical lesion

Group	n	Day 6		Day 9	
		Body weight (g)	US isolation calls/3 min	Body weight (g)	US isolation calls/3 min
Intact	14	13.2 ± 0.2	1949.2 ± 279.7	18.2 ± 0.4	1429.9 ± 167.9
Sham	12	12.6 ± 0.1	1498.4 ± 163.4	17.4 ± 0.2	1490.8 ± 182.9
Left	12	11.3 ± 0.3 [a]	1340.9 ± 229.6	15.5 ± 0.3 [a]	1493.8 ± 350.2
Right	12	11.1 ± 0.3 [a]	1511.0 ± 638.2	15.4 ± 0.3 [a]	1961.0 ± 512.6
Both	12	11.0 ± 0.3 [a]	1858.4 ± 286.9	15.3 ± 0.4 [a]	1764.0 ± 454.5

Mean ± S.E.M. *n*: males and females, equal number in each group. Left, Right and Both: unilateral lesion of the left cerebral cortex, unilateral lesion of the right cerebral cortex and bilateral cortical lesion, respectively.
[a] Difference from intact control is statistically significant at the 1% level.

cortex (according to the cortical map by Zilles, 1985) as shown in Fig. 2. No damage to the hippocampus was produced in the unilateral lesion groups.

Although no attempt was made to experimentally detect possible locomotive or behavioral defects due to the neonatal cortical lesion, no remarkable abnormal movements were noticed by gross observation of all the rats in the lesion groups. However, growth retardation was found in the lesion groups (Tables 1 and 2).

No difference in ultrasonic isolation calls between intact controls or sham-operated pups and the pups of lesion groups was detected (Table 1). Also, no systematic change in ultrasonic vocalization ascribable to the extent of lesions or to lesion side was noticed. Since essentially the same result was obtained for the data for different times after injection of diazepam, the results are shown for the data 30 min and 60 min after injection.

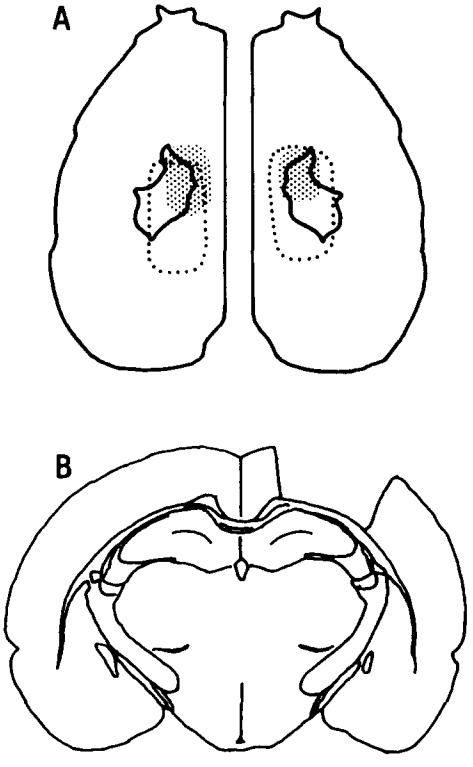

Fig. 2. Cortical surface diagram showing the extent of unilateral lesions and a typical lesion shown in coronal section. A: Hindlimb area (Zilles, 1985) is shown in solid outline. The largest lesion is shown as a broken outline. The smallest lesion is shown as a dotted area. B: A coronal section.

The ultrasonic isolation calls in pups were markedly attenuated in all the lesion groups by diazepam injection as well as in pups with an intact cortex. There was no difference in the inhibition by diazepam of the distress calls by the right and left lesion groups and the bilateral lesion group (Table 3).

None of the female adult rats emitted ultrasonic distress calls after delivery of 4 series of footshocks. Therefore, the study was continued with only male rats.

No statistically significant difference exists among the control and lesion groups in either ultrasonic distress vocalization inducibility (ratio of rats which emit ultrasounds) or in vocalization activity when the ultrasound was emitted (Table 2). Furthermore, no relationship between the above effects and the extent of the lesion or the lesion side was detectable.

In contrast to the marked attenuating effect of diazepam on the ultrasonic distress calls in the adult rats in which the cerebral cortex was intact, diazepam did not diminish the shock-elicited ultrasonic distress vo-

Table 2
Footshock-elicited ultrasonic (US) distress calls in adult male rats with and without neonatal cortical lesion

Group	Ratio of rats conditioned	Body weight (g)	US vocalization conditioned
Intact	7/9	346.6 ± 6.5	2581.5 ± 422.7
Sham	6/8	340.7 ± 7.4	2460.3 ± 593.0
Left	6/8	294.0 ± 13.6 [a]	2267.6 ± 364.1
Right	6/9	293.3 ± 16.6 [a]	2314.6 ± 384.6
Both	6/8	291.7 ± 14.3 [a]	2119.4 ± 435.7

Mean ± S.E.M. Left, Right and Both: unilateral lesion of the left cortex, unilateral lesion of the right cortex and bilateral cortical lesion, respectively.
[a] Difference from intact control is statistically significant at the 1% level.

Table 3
Effects of diazepam (1 mg/kg) on ultrasonic (US) isolation calls in rat pups with and without cortical lesion

Group	Treatment	n	US isolation calls after injection (% of pretreatment)			
			Day 6		Day 9	
			30 min	60 min	30 min	60 min
Intact	Vehicle	14	102.4 ± 4.4	101.4 ± 4.4	102.6 ± 2.9	104.7 ± 3.2
Intact	DZ	14	9.8 ± 3.3	4.5 ± 1.2	7.9 ± 2.1	7.8 ± 1.6
Sham	DZ	12	6.4 ± 1.7	4.0 ± 1.2	5.5 ± 1.4	6.4 ± 1.6
Left	DZ	12	8.8 ± 1.9	7.0 ± 2.2	9.6 ± 2.1	7.4 ± 1.7
Right	DZ	12	8.5 ± 2.3	8.8 ± 3.1	10.0 ± 3.0	8.6 ± 2.8
Both	DZ	12	8.4 ± 1.4	7.1 ± 1.1	9.7 ± 2.9	9.5 ± 2.5

Mean ± S.E.M. DZ: diazepam injection. Left, Right and Both: unilateral lesion of the left cortex, unilateral lesion of the right cortex and bilateral lesion of the cortex, respectively. Difference from intact vehicle-injected group is statistically significant in all the diazepam-injected groups at the 1% level. No statistically significant difference among diazepam-injected groups.

calization in any of the rats which had received a neonatal lesion of the cerebral cortex (Table 4).

On the other hand, 8-OH-DPAT was markedly effective as soon as 10 min after the injection to suppress shock-elicited ultrasonic distress calls in all the adult rats irrespective of the presence or the absence of the cortical lesion (Table 5). The effect at the dose of 0.01 mg/kg tended to diminish until 120 min after injection but the attenuation by the dose of 0.5 mg/kg of the ultrasonic vocalization was still almost complete 120 min after injection even in the groups with a cortical lesion (Table 5).

4. Discussion

It has been reported that neonatal removal of the motor cortex induces impairments in forelimb use in a motor task requiring sustained muscular effort and grasping precision (Plumet et al., 1990). Therefore, the retardation of growth of the rats which received a neonatal lesion of the cerebral cortex in the present study is likely to have been caused by some motor deficits.

However, the neonatal damage to the cerebral cortex, even that to the bilateral hemispheres and the hippocampus, was found not to impair ultrasonic vocalizations in the rat. The result in pups was compatible with the finding that prenatal exposure to methylazoxymethanol acetate, an agent used to ablate the neocortex, does not greatly affect the ultrasonic isolation calls in rat pups (Cagiano et al., 1987). Furthermore, the neonatal lesion of the cerebral cortex appears not to influence the susceptibility to conditioning to emit ultrasonic distress signals following averse stimuli in adult rats, and the vocalization intensity after

Table 4
Effects of diazepam (1 mg/kg) on ultrasonic (US) distress calls in adult male rats with and without neonatal cortical lesion

Group	Treatment	n	US distress vocalization after injection (% of pretreatment)	
			30 min	60 min
Intact	Vehicle	6	117.5 ± 10.4	100.8 ± 14.8
	DZ	12	13.0 ± 3.7 [a]	9.6 ± 2.7 [a]
Sham	Vehicle	6	100.4 ± 8.5	99.0 ± 8.5
	DZ	7	10.2 ± 4.2 [a]	7.2 ± 3.0 [a]
Left	Vehicle	5	107.3 ± 9.9	106.3 ± 6.9
	DZ	5	99.2 ± 10.9	105.4 ± 12.0
Right	Vehicle	5	103.9 ± 7.5	107.3 ± 7.9
	DZ	6	104.4 ± 6.1	99.2 ± 6.0
Both	Vehicle	5	103.6 ± 9.0	102.6 ± 4.6
	DZ	5	108.0 ± 10.4	98.1 ± 3.5

Mean ± S.E.M. Left, Right and Both: unilateral lesion of the left cortex, unilateral lesion of the right cortex and bilateral cortical lesion, respectively. DZ: diazepam injection.
[a] Difference from the effect of vehicle injection in each group is statistically significant at the 1% level.

Table 5
Effects of 8-OH-DPAT on ultrasonic (US) distress calls in adult male rats with and without neonatal cortical lesion

Group	Dose (mg/kg)	n	US distress vocalization after injection (% of pretreatment)	
			10 min	120 min
Intact	Vehicle	4	108.3 ± 10.3	107.3 ± 9.6
	0.01	4	36.7 ± 9.9 [a]	107.2 ± 9.1
	0.50	4	0.0 ± 0.0 [a]	0.4 ± 0.3 [a]
Sham	Vehicle	4	100.5 ± 5.2	100.4 ± 4.5
	0.01	4	38.2 ± 9.4 [a]	97.0 ± 11.4
	0.50	4	0.0 ± 0.0 [a]	0.4 ± 0.2 [a]
Left	Vehicle	4	105.1 ± 5.1	101.8 ± 7.4
	0.01	4	35.9 ± 7.1 [a]	86.0 ± 5.3
	0.50	4	0.0 ± 0.0 [a]	0.3 ± 0.2 [a]
Right	Vehicle	4	106.8 ± 5.9	101.1 ± 5.3
	0.01	4	33.9 ± 9.8 [a]	87.9 ± 3.7
	0.50	4	0.0 ± 0.0 [a]	0.3 ± 0.2 [a]
Both	Vehicle	4	99.1 ± 6.8	94.7 ± 2.5
	0.01	4	35.3 ± 6.8 [a]	79.5 ± 12.8
	0.50	4	0.0 ± 0.0 [a]	0.2 ± 0.1 [a]

Mean ± S.E.M. Left, Right and Both: unilateral lesion of the left cortex, unilateral lesion of the right cortex and bilateral cortical lesion, respectively.
[a] Difference from the effect of vehicle in each group is statistically significant at the 1% level.

induction of the vocalization response was not diminished even in the presence of the cortical lesion.

These findings indicate that the intact cerebral cortex is not essential for production of the ultrasonic vocalization response in the rat. If we assume that the shock-elicited ultrasonic distress vocalization in adult rats is an expression of fear (Tonoue et al., 1986; Kaltwasser, 1990), integrity of the brain cortex would not be necessary for the generation of such an emotion.

The attenuating effect of diazepam, at the dose employed in this study, on the ultrasonic distress calls in the normal adult rat has been repeatedly demonstrated (Sales et al., 1986; Tonoue et al., 1986; Cuomo et al., 1988; Van der Poel et al., 1989). The dose of diazepam is equal to or lower than that used in previous studies which showed that the anxiolytic effect detected in each test was not exerted through other actions of diazepam. The excessive grooming was attenuated by 1 mg/kg diazepam while no depression of locomotor activity was found at the dose of 2 mg/kg (Crawley and Moody, 1983). It was indicated that the duration of the audiogenic immobility response was, instead, shortened by diazepam at the dose of 1 mg/kg (Hård et al, 1985). It has also been reported that attenuation by 1 mg/kg diazepam of defensive burying may not be through analgesic mechanisms (Treit, 1985). The attenuation by diazepam at the dose of 2.5 mg/kg of the ultrasonic distress calls in the adult rat was not blocked by naloxone, an opiate receptor antagonist (Tonoue et al., 1987). These previous results support the possibility that the attenuation by diazepam of shock-elicited ultrasonic distress calls in the present experiments was not through the analgesic or sedative effect of diazepam. Furthermore, it has been reported that the conditioned fear in response to a signal was not abolished after chronic treatment with diazepam at the dose of 5 mg/kg (Davidson, 1990). Therefore, it is not likely that the acute effect of 1 mg/kg diazepam in the present study was through prevention of the learned anticipation of the stressful stimulus. Taken together, these previous results seem to confirm that the present finding of attenuation by diazepam of ultrasonic distress calls in the normal adult rat was a result of a so-called 'truly anxiolytic' effect of the benzodiazepine (Tripp et al., 1987).

In sharp contrast with the effect in the normal rat, diazepam, a representative anxiolytic drug, was found ineffective in rats which had neonatally received a lesion of the cerebral cortex. This finding is the first to demonstrate the difference between the mode of action of diazepam in pups and adult rats since diazepam was effective in pups with the cortical lesion as well as in normal pups.

It has been reported that diazepam at a dose effective to attenuate the ultrasonic isolation calls in the rat pup does not affect locomotive activity (Gardner, 1985a,b; Insel et al., 1986). Therefore, the attenuation by diazepam of the rat pup isolation calls is also a promising animal model of anxiolysis (Winslow and Insel, 1991b). However, it is now necessary to carefully reevaluate which of the ultrasonic isolation calls by rat pups and ultrasonic distress calls in adult rats is behaviorally compatible with human anxiety.

It seems reasonable to assume that the neonatal damage to the cerebral cortex somehow affects the receptivity to benzodiazepines in the brain during development. It has been indicated that the binding of flunitrazepam, a benzodiazepine receptor ligand, increases most sharply after birth and maintains its highest level in the frontal cortex as compared with the other regions in the rat (Rothe and Bigl, 1989). The binding of Ro15-1788, a benzodiazepine receptor antagonist, is highest in the cortex of the rat pup and the binding capacity of the sensorimotor cortex is most affected by isolating the pup (Insel et al., 1986). These findings imply a particular importance of the cerebral cortex in the anxiolytic action of benzodiazepines. The results of the present study fit the implication, and the development of the normal anxiolytic neuronal system involving benzodiazepine receptors is likely to be dependent on the integrity of the cerebral cortex itself. There are two subtypes of benzodiazepine receptors in the rat brain (Klepner et al., 1979). In the newborn rat, type 2 receptors are present at birth, whereas type 1 receptors develop after the first week (Braestrup and Nielsen, 1979). It has been suggested that the effects of benzodiazepines in the rat pup are mediated by type 2 receptors (File and Wilks, 1986; Johnston et al., 1989). Thus the present finding implies that the development of type 1 benzodiazepine receptors might be somehow impaired in the rat brain in the presence of the neonatal damage at the cerebral cortex.

Reports of the attenuation by 5-HT receptor agonists of ultrasonic vocalization in rat pups (Hård and Engel, 1988; Mos and Oliver, 1989; Winslow and Insel, 1990, 1991a) and in adult rats (Sánchez, 1993; Schreiber and De Vry, 1993) have been presented as animal models of their anxiolytic action. Ipsapirone and 8-OH-DPAT, agonists selective to 5-HT_{1A} receptor, have been shown to retain their ability to inhibit ultrasonic distress calls when injected into the dorsal raphe nucleus in adult rats which had been treated with 5,7-dihydroxytryptamine, a neurotoxin (Schreiber and De Vry, 1993). The study suggested that 5-HT_{1A} receptors located in the dorsal and medial raphe nucleus, and in particular limbic areas, are involved in the anxiolytic effects of these drugs. The present finding that 8-OH-DPAT is effective in rats with a cortical lesion, in contrast to the failure of diazepam, also suggests that the subcortical regions are important for the anxiolytic effect of 5-HT receptor agonists. It is now reasonable

to assume that the neuronal system inhibiting ultrasonic distress expression in the adult rat is not identical for all anxiolytics. The effect of diazepam is likely to require the presence of an additional neuronal system which is not essential for 5-HT receptor agonists.

The possibility that 8-OH-DPAT exerts its effect through blocking any anxiogenic mechanism is not ruled out. However, it is ruled out in the case of the effect of diazepam in the present study. The failure of diazepam to inhibit ultrasonic expression of distress in a rat that received a neonatal cortical lesion appears to indicate that a benzodiazepine-receptive neuronal mechanism other than the distress-expression mechanism and capable of blocking it, is normally formed in the presence of the intact cerebral cortex.

At present it seems too early to generalize the idea set out above to anxiogenic and anxiolytic mechanisms, because the hypothesis would be based on the effect of a single response. However, the present findings prompted us to investigate the effects of various anxiolytic drugs on several responses in rats having lesions of the cerebral cortex in order to elucidate the neurophysiology of anxiety.

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