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Maternal Behavior: Glutamic Acid Decarboxylase Activity in the Olfactory Bulb of the Rat

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MUNARO, N. I. *Maternal behavior: Glutamic acid decarboxylase activity in the olfactory bulb of the rat.* PHARMACOL BIOCHEM BEHAV **36**(1) 81–84, 1990.—Disruption of mother-pup interaction by pup deprivation induced an increase of glutamate decarboxylase (GAD) activity in the main and accessory olfactory bulbs (OB) of lactating rats. The presentation of pups to deprived mothers with restriction of suckling and tactile interaction decreased GAD activity in the main bulb to the levels of lactating rats. The accessory bulb enzymatic activity was similar to that of lactating rats when the pups were returned completely to deprived mothers. Deafferentation of OB did not modify the enzymatic activity in nonlactating rats. In lactating mothers deafferentation decreased GAD activity in the accessory OB compared to the main OB and also had less activity than the accessory OB mothers without deafferentation. Deafferentation in deprived mothers decreased GAD activity in both main and accessory OB. These results indicate a role for GABA neurotransmission in olfactory bulbs of lactating rats.

GAD activity Deprivation Lactating rat Deafferented rat GABA

IT has been demonstrated that motivational states can influence the response of olfactory neurons. In mammals the olfactory system plays an important role in behavioral events such as experimental aggressive behavior, reproductive and sexual behavior and development of maternal care (3, 4, 13, 19, 21). The removal of olfactory bulbs leads to a loss of maternal behavior and to cannibalism (24), eliminates sexual behavior (14,16) and influences aggressive behavior (23).

Several evidences indicate that GABA is an inhibitory transmitter in the olfactory bulb (15) and that GABAergic mechanisms are activated in behavioral events. For instance, changes in GABA concentration in the olfactory system have been reported in aggressive mice (6,12). Furthermore, denervation of mice olfactory bulbs affects several biochemical parameters (18). In addition, immunohistochemical procedures indicated that glutamate decarboxylase (GAD), the enzyme involved in the synthesis of GABA (17), is localized in zones rich in nerve terminals in the olfactory bulbs (1,7).

The present investigation was designed to explore rat olfactory bulbs GAD activity during maternal behavior. The activity of GAD was taken as an index of the GABAergic participation in the events under study.

METHOD

Animals

Virgin female rats weighing 200–280 g were housed in a light-(lights on from 06.00 a.m. to 07.00 p.m.) and temperature-(20–24°C) controlled room. Food and water were continuously available. Normal cycling and deafferented rats were mated in our laboratory, and upon the presence of sperm in vaginal smears, the rats were individually housed. After delivery, mothers remained with their pups until the beginning of the experiments.

Procedure

Several experimental groups were used:

1. Virgin female rats. Normal cycling rats were killed at 11.00 a.m. at any day of the estrous cycle. They received the normal handling of the housing conditions.

2. Lactating rats. Primiparous mothers were used to test for maternal behavior.

Behavioral testing. On the morning of the 2nd day after delivery the mothers were tested for maternal responsiveness and 30-min observations were made daily for 3 consecutive days. Each day 6 pups were placed in one corner of the test cage where the mother was placed 5 min earlier.

A set of maternal behavior included approaching, sniffing, licking, retrieving and crouching over any of the pups. Mothers were considered to be maternal when they displayed at least 3 of the mentioned behaviors. The mothers that displayed maternal care were submitted to several experimental situations.

a) Pup deprivation. Lactating rats were deprived of their pups

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for 4 hr in the 3rd day after 2 consecutive days of the test for maternal behavior. The mothers were placed in the test cage and at the end of the pup deprivation period they were killed by decapitation.

b) Pup deprivation plus pups' cues stimulation. Lactating rats at the end of the pup deprivation period were submitted to pups' cues (olfactory, visual and auditory stimuli) by returning the pups to the test cage for 30 min. The infants were separated from the mothers by placing a lattice in the test cage. The mothers were killed at the end of the stimulation period.

c) Pup deprivation plus pups returning. When the pup deprivation period was ended, the pups were returned to the mothers for 30 min and, thereafter, the mothers were killed.

3. Deafferented female rats. Bilateral olfactory bulb deafferentation was performed under ether anesthesia. Transsection of the olfactory tract of virgin female rats on diestrous 1 was done using a rectangular surgical blade. The blade was lowered to the base of the skull behind the cribiform plate of the ethmoidal bones, so that the peripheral afferent nerves were sectioned. Microscopic examination of the brain was routinely done to assess complete deafferentation. Sham surgeries were made by an incision on the skin, then drilling the skull and the wound closed. Sham surgeries did not induce changes on glutamic acid decarboxylase (GAD) activity compared with controls without deafferentation.

Three groups of deafferented rats were prepared.

a) Virgin deafferented females. Virgin cycling rats were deafferented at any day of the estrous cycle. Upon the reinitiation of the cycles they were killed at 11.00 hr.

b) Deafferented lactating rats. Virgin deafferented females after reinitiation of the cycle were mated in our laboratory.

c) Deafferented lactating pup-deprived rats.

For maternal behavior and pup deprivation test, the experimental procedures followed in deafferented groups were similar to that of nondeafferented rats. In the groups b and c, incomplete maternal care (30%) and/or cannibalism (18%) were present. Only mothers that presented maternal behavior were used.

Tissue Preparation

The rats were killed by decapitation, the brain was quickly removed and the olfactory bulbs were dissected out and placed on ice. Each olfactory bulb was divided into anterior and posterior parts (5) by cutting the bulb perpendicularly to the longitudinal axis at 0.5 mm of the rostral pole of the accessory bulb (8,9). The anterior block of tissue was termed main olfactory bulb and contained the anterior two-thirds of the main olfactory bulb, while the posterior part, containing the entire accessory bulb, was designated as accessory olfactory bulb. The anterior olfactory nucleus (rostral pole), which lies ventrolateral to the accessory olfactory bulb, was not included. Sample tissue from either the right or left olfactory bulb were equally used.

Glutamic Acid Decarboxylase Estimation

The activity of the enzyme GAD was determined by the production of $^{14}CO_2$ according to the method of Albers and Brady (1). The reaction was started by adding 10 μl of olfactory bulb homogenate in 25 mM potassium phosphate pH 7.0, to 10 μl of substrate containing L^{14}C glutamic acid, carried out at 37°C for 30 min, and terminated by injecting 50 μl of H_2SO_4 5 N into each tube. The $^{14}CO_2$ produced was absorbed into the test tube with methyl benzethonium hydroxide. To ensure the complete release of $^{14}CO_2$, the tubes were shaken for an additional 45 min and counted in a liquid scintillation apparatus. Boiled homogenates plus all reactants were used as blank tubes.

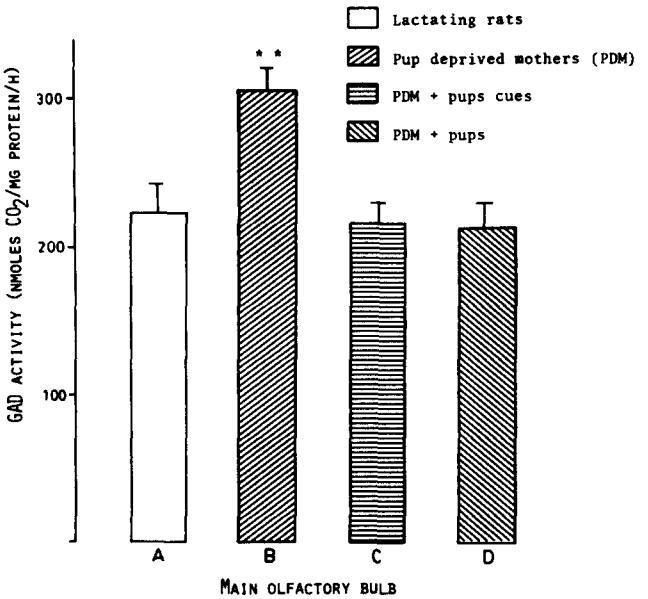

FIG. 1. Effect of different experimental conditions on GAD activity in the main olfactory bulb of: (A) lactating rats; (B) lactating rats pup deprived for 4 hr; (C) the same as B, but submitted thereafter to pups cues for 30 min; (D) the same as B with pups returned to the mother for 30 min. Each bar represents the mean of 5 to 9 rats ± S.E. of mean. B vs. A–C–D, $p<0.01$. Newman-Keuls t-test.

Enzyme activity was expressed as nmoles of $^{14}CO_2$/mg protein/hr. Protein concentration in the samples was determined by the method of Lowry *et al.* (10).

Data were analyzed using Student's t-test and paired t-test. One-way analysis of variance was used when appropriate. Post hoc comparisons between means were made by Newman-Keuls t-test. Differences between groups were considered statistically significant when $p<0.05$ (two-tailed).

RESULTS

Effect of Pup Deprivation and Restitution on GAD Activity in the Main Olfactory Bulb of Lactating Rats

Glutamic acid decarboxylase activity in the main olfactory bulb of lactating rats, measured in the 3rd day of the test for maternal behavior, increased significantly when the rats were submitted to pup deprivation for a period of 4 hr, $F(4,22)=9.78$, $p<0.01$.

After 4 hr of pup deprivation, the pups were returned to their mothers for a period of 30 min, without allowing them to suckle or touch the mother, or on the contrary, permitting the infants to have physical interaction with the mothers, the two experimental situations elicited a marked decrease of GAD activity in the main bulb of deprived mothers ($p<0.01$) (Fig. 1).

Effect of Pup Deprivation and Restitution on GAD Activity in the Accessory Olfactory Bulb of Lactating Mothers

Enzyme activity studied in the accessory olfactory bulb of lactating rats showed that similarly to that which occurred with the main bulb, pup deprivation for 4 hr in the 3rd day of the test for maternal behavior induced a marked increase of enzyme activity, $F(4,24)=13.28$, $p<0.01$. The stimulus provided by pup presentation for 30 min to 4-hr-deprived mothers did not change GAD activity in the accessory bulb, although it was increased if compared with the accessory bulb of nondeprived mothers, $p<0.05$.

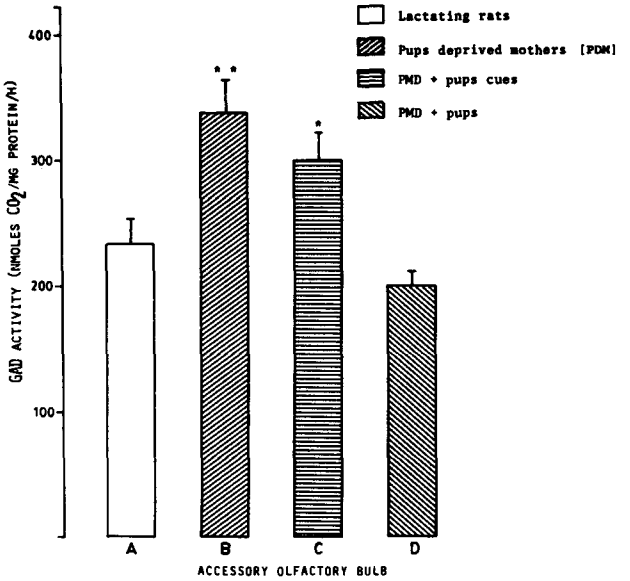

FIG. 2. Effect of different experimental conditions on GAD activity in the accessory olfactory bulb of (A) lactating rats; (B) lactating rats pup deprived for 4 hr; (C) the same as B, but submitted thereafter to pups cues for 30 min; (D) the same as B with pups returned to the mother for 30 min. Each bar represents the mean of 5 to 9 rats ±S.E. of mean. B vs. A–D, $p<0.01$. C vs. A, $p<0.05$. C vs. D, $p<0.01$. Newman-Keuls t-test.

However, pup physical interaction with the mother by restitution for 30 min of the offspring to 4-hr-deprived mothers, lowered GAD activity to the levels found in lactating rats nondeprived. Furthermore, the enzyme activity was lower than that found in the accessory bulb of pup-deprived mothers ($p>0.001$) or deprived mothers plus pup presentation ($p<0.01$) (Fig. 2).

Effect of Deafferentation on GAD Activity in the Main and Accessory Olfactory Bulb of Virgin and Lactating Rats

The values of GAD activity measured in the main and accessory bulb of virgin deafferented females were not different from that found in virgin controls rats. Deafferentation in lactating mothers failed to change GAD activity in the main bulb, while in the accessory bulb the enzyme activity was significantly lower ($p<0.02$) when compared with the values found in the same part of lactating nondeafferented rats, or that found in the main bulb of the same condition ($p<0.05$). Deafferentation, in mothers deprived of their pups for 4 hr, induced a decrease of GAD activity as well as in the main bulb ($p<0.01$), or in the accessory olfactory bulb ($p<0.001$) (Table 1).

DISCUSSION

We have measured the biochemical effects of several experimental conditions in the olfactory bulbs (OB) of primiparous mother rats. The parameter monitored was the level of the enzyme glutamic acid decarboxylase (GAD) that is a good marker for the activity of the GABAergic system (7). In regional studies GAD activity indicates inhibitory inputs for intrinsic interneurons, which probably use GABA as their transmitter (22). Pharmacological antagonisms of the actions of GABA in the olfactory bulb would indicate that GABA may act as inhibitory transmitter in their synapses (11).

We have found that the activity of the enzyme increases in the main and accessory bulbs of mothers submitted to pup deprivation for 4 hr. Similarly, depriving pup rats of the maternal care produced a decrease of the growing rate and development of the pups, and influenced the biochemical processes (20). Furthermore, our evidence demonstrates that specific cues from the pups are important factors for the presence of biochemical changes. This is evident from the decrease of GAD activity as soon as pups are presented to mothers deprived of their offspring, since GAD levels reach the values found in nondeprived mothers. The pattern of the OB response was determined by the part of the neural structure examined, since the changes were found only in the main OB (Fig. 1). The presentation of the pups to deprived mothers failed to affect GAD activity in the accessory OB, suggesting a specificity in the response of the main OB (5). In the accessory OB, a more close contact with the pups was necessary to reduce GAD levels, since only the complete restitution of the pups inhibited the activity of the enzyme. It is known that in rodents nonvolatile substances activate the accessory system (2), suggesting that the contact of the mothers with the infant rats triggers, in part, the mechanism that restores GAD activity to the levels found in nondeprived mothers.

To elucidate the influence of olfactory cues in GAD activity, we examined the effect of deafferentation in females rats.

TABLE 1

GAD ACTIVITY IN THE OLFACTORY BULBS OF DEAFFERENTED RATS

	GAD Activity (nmoles CO_2/mg prot/hr)			
	Intact		Deafferented	
Experimental Conditions	Main Bulb	Accessory Bulb	Main Bulb	Accessory Bulb
Virgin	209 ± 21.8 (5)	203 ± 22 (5)	214 ± 17.3 (5)	190 ± 22.3 (7)
Lactating	225 ± 16 (9)	234 ± 20[a] (9)	219 ± 7.9* (5)	178 ± 6.3[b] (6)
Lactating + pup deprived	308 ± 10.5[e] (5)	337 ± 20.6[c] (6)	179 ± 34.5[f] (5)	186 ± 26[d] (5)

Number of animals in parentheses. Means ± S.E. are presented. *$p<0.05$ vs. accessory bulb in the same condition. Paired t-test. a: $p<0.02$ vs. b; c: $p<0.001$ vs. d; e: $p<0.01$ vs. f. Student's t-test.

It has been reported that peripheral deafferentation did not alter the levels of GAD in the olfactory bulb (12). In our work, deafferentation of nonlactating rats did not induce changes in the levels of this neurotransmitter synthesizing enzyme (Table 1). The activation of the GABAergic system induced by pup deprivation in lactating rats in both parts of the OB (Figs. 1 and 2) was not seen in the OB of deafferented mothers (Table 1). However, in lactating deafferented rats we found a significant difference between the main and accessory OB in GAD activity (Table 1). Also, this difference was seen between the accessory OB of deafferented mothers and the same part of normal lactating mothers. These results indicate that deafferentation modified GAD activity in the accessory OB, and a possible decrease of the levels of the neurotransmitter GABA could be consistent with the alteration of maternal care that sometimes is present in deafferented mothers (24). However, the inhibitory effect on GAD activity promoted by deafferentation in the accessory bulb was also seen in both OB of deprived deafferented mothers (Table 1).

We have shown that alterations of the mother-pup interactions induced changes in the GABAergic neurotransmission in both parts of the OB depending on the presence and/or the contact of the mothers with the pups. In deafferented mothers these changes only occur in mothers not deprived of their pups. By deafferentation, olfactory stimuli from the pups is not present, suggesting that in the activation of the GABA system seen in normal deprived mothers, the olfactory cue is playing an important role in the induction of the enzymatic increase in the main bulb. This is not the case of the accessory bulb, in which mother-pup interactions seem to be more complex.

The involvement of olfactory bulb GABA in maternal behavior still leaves open how physiological mechanisms trigger the enzymatic changes described.

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