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Source: Zoological Science, 18(9) : 1225-1230

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.1225

ZOOLOGICAL SCIENCE **18**: 1225–1230 (2001)

Inhibitory Effect of Postpartum Lesions or Cuts in Median Raphe Nucleus on Maternal Behavior in Female Rats

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ABSTRACT—In order to clarify the role of the median (MRN) and dorsal (DRN) raphe nuclei in regulating maternal care (retrieving and licking behavior), radiofrequency lesions or microknife cuts were made in postpartum rats on the day after delivery. Animals were housed individually without pups after the operation. One week after the surgery, maternal behavioral test was carried out daily for 3 days using pups of 2–6 days age. The results demonstrated that rats with MRN lesions or ventral horizontal cuts of the MRN showed extremely low incidence of the maternal behavior, as compared to those in control and sham-operated groups. DRN-lesions or dorsal cuts of the MRN had no effect. In locomotor activities measured by the infrared sensor system, there was no difference between the groups. This suggest that the MRN but not DRN plays a critical role in regulating retrieving and licking behaviors and ventral outputs are involved in this function in postpartum rats.

Key words: maternal behavior, median raphe nucleus, cuts, lesions, female rat

INTRODUCTION

Parturient mammals show maternal behavior against young pups to care and keep their growing. Mother rats retrieve pups to the nest from the outside and clean the bodies by licking, especially the ano-genital area to stimulate urination and defecation. These patterns appear just after delivery and are seen until weaning in female rats (Fahrbach and Pfaff, 1982).

In the female rat forebrain, the preoptic area (POA) is thought to be a regulatory center of maternal behavior, because of severe deficit of all of maternal patterns by the lesions (Numan, 1974). The limbic system is also involved in the regulation of maternal behavior. Lesions in the cingulate cortex (Slotnick, 1967), the septal area (Fleischer and Slotnick, 1978; Korányi *et al*., 1988) and the hippocampus (Kimble *et al*., 1967) have been reported to cause disturbance of maternal care. On the other hand, damage of the accessory olfactory tract (Del Cerro *et al*., 1991) or the amygdaloid nucleus (Fleming *et al*., 1980) enhances the development of maternal behavior in nulliparous rats, therefore it is assumed that the olfactory neural system represents an inhibitory mechanism for maternal behavior.

The maternal behavior-mediating system in the forebrain is assumed to be influenced by the lower brainstem. The serotonergic projection from the raphe nuclei in the midbrain to the forebrain is important in control of reproductive process. It has been reported that female rat, in which serotonergic neurons in the median raphe nucleus were damaged by neurotoxin, could deliver pups but show low level of maternal care (Barofski *et al*., 1983). In this study, to elucidate the role of the midbrain raphe nucleus on maternal behavior in postpartum rats, the median (MRN) or the dorsal (DRN) raphe nucleus were destroyed or the fibers were transected one day after delivery, and retrieving and licking behaviors were observed.

MATERIALS AND METHODS

Female Wistar rats (180–200g) purchased from Takasugi Animal Farm (Saitama, Japan) were housed under controlled temperature (23–25°C) and photoperiod (LD 14:10, lights off at 2100 hr). Food and water were given ad libitum. Vaginal smears were taken daily. Seventy-four rats which showed more than two regular 4-day estrous cycles were used in the experiments. The present study was performed according to Guidelines for the Care and Use of Laboratory Animals in the Human Science Department of Waseda University.

Virgin female rats were placed with sexually-experienced males

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from proestrous evening to estrous morning. If sperms were detected in the vaginal smear on the morning of estrus, females were considered as pregnant. Ten days prior to expected date of parturition, the pregnant females were kept individually in a plastic cage (20–25–40 cm, h–d–w) with woodshavings.

One day after parturition (day 1), pups were taken away and all mother rats were kept solitary except 6 animals. These 6 rats were kept with own pups during the experiment (Lactating group).

Sixty rats were subject to the brain surgery under ether anaesthesia; making lesions or microknife cuts of the midbrain raphe nucleus on the day 1. Lesions in the median (MRL; n=13) or in the dorsal (DRL; n=7) raphe nucleus were made with a radiofrequency lesion generator (Radionics Inc., Burlington, MA). Rats were fixed in a stereotaxic instrument with the incisor bar set at 5 mm below the interaural line, an electrode (0.7 mm) was lowered 8.5 mm (MRL) or 6.8 mm (DRL) from the bregma level at a point 7.8 mm posterior to the bregma

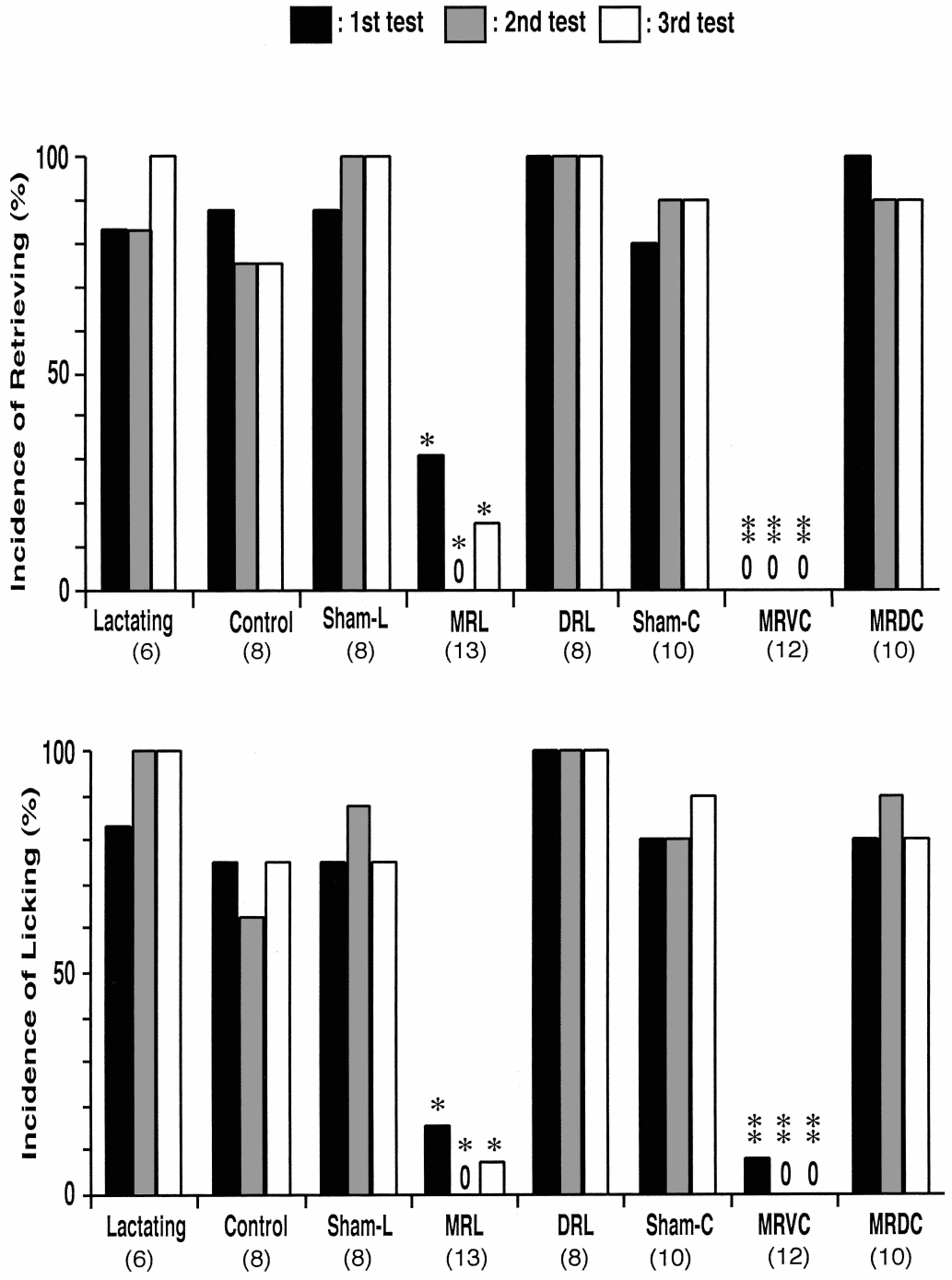

Fig. 1. Effects of the lesions or cuts of the median (MRN) or the dorsal raphe nucleus on the incidence of retrieving and licking behavior in postpartum rats. Groups: MRL, median raphe nucleus lesion; DRL, dorsal raphe nucleus lesion; MRVC, ventral cut of the MRN; MRDC, dorsal cut of the MRN. Retrieving; *p<0.05 vs. control, sham-L, lactating and DRL groups. **p<0.01 vs. control, sham-C, MRDC groups. Licking; *p<0.05 vs. control, sham-L, lactating and DRL groups. **p<0.01 vs. control, sham-C, MRDC groups.

Table 1. Effects of lesions or cuts of the median or dorsal raphe nucleus on latency (sec, median) of retrieving behavior.

	No. of rat*	1st test	2nd test	3rd test
Groups* *	with retrieving	Latency (min-max)	Latency (min-max)	Latency (min-max)
Lactating	6	35 (7–149)	12.5 (2–72)	10.5 (2–130)
Control	8	30 (14–45)	18.5 (10–66)	16 (7–468)
Sham-L	8	64 (36–244)	37.5 (11–1017)	41.5 (3–202)
MRL	5	28 (15–867)	—	197.5 (88–307)
DRL	7	33 (4–600)	22 (2–192)	28 (2–34)
Sham-C	9	56.5 (10–259)	18 (3–41)	20 (7–48)
MRVC	0	—	—	—
MRDC	10	40.5 (10–131)	22 (6–144)	16.5 (2–116)

* These include the number of rats which retrieved only one or two pups.
** Groups: see Fig.1

on the midline. To produce lesions, DC current was applied and the temperature at the electrode tip was kept at 54–58°C for 1 min. In sham-operated controls, an electrode was lowered to the same point as the MRL but current was not applied (sham-L group, n=8).

Under ether anesthesia, horizontal circle cuts were made at the ventral (MRVC, n=12) or at the dorsal (MRDC, n=10) of the MRN. The axis of an L-shaped Halász microknife with a 1.5 mm horizontal blade was adjusted to a point 7.8 mm posterior to the bregma in the midline. Then, the knife was lowered 8.7 and 7.3 mm in the MRVC and MRDC groups, respectively, and rotated 360°. In a sham-operated group, the L-shaped knife was lowered to the same point as that for the MRVC group and removed without rotation (sham-C, n=10). In addition, 8 female rats without brain surgery were housed individually as control group.

One week after the surgery (day 7), the test of maternal behavior was started and carried out daily for 3 days. On the day of the test, experiment animals in the home cage were moved to the test room 2 hr before start of the observation. Behavioral test was begun at 2 p.m. in each day under the room light condition. In the behavioral test, 3 pups (2–6 days old) were put into the home cage and retrieving and licking behaviors were observed during the next 30 min. Retrieving behavior was recorded and considered as positive when all 3 pups were retrieved. Latency was a time from introduction of pup to the first retrieving. Licking behavior was recorded and noted as positive when it was seen during 30 min.

On the next day of the last test of maternal behavior, spontaneous locomotor activities were measured continuously for 3 hrs from 1:00 p.m. using an infrared sensor system (SUPERMEX; Muromachi-Kikai, Tokyo). The locomotor activity for each rat were calculated every 15 min.

After the behavioral test, all rats were sacrificed by ether overdose. The brain was removed and fixed with 10% formalin solution. Frozen sections (100 μm) were made and stained with cresyl fast violet to determine the precise localization of the lesion or the cut in the brain.

For statistical evaluation of the incidence of behavior among the groups, Chi-square test was used. The differences in latency to retrieving were analyzed by the Mann-Whitney U-test. For all statistical tests, differences were considered significant when $p<0.05$.

RESULTS

In the first test, 88 and 75% of control females showed retrieving and licking behavior, respectively (Fig. 1). There were no difference between control and lactating groups in incidence of these behaviors. In the sham-L and the DRL groups, the incidence was comparable to that in the control group. In contrast, only 31 and 15% MRL females showed retrieving and licking, respectively ($p<0.05$, vs all other groups). When pups were put into the cage, these MRL females approached the pups but returned to the sleeping nest after sniffing but not retrieving them. In the sham-C and the MRDC groups, incidences of these behaviors were high, being comparable to those in the control group. However, none of MRVC females except one showed both retrieving and licking behavior ($p<0.01$, vs all other groups). MRVC females did not approach pups. In the latency of retrieving, there was no statistical difference among all groups (Table 1). Spontaneous locomotor activities were not affected by the experimental procedure (Fig. 2).

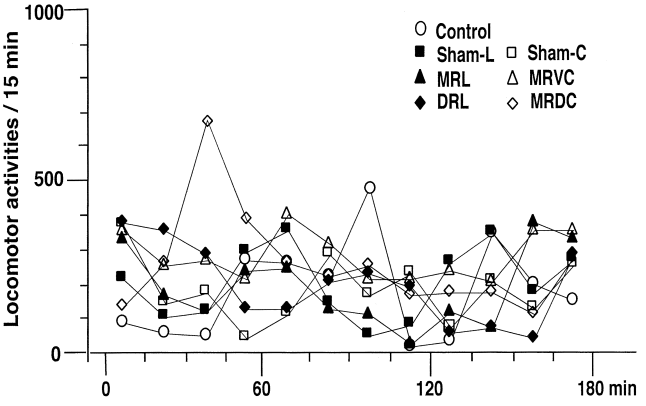

Fig. 2. Spontaneous locomotor activities in postpartum rats with lesions or cuts of the median or dorsal raphe nucleus. There are no statistical difference among the groups. Groups; see Fig.1.

The location of the lesions and the cuts were shown in Fig. 3. In the MRL group, most part of the MRN was destroyed and lesions penetrated to the decussation of the superior cerebellar peduncle. In the DRL group, main body of the nucleus except caudal part was damaged and lesions did not reach the MRN. In the MRVC group, cuts were seen in the reticulotegmental nuclei or the medial lemniscus. In the MRDC group, cuts were located below or in the decussation of the superior cerebellar peduncle.

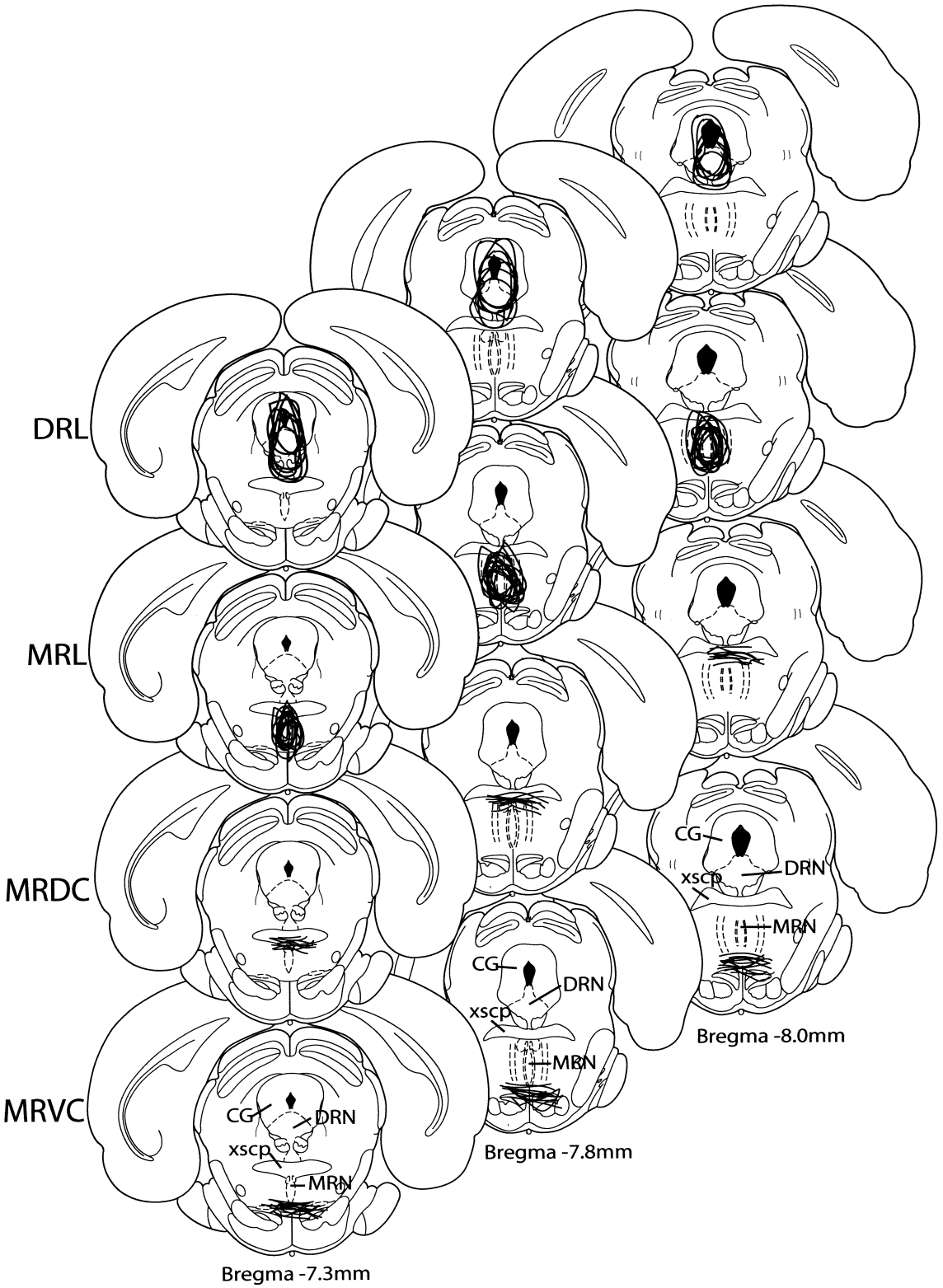

Fig. 3. Schematic representations of coronal sections of the rat midbrain at three levels showing an outline of the area of the median (MRL) or dorsal (DRL) raphe nucleus lesions and line of the ventral (MRVC) or dorsal (MRDC) cuts of the median raphe nucleus in postpartum rats. CG, central gray; DRN, dorsal raphe nucleus; xscp, decussation superior cerebellar peduncle; MRN, median raphe nucleus. The drawings are modified from the rat brain atlas of Paxinos and Watson(1997).

DISCUSSION

In this experiment, incidence of retrieving and licking behaviors in control rats, which were kept without pups, was the same level as those in the lactating group kept with pups. This suggests that the potency to express maternal behavior does not decrease one week after the removal of pups in parturient rats.

Severe deficit of retrieving and licking behavior was observed in the animals which received MRL one day after delivery , but not in the DRL rats. Locomotor activities in these groups were not different from those in control and sham-operated females. The results indicate that the MRN plays an important role in regulating maternal behavior in rats.

The MRN contains serotonergic neurons and projects the fibers to the forebrain (Törk, 1985). Similar deficiency of retrieving behavior had been reported in female rats in which the MRN serotonergic neuron was lesioned before pregnancy by a neurotoxin (Barofskiy *et al*., 1983). Thus, it can be said that suppression of maternal behavior by the MRL in the present experiment is due to damage of serotonergic function, although it can not be excluded the possibility that the other neurotransmitters contribute to control maternal care mechanism in the MRN. On the other hand, there is a report that serotonergic system has an inhibitory function in regulating maternal behavior, because a 5-HT 2A receptor agonist inhibits the incidence of maternal behavior with decrease in locomotor activity (Ferreira *et al*., 2000).

The POA which is thought to be the main regulator of maternal behavior (Numan, 1994), contains serotonin (Van de Kar and Lorens, 1979) and serotonergic axons (Vertes *et al*., 1999) from the MRN. Estrogen is thought to act on the POA and enhances maternal behavior (Numan *et al*., 1977). The serotonin receptor in this area decreases by treatment with estrogen (Biegon and McEwen, 1982). From these reports, the POA may be a possible locus of serotonergic projection originated from the MRN in regulating maternal behavior. Furthermore, the MRN sends axons including serotonergic ones to the limbic structures such as the hippocampus, the septum and the cingulate cortex (Vertes *et al*., 1999), which are included in regulating mechanism for maternal behavior (Fleischer and Slotnick, 1978; Kimble *et al*. 1967; Koranyi *et al*., 1988; Slotnick 1967). The olfactory system also receives serotonergic axons (Parent *et al*., 1981) and regulates maternal behavior in inhibitory fashion (Del Cerro *et al*., 1991). Thus, it can be speculated that the limbic and the olfactory system are also modified by the MRN via the serotonergic neuronal system.

In the present experiments, the interruption of the ventral, but not the dorsal connection of the MRN inhibited retrieving and licking behavior. The large number of the ascending fibers including serotonergic one from the MRN to the forebrain passes through the medial forebrain bundle (MFB) (Takagi *et al*., 1980). The MRVC in this experiment may interrupt such ascending fibers to the forebrain and cause impairment of maternal behavior. Although the ventral projection of the serotonergic neurons in the MRN is thought to join the MFB and terminate in the forebrain area, the cut of the MFB has been reported to cause no critical effect on retrieving behavior (Franz *et al*., 1986). Further experiment is necessary to clarify the kind of fibers which were transected by the MRVC in regulating maternal behavior in female rats.

ACKNOWLEDGMENT

This study was supported by a Grant-in-Aid from the Ministry of Education, Science, Culture and Sports of Japan (11640669), grants for special research project (2001A-611) and ARC for Human Sciences of Waseda University and the Promotion and Mutual Aid Corporation for Private School of Japan to K.Y.

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(Received June 19, 2001 / Accepted August 29, 2001)