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11 August 1970

Olfactory Bulb Removal Eliminates Maternal Behavior in the Mouse

Abstract. *Olfactory bulb removal eliminated maternal behavior in lactating and in virgin mice. The results are in contrast to the generally accepted concept of multisensory control of mammalian maternal behavior.*

Data derived principally from the rat have led to the traditional view that mammalian maternal and sexual behaviors are under multisensory control (*1*)—that is, no one sensory system is essential for the exhibition of those behaviors. This concept has recently been challenged by a report showing that olfactory bulb removal eliminates sexual behavior in the hamster (*2*). The role of the specific sensory systems in maternal behavior has not, however, been carefully examined in mammalian species other than the rat. The observations reported here clearly demonstrate that the integrity of the olfactory system is a prerequisite for maternal behavior in the mouse.

In the first experiment, 20 albino Rockland-Swiss (R-Swiss/Z) and 20 C57BL/10Gin multiparous, pregnant mice were divided into two equal groups, one of which sustained removal of the olfactory bulbs while the other underwent sham operations (*3*). Bilateral bulbectomy, performed under ether anesthesia, consisted of drilling a 2-mm hole in the skull approximately 5.0 mm anterior to the bregma and removing the tissue by suction. Sham operations consisted of everything except tissue removal. After surgery, each animal was individually housed in an 11 by 7 by 5 inch (approximately 28 by 18 by 13 cm) fiber-glass cage, the floor of which was covered with wood shavings. Animals had free access to food and water and were kept on a 12-hour light-dark cycle.

At the time of parturition, which occurred 1 to 11 days after surgery, the general bodily condition of the young and the behavior of the adult were recorded every morning and afternoon. An adult was scored as exhibiting maternal behavior if it was seen to be licking or carrying a pup, to be in a nursing posture, or to be constructing a nest. Observations were continued to weaning at 21 days or until the death of the pups. At the termination of testing, the adult animals were killed and their brains were macroscopically examined to determine the extent of the ablation. Mammary tissue was also examined.

Of 20 mice that underwent bulbectomy, 18 displayed no maternal behavior, whereas 19 of 20 sham-operated animals showed maternal behavior until weaning. This difference is statistically significant ($P < .001$). The results are summarized in Table 1.

Of the bulbectomized mice, 16 ate their young; they ate either all or part of their litters, which averaged eight pups. Cannibalism typically occurred within 12 hours of parturition, although two animals ate their young on the second postpartum day. Two ablated mice ate none of their litter but showed no maternal behavior toward any of their pups. Behaviors such as eating of the placenta and cleaning of the young, which occur at parturition, were exhibited by all animals. However, in all but two cases, bulbectomized animals were never seen to build nests or to engage in any form of maternal behavior toward the pups that were eventually eaten or to the pups that were left alone until death. Pups that were ignored died within 2 days of birth. Two adult females did place their young together and nurse the pups occasionally for about 4 hours before eating them.

Inspection of the brains revealed that the two mice in the bulbectomized group that displayed maternal behavior until weaning sustained only unilateral bulbectomies, whereas bilateral bulbectomies were sustained by the remaining subjects. Mammary tissue of the experimental subjects did not differ from that of the sham operates in terms of extent or ability to extrude milk.

These data indicate that removal of the olfactory bulbs leads to an elimination of maternal behavior and, in most instances, to an initiation of cannibalism. Furthermore, both olfactory bulbs must be removed for the effect to be seen.

Table 1. Behavior exhibited toward pups by C57BL/10Gin and R-Swiss/Z mice that either sustained olfactory bulb removal or underwent sham operations. Surgery was performed at various times throughout pregnancy.

Treatment	Ate all pups	Ate some pups; ignored the remainder	Ignored all pups	Normal maternal behavior
R-Swiss/Z mice				
Bulbectomy ($N = 10$)	7	2	0	1*
Sham ($N = 10$)	0	0	0	10
C57BL/10Gin mice				
Bulbectomy ($N = 10$)	4	3	2	1*
Sham ($N = 10$)	1	0	0	9

* Sustained incomplete bilateral bulbectomies.

Table 2. Behavior exhibited toward pups by R-Swiss/Z mice that either sustained olfactory bulb removal or underwent sham operations. Surgery was performed on day 12 of pregnancy (experiment 2) and in virgin mice (experiment 3).

Treatment	Ate all pups	Ate some pups; ignored the remainder	Ignored all pups	Normal material behavior
Experiment 2				
Bulbectomy ($N = 8$)	7	1	0	0
Sham ($N = 8$)	0	0	0	8
Experiment 3				
Bulbectomy ($N = 20$)	14	1	2	3*
Sham ($N = 21$)	1	0	1	19

* Sustained incomplete bilateral bulbectomies.

The first experiment consisted of ablating the olfactory bulbs at various times throughout the gestation period, ranging from 1 to 11 days prepartum. This procedure did not allow us to ascertain whether olfactory bulb removal caused a change in the length of the gestation period, with such a change possibly responsible for the elimination of maternal behavior. Thus, we wished to determine whether bulbectomy caused any significant deviation from the normal 19-day gestation period.

Sixteen multiparous R-Swiss/Z mice formed two equal groups, one of which was bulbectomized while the other was sham-operated. The procedure is identical to that of the previous experiment with the exception that animals were group-housed with males and that surgery was performed 12 days after the appearance of a vaginal plug. After surgery, mice were individually housed as in the first experiment.

Bulbectomized and sham operates did not differ in terms of gestation length and showed little deviation from the average 19-day gestation period (*4*). Furthermore, bulbectomized animals, in contrast to the controls, displayed no maternal behavior (see Table 2).

We next wished to determine whether the elimination of maternal behavior produced by the removal of olfactory bulbs is specific to animals that have undergone parturition and are lactating or whether it is also seen in nonparturient (virgin) mice. It is noted that virgin mice are "spontaneously maternal"—that is, they exhibit pup retrieval, genital licking, nest building, and the assumption of a nursing posture within minutes after the presentation of young (*5*).

Forty-one adult, virgin female R-Swiss/Z mice were divided into two groups, one of which was bulbectomized ($N = 20$) while the other was sham-operated ($N = 21$). After at least 7 postoperative recovery days, four 1-day-old mouse pups were placed in the cages of the adults. Every morning and afternoon the cages were inspected, and the condition of the pups and the behavior of the adult were noted. Immediately after the afternoon inspection period the pups were removed and replaced by four fresh animals. This procedure was continued for 4 days or until the death of the pups.

As summarized in Table 2, 19 of the 21 sham-operated virgin females displayed a full range of maternal behavior. In all cases, maternal activities began within 30 minutes of pup presentation and continued throughout the 4 test days. The bulbectomized mice, however, displayed a different pattern of behavior. Seventeen of them showed no maternal behavior, but three did behave maternally. The difference between the bulbectomized and the sham-operated mice is significant ($P < .001$). All but two of the bulbectomized mice that did not exhibit maternal behavior ate at least some of the pups presented to them, with cannibalism taking place within 12 hours of pup presentation. The other two mice ignored the young completely. In no case did a bulbectomized mouse build a nest.

Examination of the brains revealed that the three experimental subjects that did exhibit maternal behavior were not completely bulbectomized. Approximately 50 percent of one bulb remained intact in each mouse.

The effect of olfactory bulb removal does not, therefore, depend upon the physiological changes that accompany pregnancy and parturition. This finding agrees with evidence showing that maternal behavior in the mouse is independent of gonadal functioning since hypophysectomy does not eliminate or reduce it (*6*).

These experiments clearly demonstrate the involvement of the olfactory system in maternal behavior of the mouse (*7*). The manner in which removal of olfactory stimuli influences maternal behavior remains to be determined. One possibility may be that the mouse is unable to identify its young in the absence of olfactory stimuli from them. Another possible explanation may be that in the absence of olfaction, taste plays a critical role in the identification of food. Thus, eating of the young by bulbectomized animals may result from food sampling behavior. This may also account for tail biting, which has been reported to be induced by removal of the olfactory bulbs (*8*). Tail biting was not, however, observed in the present experiments. A further explanation may involve olfactory input into the hypothalamus. Connections have been demonstrated between the olfactory system and the hypothalamus both anatomically (*9*) and electrophysiologically (*10*). Scott and Pfaffmann electrically stimulated the olfactory bulbs in rats and found unit discharges in the lateral hypothalamus. It may be that olfactory cues emitted by the young stimulate an area of the lateral hypothalamus involved in the expression of maternal behavior. Without such stimulation, maternal behavior may not be elicited. This reasoning leads to the prediction that destruction of the lateral hypothalamus will cause a cessation of maternal behavior. This has been demonstrated (*11*). Furthermore, electrical stimulation of the lateral hypothalamus has been reported to elicit pup retrieval (*12*).

Mice that do not exhibit maternal behavior may eat their young in order to remove unwanted objects from their rather restricted environment. This behavior is seen frequently when a dead pup is given to a lactating mouse. If an animal is not disposed to exhibit maternal behavior, the young may become unwanted objects. It is also possible that cannibalism is directly elicited by the removal of the olfactory bulbs, perhaps by eliminating the ability to discern odors from the young which normally serve to inhibit cannibalism.

As in sexual behavior (*2*), the concept of multisensory control of maternal behavior in the mammal is subject to qualification.

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13. Supported in part by program project grant HD-04639 awarded to V.H.D. and M.X.Z. and by NIH postdoctoral fellowship HD-43413 awarded to R.G. We thank Dr. B. Sachs for his comments on an earlier draft of the manuscript.

27 August 1970