Maternal Behavior in the Rat: Facilitation through Gonadectomy

Abstract. *Virgin rats were ovariectomized and tested for induction of maternal behavior by being housed with neonatal young. A postoperative interval of 8 weeks yielded an average latency for the onset of maternal behavior significantly lower than that for intact controls or for virgin females ovariectomized 4 weeks before testing. Replacement of estrogen in the group ovariectomized for 8 weeks or injection of the estrogen antagonist ethamoxytriphetol (MER-25) in the group ovariectomized for 4 weeks changed the average latencies accordingly. Parallel results were obtained for males that were castrated 4 or 8 weeks before testing. It is concluded (i) that maternal behavior is under gonadal control and that this control is normally inhibitory; and (ii) that only after long-term removal of the gonads, resulting presumably in the complete clearance of estrogen and testosterone, is the maternal mediating system finally released from steroid suppression.*

A virgin female rat, when kept continuously in the presence of young, comes to behave maternally. She builds a nest, retrieves, and assumes a nursing posture, all in a manner indistinguishable from the puerperal female. However, while the puerperal female responds to her own or foster young as soon as they become available, the virgin female does not, but requires 5 or 6 days of "concaveation," or constant association with pups, before attending to them. The hormones accompanying the termination of pregnancy have been suggested as the agents responsible for the immediate responsivity of the puerperal female (*1, 2*). In contrast, the concaveation-induced maternal behavior of the virgin female has been considered free of endocrine control, since virgins subjected either to ovariectomy or hypophysectomy 14 days before the introduction of young respond not only in typical nurtural fashion but also with an average latency no different from that of intact controls (*3*). However, one can question whether, after such a relatively short postoperative period, the behavior expressed was actually free of ovarian and pituitary influence. For example, if ovarian hormones normally act in the virgin female to inhibit rather than to facilitate maternal behavior, then 2 weeks may not be long enough for this inhibition to dissipate. Thus, the ovariectomized virgin animals in the above-mentioned study may have still been under the suppressive effects of estrogen or progesterone.

Unfortunately, it is not known how long it takes for ovarian steroids to disappear from the brain of the rat following ovariectomy. Moreover, even after the steroids themselves have disappeared, cellular and subcellular trace effects may persist. On the assumption that a postoperative interval of more than 2 weeks is required for complete clearance—at least from the neuronal system mediating maternal behavior—we ovariectomized virgin females and tested for the onset of nurtural responsivity after 4 and 8 weeks. We found a highly significant latency difference contingent on the time since ovariectomy, which in turn prompted further investigation into the role of estrogen as the specific inhibitory agent.

At 70 to 90 days of age, 45 virgin female Wistar rats, reared in our laboratory, were divided into three groups. Group 4-W was bilaterally ovariectomized and tested for maternal behavior 4 weeks later; group 8-W was also ovariectomized but was tested 8 weeks later. The females of the third group remained intact and served as controls. Seven days before testing, each female was transferred from her standard laboratory cage into a large observation cage. Nest material, consisting of paper strips, was provided at 10 a.m. on the initial test day, and 4 hours later three foster pups, 1 to 3 days old, were presented. The pups remained until the following morning, when a fresh litter was substituted. This procedure continued until maternal behavior was displayed or until 7 days had elapsed. Animals not responding within this time limit were assigned latencies of 168 hours.

Table 1. Median latency to maternal behavior and quartile deviations (Q). The interval between ovariectomy (for females) or castration (for males) and testing was 4 weeks in the 4-W groups and 8 weeks in the 8-W groups. Where indicated, estrogen benzoate (EB) or testosterone propionate (TP) was given 8 days before testing, and MER-25 was given 4 days before testing.

Group	Latency (hours)	
	Median	Q
Females		
4-W	120	33
8-W	24	8
Intact control	120	51
4-W + MER-25	48	81
8-W + EB	168	63
Males		
4-W	168	42
8-W	8	34
8-W + TP	96	54
Intact control	120	72

The female was observed for 15 minutes after the pups were placed in the cage, and then checked each hour throughout the day. When a nest was discovered, and when it was evident that the pups had been retrieved to the nest, we observed the female for 30 minutes and noted whether she crouched over the young in a nursing posture. She was scored as having adopted such a posture if the pups were deployed under her and if she remained either entirely passive or engaged in no activity other than readjusting her posture in an effort to sustain the position of the young. The young themselves were then examined to determine whether they were warm and whether they had been licked, as indicated by the absence of urine.

Since each of the events or conditions just described is characteristically exhibited by the puerperal female and since each, in turn, is essential for litter survival, a female was considered "maternal" only if she built a nest, retrieved, displayed a nursing posture, licked the young, and kept them warm —in other words, only if she expressed the full spectrum of nurtural attachment. Females ovariectomized 8 weeks before testing had a significantly lower ($P < .01$) median latency to maternal behavior than either the intact control females or those ovariectomized only 4 weeks before testing (Table 1) (*4*). To determine whether estrogen, or rather the withdrawal of estrogen, was responsible for the reduced response latency of group 8-W, we instituted steroid replacement, giving estrogen at the dosage used by Moltz *et al.* (*1*) in regulating the expression of maternal behavior in the virgin animal. Beginning 49 days after castration, 15 females were injected daily with 12 μg of estradiol benzoate for 7 days (group 8-W + EB), after which they were presented with foster young as described above. These females, tested 8 weeks after ovariectomy but given estradiol benzoate during the last postoperative week, exhibited a significantly higher median response latency ($P <$

.01) than their noninjected counterparts. In contrast, they did not differ significantly from group 4-W or from the control group.

To clarify further the role of endogenous estrogen in concaveation-induced maternal behavior in the virgin animal, we used one additional group of 15 females (group 4-W + MER-25). These females were ovariectomized 4 weeks before testing, and during the last 4 days of the postoperative interval they were given daily injections of 50 mg of ethamoxytriphetol (MER-25), a drug that blocks the action of estrogen both centrally and peripherally (*5*). Our thinking was that MER-25 should reduce the effective levels of estrogen in the females ovariectomized for 4 weeks and consequently reduce the latency to maternal behavior. As expected, group 4-W + MER did not differ from group 8-W but did differ significantly from each of the remaining three groups ($P < .05$).

The adult male, like the virgin female, also responds to young if given an average of 5 or 6 days of continuous exposure. Because this concaveation-induced maternal behavior of males has also been considered free of endocrine influence on the basis of a postoperative interval of only 2 weeks (*3*), we investigated the possible inhibitory effects of testosterone. Males were tested 4 and 8 weeks after castration and compared with intact controls. In addition, steroid replacement was undertaken in 8-week castrates by giving daily injections of 0.5 mg of testosterone propionate during the last 7 days of the postoperative period. The results for males parallel those for virgin females (Table 1). Males 8 weeks after castration (group 8-W) had a significantly lower median latency to maternal behavior ($P < .01$) than did either intact controls or males 4 weeks after castration. Steroid replacement in males castrated for 8 weeks increased the median latency; these males did not differ significantly either from controls or animals castrated for 4 weeks ($P > .05$).

We have demonstrated that concaveation-induced maternal behavior in the rat is under endogenous steroid control and that this control is normally inhibitory in nature. Continuous stimulation by young can overcome the inhibition, but only after 5 to 6 days. Long-term removal of the gonads facilitates the inductive effects of concaveation, presumably by removing the suppressive action of testosterone and estrogen on the neuronal system mediating maternal behavior in the male and female, respectively.

The hormones accompanying the termination of pregnancy have been suggested as the agents responsible for the immediate responsivity of the puerperal female. Among these hormones is estrogen, which, when injected together with prolactin during progesterone withdrawal, facilitates the expression of maternal behavior in the ovariectomized virgin animal (*1*, *2*). The data reported here, in contrast, show estrogen to have an inhibitory influence. These results indicate that the particular behavioral effect exerted depends on the prevailing endocrine condition of the animal. Thus, in the virgin female subjected to high prolactin concentration and decreasing progesterone concentration (as normally occurs at the time of parturition), estrogen acts synergistically to facilitate the display of maternal behavior. On the other hand, in the normally cycling or ovariectomized female, each of which presents an hormonal picture different from that of the treated animal, estrogen inhibits the display of maternal behavior.

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References and Notes

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Transformation of Cell Cultures Derived from Human Brains

Hooks *et al.* (*1*) describe transformation of cells in culture derived from a brain of a patient with Creutzfeldt-Jakob disease. These cells (henceforth referred to as "brain cells"), maintained in parallel with many other cultures of brain cells, began forming colonies, lost contact inhibition, and exhibited an aneuploid number of chromosomes. The authors failed to observe a similar transformation in the parallel cultures, or in any other cultures of human brain cells maintained in their laboratory. Although they detected a viruslike particle in these cells by electron microscopy, they were unable to "rescue" an infectious virus from these transformed cells. It appears unlikely from the authors' careful assessment of the conditions in their laboratory that this apparent transformation resulted from contamination of their cultures either by a virus, or by stray cells from some other tissue culture. They concluded that this transformation was indeed "spontaneous," but were unable as yet to relate this change to Creutzfeldt-Jakob disease.

Their report is the third of otherwise unrelated studies documenting transformation of human brain cells in culture. We observed this phenomenon (*2*) in two such cultures derived from patients with subacute sclerosing panencephalitis (SSPE). These two cell cultures, like the one reported by Hooks *et al.*, "spontaneously" lost their contact inhibition, began forming colonies, and displayed extensive chromosomal abnormalities, but retained their identity as human cells. In our laboratory, as in that of Hooks *et al.*, other human brain cultures failed to exhibit transformation.

Webb *et al.* (*3*) studied a human fetal brain culture deliberately infected with attenuated strain of measles virus and observed transformation of these cells, characterized by loss of contact inhibition and by colony formation. They were able to recover the original infectious virus from these transformed cells.

In view of the considerable divergence of these studies and conditions in the respective laboratories, as well as the different origin of the cells (SSPE, normal human fetal brain, and Creutzfeldt-Jakob disease) it appears that the only link among these three observations is the fact that the transformed cells were derived from human