tained the 2E9A determinant would be detected. I previously showed that peptide maps of MHC from adult dystrophic PM contained many peptides in common with neonatal MHC but were not absolutely identical to the peptide map of MHC from 20-day chick PM (*8*). However, as shown in Fig. 2C, the peptides detected by 2E9A are identical in the peptide maps of MHC's from 20-day normal PM and 1-year dystrophic PM at two different *Staphylococcus aureus* V8 protease concentrations. This strongly suggests that 2E9A was reacting with the same myosin in both samples and not with a closely related isoform.

The distribution of neonatal MHC in adult dystrophic muscle fibers was then determined by immunocytochemistry. As shown in Fig. 3, 2E9A reacted with virtually all fibers in adult dystrophic muscle but with no fibers in adult normal muscle. All fibers of normal and dystrophic PM from 20-day chicks reacted with 2E9A. Thus all fibers of the dystrophic PM continued to express neonatal MHC. In a previous report I suggested from my analysis of MHC peptide maps that adult dystrophic PM contained predominantly neonatal MHC in addition to other myosins. Now, having developed a monoclonal antibody specific to the neonatal MHC, I have been able to confirm the presence of this isoform in all fibers of dystrophic PM. The present results also suggest that other myosins are present in dystrophic PM. However, since other monoclonal antibodies that will react specifically with embryonic or adult MHC's are not available, these myosins cannot now be identified.

While it is widely assumed that degeneration and regeneration is occurring in dystrophic muscle, there is little direct evidence of this. Since the amount of atrophy or hypertrophy varies in different dystrophic lines depending on background genes (*6*), regenerative processes are probably secondary effects of muscular dystrophy. In my study, virtually all muscle cells of the dystrophic PM, irrespective of fiber size, reacted with 2E9A. Thus dystrophic muscle fibers continue to accumulate neonatal MHC and fail to undergo the isoform transition from neonate to adult that is characteristic of normal muscle. This makes it unlikely that regeneration per se is responsible for the continued expression of immature isoforms, although the occasional cell that does not react with 2E9A may represent a regenerating fiber that has been shown to contain embryonic myosin (*9*). It should be pointed out that neonatal MHC has not been found in regenerating muscle (*9*), and if all the fibers in a 6-month-old dystrophic chicken are regenerating fibers (an unlikely situation), this would probably be the first demonstration of neonatal myosin in such fibers.

While other isozyme changes have been shown to be inhibited in dystrophic fibers (*3*), myosin is, to my knowledge, the only muscle protein that undergoes a transition from embryo to neonate to adult. Since the first isoform change occurs normally in dystrophic muscle, these results indicate a subsequent block in maturation 1 month after hatching. The product of the dystrophic gene has not yet been identified, but its action is consistent with its being a regulator of the expression of many gene families. One would predict that such a regulator would be present at very low levels, which may explain its elusive nature. Our knowledge of the regulation of multigene families in which different genes are expressed at different ages is very limited. It remains possible that the processes involved in turning genes on and off during normal development are impaired in dystrophic muscle, resulting in the variety of isozyme abnormalities that have thus far been identified.

Note added in proof: I have now produced a monoclonal antibody specific for the adult MHC and found a small amount of this isoform to be present in adult dystrophic PM.

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Prolactin Stimulation of Maternal Behavior in Female Rats

Abstract. *Inexperienced, hypophysectomized female rats treated with steroids were used in experiments to investigate the roles of the pituitary gland and prolactin in the expression of maternal behavior. Administration of ovine prolactin or treatment with ectopic pituitary grafts, which release prolactin into the circulation, stimulated maternal care in these females toward rat young. Steroid treatment alone, while stimulating maternal behavior in rats with intact pituitary glands, did not facilitate maternal responsiveness in hypophysectomized females. These findings indicate a stimulatory behavioral role for pituitary prolactin in the establishment of maternal care and suggest that exposure to prolactin during pregnancy helps to stimulate the immediate onset of maternal behavior at parturition.*

Studies of the relation between endocrines and behavior have indicated that changes in hormonal status during pregnancy contribute to the expression of maternal care at parturition in mammals (*1*). While the steroids estradiol and progesterone have been shown to stimulate maternal responsiveness in behaviorally inexperienced females, evidence for a role of pituitary hormones—most notably prolactin, which is secreted in large amounts during early and late stages of pregnancy (*2*)—in the induction of maternal behavior in mammals is equivocal. The early finding of Riddle *et al.* (*3*) that repeated injections of prolactin stimulated maternal behavior in female rats led to the suggestion that prolactin was a

maternal hormone. However, attempts to replicate this finding (*4*) or to stimulate maternal care by increasing the concentration of prolactin in serum of ovariectomized, nulliparous rats by means of ectopic pituitary grafts (*5*) have been unsuccessful. Furthermore, treatment of rats after surgical termination of pregnancy with drugs that inhibit prolactin secretion has not interfered with the establishment of maternal behavior (*6, 7*). Other reports have shown that prolactin or prolactin-like molecules originating in the central nervous system facilitate female sexual behavior in rats (*8*), while exogenous prolactin stimulates grooming and inhibits copulatory behavior in male rats (*9*). On the basis of these findings and the development of a hormone regimen that reliably stimulates maternal behavior in ovariectomized rats (*10, 11*), we have reexamined the roles of the pituitary gland and prolactin in maternal behavior. Our results indicate stimulatory roles for the pituitary gland and prolactin in the expression of maternal behavior in steroid-treated female rats.

In the initial experiment we determined whether treatment of rats with a steroid hormone regimen that stimulates maternal behavior in females with intact pituitary glands (*11*) would also stimulate maternal behavior in animals whose pituitary glands had been removed. Hypophysectomized and nonhypophysectomized nulliparous female rats [strain Crl:CD(SD)BR; 201 to 225 g in body weight] were obtained from Charles River Breeding Laboratories (Wilmington). Rats were housed individually in translucent polypropylene cages (20 by 45 by 25 cm) in light- and temperature-controlled rooms (lights on from 0500 to 1900 hours; 21° to 24°C). On day 1 of treatment, half of the hypophysectomized and nonhypophysectomized rats were ovariectomized and treated with subcutaneously implanted, progesterone-filled Silastic capsules (3 by 30 mm) (*11*). The remaining females were ovariectomized and given blank implants. On day 11 of treatment (from 1000 to 1200 hours), capsules were removed from all rats and estradiol-filled capsules (2 mm) were implanted into those animals that had been treated with progesterone.

Testing for maternal responsiveness (*11*) began on day 12 between 0930 and 1100 hours. The behavioral responses of the animals toward three 3- to 8-day-old foster young were recorded daily during 1-hour sessions. Animals were tested for 11 days or until they exhibited full maternal behavior (retrieving three pups and grouping them in the nest) on two consecutive test days. After testing on day 11, rats were killed. Blood was collected for determination of prolactin concentration by radioimmunoassay (rat prolactin kit, NIADDK RP-3). At autopsy the sellae turcica were examined for pituitary fragments, adrenal weights were recorded, and the presence of capsules was confirmed. Animals with detectable concentrations of prolactin in serum, pituitary fragments in the sella turcica, or elevated adrenal weights were omitted from the study before statistical analyses.

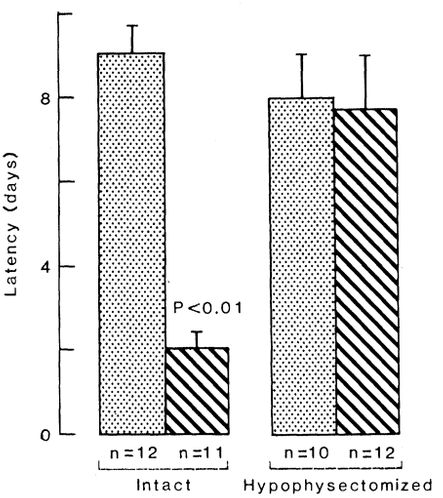

Fig. 1. Effects of hypophysectomy and steroid treatment on the expression of maternal behavior in ovariectomized, nulliparous rats. Responses are expressed as means ± standard error of the latency to onset of full maternal behavior. Striped boxes, steroid-primed females; stippled boxes, controls. Statistical analysis was by analysis of variance.

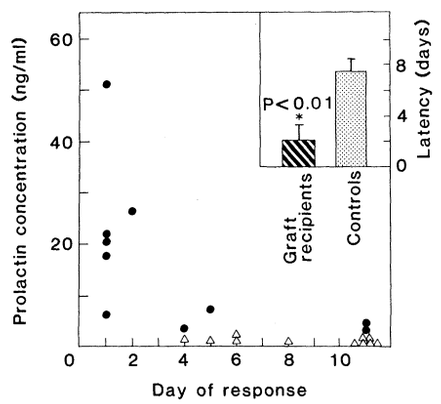

Fig. 2. Serum prolactin concentrations in steroid-treated, ovariectomized, hypophysectomized rats bearing ectopic pituitary grafts (●) or no grafts (controls △). Prolactin concentrations are shown in relation to the initial day of maternal responsiveness for each maternal animal. Concentrations were highest in graft recipients that responded rapidly to foster young (note response days 1 and 2). Values on maternal response day 11 are from eight rats that did not display maternal behavior. One of these, a graft recipient, killed test young in each of 11 test sessions; its score was not included in the calculation of behavioral latencies. As shown in the inset, graft recipients (n = 10) exhibited full maternal behavior significantly faster than did controls (n = 11). Statistical analysis was by t-test.

The effects of hypophysectomy and steroid treatment on maternal behavior are shown in Fig. 1. Sequential treatment with progesterone and estradiol stimulated a rapid onset of full maternal behavior in rats with intact pituitary glands. However, the identical hormone treatment did not stimulate maternal behavior in hypophysectomized rats. The pituitary gland, therefore, is required for this steroid-facilitated maternal behavior.

In the second experiment the effects of pituitary (prolactin) replacement therapy on the induction of maternal behavior were measured in behaviorally inexperienced female rats. Two groups of hypophysectomized, nulliparous female rats were ovariectomized and treated with subcutaneously implanted, progesterone-filled Silastic capsules (3 by 30 mm) 2 to 3 days after their arrival in our laboratory. At the time of implantation (day 1), females in one group had the anterior lobes of two pituitary glands from adult donor female rats grafted under the kidney capsule. These ectopic pituitary grafts secrete prolactin into the circulation because the pituitary lactotropes are freed from the direct inhibitory influence of the hypothalamus. Pituitary grafts remained in these animals throughout the study. Rats in the other group underwent sham operations on day 1 of treatment. On day 11 (between 1000 and 1200 hours), progesterone-filled capsules were removed from all animals and each rat was implanted with an estradiol-filled capsule (2 mm). Testing for maternal responsiveness began on day 12 between 0930 and 1100 hours and was conducted once daily as before. Blood samples were collected by jugular venipuncture from rats, under light ether anesthesia, that displayed complete maternal behavior after testing on the second consecutive day of full responsiveness. Trunk blood was collected from the remaining animals after testing on test day 11. At this time all rats underwent autopsy for validation of hypophysectomy. Serum concentrations of prolactin were determined by radioimmunoassay.

Rats given ectopic pituitary grafts displayed full maternal behavior significantly faster than did control rats (Fig. 2). Latencies for experimental and control groups to exhibit full responsiveness were 2.0 and 7.6 days, respectively. The strength of the behavioral stimulation

induced by the pituitary grafts was indicated by the observation that five of ten graft recipients retrieved, grouped, and crouched over foster young within 30 minutes during the initial test session; four of these five female responders retrieved and grouped three test young within 3 minutes. In contrast, only one control animal responded maternally during the first four test sessions. A significant positive correlation (r_s, 0.636; $P < 0.05$) was found between the rapidity of onset of maternal behavior in graft recipients and the concentration of circulating prolactin (Fig. 2). Graft recipients that responded most rapidly to foster young had the highest concentrations of prolactin in serum.

In the third experiment, the effects of prolactin administration on maternal behavior were examined. Two groups of hypophysectomized female rats were ovariectomized and treated with subcutaneously implanted progesterone-filled Silastic capsules (3 by 30 mm) on day 1 of treatment. From day 1 to day 13, rats were injected twice daily (at 0900 and 1900 hours) with either ovine prolactin (NIADDK O-PRL-16) at a dose of 500 μg per injection or 200 μl of polyvinylpyrrolidone-saline vehicle. On day 11 of treatment, progesterone implants were removed and replaced with estradiol-filled capsules (2 mm). Behavioral testing began between 1000 and 1100 hours on day 12, 22 to 24 hours after insertion of the estradiol implants and 1 hour after the injection with prolactin or vehicle. Testing was conducted daily for 11 days. After the final behavioral test rats underwent autopsy, and sera were assayed for prolactin content.

Prolactin treatment stimulated a more rapid onset of full maternal behavior than did vehicle treatment in steroid-primed, hypophysectomized females (Fig. 3). In addition, latencies of the prolactin-treated rats to carry a pup (mean latency, 2 days) and to group the young in the nest (mean latency, 3 days) were significantly shorter than those of control rats [mean latencies, 5 and 8 days, respectively; $P < 0.05$ and < 0.01 (t-tests)].

The results of these three experiments indicate a role for prolactin in the induction of maternal behavior in the rat. It appears that during pregnancy exposure to prolactin in combination with the steroids estradiol and progesterone helps to prime the female to respond maternally toward young at parturition. To reconcile the findings of earlier studies on prolactin and maternal behavior with our results, we are led to make two proposals. First, the actions of prolactin [and perhaps prolactin-like molecules (*12, 13*)] appear to depend on gonadal steroids. Second, prolactin, like estradiol and progesterone (*11*), may stimulate the induction of full maternal behavior over a prolonged period during gestation rather than just acutely during the prepartum period. If these proposals are applied to earlier reports, it is possible to account for their negative results. Specifically, the administration of prolactin by injections (*4*) or ectopic pituitary transplants (*5*) to female rats in the absence of the gonads or sufficient steroidal priming would not be expected to stimulate maternal care in inexperienced females. Likewise, acute pharmacological disruption of prolactin secretion with ergocornine (*6*), CB-154 (*6*), or apomorphine (*7*) might not disrupt the onset of maternal behavior induced by surgical termination of pregnancy because the animals had been exposed to high titers of prolactin (*2*) and prolactin-like molecules [placental lactogen (*13*)] for 16 days before surgery and behavioral testing.

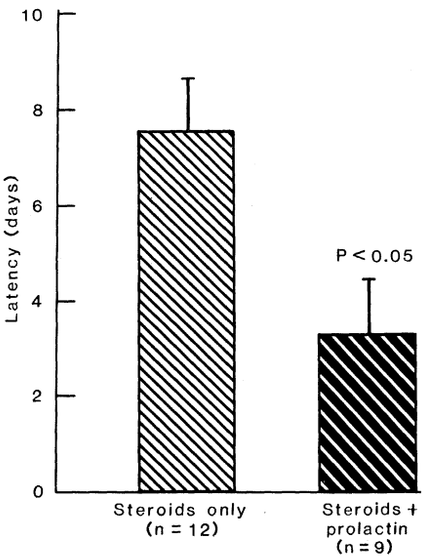

Fig. 3. Effects of prolactin treatment on the latency to onset of maternal behavior in steroid-treated, hypophysectomized, ovariectomized, nulliparous rats. Statistical analysis was by t-test.

Our findings define a role for pituitary prolactin in the induction of maternal behavior, that is, of responses oriented toward young, under controlled experimental conditions. Unlike the involvement of prolactin in the regulation of female sexual behavior, in which prolactin-like molecules apparently produced in neurons in the central nervous system affect the behavioral responses of the female (*8*), the site of origin of the prolactin stimulating maternal behavior is the pituitary gland. Thus, it appears that prolactin released by the pituitary gland feeds back on the central nervous system to affect the responsiveness of the female to young. Whether prolactin-like molecules of nonpituitary origin, such as placental lactogen or immunoreactive prolactin synthesized within the brain, mediate or supplement the actions of pituitary prolactin in the regulation of maternal behavior remains to be determined.

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