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# Central Infusions of the Recombinant Human Prolactin Receptor Antagonist, S179D-PRL, Delay the Onset of Maternal Behavior in Steroid-Primed, Nulliparous Female Rats*

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**ABSTRACT**

The expression of maternal behavior in the newly parturient rat is under endocrine regulation. Blocking endogenous PRL secretion with bromocriptine delays the normal rapid expression of maternal care shown toward foster young in steroid-primed virgin female rats. The recent development of the PRL receptor antagonist S179D-PRL, a mutant of human PRL in which the serine residue at the 179 position is replaced with aspartate, provides a potentially useful tool to examine the role of PRL in neural processing. In the present report, three experiments were conducted that examined the effects of this PRL antagonist on the induction of maternal behavior. In each experiment, ovariectomized, nulliparous rats were treated sequentially with SILASTIC capsules implanted sc with progesterone (days 1–11) and estradiol (days 11–17), a treatment that stimulates a rapid onset of maternal behavior in virgin rats. On day 11, females were implanted with Alzet miniosmotic pumps connected to cannulae directed unilaterally at the lateral ventricle (Exp 1) or bilaterally at the medial preoptic area (MPOA; Exp 2 and 3). Pumps contained either doses of S179D-PRL (0.115 or 1.15 mg/ml; Exp 1 and 2), wild-type human PRL (1.15 mg/ml; Exp 3), or the saline vehicle (Exp 1–3). Testing for maternal behavior began on day 12, a day after pump insertion, and animals were tested daily for 6 days. Latencies to contact, retrieve, and group foster test young were recorded. Administration of both the high and low doses of S179D-PRL infused into the lateral ventricle (Exp 1) or MPOA (Exp 2) significantly delayed the onset of maternal behavior. In contrast, MPOA infusions of the control hormone, wild-type human PRL, in Exp 3 did not delay the onset of maternal behavior. These findings support the concept that the effects of S179D-PRL are caused by its actions as a PRL receptor antagonist rather than by a nonspecific effect of the protein. Overall, these results demonstrate the effectiveness of S179D-PRL acting at the level of the central nervous system (and, more specifically, within the MPOA) to regulate maternal behavior, a PRL-mediated response. (*Endocrinology* **142:** 730–739, 2001)

THE RAPID ONSET of maternal behavior in the newly parturient rat is stimulated, in part, by the hormonal changes accompanying pregnancy. Whereas nulliparous adult female rats require an average of 5–6 days of continuous pup exposure to induce maternal-like responses, the parturient female displays a full complement of maternal behaviors at birth and, in some cases, before parturition (1, 2). Increases and alterations in the patterns of secretion of lactogenic hormones, *i.e.* PRL and placental lactogens, in combination with exposure to progesterone ($P_4$) and estradiol ($E_2$), are thought to stimulate the female to display maternal care post partum, *i.e.* retrieve pups to the nest, group them together, and crouch over the young to nurse and keep them warm.

Earlier studies in our laboratory demonstrated that the rapid onset of maternal behavior induced by steroid treatment in virgin rats could be delayed by the systemic administration of bromocriptine, a dopamine agonist, which suppresses endogenous PRL secretion (3). Moreover, central infusions of PRL into the lateral ventricle (LV) or medial preoptic area (MPOA) of ovariectomized, nulliparous rats, concurrently treated with steroids plus bromocriptine, facilitated a rapid onset of maternal care (4). Recent work in mice has indicated that the PRL receptor mediates the expression of maternal care (5). Specifically, female mice with null mutations for the PRL receptor exhibited deficits in maternal behavior, both as homozygous virgins and heterozygous primiparous dams.

The possible involvement of the PRL receptor in the hormonal induction of maternal behavior, however, has not been elucidated. The development of the PRL receptor antagonist, S179D-PRL, a mutant of wild-type human PRL (wt hPRL) in which the serine residue at position 179 is replaced with aspartate, provides a molecular tool to examine the role of PRL in neural processing (6). Use of S179D-PRL has the potential to bypass the possible neurological side effects of bromocriptine on brain dopaminergic systems and their possible effects on maternal care (3). Moreover, evaluation of the effectiveness of S179D-PRL within the brain will tell us whether this compound can act as a behavioral and neurobiological receptor antagonist, a finding which may prove to be clinically important.

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In the present study, three experiments were conducted that examined the possible central involvement of PRL receptors in the induction of maternal behavior. In the first study, it was asked whether direct infusions of the PRL receptor antagonist, S179D-PRL, into the ventricular system of steroid-primed, nulliparous rats would delay the onset of maternal behavior displayed toward foster young. The second study investigated whether one site of action of the PRL receptor antagonist was the MPOA. In this experiment, S179D-PRL was infused bilaterally into the MPOA of steroid-primed, nulliparous rats, and maternal behavior was measured. The third study asked whether the possible inhibitory effects of the PRL receptor antagonist were specific to the properties of the antagonist. To test this possibility, wt hPRL was infused into the MPOA, and its effects were compared with those of vehicle-treated controls. The results of these studies demonstrate that central infusions of S179D-PRL into either the ventricles or MPOA delay the onset of maternal care. The effects within the MPOA can be attributed to the biological properties of the PRL antagonist, because infusions of the wt hPRL did not interfere with the expression of maternal behavior.

## Materials and Methods

### *Animals*

Nulliparous female Sprague Dawley rats 225–250 g (Crl:CD[SD]BR) were purchased from Charles River Laboratories, Inc. (Kingston, NY). Test females were housed in polypropylene cages (45 × 25 × 20 cm) that contained 1-inch Plexiglas dividers, which separated the cage into comparable-sized quadrants. Food and water were available *ad libitum* in light (on 0500–1900 h)- and temperature (21−25 C)- controlled rooms. A separate set of lactating donor rats was maintained in our colony to provide a source of test young. All animals used in this study were maintained in accordance with the guidelines of the National Research Council.

### *Steroid treatment*

One to 2 weeks after arriving in our laboratory, all experimental animals were ovariectomized under ketamine/xylazine (100 μl/100 g) anesthesia. After a 1-week recovery period (on day 1 of steroid treatment), animals were anesthetized with Isoflurane inhalant and implanted sc with three 30-mm $P_4$-filled SILASTIC capsules. On day 11 (ketamine/xylazine anesthesia), $P_4$ implants were removed, and one 2-mm $E_2$ SILASTIC capsule was implanted. This steroid regimen has previously been shown to stimulate a rapid onset of maternal behavior in behaviorally inexperienced, nulliparous rats, reducing latencies from about 5 days to 1–2 days (7).

### *Preparation of the PRL receptor antagonist*

Both PRL preparations were produced and characterized as previously described (6). The proteins used in this study were expressed and purified in parallel and were expressed at similar levels (6). The recombinant PRLs were then tested for proliferative activity (wt hormone) in the Nb2 assay, or antagonism of this (S179D PRL). Recombinant PRL preparations were concentrated in saline using Amicon Centripreps (Amicon, Danvers, MA).

### *Experimental treatments*

*Exp 1: The effect of infusions of the PRL receptor antagonist, S179D-PRL, into the LV on the rate of induction of maternal behavior in steroid-primed, nulliparous rats.*

The goal of the first study was to determine whether central infusion of the PRL receptor antagonist, S179D-PRL, into the brain via the ventricular system would delay the rate of onset of maternal behavior. Animals (n = 9–10/group) were implanted with a sc miniosmotic pump (Model no.1007D; Alza Corp., Palo Alto, CA) connected via a 10-cm polyethylene (PE60) catheter tube to a 28-gauge cranial connector cannula (Plastics One Inc., Roanoke, VA) directed at the right LV at the time the steroid capsules were changed on treatment day 11. The stereotaxic coordinates based upon bregma for placement of unilateral cannulae in the LV was AP = −0.8; ML = −1.5; DV = +3.5 (8). The miniosmotic pumps, which were preincubated for 24 h before implantation, contained one of two doses (0.115 mg/ml or 1.15 mg/ml) of the PRL receptor antagonist [S179D-PRL (6)] or vehicle (0.85% NaCl). The infusion rate of the pumps was 0.5 μl/h. On day 12 of treatment, approximately 24 h after the miniosmotic pumps were implanted, behavioral testing began. Testing was conducted once daily for a maximum of 6 days. At the completion of testing, brains were perfused and stored at −80 C until histological verification of cannula placement sites.

*Exp 2: The effects of MPOA infusions of the PRL receptor antagonist, S179D-PRL, on the induction of maternal behavior.*

The objective of the second study was to assess whether direct infusions of S179D-PRL into the MPOA of steroid-primed, nulliparous rats would delay the onset of maternal behavior. On day 11 of treatment, at the time that $E_2$ capsules were inserted, animals were sc implanted with miniosmotic pumps (Model no. 1002), each of which delivered its contents at a rate of 0.25 μl/h for 14 days. Each pump was fitted with an 8-cm length PE60 tubing that was attached to a Y connector, which in turn was attached to the bilateral cannula. The connector cannulae were directed at the MPOA (coordinates based upon bregma: AP = −0.3; ML = ±1.0; DV = +9.0; 8). Pumps were primed before implantation, and tubing was filled with the infusate before surgery. The MPOA infusates consisted of S179D-PRL (0.115 mg/ml or 1.15 mg/ml) or vehicle (0.85% NaCl). Behavioral testing was conducted once daily for 6 days from treatment days 12–17 or until an animal reached the criterion for being fully maternal. Brains were collected at the end of testing to verify cannula placements.

*Exp 3: Effects of infusions of wt hPRL into the MPOA on the induction of maternal behavior.*

The goal of the third experiment was to determine whether infusions of a control substance, wt PRL, into the MPOA would interfere with the rate of onset of maternal behavior in steroid-primed, nulliparous rats.

Adult nulliparous female rats were implanted with $P_4$-filled SILASTIC capsules on treatment day 1, 1 week after ovariectomy. On day 11, the $P_4$ capsules were removed, a single $E_2$-filled capsule was implanted sc, and a 14-day Alzet miniosmotic pump (Model no. 1002) was connected to a bilateral connector cannula directed at the MPOA, as described in Exp 2. Pumps were filled with either wt hPRL (1.15 mg/ml) or vehicle (0.85% NaCl). Behavioral testing began on treatment day 12 and continued for a maximum of 6 days as described below. At the end of testing, brains were collected for subsequent histological analysis of cannula placement sites.

### *Testing and histology*

In each experiment, maternal behavior testing began on treatment day 12, between 0900 and 1200 h. To initiate a test session, three foster pups, 3–9 days of age, were placed in separate quadrants of the cage, avoiding the test animal's nest site. Latencies for the test female to contact, retrieve, group, and crouch over the foster test young were recorded continuously for 15 min, then checked every 15 min for pup and subject positions, throughout the daily 1-h test session (7). Subjects were tested for 6 consecutive days or until an animal displayed full maternal behavior (FMB) for 2 consecutive days. On test days 2–6, the previous test day's pups were removed from the test cage, an hour before testing.

At the end of each experiment, animals were rapidly decapitated, and

brains were removed and fixed in 10% formalin for histological examination. Brains were sectioned at 40 microns and then stained with cresyl violet before viewing cannula placements.

### *Statistical analyses*

One-way ANOVAs and Newman-Keuls tested the latencies to retrieve, group, and crouch over the test pups and display FMB. The Fisher's exact probability test was used to compare the numbers of animals displaying specific behaviors on designated test days. Statistical significance was noted by a $P$ value $\leq 0.05$.

## Results

### *Exp 1: The effect of infusions of the PRL receptor antagonist, S179D-PRL, into the LV, on the rate of induction of maternal behavior in steroid-primed, nulliparous rats*

Infusions of the PRL receptor antagonist into the LV delayed the expression of maternal behavior, most notably in animals receiving the high dose of S179D-PRL. As shown in Fig. 1A, fewer low- and high-dose females retrieved a single

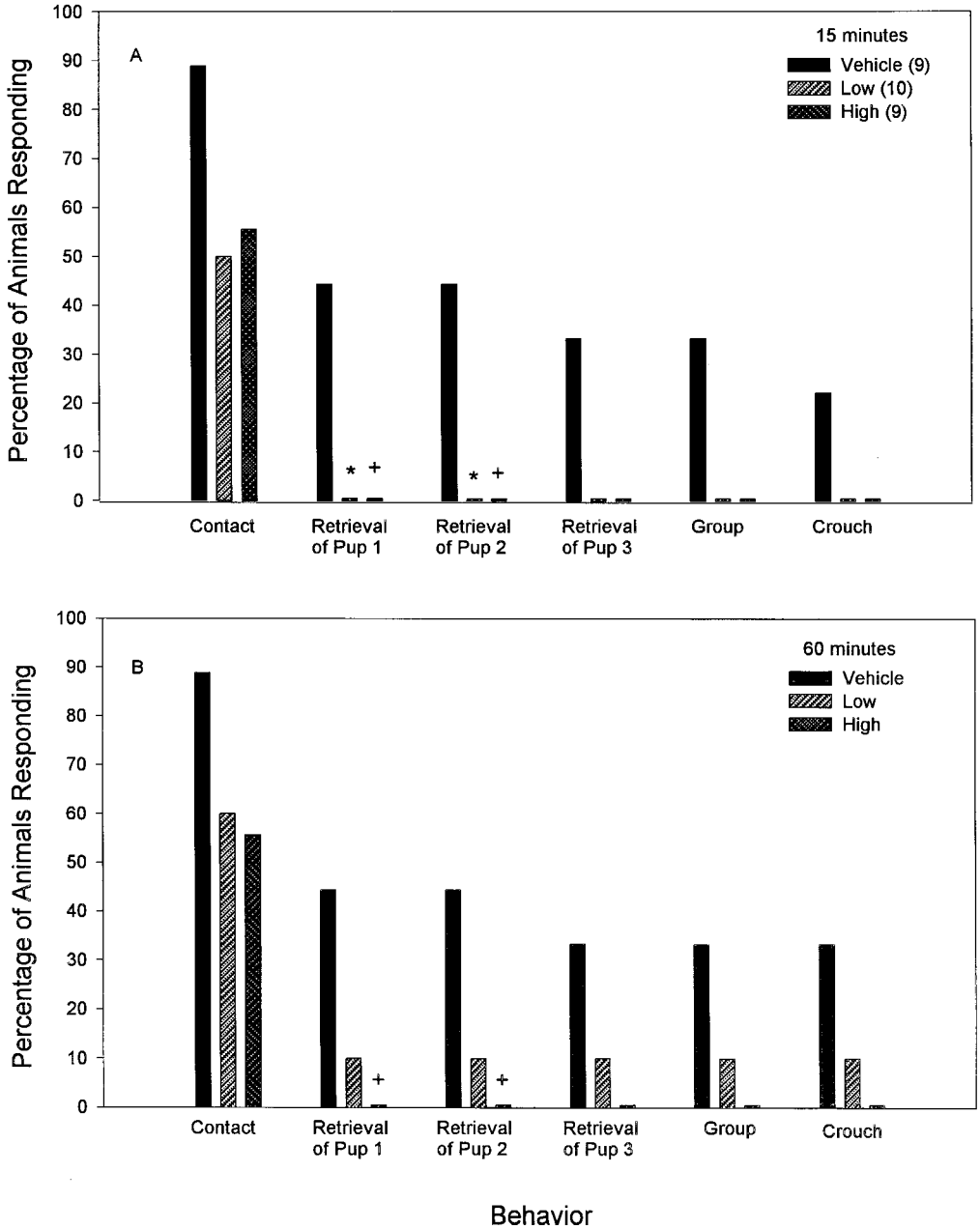


FIG. 1. Percentages of animals responding maternally within the first 15-min (A) and full 60-min (B) test periods on test day 1 (Exp 1). Subjects received intracerebroventricular infusions of vehicle (n = 9) or S179-PRL (low dose, n = 10; high dose, n = 9). As shown in A, the low dose of S179D-PRL (*) resulted in significantly fewer animals that retrieved the 1st and 2nd pup ($P = 0.033$; Fisher's exact probability test). Likewise, the high dose of S179D-PRL (+) significantly delayed the onset of retrieving the 1st and 2nd pup ($P = 0.041$). At 60 min (B), only the high dose of S179D-PRL inhibited retrieval of the 1st and 2nd pup ($P = 0.041$).

or two pups during the first 15 min of testing than did vehicle controls ($P < 0.05$). This effect persisted in the high-dose group at the end of the 1-h test session on test day 1, given that 44% of vehicle-infused animals retrieved 1 or 2 pups, compared with none of the high-dose antagonist group ($P < 0.05$; see Fig. 1B). No statistical differences were found in the other measures of maternal behavior at 15 min or 1 h on the first test day. The cumulative percentages of animals that displayed FMB over the 6 test days are shown in Fig. 2. On the second test day, significantly more vehicle-infused females (7 of 9) were fully maternal than were high-dose-treated animals (2 of 9; $P < 0.05$). Hence, infusion of the high dose of the PRL receptor antagonist directly into the ventricular system delayed the rapid onset of full maternal care on this test day.

In addition to delaying the onset of maternal behavior, central infusions of the PRL receptor antagonist also lengthened the latency of subjects to display selected aspects of maternal care on an animal's first day of responsiveness. Specifically, the latency of low-dose-treated females (mean ± SE = 933 ± 334 sec) to retrieve the second test pup was significantly longer than that of vehicle-infused animals (245 ± 61 sec; $P < 0.05$). No other behavioral measures differed between the vehicle and low-dose groups, and no differences in behavioral responses on the first day of full responsiveness were found between the vehicle and high-dose groups.

### *Exp 2: The effects of MPOA infusions of the PRL receptor antagonist, S179D-PRL, on the induction of maternal behavior*

Direct bilateral infusions of the PRL antagonist into the MPOA of steroid-primed, nulliparous rats resulted in a more profound delay in the onset of maternal behavior than that found when S179D-PRL was infused into the LV in Exp 1. As shown in Fig. 3A, fewer animals treated with the low and high doses of S179D-PRL retrieved, grouped, and crouched over the three test pups than did vehicle controls during the initial 15-min test period on treatment day 12 ($P$ values $< 0.05$–$0.02$). A similar response pattern was present at the end of the first 1-h test session (see Fig. 3B), except that only the low-dose and vehicle groups differed statistically from each other at the end of the 1-h test (8 of 11 vehicle rats retrieved 3 pups, compared with 3 of 13 low-dose animals; $P < 0.02$).

The overall full maternal responses of animals given bilateral MPOA infusions of the PRL receptor antagonist are shown in Fig. 4. Treatment with either dose of S179D-PRL blocked the expression of FMB on test day 1. On day 1 of testing, 7 of 11 vehicle-infused, steroid-primed rats were fully maternal within the 1-h test session, whereas 2 of 13 low-dose and 1 of 10 high-dose animals were fully maternal. On test day 2, only animals in the high-dose group continued to display lower incidences of FMB than did controls ($P < 0.05$). Therefore, infusions of the antagonist into the MPOA delayed the onset of maternal care, with the higher dose being more effective than the lower dose.

Comparisons of the response latencies on the first day of full responsiveness revealed that infusions of the low dose of the antagonist into the MPOA resulted in longer latencies to retrieve the second (mean ± SE = 1181 ± 348 sec) and third (1459 ± 380 sec) test pups ($P$ values $<0.05$ and $< 0.01$, respectively), group the pups together (1462 ± 380 sec; $P < 0.05$), and crouch over the young (2072 ± 420 sec; $P < 0.05$), when compared with the responses of vehicle-infused controls. The latencies of the vehicle group were the following: retrieve second pup = 182 ± 52 sec; retrieve third pup = 238 ± 47 sec; group = 254 ± 47 sec; crouch = 504 ± 141 sec. As with infusions into the ventricular system, the response latencies of the MPOA vehicle and high-dose groups did not differ on their first day

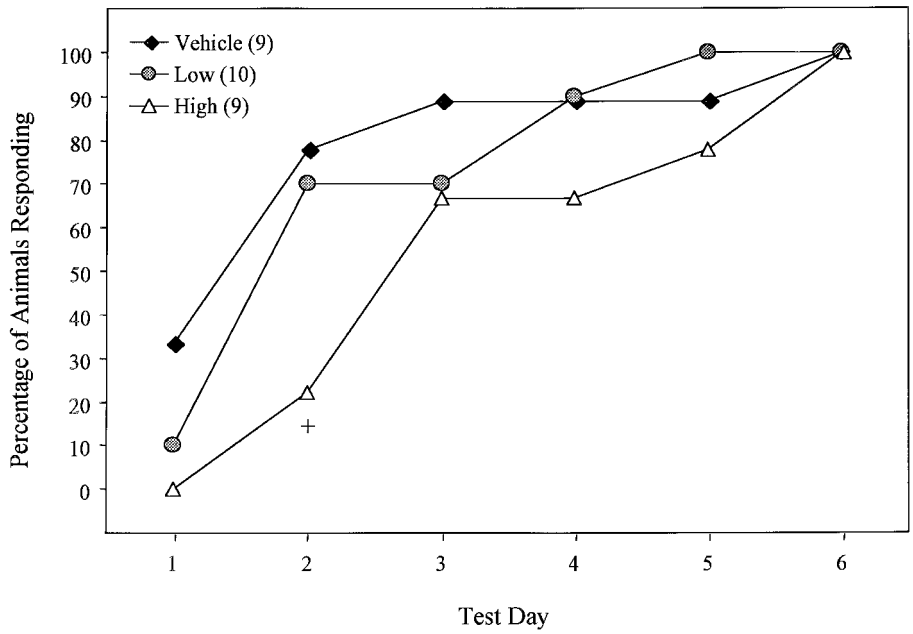


FIG. 2. Cumulative percentages of animals displaying FMB, per test day, throughout the 6 days of testing (Exp 1). Fisher's exact test revealed significant differences on day 2 of testing, when 22% of high-dose (+) animals were fully maternal *vs.* 78% of vehicle ($P = 0.027$) and 70% of low-dose ($P = 0.047$) animals.

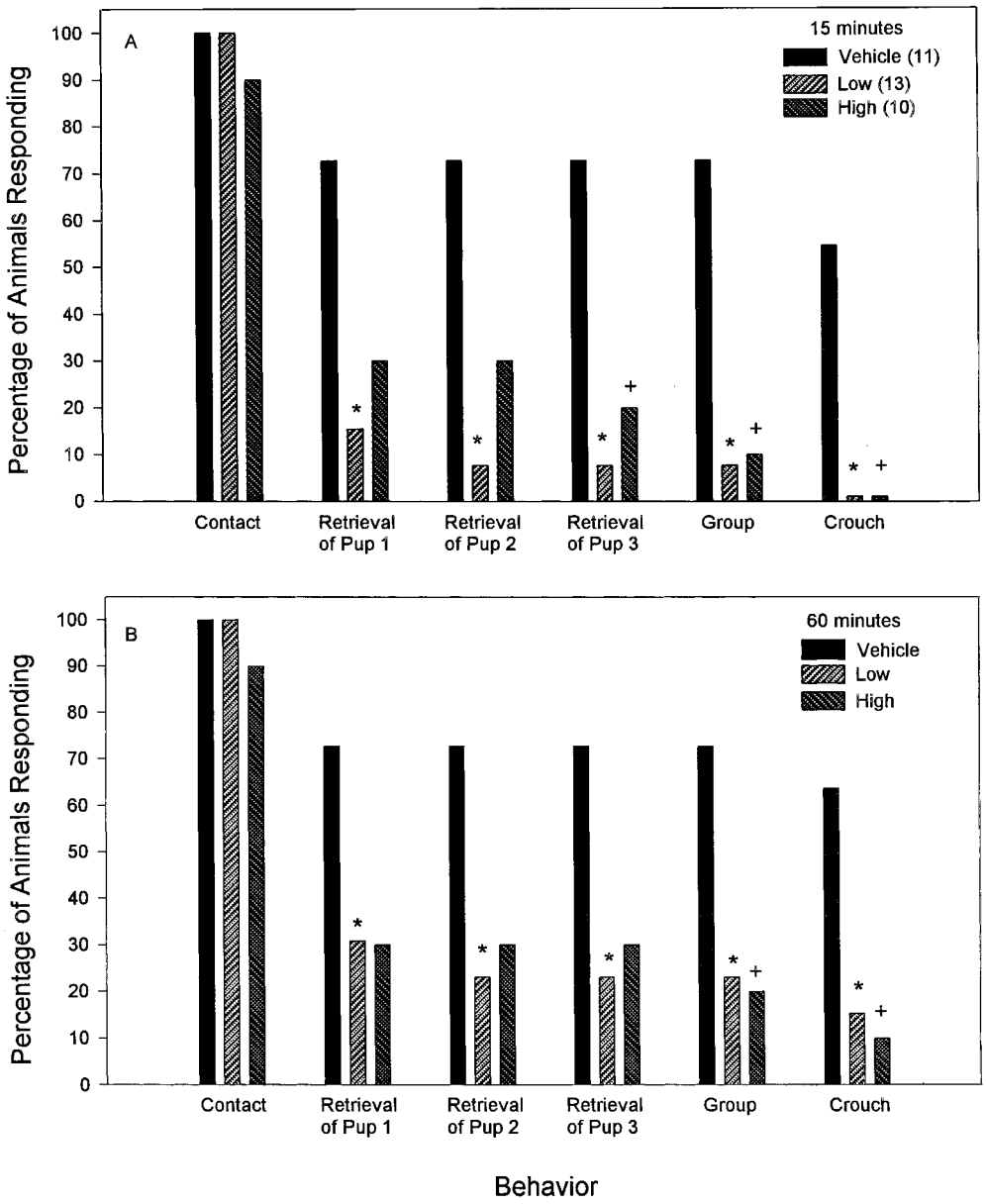


FIG. 3. Percentages of animals displaying various components of maternal responsiveness within the first 15-min (A) and full 60-min (B) test periods on test day 1, after infusions of vehicle (n = 11) or S179-PRL (low dose, n = 13; high dose, n = 10) directed at the MPOA (Exp 2). As shown in A, the low dose (*) of S179D-PRL delayed the onset of most behaviors in more antagonist-treated animals, when compared with the responses of vehicle-infused controls (Fisher exact probability): retrieve pup 1 ($P = 0.007$), retrieve pups 2 and 3 and group ($P = 0.002$), and crouch ($P = 0.003$). The high dose (+) significantly delayed the onset of the retrieval of pup 3 ($P = 0.021$), grouping ($P = 0.006$), and crouching ($P = 0.040$) behaviors. Likewise, as shown in B, a significant delay was found in both groups of animals receiving S179-PRL at 60 min; *, response was slower to retrieve pup 1 ($P = 0.044$), retrieve pups 2 and 3 and group ($P = 0.019$), and crouch ($P = 0.02$). However, only significant delays were noted in grouping ($P = 0.021$) and crouching ($P = 0.016$) behaviors in the high-dose group.

of full responsiveness, although the high-dose group tended to have slightly longer response latencies.

Cannula placements for animals in each experimental group are depicted in Fig. 5. Only data from animals with bilateral placements within or contiguous with the MPOA were included in the statistical analyses. Four females (3 high-dose and 1 low-dose) were excluded from the study because of inaccurate cannula placements. Similarly, 11 of 12 vehicle-infused animals had bilateral cannula placements within the MPOA.

*Exp 3: Effects of infusions of wt hPRL into the MPOA on the induction of maternal behavior*

Infusions of wt hPRL into the MPOA of steroid-primed, nulliparous rats did not affect the rate of onset of maternal behavior, relative to vehicle-infused controls. The percentages of animals in each group displaying various aspects of maternal behavior during the first 15 and 60 min on test day 1 are shown in Fig. 6, A and B, respectively. No behavioral differences were found in the number of animals displaying

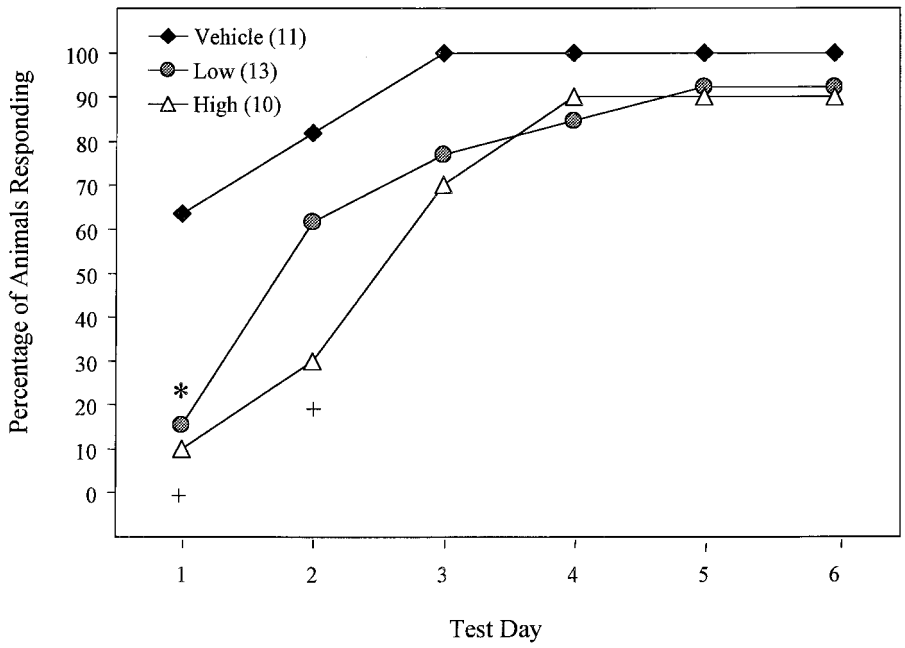


FIG. 4. Cumulative percentages of animals displaying FMB throughout the 6 days of testing (Exp 2). Fisher's exact test revealed significant differences between groups on test days 1 and 2. On day 1, 15% of low-dose (*, $P = 0.02$) and 10% of high-dose (+, $P = 0.016$) *vs.* 64% of vehicle animals were fully maternal. On day 2, a significantly slower response to display FMB was noted in the high-dose animals ($P = 0.022$) *vs.* vehicle; 30% *vs.* 82%.

various aspects of maternal behavior at either 15 or 60 min on the first test day. The cumulative percentages of animals that were fully maternal over the 6 test days are shown in Fig. 7. Again, there were no statistical differences in the proportion of animals displaying FMB between these 2 groups on any individual test day. Whereas only 1 of 12 wt hPRL MPOA-infused females was fully maternal on test day 1, by the third test day, 10 of 12 subjects were fully maternal. In comparison, 4 of 11 vehicle animals were fully maternal on day 1, whereas on test day 3, 6 of 11 were responsive. By the sixth test day, all wt hPRL and 8 of 11 vehicle MPOA-infused animals were fully responsive.

A comparison of the behavioral response latencies was also made between the wt hPRL and vehicle groups on the first day of FMB for each animal. The results of this comparison revealed that, whereas both groups contacted the test young with similar latencies, the wt hPRL infused group retrieved both the first and second test pup significantly faster than did the vehicle controls ($P = 0.007$ and $P = 0.028$, respectively; see Fig. 8). Thus, on the first day of FMB, females given MPOA infusions of wt hPRL responded faster than did controls. All bilateral cannula placements for the wt hPRL and vehicle groups are shown in Fig. 5.

## Discussion

The results of the present set of experiments demonstrate that central administration of the recombinant human PRL receptor antagonist, S179D-PRL, delays the rapid onset of maternal behavior induced by steroid treatment in nulliparous female rats. S179D-PRL has been shown to compete with wt PRL in both the stimulation of Nb2 cell proliferation (6) and signaling through janus kinase after wt hormone interaction with the PRL receptor (9). Although it is not known whether S179D-PRL acts to block the neuronal actions of PRL on a similar janus kinase pathway, recent work using nonneuronal astrocytes has shown that ethanol inhibits PRL-induced activation of the janus kinase/STAT pathway in astrocytes in culture (10). It is tempting to speculate, therefore, that the central behavioral actions of S179D-PRL are mediated through the JAK/STAT pathway.

In the present study, we administered S179D-PRL, wt hormone, or vehicle using miniosmotic pumps that continuously delivered these solutions for 1 week. Chronic infusions of S179D-PRL into the LV in $P_4$-plus-$E_2$-treated, ovariectomized rats resulted in a delay in the development of maternal behavior. Infusions of the PRL receptor antagonist into the MPOA resulted in a similar, yet slightly longer, delay in maternal care. In contrast, MPOA infusions of wt hPRL, as expected, did not affect the rate of onset of maternal behavior. These findings demonstrate that blocking access of neural PRL receptors to endogenous ligands interferes with the rapid onset of maternal care brought about by exposure to a pregnancy-like steroid regimen (7).

It was somewhat surprising that, in both Exp 1 and Exp 2, a majority of the animals treated with S179D-PRL began to display maternal behavior by the third test day. One possible explanation that might account for this escape from the inhibitory actions of S179D-PRL by test day 3 may be that test animals were exposed to progressively elevated levels of endogenous PRL from test day 1 to 6, which could override the actions of the PRL receptor antagonist. Plasma PRL levels have been shown to increase over the course of testing in the presence of $E_2$ in rats treated with this identical steroid regimen (3). Therefore, it is possible that, by test day 3, elevated titers of endogenous PRL were able to occupy a sufficient number of MPOA PRL receptors to stimulate the induction of maternal behavior. It is also possible that the onset of maternal behavior can be induced through some other neural site or is not dependent on PRL after the second day of testing. This latter possibility seems less likely to account for the behavioral responses after day 2, because continued suppression of endogenous PRL with bromocriptine prevents

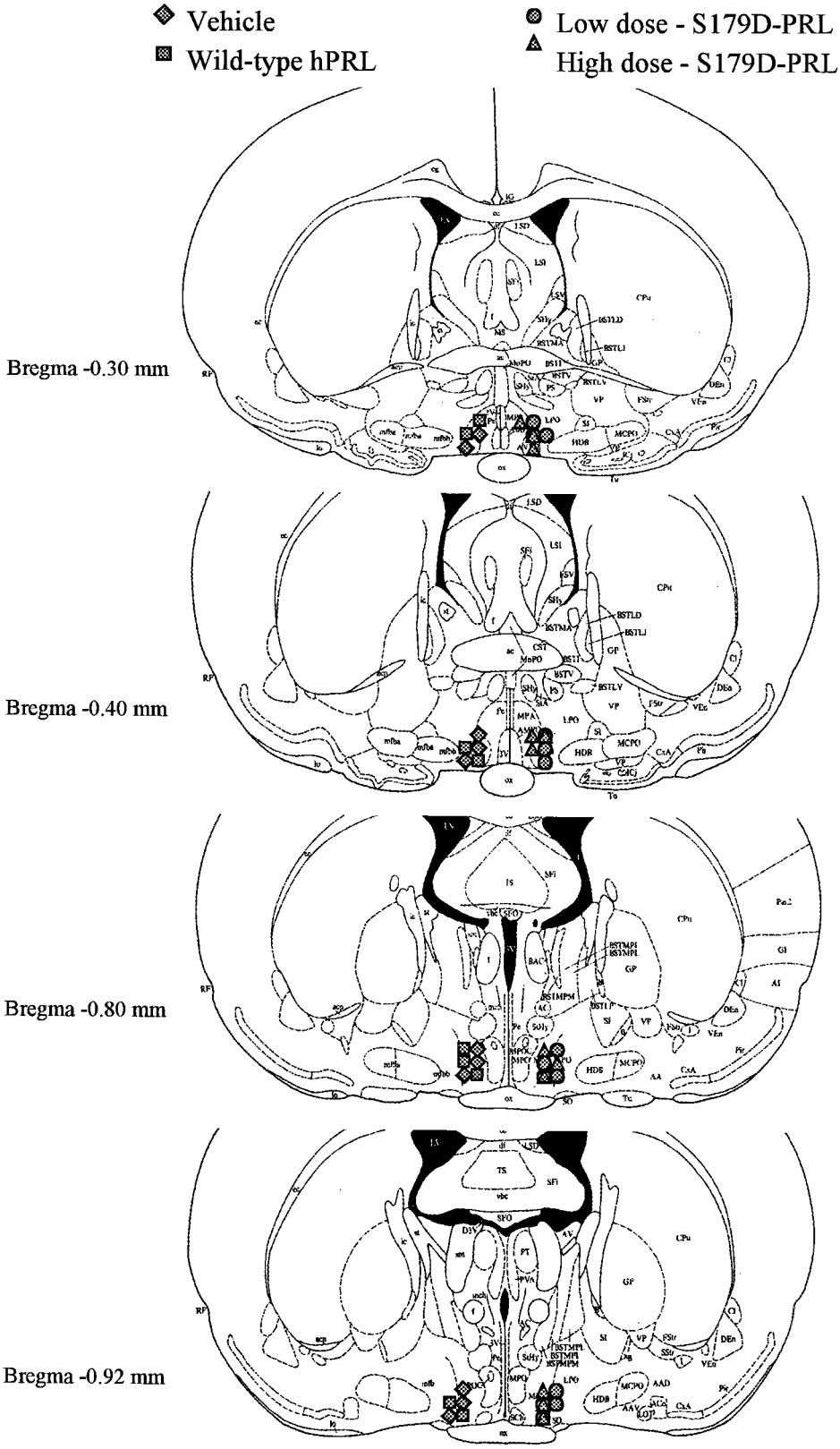


FIG. 5. Cannula placements in the MPOA for animals in each group in Exp 2 and Exp 3 receiving vehicle, the high and low doses of the PRL receptor antagonist, S179D-PRL, or wt PRL. All cannula placements were bilateral (although, for purposes of clarity, only unilateral locations are shown).

most steroid-treated females from responding maternally for 4–5 test days (3).

The possible actions of the wt hPRL on maternal behavior were examined to determine whether the inhibitory actions of S179D-PRL were specific for the purported antagonist or whether the infusion of the native protein would have similar

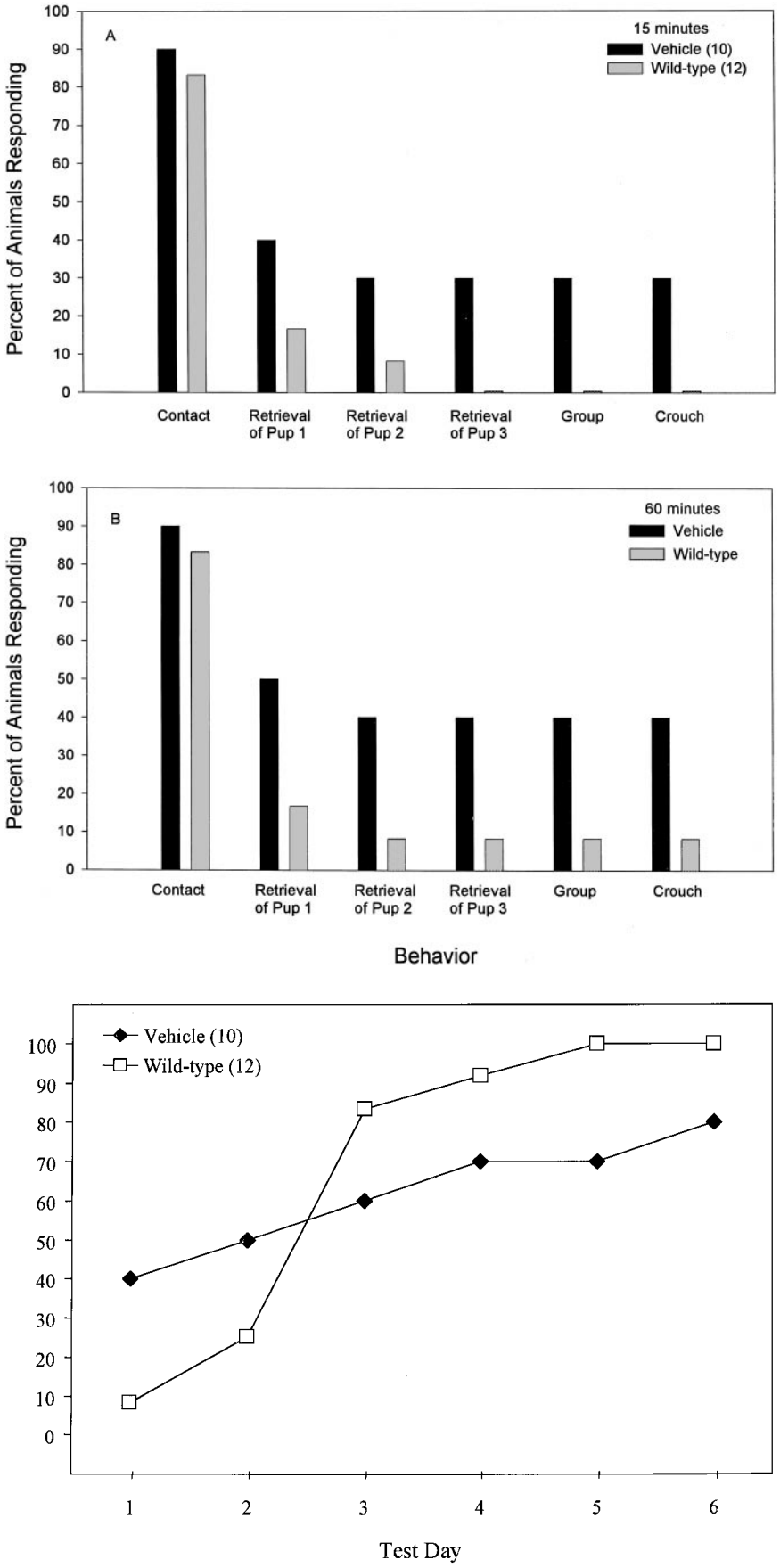


FIG. 6. Percentages of animals responding on test day 1 for maternal responsiveness within the first 15-min (A) and full 60-min (B) test periods while receiving wt hPRL (n = 12) or vehicle (n = 10) infusions directed at the MPOA (Exp 3). No statistical differences were found.

FIG. 7. Cumulative percentages of animals displaying FMB throughout the 6 days of testing (Exp 3). No statistical differences were found between groups.

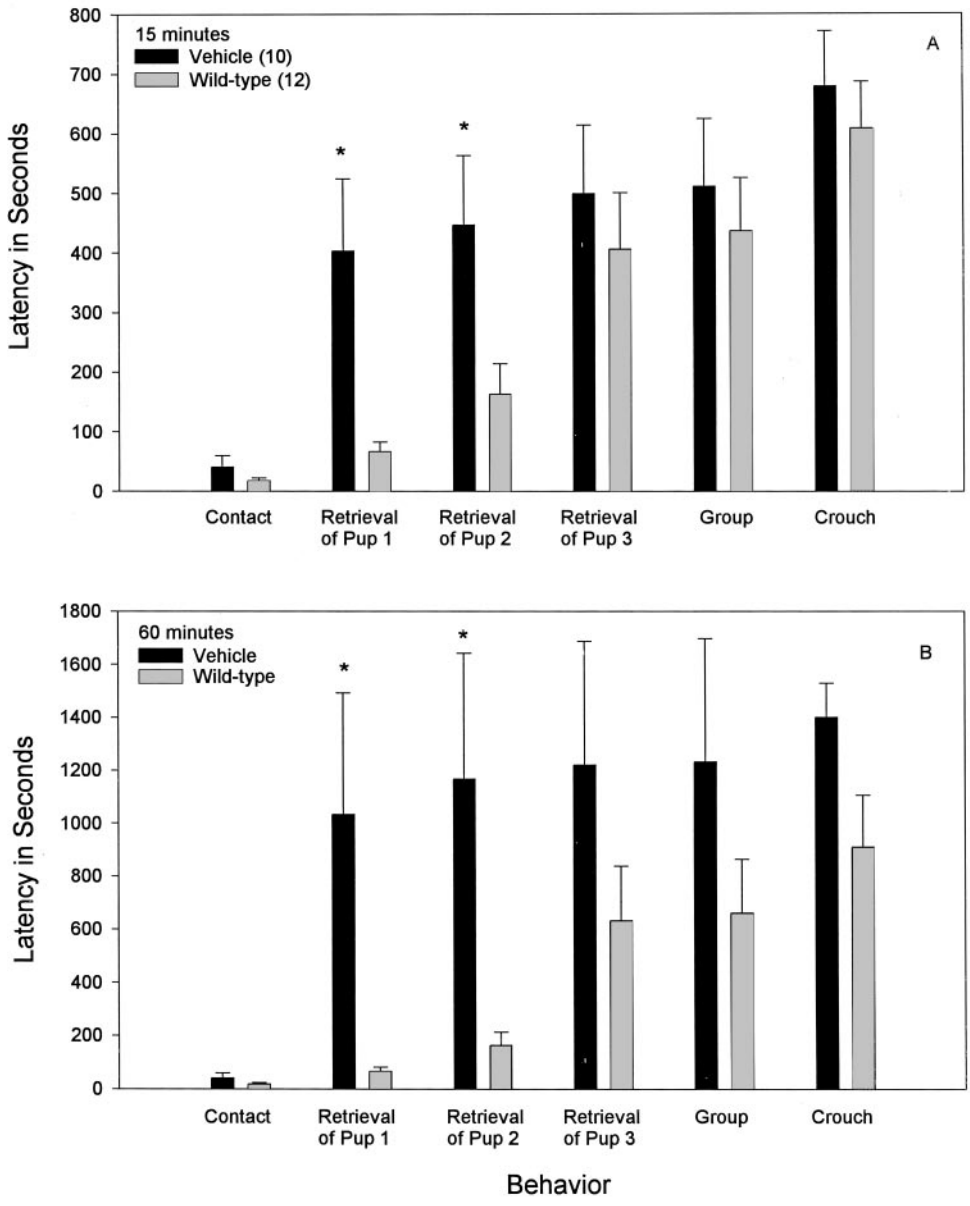


FIG. 8. Latencies to respond with FMB within the first 15-min (A) and full 60-min (B) test periods on test day 1, after receiving infusions of either wt hPRL (n = 12) or vehicle (n = 10) directed at the MPOA (Exp 3). *t* test revealed significant differences in the onset of retrieving pups 1 and 2 in vehicle *vs.* wt animals at both time points; A, retrieval of pup 1 ($P = 0.007$) and pup 2 ($P = 0.028$); B, retrieval of pup 1 ($P = 0.031$) and pup 2 ($P = 0.032$).

effects. The results indicate that the inhibitory actions of S179D-PRL in the prior experiments were attributable to its biological properties as an antagonist. Interestingly, infusion of the native hormone seemed to stimulate certain aspects of maternal behavior beyond the level induced by the steroid-treatment. Specifically, comparisons of retrieval latencies between wt and vehicle controls revealed that animals given MPOA infusions of wt hPRL retrieved the first and second test pup significantly faster than did vehicle-infused controls on the first day that animals displayed FMB. The stimulation of retrieval behavior by wt hPRL is consistent with earlier studies that found the repeated injections of ovine PRL in hypophysectomized female rats stimulated the initial induction of pup retrieval (11).

Similar comparisons of the actions of the PRL antagonist on maternal responsiveness on the first day of full maternal care indicated that animals treated with the low doses of S179D-PRL, infused into either the ventricles or MPOA, responded somewhat slower to the test pups than did vehicle-infused controls. The lack of a similar effect with the high dose of S179D-PRL may be attributable to the large degree of variability in the response latencies of high-dose animals. Further studies are needed to understand how varying doses of this antagonist act upon neurons to regulate neural processing.

The finding that central infusions of wt hPRL facilitate pup retrieval, whereas the PRL receptor antagonist delays retrieval responses, lends support to the concept that central exposure to PRL may potentiate the expression of maternal behavior once it is established, a finding that runs contrary to existing dogma in the field, *i.e.* that ongoing maternal care is regulated independent of hormonal control. It is established that cells and fibers that contain anti-PRL immunoreactive protein are present within the rat central nervous system, with high densities of immunoreactivity present in the MPOA (12), an area that is sensitive to PRL regulation

of maternal behavior (4). What the relative contributions of brain and endocrine PRL to the onset and maintenance of maternal behavior are, however, remain to be established. One possibility is that the regulation of maternal behavior may shift from an endocrine to a neural PRL dependence after parturition, a change that might help account for the diminished role for circulating hormones in regulating maternal behavior during lactation and in previously parous females.

The actions of S179D-PRL seemed to persist for a longer period when infused directly into the MPOA, a neural site known to regulate PRL and placental lactogen-stimulated maternal behavior (4, 13), than when infused into the ventricular system. Although the present results have established that the MPOA is one neural site involved in PRL receptor-mediated regulation of maternal behavior, an examination of other possible neural sites of PRL action is warranted. Given the recently identified role of the ventromedial hypothalamus (VMH) in the neural regulation of maternal behavior (14), the abundance of PRL receptors present in the VMH (15), and the role of the VMH in feeding (16, 17), it would be of interest to determine whether this region might be a site of PRL regulation of feeding behavior during lactation.

One significant advantage in using this PRL receptor antagonist to examine the role of PRL is that S179D-PRL presumably acts directly on the PRL receptor to interfere with maternal care, rather than acting indirectly via the dopaminergic system to suppress PRL secretion (3). Given that maternal behavior and many other behaviors, including locomotion and appetitive behaviors (18), can be affected by the activity of the endogenous dopaminergic system, use of this new PRL receptor antagonist may bypass the possible confound that could result from a more general stimulation of neural dopaminergic activity after systemic bromocriptine administration.

Finally, S179D-PRL is a molecular mimic of phosphorylated PRL, which is a natural product of the rat pituitary gland (19–21). Phosphorylated PRL itself acts as a PRL antagonist under certain conditions (22). The proportion of unmodified-to-phosphorylated PRL is physiologically regulated (23, 24), with higher proportions of unmodified PRL being characteristic of the latter two-thirds of pregnancy (23). This proportional change during pregnancy is dependent on ovarian rather than placental hormones (23) and can be reproduced by the administration of estrogen to ovariectomized animals (A. M. Walker, unpublished data). At least part of the estrogen priming effect on maternal behavior, therefore, could be due to changes in the form of PRL produced. On the basis of these studies, one would also suggest that appropriate changes in the form of PRL during pregnancy and lactation may be necessary for normal maternal behavior and that abnormal ratios of the two forms of PRL could be responsible for diminished maternal behavior in some animals.

In summary, the results of the present study demonstrate that S179D-PRL can act within the brain to alter the expression of a PRL-regulated behavior. Chronic infusion of S179D-PRL into the LV or MPOA of steroid-primed virgin rats delayed the onset of maternal care. Central administration of this PRL receptor antagonist provides researchers with an endocrine preparation with which to assess the central actions of PRL and other lactogenic molecules that bind to PRL receptors.

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