Prolactin-Induced Mitogenesis in the Subventricular Zone of the Maternal Brain during Early Pregnancy Is Essential for Normal Postpartum Behavioral Responses in the Mother

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High prolactin during pregnancy, which is essential for normal postpartum maternal behavior, increases neurogenesis in the subventricular zone of the lateral ventricle (SVZ) of the maternal brain. Because SVZ mitogenesis generates new olfactory neurons and may contribute to perception of novel odorants, we hypothesized that the prolactin-induced increase in SVZ mitogenesis during pregnancy might be important for normal maternal interactions with pups. To investigate this hypothesis, prolactin secretion was suppressed for 3 d early in pregnancy in mice, using a carefully timed dose of bromocriptine. The bromocriptine-induced reduction in prolactin prevented the normal increase in generation of neural progenitors in the SVZ of the maternal brain. Another group of bromocriptine-treated animals were allowed to continue their pregnancy until term, and then maternal behaviors were evaluated postpartum. Low prolactin during early pregnancy, and the consequent suppression of mitogenesis in the SVZ of the maternal brain, was subsequently followed by increased postpartum anxiety and markedly impaired maternal behavior. In another group of pregnant females, injections of the mitotic inhibitor methylazoxymethanol to specifically suppress neurogenesis in the mother during early pregnancy without affecting prolactin secretion also caused postpartum anxiety and impaired maternal behavior. These data demonstrate that prolactin-induced increase in generation of neural progenitors in the SVZ of the maternal brain during early pregnancy is required for normal expression of postpartum maternal behaviors. **(*Endocrinology* 151: 3805–3814, 2010)**

Multiple adaptations occur in the maternal brain to prepare the mother for the changing physiological and psychological demands that occur during pregnancy and lactation (1). The anterior pituitary hormone prolactin and/or the related placental lactogen hormones are secreted at high levels during pregnancy and lactation (2, 3). Prolactin acts in the brain to stimulate the onset of maternal behaviors in rodents (4–6), and prolactin receptor-deficient mice have markedly impaired maternal behavior (7). Prolactin is also implicated in the increased appetite that occurs during pregnancy (8, 9) and lactation (10). Moreover, prolactin has been shown to have acute antistress and anxiolytic actions (11) and may be involved in the well-characterized changes that occur in stress responses and anxiety in the postpartum period (12). Therefore, the increase in lactogenic activity during pregnancy and lactation may be critical in mediating a number of the adaptive changes that occur in maternal physiology at this time (13, 14).

Recently, it was shown that in the mouse, high prolactin levels during pregnancy promote adult neurogenesis in the subventricular zone of the lateral ventricle (SVZ) of the maternal brain (15). Similar pregnancy-induced increases in SVZ neurogenesis have also been reported in rats (16). Newly generated SVZ neurons migrate via the rostral migratory stream and are incorporated into the olfactory bulb (17), where they may contribute to olfactory learning of novel odorants (18, 19). Because the presence of pups

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Abbreviations: BrdU, Bromodeoxyuridine; EPM, elevated plus maze; MAM, methylazoxymethanol; SVZ, subventricular zone of the lateral ventricle.

is a novel olfactory experience for the primiparous mother, we hypothesized that the prolactin-induced increase in SVZ mitogenesis during pregnancy may play an important permissive role in postpartum maternal behaviors. To evaluate this hypothesis, we suppressed prolactin secretion during early pregnancy in mice to reduce mitogenesis in the SVZ and subsequently evaluated anxiety-like behavior and maternal behavior in the postpartum period. Because prolactin treatment, *per se*, may influence a range of maternal behaviors (2), we also evaluated whether pharmacological ablation of adult neurogenesis during early pregnancy, without affecting prolactin secretion, also influences these essential adaptive responses in the mother.

Materials and Methods

Animals

Maternally naive virgin C57BL/6J mice (6–9 wk old) were housed in 32 × 16 × 18 cm individual cages (all groups, n = 6). Some females were paired with males and were considered d 1 pregnant when mating was confirmed by sperm being present in a vaginal smear. Mice were housed under a 12-h light, 12-h dark cycle (lights on at 0700 h), with *ad libitum* access to food and water. The University of Otago Animal Ethics Committee approved all experiments.

Bromocriptine treatment

To reduce serum prolactin levels in early pregnancy required a carefully timed low dose of bromocriptine to avoid termination of pregnancy. Based on our preliminary work identifying the timing of the prolactin surges in early pregnancy in mice (Fig. 1), bromocriptine was injected once daily for 3 d starting at 1630 h on d 1 of pregnancy. With this injection regime, a small rise in serum prolactin occurred before each injection (Fig. 2A). This was sufficient to maintain the corpus luteum, and thus the pregnancy, although still significantly decreasing serum prolactin levels at other time points. The same injection daily at 1300 h ablated the prolactin surges and terminated the pregnancy (data not shown). For each injection, 50 μg bromocriptine mesylate (Sigma, St. Louis, MO) was dissolved in a minimum amount of ethanol and made up to 0.1 ml with sesame oil and then injected sc. To replace prolactin in bromocriptine-treated mice, mice were injected sc with prolactin (50 μg purified ovine prolactin in saline) at 1645 h from d 1–3 of pregnancy (to mimic the early pregnancy surges of prolactin).

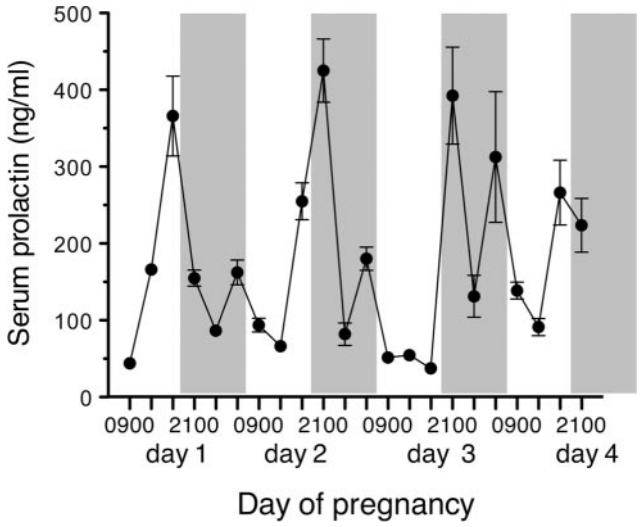

FIG. 1. Pattern of prolactin secretion in untreated mice during the first 4 d of pregnancy. Groups of mice were killed every 4 h from 0900 h on d 1 of pregnancy. *Gray shaded regions* represent dark periods of the light-dark cycle. Note the twice-daily prolactin surges, with an afternoon peak occurring immediately before lights off, and a smaller nocturnal surge occurring approximately 1 h before lights on.

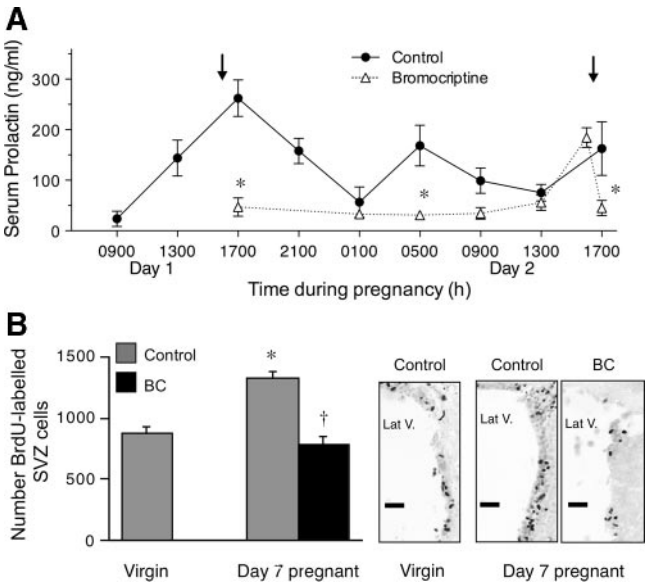

FIG. 2. Suppression of prolactin secretion during early pregnancy reduces levels of neural progenitors in the SVZ of the maternal brain. A, Serum prolactin during early pregnancy. Bromocriptine treatment in early pregnancy prevents the twice-daily surges of prolactin (*, $P < 0.05$). *Arrows* indicate times of bromocriptine injection. B, Day 7 pregnant mice had a significant increase in the number of BrdU-labeled cells in the SVZ compared with virgin controls (*, $P < 0.05$). Bromocriptine treatment (BC) from d 1–3 of pregnancy caused a significant decrease in BrdU-labeled cells in the SVZ on d 7 of pregnancy compared with pregnant controls (†, $P < 0.05$). Micrographs show representative images from the SVZ in the three treatment groups, with black staining representing BrdU-labeled nuclei within recently divided cells. Lat. V., Lateral ventricle.

Serum prolactin levels in early pregnancy

Pregnant mice, either treated with bromocriptine or vehicle controls, were killed for collection of trunk blood every 4 h from 0900 h on d 1 of pregnancy for measurement of prolactin by RIA using National Institutes of Health (Bethesda, MD) reagents (20). Sensitivity was 2 ng/ml, and all samples were run in a single assay, with an intra assay coefficient of variation of 2.5%.

Mitogenesis in the SVZ

Virgin diestrous female mice, or d 7 pregnant mice exposed to bromocriptine or vehicle were injected with bromodeoxyuridine (BrdU) every 2 h from 0500–1500 h (six injections of 12 mg/100 g body weight, dissolved in phosphate buffer) (20). Mice were then perfused with 4% paraformaldehyde either at 1700 h the same day for evaluation of the SVZ, or on d 2 postpartum for

evaluation of the olfactory bulb. BrdU-labeled cells were detected using immunohistochemistry (mouse monoclonal anti-BrdU, 1:200; Dako, Carpinteria, CA). Dual-label immunofluorescence histochemistry was performed to determine whether the BrdU-immunopositive cells in the SVZ were neural progenitor cells using doublecortin as a marker (goat anti-doublecortin, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA). For quantification, SVZ cells were observed under ×20 magnification. BrdU-immunopositive cells were counted in a systematic manner after a random start, such that all labeled nuclei were counted in one-in-six serial 20-μm sections (*i.e.*120 μm apart) for a total of 14 sections throughout the SVZ (approximately Bregma +1.94–3.9 mm). For the olfactory bulb, one-in-three serial 20-μm sections throughout the entire olfactory bulb were analyzed and all labeled cells counted. Data are presented as the total counts collected and are not corrected for the total number of sections.

Methylazoxymethanol treatment

To reduce levels of adult mitogenesis in the brain, pregnant females were injected ip with methylazoxymethanol (MAM) (5 mg/kg, F0040; Midwest Research Institute, Rockville, MD) or vehicle daily at 1100 h, from d 4–7 of pregnancy in one experiment and d 1–3, d 4–8, or d 1–8 of pregnancy in a subsequent experiment. To mitigate the concern that MAM might have adverse side effects in the mother, or affect fetal brain development, our experimental design included the following features: 1) The dose of MAM used was low, based on data showing that it would transiently decrease adult neurogenesis without making animals sick (21, 22), and 2) the timing of administration was very early in pregnancy (maximum from d 1–8 of gestation), before critical periods (d 12–20) where MAM might influence fetal brain development (23). Maternal body weights throughout pregnancy and the weight of the pups at birth were recorded and compared with controls.

Anxiety testing

Day 2 postpartum or virgin females were assessed for anxiety on the elevated plus maze (EPM) or in the light-dark box. All testing was carried out between 0930 and 1030 h in the room where the animals were usually housed. To avoid habituation to stress, the animals were not handled before the experiment and were used in only one experiment. At the end of each trial, the apparatus was cleaned with gauze swabs soaked in 100% ethanol to eliminate odors from previous mice.

EPM testing

The EPM had two opposing enclosed arms (30 × 5 × 5 cm), two opposing open arms (30 × 5 cm), and a square uncovered central platform (5 × 5 cm), all on a 1-m-high hidden stand. To assess anxiety, the mouse was placed on the central platform facing an open arm and allowed to roam at will for 5 min. All arm entries and exits were recorded. Once a mouse had all four paws on an arm of the maze, it was counted as having entered that arm, whereas placing two paws out of an arm was counted as an exit.

Light-dark box testing

The light-dark box had an enclosed dark box (18 × 27 × 27 cm; black Perspex sides, bottom and top, ~30 lux) separated by a partition with a hole (6 cm) from a light box (27 × 27 × 27 cm; clear Perspex, with a transparent cover with ventilation holes in it; lit 56 cm from above with a 100-W light bulb, ~753 lux). Mice were placed at the entrance to the dark box and allowed to roam at will for 5 min. All entries and exits to the light or dark box were recorded. Once a mouse had all four paws within a box, it was counted as having entered that box, whereas placing two paws out of a box was counted as an exit.

Maternal behavior testing

Day 2 postpartum mice had their pups removed from the home cage. Immediately, three foster pups were placed into the cage and maternal behaviors recorded (20). To assess maternal behavior in an anxiogenic situation, another group of postpartum d 2 females were placed into a clean novel cage into which three foster pups had been placed, and any behavior was recorded. Full maternal behavior was defined as being when the mouse had gathered all three pups to a nest and was crouching over them. Testing was performed between 0930 and 1130 h and continued until full maternal behavior had been displayed or for a maximum of 60 min.

Statistical analysis

The data are expressed as mean ± SEM. The amount of time on the open arms of the EPM is expressed as a percentage of total time on the open and closed arms [open time/(open + closed time) × 100]. Data were compared by one-way ANOVA and where a significant F statistic was obtained, *post hoc* comparisons were made using Newman-Keuls multiple-comparison test (Graphpad Prism Software, La Jolla, CA), and $P < 0.05$ was used as the level of significance.

Results

Low prolactin in early pregnancy reduced numbers of newly generated neural progenitors in the SVZ during pregnancy

In rodents, mating induces twice-daily surges of prolactin to maintain the function of the corpus luteum and establish the pregnancy (24). To reduce serum prolactin levels during this time without causing termination of the pregnancy, we first had to establish the timing of prolactin surges in our mouse model. As shown in Fig. 1, mice had twice-daily surges of prolactin secretion in early pregnancy, one large diurnal surge peaking about the time of lights off, then a second, smaller nocturnal surge. The nocturnal surge was seen from d 1–4 of pregnancy but became less distinct on d 5 and was absent by d 6 of pregnancy (data not shown). The daily diurnal surge of prolactin remained until d 9 of pregnancy, which was the limit of sampling undertaken in this study. We administered a carefully timed low dose of the dopamine D2-receptor agonist bromocriptine, which blocks prolactin secretion from the anterior pituitary gland. Bromocriptine was injected once daily for 3 d starting at 1630 h on d 1 of pregnancy. This rapidly truncated the afternoon prolactin surge and blocked the subsequent nocturnal surge of prolactin but allowed a brief elevation of prolactin the fol-

lowing afternoon that was sufficient to maintain pregnancy (Fig. 2A). Prolactin levels in bromocriptine-treated pregnant females were significantly lower than controls [$F_{(15,80)} = 17.34$; $P < 0.05$; Fig. 2A] at all time points after the injection apart from 0100 and 1300 h, the nadir of prolactin secretion, and at 1630 h (just before the next bromocriptine injection). There was no effect of bromocriptine during early pregnancy on maternal health, with no significant differences observed in maternal weight throughout pregnancy, timing of parturition, litter size or pup mortality, or pup weights and growth rates during lactation (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org).

To investigate the effects of our treatments on mitogenesis in the SVZ, groups of nonpregnant and bromocriptine-treated (low prolactin) and vehicle-treated (control) mice on d 7 of pregnancy were treated with a series of BrdU injections and then killed (after 12 h of BrdU treatment) for assessment of mitogenesis, as described previously (20). To ensure animals had remained pregnant, the uterus was checked at this time for the presence of healthy conceptuses (Supplemental Fig. 1). Postpartum control mice had significantly increased numbers of BrdU-immunoreactive cells in the SVZ on d 7 of pregnancy compared with virgin controls (15) and with bromocriptine-treated mice [$F_{(2,15)} = 31.91$; $P < 0.0001$; Fig. 2B]. The normal pregnancy-induced increase in SVZ cell division on d 7 of pregnancy was completely abolished in the low-prolactin mice, such that they were now not significantly different from virgin controls (Fig. 2B, $P = 0.2917$). There was no change in the proportion of those cells that were doublecortin positive, marking them as neural progenitors (control, 90 ± 6%; low prolactin, 93 ± 4%, Supplemental Fig. 2A). In accordance with other studies that have found that neurogenesis in the hippocampus is unaffected by the hormonal changes of pregnancy (15, 16), suppression of prolactin levels from d 1–3 of pregnancy did not affect numbers of BrdU-immunoreactive cells in the subgranular zone of the dentate gyrus on d 7 of pregnancy. Consistent with the reduced mitogenesis in the SVZ during pregnancy, on postpartum d 2, there was a significant decrease in the number of BrdU-immunoreactive cells in the olfactory bulb in low-prolactin mice compared with control mice ($P < 0.0001$; Supplemental Fig. 2B).

Low prolactin during early pregnancy was associated with a subsequent increase in postpartum anxiety

Groups of low-prolactin and control mice were allowed to run their pregnancy to term, and anxiety-like behavior was evaluated on d 2 postpartum, using both an EPM and a light-dark box, two well-characterized tests of anxiety in mice (25, 26). Low prolactin during early pregnancy prevented the normal pregnancy-induced suppression of anxiety that is seen in rodents (27), resulting in mothers that spent significantly less time on the open arms of the EPM on d 2 postpartum compared with postpartum controls [$F_{(2,15)} = 5.746$; $P = 0.0140$; Fig. 3A]. Indeed, the low-prolactin animals showed levels of postpartum anxiety that were more typical of that seen in nonpregnant females. There were no significant differences in the number of entries into the open arms, but low-prolactin animals also showed significantly increased number of entries into the closed arms compared with postpartum controls ($P = 0.0274$; Supplemental Table 2). To determine whether the effects observed in the low-prolactin mice could be rescued by prolactin, another group of bromocriptine-treated mice were injected with prolactin at 4.45pm daily from d 1–3 of pregnancy, to restore the normal afternoon surge of prolactin (high-prolactin). Prolactin treatment in early pregnancy restored the normal pregnancy-induced suppression of anxiety in low-prolactin mice (Fig. 3A). This demonstrates that the effect of bromocriptine during early pregnancy on anxiety postpartum was specifically due to the inhibition of prolactin secretion, and not any other possible effects of bromocriptine. Data from the light-dark box showed a similar increase in post partum anxiety in the low-prolactin animals, which was completely reversed in the high-prolactin animals [$F_{(2,15)} = 23.30$; $P < 0.0001$; Supplemental Fig. 3].

Low prolactin during early pregnancy was associated with a subsequent impairment of maternal behavior

To determine whether low prolactin during early pregnancy also affected postpartum maternal behavior, we measured expression of maternal behavior using a pup retrieval paradigm. As has been documented previously (28), postpartum females were significantly faster to express full maternal behavior compared with virgin mice [$F_{(2,15)} = 53.55$; $P < 0.0001$; Fig. 3B]. When tested in their home cage, low-prolactin mice were not significantly different in retrieving pups compared with postpartum controls in their home cage. Despite the time of pup retrieval being apparently normal in the low-prolactin mice in the home cage, however, the mothers appeared more nervous and readily left the nest if disturbed, indicating a potential difference in maternal behavior if the mouse was stressed. Hence, additional groups of mice were tested for pup retrieval in a novel cage to evaluate their behavior when placed in an anxiogenic situation. Under these conditions, all groups tested took significantly longer to express full maternal behavior compared with that in the home cage.

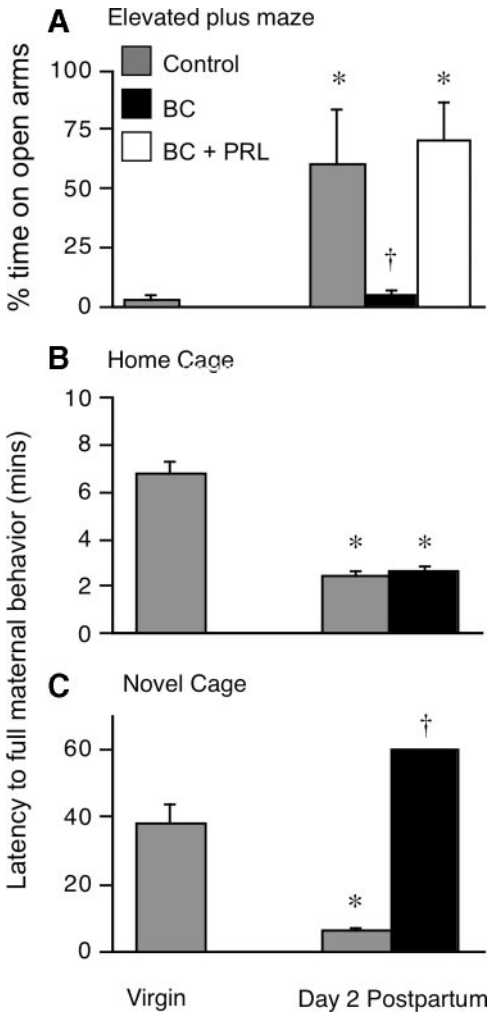

FIG. 3. Suppression of prolactin secretion during early pregnancy was associated with subsequent postpartum anxiety and impaired maternal behavior. A, Time spent on open arms of the EPM. Day 2 postpartum controls spent significantly more time on the open arms of an EPM compared with virgin mice (*, $P < 0.05$). Mice treated with bromocriptine (BC) during early pregnancy spent significantly less time on the open arms of the EPM compared with d 2 postpartum controls (†, $P < 0.05$) and therefore displayed a significant increase in anxiety. Prolactin treatment for 3 d during early pregnancy completely reversed the effect of bromocriptine. B, Maternal behavior in a home cage. Day 2 postpartum mice were significantly faster to express maternal behavior to foster pups compared with virgin controls (*, $P < 0.05$). Bromocriptine treatment had no effect on the expression of maternal behavior in the home cage. C, Maternal behavior in a novel cage. All animals took significantly longer to express maternal behavior to foster pups, but d 2 postpartum mice continued to be significantly faster than virgin controls (*, $P < 0.05$). Unlike in the home cage, in the anxiogenic situation of the novel cage, mice treated with bromocriptine during early pregnancy displayed significantly impaired maternal behavior to foster pups compared with postpartum controls (†, $P < 0.05$), and none of the mice retrieved all three pups within the 1-h observation period.

Day-2 postpartum control mice remained significantly faster to express full maternal behavior compared with virgin controls ($P < 0.001$; Fig. 3C) and were faster than all tested groups [$F_{(2,15)} = 59$; $P < 0.0001$; Fig. 3C]. In contrast, however, low-prolactin mice displayed dramatically impaired maternal behavior when tested in a novel cage compared with both virgin mice and postpartum controls [$F_{(2,15)} = 59$; $P < 0.0001$; Fig. 3C]. These animals investigated the pups occasionally but did not sit with or retrieve the pups. Instead, they made a nest some distance away from the pups, and none of the mice expressed full maternal behavior within the designated 60-min observation period (see Supplemental Video 1).

Reduction in neurogenesis during early pregnancy with the mitotic inhibitor MAM was also associated with increased postpartum anxiety and impaired maternal behavior

The above data were consistent with the hypothesis that changes in mitogenesis in the SVZ mediated the behavioral effects of low prolactin but were correlative only and could not rule out the possibility that another distinct action of prolactin in the brain was involved. To specifically test the role of neurogenesis during pregnancy on postpartum anxiety and maternal behavior without manipulating prolactin levels, groups of mice were injected with the mitotic inhibitor MAM (29) from d 4–7 of pregnancy. This treatment prevented the pregnancy-induced increase in neural progenitors in the SVZ observed in pregnant controls [$F_{(2,15)} = 29.37$; $P < 0.0001$; Fig. 4A] and also reduced neural progenitors in the dentate gyrus of the hippocampus to almost nondetectable levels ($P < 0.001$; Supplemental Fig. 4A). There were no adverse effects on the pregnancy, as measured by maternal body weight, length of pregnancy, and the number of live pups delivered (Supplemental Fig. 4B). Importantly, there was no effect of MAM treatment on levels of serum prolactin (*e.g.* 1300 h on d 2 of pregnancy MAM, 55.7 ± 0.9, and vehicle controls, 59.5 ± 7.4; 1700 h on d 3 of pregnancy MAM, 228.4 ± 31.4, and vehicle controls, 255.8 ±. 59.9). As seen in low-prolactin animals, the MAM-treated mice displayed apparently normal maternal behavior in their home cage, but when placed into the novel cage, these mice had impaired maternal behavior [$F_{(2,15)} = 59$; $P < 0.0001$; Fig. 4C]. Unlike the reduction in mitogenesis in the SVZ caused by low prolactin, however, the short-term MAM-induced reduction in mitogenesis had no effect on postpartum anxiety-like behaviors ($P = 0.3440$; Fig. 4B). MAM-treated animals showed the normal pregnancy-induced decrease in anxiety when measured on the EPM (Fig. 4B) or the light-dark box (Supplemental Fig. 2). To determine whether the failure of MAM to affect anxiety-like behavior was due to the timing or duration of treatment, additional groups of mice were treated with MAM from d 1–3, 4–8, or 1–8 of pregnancy, and then anxiety-like behavior was tested on the EPM on d 2 postpartum. As seen in the previous experiment, the shorter durations

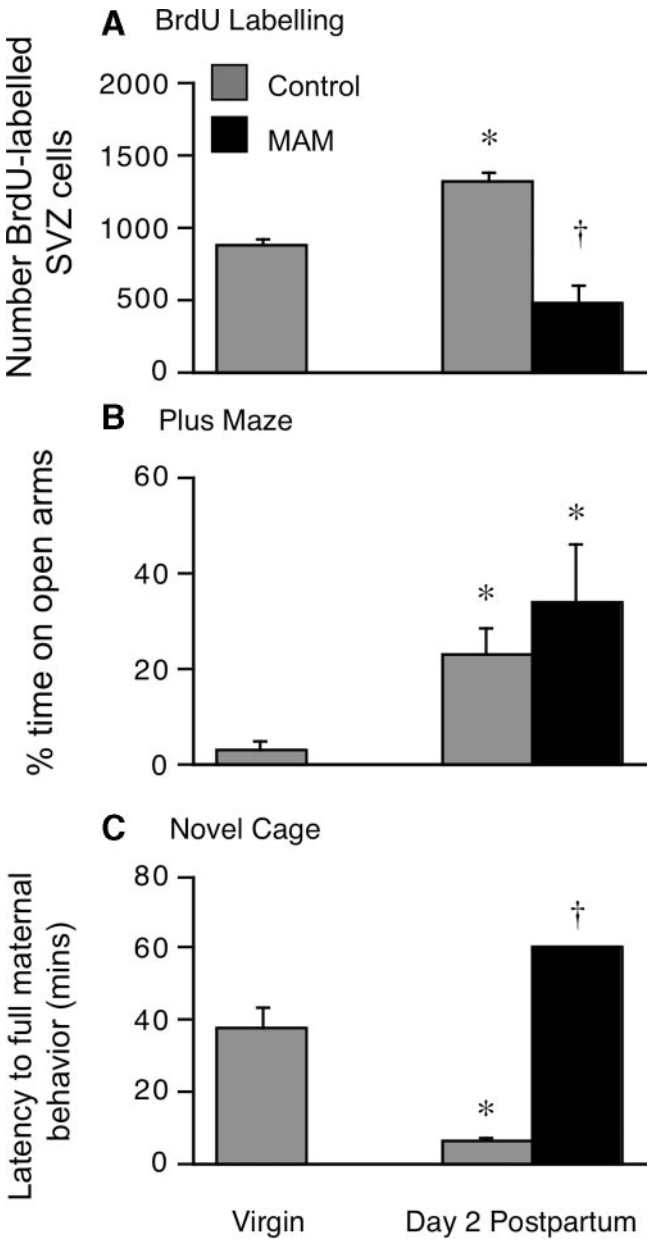

FIG. 4. Pharmacological suppression of SVZ neural progenitors during early pregnancy was associated with impaired maternal behavior. A, SVZ neural progenitors. Day 7 pregnant mice had a significant increase in the number of BrdU-labeled cells in the SVZ compared with virgin controls (*, $P < 0.05$). Treatment with the mitotic inhibitor MAM from d 4–7 of pregnancy caused a significant decrease in BrdU-labeled cells, completely preventing the normal pregnancy-induced increase in neural progenitors (†, $P < 0.05$). B, Suppression of neural progenitors from d 4–7 of pregnancy does not affect anxiety postpartum. Day 2 postpartum controls spent significantly more time on the open arms of an EPM compared with virgin mice (*, $P < 0.05$). Injection of MAM from d 4–7 of pregnancy to suppress levels of neural progenitors had no effect on anxiety postpartum. C, Suppression of neural progenitors impairs maternal behavior in a novel cage. Day 2 postpartum mice were significantly faster to express maternal behavior to foster pups in a novel cage compared with virgin controls (*, $P < 0.05$). Mice in which generation of neural progenitors was inhibited with MAM from d 4–8 of pregnancy displayed significantly impaired maternal behavior to foster pups compared with postpartum controls (†, $P < 0.05$), and none of the mice retrieved all three pups within the 1-h observation period.

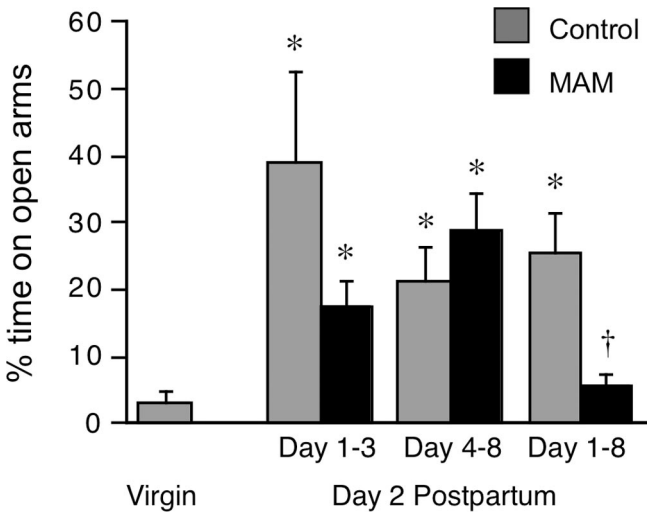

FIG. 5. Suppression of neural progenitors from d 1–8 of pregnancy was associated with postpartum anxiety. Day 2 postpartum controls spent significantly more time on the open arms of an EPM compared with virgin mice (*, $P < 0.05$). Mice treated with MAM from d 1–8 of pregnancy displayed significantly impaired anxiety on the EPM compared with postpartum controls (†, $P < 0.05$). Mice injected with MAM over other time periods were not significantly different from d 2 postpartum controls.

of treatment did not affect postpartum anxiety, although in females treated from d 1–3 of pregnancy, there was a trend of an increase in anxiety that was very close to being significantly different from anxiety in postpartum controls ($P = 0.051$; Fig. 5). When the treatment was maintained throughout d 1–8 of pregnancy, however, there was a significant decrease in the time spent on the open arms of the EPM [$F_{(2,15)} = 29.37$; $P = 0.0071$; Fig. 5], similar to that seen in the low-prolactin animals, indicative of increased anxiety postpartum compared with postpartum control mice.

Discussion

There have been many previous studies documenting a role for prolactin in maternal behavior (4, 5, 7, 30, 31). To date, these studies have focused on the role of relatively acute changes in prolactin secretion around the time of onset of maternal behavior at parturition. Similarly, acute anxiolytic actions of prolactin have also been reported (11), apparently mediated through actions in the hypothalamus. It seems unlikely that such acute actions of prolactin are involved in the effects seen in the present study. Here, manipulations of prolactin very early in pregnancy had effects on maternal mood and behavior over 2 wk later, after the animals had given birth. Such a long-lasting action of prolactin was unexpected and surprising. Our data provide novel evidence that the prolactin-induced increase in generation of neural progenitors in the SVZ in

early pregnancy is a critical part of the normal adaptive responses that result in changes in mood and behavior in the postpartum period.

The pattern of prolactin secretion observed during early pregnancy was similar to that reported in a number of previous studies. We found that serum prolactin levels markedly increased in a pattern of twice-daily surges, with a larger diurnal increase occurring just before lights off and a smaller nocturnal surge occurring midway through the dark period. After 4–5 d, the nocturnal surge was lost, but the diurnal surge persisted until at least d 9 of pregnancy. This is reminiscent of the pattern of prolactin secretion that has been documented in rats, although in rats, the larger of the two surges occurs during the dark period, and the nocturnal surge also persists longer (32, 33). These surges are initiated by the cervical stimulation associated with mating (24) and can also occur after cervical stimulation in the absence of a fertile mating, a condition known as pseudopregnancy (34–38). We have previously shown that by d 6 of pseudopregnancy in mice, only one large daily surge of prolactin occurs when the lights go off (38). Other studies have also found that there is one surge of prolactin in mice at this stage of pregnancy (34, 39). Thus, although the pattern of twice-daily prolactin surges in early pregnancy is similar in rats and mice, the duration of the two surges seems to be quite different, emphasizing subtle but distinct differences in hormone secretion between the two species.

The increased postpartum anxiety in the low-prolactin mice was observed in two independent tests, the EPM and the light-dark box, both of which are well-validated tests of anxiety-like behavior (25, 26). Interestingly, the C57BL/6J mice that were used in this study are known to be a low-anxiety strain of mice compared with other strains, displaying less responsiveness to anxiety on both of these tests (40, 41). We selected C57BL/6J mice for this study because many genetically modified mice are developed in this highly fertile strain, and such mice may be incorporated into future studies. The large increase in anxiety-like behavior seen postpartum in this low-anxiety stain after the decrease in prolactin-induced mitogenesis in the SVZ during early pregnancy was, therefore, even more remarkable.

The mechanisms underlying the normal postpartum decrease in anxiety are unknown. Although acute increases in prolactin are known to be anxiolytic (11), and women who have recently experienced breastfeeding-induced increases in serum prolactin levels are less anxious in response to stress (42), suckling-induced hormones are apparently not essential for postpartum changes in anxiety (43). The results shown here indicate that decreased serum prolactin levels specifically in early pregnancy can initiate increased postpartum anxiety over 2 wk later by decreasing the generation of SVZ neural progenitors. A number of previous studies have linked neurogenesis to pathological changes in mood, including depression and anxiety (44, 45). The primary focus of these studies, however, has been adult neurogenesis in the dentate gyrus of the hippocampus (46), and this is not thought to change during pregnancy (15, 16). There may be an increase in cell survival and migration in the hippocampus, however, close to parturition (47). Recent data suggest that prolactin might enhance neuronal survival in the hippocampus under stressful conditions (48), but this is likely to be through a different mechanism from cell proliferation. Because MAM treatment affected hippocampal as well as SVZ neurogenesis, it is possible that some hippocampal-dependent functions have also been affected. In rats, hippocampal volume decreases during pregnancy (49), but dendritic spine density in the hippocampus is increased in lactation (50). These changes might be important for learning and memory of maternal tasks (51–55). The effect of prolactin on neurogenesis during early pregnancy, however, was specific to the SVZ (15). Thus, the present data are the first to link decreased generation of neural progenitors in the SVZ to changes in anxiety.

We were specifically interested in assessing the impact of increased anxiety in the postpartum period on behaviors that are critical for effective maternal care, such as maternal responsiveness to pups. Although there were no differences between the two groups in a standardized test of pup retrieval, we did observe some behavioral abnormalities, such as a tendency to leave the nest more readily or to be easily distracted from looking after the pups. This suggested that although basic maternal behaviors, such as pup retrieval, may be preserved in the anxious mothers, the quality of maternal care in an anxiogenic situation may be altered. To test this idea, we decided to place the mice into a novel cage, a mild stressor that induces changes in stress responsiveness that the mouse does not become habituated to (56). Under these conditions, all of the mice took longer to retrieve the pups, indicating that the stress of a novel cage environment does affect maternal responsiveness to pups in mice. The anxious mothers were extremely adversely affected, however, with most of these mice displaying no overt signs of maternal responsiveness to pups within the test period. Thus, the anxiogenic situation revealed an underlying deficit in the maternal behavior that was not immediately apparent in the home cage. This suggests that testing maternal behavior in a novel cage environment is a sensitive measure of the effect of anxiety on maternal behavior and demonstrated that anxiety in mice postpartum affects maternal care of offspring.

We observed that suppression of neurogenesis by MAM treatment for 8 d was required to mimic the effect of low prolactin, whereas MAM administration from d 4–7 of pregnancy impaired maternal behavior but did not affect postpartum anxiety. The requirement for 8 d of MAM may simply reflect the relatively short half-life of this drug (57, 58). Bromocriptine treatment significantly suppressed prolactin for over 20 h, and thus daily injection of this drug provided a prolonged reduction in prolactin throughout early pregnancy. Indeed, treatment only on d 1–3 of pregnancy had an effect on mitogenesis in the SVZ that could be observed on d 7 of pregnancy. MAM, on the other hand, has a transient, short-lived action, and at the doses used, is likely to only be suppressing neurogenesis for a few hours of each day. The additive effect of this treatment, over 8 d, provided a similar long-term consequence. The fact that maternal behavior was affected by a shorter duration of treatment, however, suggests that there may be two separate mechanisms regulating anxiety and maternal behavior that exist downstream of the generation of neural progenitors or that maternal behavior may be more sensitive to a decrease in levels of neural progenitors. Nevertheless, it seems clear that the impaired maternal behavior was not simply a consequence of increased anxiety.

The mechanism by which increased neurogenesis in the SVZ could affect complex behaviors in the postpartum period remains to be determined. Genetic tracing studies suggest that most SVZ neurons migrate in the rostral migratory stream, contributing to granule cell turnover and replacement in the olfactory bulb (17). The new granule cells become morphologically mature as early as 2 wk after initial cell division in the SVZ, although they show markedly different electrophysiological responses to existing granule cells (59). Thus, an increase in mitogenesis in the SVZ during early pregnancy would be expected to result in new mature granule cells being present in the maternal olfactory bulb at approximately the time of parturition. Consistent with this, we observed that reduced mitogenesis in the SVZ during early pregnancy in low-prolactin animals was subsequently associated with reduced numbers of BrdU-immunopositive cells in the olfactory bulb on d 2 postpartum. Because there is evidence that granular cell turnover facilitates olfactory learning of novel odorants (18, 19), this could be important for fine tuning the olfactory response to pups and thus influencing maternal behavior (20, 60). The olfactory bulb provides important afferent inputs into the amygdala and subsequently into the medial preoptic area (61). These brain regions have been shown to be essential for maternal behavior (4, 62), and thus plasticity in afferent inputs may also be critical for appropriate postpartum adaptive responses. In addition to the olfactory bulb, the newly generated SVZ neural progenitors may also migrate to other areas of the brain, such as the cerebral cortex (63), and thus might contribute to plastic changes in other circuits involved in postpartum behavioral responses.

These data suggest that prolactin actions early in gestation play a critical role in establishing appropriate adaptive responses in the mother that subsequently alter behavioral responses postpartum. At least some of these adaptive responses are mediated by a prolactin-induced increase in neural progenitors in the SVZ of the maternal brain. Failure of these adaptive changes might result in postpartum mood disorders or an inability to cope with stressful situations during lactation. During the postpartum period, there is increased incidence of dysfunctional mood disorders (64), with pathological anxiety the most common (65, 66). Previous studies examining the hormonal regulation of mood during the peripartum period have focused on acute changes in hormones at that time, and no clear role of hormones in the control of mood has emerged. The present data provide novel insights into the effect of pregnancy hormones on anxiety, identifying a critical period much earlier in gestation than previously suspected. Hence, factors influencing prolactin (and potentially other hormones) early in pregnancy, such as stress, nutrition, or medication, might have an important and previously unsuspected impact on mood in the postpartum period.

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