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# Postpartum inhibition of ovarian steroid action increases aspects of maternal caregiving and reduces medial preoptic area progesterone receptor expression in female rats

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ABSTRACT

The rapid peripartum onset of maternal caregiving involves progesterone synergizing with estradiol, but prolonging progesterone exposure past this time can prevent the emergence of mothering. Interestingly, there is a 7–10 day-long rise in progesterone during mid-lactation, but its effects on mothering are unknown. Given progesterone's potential to inhibit mothering onset, this mid-lactational rise may contribute to the normal attenuation of caregiving behaviors across lactation. To evaluate this, recently-parturient rats were ovariectomized and caregiving observed from postpartum days (PPD) 7–18. Ovariectomized dams were found to lick, hover over, and nurse in kyphosis more frequently than controls. Ovariectomy also decreased medial preoptic area (mPOA) progesterone receptor (PR) mRNA, which was negatively correlated with pup licking and kyphosis, but it did not affect mPOA levels of oxytocin receptor or vasopressin V1a receptor mRNAs. In a second study, gonadally intact dams were given the PR antagonist, RU 486, and were found to display more kyphosis and less supine nursing compared to controls. Finally, progesterone sensitivity across lactation was examined by measuring numbers of PR immunoreactive (PR-ir) cells in the mPOA, ventral bed nucleus of the stria terminalis (BSTv) and periaqueductal gray (PAG). PR-ir was higher in the mPOA at parturition compared to virgins, while PR-ir in the mPOA and BSTv dropped from parturition to PPD 7 and remained low through PPD 18. The number of PR-ir cells in the PAG was constant. Thus, in addition to their well-known prepartum effects, ovarian hormones limit the display of some maternal behaviors during mid-to-late lactation and contribute to their decline as weaning approaches.

## 1. Introduction

The onset of maternal behaviors in many female mammals is mediated by the hormonal milieu of pregnancy and parturition, with particular importance of the ovarian hormones estradiol and progesterone (Bridges, 2015). Pregnant laboratory rats have relatively low levels of circulating estradiol until a few days before parturition. In contrast, plasma progesterone slowly rises throughout the first two weeks of pregnancy, peaks in the third week, and then precipitously declines before dams give birth (Bridges, 2015). Many experimental paradigms have shown that this pattern of rising estradiol against a background of rising then falling progesterone potently stimulates maternal caretaking behaviors in laboratory rats (see Bridges, 2015; Lonstein et al., 2014 for reviews). The withdrawal of progesterone is critical, because artificially maintaining high circulating progesterone at the end of pregnancy or in pregnancy-terminated female rats interferes with the onset of maternal behavior (Bridges and Feder, 1978; Bridges et al., 1978; Herrenkohl, 1974; Moltz et al., 1969; Numan, 1978). Progesterone's inhibitory action on caretaking is mediated by its nuclear receptor (PR), because progesterone-mediated inhibition of maternal behavior is prevented in female rats by the PR antagonist RU 486 (Numan et al., 1999) and null mutation of PRs in male mice increases their positive responses to pups (Schneider et al., 2003). Thus, even though progesterone is probably best known to synergize with estradiol to promote maternal behaviors (Bridges, 1984; Doerr et al., 1981), progesterone signaling can also inhibit caregiving.

In contrast to the well-established endocrine basis for the onset of maternal behaviors in laboratory rats and many other animals, it has long been thought that ovarian and other steroid hormones have little to no role after parturition in maintaining the display of maternal behaviors, and that mothering is instead controlled by sensory cues that dams receive from pups (Erskine et al., 1980; Hansen and Ferreira,

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1986; Lonstein, 2005; Stern and Johnson, 1989, 1990). This is reasonable given that circulating progesterone and estradiol are both very low during the first few days postpartum (Grota and Eik-Nes, 1967; Hansen et al., 1983; Smith and Neill, 1977; Taya and Greenwald, 1982), and that an early study indicated that ovariectomy either one or two days before parturition did not appear to affect maternal behavior in many female rats (Moltz and Wiener, 1966). However, two studies re-examining the influence of postpartum circulating steroids on maternal behaviors produced intriguing results. First, De Sousa et al. (2010) found that ovariectomizing primiparous rats the day after parturition decreased maternal licking of pups when observed over the next six days. Second, Rees et al. (2004) found that adrenalectomy in late pregnancy also decreased early postpartum maternal licking, as well as decreased the amount of time dams spent in their nests.

There has been no examination, however, of how any steroid hormone influences the details of maternal caregiving during mid-to-late lactation. These times of the postpartum period (i.e., the 2nd and 3rd weeks postpartum in rats) seem most relevant to examine the effects of ovarian hormones, particularly progesterone, because there is a rarely-studied rise in ovarian-produced progesterone in female rats starting a few days after parturition, which reaches a peak on postpartum days 10–12 and returns to premating levels about 7 days later (Hansen et al., 1983; Smith and Neill, 1977). On the other hand, estradiol levels are very low throughout almost all of lactation, with a rise at the very end when suckling by the soon-to-be weanlings has mostly ceased and inhibition of gonadotropin release is alleviated (Smith and Neill, 1977; Taya and Greenwald, 1982). This pattern of postpartum ovarian hormone release in many ways looks quite similar to what is seen during pregnancy - a rise and fall of progesterone against a background of low estradiol. Given that maternal behaviors, at least during the early postpartum period, remain sensitive to ovarian hormone signaling (Champagne et al., 2003; de Sousa et al., 2010; Herrenkohl, 1974), it is possible that ovarian hormones retain their capacity to influence maternal behaviors even through the late postpartum period.

To examine the influence of postpartum ovarian hormones, and particularly progesterone, in the display of maternal caregiving we first ovariectomized recently-parturient rats and observed their maternal behaviors throughout mid-to-late lactation. In a second study, we specifically targeted PRs during late lactation with the antagonist RU 486 and observed dams' caregiving behaviors. Given progesterone's ability to interfere with the onset of caregiving behaviors (Bridges and Feder, 1978; Bridges et al., 1978; Numan, 1978), we predicted that postpartum ovariectomized or RU 486-treated dams would display more maternal behavior compared to controls. Because the medial preoptic area (mPOA) mediates the effects of many hormones on maternal behavior (Numan, 2006), we determined the effects of postpartum ovariectomy on mPOA expression of the mRNAs for the PR isoforms A and B, as well as for the oxytocin receptor (OTR) and vasopressin V1a receptor. Central OTR and V1a receptor expression is positively associated with maternal caregiving (Bosch and Neumann, 2012; Bosch and Neumann, 2008; Champagne et al., 2001; Francis et al., 2000) and regulated by ovarian hormones (Caldwell et al., 1994; Johnson et al., 1989; Kalamatianos et al., 2004; Patchev et al., 1993; Quiñones-Jenab et al., 1997; Schumacher et al., 1989). It could be possible that postpartum ovarian hormone manipulations affect maternal behaviors by modulating OTR or V1a receptor expression, in addition to PR expression. Because we did find that ovariectomy and RU 486 each increased the display of some maternal behaviors, we quantified the number of PR-ir cells in the mPOA and two other progesterone-sensitive brain sites involved in maternal caregiving (i.e. bed nucleus of the stria terminalis (BST) and midbrain periaqueductal gray (PAG)) (Numan and Numan, 1996; Lonstein and Stern, 1997a, 1997b) from early to late postpartum to gain insight into when progesterone signaling though PR in these sites could have its maximal effects on mothering.

## 2. Methods

### 2.1. Subjects

Subjects were female Long-Evans rats born and raised in our colony, descended from rats purchased from Harlan Laboratories (Indianapolis, IN). Females were housed after weaning with 1 or 2 same-sex littermates in clear polypropylene cages (48 cm × 28 cm × 16 cm) containing wood chip bedding. Food (Tekland rat chow, Indianapolis, IN) and water were available ad libitum, and the room was maintained on a 12:12 light/dark cycle (lights on at 0700 h) with temperature at 22 ± 1 °C and relative humidity of 40–50%. After reaching 65 days of age, estrous cycles were monitored daily by vaginal smearing. On a day of proestrus, subjects to be used for the postpartum groups were placed overnight with a sexually-experienced male from our colony. Pregnancy was confirmed the next day by the presence of semen in a vaginal smear or the presence of a vaginal plug. Subjects were then housed with 1–2 other pregnant females until 5–7 days before expected parturition, after which they were singly housed. The females used for observations of maternal behavior were singly housed in larger polypropylene cages (48 cm × 38 cm × 20 cm) containing wood chip bedding and continually replenished ENVIRO-DRI™ strips bedding material that they could use to build nests (Shepherd Specialty Papers, Richland, MI). Compared to our standard housing cages, these larger cages allowed females more choice of whether or not to spend time in contact with the litter. Litters were culled to contain 8 pups (4 males, 4 females) soon after parturition. All procedures were performed in accordance with the principles of the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Michigan State University.

### 2.2. Ovariectomy

In the first experiment, two or three days following parturition, litters were removed from their dams and placed in an incubator set at nest temperature (34 °C). Dams were weighed and anesthetized by an intraperitoneal injection of 90 mg/kg ketamine (Henry Schein Animal Health, Dublin, OH) followed by an intraperitoneal injection of 8 mg/kg xylazine (Lloyd laboratories, Shenandoah, IA). Incisions were made through the skin and underlying muscle in the dorsolateral flanks and the ovaries were externalized. In the experimental group (ovariectomized (OVX); $n = 8$), the distal portion of the uterine horns were tied with silk suture and the ovaries removed. In the control group (Sham; $n = 8$), the externalized ovaries and uterine horns were placed back into the body cavity. Incisions in the muscle layer were sutured closed and incisions in the skin closed with surgical staples. After surgery, subjects were placed on a heating pad within their home cage until they recovered from anesthesia. Their litters were then given back and the cages returned to the colony room. Beginning the day after maternal ovariectomy or sham surgery, the litters were cross-fostered daily between the subjects and surrogate dams from our colony for the duration of the experiment in case there were effects of maternal ovariectomy on lactation, to ensure that all dams interacted with healthy litters each day. Cross-fostering was done after daily behavior observations, and litters were weighed at that time on postpartum days (PPDs) 8, 13, and 19.

### 2.3. RU 486 injections

In the second experiment, each dam received a subcutaneous injection of the PR antagonist, RU 486 (10 mg of RU 486 in 0.4 ml of 80% sesame oil, 15% benzyl benzoate, 5% ethanol (Numan et al., 1999; $n = 7$), daily between 0700 and 0730 h on PPDs 15–20. Control dams were injected with vehicle ($n = 8$). The researchers performing the injections were blind to what was being administered to the subjects.