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Oxytocin: An emerging regulator of prolactin secretion in the female rat

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Abstract

In the female rat, a complex interplay of both stimulatory and inhibitory hypothalamic factors controls the secretion of prolactin. Prolactin regulates a large number of physiological processes from immunity to stress. In the following review, we have chosen to focus on the control of prolactin secretion in the female rat in response to suckling, mating and ovarian steroids. In all three of these states, dopamine, released from neurones in the mediobasal hypothalamus, is a potent inhibitory signal regulating prolactin secretion. Early research has determined that the relief of dopaminergic tone is not enough to account for the full surge of prolactin secretion observed in response to the suckling stimulus, launching a search for possible prolactin-releasing factors. This research has since broadened to include searching for prolactin-releasing factors controlling prolactin secretion following mating or ovarian steroids. A great deal of literature has suggested that this prolactin-releasing factor may include oxytocin. Oxytocin receptors are present on lactotrophs. These oxytocin receptors respond to exogenous oxytocin and antagonism of endogenous oxytocin inhibits lactotroph activity. In addition, the pattern of oxytocin neuronal activity and oxytocin release correlate with the release of prolactin. Here we suggest that not only is oxytocin stimulating prolactin secretion, but we also hypothesize that prolactin secretion is controlled by a complex network of positive (oxytocin) and negative (dopamine) feedback loops. In the present review, we will discuss this literature and attempt to describe the circuitry we believe may be responsible for controlling prolactin secretion.

Prolactin, so named for its ability to stimulate lactation and mammary gland development, is a protein hormone which is primarily synthesized in and secreted from lactotrophs in the anterior pituitary gland (1). In rats, the translation of the prolactin gene results in a 197 amino acid polypeptide (2), which influences over 300 physiological events including osmoregulation, immunomodulation, behaviour, reproduction, growth and development (3). Although prolactin exerts its effects on a number of biological events, the physiological control of its secretion is not wholly defined. Furthermore, while prolactin plays an undeniable role in pregnancy and stress, the role of oxytocin in these states is not yet clear. Therefore, in the following sections, we focus on the control of prolactin secretion in response to suckling, mating and ovarian steroids. We include evidence for oxytocin’s stimulatory and dopamine’s inhibitory roles on prolactin secretion. In addition, we discuss prolactin’s stimulatory role on oxytocin secretion, suggesting an oxytocin-prolactin positive feedback in conjunction with the classical dopamine-prolactin negative feedback in the control of prolactin secretion.

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Prolactin Secretion

Prolactin is secreted 1) in response to suckling 2) in response to mating and 3) on pro-oestrus evening in response to ovarian steroids (3) (Fig. 1). The suckling of rat pups initiates a surge of prolactin concentration that persists in the lactating rat as long as the suckling stimulus is maintained (4). Prolactin increases within 5 minutes of the suckling stimulus and reaches peak levels by 30 minutes. Upon the withdrawal of this stimulus, prolactin secretion subsides within 10 minutes. Increasing the number of suckling pups further increases the magnitude of prolactin secretion (4). This is a classical neuroendocrine reflex; an acute stimulus tightly accompanied by a transient secretory response that persists as long as the stimulus is applied. Following the mating stimulus, the female rat secretes a nocturnal (peaking between 0300-0500h) and a diurnal (peaking between 1700-2000h) surge of prolactin (5-8). The nocturnal surge is consistently greater in magnitude compared to the diurnal surge (5, 8). These surges function to maintain progesterone secretion by rescuing the short-lived corpus luteum. Progesterone induces changes of the endometrium allowing implantation of the blastocyst and is responsible for maintaining uterine quiescence throughout gestation. If the mating was fertile, the prolactin surges persist for 10 days (8, 9) at which point a prolactin-like luteotrophic factor, secreted from the placenta, maintains progesterone secretion for the remainder of pregnancy. If the mating was sterile, the female rat enters into a period of pseudopregnancy and these prolactin surges recur for 12 days (6, 8). Artificial stimulation of the uterine cervix is a copulomimetic model in ovariectomized rats, in which the female secretes surges of prolactin for 12 days even in the absence of a continued stimulus. Because these rats lack ovaries, this demonstrates that ovarian steroids are not required for initiation or maintenance of this unique secretory response. Conversely, in response to rising titers of oestradiol during the 4-day oestrous cycle of the female rat, prolactin secretion is sharply elevated on the evening of pro-oestrus just prior to the luteinizing hormone surge (6). Prolactin returns to basal levels the following morning and remains there throughout the rest of the oestrous cycle. In ovariectomized animals, the administration of a bolus injection of oestradiol initiates a surge of prolactin release, which is similar in timing and magnitude to that secreted on pro-oestrus (9). The administration of progesterone amplifies and anticipates this surge (10).

Neuroendocrine Dopaminergic Neurones Tonically Inhibit Prolactin Secretion

During periods of non-stimulation, lactotrophs release prolactin in a constitutive manner. For this reason, neuroendocrine dopaminergic neurones tonically inhibit prolactin secretion from the lactotrophs. The coordinated release of dopamine from three populations of dopaminergic neurones arising from the mediobasal hypothalamus produces this tonic inhibition. The 1) tuberoinfundibular dopaminergic (TIDA) neurones (A12) are located in the dorsomedial arcuate nucleus and project to the external zone of the median eminence where they terminate on a fenestrated capillary bed that drains into long portal vessels, carrying dopamine to the anterior lobe of the pituitary gland. 2) The tuberohypophyseal dopaminergic (THDA) neurones (A12) are located throughout the rostral arcuate nucleus and project through the internal zone of the median eminence before terminating on short portal vessels in the neural and intermediate lobe of the pituitary. Likewise, the 3) periventricular hypophyseal dopaminergic (PHDA) neurones (A14) are located in the periventricular region of the hypothalamus and project through the internal layer of the median eminence before terminating on short portal vessels in the intermediate lobe. Dopamine released from the THDA and PHDA neurones arrives at fenestrated short portal vessels, which then drain into the anterior lobe. These dopamine neurones are often referred to as neuroendocrine dopaminergic neurones, as dopamine arrives at the lactotroph through

a blood-borne pathway. Upon arrival at the lactotroph, dopamine binds to the D₂ receptors (11), resulting in a decrease in prolactin secretion (for reference see (3)).

In response to suckling, oestradiol or mating stimuli, the amount of dopamine arriving at the anterior pituitary decreases. Following a short time delay, prolactin secretion is elevated before negatively feeding back to stimulate dopamine release into portal blood by crossing an active transport system in the choroid plexus (12) and binding to prolactin receptors on neuroendocrine dopaminergic neurones (13-15). The interactions between dopamine and prolactin constitutes a negative feedback loop in which prolactin controls its own secretion (16). Inhibition of dopamine secretion into portal blood is most likely the main mechanism involved in controlling prolactin secretion (17), making prolactin a unique hormone in that its main source of control is inhibitory. However, previous studies suggest that the reduction of dopamine alone may not account for the entire suckling-induced surge of prolactin (18), suggesting that a prolactin-releasing factor is involved in this secretory response.

Oxytocin as a Prolactin-Releasing Hormone

Many prolactin-releasing factors have been suggested, (among them, thyrotropin-releasing hormone, vasoactive intestinal polypeptide, vasopressin, serotonin, angiotensin II (3), and prolactin-releasing peptide (19)). However, oxytocin has emerged as the strongest candidate of a prolactin-releasing factor, as determined using Geoffrey Harris' criteria for hypophysiotrophic hormones (Table 1). Like prolactin, oxytocin plays various roles in coordinating reproduction and it is not entirely surprising that prolactin and oxytocin may interact to orchestrate this vital biological process. Mainly produced in neurones of the paraventricular nucleus and supraoptic nucleus of the hypothalamus, oxytocin is a nonapeptide classically known for its role in milk ejection and parturition. Oxytocin is released from the posterior pituitary, into the peripheral circulation to stimulate contractions of the uterine myometrium or myoepithelium of the mammary glands (20, 21). Centrally, oxytocin has been implicated in determining maternal behaviour (22). While the majority of oxytocin neurones project to the posterior pituitary, oxytocin projections to the median eminence are also present (23). Therefore, oxytocin reaches the lactotrophs in the anterior pituitary primarily through 1) the long portal vessels (from the median eminence to the anterior lobe) 2) through short portal vessels (from the posterior lobe to the anterior lobe) and 3) from the peripheral circulation following release from the posterior pituitary. Here, oxytocin is capable of binding to oxytocin receptors found on the cell membrane of lactotrophs (fulfilling Table 1, Criterion [1]) (24). Experiments done *in vitro* have shown that administration of oxytocin to lactotrophs initiates prolactin release (fulfilling Table 1, Criterion [2]) that is preceded by intracellular Ca²⁺ release, suggesting that oxytocin stimulates prolactin secretion via a Ca²⁺-dependent mechanism (25).

Oxytocin and Suckling-Induced Prolactin Secretion

A number of studies have shown a relationship between oxytocin and prolactin secretion in response to suckling, mating and ovarian steroids. In response to suckling, a rise in oxytocin in the peripheral plasma precedes the increase in suckling-induced prolactin secretion (fulfilling Table 1, Criterion [4]) (26, 27). Lesions of the paraventricular nucleus suppress suckling-induced prolactin secretion (28, 29). Additionally, peripheral immunoneutralization of oxytocin has been shown to attenuate the suckling-induced rise in prolactin secretion (fulfilling Table 1, Criterion [3]) (27). In agreement with Samson et al., data from our lab (Fig. 2) (30) has shown that administration of an oxytocin antagonist through an osmotic mini-pump (allowing constant dosing) effectively blocks the suckling-induced rise in prolactin secretion. This antagonist was not capable of passing the blood-brain-barrier, suggesting that its site of action was at the lactotroph. This is not without debate; a second

study from the Negro-Vilar laboratory (31) in which an oxytocin antagonist was used does not support these findings. This may have been due to methodological differences in the study; lower doses of an alternate antagonist were injected intravenously. This may suggest that the difference between the data in the Negro-Vilar study and the Freeman study (30) could have been attributed to a missed critical time window, an ineffective oxytocin antagonist, an ineffective dose of the oxytocin antagonist or some combination of these factors.

Interestingly, prolactin stimulates oxytocin secretion during lactation (32, 33). How this occurs is not yet clear. The number of oxytocin neurones in the paraventricular nucleus expressing the long form of the prolactin receptor increases during lactation (34). We hypothesize that this couples a positive feedback loop between prolactin and oxytocin leading to increased release of oxytocin, which can then act at the lactotroph to stimulate prolactin secretion (27, 30). This feedback loop would be mutually beneficial, and would guarantee the necessary partnership of milk production and letdown. During early lactation, in response to the both the cessation of the suckling stimulus and high levels of prolactin, the dopamine-prolactin negative feedback loop is triggered, since a decrease in dopamine release is also observed in response to the suckling stimulus (35, 36). However, by day 13 of lactation, prolactin is no longer able to activate tuberoinfundibular dopaminergic neurones, suggesting that the dopamine-prolactin negative feedback loop is uncoupled during lactation (37).

Oxytocin and Mating-Induced Prolactin Secretion

In response to mating stimuli, oxytocin neurones are activated resulting in an immediate surge of oxytocin secretion (38, 39). This release of oxytocin is consistently followed by rhythmic prolactin secretion in rats (fulfilling Table 1, Criterion [4]) (40). Furthermore, there is much evidence of oxytocin's stimulatory role in the prolactin secretory rhythm induced by cervical stimulation. Oxytocin neurones in the paraventricular nucleus and supraoptic nucleus are activated during times of the surges (41-43) and a peripheral bolus injection of oxytocin initiates the prolactin secretory rhythm induced by cervical stimulation (fulfilling Table 1, Criterion [2]; Fig. 3) (44). In addition, blocking peripheral oxytocin receptors, prior to or after a mating stimulus, blocks these surges (fulfilling Table 1, Criterion [3]; Fig. 4) (45, 46). Once the peripheral oxytocin receptors are no longer blocked, the daily prolactin rhythm induced by cervical stimulation is initiated (46). Therefore, oxytocin likely stimulates prolactin secretion through direct lactotroph activity.

In addition, when an oxytocin antagonist blocks rhythmic prolactin secretion, rhythmic dopamine release is disrupted as well (46). For example, prior to the prolactin surge induced by cervical stimulation, THDA dopaminergic activity is elevated. However, in the presence of an oxytocin antagonist, and hence, low prolactin levels, this dopaminergic activity is significantly decreased. This may seem counterintuitive since elevated dopamine release is expected when prolactin secretion is low. However, prolactin secretion is required to stimulate dopamine release with a time delay. Therefore, without an increase in prolactin, there may be no stimulation of dopamine to negatively feedback and decrease prolactin secretion, thereby also altering the time delay. However, dopamine activity of TIDA neurones remained elevated prior to the expected prolactin surge. Therefore, there may be differential regulation by the neuroendocrine dopamine neurones populations in controlling prolactin secretion (47, 48). One may explain these data by suggesting that the oxytocin antagonist is directly disrupting the activity of dopamine, thereby inhibiting prolactin secretion. However, this is unlikely as oxytocin receptors have not yet been described on neuroendocrine dopaminergic neurones, suggesting instead that the disruption in dopamine release is likely indirect, via its effects on prolactin secretion.

Conversely, a central bolus injection of oxytocin does not trigger a daily secretory prolactin rhythm (D. McKee, unpublished data). If we consider the possibility that the amount of oxytocin required to stimulate prolactin secretion, which is unknown, did not/could not reach the lactotroph, this finding could support the hypothesis that oxytocin acts directly at the lactotroph to stimulate prolactin secretion. Regardless, these data suggest that oxytocin does not act centrally to trigger the daily secretory prolactin rhythm, instead suggesting that oxytocin acts peripherally to stimulate prolactin secretion. Interestingly, central or peripheral ovine prolactin delivery initiates prolactin surges in rats (49). Blocking central prolactin receptors disrupts the prolactin secretory rhythm induced by ovine prolactin and cervical stimulation. However, similar to blocking oxytocin receptors, once the prolactin antagonist has cleared, the daily prolactin rhythm is restored. These findings may reflect the reported stimulatory effect of central prolactin on oxytocin secretion (50), thus supporting our proposed mechanism of an oxytocin-prolactin positive feedback loop. Presumably, central prolactin stimulates oxytocin neurones, thus oxytocin secretion to the lactotroph. Oxytocin then stimulates prolactin secretion, creating a positive feedback loop. Once a threshold of prolactin is reached, prolactin stimulates dopamine release triggering the dopamine-prolactin negative feedback loop. Therefore, blocking central prolactin receptors, located on oxytocin neurones (34), prevents oxytocin secretion and the oxytocin-prolactin positive feedback loop. Consequently, prolactin levels remain basal, and there is no initiation of the dopamine-prolactin negative feedback loop.

Conversely, the dopamine-prolactin negative feedback loop is initiated when the prolactin secretory rhythm is induced by ovine prolactin, and it is disrupted when the prolactin secretory rhythm is blocked by a prolactin antagonist (46). When the prolactin secretory rhythm is initiated by oxytocin or ovine prolactin (Fig. 5), a decrease in dopamine release coincides with elevated prolactin levels (44, 49). These data are consistent with our oxytocin-prolactin positive feedback loop hypothesis and further suggests that this hypothetical loop is capable of overcoming the inhibition of dopamine; at least until the prolactin threshold is reached. These data confirm the significance of prolactin-dopamine negative feedback and that oxytocin, indeed, stimulates the daily prolactin secretory rhythm. More so, these data support our hypothesis that an oxytocin-prolactin positive feedback loop is important in triggering the daily prolactin secretory rhythm.

Oxytocin and Ovarian Steroid-Induced Prolactin Secretion

Ovarian steroids influence both oxytocin neuronal activity and prolactin secretion. Plasma oxytocin concentrations are highest in hypophyseal portal blood on the afternoon of pro-oestrus (51) just prior to the pro-oestrous surge of prolactin (fulfilling Table 1, Criterion [4]) (6). Likewise, lactotrophs obtained from pro-oestrous rats are more responsive to oxytocin than those obtained from rats in dioestrus (52). In the supraoptic nucleus, oxytocin gene expression increases throughout the oestrous cycle before peaking on oestrus (53). Likewise, the number of oxytocin neurones expressing fos-related antigen in the paraventricular and periventricular nuclei of oestradiol-treated animals gradually rises across the day, peaking at approximately the same time as the oestradiol-induced surge of prolactin, suggesting that oxytocin is modulated by oestradiol and may be stimulating prolactin secretion (54). Oxytocin immunoneutralization or application of an oxytocin antagonist attenuates the pro-oestrous and the oestradiol-induced surges of prolactin (fulfilling Table 1, Criterion [3]) (27, 30, 31, 55). Additionally, plasma oxytocin concentrations increase in response to exogenous oestradiol administration (56). Therefore, a possible permissive role of oestradiol on prolactin secretion may be its ability to influence oxytocin activity.

In the rat (57) and the mouse (58), oxytocin receptors are influenced by oestradiol and their promoters contain an oestrogen response element. In the anterior pituitary, oxytocin receptor

mRNA has been localized to lactotrophs and oestradiol has been shown to increase this population of oxytocin receptor mRNA 6-fold in ovariectomized rats (24, 59). Because oxytocin receptors are robustly upregulated in response to oestradiol, it is not surprising that a lower dose of oxytocin antagonist is necessary to block the surges of prolactin in cervically stimulated-ovariectomized rats compared to oestradiol-treated ovariectomized rats (Fig. 6) (30, 46).

In ovariectomized animals, the administration of a bolus injection of oestradiol initiates a surge of prolactin release, which is similar in timing and magnitude to that secreted on pro-oestrus (9). The administration of progesterone amplifies and anticipates this surge (10). Interestingly, the estradiol + progesterone-induced surge of prolactin is not prevented by oxytocin antagonist administration (30). In the anterior pituitary, oxytocin receptor gene expression increases in response to oestradiol, but no further increase is observed in response to progesterone (60). Likewise, in the anterior pituitary (where oxytocin receptors have been localized to lactotrophs (59), progesterone has no effect on oestradiol-induced upregulation of oxytocin receptor density (30), suggesting that progesterone acts through other mechanisms to stimulate prolactin secretion. One suggested mechanism might be through the inhibition of dopamine. Short-term progesterone treatment decreases dopamine synthesis and release, as well as tyrosine hydroxylase activity and mRNA (47, 61-68). This results in the prolongation and amplification of the prolactin surge (61, 64, 65). While progesterone receptors are not localized to lactotrophs in the rat (69), they have been localized to tyrosine hydroxylase-positive neurones found in the periventricular and ARN of the hypothalamus (69-71). TIDA, THDA and PHDA neurones (72) arise from these regions and secrete dopamine into hypophyseal blood which binds to D₂ receptors found on lactotrophs and inhibits the secretion of prolactin (11). Therefore, in the presence of progesterone, prolactin secretion may not involve oxytocin; instead, it may simply involve the inhibition of dopamine, thereby allowing for a prolactin surge. Regardless, this underscores the complex interplay between the physiological regulation of prolactin by its modulators, dopamine and oxytocin.

The stimulatory effects of prolactin on oxytocin neurones has been described, however, recent data has added a novel dimension to this proposed model. Prolactin has been shown to decrease firing of oxytocin neurones in the supraoptic nucleus in non-pregnant rats (stage of oestrous cycle not described) (34), cause a hyperpolarizing effect on magnocellular oxytocin neurones in the paraventricular nucleus of pubertal females (stage of oestrous cycle not described) (73) and induces both inhibitory and stimulatory effects in male rats. This intriguing effect suggests that prolactin is capable of controlling its secretion through two routes; prolactin is capable of feeding back to dopaminergic neurones to increase their firing which ultimately decreased prolactin release and now there is evidence that prolactin is able to inhibit its stimulatory factor when prolactin levels are high. It would be interesting to see if prolactin has a differential effect on oxytocin neurone activity across (1) different stages of the estrous cycle, (2) following mating or (3) during pregnancy and lactation. Due to the tight regulation of both prolactin and oxytocin by estradiol and progesterone, different physiological conditions may possibly affect the firing rate of oxytocin neurones following prolactin treatment. These studies add an exciting new direction for this field; prolactin may play both a stimulatory role on oxytocin activity as well as an inhibitory one, suggesting that the control of prolactin secretion is even more complicated than previously described.

Implications in humans

Evidence suggests that this circuitry may extend from rats to humans. For example, intranasal oxytocin administration increases the amount of milk collected from primipara women who have delivered prematurely (74). Although prolactin secretion was not directly

measured, this would suggest that oxytocin stimulated milk production. The other possibility of course is that oxytocin stimulated milk ejection alone, but this is less likely since a 3.5 times enhancement in milk occurred. In addition, a positive correlation between oxytocin and prolactin secretion is observed during breastfeeding, even though differential patterns of oxytocin (pulsatile) and prolactin (continuous) secretions are present (75). More so, oxytocin significantly increases ten minutes prior to elevation of prolactin secretion, consistent with our hypothesis that oxytocin stimulates prolactin secretion in response to suckling (75). Following mating, there is an increasing evidence of the interactions between oxytocin and prolactin following orgasm in humans. Interestingly, briefly following orgasm, oxytocin and prolactin begin to elevate (76, 77). Oxytocin increases in response to sexual arousal and is highest following orgasm (78). Following orgasm, an acute surge of prolactin occurs and remains elevated for 60 minutes and begins to decline by 100 minutes in humans (79). During the menstrual cycle, oxytocin's role is not clear.

In some cases, oxytocin and prolactin secretion show no correlation during the menstrual cycle (80). However, it appears that oxytocin may indirectly stimulate prolactin secretion by enhancing vasoactive intestinal polypeptide-induced prolactin secretion (81). Interestingly, neuroendocrine dopaminergic neurones and oxytocin neurones both express vasoactive intestinal polypeptide 2 receptors (25, 82), suggesting a complex set of negative and positive feedback loops. It seems reasonable to hypothesize that in response to oxytocin, vasoactive intestinal polypeptide is released which binds to vasoactive intestinal polypeptide 2 receptors on oxytocin neurones leading to oxytocin release into the portal vasculature ultimately stimulating prolactin secretion from the lactotroph.

Conclusion

The complexity of prolactin secretion is undeniable. This may be because prolactin plays such an essential role in successful rat gestation. This review proposes mechanisms involved in the control of prolactin secretion in response to suckling, mating stimuli, and ovarian steroids. In these three conditions, we suggest that oxytocin stimulates prolactin secretion initiating an oxytocin-prolactin positive feedback loop. This positive feedback loop produces a sufficient amount of prolactin secretion to induce a dopamine-prolactin negative feedback loop, resulting in two independent loops with indirect interactions (Fig 7). It is possible that prolactin is variably controlled in order to maintain the success of reproduction. One thing that remains consistent, however, is the stimulatory effect that oxytocin has on prolactin secretion and in turn the inhibitory tone that neuroendocrine dopaminergic neurones must sustain in order to maintain the quiescence of the lactotrophs. It seems that oxytocin's physiological role is to directly stimulate prolactin secretion, which then triggers dopamine release. This pattern varies according to the physiological state, yet the basic mechanism is similar. Future work should investigate the direct relationship between oxytocin, prolactin and neuroendocrine dopamine in order to have a more thorough understanding of the control of prolactin secretion.

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References

1. Riddle O, Bates RW, Dykshorn SW. The preparation, identification and assay of prolactin--a hormone of the anterior pituitary. *Am J Physiol.* 1933; 105(1):191–216.

2. Cooke NE, Coit D, Weiner RI, Baxter JD, Martial JA. Structure of cloned DNA complementary to rat prolactin messenger RNA. *J Biol Chem.* 1980; 255(13):6502–10. [PubMed: 6993473]
3. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: Structure, Function, and Regulation of Secretion. *Physiol Rev.* 2000; 80(4):1523–631. [PubMed: 11015620]
4. Grosvenor CE, Whitworth N. Evidence for a steady rate of secretion of prolactin following suckling in the rat. *J Dairy Sci.* 1974; 57(8):900–4. [PubMed: 4851936]
5. Freeman ME, Smith MS, Nazian SJ, Neill JD. Ovarian and hypothalamic control of the daily surges of prolactin secretion during pseudopregnancy in the rat. *Endocrinology.* 1974; 94(3):875–82. [PubMed: 4813686]
6. Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology.* 1975; 96(1):219–26. [PubMed: 1167352]
7. Gunnet JW, Freeman ME. The mating-induced release of prolactin: a unique neuroendocrine response. *Endocr Rev.* 1983; 4(1):44–61. [PubMed: 6339213]
8. Freeman ME, Neill JD. The pattern of prolactin secretion during pseudopregnancy in the rat: a daily nocturnal surge. *Endocrinology.* 1972; 90(5):1292–4. [PubMed: 5012741]
9. Neill JD. Sexual differences in the hypothalamic regulation of prolactin secretion. *Endocrinology.* 1972; 90(5):1154–9. [PubMed: 5062474]
10. Caligaris L, Astrada JJ, Taleisnik S. Oestrogen and progesterone influence on the release of prolactin in ovariectomized rats. *J Endocrinol.* 1974; 60(2):205–15. [PubMed: 4815977]
11. Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. *Endocr Rev.* 2001; 22(6):724–63. [PubMed: 11739329]
12. Walsh RJ, Slaby FJ, Posner BI. A receptor-mediated mechanism for the transport of prolactin from blood to cerebrospinal fluid. *Endocrinology.* 1987; 120(5):1846–50. [PubMed: 3569115]
13. Lerant A, Freeman ME. Ovarian steroids differentially regulate the expression of PRL-R in neuroendocrine dopaminergic neuron populations: a double label confocal microscopic study. *Brain Res.* 1998; 802(1-2):141–54. [PubMed: 9748546]
14. Arbogast LA, Voogt JL. Prolactin (PRL) receptors are colocalized in dopaminergic neurons in fetal hypothalamic cell cultures: effect of PRL on tyrosine hydroxylase activity. *Endocrinology.* 1997; 138(7):3016–23. [PubMed: 9202247]
15. Grattan DR. The actions of prolactin in the brain during pregnancy and lactation. *Prog Brain Res.* 2001; 133:153–71. [PubMed: 11589128]
16. DeMaria JE, Lerant AA, Freeman ME. Prolactin activates all three populations of hypothalamic neuroendocrine dopaminergic neurons in ovariectomized rats. *Brain Res.* 1999; 837(1-2):236–41. [PubMed: 10434008]
17. Gibbs DM, Neill JD. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. *Endocrinology.* 1978; 102(6):1895–900. [PubMed: 570492]
18. de Greef WJ, Visser TJ. Evidence for the involvement of hypothalamic dopamine and thyrotrophin-releasing hormone in suckling-induced release of prolactin. *J Endocrinol.* 1981; 91(2):213–23. [PubMed: 6795296]
19. Maixnerova J, Spolcova A, Pychova M, Blechova M, Elbert T, Rezacova M, Zelezna B, Maletinska L. Characterization of prolactin-releasing peptide: Binding, signaling and hormone secretion in rodent pituitary cell lines endogenously expressing its receptor. *Peptides.* 2011; 32(4):811–7. [PubMed: 21185342]
20. Kiss A, Mikkelsen JD. Oxytocin--anatomy and functional assignments: a minireview. *Endocr Regul.* 2005; 39(3):97–105. [PubMed: 16468232]
21. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev.* 2001; 81(2):629–83. [PubMed: 11274341]
22. Kendrick KM. Oxytocin, motherhood and bonding. *Exp Physiol.* 2000; 85(Spec No):111S–24S. [PubMed: 10795913]
23. Zimmerman EA, Nilaver G, Hou-Yu A, Silverman AJ. Vasopressinergic and oxytocinergic pathways in the central nervous system. *Fed Proc.* 1984; 43(1):91–6. [PubMed: 6690342]

24. Chadio SE, Antoni FA. Characterization of oxytocin receptors in rat adenohypophysis using a radioiodinated receptor antagonist peptide. *J Endocrinol.* 1989; 122(2):465–70. [PubMed: 2549159]
25. Egli M, Bertram R, Sellix MT, Freeman ME. Rhythmic secretion of prolactin in rats: action of oxytocin coordinated by vasoactive intestinal polypeptide of suprachiasmatic nucleus origin. *Endocrinology.* 2004; 145(7):3386–94. [PubMed: 15033917]
26. Grosvenor CE, Shyr SW, Goodman GT, Mena F. Comparison of plasma profiles of oxytocin and prolactin following suckling in the rat. *Neuroendocrinology.* 1986; 43(6):679–85. [PubMed: 3762864]
27. Samson WK, Lumpkin MD, McCann SM. Evidence for a physiological role for oxytocin in the control of prolactin secretion. *Endocrinology.* 1986; 119(2):554–60. [PubMed: 3732136]
28. Kiss JZ, Kanyicska B, Nagy GY. The hypothalamic paraventricular nucleus has a pivotal role in regulation of prolactin release in lactating rats. *Endocrinology.* 1986; 119(2):870–3. [PubMed: 3732147]
29. Bodnar I, Banky ZS, Toth BE, Nagy GM, Halasz B. Brain structures mediating the suckling stimulus-induced release of prolactin. *J Neuroendocrinol.* 2002; 14(5):384–96. [PubMed: 12000544]
30. Kennett JE, Poletini MO, Fitch CA, Freeman ME. Antagonism of oxytocin prevents suckling- and estradiol-induced, but not progesterone-induced, secretion of prolactin. *Endocrinology.* 2009; 150(5):2292–9. [PubMed: 19106214]
31. Johnston CA, Negro-Vilar A. Role of oxytocin on prolactin secretion during proestrus and in different physiological or pharmacological paradigms. *Endocrinology.* 1988; 122(1):341–50. [PubMed: 3335212]
32. Parker SL, Armstrong WE, Sladek CD, Grosvenor CE, Crowley WR. Prolactin stimulates the release of oxytocin in lactating rats: evidence for a physiological role via an action at the neural lobe. *Neuroendocrinology.* 1991; 53(5):503–10. [PubMed: 1883415]
33. Ghosh R, Sladek CD. Prolactin modulates oxytocin mRNA during lactation by its action on the hypothalamo-neurohypophyseal axis. *Brain Res.* 1995; 672(1-2):24–8. [PubMed: 7749745]
34. Kokay IC, Bull PM, Davis RL, Ludwig M, Grattan DR. Expression of the long form of the prolactin receptor in magnocellular oxytocin neurons is associated with specific prolactin regulation of oxytocin neurons. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290(5):R1216–25. [PubMed: 16410399]
35. Selmanoff M, Gregerson KA. Suckling decreases dopamine turnover in both medial and lateral aspects of the median eminence in the rat. *Neurosci Lett.* 1985; 57(1):25–30. [PubMed: 4041019]
36. Rondeel JM, de Greef WJ, Visser TJ, Voogt JL. Effect of suckling on the in vivo release of thyrotropin-releasing hormone, dopamine and adrenaline in the lactating rat. *Neuroendocrinology.* 1988; 48(1):93–6. [PubMed: 3140046]
37. Arbogast LA, Voogt JL. The responsiveness of tuberoinfundibular dopaminergic neurons to prolactin feedback is diminished between early lactation and midlactation in the rat. *Endocrinology.* 1996; 137(1):47–54. [PubMed: 8536641]
38. Flanagan LM, Pfaus JG, Pfaff DW, McEwen BS. Induction of FOS immunoreactivity in oxytocin neurons after sexual activity in female rats. *Neuroendocrinology.* 1993; 58(3):352–8. [PubMed: 8255396]
39. Sansone GR, Gerdes CA, Steinman JL, Winslow JT, Ottenweller JE, Komisaruk BR, Insel TR. Vaginal stimulation releases oxytocin within the spinal cord in rats. *Neuroendocrinology.* 2002; 75(5):306–15. [PubMed: 12006784]
40. Freeman ME, Serman JR. Ovarian steroid modulation of prolactin surges in cervically stimulated ovariectomized rats. *Endocrinology.* 1978; 102(6):1915–20. [PubMed: 744058]
41. Arey BJ, Freeman ME. Activity of oxytocinergic neurons in the paraventricular nucleus mirrors the periodicity of the endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology.* 1992; 130(1):126–32. [PubMed: 1727695]
42. Polston EK, Erskine MS. Patterns of induction of the immediate-early genes c-fos and egr-1 in the female rat brain following differential amounts of mating stimulation. *Neuroendocrinology.* 1995; 62(4):370–84. [PubMed: 8544951]

43. Polston EK, Centorino KM, Erskine MS. Diurnal fluctuations in mating-induced oxytocinergic activity within the paraventricular and supraoptic nuclei do not influence prolactin secretion. *Endocrinology*. 1998; 139(12):4849–59. [PubMed: 9832421]
44. Egli M, Bertram R, Toporikova N, Sellix MT, Blanco W, Freeman ME. Prolactin secretory rhythm of mated rats induced by a single injection of oxytocin. *Am J Physiol Endocrinol Metab*. 2006; 290(3):E566–72. [PubMed: 16467487]
45. Arey BJ, Freeman ME. Oxytocin, vasoactive-intestinal peptide, and serotonin regulate the mating-induced surges of prolactin secretion in the rat. *Endocrinology*. 1990; 126(1):279–84. [PubMed: 2136724]
46. McKee DT, Poletini MO, Bertram R, Freeman ME. Oxytocin action at the lactotroph is required for prolactin surges in cervically stimulated ovariectomized rats. *Endocrinology*. 2007; 148(10):4649–57. [PubMed: 17615142]
47. DeMaria JE, Livingstone JD, Freeman ME. Ovarian steroids influence the activity of neuroendocrine dopaminergic neurons. *Brain Res*. 2000; 879(1-2):139–47. [PubMed: 11011015]
48. Lerant AA, DeMaria JE, Freeman ME. Decreased expression of fos-related antigens (FRAs) in the hypothalamic dopaminergic neurons after immunoneutralization of endogenous prolactin. *Endocrine*. 2001; 16(3):181–7. [PubMed: 11954661]
49. Helena CV, McKee DT, Bertram R, Walker AM, Freeman ME. The rhythmic secretion of mating-induced prolactin secretion is controlled by prolactin acting centrally. *Endocrinology*. 2009; 150(7):3245–51. [PubMed: 19282380]
50. Donner N, Neumann ID. Effects of chronic intracerebral prolactin on the oxytocinergic and vasopressinergic system of virgin ovariectomized rats. *Neuroendocrinology*. 2009; 90(3):315–22. [PubMed: 19546517]
51. Sarkar DK, Gibbs DM. Cyclic variation of oxytocin in the blood of pituitary portal vessels of rats. *Neuroendocrinology*. 1984; 39(5):481–3. [PubMed: 6542626]
52. Tabak J, Gonzalez-Iglesias AE, Toporikova N, Bertram R, Freeman ME. Variations in the response of pituitary lactotrophs to oxytocin during the rat estrous cycle. *Endocrinology*. 151(4):1806–13. [PubMed: 20176719]
53. Van Tol HH, Bolwerk EL, Liu B, Burbach JP. Oxytocin and vasopressin gene expression in the hypothalamo-neurohypophyseal system of the rat during the estrous cycle, pregnancy, and lactation. *Endocrinology*. 1988; 122(3):945–51. [PubMed: 3342761]
54. Kennett JE, Poletini MO, Freeman ME. Vasoactive intestinal polypeptide modulates the estradiol-induced prolactin surge by entraining oxytocin neuronal activity. *Brain Res*. 2008; 1196:65–73. [PubMed: 18234164]
55. Sarkar DK. Immunoneutralization of oxytocin attenuates preovulatory prolactin secretion during proestrus in the rat. *Neuroendocrinology*. 1988; 48(2):214–6. [PubMed: 3221983]
56. Yamaguchi K, Akaishi T, Negoro H. Effect of estrogen treatment on plasma oxytocin and vasopressin in ovariectomized rats. *Endocrinol Jpn*. 1979; 26(2):197–205. [PubMed: 477611]
57. Bale TL, Dorsa DM. Cloning, novel promoter sequence, and estrogen regulation of a rat oxytocin receptor gene. *Endocrinology*. 1997; 138(3):1151–8. [PubMed: 9048622]
58. Kubota Y, Kimura T, Hashimoto K, Tokugawa Y, Nobunaga K, Azuma C, Saji F, Murata Y. Structure and expression of the mouse oxytocin receptor gene. *Mol Cell Endocrinol*. 1996; 124(1-2):25–32. [PubMed: 9027321]
59. Breton C, Pechoux C, Morel G, Zingg H. Oxytocin receptor messenger ribonucleic acid: characterization, regulation, and cellular localization in the rat pituitary gland. *Endocrinology*. 1995; 136(7):2928–36. [PubMed: 7540544]
60. Breton C, Zingg HH. Expression and region-specific regulation of the oxytocin receptor gene in rat brain. *Endocrinology*. 1997; 138(5):1857–62. [PubMed: 9112379]
61. Arbogast LA, Ben-Jonathan N. The preovulatory prolactin surge is prolonged by a progesterone-dependent dopaminergic mechanism. *Endocrinology*. 1990; 126(1):246–52. [PubMed: 1967162]
62. Morrell JI, Rosenthal MF, McCabe JT, Harrington CA, Chikaraishi DM, Pfaff DW. Tyrosine hydroxylase mRNA in the neurons of the tuberoinfundibular region and zona incerta examined after gonadal steroid hormone treatment. *Mol Endocrinol*. 1989; 3(9):1426–33. [PubMed: 2575219]

63. Arbogast LA, Voogt JL. Progesterone induces dephosphorylation and inactivation of tyrosine hydroxylase in rat hypothalamic dopaminergic neurons. *Neuroendocrinology*. 2002; 75(5):273–81. [PubMed: 12006780]
64. Arbogast LA, Voogt JL. Progesterone suppresses tyrosine hydroxylase messenger ribonucleic acid levels in the arcuate nucleus on proestrus. *Endocrinology*. 1994; 135(1):343–50. [PubMed: 7912184]
65. Yen SH, Pan JT. Progesterone advances the diurnal rhythm of tuberoinfundibular dopaminergic neuronal activity and the prolactin surge in ovariectomized, estrogen-primed rats and in intact proestrous rats. *Endocrinology*. 1998; 139(4):1602–9. [PubMed: 9528940]
66. Babu GN, Vijayan E. Hypothalamic tyrosine hydroxylase activity and plasma gonadotropin and prolactin levels in ovariectomized-steroid treated rats. *Brain Res Bull*. 1984; 12(5):555–8. [PubMed: 6147181]
67. Beattie CW, Rodgers CH, Soyka LF. Influence of ovariectomy and ovarian steroids on hypothalamic tyrosine hydroxylase activity in the rat. *Endocrinology*. 1972; 91(1):276–9. [PubMed: 4402053]
68. Arbogast LA, Murai I, Ben-Jonathan N. Differential alterations in dopamine turnover rates in the stalk-median eminence and posterior pituitary during the preovulatory prolactin surge. *Neuroendocrinology*. 1989; 49(5):525–30. [PubMed: 2725844]
69. Fox SR, Harlan RE, Shivers BD, Pfaff DW. Chemical characterization of neuroendocrine targets for progesterone in the female rat brain and pituitary. *Neuroendocrinology*. 1990; 51(3):276–83. [PubMed: 1970131]
70. Lonstein JS, Blaustein JD. Immunocytochemical investigation of nuclear progesterin receptor expression within dopaminergic neurones of the female rat brain. *J Neuroendocrinol*. 2004; 16(6):534–43. [PubMed: 15189328]
71. Sar M. Distribution of progestin-concentrating cells in rat brain: colocalization of [3H]ORG.2058, a synthetic progestin, and antibodies to tyrosine hydroxylase in hypothalamus by combined autoradiography and immunocytochemistry. *Endocrinology*. 1988; 123(2):1110–8. [PubMed: 2899499]
72. DeMaria JE, Zelena D, Vecsernyes M, Nagy GM, Freeman ME. The effect of neurointermediate lobe denervation on hypothalamic neuroendocrine dopaminergic neurons. *Brain Res*. 1998; 806(1):89–94. [PubMed: 9739113]
73. Sirzen-Zelenskaya A, Gonzalez-Iglesias AE, Boutet de Monvel J, Bertram R, Freeman ME, Gerber U, Egli M. Prolactin induces a hyperpolarising current in rat paraventricular oxytocinergic neurones. *J Neuroendocrinol*. 2011; 23(10):883–93. [PubMed: 21851427]
74. Ruis H, Rolland R, Doesburg W, Broeders G, Corbey R. Oxytocin enhances onset of lactation among mothers delivering prematurely. *Br Med J (Clin Res Ed)*. 1981; 283(6287):340–2.
75. Jonas K, Johansson LM, Nissen E, Ejdeback M, Ransjo-Arvidson AB, Uvnas-Moberg K. Effects of intrapartum oxytocin administration and epidural analgesia on the concentration of plasma oxytocin and prolactin, in response to suckling during the second day postpartum. *Breastfeed Med*. 2009; 4(2):71–82. [PubMed: 19210132]
76. Kruger TH, Haake P, Chereath D, Knapp W, Janssen OE, Exton MS, Schedlowski M, Hartmann U. Specificity of the neuroendocrine response to orgasm during sexual arousal in men. *J Endocrinol*. 2003; 177(1):57–64. [PubMed: 12697037]
77. Blaicher W, Gruber D, Bieglmayer C, Blaicher AM, Knogler W, Huber JC. The role of oxytocin in relation to female sexual arousal. *Gynecol Obstet Invest*. 1999; 47(2):125–6. [PubMed: 9949283]
78. Carmichael MS, Humbert R, Dixen J, Palmisano G, Greenleaf W, Davidson JM. Plasma oxytocin increases in the human sexual response. *J Clin Endocrinol Metab*. 1987; 64(1):27–31. [PubMed: 3782434]
79. Kruger TH, Hartmann U, Schedlowski M. Prolactinergic and dopaminergic mechanisms underlying sexual arousal and orgasm in humans. *World J Urol*. 2005; 23(2):130–8. [PubMed: 15889301]
80. Salonia A, Nappi RE, Pontillo M, Daverio R, Smeraldi A, Briganti A, Fabbri F, Zanni G, Rigatti P, Montorsi F. Menstrual cycle-related changes in plasma oxytocin are relevant to normal sexual function in healthy women. *Horm Behav*. 2005; 47(2):164–9. [PubMed: 15664019]

81. Chiodera P, Volpi R, Capretti L, Coiro V. Oxytocin enhances the prolactin response to vasoactive intestinal polypeptide in healthy women. *Fertil Steril*. 1998; 70(3):541–3. [PubMed: 9757887]
82. Gerhold LM, Horvath TL, Freeman ME. Vasoactive intestinal peptide fibers innervate neuroendocrine dopaminergic neurons. *Brain Research*. 2001; 919(1):48–56. [PubMed: 11689162]

Figure 1.

Prolactin is secreted 1) in response to suckling 2) in response to mating and 3) on pro-oestrus evening in response to ovarian steroids (3) (Fig. 1). The suckling of rat pups initiates a surge of prolactin concentration that persists in the lactating rat as long as the suckling stimulus is maintained (4). Prolactin increases within 5 minutes of the suckling stimulus and reaches peak levels by 30 minutes.

Figure 2. Suckling-induced prolactin (PRL) secretion. A) Mean concentration ($\text{ng/mL} \pm \text{SEM}$) of serum prolactin in lactating animals 5 days after parturition ($n=6$) following the infusion of saline or oxytocin antagonist (OTA) ($1.25 \text{ } \mu\text{g/L}$ or $12.5 \text{ } \mu\text{g/L}$). Upon pup replacement, $12.5 \text{ } \mu\text{g/L}$ of OTA prevented the suckling-induced rise in prolactin secretion by 20 minutes (two-way ANOVA, followed by Bonferroni's test)

Figure 3.

Prolactin secretory rhythm induced by oxytocin. Prolactin secretion in ovariectomized (rats) after a single intravenous injection (black arrow) of either oxytocin (solid line) or saline (dotted line with open circles). *Significantly higher prolactin levels for oxytocin-injected animals compared with saline-injected animals ($P < 0.05$); #Significantly higher prolactin levels for cervically-stimulated animals (dotted line with closed circles) compared with saline-injected animals ($P < 0.05$). (Adapted from Egli 2006)

Figure 4.

Prolactin (PRL) secretory rhythm induced by cervical stimulation (CS). The prolactin secretory rhythm induced by cervical stimulation is blocked by antagonizing peripheral oxytocin receptors. Oxytocin antagonist (OTA) (dotted line) or saline (solid line) in ovariectomized rats. Values are expressed as mean ng/mL of prolactin $\pm$ SE ($n=3-10$ serial samples/point). *Significantly lower prolactin levels than corresponding times in saline infused rats ($P < 0.05$). There was no significant elevation in prolactin secretion at any time in the oxytocin antagonist-treated group. aStatistical difference from all other time points within the saline infused rats.

Figure 5.

Intracerebroventricular (icv) injection or systemic ovine prolactin (oPRL) administration initiates a daily prolactin secretory rhythm. Ovariectomized rats were injected with ovine prolactin or vehicle intracerebroventricular (0.15 %g) (A) or ovine prolactin systemically (15 or 150 %g) (B) at 2200 h of day 0 (arrow). Blood samples were withdrawn during the next 2 days to determine the presence of the prolactin diurnal and nocturnal surges ($n = 5-11$). Data are presented as mean $\pm$ SEM. #, $P < 0.05$; ###, $P < 0.001$ vs. vehicle-injected group at the same time point. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. basal prolactin on 2100 h of day 1 in the same experimental group.

Figure 6. Oestradiol-induced prolactin (PRL) secretion. Mean concentration (ng/mL /mL±SEM) of serum prolactin in ovariectomized animals treated with oestradiol (n=6) following the infusion of oxytocin antagonist (solid line) or saline (dotted line). A 24 hour infusion of 12.5 µg/L of oxytocin antagonist attenuated the estradiol-induced prolactin surge at 1700h, whereas 1.25 µg/L and 3.75 µg/L did not affect the prolactin surge (two-way ANOVA, followed by Bonferroni's test)

Figure 7.

Proposed Model. The proposed model for the control of prolactin secretion in the female rat in response to three physiological conditions: suckling, mating stimuli, ovarian steroids. This includes the classical negative dopamine-prolactin feedback loop and the positive oxytocin-prolactin feedback loop. We hypothesize that oxytocin stimulates (+) prolactin secretion triggering a positive feedback loop until a threshold of prolactin secretion is reached to stimulate dopamine release. Dopamine in turn inhibits (-) prolactin secretion. DA=dopamine, OT=oxytocin, PRL=prolactin.

Table 1

[1]	The presence of receptors on the target cell.
[2]	The response of the target cell to exogenous peptide.
[3]	An inhibitory effect observed following antagonism of endogenous peptide.
[4]	A pattern of secretory activity of the endogenous peptide that correlates with the activity of the target cell.