Effects of Lactation, Diet Condition, and Testing Situation on Estrogen Induced Sexual Responses in Rats.

Guinevere Arciszewska

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ABSTRACT

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The experiments presented in this thesis investigated the combined effects of lactation and food restriction in rats. The ability of exogenous estrogen administration to stimulate both receptive and proceptive behaviours were examined in addition to both active and passive rejection responses. In experiment 3, the effect of testing situation was studied. Females in this experiment, unlike either Experiment 1 or 2, were allowed to pace their copulatory contacts with the male. The results of these experiments demonstrate that lactating females are able to respond to the stimulatory effects of exogenous estrogen administration on the induction of the lordosis reflex. Interestingly though, females tested on Day 15 postpartum responded with much lower lordosis quotients compared to ovariectomized controls. Overall, unlike receptive behavior, proceptive behaviors were observed to increase across days postpartum. Finally, in Experiment 3, females that were allowed to pace their contacts with the male, showed no differences in passive rejection responses, receptivity or proceptivity. Active rejection responses followed a different pattern in Experiment 3 from that obtained in Experiment 2.

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GENERAL INTRODUCTION

Offspring of different species vary in their level of maturity at birth. These variations in precocity represent a trade-off between the energy invested in the production of offspring and the energy invested in the later care of young. Prococial young require more initial transfer of energy from the mother during fetal development in order to reach their more advanced stage at birth, but require relatively little parental care after birth. Altricial young, on the other hand, do not require as much energy during fetal development, but require a great amount of energy in the form of parental care after birth to ensure their survival (Nelson, 2000).

Those animals that provide parental care (parental investment) display behaviors that increase the survival potential of their young such as providing shelter, food, and protection from potential harm, such as predation. It is in the best interest to the parent to provide care to current offspring until such a point that they can survive on their own (Mealey, 2000). On the other hand, parents should terminate parental investment in a particular offspring whenever the costs of sacrificing future reproductive efforts outweigh the benefits of increased survival of the current offspring (Trivers, 1974). Although parental behavior can be observed in both sexes, in mammals, the majority of parental investment is provided by the mother. In mammals a crucial form of parental investment comes from the mother's ability to produce and deliver milk for her young, by lactating. Lactation is unique to mammals and the female sex (Nelson, 2000).

Providing sustenance to offspring through the production and delivery of milk is the most energetically demanding undertaking in a female's life (McNeilly, 1994; Canas,

Romero, & Baldwin, 1982). To keep up with these energetic demands, rats, for instance, increase their food consumption, decrease their metabolic rate, and utilize their fat stores (Canas et al., 1982; Fleming, 1976; Vernon, 1980, 1989; Vernon et al., 1995). Ample fat stores are required in order to maintain reproductive function in female mammals. It has been shown that a bout of acute food deprivation or chronic food restriction delays the onset of puberty, lengthens estrous cycles and causes a reduction in sexual behavior in rodents (Bronson, 1986; Bronson, 1987; Jones & Wade, 2002; Wade & Schneider, 1992).

Not surprisingly then, given the high energetic demands of lactation, many mammal species experience a period of infertility concurrent with lactation called lactational anovulation (Lamming, 1978). The duration of lactational anovulation is influenced by such factors as food intake as well as the number of young nursed. Intense ventral stimulation from pups, in the form of suckling behavior, is required in order to maintain lactational diestrus in rats (Rothchild, 1960). The intensity of this ventral stimulation varies according to the number of pups nursing as well as the frequency of nursing bouts (Woodside et al., 2001). Removal of pups, results in the quick resumption of ovulation and sexual behavior (Rothchild, 1960; Sodersten, Hansen & Eneroth, 1983; Woodside, 1991). Additionally, it has been shown, that further tapping the females available resources through a regimen of food restriction in the first two weeks of lactation to 50% of that given to ad libitum fed rats prolongs lactational anovulation for approximately seven days (Woodside, 1991; Woodside & Jans, 1995).

This state of lactational infertility could be viewed as an adaptive response by the female to changes in the immediate environment. A female that lacks proper nutrition, at any time during her reproductive lifetime, may be able to delay investing in the

production of offspring until her condition improves. A mechanism that allows for the suspension of ovulation and display of sexual behaviors during times of food deprivation ensures that offspring will not be produced during particularly lean times. Certainly, females who are both sexually unresponsive and anovulatory will not be producing young. Furthermore, a mechanism that allows for the cessation of reproductive behavior lets the female concentrate her efforts on current young without the added hardship of producing new young, increasing the survival potential of both sets of offspring.

Considerable evidence has accrued about the alterations in hormonal profile and neural states that occur during lactational diestrus and food restriction. The majority of research, however, has focused on the mechanisms that lead to ovulation rather than the induction of sexual behavior. The aim of this thesis is to investigate the resumption of sexual responsiveness in lactating rats together with its modulation by food restriction. To provide a background for this research, in the following sections, a brief synopsis of rat sexual behavior will be provided. In addition, the neuroendocrine events that lead to both ovulation and sexual behavior in cycling females will be presented. Finally, the changes in neuroendocrine status that result in lactational diestrus will also be discussed.

Sexual behaviors in female rats

Sexual behavior in female rats is comprised of two main components, proceptive behavior and receptive behavior. Proceptive behaviors are those appetitive activities that manifest themselves in solicitations of a male by a female. Receptivity, on the other hand, represents the consummatory phase of a bout of sexual activity and is indicated by a species-specific mating posture (Beach, 1976). Two proceptive behaviors that demonstrate a female's interest in initiating sexual contact with a male are ear wiggling

(Madlafousek & Hlinak, 1978; Erskine, 1989) and hop-darting (McClintock & Alder, 1978). Ear wiggling is defined as rapid vibration of the ears that is usually accompanied by an upward head toss. Hop-darting involves a pattern of alternating approaches and withdrawals from a male by an estrus female. Hopping behavior consists of a short leap with the female landing on all fours which is followed by a crouching posture, darting on the other hand, is defined as a run consisting of several steps, abruptly terminated by the crouching position (Madlafousek & Hlinak, 1978). Hopping and darting behavior generally occur together. Receptive behavior in female rats takes the form of the lordosis posture. The lordosis posture is achieved through extreme dorsiflexion by the female, in which her hind end is raised and her tail is deflected in order to facilitate copulation. The lordosis response is most often measured by calculating the lordosis quotient, which is calculated by dividing the total number of lordosis responses by the total number of mounts by the male (Beach, 1943).

The lordosis posture is a robust spinal reflex that can be induced by means of flank stimulation, usually by a male (Schwartz-Giblin &Pfaff, 1990). Because of the robust nature of the lordosis reflex, females who are provided with proper flank stimulation will generally show lordosis in testing conditions where proceptive behaviors may be difficult or impossible for the female to display. Given the nature of the reflex, females do not require much room in order to display lordosis. Proceptivity, specifically hopping and darting behaviors, and pacing behavior (an alternating pattern of approach and withdrawal) necessarily requires that there be ample room in order for the female to engage in such behaviors (Pfaus et al., 1999). In the wild or semi-natural laboratory environments, estrus females are able to control the pace of copulation through this

approach and withdraw behavior. When females are not allowed to control the rate of copulation, as is the case in small single level chambers, they often use defensive behaviors to enforce their preferred rate of male contacts (Pfaus et al., 1999). Females that are tested in such small single level chambers are unable to escape the pursuing male, and hence are not able to properly control the pace of mating. Females that are allowed a means to escape a pursuing male, and therefore control the rate of copulation, often display more complex patterns of solicitation and pacing behaviors compared to those who cannot (Erskine, 1985, 1989).

Neural and hormonal correlates of ovulation and sexual behavior

The hormonal events underlying the rat estrous cycle involves the release of hormones at the hypothalamic, pituitary and ovarian levels of the reproductive axis (Long & Evens, 1922). The ovarian steroid hormones estradiol (E2) and progesterone (P4) interact to induce female reproductive behavior and ovulation during the rat estrous cycle (Beach, 1942). Every fourth or fifth day the rat comes into behavioral estrus and ovulates. Gonadotropin releasing hormone (GnRH) is secreted in a pulsatile manner from the hypothalamus, which, in turn, stimulates cells within the anterior pituitary to release follicle stimulating hormone (FSH) and lutinizing hormone (LH). FSH causes growth of ovarian follicles and as the follicles develop they produce estrogen. The initial production of estrogen results in the down regulation of the gonadotropins through a negative feedback mechanism. However, as the follicles continue to grow there is a rapid increase in estrogen release during pro-estrus and this negative feedback mechanism is momentarily disabled. The rapid rise in estrogen also stimulates an LH surge via a positive feedback mechanism. In turn, the LH surge stimulates ovulation (Pfaff, 1985).

The ovarian hormones that stimulate the LH surge and ovulation also stimulate sexual behavior (Pfaff, 1985). Estrogen is the principle hormone involved in the stimulation of female rat receptive behavior. Sufficiently high doses of estrogen are able to induce sexual receptivity without progesterone stimulation. For example, high levels of estradiol have been shown to induce sexual receptivity in ovariectomized rats independent of progesterone (Kow & Pfaff, 1977). As well, ovariectomized rats that are exposed to estradiol for prolonged periods may display sexual behavior in the absence of progesterone administration (Albert, Jonik, Gorzalka, Newlove, Webb & Walsh, 1991). Lordosis is also seen in rats that have been ovariectomized and adrenalectomized, eliminating all endogenous sources of progesterone, further illustrating that the expression of estrogen-induced lordosis is not dependent on the presence of progesterone (Davidson, Rogers, Smith & Bloch, 1968). Furthermore, Tennent et al. (1980) showed that estrogen priming (2.5 μg/ 100g body wt) both ovariectomized and ovariectomized adrenalectomized rats for 7 days resulted in low levels of proceptivity in both groups of rats. Rats who were ovariectomized and adrenalectomized, however displayed significantly fewer proceptive behaviors than ovariectomized rats. Progesterone administration after estrogen priming resulted in a significant increase in proceptive behaviors compared to estrogen priming alone.

Progesterone is required for the stimulation of full-blown proceptive behaviors such as ear wiggling and hop darting and progesterone also facilitates sexual receptivity in female rats (Parsons & Pfaff, 1985). Paradoxically, it appears that progesterone is also responsible for the prevention of proestrus re-induction of sexual receptivity and for the cessation of sexual receptivity during vaginal estrus. Estrogen concentrations fall rapidly

during vaginal estrus, but high levels of progesterone fall more gradually, especially if mating has occurred (Moguilewsky & Raynaud, 1979).

Ovariectomized rats, show neither receptive nor proceptive components of sexual behavior (Boling & Blandau, 1939). Both the facilitative and inhibitory actions of progesterone on sexual behavior can be mimicked by the administration of estrogen and progesterone to ovariectomized rats, however (Sodersten, 1985). Changing the relative timing of estrogen and progesterone administration determines whether progesterone facilitates or inhibits the stimulatory effects of estrogen on sexual behavior. For example, in ovariectomized rats, an injection of progesterone given 24 hours after EB priming induces sexual receptivity (Schwartz, Blaustein & Wade, 1979). If a sufficiently low dose of progesterone is used (less than or equal to 0.5 mg), another period of sexual receptivity can be induced with a second injection of progesterone administered 24 hours after the initial progesterone injection (48 hours after EB). If progesterone levels are high coincident with the increase in estrogen then a subsequent progesterone injection no longer stimulates proceptive behavior. For instance, if a large dose of progesterone (greater than or equal to 1 mg) is administered 24 hours after EB priming, there is a refractory period during which a second progesterone injection will not induce estrous behavior (sequential inhibition). Similarly, inhibition of sexual behavior can be obtained by the injection of a large dose of progesterone at the time of estrogen priming. An additional injection of progesterone 24-48 hours later fails to induce estrous behavior (concurrent inhibition, akin to pregnancy or pseudopregnancy) (Blaustein & Wade, 1977). Although administration of estradiol alone is able to reinstate female rat receptive behavior, pre-treatment with estradiol is necessary in order for progesterone to induce

proceptive behavior. Sequential treatment of estradiol and progesterone significantly increases the probability that the female will engage in proceptive as well as receptive behavior (Blaustein, Mani & O'Malley, 1997).

Changes in progestin receptor levels in the hypothalamus and anterior hypophysis play a critical role in the facilitative and inhibitory effects of progesterone on female rat sexual behavior. Initially increases in estrogen levels induce an increase in progestin receptors in areas of the brain implicated in the control of sexual behavior (Moguilewsky & Raynaud, 1979). Activation of these receptors results in the stimulation of proceptive behavior and the facilitation of the lordosis reflex. However, progesterone binding to its receptor also results in the targeting of those receptors for enzymatic degradation resulting in a downregulation of receptor number and hence refractoriness to subsequent activation (Turgeon & Waring, 2000).

Changes in the reproductive axis during lactation

Lactation is associated with a unique hormonal profile. Pulsatile GnRH release from the hypothalamus is reduced resulting in the suppression of LH release from the pituitary. As a consequence follicular growth and levels of circulating estrogen are greatly diminished (Smith & Neill, 1977). On the other hand, the release of FSH is suppressed only during early lactation (Taya & Greenwald, 1982). Estrogen levels increase gradually over lactation and by approximately day 14 postpartum are comparable to estrogen levels seen in cycling females on diestrus (Smith & Neill, 1977). Progesterone levels on the other hand, are relatively high in lactating females as corpora lutea function is resumed after postpartum ovulation. These high progesterone levels peak at around days 10-13 postpartum and decline thereafter (Grota & Eik-Nes, 1967). The

low basal levels of LH during lactation contribute to the anovulatory state by preventing follicular growth and hence sufficient estradiol release to stimulate an ovulatory surge in LH.

Previous research has investigated the ability of the hypothalamus to respond to estradiol with an LH surge during lactation by exogenous administration of the hormone. For example, it has been shown that estrogen administration that is able to produce an LH surge in non-lactating rats is unable to induce an LH surge in lactating rats on days 5, 10 or 15 postpartum (Smith, 1981). Such changes within the reproductive axis of lactating rats have been shown to persist during food-restriction. In ad libitum fed lactating rats, positive feedback in response to exogenous estrogen is partially restored by day 20 postpartum, whereas positive feedback in response to estrogen treatment is not seen until day 25 postpartum in food-restricted females (Abizaid et al., 2003).

However, lactating rats not only have reduced levels of endogenous estrogen, but also experience elevated levels of progesterone caused by the resumption of corpora lutea function after postpartum ovulation. Removing the endogenous source of progesterone through ovariectomy of lactating dams results in larger estrogen induced LH surges on days 10 and 15 postpartum compared to intact lactating rats (Hodson, Simpkins, Meites, 1978). Additionally, blocking the actions of progesterone with a progesterone receptor antagonist (RU 486) in lactating rats, shortens the duration necessary for estrogen treatment to induce positive feedback following pup removal (Lee, Haisenleder, Marshall & Smith, 1989), and significantly reduces the duration of lactational diestrus (van der Schoot, Uilenbroek & Slappendel, 1989). Abizaid et al., (2003), demonstrated that ovariectomy or RU486 treatment restores the ability of estrogen to induce LH surges in

lactating ad libitum fed and food restricted rats. Furthermore, chronic exposure to progesterone caused a reduction in the ability of estrogen administration to induce an LH surge in ad libitum fed ovariectomized rats.

The ability of the gonadal steroid hormones, estradiol and progesterone to mediate sexual behavior in both food restricted and lactating rats led us to investigate further the combined effects of lactation and food restriction on the induction of both receptive and proceptive behaviors. The combined effects of lactation and food restriction have previously been examined by Abizaid et al., (2003), but the ability of exogenous estrogen administration to generate an LH surge was studied rather than the induction of sexual behaviors. In this study it was found that high circulating progesterone levels in lactating females attenuates the ability of estrogen to induce progesterone receptors within the AVPV (Abizaid et al., 2003). Although extensive research has documented the effects of either lactation or food restriction on the induction of sexual receptivity by exogenous estrogen administration, the combined effects of both lactation and food restriction on the induction of both receptive and proceptive behaviors in the rat has not yet been examined. The experiments in this thesis will investigate the effects of this combined manipulation on the induction of both sexual receptivity and proceptivity in rats. Furthermore, the effects of testing situation on the induction of sexual and avoidance behaviors will also be examined.

EXPERIMENT 1- Ability of estrogen to induce sexual behavior in lactating rats

The attenuated response of the reproductive axis to the stimulatory effects of estrogen during lactation is well documented, but less attention has been paid to the ability of estrogen to stimulate sexual behavior at this stage of the reproductive cycle. Sodersten et al., (1983) reported that lactating rats on day 6 postpartum are refractory to the stimulatory effects of estrogen administration on sexual behavior. Even large doses of exogenous estrogen are unable to compensate for low circulating estrogen levels in lactating rats. For example, administration of estradiol benzoate (EB), (250 μg/ 0.1 ml oil) fails to induce sexual behavior in lactating rats six days after parturition (Sodersten, Hansen & Eneroth, 1983). Clarke et al., (1984), however, using a different dosing regimen (10 μg estrogen/0.1 ml oil daily for 3 days) induced high levels of receptivity in both ovariectomized and lactating rats on days 5-9 postpartum. These data are interesting because they suggest that lactation has differential effects on the mechanisms underlying the LH surge and the induction of the lordosis reflex.

To further explore changes in sensitivity to estrogen, lactating rats were tested for estrogen induced sexual behaviors on days 10, 15, 20 & 25 of lactation. The dosing regimen described by Clarke et al. (1984) was used, and the above test days were chosen because they include the time period during which the responsiveness to the positive feedback effects on LH release is restored (Abizaid et al., 2003). Clarke has also shown that progesterone administration prior to testing caused a significant reduction in sexual

receptivity in estrogen-primed lactating rats. Thus, it was of interest to determine the contribution of circulating P levels to the reduction of estrogen stimulated sexual behavior in lactation. Progesterone levels are generally high in lactating dams because suckling-induced prolactin maintains the corpora lutea that are produced after the postpartum ovulation (Smith, 1980). Peak circulating P levels are reached between approximately days 10-13 postpartum and then decrease to cycling levels by approximately day 16 postpartum (Woodside, 1991). Thus, the test days chosen encompass the period of changes in circulating progesterone levels. The proceptive behaviors measured in this experiment were ear wiggles and hop-darts. Receptivity was determined by calculating the lordosis quotient for each animal.

GENERAL METHODS

Animals and housing

Female Wistar rats weighing 220-240 grams were obtained from Charles River Canada Inc, St.Constant, Quebec. Rats were allowed a one week acclimatization period prior to group mating with a male or bilateral ovariectomy. Immediately after surgery, as soon as pregnancy became obvious, or after 19 consecutive days housed with a male, females were individually housed in polypropylene cages (45 x 25 x 20 cm) with Beta chip bedding. The day of birth was assigned as day 1 of lactation. Pups were culled to eight on day 1 or 2 of lactation. The body weight of all females and their litters were recorded throughout the experiments. Lactating females were kept with their litters for the duration of the experiment.

Intact male Wistar rats served as stimulus males and received 10 tests of sexual behavior with a receptive female. Stimulus males were selected based on their ability to initiate vigorous copulatory activity with females within 1 minute of being placed inside the testing chamber. All training sessions took place within the testing chamber prior to the beginning of experimentation.

All animals were maintained under a 12: 12 light dark cycle with lights on at 2400h and off at 1200h, at constant temperature (20 +/- 1 c) and humidity. Except where otherwise stated all animals had ad libitum access to food and water throughout the experiment. All experimental procedures were approved by the Concordia University Animal Care Committee according to the guidelines set by the Canadian Council of Animal Care.

Food-restriction

Ad libitum fed females and stimulus males had free access to rat chow (Agway Ltd.). Females in the food-restricted groups were given a daily ration of 50% of a previously determined ad libitum intake beginning on day 1 of lactation through to day 14 of lactation. All animals had free access to water.

Materials

Sexual behavior was recorded in an open field (Experiments 1 and 2) (H: 48 cm, W: 49 cm, L: 55 cm) lined with Beta chip, using a Toshiba Television, Sears electronic video cassette recorder, and a Panasonic video camera mounted on a tripod which allowed the inside of the testing chamber to be viewed. In experiment 3 a pacing chamber was used (H: 48 cm, W: 49 cm, L: 68 cm, 34 cm per chamber) instead of the open field. Aside from differences in overall size, the pacing chamber was similar in appearance to the original open field chamber. The pacing chamber was also divided into two equal sized chambers by a dividing board with 3 equally spaced holes through which test females but not stimulus males could easily pass.

Ovariectomies

Control females were anesthetized via intraperitoneal injection using a mixture of ketamine hydrochloride and xylazine hydrochloride (6mg ketamine and 0.86mg xylazine/100g of body weight). The ovaries of the anesthetized females were bilaterally removed through flank incision. Flank incisions were closed using stainless steel wound clips. Ovariectomized females were allowed at least two weeks of post surgical recovery before the beginning of hormonal priming and subsequent behavioral testing.

Estrogen treatment

Lactating females and controls received three daily subcutaneous injections (12 p.m.) of 10μg estradiol benzoate (EB) in 0.1 ml sesame oil and were tested for sexual behaviors on the fourth day.

Tests of sexual behaviors

Testing began during the dark phase. On the assigned day of testing females were placed in an open field chamber with a stimulus male. Behaviors were recorder for a period of 30 minutes. Receptive as well as proceptive behaviors were scored without knowledge of group assignment. Receptive behavior (lordosis) was scored on a scale of 0 (no lordosis) to 3 (exaggerated lordosis) (Hardy & DeBold, 1971) and the lordosis quotient (LQ) was calculated for each female by dividing the number of lordosis responses (a score of 2 or above) by the total number of mounts and multiplying by 100. The proceptive behaviors scored were the frequency of ear wiggles as well as the frequency of the hop/dart gait observed in a test of either 30 or 15 minutes.

Experiment 1

Procedure

Five groups of females were included in this experiment. Four groups of ad libitum fed lactating females: i) ad libitum day 10 (AL D10, n=10); ii) ad libitum day 15 (AL D15, n=10); iii) ad libitum day 20 (AL D20, n=10); ad libitum day 25 (AL D25, n= 10); and iv) a group of nulliparous ovariectomized controls (n=10).

Statistical analysis

All parametric tests were analyzed using the Statistical Package for the Social Sciences (SPSS). When appropriate post hoc tests were used for pairwise comparisons. The significance level was set at alpha = 0.05. Three separate One-way Analysis of Variance (Anova) were used to compare; i) the Lordosis Quotient across groups, ii) the frequency of ear-wiggles across groups, and iii) the frequency of the hop/dart gate across groups. Post hoc comparisons were made by means of Tukey HSD.

RESULTS

Lordosis quotient

As can be seen in figure 1, a significant effect was obtained for lordosis quotient ($F(4, 45) = 2.894$, $P < 0.05$). Post-hoc comparisons indicated that day 15 postpartum lactating rats displayed significantly lower lordosis quotients than day 25 lactating rats (*Tukey HSD. P = 0.032.* $P < 0.05$). Interestingly, mean lordosis quotients did not differ significantly between lactating rats and ovariectomized controls with the exception of a trend (*Tukey HSD. P = 0.063*) for day 15 lactating rats to display lower lordosis quotients than ovariectomized controls.

Proceptive behaviors

Both frequency of ear-wiggles and hop-dart gait increased across groups ($F(4, 45) = 4.562$. $F(4,45) = 8.908$, $P < 0.05$). Day 25 lactating rats displayed a significantly greater number of ear wiggles compared to ovariectomized controls (*Tukey HSD* = 0.025), day 10 lactating rats (*Tukey HSD* = 0.014) and day 15 lactating rats (*Tukey HSD* = 0.005). This same pattern emerged with hop darting behavior. For this measure, day 25 lactating rats showed a higher frequency of hop-darts then ovariectomized controls (*Tukey HSD* = 0.001), day 10 (*Tukey HSD* = 0.000) and day 15 (*Tukey HSD* = 0.000). (see Figures 2 and 3).

DISCUSSION

Lactating rats on Days 10, 20 and 25 postpartum responded to exogenous estrogen administration with similar levels of receptive behavior to those seen in ovariectomized nulliparous animals. These results support those reported by Clarke et al (1984) who used similar doses of estrogen administration and examined the behavior of rats on Days 5-9 postpartum. Together these data suggest that estrogen is able to stimulate receptive behavior throughout much of lactation. There was a trend for lactating rats on Day 15 postpartum to have lower lordosis quotients than those on Day 25 postpartum and ovariectomized animals, however, suggesting that peak lactation and hence peak energy drain might modulate lordosis behavior even in the presence of exogenous estrogen administration.

The pattern of results obtained for the proceptive behaviors, ear wiggling and hop-darting, differed from that obtained for receptive behavior. As expected ovariectomized nulliparous rats showed low levels of both ear wiggling and hop-darting behavior and similarily, low rates of these behaviors were seen in lactating rats tested on Day 10 and Day 15 postpartum. Higher levels of these behaviors were seen when rats were tested on Day 20 or Day 25 postpartum. The low levels of proceptive behavior seen in ovariectomized rats are consistent with earlier results showing that P is necessary to stimulate proceptive behavior. Given that progesterone levels peak between Days 10-13 in lactating rats the low level of proceptivity obtained in E-treated rats tested on Days 10 and 15 postpartum is unlikely to have resulted from lack of P. It is more likely that prolonged exposure to high circulating P levels prior to testing acted to suppress

proceptivity. During lactation progesterone levels fall to those typical of cycling rats in the third week postpartum and thus rats on Day 20 and 25 postpartum are capable of responding to the combination of exogenous estrogen and endogenous P and hence show higher levels of proceptive behavior.

When the pattern of results obtained here are compared with those of other studies examining increases in basal LH levels and the ability of estrogen to stimulate an LH surge, it becomes clear that the resumption of reproductive function during lactation is not a unitary process. Indeed, the absence of sexual receptivity during lactation appears to reflect only the absence of sufficient estrogen. There is a greater similarity in the time course of the resumption of responsivity to the positive feedback effects of estrogen on LH release and restoration of proceptive behavior, which probably reflects the inhibitory effects of progesterone on both processes. Given that food restriction during lactation results in prolonged exposure to even higher circulating levels of progesterone than those seen in ad libitum fed lactating rats (Woodside, 1991) one would predict that food restricted rats would show a prolonged suppression of proceptive behaviors. Because receptive behavior seems only to depend on sufficiently high levels of E one might predict that lordosis quotient would not differ between ad libitum and food restricted rats across lactation. The tendency for receptivity to be lower on Day 15 postpartum, which represents the time of peak lactation and hence greatest energy drain, than the other test days, however, raises the possibility that receptive behavior might be modulated by metabolic state. Given the effects of food restriction in lactating rats persist for at least 5 days after re-feeding (Walker et al, 1995, Abizaid et al, 2003) one might expect that the

differences between ad libitum fed and food restricted rats would be greatest on Day 20 postpartum. These possibilities were tested in Experiment 2.

EXPERIMENT 2 - The effects of diet condition on sexual behavior

The effects of diet condition on reproductive behavior have been noted throughout history. Food restriction has been found to delay the onset of puberty as well as disrupt ovulatory cycles and sexual behavior in domestic animals (Bronson, 1989; Booth, 1990; Schillo, 1992), rodents (Bronson, 1989; Wade & Schneider, 1992) and primates (Steiner, 1987; Warren, 1983). For instance, 48 hours of food deprivation has been shown to result in a decrease in plasma LH levels (Briski & Sylvester, 1998), as well as decreases in both the amplitude (Nagatani, Bucholtz, Murahashi & Estacio, 1996) and frequency of LH pulsatility (Murahashi, Bucholtz, Nagatani, Tsukahara, Tsukamura, Foster & Maeda, 1996). Furthermore, this same length of food deprivation also disrupts estrous cycles in Syrian hamsters (Morin, 1986) as well as hormone induced sexual behavior in ovariectomized Syrian hamsters (Dickerman, Li & Wade, 1993). Additionally, Jones and Wade (2002), have demonstrated that a 74 hour fast decreases the lordosis quotients in ovariectomized steroid primed rats in comparison to ad libitum fed ovariectomized rats given the same hormone treatment.

These effects of food restriction have also been extended to include lactating rats. For example, food-restricting rats to 50% of an ad libitum ration for the first 14 days postpartum prolongs the period of lactational anovulation by about 7 days (Woodside, 1991). In this study, the effect of this manipulation on sexual behavior was examined by comparing the ability of exogenous estrogen administration to stimulate both proceptive and receptive behavior in ad libitum fed and food restricted lactating rats. Given the prolonged exposure to high circulating P levels experienced by food restricted females, it

was expected that they would show low levels of proceptive behavior for a longer period postpartum than would ad libitum fed rats. If the reduction of lordosis behavior obtained in Experiment 1 primarily depends on metabolic cues then one would predict that the effect of food restriction may prolong this reduction of receptive behavior to include day 20 postpartum.

METHODS

The subjects, housing conditions and general procedures used in these experiments were described in the General Methods section.

Procedure

In order to compare the ability of estrogen to induce sexual behavior in ad libitum fed and food-restricted lactating rats eight groups were formed based on diet condition and the day postpartum on which tests of sexual behavior occurred. Groups were as follows: i) ad libitum day 10 (AL10, n = 8), ii) ad libitum day 15 (AL15, n = 8), iii) ad libitum day 20 (AL20, n = 8), iv) ad libitum day 25 (AL25, n = 8), v) food-restricted day 10 (FR10, n = 8), vi) food-restricted day 15 (FR15, n = 8), vii) food-restricted day 20 (FR20, n = 8) and viii) food-restricted day 25 (FR25, n = 8).

Tests of sexual and avoidance behaviors

Tests of sexual behavior were as described earlier except that the duration of the test was reduced to 15 minutes from 30. In addition, another behavioral measure, active rejection, was scored. This measure was a composite frequency of the number of head-wise fighting stances, boxing, kicking, and sideways threat postures displayed by the female toward the male.

Statistics

A 2 x 4 Analysis of Variance (ANOVA) with diet (ad-lib or food-restricted) and day (day 10, 15, 20 and 25 of lactation) as the between subjects variables was used to

analyze group differences in each measure of sexual behavior as well as active avoidance. Post-hoc comparisons were carried out using Fisher's LSD.

RESULTS

Lordosis quotient

A significant main effect for day of lactation was obtained, ($F(7,56) = 7.837$, $P < 0.05$). In contrast, no significant main effect was found for diet condition ($F(7,56) = 2.864$, P, 0.05). Post hoc analysis revealed that, as in experiment 1, females were less receptive on day 15 of lactation than on days 10, 20 or 25. No interaction effects between diet condition and day of lactation on the display of receptivity were obtained in this experiment. Because of the specific prediction that day 20 postpartum would most likely yield a diet condition effect, a planned comparison was carried out to compare ad libitum fed and food restricted lactating females on postpartum day 20. The results of this planned comparison showed that day 20 ad libitum fed lactating rats were significantly more receptive than day 20 food restricted lactating rats ($t(14) = 2.147$, $P = 0.05$). (see Figure 4.)

Proceptivity

A significant main effect was found for day of lactation on the proceptive behavior of ear wiggling ($F(7,56) = 4.6$, $P < 0.05$), and hop-darting ($F(7,56) = 2.988$, $P < 0.05$). No significant effects of diet condition for either ear wiggle or hop darting frequencies were obtained. No significant interaction was obtained between day and diet condition on the frequency of either proceptive behavior. Post hoc comparisons showed that: the frequency of ear wiggling was significantly greater in day 25 lactating rats than other days tested and, furthermore, day 15 lactating rats displayed reduced proceptive behavior in the form of hop-darting in comparison to both day 20 & 25 lactating rats.

Because of the specific prediction that food restricted females would maintain low levels of proceptivity for a longer duration than ad libitum fed females, a planned comparison was carried out for day 20 in ad libitum fed and food restricted rats. The results of this planned comparison showed that day 20 food restricted females displayed significantly fewer ear wiggles compared to day 20 ad libitum fed rats ($t(14) = 2.392$, $P < 0.05$). No significant difference was obtained for the frequency of hop-darting behavior between day 20 food restricted and ad libitum fed females ($t(14) = 0.990$, $P > 0.05$). (see figures 5 and 6).

Active rejections

As can be seen in figure 7, significant main effects were obtained for both day of lactation and diet condition on the number of active rejection behaviors made by the females towards males ($F(7,56) = 5.513$. $F(7,56) = 7.954$, $P < 0.05$). There was no significant interaction effect for day and diet condition on the occurrence of active rejection responses. Post hoc tests revealed that, overall, day 10 lactating females displayed significantly more active rejections than either day 20 or 25 females. Furthermore females on day 15 of lactation were found to be more aggressive in their attempts to fight off the male than day 25 females. Additionally, overall, females in the food restricted group were found to be significantly more actively rejecting of the stimulus male than those females in the ad libitum fed condition.

DISCUSSION

Overall, there was no main effect of food restriction on sexual receptivity. This supports the suggestion that the stimulation of receptive behavior is primarily dependent on circulating estrogen levels and not progesterone. Progesterone titers are higher in food-restricted rats than ad libitum fed rats, but as all females were administered the same high dose of estrogen, estrogen titers should not differ significantly between food restricted and ad libitum fed females. As in Experiment 1, day 15 lactating rats displayed significantly lower levels of receptivity than the other groups tested. However, a planned comparison showed that food restriction extended the duration of reduced receptive behavior to include day 20 postpartum in food restricted rats. This finding is supported by past research that has shown that the effects of food restriction in lactating rats are extended to at least 5 days after re-feeding (Walker et al., 1995; Abizaid et al., 2003).

Proceptive behaviors in this experiment differed somewhat from those obtained in Experiment 1, but the basic pattern remained unchanged. There was a significant effect of day postpartum on the display of proceptive behaviors, generally, as in Experiment 1 proceptive behavior was seen to increase across days of lactation. Additionally, there was an effect of diet condition on the prolongation of reduced levels of proceptive behaviors until at least day 20 postpartum. Ad libitum fed females on day 20 postpartum displayed significantly more proceptive behavior in the form of ear wiggling than food restricted rats tested on day 20 postpartum. This finding suggests that like receptivity, diet condition also has an effect on the display of proceptive behavior across lactation.

The occurrence of active rejection behaviors (head-wise fighting stances, boxing, kicking, and sideways threat postures)(Barnett, 1963) was found to vary significantly between the two diet conditions. Not only were acts of active rejection found to decrease across days of lactation, but food restricted females displayed significantly more active rejections of the stimulus male than ad libitum fed females.

The incidences of active rejection observed in this study may have occurred as a result of the testing apparatus used. Females were placed in a single level chamber that provided them with little or no means to escape the stimulus male's advances. Under these circumstances it is difficult to determine whether or not the females were experiencing difficulty pacing their contacts with the male and so used active rejection to force their desired rate of copulation with the male. Alternatively, the levels of active rejection observed might be indicative of an underlying hormonal state.

Female displays of active rejection can lead to ambiguities in the understanding of female sexual behaviors. For instance, a female that is forced to use active rejections to enforce intervals between intromissions may signal to the pursuing male that she is not receptive. It has been suggested that the display of active rejections may be an indicator of estrous termination (Pfaus et al., 1999), which may act as a signal to a sexually experienced male that perhaps now is not the best time to be actively pursuing the female in question (Pfaus & Pinel, 1989). The finding that food restricted females display more acts of active rejection than ad lib fed females is of interest because progesterone levels are higher in food restricted lactating rats in comparison to ad libitum fed lactating rats. These high basal levels of progesterone may be acting to inhibit estrus behavior in these females as they do to terminate post estrus sexual behavior in cycling rats.

EXPERIMENT 3 - The effects of female choice on sexual and avoidance behaviors

The results obtained in experiment 2 demonstrated that overall lactating females display fewer instances of active rejection as a function of increasing days across lactation and that food restricted females display more acts of active rejection in comparison to ad libitum fed females. These differences may have occurred as a result of the testing apparatus used. In Experiments 1 and 2 a single level chamber was used which made escape from the male impossible. Active rejection responses may have been exacerbated as a direct result of the females attempting to enforce the rate of copulatory contact with the stimulus male. On the other hand, however, such differences may not be mediated by the fact that the female has difficulty pacing under such circumstances, but may instead be a factor of the underlying hormonal profile of these females.

Differences in sexual responsiveness have been shown between females who are allowed to pace their copulatory contacts with the male and those that cannot. For instance females that do not pace their contacts with the male may not provide enough stimulation for the male and hence be devalued as a sexual partner, leading to a reduction or cessation of copulation by the male (Everitt, 1990). Lack of pacing behavior not only discourages the male to pursue the female, but females who cannot pace their copulatory contacts may experience difficulties reproducing. The temporal pattern of male intromissions effect reproductive behavior in females. Multiple intromissions are required to induce corpora lutea development in rats (Gunnet & Freeman, 1983). Corpora lutea development is necessary for ova to implant into the uterine wall as well as support pregnancy through the production of progesterone (Gunnet & Freeman, 1983). In mating

tests where females cannot control the pace of male contacts approximately twice as many intromissions are required to induce corpora lutea development in comparison to females that can pace their copulatory contacts. Furthermore, it has been shown that more females are impregnated in pacing conditions as opposed to standard non-pacing conditions (Erskine, 1985).

In order to determine whether relatively high levels of active rejection seen in food restricted females would be reduced if females were given the opportunity to avoid the male, in the next Experiment, females were given the opportunity to escape the pursuing male via a pacing chamber.

METHODS

General methods were as previously described except an additional behavioral measure, passive avoidance was scored and a pacing chamber rather than the open field was used in this experiment. The pacing chamber is a single level chamber divided into two equal parts by a partition containing three holes through which females, but not males can pass. The proportion of total test time that females displayed passive rejection (retreating to the side of the pacing chamber that does not contain the male) was scored as a measure of female passive avoidance of the stimulus male (Pfaus et al, 2000).

Procedure

Four groups were formed based on diet condition and the day postpartum on which tests of sexual behavior were performed. Groups were as follows: i) ad libitum day 15 (AL15, n = 8), ii) ad libitum day 20 (AL20, n = 8), iii) food-restricted day15 (FR15, n = 8) and iv) food-restricted day 20 (FR20, n = 8). Days 15 and 20 postpartum were chosen because they represent the days postpartum where estrogen administration has its greatest difference in the induction of estrogen positive feedback in lactating females.

Statistical analysis

A 2 x 2 Analysis of Variance (ANOVA) with diet (ad-lib or food-restricted) and day (day 15 and 20 of lactation) as the between subjects variables was used to analyze group differences in each measure of sexual behavior, as well as active rejections and passive rejection.

RESULTS

Lordosis quotient

No significant main effects were found for either test day or diet condition; ($F(3,28) = 0.532$. $F(3,28) = 2.672$, $P > 0.05$). No significant interaction effect between days postpartum and diet condition was obtained for the measure of lordosis. (see figure 8).

Proceptivity

There were no significant differences for either days across lactation or diet condition in the occurrence of ear wiggles ($F(3, 28) = 0.383$. $F(3,28) = 1.531$, $P > 0.05$). Similarly the frequency of hop-darts did not differ across test days or diet condition ($F(3,28) = 0.551$. $F(3,28) = 0.812$, $P > 0.05$). No interaction effect was obtained between test days and diet condition on either the frequency of ear wiggles or hop-darts. (see figures 9 and 10).

Active rejections

As can be seen in figure 11, the results of this experiment revealed significant main effects for both test days and diet condition ($F(3,28) = 4.904$. $F(3,28) = 4.751$, $P < 0.05$). These main effects were modified by a significant interaction effect between test days and diet condition ($F(3,28) = 21.675$, $P < 0.05$). On day 15 postpartum food restricted rats showed much higher levels of active rejection than did ad libitum fed rats. This pattern was reversed on day 20 postpartum where rats in the ad libitum fed group showed more active rejection than those in the food restricted group.

Passive rejections

No significant differences were found in the amount of time females spent passively avoiding the stimulus male in either diet condition or days across lactation ($F(3,28) = 1.895$. $F(3,28) = 0.149$, $P > 0.05$). There were also no interaction effects for day of lactation and diet condition on the occurrence of passive rejections. (see figure 12).

DISCUSSION

In this experiment no significant differences were obtained for diet condition or days of lactation on the occurrence of sexual behavior or passive avoidance. As well, no interaction effect was obtained between diet condition and days of lactation for sexual behavior or passive avoidance. As expected, hop-darting behavior was observed very infrequently in the pacing condition. Females tend to use the hop-dart gait in situations where pacing male contacts is difficult. Significant effects were obtained, however, for the measure of active rejection. One particularly interesting finding of this study was that day 15 ad libitum fed rats displayed fewer acts of active rejection than both day 20 ad libitum fed lactating rats and day 15 food restricted rats. This finding is of interest because it suggests that ad libitum fed females on day 15 postpartum are less actively rejecting of the stimulus male if they are allowed to pace their copulatory contacts with the male in comparison to both day 20 ad libitum fed females and day 15 food restricted females. Additionally, day 15 food restricted females were found to display more acts of active rejection towards the stimulus male than day 20 food-restricted females. This difference together with the rather high levels of active rejection seen in ad libitum fed rats on day 20 postpartum may reflect differences in endogenous levels of progesterone and/or other hormones among the groups tested.

GENERAL DISCUSSION

The results of this thesis confirm the data by Clarke et al. (1984), showing that lactating females are able to respond to the stimulatory effects of estrogen administration in the induction of receptive behavior. This finding was extended to include both ad libitum fed and food-restricted lactating females. Somewhat surprising was the finding, in both Experiments 1 and 2, that day 15 lactating rats displayed significantly lower levels of receptivity than females tested earlier or later in lactation. Moreover, this reduction in receptivity was extended by at least five days in food-restricted females so that food restricted females tested on day 20 of lactation were significantly less receptive than ad libitum fed females on day 20 postpartum. This finding is in agreement with previous data that shows that the effects of food restriction on the reproductive axis persist for at least five days after re-feeding (Walker et al., 1995; Abizaid et al., 2003).

The finding that both ad libitum fed and food restricted lactating rats respond to the stimulatory effects of estrogen on the lordosis reflex is consistent with evidence showing that large doses of exogenous estrogen are able to induce the lordosis reflex in the absence of progesterone (Kow & Pfaff, 1977). However, the females used in this study were not experiencing a lack of endogenous progesterone, but rather had very high levels of endogenous P. The mechanism involved in the induction of sexual receptivity in this study, therefore, is most likely different than those responsible for estrogen induction of receptivity in the absence of progesterone. Furthermore, it is important to note that the doses and timing of estrogen used in the current study are likely to be supraphysiological. The fact that lactating rats respond to this pattern of estrogen administration, therefore,

does not mean that the neural circuitry underlying the lordosis reflex is unaltered during lactation and further experiments will be required to examine this issue.

The tendency for receptive behavior to be reduced at those points in lactation associated with the greatest energy drain, suggests that lordosis may be directly affected by metabolic cues. Consistent with this hypothesis, neuropeptides that are increased by food restriction have also been shown to suppress the lordosis reflex. For example, central administration of neuropeptide Y (NPY) (Clark, Kalra & Kalra, 1985; Corp, Greco, Powers, Bivens & Wade, 2001) and corticotropin-releasing hormone (CRH) (Jones, Keene, Pick, Corp & Wade, 2001; Sirinathsinghji, Rees, Rivier & Vale, 1983) have been shown to suppress estrous behavior in hamsters and rats within minutes.

In contrast to the relatively high levels of receptive behavior seen on most of the test days, the ability of exogenous estrogen to stimulate proceptive behaviors increased across days 15-25 postpartum. These findings emphasize the dissociation between the mechanisms involved in receptive and proceptive behaviors. Receptivity is primarily regulated by estrogen, with progesterone playing only a facilitative role (Sodersten, 1985). Full-blown proceptive behaviors on the other hand, depend on the presence of progesterone (Tennent et al., 1980), and no proceptive behavior is seen when progesterone is administered without prior estrogen administration (Blaustein, Mani & O'Malley, 1997). When progesterone levels are high at the time of estrogen administration however, progesterone suppresses proceptive behavior (concurrent inhibition) (Blaustein & Wade, 1977). This mechanism may explain the finding that proceptive behaviors tend to increase across days of lactation coincident with the fall in endogenous progesterone levels that typically occurs in the third week of lactation. The

fact that on day 20 postpartum food restricted lactating rats displayed fewer earwiggles than did ad libitum fed females is consistent with this mechanism because progesterone levels remain elevated in food restricted rats even after re-feeding (Woodside, 1991; Walker et al., 1995).

The neural substrates for proceptive and receptive behavior, although overlapping, are distinct. Rather more is known about the neural circuitry underlying the lordosis reflex than that of proceptive behavior. The reason for this difference is most likely due to the fact that proceptive behaviors are much more variable than the spinal reflex of lordosis. The ventomedial nuclei of the hypothalamus (VMH) are critical for the induction of the lordosis reflex (Parsons & Pfaff, 1985). VMH lesions, or destruction of afferent or efferent projections from the VMH, have been demonstrated to reduce the lordosis response (Clark et al., 1981; Kennedy, 1964; Yamanouchi, 1980; Emery et al., 1984). VMH neurons innervate the periaqueductal gray (PAG), which project to the premotor neurons in the medullary reticular formation (MRF). These premotor neurons act on the motor neurons of the spinal cord that innervate the lumbar epaxial muscles that produce the lordosis reflex (Pfaff, 1980). Additionally, the midbrain central gray (MCG) is known to be part of the neural circuitry regulating the lordosis reflex. Electrolytic lesions of the MCG greatly reduce or abolish the lordosis reflex seemingly permanently (Pfaff, 1980; Pfaff, Schwartz-Giblin, McCarthy & Kow, 1994); electrical stimulation of the MCG has been shown to strengthen the intensity of the lordosis reflex during flank palpation (Pfaff, 1980; Pfaff, Schwartz-Giblin, McCarthy & Kow, 1994), and bilateral lesions of the tracts that connect the VMH to the MCG severely disrupt lordosis (Hennessey, Camak, Gordon & Edwards, 1990; Malsbury, Kelley & Pfaff, 1972; Pfaff,

1980). Furthermore, destruction of the midbrain ascending ventral noradrenergic bundle (VNAB) completely abolishes the lordosis reflex (Hansen et al., 1980, 1981).

Although the display of proceptive behavior is reduced by VMH lesions (Clark & Pfeifle, 1981) and estrogen primed ovariectomized rats that received progesterone implants into the VMH displayed full-blown proceptive behaviors including ear wiggling and hop darting (Rubin and Barfield, 1983), there is evidence implicating other brain areas in the control of proceptive behavior. The medial preoptic area of the hypothalamus (MPOA) is believed to inhibit lordosis behavior and facilitate pacing or proceptive behaviors (Hoshina, Takeo, Nakano, Sato & Sakuma, 1994;Modianos, Delia & Pfaff, 1976; Whitney, 1986). Electrolytic lesions of the MPOA have been shown to facilitate lordosis responses (Pfaff & Sakuma, 1979; Powers & Valenstein, 1972), whereas, electrical stimulation of the MPOA has been shown to inhibit lordosis (Takeo, Chiba & Sakuma, 1993). Furthermore, excitotoxic lesions of cell bodies, but not afferent or efferent fibers within the MPOA, have been shown to facilitate lordosis, but severely diminish or abolish proceptive behavior (Hoshina, Takeo, Nakano, Sato & Sakuma, 1994). In addition, Whitney (1986) showed that, the effects of MPOA lesions on the induction of lordosis, is determined by the test situation. Females who were not allowed an exit from the pursuing male displayed increased lordosis quotients, whereas females that were allowed an escape did not show elevated lordosis quotients, illustrating that MPOA lesions do not increase the female's motivation to engage in copulatory behavior with the male.

Although the midbrain is crucial for the induction of lordosis, it appears to be less critical for the induction of proceptive behaviors (Erskine, 1989). Proceptive behaviors

remain intact and in some instances are enhanced by destruction of the VNAB, which abolishes the lordosis reflex (Hansen et al., 1980, 1981). Another region shown to have effects on the induction of both receptive and proceptive behavior is the lateral septum. Electrolytic lesions or destruction of afferent and efferent fibers of the lateral septum facilitates both lordosis and solicitations in female rats (Nance, Shryne & Gorski, 1975; Nance, Shryne & Gorski, 1975; Yamanouchi & Arai, 1990). On the other hand, electric stimulation of the septum results in inhibition of lordosis and proceptive behavior in female hamsters (Zasosrin, Malsbury & Pfaff, 1975). The septum sends efferent fibers to a number of brain areas including the MPOA, BNST and lower brain stem that play a role in regulating female sexual behaviors (Nance, Shryne & Gorski, 1975; Veening, Swanson, Cowan, Nieuwenhuys & Geerdts, 1982).

Further support for the dissociation of neural substrates that mediate receptive and proceptive behavior comes from studies of neural activation in response to different types of induced sexual behavior in female rats. It has been shown that copulation with intromission or manual vaginocervical stimulation (VCS) induces c-Fos (mRNA or protein product of immediate early genes correlated with neuronal activity) in female rats and hamsters in the MPOA, ventrolateral septum, BST, PVN, ventrolateral VMH, arcuate nucleus, lateral habenula, PMV, VTA, MCG, and peripeduncular nuclei (Dudley, Rajendren &Moss; Dudley & Moss, 1994; Erskine, 1993; Flanagan-Cato & McEwen, 1995; Joppa, Meisel & Garber, 1995; Pfaus, Kleopoulos, Mobbs, Gibbs & Pfaff, 1993; Pfaus, Marcangione, Smith, Manitt & Abillamaa, 1996; Polston & Erskine, 1995; Ramos & DeBold, 1995; Rowe & Erskine, 1993; Tetel, Getzinger & Blaustein, 1993; Tetel Getzinger & Blaustein, 1994; Tetel, Celentano & Blaustein, 1994; Wersinger, Baum &

Erskine, 1993). Lordosis behavior does not induce Fos by itself (Joppa, Meisel & Garber, 1995; Pfaus, Kleopoulos, Mobbs, Gibbs & Pfaff, 1993), but it is not yet known whether or not other sexual behaviors such as proceptivity or pacing lead to Fos induction in discrete nueral loci (Pfaus & Heeb, 1997). Fos induction is observed in the cingulate cortex of females that copulate actively in bilevel chambers (where they demonstrate high levels of pacing and proceptive behavior), but not when they receive either manual flank palpation or VCS (Pfaus, Kleopoulos, Mobbs, Gibbs & Pfaff, 1993). VCS has also been shown to induce excitation of MPOA neurons (Blake & Sawyer, 1972; Dafny & Terkel, 1990). VCS during copulation appears to induce the cessation of lordosis and stimulate pacing behavior (Pfaus, Smith & Coopersmith, 1999). Therefore, activation of the MPOA during copulation in female rats may inhibit lordosis and stimulate pacing or proceptive behaviors (Pfaus & Heeb, 1997).

Though the neural substrates on which steroid hormones act to induce receptive and proceptive behaviors might differ their actions at all sites are mediated through effects on their specific receptors. Steroid receptors are nuclear transcription factors that are mediated by the binding of specific hormones. In the regulation of transcription, hormone receptors interact with specific cofactors to activate the transcriptional machinery (DeMayo, Zhao, Takamoto & Tsai, 2002). For instance, estradiol administered to ovariectomized rodents causes an increase in the concentration of cytoplasmic progestin-specific receptors in the hypothalamus and preoptic area (MacLusky & McEwen, 1980). Progesterone treatment after estrogen priming decreases the concentration of cytoplasmic progestin receptors and results in the accumulation of progestin receptors within the cell nuclei (Blaustein & Feder, 1980). Facilitation of

female rat sexual behavior by progesterone requires the accumulation of nuclear progestin receptors within the hypothalamus (Blaustein & Brown, 1985).

It is most likely that the differential effects on sexual behavior induced by estrogen administration in lactating rats observed in the current study reflect changes in steroid receptor availability. For instance, the reduction in receptivity observed in females tested on day 15 postpartum most likely reflects a reduction of ER-alpha in brain areas known to mediate the lordosis reflex. On the other hand, the finding that proceptivity increases across days post partum suggests that progesterone receptors are being upregulated at the end of lactation, hence, one would expect to see changes in the number of progestin receptors across different days postpartum in brain areas that mediate proceptive behaviors. A number of studies have examined the effects of reducing food availability on estrogen receptor content in brain areas known to sub-serve sexual behavior. In cycling hamsters, for example, food deprivation, or treatment with metabolic fuel inhibitors, causes a reduction in the number of estrogen receptors-alpha in the VMH but increases the number of ER-alpha in the MPOA (Li et al., 1994). Furthermore, food restricting Syrian hamsters causes an increase in the number of neural estrogen receptor-alpha immunoreactive cells in the MPOA, paraventricular nucleus (PVN) and arcuate nucleus (ARC), but decreases in the VMH (Early, Wade & Lempicki, 1999; Li, Wade & Blaustein, 1994; Panicker, Mangels, Powers, Wade & Schneider, 1998; Panicker & Wade, 1998). Food restricting prepubertal female mice for 7 days has also been shown to cause reduced ERIR in the MPOA and VMH, but not in the ARC (Roemmich, Li, Rogol & Rissman, 1997).

Although, the above data shows that food deprivation causes a reduction in the number of ERIR positive cells within the VMH, a principle site of estrogen action on the induction of receptive behavior, in both hamsters and mice, the data available for rats is somewhat contradictory. Previous research has demonstrated that 48 hours of food deprivation or 1 hour of 2-deoxy-D-glucose (2DG) administration causes significant increases in ERIR in the PVN and the A1 and A2 regions of the hindbrain, but has no effect on ERIR within the VMH (Estacio, Tsukamura, Yamada, Tsukahara, Hirunagi & Maeda, 1996; Estacio, Yamada, Tsukamura, Hirunagi & Maeda, 1996; Reyes, Estacio, I'Anson, Tsukamurs & Maeda, 2001). Additionally, 48 hours of food deprivation does not alter ER-alpha mRNA in the PVN, POA, ARC or VMH according to (Cunningham, Clifton & Steiner, 1999). Recently however, Jones and Wade (2002) have demonstrated that a 74 hour fast caused a significant reduction of ERIR in the ARC, PVN, VMH and the ventral bed nucleus of the stria terminalis (BST).

In addition, Abizaid et al., (2003) found a reduction in progesterone receptor immunoreactivity in food restricted lactating rats compared to ad libitum fed lactating rats within the anterioventralperiventricular area (AVPV) and VMH (Abizaid, Service, & Woodside, 2002). It was concluded that high circulating progesterone levels in food restricted dams led to an attenuation of the ability of estrogen to induce progesterone receptors within both the AVPV and VMH. Neural loci mediating proceptivity, such as the MPOA, were not a consideration of this study.

All of the results described above, except for the PR data, were obtained from cycling or ovariectomized nulliparous females. In the current context it would be interesting to determine how these substrates are affected by lactation and particularly

how lactation alone or in combination with food restriction might modulate estrogen, progestin and cofactor receptor levels in these areas. An obvious primary site of interest is the VMH. This region is rich in estrogen receptors and implants of estradiol into the VMH of ovariectomized rats induce low frequency lordosis responses (Rubin & Barfield, 1983). Proceptivity, however, seems to be primarily regulated by progesterone at the MPOA. Progestin receptor levels are likely to fluctuate across days postpartum given that high progesterone titers begin to decline in the third week of lactation. An area of interest to look for these changes would therefore be the MPOA. If subsequent experiments show that lactating rats are less responsive to lower doses of estrogen than nulliparous females then one could examine whether lactation and concurrent food restriction modulate estrogen, progestin or cofactor receptor levels in these areas. As was mentioned previously, hormones receptors interact with specific cofactors in order to activate transcriptional machinery. The Steroid Receptor Coactivator (SRC) family of cofactors serves to modulate the transcriptional activity of the hormone receptors (DeMayo, Zhao, Takamoto & Tsai, 2002).

The findings of this thesis have demonstrated that regardless of test condition both ad libitum fed and food restricted lactating rats display high levels of active rejection responses, with food restricted females displaying greater numbers of active rejections towards the stimulus male than did ad libitum fed females in Experiment 2. Furthermore, these acts of active rejection were seen to decrease across days postpartum in both ad libitum and food restricted lactating rats. This pattern of results suggests that the increased levels of estrogen in combination with falling progesterone levels at the end of

lactation promote the induction of proceptive behaviors, and at the same time reduce the amount of active rejection responses.

The findings of Experiment 3 differed from those obtained in Experiment 2. In Experiment 2 females were not allowed to pace their contacts with the male, in Experiment 3, however, a pacing chamber was used which made it possible for females to escape the male. In this context, females displayed a much different pattern of active rejections than females in the non-pacing condition. For example, active rejections were not seen to decrease across days postpartum in ad libitum fed females. Day 15 ad libitum fed females displayed significantly fewer acts of active rejection towards the stimulus male than did day 20 ad libitum fed females. This finding suggests that if given the opportunity to pace day 15 ad libitum females are less rejecting of the male's advances. On the other hand however, Food restricted lactating rats tested on day 20 postpartum were found to be significantly less actively rejecting of the male than day 15 food restricted females. The pattern of results in the food restricted group resembles that of Experiment 2 in that active rejection responses were reduced across days postpartum.

Interestingly, females in Experiment 3 displayed the same amount of passive rejections of the stimulus male across groups. This suggests that lactating females who are given the means to escape a pursuing male, do not, instead it appears that they prefer to actively reject them. Although speculative, perhaps maternal aggression is being activated by the presence of a novel male rat even though pups are not present during the testing phase. Maternal aggression in rats is thought to be triggered by changes in endocrine pattern associated with late gestation, parturition and lactation (Mayer & Rosenblatt, 1993). Previous research in this area has shown that changes in ovarian

hormones (Mayer, Ahdieh & Rosenblatt, 1990; Mayer, Reisbick, Siegel & Rosenblatt, 1987; Mayer & Rosenblatt, 1984; Mayer & Rosenblatt, 1987), prolactin (Svare & Mann, 1983) and oxytocin (Consiglio & Lucion, 1996; Giovenardi, Padoin, Cadore & Lucion, 1998; Hansen & Ferreira, 1986; Olazabal & Ferriera, 1997) are related to the expression of maternal aggression. Because food restricted dams showed elevated levels of active rejection responses compared to ad libitum fed rats, it might be expected that the profile of circulating hormones and peptides would differ between females in the two diet conditions.

In summary, the experiments in this thesis were designed to investigate the combined effects of lactation and food restriction on the ability of exogenous estrogen administration to induce sexual behavior on days 10, 15, 20 and 25 of lactation. We found that exogenous estrogen administration during the postpartum period mentioned above induced lordosis responses similar in magnitude to ovariectomized controls on all test days but day 15. The reduction in lordosis quotient seen on day 15 postpartum suggests that lordosis may be mediated by metabolic cues. Furthermore, this reduction in lordosis was also observed to extend to at least day 20 in food restricted females. Unlike receptivity, proceptivity increased across days postpartum in both ad libitum and food restricted lactating rats. This suggests, that rising estrogen levels and dropping progesterone levels at the end of lactation, promote the induction of proceptive behaviors. Additionally, reduced proceptivity was extended to day 20 postpartum in food restricted lactating dams. Active rejection responses in females within both diet conditions decreased across days postpartum, and like proceptive behaviors, increased levels of

estrogen in combination with falling levels of progesterone at the end of lactation are most likely involved in the reduction of avoidant behavior.

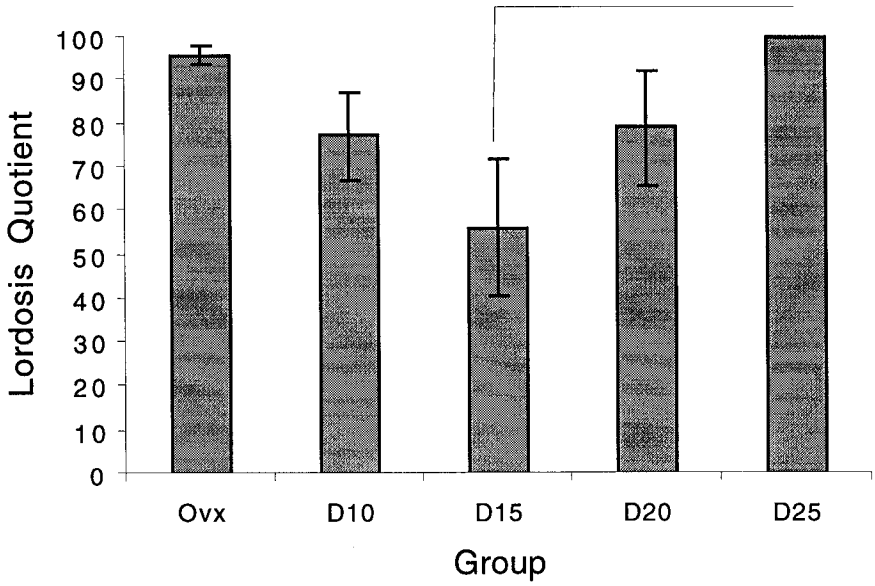

Figure 1. Lordosis Quotients (mean ± standard error). Lines show significant differences between groups.

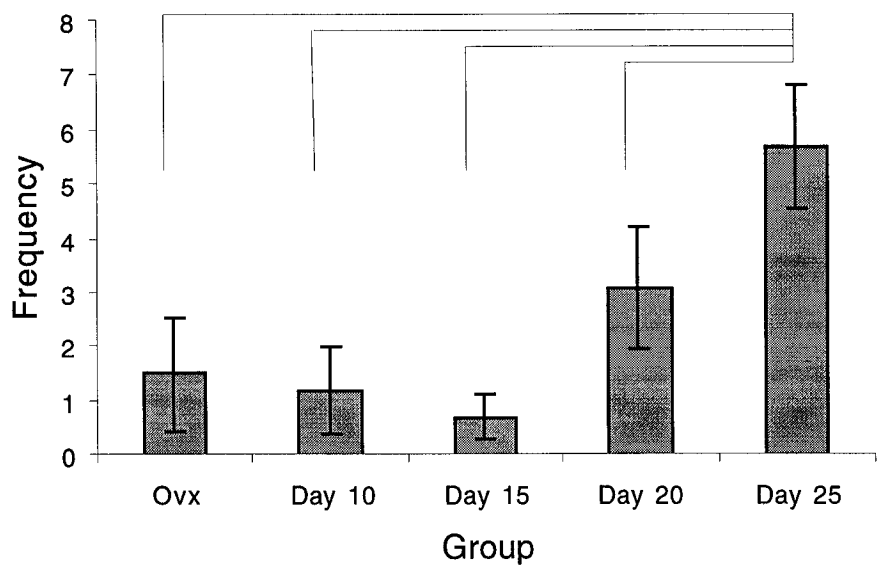

Figure 2. Frequency of earwiggling behaviour (means ± standard error). Lines show significant differences between groups.

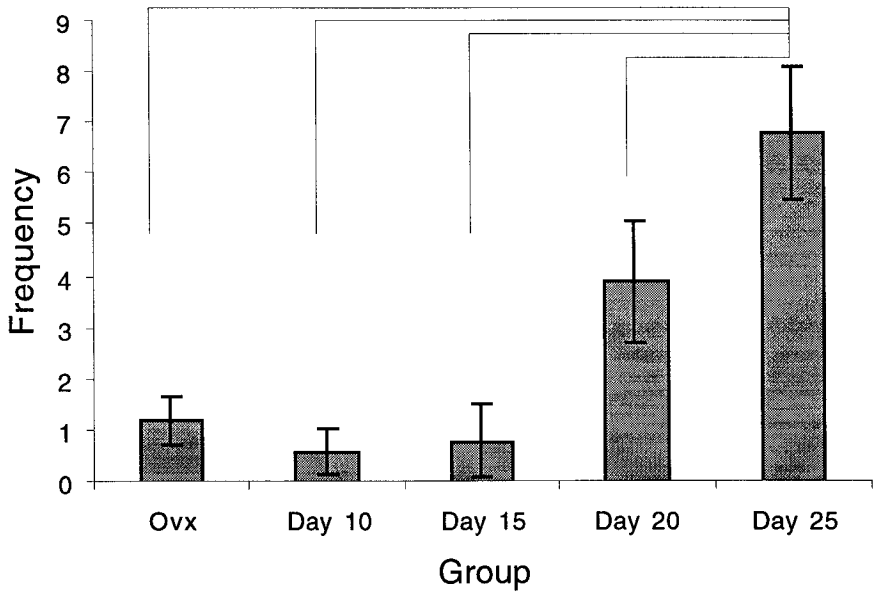

Figure 3. Frequency of the hop-dart gait (Means ± standard error). Lines show significant differences between groups.

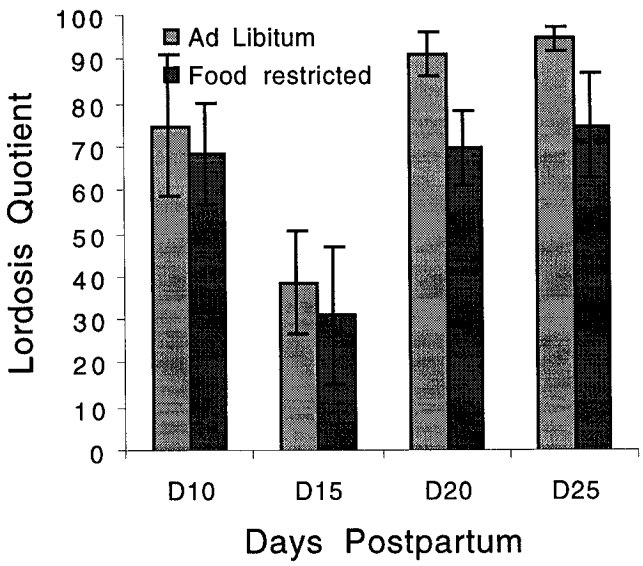

Figure 4. Lordosis Quotients (means ± standard error).

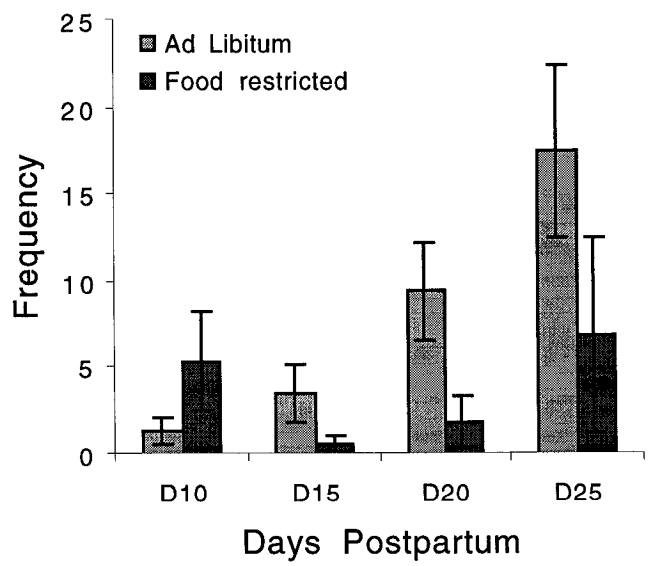

Figure 5. Frequency of earwiggling behaviour (means ± standard error).

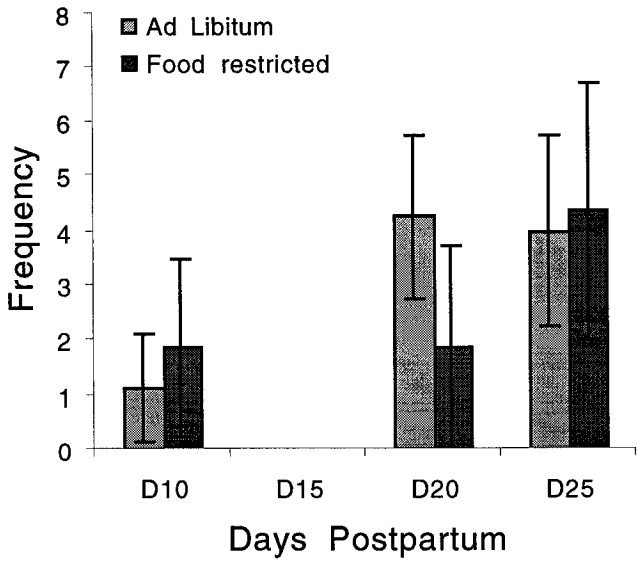

Figure 6. Frequency of the hop-dart gait (means ± standard error).

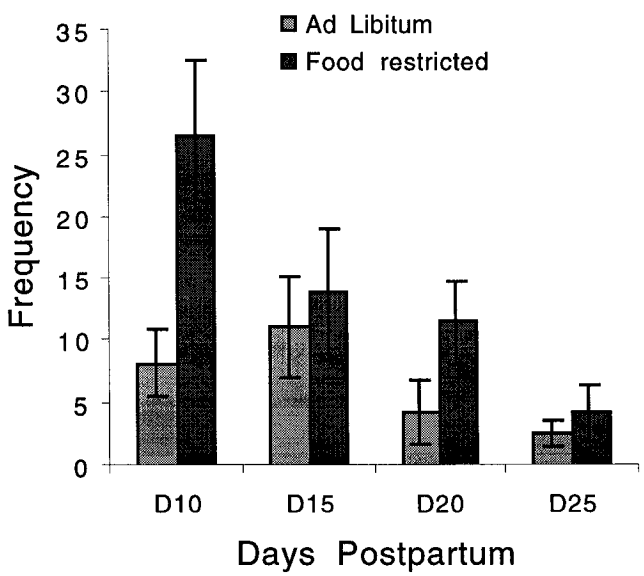

Figure 7. Frequency of active rejection responses (means ± standard error).

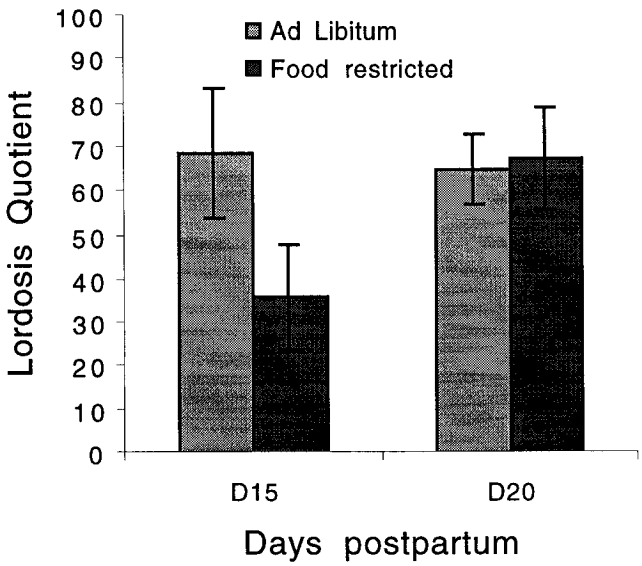

Figure 8. Lordosis Quotients (means ± standard error).

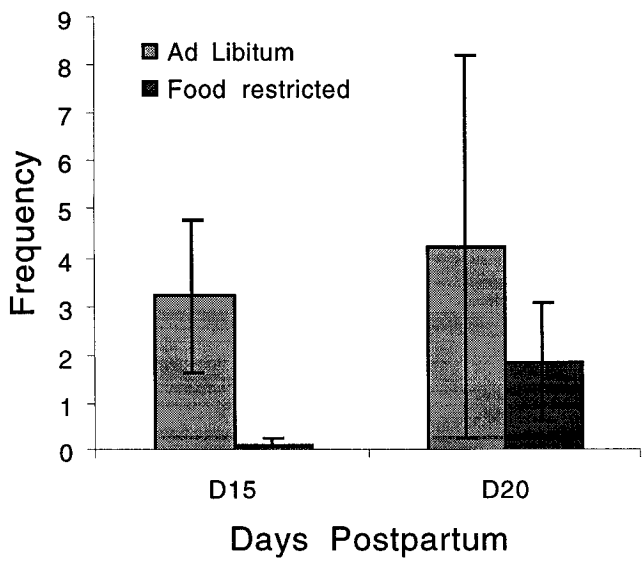

Figure 9. Frequency of earwiggling behaviour (means ± standard error).

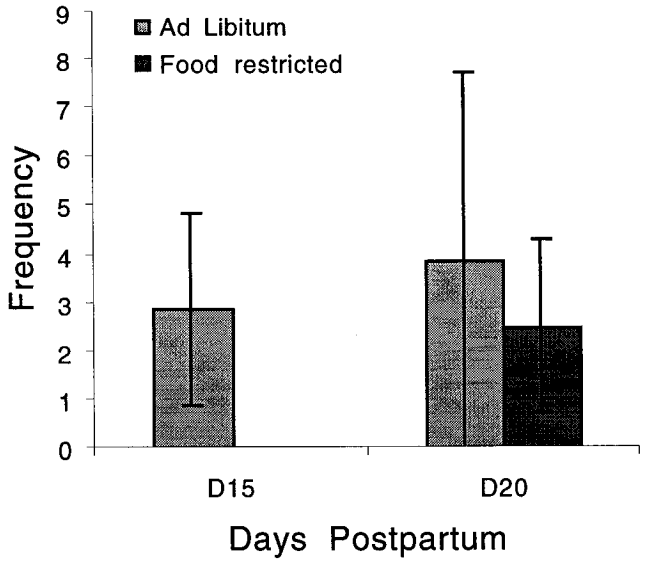

Figure 10. Frequency of the hop-dart gait (means ± standard error).

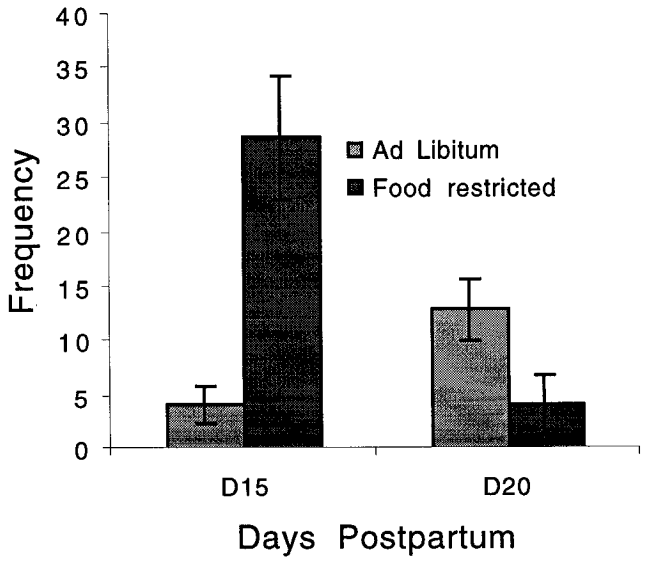

Figure 11. Frequency of active rejection responses (means ± standard error).

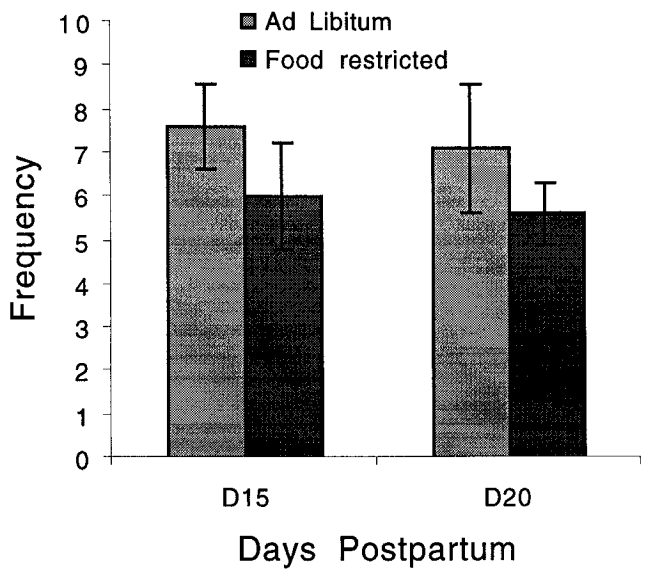

Figure 12. Frequency of passive rejection responses (means ± standard error).

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