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THE EFFECT OF MULTIPLE ELECTRICAL STIMULATION IN THE
MEDIAL PREOPTIC AREA AND THE MEDIAL AMYGDALA
ON MATERNAL BEHAVIOR IN THE FEMALE RAT

by

Hywel David Morgan

A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Graduate Department of Psychology
University of Toronto

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THE EFFECT OF MULTIPLE ELECTRICAL STIMULATION IN THE
MEDIAL PREOPTIC AREA AND THE MEDIAL AMYGDALA
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PhD Thesis of Hywel David Morgan, 1999
Department of Psychology, University of Toronto

A set of experiments was conducted to investigate the functions of the medial preoptic area (MPOA) and the medial amygdala (MedAmyg) and their relationship with regards to maternal (and related) behavior(s) using electrical (kindling-like) stimulation. This procedure is thought to enhance the function of the substrate being stimulated and has never been used before to investigate maternal behavior. A program of repeated electrical stimulation was applied daily for 14 days using 2 second trains of biphasic square wave pulses at 60 Hz, 1 ms duration and 300-500μA. Confirmation of afterdischarge (AD) using these parameters was established. Electrophysiological data showed strong transfer of AD from the MedAmyg to the MPOA, but not in the other direction (suggesting unidirectional circuitry). In the first experiment, maternally experienced (but not post-partum) MedAmyg stimulated animals became maternal more slowly than did MedAmyg not stimulated animals or than MPOA stimulated animals. In the second experiment, virgin animals were used. MPOA stimulation enhanced the female's preference for pup associated environments in the conditioned place preference (CPP) paradigm. MedAmyg stimulation had no effect on CPP performance, but produced a decreased preference for pup odors in a modified hole board test and increased 'anxiety' in the open field. The third experiment investigated the interaction of electrical stimulation
and pup exposure in virgin females. MPOA stimulation interacted with pup exposure to further reduce the latency to become maternal. Finally, the forth study investigated the effects of MPOA/MedAmyg stimulation in stria terminalis lesioned maternally experienced females. Results were consistent with above studies, except MedAmyg stimulation only partially attenuated maternal behavior in the lesion condition. Generally, these results confirm that the MPOA and the MedAmyg are involved in facilitating and attenuating maternal responsiveness and related (precursor?) behaviors, respectively. It appears that chronic (kindling-like) stimulation of these neural substrates enhances their functions. MPOA stimulated facilitation is further enhanced by pup experience. Also, the attenuation of maternal behavior appears to be (at least somewhat) dependent on the MedAmyg connections with the MPOA. This set of experiments clarifies our understanding of MPOA/MedAmyg functioning and their relationship with regards to maternal behavior. The technique used in this experiment is a useful tool for investigating the function of neural substrates.
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Maternal responsiveness in rats can be divided into two components: the onset of the behavior and its subsequent maintenance. Both of these depend on a change in the hormonal/neurochemical status and the activation/change of particular neural substrates. In the naturally parturient female, the immediate maternal responses are controlled by hormones acting on the brain (Bridges, 1996; Bridges & Freemark., 1995; Bridges, 1990; Bridges et al., 1990; Fahrbach, Morrell & Pfaff, 1985; Numan, 1994; Numan, Rosenbaltt & Komisaruk., 1977). The continuation of maternal behavior past the period of hormonal priming is based on the activation of sensory and memory processes and their neural substrates.

The role of various neural substrates in the onset of maternal behavior in animals has been studied extensively using lesion and hormone implant techniques (Bridges et al., 1990; Fleming, Cheung, Myhal & Kessler., 1989; Fleming, Vaccarino & Leubke, 1980; Numan & Smith, 1984; Numan et al., 1977). This thesis uses repeated
electrical neural stimulation (akin to kindling) to analyze the function and relationship between certain substrates of maternal behavior. The relatively permanent change that results from this type of stimulation provides a new method by which to study the onset and maintenance of maternal behavior.

This type of stimulation can be used as a tool to reveal neural mechanisms underlying both the onset of maternal behavior and the long-term neural changes underlying its maintenance. Specifically, in this thesis, the role of the medial preoptic area of the hypothalamus (MPOA) and the medial amygdala (MedAmyg) in the onset of maternal behavior, and related affective/motivational changes (such as attraction to distal pup cues, and reinforcing qualities of pups) will be investigated using electrical stimulation. Finally, these behaviors will be evaluated at a time removed from the stimulation to investigate the relative permanence of any changes.

The remainder of this introduction provides first a
review of the current state of knowledge of the onset and maintenance of maternal behavior. Next the rationale for using the electrical stimulation procedure is discussed. Finally, the last section introduces the experiments.

The Onset of Maternal Behavior

Description of maternal behavior. Female rats exhibit maternal responsiveness towards their pups at birth (Rosenblatt & Lehrman, 1963). Within 30 minutes after birth, the new mother performs the entire spectrum of maternal behaviors: she retrieves them, licks them and assumes a lactating posture over them (Fleming & Rosenblatt, 1974a). The virgin animal, however, is not spontaneously maternal towards pups. Upon initial exposure to pups, virgin females may ignore them, move away from them or even cannibalize them (Terkel & Rosenblatt, 1971). This changes with continuous exposure to pups; the virgin will eventually become maternally responsive to them within 5 to 10 days (Rosenblatt,
Hormonal control of the onset of maternal behavior.

The onset of maternal behavior is thought to be controlled by hormones in the female rat. At the end of pregnancy there is a relatively sharp decrease in progesterone levels and an increase in estrogen levels (MacDonald & Matt, 1984; Davies & Ryan, 1972). Other neurohormones are released at parturition as well, including oxytocin, and prolactin (Bridges, 1996; Bridges, 1990; Rosenblatt, 1990, Insel, 1990). When a regimen of these hormones, designed to mimic their natural release during pregnancy and parturition, is given to virgins, it induces a rapid onset of maternal responsiveness (Moltz, Lubin, Leon & Numan, 1970; Rosenblatt, 1990; Bridges, 1990; Insel, 1990).

It seems that each hormone has a number of different behavioral effects. These effects may be the result of their actions on a number of different neural structures.
The most widely studied hormones, for instance, estrogen and progesterone, can facilitate maternal responsiveness by promoting changes in the female's attraction to the odors of pups, perhaps by reducing her 'fearfulness' and neophobia in their presence, and by augmenting the pups' reinforcing value to her (Fleming et al., 1989; Fleming & Corter, 1995).

The change in the female's attraction to pup odors is nicely illustrated by the observation that new mothers, without experience with pups, prefer nest material from the nest of a new mother and her pups over from any other nesting material (Fleming et al., 1989). Virgins given a hormonal regimen designed to mimic the parturitional changes in progesterone and estrogen also exhibit a preference for pup odors (Fleming et al., 1989).

Hormonal changes also appear to reduce neophobia in the new mother. Naturally parturient females are less avoidant when presented with pups than are virgin
animals. Indeed, they are also more willing to approach an unfamiliar intruder and to explore a novel environment (Fleming & Luebke, 1981). Since regimens of progesterone and estrogen that facilitate maternal behavior in the virgin female also reduce pup-avoidance and measures of timidity in an open field apparatus, these emotionality differences would appear to be hormonally mediated (Fleming et al., 1989).

Hormones may act directly to alter the reinforcing value of pups to the mother. Fleming, Korsmit & Deller (1994) found that postpartum females learn quickly to associate pups with a particular location in a conditioned place preference paradigm, whereas virgin females (even those induced to become maternal) will not. However, unravelling the specific hormones that change the animal's behavior, and how they do it is a complex problem. The following outlines the specific roles for the most important hormonal and neurochemical substances involved in maternal behavior.
**Estrogen.** Estrogen appears to be the key hormone in the induction of maternal behavior. Siegel & Rosenblatt (1975) showed that virgin females, given a hysterectomy and ovariectomy (to eliminate the source of sex steroids) and subsequently injected with estradiol, showed short latencies to the onset of maternal behavior when compared to vehicle treated controls. Further, Moltz, Lubin, Leon & Numan (1970) found that injections of progesterone and prolactin without estrogen failed to stimulate maternal care in the ovariectomized virgin rat. It is currently unknown whether estrogen's action occurs directly on the brain, independent of progesterone and prolactin to stimulate maternal behavior, or whether it indirectly allows progesterone and/or prolactin to stimulate the neural mechanisms mediating its expression.

Numan, Rosenblatt & Komisaruk (1977) have shown that the medial preoptic area (MPOA) is a possible site of action for estrogen in the stimulation of maternal behavior. This region is crucial for the expression of
maternal behavior (see discussion below; Numan, 1990). They found that the application of estrogen to the MPOA of 16-day pregnant, hysterectomized-ovariectomized rats stimulates an almost immediate onset of maternal behavior. Further, Rosenblatt, Wagner & Morrell (1994) found that estrogen receptor concentrations in the MPOA are increased during pregnancy and postpartum. Also, Wagner and Morrell (1995) found mRNA for estrogen receptors increased in the ventromedial hypothalamus (VMH) just prior to parturition (although the actions of estrogen in the VMH in controlling maternal behavior have not been established). More recently, Wagner and Morreil (1996) investigated the levels of estrogen receptor immunoreactivity in behaviorally relevant brain regions in rats during pregnancy. They found significantly greater levels of estrogen receptor proteins on days 16 and 22 of pregnancy than on day 8 of pregnancy or postpartum day 1 (parturition typically occurs on or around day 22). They also found higher levels of estrogen
receptor proteins in the VMN on day 22 of pregnancy than day 16 or day 1 postpartum, higher levels in the bed nucleus of the stria terminalis (BNST) on day 1 postpartum than during pregnancy and no significant changes in the medial amygdala. They suggested that regionally and temporally specific changes in these brain structures underlie the expression of maternal behavior.

**Progesterone.** The onset of parturition is prefaced by precipitous decline in serum levels of progesterone (about 24-48 hours prior to parturition) (Bridges, 1984). This decline is important in regulating the timing of maternal behavior at the end of pregnancy. If circulating levels of progesterone are held high (after hysterectomy-ovariectomy at day 17 of pregnancy), the rapid onset of maternal behavior induced by progesterone withdrawal is prevented (Bridges, Rosenblatt & Feder, 1978). Also, the rapid expression of maternal behavior in estrogen treated virgin rats is blocked by progesterone (Doerr, Siegel & Rosenblatt, 1981). Unfortunately, the neural site of
progesterone’s action has not been located. Numan (1978) was unable to affect the onset of maternal behavior with the application of progesterone to any one site. He suggested that progesterone’s inhibitory action may occur at multiple dependant sites.

More recent work on the role of progesterone in maternal behavior suggests its importance in the onset of maternal behavior in primiparous mice. Wang, Crombie, Hayes & Heap (1995) administered progesterone antibodies (subcutaneously) at different times pre- and postpartum. They found an impairment in postpartum maternal care if administered at either day 2 or day 17 prepartum, but no impairment if administered during the postpartum period. A further study found this effect in primiparous but not multiparous animals (Crombie, Hayes, Heap & Wang, 1995).

**Prolactin.** Recently, strong evidence has emerged showing a role of prolactin and lactogenic hormones in the induction of maternal behavior. Initially, Bridges, DiBiase, Loundes & Doherty, (1985) found that
hypophysectomized rats responded to the stimulatory effects of sequential exposure to progesterone (at days 1-11) and estrogen (days 11 to end of testing) only when also given prolactin. Further, the onset of maternal behavior in ovariectomized steroid treated rats was delayed when endogenous prolactin was suppressed compared to controls (Bridges & Ronsheim, 1990). The administration of prolactin to the MPOA was found to reverse this effect (Bridges et al., 1990). It turns out that infusions of rat prolactin into the MPOA of ovariectomized virgin rats only stimulates maternal behavior when rats are also treated sequentially with progesterone and estrogen (Bridges & Freemark, 1995). Finally, the actions of prolactin on maternal behavior are shared by other lactogenic molecules, including ovine growth hormone, human placental lactogen, and rat placental lactogens (Bridges & Millard, 1988; Bridges et al., 1994; Bridges & Freemark, 1995).
Peptidergic and neurotransmitter control of the onset of maternal behavior. A number of peptides have been shown to affect the onset of maternal behavior in the female rat. Oxytocin and cholecystokinin (CCK) stimulate the onset of maternal behavior, while corticotrophin releasing factor (CRF) disrupts it. Although the actions of peptides seems to be acute, the opioids appear to affect the maintenance and the onset of maternal behavior differentially. The involvement of specific neurotransmitters in the control of maternal behavior has received limited attention in the literature. However, the roles of dopamine and norepinephrine in maternal care will be reviewed in this section.

Oxytocin. Ovarian steroids may activate maternal behavior by exerting effects on oxytocin systems in the brain. Early studies have shown that icv administration of oxytocin was observed to stimulate maternal behavior rapidly in virgin rats (Pedersen & Prange, 1979).
Ovariectomy abolished and estrogen priming reinstated sensitivity to this effect (Fahrbach & Morrell, 1984). Further, icv administration of an oxytocin antagonist blocked the onset of maternal behavior in parturient rats (VanLeengoed, Kerker & Swanson, 1987). This treatment had no effect on rat mothers that had been allowed several or more days of pup experience. Also, both electrolytic and kainic acid lesions of the paraventricular nucleus, the main source of oxytocin projections within the brain, disrupt maternal behavior (Insel & Harbaugh, 1989; Olazabal & Ferreira, 1997). These findings suggest oxytocin interacts with ovarian steroids.

The site of action of oxytocin has been difficult to establish. No appreciable oxytocin binding has been found in the MPOA, ventral tegmental area (VTA) or the BNST using autoradiography (Insel, 1990; Insel, 1986; Tribolet et al., 1988). However, Fahrbach, Morrell & Pfaff (1985) found that oxytocin infusion into the VTA and BNST significantly stimulated maternal behavior in estrogen
primed virgin rats. Also, using radio ligand assays on dissected out nuclei, Pedersen et al. (1994) found considerable oxytocin binding in the MPOA, which was highest during the mid-parturition period. They also found similar results for ventral midbrain structures (including the VTA). The reason for these discrepant findings is not clear at this time.

More recent studies have also shown contradictory results. Young, Muns, Wang & Insel (1997) demonstrated elevated oxytocin mRNA in the MPOA mid-pregnancy compared to day of parturition and elevated estrogen was also associated with increased oxytocin mRNA in the VMH. However, studies of knockout mice, that lack the nucleotide sequences encoding the oxytocin peptide, show normal maternal behavior (Nishimori et al., 1996; Young et al., 1997). This finding suggests that oxytocin is not necessary for the expression of maternal. However, it should also be noted that, unlike rats, mice are spontaneously maternal and there is no shift in behavior
at parturition. Therefore, it is not surprising that oxytocin deficient mice show normal maternal behavior.

**CCK and CRF.** Systemic infusions of CCK into estrogen treated Wistar virgin rats induce a more rapid onset of maternal behavior in experimental rats than in controls (Linden, Uvnas-Moberg, Enroth & Sodersten, 1989) However, this may be strain specific since there was no difference in Sprague-Dawley animals (Mann, Felicio & Bridges, 1995). However, CCK antagonists disrupt maternal behavior in postpartum lactating rats and direct infusions of CCK into the MPOA block the disruptive effects of b-endorphins in postpartum Sprague-Dawley rats (Mann et al., 1995). These findings support an involvement of CCK in the maintenance, but not the onset of maternal behavior.

Pedersen, Caldwell, McGuire & Evans (1991) have reported that icv infusions of CRF into virgin rats resulted in increased incidences of pup killing and lower incidences of maternal care. It is not clear at this
time, exactly what are the normative CRF release patterns during the peri-partum period.

**Opioids.** The endogenous opioid peptide, β-endorphin, appears to have two roles in the expression of maternal behavior. It may help stimulate maternal behavior during the peri-partum period, while in lactating rats it may inhibit maternal care. The studies of Mayer, Faris, Komisaruk & Rosenblatt (1985) and Thompson and Kristal (1996) indicate that opioids may be stimulatory to the induction of maternal behavior. Injections of naloxone (an opioid antagonist) blocked placentophagia at parturition (Mayer et al., 1985). Also, infusions of morphine into the VTA stimulated the onset of maternal behavior in virgin rats (Thompson & Kristal, 1992). Zaias et al. (1996) have found that naltrexone (opiate antagonist) delayed that expression of parental care in both female and male juvenile rats, although Wellman et al. (1997) found morphine infusions into the MPOA disrupted the onset of maternal behavior in juvenile
rats.

There are physiological data regarding the endogenous opiate system that can be interpreted as support for a stimulatory role of opioids around the time of parturition. First, opioid receptor densities increase in the MPOA around the time of parturition (Dondi et al., 1991; Hammer, Mateo & Bridges, 1992). Also, pain thresholds increase during gestation (Gintzler, 1980).

Opioids appear to interfere with the maintenance of ongoing maternal behavior, by blocking retrieval and grouping responses (Kinsley & Bridges, 1988; Mann, Kinsley & Bridges, 1991), possibly by decreasing the olfactory attraction of the pups (Kinsley & Bridges, 1990). Mann et al. (1991) found that the icv infusion of β-endorphin into lactating primiparous animals interfered with maternal behavior for up to three hours. Further, a recent study shows that icv infusion of morphine on day 5 or 6 of lactation reduced preference for pup odors
(Kinsley et al., 1995). There is some evidence that this reduction in maternal responsiveness involves the MPOA. A possible interpretation of this phenomena is that the release of β-endorphins serves to quiet the female rat which would be advantageous during nursing bouts.

**Dopamine.** A number of studies suggest a stimulatory role for dopamine in maternal care. Szechzman, Siegel, Rosenblatt & Komisaruk (1977) first suggested that tail-pinching, a procedure thought to activate the nigrostriatal dopaminergic system, shortened retrieval latencies. Numan and Nagle (1983) found that lesioning the substantia nigra, a rich source of dopaminergic neurons, disrupted retrieval behavior but not nursing behavior. Likewise, Hansen et al. (1991) showed that infusions of the neurotoxin 6-hydroxydopamine (6-OHDA) into the ventral striatum (depleting mesolimbic dopamine) produced retrieval deficits in lactating rats. Interestingly, this deficit could be overcome if the female dams were separated from pups for an extended
period of time. Hansen (1994) suggested this separation caused an increase in motivation, overcoming some of the lesion deficits. Finally, systemic injections of haloperidol, a dopamine antagonist, interferes with pup retrieval (Giordano, Johnson & Rosenbaltt, 1990; Stern & Taylor, 1991).

**Norepinephrine.** Initial work on the role of the noradrenergic system on maternal behavior suggests it is involved in the onset rather than the maintenance of maternal behavior. Moltz, Roland, Steele & Halaris (1975) found an increase in hypothalamic norepinephrine turnover at parturition. Also, Rosenberg, Halaris & Moltz (1977) found that ivc administration of 6-OHDA interfered with maternal behavior if given 2 days postpartum, but not if given 4 days postpartum.

More recent work has built on the concept of noradrenergic involvement in learning, and hence the maintenance of maternal behavior. There is considerable evidence that the noradrenergic system is involved in the
consolidation of a wide variety of learning tasks (Decker, Gill & McGaugh, 1990; McGaugh, Introini-Collison & Nagahara, 1988; Rosser & Keverne, 1985). Also, there is evidence in the sheep that maternal bonding can be prevented by the 6-OHDA lesions of noradrenergic projections to the olfactory bulbs (Levy, Gervais, Kinderman & Orgeur, 1990; Pissonnier et al., 1985). Moffat, Suh & Fleming (1993) showed that females injected with a noradrenergic antagonist during a one hour experience within 36 hours of parturition showed longer latencies to become maternal 10 days later than control animals injected with saline or a noradrenergic agonist.

The neural control of maternal behavior. A number of past studies have explored the possible neural sites where hormones might act to influence maternal behavior in rodents (Bridges, 1990; Palka, Ramirez & Sawyer, 1966; Numan, 1994; Toubeau, Desclin, Parmentier & Pasteels, 1979). The following outlines the current research on the
most important sites controlling maternal behavior. Specifically, the discussion will focus on the MPOA, the amygdala, their relationship, and their afferents and efferents.

**MPOA.** As eluded above, Numan et al. (1977) implanted estradiol into the medial preoptic area (MPOA) and was able to facilitate maternal behavior in rats that were hysterectomized and ovariectomized on day 16 of pregnancy, whereas estrogen implants to other neural sites were without effect. The MPOA contains a high concentration of estrogen and progesterone binding neurons (Pfaff & Keiner, 1973) and seems to be very important for the expression of maternal behavior. Numerous studies show that lesioning the MPOA disrupts maternal behavior in the rat, which includes a disruption in retrieving, nest building, and nursing behavior (Numan, 1974; Jacobson, Terkel, Gorski & Sawyer, 1980; Gray & Brooks, 1984). Other parturient hormones also facilitate maternal behavior by their action on the MPOA.
MPOA implants of oxytocin and prolactin facilitate maternal responding (Pedersen, 1997; Pedersen et al., 1994; Bridges & Freemark, 1995; Insel, 1990; Numan, 1990).

It is not clear how the MPOA coordinates the display of maternal behavior. It has been suggested that the preoptic area is involved in the regulation of pituitary gland function (Clemens, Samlstig & Sawyer, 1976; Freeman & Banks, 1980), and the disruption of maternal behavior following preoptic damage results in a neuroendocrine imbalance. This seems unlikely since this disruption occurs in both hormonally primed animals and pup-stimulated (sensitized) animals. Another suggestion is that the MPOA acts as an integrator of sensory information necessary for the display of maternal behavior. Finally, the MPOA might be involved in 'motivating' the animal towards pup stimuli (Numan, 1990). This latter proposal is particularly intriguing in the light of recent evidence suggesting that the MPOA is involved in sexual motivation in male rats; MPOA lesions
reduce sexual motivation (Paredes, Highland & Karam, 1993), infusion of naloxone into the MPOA blocks sexual reinforcement (Agmo & Gomez, 1993), and dopamine antagonists infused into the MPOA decrease sexual motivation (Hull, Lorrain & Matuszewick, 1995; Moses et al., 1995; Warner et al., 1991).

**Amygdala.** The amygdala seems to have an inhibitory function. Lesions here do not disrupt its display (Fleming et al., 1980; Numan, 1994; Slotnick & Nigrosh, 1975). Fleming et al. (1980) showed that virgin females with medial amygdala lesions showed significantly shorter latencies to the onset of maternal behavior. They suggest that the medial amygdala is responsible for neophobia to pups, which can be eliminated by repeated exposure to pups or by ablation of the nucleus. They further suggested that olfactory input may inhibit maternal behavior in rats via its connections with the medial amygdala. Indeed, lesions to the main olfactory system or the vomeronasal nerve causes a similar facilitation of
maternal behavior (Fleming, Vaccarino, Chee & Tambosso, 1979). There is no evidence at this time to suggest that the amygdala is sensitive to parturient hormonal changes, although like the MPOA, it contains a high concentration of estrogen binding neurons (Pfaff & Keiner, 1973).

One hypothesis for a neural circuit for maternal behavior asserts that main and accessory olfactory input inhibits maternal behavior in virgins by activating amygdaloid inhibition of the MPOA via the stria terminalis (Fleming et al., 1980). Indeed, lesions to the stria terminalis, the major efferent pathway to the MPOA (DeOlmos & Ingram, 1972; Watson et al., 1983) have a similar effect on maternal behavior as amygdala lesions (Fleming et al., 1980).

It is likely that the decrease in neophobia seen in hormonally induced or pup-exposure induced maternal behavior is mediated via the amygdala. The amygdala would appear to be particularly important in controlling affective changes in the newly parturient rat. In
addition to facilitating maternal behavior in virgin females, medial amygdala lesions resulted in animals that behaved considerably less fearfully in a series of emotionality tests (Fleming et al., 1980). The amygdala has been implicated in emotion, as well as stimulus reward conditioning, fear conditioning, anxiety, motivational context and of associative pairing of neural cues with biologically-relevant cues (Cahill & McGaugh, 1996; Davis, 1992; Gaffan, 1992; LeDoux, 1992; McGaugh et al., 1992; Otto & Eichenbaum, 1992; Pitkanen, Savander & LeDoux, 1997; Phelps & Anderson, 1997; Simonov, 1997; Tucker, Luu & Pribram, 1995). Also, recent evidence has implicated the amygdala in male sexual motivation (Ohkura, Fabre-Nys, Broad & Kendrick, 1997; van Furth, van Emst & van Ree, 1995). It is suggested that initial avoidance of the pups is due to a natural neophobia in virgin animals, which is reduced by repeated pup exposure.

It should be noted, however, there is little
evidence elucidating which sub-nuclei of the amygdala are responsible for behavior associated with maternal responsiveness. Fleming et al. (1980) found that maternal behavior could be elicited by medial amygdala lesions. They suggest that these lesions attenuated the affective state of the animal so that pup stimuli were not as aversive. However, other nuclei of the amygdala may have control of similar affective qualities. McDonald and White (1993) have proposed that the basolateral amygdala is responsible for performance on behavioral tasks that require associations of neutral stimuli with incentive stimuli. Adamec & Morgan (1994) suggest there is a dissociation between the medial and lateral amygdala, the former being involved in anxiogenic behavior and the later being involved in anxiolytic behavior.

**Sensory afferents.** Studies of the sensory factors necessary for the performance of maternal behavior have focused on olfactory, somatosensory and (to a lesser degree) auditory pup stimuli. Although ultrasonic
vocalizations from pups influence maternal behavior in rats (Allin & Banks, 1972), the perception of infant vocalizations is not essential for maternal responsiveness (Herrenkohl & Rosenberg, 1972). The rest of the discussion will consider olfactory and somatosensory inputs.

The early studies of Beach and Jaynes (1956) showed that anosmia produced by olfactory bulbectomy does not interfere with maternal behavior in rats. However, subsequent work has shown that anosmia facilitates the maternal reactions of virgin females towards pups (Fleming & Rosenblatt, 1974a,b; Mayer and Rosenblatt, 1977). Both the main and accessory olfactory systems appear to be involved in the suppression of maternal responsiveness in virgin females (Fleming et al., 1979). It is suggested that hormones during parturition act to reduce the aversion toward novel pup odors (Fleming & Rosenblatt, 1974a,b; Fleming & Luebke, 1981; Hard & Hansen, 1985; Pietras & Moulton, 1974).
Recently, the importance of somatosensory inputs for maternal behavior has come to light. Kenyon, Cronin & Keeble (1981, 1983) initially found that occluding sensation from the mystacial pads of the snout (perioral) can interfere with retrieving behavior for up to 24 hours. Also, Stern has shown that perioral and ventral sensitivity are important for the full range of maternal behaviors (Stern, 1996 for review). Further, there is a behavioral link between perioral stimulation and the display of a full nursing posture. This link is paralleled by a mechanistic link because there is a projection from the brainstem trigeminal complex to the afferent milk-ejection pathway in the mesencephalic lateral tegmentum (Dubois-Dauphin, Armstrong, Tribolett & Dreifuss, 1985a,b). They have also shown that there are cortical changes associated with ventral stimulation suggesting the formation of maternal memories (Xerri, Stern & Merzenich., 1994).

It would appear that olfactory and somatosensory
manipulation have effects on maternal behavior via the MPOA. Perioral occlusion affects retrieval behavior much the same as MPOA lesions do. This is not surprising since trigeminal afferents interact with the MPOA via the nucleus of the solitary tract (Numan & Numan, 1991). Also, olfactory lesions reduce pup aversion in much the same way as do amygdala lesions (Fleming et al., 1981). It is possible that olfactory stimuli act to inhibit maternal behavior via (olfactory bulb) projections to the amygdala in the virgin female, and somatosensory stimuli act to facilitate maternal behavior via (cortical) connections to the MPOA. These functions appear to be modulated by hormones.

**Maternal circuit and effector system.** The MedAmyg projects to the MPOA via the ST (DeOlmos & Ingram, 1972; Watson et al., 1983). Also, MedAmyg lesions and ST lesions facilitate maternal behavior, possibly by removing vomeronasal inhibition from the MPOA (Fleming et al., 1980; Numan, 1994). More recently the connections
between the amygdala and the ventromedial hypothalamus (VMH) via the ST have been investigated. Bridges and Mann (1994) have suggested that the VMN may inhibit maternal behavior in that damage to the area appeared to be facilitative. There is evidence that the VMN participates in an amygdalo-hypothalamic-brainstem circuit mediating fear and anxiety (Roeling et al., 1994). Also the VMN projects to the PAG, a region that is critically involved in regulating reactions related to fear and anxiety (Behbehani, 1995; Roeling et al., 1994). Further, Sheehan and Numan (1997) have shown that an anxiolytic receptor antagonist (tachykinin) has potent inhibitory effects on maternal behavior when applied directly to the VMN. Numan & Sheehan (1997) suggest that a separate amygdala-to-ventromedial circuit could regulate motivational avoidance of pups.

Maternal behavior is disrupted by MPOA lesions (Numan, 1974). Disruption is also caused by damage to efferent connections of the MPOA including: the ventral
bed nucleus of the stria terminalis (vBNST) (Numan & Numan, 1996), the ventral tegmental area (VTA), lateral habenula (Lhb) and the nucleus accumbens (Nacc) (Numan, 1994), the lateral hypothalamus (Avar & Monos, 1969a,b) and the periaqueductal grey (PAG) (Lonstein and Stern, 1997a).

The most important outputs from the MPOA for maternal behavior appear to involve its lateral connections. Knife cuts that sever lateral connections of the MPOA disrupt maternal behavior (Numan, McSparren & Numan, 1990; Numan & Callahan, 1980). Further, Numan, Morrell & Pfaff (1985) showed that projections to the VTA via the lateral preoptic area (LPOA) are important in maternal behavior. However, it is not clear at this point whether the nucleus itself or fibers passing through are involved (Numan & Numan, 1991; Numan, 1990). The VTA contributes neurons to the mesotelencephalic dopamine system, with major termination sites in ventral striatal structures, which include the nucleus accumbens.
(Blackburn, Pfaus & Phillips, 1992). This finding is speculated to be important for two reasons: 1) the nucleus accumbens in turn projects to the ventral pallidum which in turn projects to brainstem motor structures (Mogenson, Swanson & Wu, 1983; Swanson, Mogenson, Gerfen & Robinson, 1984) and 2) this system is involved in motivational aspects of a variety of behaviors, and the system acts to potentiate the ability of biologically relevant stimuli to activate appropriate responses (Everitt, 1990; Blackburn et al., 1992). Other evidence also supports the importance of the VTA dopaminergic systems in maternal behavior. Notably, the application of 6-OHDA to either the VTA or the Nacc disrupts retrieval behavior (Hansen, 1994; Hansen et al., 1991a,b). This disruption does not occur if mother and pups are separated for three hours. This finding, in particular, suggests that this system may regulate motivation rather than the ability to retrieve. Although damage to the ventral striatum does not interfere with
maternal behavior under all conditions, damage to the MPOA and vBNST efferents do appear to permanently abolish retrieval behavior (Numan, 1990). This suggests that these efferents may project to other regions in addition to the VTA to influence maternal behavior. Numan and Numan (1991) suggest this may include more caudal brain stem structures such as the retrorubal field (which in turn also projects to the ventral striatum [Deutch, Goldstein, Baldino & Roth, 1988]).

The vBNST forms a junction between the medial and lateral preoptic area and damage to this area also causes retrieval deficits (Numan & Numan, 1996). Numan & Numan (1997) recently conducted an extensive study tracing the output circuit from the MPOA/vBNST and speculated how these structures regulate excitatory influences over maternal behavior. They found that the MPOA projected most strongly to the medial hypothalamus at the level of the VMN and to the lateral septum. The vBNST projected most strongly to the retrorubal field, ventral tegmental
area and medial hypothalamus. Also, a large proportion of neurons from the MPOA and vBNST projected to the PAG.

**Fos studies.** As part of the primary genomic response to external stimulation, transcriptional activity of the immediate early gene, c-fos, is triggered in many cell types by a wide variety of excitors, including different stimulus situations and behavioral states (Morgan & Curran, 1991; Sagar, Sharp & Curran, 1988). When produced in conjunction with other proto-oncogene proteins, Fos protein 'turns on' other genes within the cell that may lead to long-term structural or functional changes in the brain (Morgan & Curran, 1991). By exploring which cell groups within the brain express the Fos protein in response to stimulation or during ongoing behavior, one can trace the neural circuitry relevant to the behavior. This method has proven extremely useful in investigating the functional neuroanatomy of various behaviors in rats (eg. Baum & Everitt, 1992; Erskine & Rowe, 1992; Pfaus et al., 1992). Also, since the process may reflect long-term
changes in the brain, it has also been speculated to be a mechanism of learning at the cellular level (Morgan & Curran, 1991).

Numan & Numan (1994) showed that virgin females that showed maternal behavior had more Fos-labeled cells in the MPOA and the vBNST than virgin females that were not maternally responsive. They also found that postpartum rats had more Fos-labeled cells when exposed to pups than when exposed to non-pup stimuli. Fleming, Suh, Korsmit & Rusak (1994) found similar results in postpartum animals exposed to pups, conspecifics, food, or no stimuli. They also found increased levels of Fos in the olfactory bulbs, and in the cingulate and somatosensory cortices for the pup-exposed group, and higher levels of Fos in the pup-exposed and conspecific groups for the medial and cortical amygdaloid nuclei (than other groups). Additional studies by Lonstein and colleagues (Lonstein & Stern, 1997; Lonstein, Simmons, Swann & Stern, 1998) have investigated the activation of neural substrates
given distal pup experience, and proximal pup experience with or without suckling. Consistent with the above studies, and current theory about the maternal circuit, they found activation of the MPOA, Nacc, lateral septum, Lhb and BNST with proximal interaction with pups. Distal cues did not elicit differential activation of c-fos than control groups, also consistent with other findings (Fleming & Walsh, 1994; Walsh, Fleming, Lee & Magnusson, 1996). However, proximal interaction, with suckling, activated c-fos in the PAG, whereas interaction without suckling did not. Lonstein and colleagues suggest this area is important for the expression of a full lactating posture (called kyphosis).

Walsh et al. (1996) examined Fos in postpartum rats that received various sensory desensitizations (including zinc sulphate induced anosmia, topically anesthetized perioral and ventral regions and combinations of these treatments) and then were exposed to pups for 1 or 2 hours. Interestingly, there were no decrements in Fos in
the MPOA in any of the desensitized groups. In contrast, in animals sustaining chemosensory desensitizations, there were Fos decrements in the medial and cortical nuclei. Further, there was a summative effect of denervations in the basolateral nucleus, where manipulations of both somatosensory and chemosensory systems showed the greatest reduction in Fos. This region is believed to receive multi-modal input and to mediate the formation of associations. It is possible that this structure is involved in sensory integration necessary for the coordinated maternal response.

The Maintenance of Maternal Behavior

Whereas hormones are particularly important in initiating maternal behavior, they do not seem to play an important role in its continuance (Fleming, Morgan & Walsh, 1996; Numan, 1994). If pups are removed at birth, levels of progesterone and estrogen return to normal (cycling) levels soon after birth, but mothering behavior
continues for sometime afterwards in the rat (Moltz & Wiener, 1966; Erskine, Barfield & Goldman, 1980). Although lactational hormones, including prolactin and oxytocin may well contribute to the patterning of the dam's nursing behavior, maternal responsiveness will be maintained following the decline of parturitional hormones (Leon, Coopersmith, Beasley & Sullivan, 1990). Hence, it is assumed that maintenance of the behavior is based on stimulation provided by pup experience (Orpen & Fleming, 1987; Stern, 1983).

The effects of maternal experience. The effects of experience with pups on subsequent maternal responsiveness can be demonstrated by removing pups at various intervals and testing for maternal behavior to foster pups after a period of separation (Bridges, 1975; Jackubowski & Terkel, 1986; Rosenbaltt & Lehrman, 1963; Seigel & Greenwald, 1978). Fleming and colleagues (Fleming & Rosenblatt, 1974a; Orpen and Fleming, 1987) have found that if pups are removed at parturition, then
10 days later the dam will not be maternally responsive to foster pups. They will show the approximately the same latency to become maternal as do virgin animals (Fleming & Rosenblatt, 1974a). If the dams are permitted to interact for a short period at parturition, their latencies to become maternal 10 days later will decrease (Orpen & Fleming, 1987; Bridges, 1975). How rapidly dams respond to pups at retention testing is directly related to the duration of the initial experience, and inversely related to the interval between experience and test (Fleming & Sarker, 1990).

The role of hormones. Although hormones are not necessary for the maternal experience to produce a long-term effect, the postpartum state does enhance the effectiveness of that experience. Experience with pups during a period of hormonal priming reduces the latency to become maternal upon re-exposure (Fleming & Sarker, 1990). Thus hormones appear to enhance and promote the retention of maternal experiences. There are a number of
ways hormones could be acting to promote the experience effects. They could be increasing the salience of associative cues (eg. olfactory cues from pups), they could be acting to strengthen the association between the conditioned and unconditioned pup-associated cues, or they could be producing internal cues that act themselves as conditioned stimuli, or all three.

The role of sensory input. A robust retention of responsiveness usually requires that the mother physically interact with pups during the postpartum period. This appears to involve chemosensory and somatosensory stimulation that occurs in the context of the initiated behavior, during nosing, licking and nursing of pups (Stern, 1996; Stern, 1989; Stern, Dix, Pointek & Thramann, 1990). Lesioning the olfactory system affects neither the onset nor maintenance of maternal behavior in postpartum rats, and chemosensory pup stimuli on its own is insufficient to maintain maternal responsiveness in rats (Fleming, Garvath & Sarker, 1992;
Jakubowski & Turkel, 1986; Orpen & Fleming, 1987). Only the elimination of both perioral and ventral somatosensory stimulation has been found to effectively disrupt the retention of maternal behavior (Morgan, Fleming & Stern, 1992). However, a study using the conditioned place preference paradigm (where the dam learns to associate pup cues with a particular location) showed that postpartum animals learned a preference for a location associated with pup experience, but not if that experience was gained when the dam received any of: ventral anesthetization, perioral anesthetization, or zinc sulphate induced hyposmia (Magnusson & Fleming, 1995). This suggests that both chemosensory and somatosensory stimulation provide the dam with experience enhancing (reinforcing) stimuli.

**Neurochemistry of maternal experience.** Because the maternal experience effect reflects a long term change in behavior, similar to other forms of learning, there must occur mediating structural or functional changes.
Fleming, Cheung & Barry (1990) and Malenfant, Barry & Fleming (1991) found that if a drug that inhibits protein synthesis (cycloheximide) was injected into dams immediately after a pup exposure, long term retention was blocked (whereas injection 24 hours after exposure had no effect). This procedure has been postulated to inhibit the consolidation of memories at a molecular level (Davis & Squire, 1984), although it is not clear whether this involves altering proteins, enzymes, or neurotransmitters.

One possible neurotransmitter system that may be involved in forming maternal memories is the dopamine system. This neurotransmitter has been implicated in processes of reinforcement within several behavioral contexts including feeding and sex (Bechara, Harrington, Nader & van derkooy, 1992; Wise & Rompre, 1989). Because rat pups become more reinforcing to the mother rat through interactive experience with them, it seems likely that dopamine would play a role. Fleming, Korsmit &
Deller (1994) explored the effects of blocking dopamine on the development of a conditioned place preference (for pups). They found that a systemically administered dopamine antagonist eliminated the conditioned place preference effect. It is interesting to note that the dopaminergic mesolimbic system originates in the VTA, an area also thought to be important in maternal behavior. Also, there are extensive neural connections from this system to the amygdala (Everitt & Robbins, 1992). This is notable for a number of reasons: 1) the amygdala is rich in noradrenergic innervation (a neurotransmitter system thought to be involved in learning and maternal; see above), 2) lesioning the amygdala elicits quick onset of maternal behavior in virgin female rats (also see above), and 3) the amygdala is thought to be involved in the memory for events with affective valence (Cahill & Mcgaugh, 1996; Phelps & Anderson, 1997).

**Neuroanatomy of maternal experience.** Recently there has been an effort to identify the structures involved in
the maternal experience effect. It seems likely that the experience effect involves many of the same processes involved in learning and memory in other behavioral contexts. Modney and Hatton (1990) have found structural and functional changes in the supraoptic nucleus that occur with suckling experience. Specifically, they found the formation of new synapses and an increase in electrical coupling among neurons in this nucleus. Further, the development of these changes appeared to correlate with the onset of maternal behavior. Also, Xerri et al. (1994) have found that there are alterations of the cortical representation of the rat ventrum induced by nursing behavior.

Fleming and colleagues have attempted to lesion or block the activation of neural sites (with cycloheximide) thought to be involved in memories within other behavioral contexts (including the dorsal hippocampus, the prefrontal cortex, and the central and basolateral nuclei) as well as amygdaloid sites that are thought to
process sensory information and are responsive to hormones (medial and cortical nuclei) (Fleming et al., 1996). They have been mostly unsuccessful in their attempts to block the maternal experience effect with lesions or cycloheximide infusions to any one site, although some recent evidence (Lee & Fleming, in press) suggests that cortical amygdala lesions increase reinduction latencies in maternally experienced animals. However, studies showing the activation of multiple brain sites using c-fos staining (see above) would suggest that either maternal learning is mediated by different systems than are other types of learning or there is considerable redundancy in control mechanisms. Alternately, because of the complexity of the pup cues and of the task, multiple systems may be activated to consolidate the experience.

Fleming and colleagues have also used the c-fos paradigm to explore which brain regions might be involved in the storage or retrieval of a maternal memory (Fleming & Korsmit, 1996). They allowed animals to interact with
pups for 1 hour (experienced) or not, one day postpartum. At day 5 postpartum they exposed the dam to pups or pup-associated cues (hence, only conditioned stimuli) for another 2 hours (retention phase), after which brains were removed and c-fos immunohistochemistry was performed. They found Fos expression in the MPOA, basolateral amygdala and parietal cortex only in postpartum animals that had had a previous pup experience and interacted with pups at retention. Subsequently, they extended this study by adding a group receiving distal pup cues and providing 4 hours of experience 10 days after initial exposure. The results were essentially the same as the previous findings. However, there was also elevated Fos expression in experienced animals in response to distal pup cues. They interpreted this result to mean that through an association with the unconditioned (somatosensory?) stimuli present during physical interactions with pups, distal pup cues become conditioned stimuli than can then activate "the final
common path" for the expression of maternal behavior. This result led them to question whether the MPOA is part of the effector system for the expression of maternal behavior, or part of the motivational system.

In summary, it seems logical to infer that the maintenance of maternal behavior must be mediated through long term changes in neural structures important for maternal behavior elicited by the interaction of sensory input and hormonal priming, or prolonged sensory input alone. This probably involves a mechanism of cellular changes. The next section introduces a procedure that induces cellular changes thought to be similar to those involved in memory formation.

**The Stimulation Procedure**

The studies herein use a stimulation procedure following the parameters necessary to partially kindle neural substrates. Kindling is a procedure of systematic administration of electrical or chemical stimulation of
the brain that cumulatively over time produces epileptiform electrical afterdischarges and corresponding motor seizures (Cain, 1985). The term kindling, coined by Goddard, refers to the progressive nature of motor seizures that vary systematically with successive stimulations (Goddard, McIntyre & Leech, 1969; Racine, 1978). Traditionally, the procedure is to unilaterally stimulate a specific site once or many times a day until at least one class five motor seizure (motor seizure with the loss of balance) or characteristic afterdischarges are recorded (Racine, 1972). Most brain sites will produce the kindling phenomena, but they vary in the number of stimulations necessary to produce the class five motor seizure (Majkowski, 1986; Racine, 1978). Kindling has been used as a model of epilepsy and plasticity in the brain (Cain, 1992; Majkowski, 1986; Morrell & de Toledo-Morrell, 1986; Racine, 1991).

Kindling serves as a model of neural plasticity because the repeated administration of an initially sub-
threshold stimulation becomes sufficient to produce motor seizures and this lowering of threshold remains for many months if not for the lifetime of the subject (Cain, 1992; Racine, 1978; 1972; Moshe & Albala, 1982; Goddard et al., 1969). Synaptic changes that are thought to occur as a result of kindling have been compared to the cellular changes that occur under a long-term potentiation paradigm (Racine, 1991; Majewski, 1986). Differences exist between long-term potentiation and kindling, in terms of stimulation frequency, duration, and responses. However, the basic underlying mechanisms involved in these two examples of neural plasticity are thought to be similar (Berman, 1991; Cain, 1989). Both mechanisms are thought to somehow enhance the synaptic connections between the neurons. Some of these possible cellular mechanisms have been suggested to be: increased neurotransmitter release/production; changes in the synthesis of specific macromolecules; or increased protein synthesis (Cain, 1990; 1989; Racine, 1991; 1978).
These ideas will be expanded on in the next section.

The cellular and molecular basis of kindling development. Kindling induces an enhanced propagation of afterdischarge to and a subsequent reduced threshold of current necessary to produce afterdischarges in cells adjacent to the kindling focus. This hyperexcitability of cells in the kindled brain is thought to involve a modification of synapses at the kindling focus (McNamera, 1995). Modification of these synapses could represent altered efficacy of pre-existing synapses and/or formation of novel synapses. Evidence for both of these possibilities has emerged. In regard to altered efficacy of preexisting synapses, evidence has emerged implicating enhanced function of glutamatergic synapses in the kindled brain (McNamera, 1994; McNamara, Bonhaus, Nadler & Yeh, 1990). An alternate, and not mutually exclusive, possibility is that structural rearrangements underlie hyperexcitability. Sutala and colleagues (Sutula et al., 1998; Sutula, He, Cavazos & Scott, 1988) and Nadler,
Perry & Cotman (1980) have discovered that kindling is accompanied by sprouting of the mossy fiber axons of the dentate granule cells of the hippocampus. Whether such sprouting is unique to the granule cell axons is unclear, although it seems likely that such sprouting exists in all sites of kindling focus (but may be more difficult to detect).

Included in the synaptic events undoubtedly occurring during an afterdischarge is the activation of glutamate receptors, since glutamate receptor antagonists can block focal seizures (Traynesis & Dingleidne, 1988). A recent study, using microdialysis, showing increased levels of glutamate during seizures, is also compelling evidence for the involvement of the excitatory neurotransmitter glutamate in kindling (Minamoto et al, 1992). Further, the particular receptor involved in kindling development appears to be the N-methyl-D-aspartate (NMDA) subtype, since kindling development is retarded in the presence of NMDA receptor agonists (Cain,
Desborough & McKitrick, 1988; Vezzanni, Wu, Moneta & Samanin, 1988; McNamera et al., 1990). Morgan & Curren (1986) and Goelet, Castellucci, Schacher, Kandel (1986) have suggested that the activation of NMDA receptors may induce long-term structural and functional changes indicative of kindling through the transcriptional activation of intermediate early genes (such as c-fos). A number of labs have found that the induction of even minimal afterdischarges can elicit Fos expression (Morgan, Cohen, Hempstead & Curran, 1987; Dragunow & Robertson, 1987; Simonato et al., 1991).

Another mechanism proposed to account for the kindling phenomena is that inhibitory systems have failed. One of the most reliable ways of inhibiting epileptiform discharges is by using GABA agonists (Meldrum, 1975). However, kindling studies have found that GABA antagonists do not facilitate kindling (Burnham, 1989; Kalichman, Livingston & Burnham, 1982), and there is little change in extracellular GABA levels.
during or after stimulation (Minamoto et al., 1992).

A number of investigators have suggested that the cellular and molecular mechanisms by which physiologic activity lead to normal synapse formation in the developing or learning nervous system provides a useful framework for thinking about mechanisms underlying formation of kindling (Morrell & DeToledo-Morrell, 1986; Racine, 1991; Majowski, 1986). Majkowski (1986) suggests that the neuronal plasticity produced by kindling and expressed in evoked potential changes represents a more general process, like in long term potentiation, and similar to changes related to the process of learning and memory. Further, the neurophysiological changes occurring during kindling appear to be similar to the processes occurring in long-term potentiation.

Long-term behavioral changes associated with kindling stimulation. Lasting behavioral changes have been observed in animals following kindling (Adamec, 1997; Adamec & Morgan, 1994; Adamec, 1975; Goddard,
1980). In particular emotional changes have been induced by kindling neural structures thought to be involved in emotion. Adamec and Stark-Adamec (1983) found that the spread of kindled seizure activity to the amygdala and the amygdala-ventromedial hypothalamic pathway (AM-VMH) is critical for the development of enhanced defensiveness. Pinel, Treit & Rovner (1977) found that kindling of the rat amygdala resulted in an increase in reactive response to tail tap or resistance to handling, and Adamec (1990) found that rats kindled in the medial amygdala were less likely to explore the open areas of an elevated plus maze. More recently, Adamec and Morgan (1994) demonstrated that kindling the medial amygdala was anxiogenic and kindling the lateral amygdala was anxiolytic in male rats, and Kalynchuk, Treit & Kippin (1997) have shown that kindling either the basolateral amygdala or the central amygdala induced anxiolysis in the rat. Kindling has also been used to facilitate other behaviors such as aggression, passive avoidance,
muricide, and ranicide (Bawden & Racine, 1979) and recently, the acoustic startle response (N’Gouemo & Faingold, 1997).

Behavioral changes have also been induced using partial kindling, a procedure whereby the neural substrate is induced to produce afterdischarges without (and usually before) the display of motor seizures. Adamec (1991) caused suppression of feline aggression by partial kindling of the amygdala, and the results were interpreted as altering the emotionality and personality of the subjects. Also, Paredes et al. (1990) investigated whether widespread modification of brain function produced by kindling could induce sexual behavior in non-copulating rats. They found that male rat copulatory behavior was facilitated in previously non-copulatory male rats by partially kindling the MPOA. They proposed that sexual behavior was facilitated in these rats by local neural changes produced by (partial) kindling. These results are of particular interest to the proposed
research. Since Paredes and colleagues found that sexual behavior could be induced by partial kindling, and the MPOA is also important for the expression of maternal behavior in female rats, it is proposed that partially kindling the MPOA may enhance the expression of maternal behavior.

In view of the plastic changes resulting from kindling and changes that have been produced in non-convulsant behaviors we predicted that electrical stimulation using parameters used to induce partial kindling in rats would produce changes in maternal responsiveness.

**The Present Study**

Based on the literature presented above on maternal behavior there are a number of interesting issues that remain unanswered and some of these issues constitute the focus of this thesis.

The present studies investigated the functions of
the medial amygdala and the MPOA using a partial kindling stimulation procedure. These two nuclei were selected because they exert inhibitory and excitatory roles (respectively) on maternal behavior. However, the precise function of these sites is not clear. With regards to this statement, I address four questions that constitute the focus of this thesis: (1) is the MPOA involved in simply coordinating an effector response or changing the animal's affective and/or motivational response (toward pup stimuli), or both? (2) is the medial amygdala involved in the general change in affective/motivational responses or is the affective/motivational change pup specific, (3) how do long term changes in these nuclei affect the expression of either (or both) of these (above) responses and (4) how are the MPOA and the MedAmyg related?

Specifically, this thesis examined whether chronic stimulation of the MPOA or the MedAmyg can induce an onset or long term change in maternal behavior and in
associated responses toward pup cues.

Since the stimulatory changes that are induced by the kindling-like procedure of stimulation are proposed to be relatively permanent (Cain, 1992; Racine, 1978; 1972; Moshe & Albala, 1982; Goddard et al, 1969), I looked at the relatively long-term changes in maternal responsiveness that this stimulation might produce. Also, there are other functions in which the MPOA and MedAmyg are implicated which affect maternal behavior (i.e. affective changes, changes in emotion-based memories, and exploratory tendencies).

The initial studies were conducted to assess the validity of the hypothesis that kindling-like stimulation can activate and change the substrates and their associated behaviors: the function of both the MPOA and the MedAmyg in the onset and retention of maternal behavior. The first study assessed whether stimulating the MPOA or the MedAmyg can facilitate and inhibit, respectively, the onset of maternal behavior in
previously experienced and hormonally primed animals. The second experiment investigated whether stimulating neural substrates of maternal behavior (MPOA or MedAmyg) facilitated or inhibited maternal behavior in inexperienced (virgin) and non-hormonally primed animals. To assess whether the MPOA and the MedAmyg may act with respect to 'motivational', as well as simple effector processes, the animals were also be assessed for the attractive and reinforcing value of the pups and other behaviors thought to reflect the motivational or emotional state of the animal.

Throughout this paper use of the term 'emotionally' refers to the performance on the open field task. This task involves a well established method for measuring anxiety in the rat. This measure has been found to be sensitive to known anxyolotic drug administration in rats (Angrini, Leslie & Shephard, 1998; Broaderick, Hope & Jeannot, 1998; Nazar Jessa & Plaznik, 1997). It has recently been used to investigate the effects of
References to maternal 'motivation' reflect performance on the hole-board test, and the conditioned-place preference test. The validity of head-dipping as a measure of motivation to explore was reviewed by File and Wardill in 1975. This measure has been used to investigate exploratory motivation in animal models of depression (O'Connor & Leonard, 1998), and anxiety (Adamec & Morgan, 1994; Adamec, 1990; Crawley, 1985; Takeda, Tsuji & Matsumiya, 1998). Recently, Mayer and Rosenblatt (1993) modified this apparatus to investigate a rat's motivation to seek pup odors. They found that lactating females preferred to head-dip in holes containing pup odors (versus non-odored holes in the same apparatus). The conditioned place preference test is a well established measure of learned associated preference.
for location. Animals have been trained, using this apparatus to prefer sections of the apparatus associated with opioid administration (Kim et al., 1998; Kim et al., 1997; Kim, Jang & Park, 1996; Vezina & Stewart, 1987) and sexual partners (Agmo, Rojas & Vasquez, 1992; Meisel, Joppa & Rowe, 1996; Miller & Baum, 1987). Recently, Fleming, Kuchera, Lee & Fleming (1994) used this apparatus to measure the reinforcement value of rat pups to their mother (dam). They found that the dam learned to prefer a box where she had been allowed pup contact versus a similar, but distinct she had experienced without pup contact. The same procedures were used in this experiment.

Experiment three was designed to investigate the interaction of pup experience and electrical stimulation on the neural substrates involved in the maternal sensitization of virgin (non- hormonally primed) rats. It was hypothesized that pup exposure and MPOA stimulation would have an additive effect, inducing a quicker onset
of maternal responsiveness. Experiment three also investigated the specificity of motivational changes. We wanted to find out if MedAmyg and MPOA stimulation would change the animals' motivation toward pup and other biologically relevant stimuli. Finally, experiment four was designed to investigate the functional relationship between the MPOA and the medial amygdala on maternal behavior (in previously experienced hormonally primed rats) using electrical stimulation of these nuclei in isolation of each other. If MedAmyg attenuation of maternal behavior is necessary on its connections with the MPOA, then stimulating the MedAmyg in isolation would have no effect on maternal responsiveness.

General Methods

Subjects

Subjects were Sprague-Dawley females, with body weights varying from 250-300g at the beginning of the experiment. Subjects were bred at Erindale College,
University of Toronto, from a stock obtained from Charles
River Laboratories (Quebec, Canada).

Females were housed in plastic cages (22x45x15cm) with wood shavings as bedding. Purina rat chow and water were continuously available. Temperature in the room was thermostatically controlled at 22 degrees Celsius with a 12:12 hour light cycle (lights on at 8am). One day prior to maternal induction testing, the subjects were transferred to larger plastic cages (37x47x21cm), wood shavings served as bedding and two pieces of paper towel were added for nesting material.

Surgeries and Electrical Stimulation Procedures

All groups received right unilateral chronic electrode implants. Electrodes were bipolar coated stainless steel obtained from Plastics One, with a wire diameter of 0.2mm (model MS303/2; Roanoke, Va.). The electrodes were affixed with dental cement and three stainless steel screws (screwed to the skull). Somnitol
60mg/100mLs (Astra Pharmaceutical, Mississauga, Canada) was administered to the subjects (1mL/kg) in preparation for surgery. Stereotaxic coordinates were calculated using Paxinos & Watson (1986) atlas: MedAmyg - ML -3.1, DV -9.2 (from skull), AP -2.5 and MPOA - ML +0.3, DV -8.6 (from skull), AP -0.4. All coordinates were from bregma. A seven day recovery period was given following the surgery.

Stimulation was given once daily for each subject. Animals were individually transferred from their home cages to the stimulation cages for each session. Control (not stimulated) subjects were connected to the stimulator but no current was delivered. A Grass Instruments (Boston, Mass.) SD9 stimulator, modified for constant current delivery, was used. Stimulation consisted of a 2 second biphasic pulse train with an initial current of 300 µA increased to 500 µA after the first five stimulations. The frequency of the pulse was 60 Hz and a pulse width of 1 ms. Stimulations were
continued for 14 consecutive days. Animals that exhibited a stage 4 seizure (according to Racine's classification) or greater at any point during the stimulation treatment were eliminated from the study. After the 14th day of stimulation subjects were permitted to rest for 7 days.

**Calibration of Stimulation Regimen**

Electrophysiological monitoring of the stimulation was performed in two additional groups of animals. Virgin females from the same colony were either stimulated in the MedAmyg (n=4) or the MPOA (n=3). Recording electrodes were also placed in both the MPOA and the MedAmyg in all animals. Generalized epileptiform afterdischarges (ADs) were recorded using a Grass Model 7 polygraph.

For MPOA stimulated animals the duration of ADs in the MPOA ranged from 0-6 seconds at day 1 of stimulation to 11-20 seconds at day 14. There was a change in AD from 0 seconds at day one to a range of 0-10 seconds at day 14 from the MedAmyg in MPOA stimulated animals (see figure
91

1 for a graph of the medians; the top of figure 2 shows
an example of AD from the MPOA and MedAmyg at day 7 and
14). One animal showed no AD from either site at day 14
and no animals showed behavioral seizures.

There was a larger increase in AD duration in
MedAmyg kindled animals. Recording from the MedAmyg there
was an increase from 0-10 seconds at day 1 to 45-60
seconds at day 14. There was a parallel development of
increase in AD duration in the MPOA for MedAmyg
stimulated animals from 0-10 seconds at day 1 to 45-55
seconds at day 14 (fig. 1 shows medians; the bottom of
figure 2 shows an example of AD from the MedAmyg and MPOA
at day 14). All animals kindled in the MedAmyg showed ADs
at day 14 and two animals showed behavioral seizures
(stage 3).

____________________

Insert figures 1 & 2 about here

____________________

The results of this kindling calibration suggest
Figure 1
REPRESENTATIVE AFTER DISCHARGES

MED AMYG STIM

MPOA

MED AMYG

DAY 7

2 sec.

DAY 14

MPOA

MED AMYG

MPOA STIM

MED AMYG

MPOA

DAY 7

DAY 14

Figure 2
that the stimulation parameters used in this study produce reliable ADs. However, the MedAmyg more readily kindled than the MPOA. Further, the kindling stimulation of the MedAmyg is more likely to carry-over (in AD) to the MPOA than vice versa. This suggests that there are stronger connections from the medial amygdala to the MPOA than the other way around.

**Behavioral Testing**

**Pup induction testing.** In order to test the latencies to become maternal, pup inductions were performed according to the procedure used by Orpen and Fleming (1987). One day after being transferred to larger observation cages, females were tested for maternal behavior on each subsequent morning. Daily testing was continued until a female was designated as maternal or for 10 days. Prior to each test for maternal behavior, any foster pups from the previous day were removed. Then, six recently fed foster pups, 1-4 days of age were taken
from a donor mother and placed in the front right quadrant (or in the quadrant opposite to the nest site if there was one) of the test female's cage. The behavior of the test female was recorded for the following eight minutes using a checklist. The eight minute checklist was divided into 96 intervals lasting 5 seconds in which the following behaviors were recorded using a 1/0 sampling procedure: retrieve pups (pups were picked up and moved to the nest site), groom (dam was observed grooming self), lick (dam was observed licking pups), genital lick pup (dams were observed holding pup’s posterior dorsal end up and licking pup’s anogenital region), crouch over pup (animal is observed with all six pups under her ventrum, but not necessarily in a lactating posture), sniff pup (dam was observed to approach and sniff pup) sniff air (dam was observed to sniff air, bedding or dig), nest build (dam was observed to use bedding, or paper towel to construct a nest site in one quadrant), rest/sleep (dam was observed to settle, lie or sleep away
from pups. Spot-checks for maternal behavior were conducted at 3 hours and 6 hours after the initial 8 minute observation (during which the dam was noted to be either in a crouch posture over the pups or not). An animal was considered maternal if she retrieved all 6 pups during the eight minute observation period on two consecutive days and was observed in a crouch posture (either during the observation period or spot check) on at least one of those days. Animals were given a maternal rating that corresponds to the first day of the two day maternal criteria. The first day of maternal induction testing was designated as day zero, animals not designated as maternal by the 10 day limit are assigned a maternal rating of day 11.

**Emergence and open field testing.** An open-field apparatus was used to test 'anxiety' and involved exploration of a novel environment. This consisted of a start box, with a 11x13 cm gate leading directly into a 150x150 cm open-field arena with a black plexiglass floor
and black wooden walls. The floor area was marked off into 100 15 cm squares. Illumination was provided by a single 40-W light bulb centered over the field and suspended 200 cm from the floor of the field. Prior to each test, the start box, sliding door, and arena walls were thoroughly wiped with 70% alcohol.

Emergence test. The subject was placed into the start box for a five minute period, with the gate closed. The emergence test started when the gate was raised and the female was permitted to enter the open-field. Once the female entered the field with all four feet the gate was closed and the open-field test was initiated. If the female had not entered the field within 15 minutes, she was placed into the field in front of the closed door and the open-field test was begun. The time it took the female to enter the field with all four feet was called the emergence latency. If the female failed to enter the field a maximum score of 901 seconds was given. Longer latencies were considered to reflect greater timidity or
'anxiety'.

**Open field test.** The movements of the female were monitored using a data sheet that was divided into 100 squares for a 5 minute period. An animal was said to enter a square when all four feet had crossed over from an adjoining square. It was assumed that the higher the ambulation score obtained, the less 'neophobic' the animal was. Further analysis investigated the ratio of central squares crossed (squares not touching the walls of the box) with all squares (peripheral squares – squares along the walls of the box plus central squares). The animal was also considered less neophobic if it was more willing to cross the center squares.

**Conditioned place preference testing.** The conditioned place preference test was designed to measure the reinforcement value of the pups to the female subject (Fleming et al., 1994). The apparatus consisted of two white plexiglass boxes (21.7x39.5x30.2 cm). Horizontal and vertical environments were created within the CPP
boxes using black electrical tape spaced 2 cm apart along the walls and roof of the box allowing for an equal amount of 'darkness' to be experienced in both boxes. A complete CPP box consisted of one horizontal environment, one vertical environment, and one narrow section between them (this section had no pattern). The three sections were separated by dividers. On test days the dividers were removed allowing the mother uninterrupted exposure to both environments.

**Exposure phase.** Females were randomly assigned to either the horizontal or the vertical box on their first day of being run in the CPP boxes. On days 1 and 3, females were placed in the CPP boxes with pups for 1 hour. Spot checks were made every 10 minutes. On days 2 and 4, females were placed in CPP boxes, which were the opposite ones from days 1 and 3, without pups for 1 hour but did receive exposure to pups in their home cages for 1 hour later in the day. An observation sheet was used to record the location of the female (and behaviors she was
performing) and pups while in the box. The following behaviors were recorded: crouch over pups (lactating posture or other), lie in near pups, and settle (away from pups).

**Testing phase.** Females were tested for the development of a pup preference on day 5 by removing the dividers between boxes thus allowing them access to both boxes so that they could indicate their preference for either the pup-box or the no-pup-box. The duration of the preference test was 10 minutes during which time the frequency of the female's entries into the boxes and time spent in each box was recorded. If the female spent more time in the box associated with pups than the alternate box she was considered to have a preference for pup stimuli.

**Hole board testing.** The hole board was used to measure exploratory behavior and attraction to pup odor cues. The test consisted of a square wooden box, 60 cm on a side, with four sides rising 35 cm above the floor of
the box. There were 4 evenly spaced holes drilled in the floor of the box, which was elevated 12 cm above the ground. The holes were drilled at the corners of a square drawn on the inside of the box whose sides were 14 cm from the walls of the hole board. The box was painted with flat grey enamel paint.

The hole board was originally designed to measure activity and exploratory behavior (File & Wardill, 1975), however, it was modified to test for preference of distal pup cues in a manner similar to that explained by Mayer & Rosenblatt (1993).

Testing began when the female was placed in the center of the hole board. A number of behavioral measures were taken: 'head dipping' (a measure of exploratory tendency taken as number of times head was placed into any hole in the board), and 'ratio time center' (the ratio of time animal spends in the center of the board versus along the sides is taken as an emotionality measure). In the modified version of the test two of the
holes (diagonally situated) have bedding with pup odors under them and the other two holes have non-scented bedding under them (placement is rotated after each test). The ratio of both frequency and time spent head dipping in the pup-scented versus the non-scented holes was called 'odor head dip'. The more an animal head dipped into a hole that had pup-odored bedding, the more attracted she is to pup-cues.

**Histology**

After all behavioral testing concluded, females were anaesthetized with a lethal dose (1 mL) of somnitol 60mg/100mL and perfused. Their brains were removed for histological confirmation of stimulation locus. Electrode placement was considered on target if the lowest and most central point was measured within .2mm (+/−) of intended coordinates. Given these parameters, for experiment 1, 73% (or 16 of 22) of MedAmyg animals and 64% (or 14 of 22) of MPOA animals were on target. In experiment 2, 82%
(or 23 of 28) of MedAmyg animals and 74% (or 29 of 37) of MPOA animals were on target. In experiment 3, 92% (or 24 of 26) of MedAmyg animals and 83% (or 24 of 29) of MPOA animals were on target. In experiment 4, 83% (or 25 of 30) of MedAmyg animals and 74% (or 23 of 31) of MPOA animals were on target. Animals not on target were eliminated from the study for both stimulated and not stimulated conditions.

Also, to rule out the occurrence of seizure-induced neurotoxic effects, hippocampal atrophy was analyzed (since the hippocampus is particularly susceptible to seizure-induced brain damage). Three randomly selected animals in each condition (not stimulated, MPOA stimulated, MedAmyg stimulated) were analyzed in each experiment for area and cellular density (at AP coordinate -2.8).

The hippocampus of each section was examined under a light microscope on a magnification of 10x15. A reticule containing a grid measuring 10x10 squares (1mm²)
was inserted into the microscope. Using grid measurements based on the stereotaxic atlas of Paxinos and Watson (1986) the area of the hippocampus was measured at AP coordinate -2.8. Also, placing the right edge of the grid against the midline and the bottom edge touching the most ventral boundary of the dentate gyrus, a count was made of stained cell bodies in a section of the left hippocampus. There were no differences between any groups in any experiment.
Experiment One: The Effects of Multiple Electrical Stimulation of the MPOA and the MedAmyg on the Long-term Maternal Responsiveness of Postpartum Female Rats.

Animals that have previously become maternally responsive will become responsive again more rapidly than maternally naive animals (Fleming et al., 1996; Numan, 1994). We have found that postpartum pup experience will reduce the reinduction of maternal behavior one month later compared to postpartum animals with no pup experience. This first study investigated whether stimulation of a previously primed substrate can reactivate its associated maternal behavior (whether inhibitory or facilitatory). It was hypothesized that stimulation within the kindling parameters would reactivate the neural substrate and that reactivation would be long lasting. Specifically, it was predicted that kindling-like stimulation of the MPOA would facilitate maternal responsiveness quickly in cycling
multiparous animals that had not been with pups for at least 30 days; inhibitory influences of the MedAmyg on maternal behavior were expected to follow stimulation, producing a longer latency to become maternal.

**Method**

All of the animals in the following methods were allowed 5 days of exposure to their pups immediately after birth. This time period was based on pilot testing. Postpartum animals who were exposed to pups for 5 days immediately after birth had higher reinduction latencies than animals with 10 days experience, but lower latencies than animals without any pup exposure one month later. Therefore, 5 days experience with pups postpartum was considered to be the optimal period to display an increase or decrease in latencies by stimulation (see Appendix 1).

The methods of this experiment followed those outlined in the General Methods section. Forty-four
female rats (250-300g) were assigned to one of four electrode implant groups (n=11). Fourteen animals were excluded from the experimental results on the basis of histological analysis. Final groupings are displayed in figure 3: MPOA stimulated (n=8), MPOA not stimulated (n=8), MedAmyg stimulated (n=8), and MedAmyg not stimulated (n=6). All animals were impregnated, and after birth were allowed 5 days of unrestricted contact with their pups. On day 6 postpartum the pups were removed and right unilateral bipolar electrodes were implanted; this was called experimental day 1. After surgery the animals were given a one week recovery period. The animals were given kindling-like stimulation from experimental days 7-21. On experimental day 30 maternal induction testing was initiated (using foster pups) to determine level of maternal responsiveness.

Insert figure 3 about here
EXPERIMENT 1

PARTURITION

5 DAYS CONTACT WITH PUPS

DAY 1
PUPS REMOVED
ELECTRODES IMPLANTED

GROUPS

MPOA STIM (n=8)
MPOA NOT STIM (n=8)
MED AMYG STIM (n=8)
MED AMYG NOT STIM (n=6)

DAY 7 - 21
ELECTRICAL STIMULATION

DAY 30
MATERNAL INDUCTION TEST

Figure 3
Two-way analyses of variance (ANOVA) were conducted to test for the effects of stimulation status (stimulated/not stimulated); electrode location (MPOA/MedAmyg) and their interaction on maternal behaviors. The data were further explored by one way analyses of variance (provided in {braces}). Bonferroni post-hoc tests were conducted to assess differences between groups. Bonferroni post-hoc tests perform pairwise comparisons between group means, but controls overall error rate by setting the error rate for each test to the experimentwise error rate divided by the total number of tests. Hence, the observed significance level is adjusted for the fact that multiple comparisons are being made.

Results

Figure 4 illustrates that electrode location and stimulation status both affected latency to become maternal \( F[1,29]=7.96; p<.01 \), one-way
ANOVA: (F[3, 26] = 3.73; p < .03). Post-hoc testing revealed that the MedAmyg stimulated animals showed longer latencies to become maternal than did the MPOA stimulated animals (p < .05) and than did MedAmyg not stimulated animals (p < .05). However, there were no significant differences between MPOA stimulated and MPOA not stimulated groups. Also, there were no significant differences between the not stimulated groups. There was a similar interaction for latency to retrieve pups (F[1, 29] = 5.47; p < .03; figure not shown) (one-way ANOVA: F[3, 26] = 3.8; p < .02); post-hoc tests yielded the same group differences.

An interaction was also observed for latency to crouch or hover over pups observed during the spot-checks (F[1, 29] = 8.57; p < .01, figure 5) (one-way ANOVA: F[3, 26] = 3.81; p < .02). The MedAmyg stimulated animals showed longer latencies to crouch over pups than MedAmyg not stimulated animals (p < .05), and than MPOA stimulated animals (p < .05). There were no differences between the
MPOA stimulated and MPOA not stimulated groups, and the MPOA groups did not differ from the MedAmyg not stimulated group.

Insert figures 4 & 5 about here

A comparison of the mean frequency for behaviors observed during maternal latency testing (Table 1) showed that there was a significant interaction for the amount of licking performed in the last two days of induction ($F[1,29]=4.25; p<.05$) (one-way ANOVA: $F[3,26]=3.1p<.05$). The MedAmyg stimulated animals were licking pups more than MedAmyg not stimulated (p<.05) and MPOA not stimulated animals (p<.05). It seems that although the MedAmyg stimulated animals showed longer latencies to become maternal they were licking the pups more than the other groups at the end of the induction period.
LATENCY TO BECOME MATERNAL

![Bar chart showing latency in days to become maternal for STIM and NOT STIM conditions in MPOA and MED AMYG regions.](chart)

Figure 4
LATENCY TO CROUCH OR HOVER OVER PUPS

Figure 5
Histological analysis of the brain tissue indicated that the location of the electrode placements was fairly well restricted to the intended sites (Figure 6).

One animal in the MedAmyg kindled group showed a stage 2 seizure during the stimulation period. Analysis was re-conducted without this animal. The results did not differ except the effect on pup-licking became marginally significant (p=.1). No animals had any seizures after the stimulation period.

**Discussion**

These results indicate that kindling-like
Table 1: Daily mean frequencies (+SEM) for behaviors observed during maternal latency (induction) testing in Experiment One (for first two [1st] and last two days [fin]).

<table>
<thead>
<tr>
<th></th>
<th>MPOA/ stim</th>
<th>MPOA/ not stim</th>
<th>MedAmyg/ stim</th>
<th>MedAmyg/ not stim</th>
<th>Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniff air (1st)</td>
<td>58.2 (4.9)</td>
<td>70.0 (3.8)</td>
<td>62.9 (4.3)</td>
<td>66.7 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Sniff air (fin)</td>
<td>50.9 (3.1)</td>
<td>53.3 (2.7)</td>
<td>45.6 (4.5)</td>
<td>55.7 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Sniff pup (1st)</td>
<td>5.3 (1.7)</td>
<td>3.2 (1.4)</td>
<td>2.6 (1.6)</td>
<td>3.2 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Sniff pup (fin)</td>
<td>9.0 (1.8)</td>
<td>10.5 (1.8)</td>
<td>12.5 (3.6)</td>
<td>4.4 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Groom (1st)</td>
<td>9.8 (2.3)</td>
<td>10.1 (2.2)</td>
<td>9.7 (2.6)</td>
<td>12.2 (4.5)</td>
<td></td>
</tr>
<tr>
<td>Groom (fin)</td>
<td>12.1 (2.6)</td>
<td>8.5 (2.0)</td>
<td>13.8 (3.1)</td>
<td>14.7 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Lick (1st)</td>
<td>5.9 (1.1)</td>
<td>5.0 (1.5)</td>
<td>2.3 (1.8)</td>
<td>3.3 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Lick (fin)</td>
<td>8.2 (1.8)</td>
<td>10.0 (1.3)</td>
<td>16.1 (3.5)</td>
<td>8.9 (2.9)</td>
<td>MA/S&gt; MA/NS, MPOA/NS (p&lt;.05)</td>
</tr>
<tr>
<td>Genital lick (1st)</td>
<td>1.8 (1.1)</td>
<td>0.7 (0.5)</td>
<td>0.9 (0.8)</td>
<td>2.2 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Genital lick (fin)</td>
<td>4.1 (1.6)</td>
<td>2.8 (0.8)</td>
<td>4.8 (1.6)</td>
<td>3.7 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Retrieve (1st)</td>
<td>3.6 (0.8)</td>
<td>2.7 (0.9)</td>
<td>2.2 (1.1)</td>
<td>4.3 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Retrieve (fin)</td>
<td>5.2 (0.6)</td>
<td>6.3 (0.2)</td>
<td>5.5 (1.3)</td>
<td>6.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Crouch (1st)</td>
<td>2.1 (2.0)</td>
<td>1.6 (1.8)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Crouch (fin)</td>
<td>5.7 (3.4)</td>
<td>6.5 (4.9)</td>
<td>19.8 (8.9)</td>
<td>3.8 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Nest build (1st)</td>
<td>1.0 (0.6)</td>
<td>0.2 (0.2)</td>
<td>0.0 (0.0)</td>
<td>0.7 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Nest build (fin)</td>
<td>1.8 (0.6)</td>
<td>3.6 (1.5)</td>
<td>1.7 (0.7)</td>
<td>1.7 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Settle (1st)</td>
<td>0.0 (0.0)</td>
<td>3.6 (3.5)</td>
<td>10.3 (6.9)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>
| Significant
Differences |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOA/ stim</td>
</tr>
<tr>
<td>Settle (min)</td>
</tr>
</tbody>
</table>

MPOA - medial preoptic area
MA - medial amygdala
S - stimulated
NS - not stimulated
stimulation of the MedAmyg inhibits the emergence of maternal behavior in maternally experienced animals. These findings are consistent with previous research indicating that the medial amygdala has an inhibitory function. Apparently, this function was enhanced by the stimulation pretreatment. The MPOA stimulation did not produce a significant functional enhancement, although the stimulated animals did show a trend to lower latencies, suggesting that an initial pup experience has primed the substrate for more efficient reactivation at another time.

The inability of MPOA stimulation to significantly reduce maternal latencies may have a number of explanations. First, this may reflect a decreased responsiveness to the stimulation (the MPOA kindles more slowly than the MedAmyg). Had we stimulated the structure over a longer period of time, behavioral facilitation may well have occurred. Second, the absence of a significant MPOA effect may reflect a floor effect since the MPOA
animals that were not stimulated showed very low maternal onset latencies, leaving little room for further enhancement. Third, it is also possible that the electrode tract for MPOA animals partially lesioned the nucleus. This may have resulted in a decrease in maternal responsiveness that was in turn partially compensated for by stimulating the nucleus. Finally, it is possible that the electrical stimulation had no enduring effect for a significant number of MPOA stimulated animals. Indeed, it should be pointed out that one third of the MPOA stimulated animals in the calibration phase developed no afterdischarges.

MedAmyg stimulated animals did not have longer latencies to onset of maternal behavior than did MPOA not stimulated animals. It is possible that lowering the electrode into the MPOA may partially damage some neurons in the nucleus for this group, also. Hence, electrode placement without stimulation may have had a minor inhibitory effect. The inclusion of a control group
without implants may have addressed this problem. Unfortunately, this group was not included.

The fact that MedAmyg animals licked pups more may reflect the condition of the animal: that is, an animal that is an experienced mother (and therefore somewhat responsive to pup stimuli), but stimulated to find pup stimuli aversive. Hence we see an uncoordinated response, an animal that will not retrieve pups but will lick them; further, a greater amount of licking may be reflective of a compensatory behavior to reduce the animal's inclination to behave maternally.

To test whether kindling-like stimulation of the MPOA or MedAmyg of a 'non-primed' or inexperienced animal (both hormonally and with pups) can facilitate or attenuate the expression of maternal behavior, the next experiment used the same paradigm to investigate maternal behavior in virgin animals.
Experiment Two: The Effects of Multiple Electrical Stimulation of the MPOA and the MedAmyg on the Induction of Maternal Motivation and Behavior of Nulliparous (virgin) Female Rats.

This experiment is designed to address the issue of whether the onset of maternal behavior in nulliparous animals can be facilitated or attenuated by kindling-like stimulation in the MPOA and MedAmyg respectively. Can electrical stimulation of a 'non-primed' substrate facilitate its function?

Kindling-like stimulation of a non-primed substrate also provides an excellent opportunity to explore the 'hard wired' function of that substrate. There is some controversy as to whether the MPOA is involved in attracting the animal to pup stimulus, or is part of an effector system (Numan, 1994). To address these questions, animals were tested for their attraction to pup odors as well as their maternal behavior during
induction testing.

Additionally, virgins induced to become maternal find pups to be less reinforcing and hence show less preference for pup cues than postpartum females in a conditioned-place paradigm (Fleming, Kuchera, Lee & Winocur, 1994). Perhaps by stimulating the MPOA in virgins this difference would be attenuated, suggesting that the MPOA is involved in some of the 'motivational' aspects of maternal behavior. This was tested using a conditioned place preference test for pups.

It is also unclear as to what information the medial amygdala mediates in this context. The MedAmyg is thought to be involved in adding emotional significance to stimuli (LeDoux, 1992). Specifically, it has been implicated in anxiogenic behavior in rats (Adamec & Morgan, 1994; Davis, 1992; Henke & Sullivan, 1985). The MedAmyg would appear to be particularly important in controlling affective changes in the newly parturient rat. Fleming et al. (1980) found that maternal behavior
could be elicited and open field activity could be enhanced by MedAmyg lesions. To investigate the emotional changes mediated by the MedAmyg, the open field test was also added.

Finally, intermittent pup-exposure or short term exposure to pups can also contribute to maternal responsiveness in virgin rats, although the onset of fully expressed maternal behavior takes longer than if the rat was constantly exposed to pups (Fleming & Luebke, 1981). We hypothesized that MPOA stimulation would enhance the effects of short term pup exposure and MedAmyg stimulation would attenuate or eliminate it.

**Method**

The general methods of this experiment followed those outlined in the General Methods section. Nulliparous virgin females (250-300g) were surgically implanted with bipolar electrodes in the right hemisphere. Animals were assigned to one of four groups as in
experiment one. The groups were further subdivided into pup-exposed and non-exposed groups to assess the possible additive effect of exposure to pup stimuli with kindling-like simulation. Pup exposed groups were presented with six pups in the stimulation cage for 10 minutes immediately after stimulation. Non-exposed groups were left in the stimulation cage for 10 minutes but received no pups. Thus, a total of eight groups were formed using 72 animals (n=9). Of this total, twenty animals were eliminated from the experiment: seven animals pulled out their electrodes during testing and thirteen on the basis of histological analysis. The final groupings were as follows (see figure 7): MPOA stimulated pup-exposed (n=5), MPOA stimulated not exposed (n=7), MPOA not stimulated pup-exposed (n=9), MPOA not stimulated not exposed (n=8), MedAmyg stimulated pup-exposed (n=5), MedAmyg stimulated not exposed (n=6), MedAmyg not stimulated pup-exposed (n=6), and MedAmyg not stimulated not exposed (n=6).
All animals were implanted with electrodes and given a one week recovery period. Stimulation was given days 7-21 post surgically. After stimulation the following tests were conducted (in order): at day 29 the modified hole board test and open field test; at day 30 maternal induction testing; and the conditioned place preference test was conducted one day after the female became maternal or after 11 days in the induction testing (days 37-47).

Two-way analyses of variance were conducted to test for the effects of stimulation status (stimulated/not stimulated); electrode location (MPOA/MedAmyg) and their interaction on maternal behaviors. The data were further explored by one way analyses of variance (results given in {braces}) and chi-square comparisons (for latency and CPP data). Bonferroni post-hoc tests were conducted to
EXPERIMENT 2

D1
ELECTRODES IMPLANTED

GROUPS

MPOA STIM (n=12)
MPOA NOT STIM (n=17)
MED AMYG STIM (n=11)
MED AMYG NOT STIM (n=12)

D7-21
ELECTRICAL STIMULATION
(AND PUP EXPOSURE)

D29
HOLE BOARD TEST
OPEN FIELD TEST

D30
MATERNAL INDUCTION TEST
(POST INDUCTION CPP TEST)

Figure 7
assess differences between groups.

Results

Maternal Induction testing

There were no differences between pup-exposed and not exposed groups for any behavior so they were combined for all subsequent analyses (see appendix 2 for a comparison of group means). A two-way analysis of variance yielded no differences for latency to become maternal within the 10 days of induction testing. However, a 2 (location) x 2 (stimulation status) chi-square analysis of the percentage animals that became maternal during the induction period yielded a significant result: a greater percentage of MPOA stimulated animals became maternal than did MedAmyg stimulated animals ($\chi^2=17.2; p<.05$, figure 8).

Insert figure 8 about here
PERCENT OF ANIMALS BECOMING MATERNAL WITHIN 10 DAYS

Figure 8
A comparison of the mean frequency for behaviors observed during maternal latency testing (Table 2) showed that there was a significant interaction for the amount of sniffing pups performed in the first two days of induction \((F[1,51]=2.55,p<.05)\) {one-way ANOVA: \(F[3,48]=5.94,p<.01\)}. The MPOA stimulated group was sniffing pups more than all the other groups \((p<.05)\). There was a similar effect during the last two days of testing \((F[1,51]=5.3,p<.05)\) {one-way ANOVA: \(F[3,48]=8.91;p<.01\)} where the MPOA stimulated group was sniffing pups more than the MedAmyg stimulated group \((p<.05)\).

Insert table 2 about here

Hole Board and Open Field testing

For measures of exploratory behavior and pup odor preference the primary effects were mediated by stimulating the MedAmyg, and were inhibitory. An
Table 2: Daily mean frequencies (+SEM) for behaviors observed during maternal latency (induction) testing in Experiment Two (for first two [1st] and last two days [fin]).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>MPOA/stim</th>
<th>MPOA/not stim</th>
<th>MedAmyg/stim</th>
<th>MedAmyg/not stim</th>
<th>Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniff air (1st)</td>
<td>60.0 (6.6)</td>
<td>59.3 (2.6)</td>
<td>62.5 (4.9)</td>
<td>59.9 (5.5)</td>
<td></td>
</tr>
<tr>
<td>Sniff air (fin)</td>
<td>49.9 (4.6)</td>
<td>52.2 (5.1)</td>
<td>59.7 (6.8)</td>
<td>52.6 (5.8)</td>
<td></td>
</tr>
<tr>
<td>Sniff pup (1st)</td>
<td>23.0 (5.6)</td>
<td>11.6 (2.4)</td>
<td>5.5 (1.1)</td>
<td>6.7 (1.2)</td>
<td>MPOA/S &gt; MPOA/NS, MA/S, MA/NS (p&lt;.05)</td>
</tr>
<tr>
<td>Sniff pup (fin)</td>
<td>22.8 (5.9)</td>
<td>14.9 (2.6)</td>
<td>5.4 (0.9)</td>
<td>11.3 (5.6)</td>
<td>MA/S &lt; MPOA/S (p&lt;.05)</td>
</tr>
<tr>
<td>Groom (1st)</td>
<td>13.7 (1.6)</td>
<td>15.7 (2.8)</td>
<td>10.2 (2.2)</td>
<td>11.9 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Groom (fin)</td>
<td>20.6 (4.2)</td>
<td>9.3 (2.6)</td>
<td>13.2 (5.4)</td>
<td>13.8 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Lick (1st)</td>
<td>0.1 (0.0)</td>
<td>0.2 (0.1)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Lick (fin)</td>
<td>5.1 (2.6)</td>
<td>3.6 (1.0)</td>
<td>0.0 (0.0)</td>
<td>1.8 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Genital lick (1st)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Genital lick (fin)</td>
<td>1.6 (1.0)</td>
<td>1.0 (0.4)</td>
<td>0.0 (0.0)</td>
<td>1.1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Retrieve (1st)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Retrieve (fin)</td>
<td>1.7 (0.8)</td>
<td>1.1 (0.5)</td>
<td>0.0 (0.0)</td>
<td>1.3 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Crouch (1st)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Crouch (fin)</td>
<td>6.7 (3.5)</td>
<td>3.2 (2.1)</td>
<td>0.0 (0.0)</td>
<td>5.0 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Nest build (1st)</td>
<td>1.3 (0.9)</td>
<td>0.7 (0.5)</td>
<td>0.1 (0.1)</td>
<td>1.6 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Nest build (fin)</td>
<td>0.2 (0.2)</td>
<td>1.2 (0.6)</td>
<td>0.0 (0.0)</td>
<td>0.1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Settle (1st)</td>
<td>25.8 (6.9)</td>
<td>19.8 (5.5)</td>
<td>10.9 (3.8)</td>
<td>11.5 (3.9)</td>
<td></td>
</tr>
</tbody>
</table>
### Experiment Two

<table>
<thead>
<tr>
<th></th>
<th>MPOA/ stim</th>
<th>MPOA/ not stim</th>
<th>MedAmyg/ stim</th>
<th>MedAmyg/ not stim</th>
<th>Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Settle (min)</td>
<td>31.0 (6.4)</td>
<td>15.7 (5.7)</td>
<td>14.9 (5.4)</td>
<td>17.6 (5.6)</td>
<td></td>
</tr>
</tbody>
</table>

MPOA - medial preoptic area
MA - medial amygdala
S - stimulated
NS - not stimulated
interaction between electrode location and stimulation was observed for proportion of time sniffing pup odors in the modified hole board (F[1,51]=5.72;p<.05, figure 9){one-way ANOVA: F[3,48]=4.57;p<.05}. The MedAmyg stimulated animals spent less time sniffing in pup-odored holes than did the other three groups (p<.05). The MPOA stimulated and MPOA not stimulated groups did not differ from each other, and the MPOA groups did not differ from the MedAmyg not stimulated group. There was no correlation (Spearman) between maternal induction status (whether the animal became maternal or not) and amount of time spent sniffing pup odors in the hole board. There were no group differences for frequency of head-dipping.

Insert figure 9 about here

There was also a marginally significant interaction for the open field (F[1,51]=3.48;p=.06, figure 10){one-way ANOVA: F[3,48]=5.1;p<.03}, where MedAmyg stimulated
HOLE BOARD: PROPORTION OF TIME SNIFFING IN PUP-ODOURED HOLES

Figure 9
animals crossed proportionally fewer central squares in the open field than did the other three groups \((p < .05)\). The MPOA stimulated and MPOA not stimulated groups did not differ from each other, and there was no difference between the MPOA and MedAmyg not stimulated groups. There were no differences between any groups for the emergence test (all animals remained in the start box for the duration). Comparison between the hole board and the open field showed that there was a significant positive correlation between the number of central squares crossed in the open field and the number of head dips in the hole board (Spearman, \(r_s = .31, \ p < .05\)). There was no relationship between maternal induction status and number of squares crossed in the open field.

_______________________________

Insert figure 10 about here

_______________________________

To Determine whether there was any indication of stimulation induced effects on general activity level in
OPEN FIELD: PROPORTION OF CENTRAL SQUARES CROSSED

Figure 10
either the open field or hole board tasks, the groups were compared for total number of squares crossed in the open field and total number of head dips in the hole board. There were no significant group differences on any of the measures, indicating general motility was not affected by stimulation of either brain site.

**Conditioned Place Preference testing**

In contrast to the hole board and open field, in the CPP test the primary effect was seen in the MPOA stimulated group, as an enhancement of preference. A one-way analysis of variance yielded a significant effect for proportion of time spent in the pup-associated box \( F[3,48]=2.65; p<.05 \) (figure not shown), where the MPOA stimulated animals were spending more time in the pup-associated box than the MedAmyg stimulated animals \( (p<.05) \). A chi-square analysis showed a significantly greater percentage of MPOA stimulated animals showed a preference for pup associated boxes in the conditioned place preference paradigm than did each of the other
groups ($\chi^2=21.2; p<.05$, figure 11). The MPOA stimulated animals spent more time in the pup associated box, whereas the other groups showed no preference for the pup associated box. Also, there was a significant correlation between maternal status in the induction testing and the CPP training, where animals that became maternal in the induction testing were more likely to crouch over the pups in the CPP apparatus during training ($rs=.38, p<.005$). There were no correlations between crouching over pups in the CPP and either preference for pup odor in the hole board test or number of squares crossed in the open field.

Histological analysis of the brain tissue indicated that the location of the electrode placements was fairly well restricted to the intended sites (Figure 12). No animals showed any seizures during stimulation or
Figure 11

CPP: PERCENT OF ANIMALS PREFERING PUP-ASSOCIATED BOX

<table>
<thead>
<tr>
<th>(percents</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPOA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MED AMYG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
afterward.

Discussion

The latency to become maternal is not affected by MPOA stimulation in nulliparous animals, although a greater percentage of MPOA stimulated animals became maternal during the induction period than did MedAmyg stimulated animals. Moreover, an examination of behavioral frequencies during the first two and last two days of induction testing also suggested that MPOA stimulation facilitated and MedAmyg stimulation attenuated maternal behavior. During the first two days of induction testing more MPOA stimulated animals were sniffing pups more than the other groups suggesting that they were initially more motivated toward pup stimuli. During the last two days of testing MPOA stimulated
Figure 12
animals were again sniffing pups more than MedAmyg stimulated animals. This may suggest that the effect of the stimulation treatment on maternal responsiveness extended past the initial onset period, where MPOA animals continued to be more attracted to pup odors and MedAmyg animals continued to find pup stimuli aversive. It could also be that the earlier experience during the first two days affected the later measures.

Electrical stimulation of the MedAmyg inhibits and stimulation of the MPOA facilitates 'emotional' changes associated with maternal behavior. These changes may involve the 'hard-wired' functions of the substrates being stimulated. The MedAmyg, which is thought to be involved in anxiety, produced anxiogenic effects when stimulated in this study. This is consistent with Adamec and Morgan’s (1994) findings that MedAmyg kindling is anxiogenic and the findings that MedAmyg lesions are anxiolytic (Fleming et al., 1980). It is likely that initial phobia to pup stimuli is mediated by activation
of the MedAmyg. Related to this is the finding that MedAmyg stimulated groups spent less time head-dipping in pup-odored holes, of the hole board test, than the other groups. Since the MedAmyg receives olfactory input, it is likely that this input is assessed for emotional significance here. Since Fleming et al. (1980) found a decrease in anxiety towards olfactory pup stimuli with lesions to the MedAmyg, it is not surprising that chronic stimulation of this structure would cause an increase in aversion to olfactory pup stimuli.

Motivational changes in the animals that received electrical stimulation of the MPOA were also observed. A preference for a learned pup-associated box was developed in MPOA stimulated animals. Interestingly, this change was seen only in animals that became maternal during the induction phase, suggesting the creation of some sort of rewarding value for pups by an interaction between MPOA stimulation and pup experience. Since there are strong projections from MPOA neurons to areas that are part of
the reward systems (Numan & Numan, 1991; Conrad & Pfaff, 1976), it is possible that the MPOA is important for mediating the integration of sensory input with reward systems.

The effect of pup experience on pup preference in MPOA stimulated animals occurred only after stimulation and not during. There was no effect of pup exposure during stimulation on any subsequent behaviors. This seems unusual given that the results of these two studies clearly suggest an interaction between stimulation and pup experience. It is possible that experience only during stimulation has no effect, or it may be that the experience the animals received in this paradigm was insufficient or below threshold (only 10 minutes each day for 14 days). We further investigated this issue in experiment three using longer pup-exposures during the stimulation phase in virgin animals.
A Further Comparison of Experiment 1 and Experiment 2
(postpartum and virgin animals)

The results of the first two experiments show that electrical stimulation of the MPOA and the MedAmyg produce changes in maternal responsiveness in both postpartum and nulliparous (virgin) animals. Experiment one showed that stimulating the MedAmyg reduces maternal responsiveness in maternally experienced animals; latencies to retrieve and crouch over pups were significantly longer than in the appropriate control. MPOA stimulation did not induce a significantly shorter latency to onset of maternal behavior within the 10 day testing period. However, there was evidence that MPOA stimulation enhanced responsiveness and probability of expression of maternal behavior in the more responsive animals, indicated by a stimulation induced increase in the number of virgin animals showing maternal behavior within the 10 day induction period (experiment two).

MPOA stimulation enhanced the rewarding (as measured
by the CPP test) properties of pup stimuli acquired through pup exposure, in animals that have not yet expressed maternal behavior. The ability of repeated electrical stimulation to enhance reward has also been shown for brain stimulation reward (Corcoran, 1988). In contrast, stimulating the MedAmyg in virgin animals decreased the preference for pup stimuli when compared to all other groups. In addition, MedAmyg stimulation appears to increase the animal's anxiety, an 'emotional' state normally expressed by non-maternal virgin animals. It is possible that all of these changes must occur in the animal before a coordinated maternal response can be expressed.

In experiment two there was a dissociation between the hole-board/open field and preference learning (CPP) tests. Stimulating the MPOA enhanced preference learning whereas stimulating the MedAmyg enhanced the 'emotional' and 'motivational' tests. This may represent a functional dissociation. This is further supported by the
observation that measures on the hole-board and open field tests were correlated with each other, but not with the measures from the CPP test. The absence of depression in overall group activity and sniffing in the open field and hole board tests after stimulation suggests those stimulation effects are not due to a nonspecific disruption of the animal's motility or activity.

Although we did not find an effect of MPOA stimulation on maternal latency in virgin animals, we did find a more subtle change in maternal 'motivation'. We speculate that these less pronounced changes are due to the fact that we are stimulating a 'non-primed' neural substrate. Kindling-like stimulation of a neural substrate in the circuit for maternal behavior may interact with experience to facilitate its function. It is likely that prior experience with pups, or hormonal changes associated with parturition change the neural substrates involved in maternal behavior. Multiparous animals do become maternal more quickly, suggesting a
more permanent neural reorganization. Therefore, it may require little exogenous stimulation to reactivate the maternal circuit in the postpartum animal, but a lot of stimulation to activate the circuit for the first time. More subtle changes require more sensitive tests.
Experiment Three: The Effects of Multiple Electrical Stimulation of the MPOA and the MedAmyg and Pup Exposure on the Induction of Maternal Motivation and Behavior in Nulliparous (virgin) Female Rats.

Virgin female rats retrieve pups in the apparent absence of hormonal influence, after exposure to pups for several days (Rosenblatt, 1967). Through experience, the animal learns about the eliciting stimuli; familiarity with pup odors results in a reduction in the animal’s natural phobic responses to these cues, eventually ‘allowing’ her to become maternal (Fleming & Luebke, 1981). This change occurs within a number of days in albino rats if given constant proximal exposure to pups. Intermittent exposure or short-term exposure to pups also contributes to maternal responsiveness in virgin female rats. It is not clear whether this is a general motivational change or if it is pup specific. There is some evidence that olfactory stimulation from the pups
induces brain changes in the virgin rat (Modney & Hatton, 1990). It is not clear how much sensory stimulation is required to cause brain changes that lead to maternal responsiveness. However, this plasticity is possibly evidence for an experience-based neural re-organization.

The previous experiments found that stimulating the MPOA enhances maternal motivation and stimulating the MedAmyg attenuates it. These changes were greater in postpartum animals that had previously experienced pups under the influence of hormones (than virgin animals). There is now evidence to show that the onset and maintenance of maternal behavior can be influenced by hormones, pup experience and electrical stimulation of neural substrates involved in maternal behavior. It appears that these three factors can interact to reach an onset threshold, although the nature of that interaction is not clear.

The previous experiments also suggested that there are motivational changes associated with stimulating the
both the MPOA and the MedAmyg. MPOA stimulation enhanced preference for pup associated cues and MedAmyg stimulation decreased it. Also, MedAmyg stimulation increased anxiety. However, it is still not clear whether these motivational changes are general or pup-specific.

Finally, the results of the previous two experiments suggest an interaction between stimulation and pup experience. However, a 10 minute experience during stimulation has no effect on subsequent latencies in experiment two. We further investigated this issue using longer pup-exposures during the stimulation phase in virgin animals.

This experiment investigated the interactive effects of multiple electrical stimulations of the MPOA and the MedAmyg and brief pup exposure during stimulation on maternal responsiveness and associated behaviors in virgin rats. Also, the specificity of these changes was investigated. We used the same systematic administration of electrical stimulation that has been found to produce
epileptiform afterdischarges and has been used as a model of plasticity in the brain (Cain, 1992).

In view of the plastic changes that result from this procedure and prior pup experience, and based on our previous results, we predicted that electrical stimulation would enhance the function of neural substrates associated with maternal behavior (producing the opposite effects of lesions): MPOA stimulated animals were expected to show an attraction to pup-cues quickly, whereas MedAmyg stimulated animals were expected to show the opposite effect as well as an enhancement in neophobia. It was further predicted that pup exposure (or an experience with pups) during stimulation would interact with neural site of stimulation, such that MPOA stimulated, pup-exposed animals would show a faster onset of maternal responsiveness than the other groups.

Method

The general methods of this experiment followed
those outlined in the General Methods section. Nulliparous females (250-300g) were surgically implanted with bipolar electrodes in the right hemisphere. Fifty-six animals were assigned to one of eight groups (n=7). Of this total, eight animals were eliminated from the experiment: one animal pulled out its electrode during testing and seven on the basis of histological analysis. The final groupings were as follows (see figure 13): MPOA stimulated (n=7) and MPOA not stimulated (n=6) without pup exposure, MedAmyg stimulated (n=6) and MedAmyg not stimulated (n=6) without pup exposure, MPOA stimulated (n=6) and MPOA not stimulated (n=5) during pup exposure, and MedAmyg stimulated (n=6) and MedAmyg not stimulated (n=6) during pup exposure.

Females were stimulated on days 7-21 post-implantation. In the pup exposed condition, animals were moved to a different room and placed in another cage with foster pups each morning for a one hour period. At the end of this period they were returned to their home cage
and room. In the not exposed condition animals were simply moved to a different room and cage (without pup stimuli) for the one hour period. Stimulation proceeded in the afternoon. At day 29 the hole board test was used to measure the motivation to investigate pup nest odors by placing nest odors under the holes of an elevated square platform. To investigate the specificity of this motivational change, two additional tests were conducted in the hole board: first to measure motivation to investigate bedding odors from a male rat’s cage (a different reproductively relevant stimuli), and a second, a novel food odor (ground fruit loops, biologically, but not reproductively relevant). The order of presentation of each of these hole board tests was randomized to eliminate the possibility of an order effect or habituation to the apparatus. The open field test was used to measure investigatory behavior in a strange environment. At day 30 maternal induction testing was initiated to determine level of maternal responsiveness.
(reflected in latency to show maternal behavior to foster pups). CPP testing was not conducted in this experiment.

Three way analyses of variance were conducted on the data for the following factors: stimulation (stimulated/not stimulated); location (MPOA/MedAmyg); pup exposure (pup exposed/not pup exposed). A chi-square planned comparison was conducted for latency data. The data were further explored by one way analyses of variance (provided in {braces}) and chi square analysis (for latency data). Bonferroni post-hoc tests were conducted to assess differences between groups.

Results

Maternal Induction testing

A three way analysis of variance yielded a significant main effect of exposure ($F[1,47]=8.92, p<.013$;
EXPERIMENT 3

**DAY 1**
ELECTRODES IMPLANTED

GROUPS

- **PUP EXPOSED**
  - MPOA STIM (n=6)
  - MPOA NOT STIM (n=5)
  - MED AMYG STIM (n=6)
  - MED AMYG NOT STIM (n=6)

- **PUP NOT EXPOSED**
  - MPOA STIM (n=7)
  - MPOA NOT STIM (n=6)
  - MED AMYG STIM (n=6)
  - MED AMYG NOT STIM (n=6)

**DAY 7 - 21**
ELECTRICAL STIMULATION
(AND PUP EXPOSURE)

**DAY 29**
HOLE BOARD TEST
OPEN FIELD TEST

**DAY 30**
MATERNAL INDUCTION TEST

Figure 13
figure 14) for latency to become maternal. Animals exposed to pups during stimulation showed lower latencies to become maternal at induction than not exposed animals. There were no other main effects or interactions for latency measures.

However, a comparison of the percentage of animals becoming maternal within the 14 day induction period yielded a significant effect ($\chi^2=16.5, p<.05; \text{figure 15}$), where a greater percentage of MPOA stimulated pup exposed animals became maternal within the induction period than all other groups. There were also significantly more MPOA stimulated animals (pup exposed only) behaving maternally than other groups during the pup exposure/stimulation period ($\chi^2=12.3, p<.05; \text{figure 15}$). There was also a non-significant correlation between latency to become maternal in the induction testing and maternal responsiveness at exposure.
A comparison of the mean frequency for behaviors observed during maternal latency testing (Table 3) showed that there was a significant 2-way interaction (stimulation by location) for the amount of sniffing pups performed in the first two days of induction ($F[1,47]=5.25; p < .05$) (one-way ANOVA: $F[7,40]=10.25; p < .01$). The MPOA stimulated animals were sniffing pups more than the other groups for both the pup exposed ($p < .05$) and not exposed conditions ($p < .05$). There was a 3-way interaction (stimulation by location by exposure) for sniffing pups during the last two days of induction ($F[1,47]=5.3, p < .05$) (one-way ANOVA: $F[7,40]=7.9=7.98; p < .04$) where MedAmyg stimulated not exposed animals were sniffing pups less than MPOA stimulated not exposed ($p < .05$) and MedAmyg not stimulated pup exposed animals ($p < .05$). There was also a main effect
LATENCY TO BECOME MATERNAL

Figure 14
PERCENT OF ANIMALS BECOMING MATERNAL DURING EXPOSURE

<table>
<thead>
<tr>
<th>PERCENT OF ANIMALS</th>
<th>PUP EXPOSED</th>
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<tr>
<td>MPOA/STIM</td>
<td>25</td>
</tr>
<tr>
<td>MPOA/NOT STIM</td>
<td>15</td>
</tr>
<tr>
<td>MEDAMYG/STIM</td>
<td>10</td>
</tr>
<tr>
<td>MEDAMYG/NOT STIM</td>
<td>5</td>
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</table>

PERCENT OF ANIMALS BECOMING MATERNAL WITHIN 10 DAYS

<table>
<thead>
<tr>
<th>PERCENT OF ANIMALS</th>
<th>PUP EXPOSED</th>
<th>NOT PUP EXPOSED</th>
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</thead>
<tbody>
<tr>
<td>MPOA/STIM</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>MPOA/NOT STIM</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>MEDAMYG/STIM</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>MEDAMYG/NOT STIM</td>
<td>50</td>
<td>10</td>
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</table>

Figure 15
of experience for pup licking ($F[1,47]=4.6, p<.05$), genital licking ($F[1,47]=3.9, p<.05$), and retrieval ($F[1,47]=3.7, p<.05$), during the last two days of induction testing, where not exposed animals were performing these behavior less than the pup exposed groups. There were no other effects.

Insert table 3 about here

Hole Board and Open Field testing

Analysis of variance yielded no effects of head-dipping for pup-odors, male odors or food odors (see figures 16 & 17). There were no significant groups differences for any of the hole board measures.

Insert figures 16 & 17 about here

However, there was a significant positive correlation between approach to pups during the exposure
Table 3: Daily mean frequencies (+SEM) for behaviors observed during maternal latency (induction) testing in Experiment Three (for first two [1st] and last two days [fin]).

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<tbody>
<tr>
<td>Sniff air</td>
<td>64.1 (4.2)</td>
<td>6.2 (5.3)</td>
<td>6.6 (7.2)</td>
<td>64.1 (10.0)</td>
<td>69.0 (10.0)</td>
<td>63.1 (3.9)</td>
<td>70.5 (5.9)</td>
<td>70.7 (5.4)</td>
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<tr>
<td>(1st)</td>
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</tr>
<tr>
<td>Sniff air</td>
<td>48.7 (8.9)</td>
<td>62.3 (14.6)</td>
<td>45.6 (6.6)</td>
<td>42.2 (3.4)</td>
<td>52.2 (5.9)</td>
<td>63.3 (8.5)</td>
<td>54.3 (10.4)</td>
<td></td>
<td>MPOA/S/E &gt; other E groups (p&lt;.05)</td>
</tr>
<tr>
<td>(fin)</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Sniff pup</td>
<td>22.0 (4.2)</td>
<td>7.0 (3.7)</td>
<td>9.0 (2.6)</td>
<td>8.5 (2.4)</td>
<td>39.2 (7.4)</td>
<td>4.2 (1.5)</td>
<td>10.5 (3.6)</td>
<td>7.3 (2.3)</td>
<td>MA/S/NE &lt; MPOA/S/NE, MA/NS/E (p&lt;.05)</td>
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<tr>
<td>(1st)</td>
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<td></td>
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<tr>
<td>Sniff pup</td>
<td>11.2 (0.9)</td>
<td>-9.6 (2.7)</td>
<td>13.3 (3.0)</td>
<td>17.5 (3.9)</td>
<td>22.1 (6.6)</td>
<td>18.2 (3.9)</td>
<td>4.0 (0.7)</td>
<td>13.9 (1.9)</td>
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<tr>
<td>(fin)</td>
<td></td>
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<td></td>
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<tr>
<td>Groom</td>
<td>13.3 (4.7)</td>
<td>6.6 (2.7)</td>
<td>11.6 (2.6)</td>
<td>13.1 (4.2)</td>
<td>11.5 (3.8)</td>
<td>11.0 (3.8)</td>
<td>9.8 (3.8)</td>
<td>11.2 (3.5)</td>
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<tr>
<td>(1st)</td>
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## Experiment Three

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<tr>
<td>Groom (fin)</td>
<td>12.0 (3.6)</td>
<td>5.6 (0.6)</td>
<td>9.9 (2.4)</td>
<td>6.8 (1.6)</td>
<td>10.3 (1.3)</td>
<td>10.7 (3.6)</td>
<td>7.4 (4.8)</td>
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<td></td>
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<tr>
<td>Lick (1st)</td>
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<td>0.0 (0.0)</td>
<td>2.1 (2.0)</td>
<td>0.2 (0.2)</td>
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<tr>
<td>Lick (fin)</td>
<td>16.8 (10.0)</td>
<td>19.6 (11.9)</td>
<td>14.3 (5.0)</td>
<td>19.1 (4.6)</td>
<td>12.6 (3.9)</td>
<td>11.7 (7.2)</td>
<td>0.0 (0.0)</td>
<td>14.7 (3.2)</td>
<td>NE&lt;E (p&lt;.05)</td>
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<td>Genital lick (1st)</td>
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<td>0.6 (0.4)</td>
<td>0.0 (0.0)</td>
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<tr>
<td>Genital lick (fin)</td>
<td>7.2 (4.2)</td>
<td>8.3 (2.6)</td>
<td>2.8 (2.0)</td>
<td>5.6 (2.0)</td>
<td>4.7 (2.0)</td>
<td>12.1 (12.3)</td>
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<td>2.1 (2.0)</td>
<td>NE&lt;E (p&lt;.05)</td>
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<tr>
<td>Retrieve (1st)</td>
<td>0.0 (0.0)</td>
<td>0.1 (0.1)</td>
<td>0.6 (0.4)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
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<td>0.0 (0.0)</td>
<td>0.5 (0.5)</td>
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<tr>
<td>Retrieve (fin)</td>
<td>3.0 (1.7)</td>
<td>5.0 (1.7)</td>
<td>4.0 (1.2)</td>
<td>4.7 (0.8)</td>
<td>3.7 (1.4)</td>
<td>2.7 (1.6)</td>
<td>0.0 (0.0)</td>
<td>2.0 (1.2)</td>
<td>NE&lt;E (p&lt;.05)</td>
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<tr>
<td>Crouch (fin)</td>
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<td>6.4 (6.3)</td>
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<td>0.5 (0.3)</td>
<td>0.0 (0.0)</td>
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<td>3.0 (1.9)</td>
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<tr>
<td>Nest build (fin)</td>
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<td>0.0 (0.0)</td>
<td>0.8 (0.4)</td>
<td>1.5 (1.5)</td>
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<td>0.0 (0.0)</td>
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<tr>
<td>Settle (1st)</td>
<td>0.5 (0.5)</td>
<td>15.3 (3.8)</td>
<td>0.0 (0.0)</td>
<td>0.9 (0.3)</td>
<td>0.0 (0.0)</td>
<td>14.7 (7.3)</td>
<td>6.0 (3.7)</td>
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<tr>
<td>Settle (fin)</td>
<td>4.2 (4.1)</td>
<td>34.9 (8.0)</td>
<td>19.0 (12.5)</td>
<td>0.0 (0.0)</td>
<td>8.7 (8.5)</td>
<td>0.0 (0.0)</td>
<td>10.0 (3.7)</td>
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</table>

MPOA - medial preoptic area
MA - medial amygdala
S - stimulated
NE - not exposed

E - pup exposed
NS - not stimulated
HOLE BOARD (PUP ODORS)

Figure 16
and the frequency of head-dipping in pup odored holes in the hole board (Spearman, rs=.3341, p<.03), but there was no correlation between maternal behavior during exposure and sniffing male odors or novel food odors.

Finally, a three-way analysis of variance yielded a significant main effect for exposure when comparing groups in the open field test (F[1,47]=3.66, p<.05; figure 18) (one-way ANOVA: F[7,40]=4.8; p<.02), where pup exposed groups were spending proportionally more time in the central squares of the open field. There were no other group differences. There was no correlation between open field behavior and maternal responsiveness during the exposure period. There were no differences between groups for emergence testing.

Histological analysis of the brain tissue indicated that the location of the electrode placements was fairly
Figure 18
well restricted to the intended sites (figure 19). One animal experienced a stage 4 seizure during stimulation. Analysis was conducted without this animal.

Insert figure 19 about here

Discussion

The results show that exposure to pups during the stimulation phase reduced the latency to become maternal in all groups. However, MPOA stimulation interacted with pup exposure to further reduce the latency to become maternal. Stimulating the MPOA did not reduce the latency to become maternal in the not pup-exposed condition. Surprisingly, MedAmyg stimulation did not increase the latency to become maternal in either condition.

An examination of behavioral frequencies during the first two and last two days of induction testing also showed a strong effect of the exposure treatment, where
Figure 19
EXPERIMENT 3

MPOA

X = STIMULATED (n=6)

PUP EXPOSED

O = NOT STIMULATED (n=5)

MED AMYG

X = STIMULATED (n=6)

O = NOT STIMULATED (n=6)
the pre-induction exposure to pups appeared to inoculate the animals from the attenuating effect of MedAmyg stimulation. Animals in the exposed groups licked, genital licked and retrieved pups more than the not exposed groups during the last two days of induction. However, animals in the not exposed MedAmyg stimulated group sniffed pups less during the last two days of induction, suggesting that this group experienced a long term reduction in maternal responsiveness. This also is a finding consistent with the previous study. Also consistent with the previous study was the finding that MPOA stimulated animals were sniffing pups more in the first two days of induction. This was the case in both pup exposed and not exposed conditions.

MedAmyg stimulation had no effect on head-dipping behavior for either pup exposed or not exposed animals. This is not consistent with the previous experiment which showed that MedAmyg stimulation attenuated head-dipping for pup odors. It is possible that a difference in
procedure between the two experiments may account for this discrepancy. This experiment administered the hole board test to each animal three times (tests for head-dipping for pup odors, male odors and food odors). The order of presentation was randomized for each animal. The animal may have become habituated to the task upon subsequent administrations, affecting the results for all three head-dipping tests.

The open field data showed that pup-exposed animals were less anxious than not exposed animals. Our previous findings have shown that MedAmyg stimulation enhances anxiety (as measured in the open field). This was not the case in this experiment, although there was a trend toward MedAmyg stimulated anxiolysis in the pup exposed animals.

This experiment did not show the same significant motivational and affective changes that were observed in experiment two, although there were trends in the same directions. It is possible that differences in procedure
sensitized the animals in a way that masked the stimulation effects. For example, animals were moved to different environments and cages during pup exposure. Also, the hole board test was administered more than once. These procedures increased the amount of handling time (over experiment two) for each animal. This extra handling time may have further ‘tamed’ the animal, resulting in an animal with a different affective constitution than animals in experiment two. This is supported by the observation that all experiment three animals spent more time in the center squares of the open field than animals from experiment two.

The issue of affective and motivational changes caused by MedAmyg and/or MPOA stimulation is further explored in the next experiment.

**A Further Comparison of Experiment 2 and Experiment 3**

*(virgin animals)*

Experiments two and three investigated the effects
of stimulating the MPOA and the MedAmyg in virgin animals. Further, the interaction of experience with stimulation and whether the motivational changes seen as a result of stimulation were specific to pup stimuli were investigated (in experiment three).

Both experiments two and three were unable to show an attenuation or facilitation of the full expression of maternal behavior with MPOA or MedAmyg stimulation. However, both experiments showed that MPOA stimulation facilitated the onset of maternal responsiveness within the 10 day induction period. In experiment three, there was a main effect of pup exposure, where animals that were exposed to pups during the stimulation period became maternal more rapidly during the induction period. Further, there was an additive effect of MPOA stimulation and exposure, where more MPOA stimulated pup exposed animals became maternal during the 10 day induction period.

In both experiments two and three, MPOA stimulation
facilitated pup sniffing in the first two days of induction (latency) testing. This suggests that these animals were stimulated to display precursor maternal behaviors: approach and explore pup stimuli. Although a crude measure, this may further indicate a change in the 'motivational' status of the animal, with MPOA stimulation.

Unfortunately, the results of experiment three were unable to clarify the specificity of motivational changes seen with stimulation (in experiment two). This may have been the result of procedural differences in experiment three (see discussion above).

Comparing experiments two and three for the effects of pup-exposure during stimulation shows that daily one hour pup-exposures (in experiment three) decreased the latencies to become maternal, and the expression of anxiety in the open field. Also, pup experience increased the frequencies licking, genital licking and retrieval behaviors during the last two days of induction testing.
This suggests that the exposed animals attained a higher level of maternal responsivity at the end of the 10 day induction testing than the not exposed animals.
Experiment Four: The Effects of Multiple Electrical Stimulation of the MPOA and MedAmyg on the Long-term Maternal Motivation and Behavior in Postpartum Female Rats with Stria Terminalis Lesions.

Maternal behavior is attenuated by lesions to the medial preoptic area MPOA in both postpartum and virgin rats (Fleming et al., 1980; Numan et al., 1977; Numan & Callahan, 1980). Moreover, repetitive electrical stimulation of the MPOA enhances maternal behavior. The MPOA is connected to the MedAmyg via the stria terminalis (ST), a pathway containing fibers that project in both directions (DeOlmos & Ingram, 1972). Maternal behavior is facilitated by lesions to the MedAmyg in virgin rats (Fleming et al., 1980). Amygdala lesions have been found not to disrupt maternal behavior in mice (Slotnick & Nigrosh, 1975), and lesions to the stria terminalis do not disrupt the maternal behavior of postpartum female
rats (Numan, 1974). Also, repetitive MedAmyg stimulation delays maternal behavior and increases anxiety in both virgin and postpartum animals. Although the MPOA and the MedAmyg are both implicated in the expression of maternal behavior, how they influence one another is not clear. Electrophysiological and anatomical evidence suggest that the strongest projections are from the MedAmyg to the MPOA, rather than in the other direction (see calibration section of General Methods; DeOlmos, 1972). Whether these neurons are predominantly inhibitory or excitatory is not known. Further, the inhibitory effect of the MedAmyg activation on maternal behavior could act indirectly by activating the fear system.

This study investigated the functional relationship between the MPOA and the MedAmyg, by comparing maternal behavior (the latency to become maternal test), motivation to seek pup related odors (using the pup-odor preference test) and fear mediated behavior (using the open field test) in different groups of postpartum
Method

The general methods of this experiment followed those outlined in the General Methods section. Cycling female rats (250-300g) were impregnated, and after birth were allowed five days contact with their pups. On day five postpartum pups were removed; bilateral ST electrolytic lesions or sham lesions were done and right unilateral bipolar electrodes were surgically implants into either the MPOA or the MedAmyg. All animals were given seven days to recover. Half of the animals were stimulated and the other half were sham stimulated. There were a total of sixty-four animals in eight groups (n=8). Of this total, sixteen animals were eliminated from the experiment: three animals pulled out their electrodes during testing and thirteen on the basis of histological analysis. The final groupings were as follows (see figure
20): MPOA/ST lesion/stimulated (n=6), MPOA/ST lesion/not stimulated (n=5), MPOA/sham lesion/stimulated (n=6), MPOA/sham lesion/not stimulated (n=6), MedAmyg/ST lesion/stimulated (n=6), MedAmyg/ST lesion/not stimulated (n=6), MedAmyg/sham lesion/stimulated (n=7) and MedAmyg/sham lesion/not stimulated (n=6). Stimulated animals were stimulated once a day for 14 days. Animals experiencing seizures during the stimulation or afterward were eliminated from the experiment and replaced with new subjects.

At day 29 postpartum animals were given the pup odor preference test and the open field test. The pup odor test measured the animal’s preference for head dipping in pup odored holes versus non-odored holes in a hole board apparatus. The open field test measured the animal’s willingness to explore an anxiety-provoking (open) environment. The next day maternal induction testing was initiated to determine level of maternal responsiveness, reflected in latency (in days) to show maternal behavior.
to foster pups. CPP testing was not conducted in this experiment.

Three way analyses of variance were conducted on the data for the following factors: stimulation (stimulated/not stimulated); location (MPOA/MedAmyg); lesion (ST lesion/sham lesion). The data were further explored by one way analyses of variance (provided in {braces}). Bonferroni post-hoc tests were conducted to assess differences between groups.

Histological analysis was conducted to assess the accuracy of electrode placements and lesions. Lesions were considered accurate if they obliterated the entire ST at AP level -0.4mm. The accuracy of all the ST lesions is displayed in Appendix 3.
EXPERIMENT 4

1. **PARTURATION**

2. **5 DAYS CONTACT WITH PUPS**

   - **DAY 1**
     - PUPS REMOVED
     - ELECTRODES IMPLANTED

   - **GROUPS**
     - **ST LESION**
       - MPOA STIM (n=6)
       - MPOA NOT STIM (n=5)
       - MED AMYG STIM (n=7)
       - MED AMYG STIM (n=6)
     - **SHAM LESION**
       - MPOA STIM (n=6)
       - MPOA NOT STIM (n=6)
       - MED AMYG NOT STIM (n=6)
       - MED AMYG NOT STIM (n=6)

3. **DAY 7 - 21**
   - ELECTRICAL STIMULATION

4. **DAY 29**
   - HOLE BOARD TEST
   - OPEN FIELD TEST

5. **DAY 30**
   - MATERNAL INDUCTION TEST

*Figure 20*
Results

Maternal Induction testing

A three way analysis of variance yielded a significant main effect of lesion ($F[1,47]=5.99, p<.01$) and an interaction of stimulation (stimulated/ not stimulated) and location (MPOA/ MedAmyg) ($F[1,47]=7.39, p<.01$) (one-way ANOVA: $F[7,40]=2.22; p<.05$) for latencies to reinduced maternal responsiveness (according to criteria; figure 21). Post-hoc testing showed that MPOA stimulated sham lesioned animals showed lower latencies to become maternal than MPOA not stimulated ST lesioned animals ($p<.05$).

Insert figure 21 about here

A three way analysis of variance yielded a significant 3-way interaction (stimulation by location by lesion) ($F[1,47]=8.76, p<.01$; figure 22) (one-way ANOVA: $F[7,40]=2.30; p<.05$) for latency to either retrieve pups
LATENCY TO BECOME MATERNAL BEHAVIOR (retrieve and hover over pups)

Figure 21
or hover over them. MedAmyg stimulated animals in the sham lesion condition showed greater latencies to become maternal than both MPOA stimulated sham lesion animals (p<.05) and MPOA stimulated ST lesion animals (p<.05). Also, MPOA not stimulated ST lesion animals showed greater latencies than MPOA stimulated sham lesion animals (p<.05).

A comparison of the mean frequency for behaviors observed during maternal latency testing (Table 4) showed that there was a significant 3-way interaction (stimulation by location by lesion) for the amount of sniffing pups performed in the first two days of induction (F[1,47]=3.9;p<.05) (one-way ANOVA: F[7,40]=2.83;p<.02). The MPOA stimulated sham lesion animals were sniffing pups more than the other groups (p<.05). There was also a main effect of pup sniffing
Figure 22

Latency in days (mean ± sem) to hover or retrieve.
during the last two days of induction, where the ST lesion groups were sniffing pups less than the sham lesion groups \((F[1,47]=5.0,p<.05)\). There was a 3-way interaction (stimulation by location by lesion) for nest building \((F[1,47]=5.8,p<.05)\) during the first two days, where the MPOA not stimulated ST lesioned animals were performing less nest building than the other groups \((p<.05)\).

Finally, there was a marginally significant main effect for retrieval during the last two days of induction \((F[1,47]=3.6,p<.06)\) \(\text{(one-way ANOVA: } F[7,40]=2.58;p<.03\)}\) during the first two days, where the ST lesion animals were performing more retrievals than sham lesion animals.

______________________________

Insert table 4 about here

______________________________

Hole Board and Open Field testing

A three way analysis of variance yielded a significant 3-way interaction of stimulation (stimulated/
Table 4: Daily mean frequencies (+SEM) for behaviors observed during maternal latency (induction) testing in Experiment Four (for first two [1st] and last two days [fin]).

<table>
<thead>
<tr>
<th></th>
<th>Experiment Four</th>
<th></th>
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<th>Signif. Diff's</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MPOA/ stim/ ST lesion</td>
<td>MPOA/ not stim/ ST lesion</td>
<td>MedAmyg / stim/ ST lesion</td>
<td>MedAmyg / not stim/ ST lesion</td>
<td>MPOA/ stim/ sham lesion</td>
<td>MPOA/ not stim/ sham lesion</td>
<td>MedAmyg / stim/ sham lesion</td>
<td>MedAmyg / not stim/ sham lesion</td>
<td></td>
</tr>
<tr>
<td>Sniff air (1st)</td>
<td>55.6 (5.3)</td>
<td>51.7 (3.3)</td>
<td>56.6 (2.7)</td>
<td>49.1 (5.5)</td>
<td>42.0 (2.9)</td>
<td>52.6 (7.4)</td>
<td>58.6 (4.1)</td>
<td>52.1 (6.0)</td>
<td>MPOA/SL&gt;all other groups (p&lt;.05)</td>
</tr>
<tr>
<td>Sniff air (fin)</td>
<td>37.3 (8.8)</td>
<td>39.0 (6.3)</td>
<td>35.0 (8.6)</td>
<td>39.9 (4.8)</td>
<td>54.8 (8.6)</td>
<td>31.7 (9.8)</td>
<td>36.7 (7.0)</td>
<td>45.2 (10.2)</td>
<td>ST&lt;SL (p&lt;.05)</td>
</tr>
<tr>
<td>Sniff pup (1st)</td>
<td>15.1 (5.5)</td>
<td>15.4 (1.7)</td>
<td>13.8 (1.9)</td>
<td>20.2 (1.7)</td>
<td>29.1 (5.3)</td>
<td>11.0 (2.8)</td>
<td>12.0 (3.5)</td>
<td>10.7 (3.7)</td>
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</tr>
<tr>
<td>Sniff pup (fin)</td>
<td>18.9 (3.8)</td>
<td>13.8 (2.6)</td>
<td>15.5 (2.9)</td>
<td>33.1 (13.2)</td>
<td>11.1 (1.9)</td>
<td>17.9 (1.8)</td>
<td>11.2 (3.3)</td>
<td>10.2 (4.3)</td>
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</tr>
<tr>
<td>Groom (1st)</td>
<td>7.4 (1.8)</td>
<td>9.3 (3.3)</td>
<td>8.5 (2.0)</td>
<td>5.6 (0.8)</td>
<td>9.0 (2.4)</td>
<td>13.5 (5.6)</td>
<td>10.8 (1.9)</td>
<td>10.9 (4.2)</td>
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<tr>
<td>Groom (fin)</td>
<td>6.8 (2.6)</td>
<td>6.9 (1.1)</td>
<td>5.1 (1.6)</td>
<td>5.9 (0.9)</td>
<td>2.4 (0.9)</td>
<td>7.9 (1.8)</td>
<td>7.2 (2.1)</td>
<td>8.0 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Lick (1st)</td>
<td>3.5 (1.6)</td>
<td>1.2 (1.0)</td>
<td>3.4 (1.8)</td>
<td>3.3 (2.0)</td>
<td>3.8 (5.7)</td>
<td>3.2 (1.6)</td>
<td>0.7 (0.4)</td>
<td>3.0 (1.6)</td>
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<tr>
<td></td>
<td>MPOA/N</td>
<td>S/SST,</td>
<td>MA/S/SST,</td>
<td>MA/NS/S</td>
<td>T-cell SL</td>
<td>gr &lt; 0.05</td>
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<tr>
<td>Lick (min)</td>
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<tr>
<td>Genital lick (1st)</td>
<td>0.4 (0.2)</td>
<td>1.9 (1.0)</td>
<td>6.4 (3.9)</td>
<td>9.4 (2.2)</td>
<td>4.0 (3.9)</td>
<td>6.6 (6.5)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Genital lick (2nd)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Retrieve (1st)</td>
<td>2.7 (2.7)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Retrieve (2nd)</td>
<td>2.7 (2.7)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
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<tr>
<td>Crouch (1st)</td>
<td>2.7 (2.7)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
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<td></td>
<td></td>
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<tr>
<td>Crouch (2nd)</td>
<td>2.7 (2.7)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.0 (1.4)</td>
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</tbody>
</table>

Experiment Four

<p>| MedAmyg / stim/ sham lesion | 2.4 (1.1) | 0.5 (0.3) | 3.6 (2.8) | 0.9 (0.6) | 5.3 (1.0) | 0.4 (0.4) |
| MedAmyg / not stim/ST lesion | 4.1 (1.8) | 1.1 (0.7) | 1.6 (1.0) | 0.1 (0.1) | 3.5 (0.5) | 0.4 (0.1) |
| MPOA/ S/SST sham lesion | 5.6 (5.4) | 1.1 (0.7) | 1.9 (0.9) | 1.6 (0.8) | 5.3 (1.1) | 2.8 (2.6) |
| MPOA/ S/SST not stim/ST lesion | 9.4 (3.3) | 0.7 (0.5) | 2.3 (0.4) | 1.0 (0.1) | 12.2 (4.6) | 3.1 (1.4) |
| MPOA/ S/SST stim/ ST lesion | 9.5 (4.8) | 0.0 (0.0) | 2.7 (2.7) | 0.0 (0.0) | 7.8 (3.4) | 3.1 (2.0) |
| MPOA/ S/SST not stim/ST lesion | 8.8 (3.0) | 4.0 (2.2) | 7.8 (3.4) | 4.0 (2.2) | 6.6 (6.5) | 1.9 (1.1) |</p>
<table>
<thead>
<tr>
<th>Experiment Four</th>
<th>MPOA/stim/ST lesion</th>
<th>MPOA/not stim/ST lesion</th>
<th>MedAmyg/stim/ST lesion</th>
<th>MedAmyg/not stim/ST lesion</th>
<th>MPOA/stim/sham lesion</th>
<th>MPOA/not stim/sham lesion</th>
<th>MedAmyg/stim/sham lesion</th>
<th>MedAmyg/not stim/sham lesion</th>
<th>Signif. Diff's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nest build (1st)</td>
<td>0.6 (0.5)</td>
<td>1.7 (1.6)</td>
<td>0.2 (0.2)</td>
<td>0.4 (0.2)</td>
<td>3.6 (1.0)</td>
<td>0.5 (0.3)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.8)</td>
<td>MPOA/S/SL &gt; all other groups (p&lt;.05)</td>
</tr>
<tr>
<td>Nest build (fin)</td>
<td>1.6 (0.7)</td>
<td>1.1 (0.8)</td>
<td>1.2 (0.6)</td>
<td>1.8 (0.7)</td>
<td>0.6 (0.4)</td>
<td>0.7 (0.3)</td>
<td>0.6 (0.4)</td>
<td>0.3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Settle (1st)</td>
<td>5.6 (5.5)</td>
<td>5.1 (3.8)</td>
<td>0.0 (0.0)</td>
<td>2.6 (1.5)</td>
<td>4.7 (3.4)</td>
<td>1.7 (1.6)</td>
<td>1.0 (1.0)</td>
<td>0.6 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Settle (fin)</td>
<td>0.6 (0.5)</td>
<td>3.3 (2.8)</td>
<td>0.4 (0.4)</td>
<td>0.9 (0.7)</td>
<td>3.2 (2.3)</td>
<td>0.3 (0.3)</td>
<td>0.0 (0.0)</td>
<td>0.4 (0.4)</td>
<td></td>
</tr>
</tbody>
</table>

MPOA - medial preoptic area  
MA - medial amygdala  
S - stimulated  
NS - not stimulated  
ST - stria terminalis lesion  
SL - sham lesion
not stimulated), location (MPOA/MedAmyg) and lesion (ST lesion/ sham lesion) for proportion of time spent sniffing in pup odored holes in the hole board (F[1,47]=4.65, p<.05; figure 23) (one-way ANOVA: F[7,40]=3.61; p<.01). Post-hoc testing showed that MedAmyg stimulated sham lesion group sniffed in pup odored holes less than the MPOA stimulated ST lesion (p<.05), MedAmyg stimulated ST lesion (p<.05), MedAmyg not stimulated ST lesion (p<.05) and MPOA stimulated sham lesion (p<.05) groups. There were no differences between groups for the frequency of head dips in pup-odored holes. Also, there were no differences between any groups for the total number of head dips suggesting that the odor preference differences between groups are not due to differences in activity levels.

Insert figure 23 about here

A three way analysis of variance yielded a
Figure 23

HOLE BOARD

ST lesion

PROPORTION OF TIME (mean+se)

MPOA

AME

SHAM

STIM

NOT STIM

MPOA

AME
significant interaction of stimulation (stimulated/ not stimulated) and location (MPOA/MedAmyg) \( (F[1,47]=3.6,p<.05; \text{figure } 24) \) (one-way ANOVA: \( F[7,40]=3.6;p<.05 \)) for the open field test. Post-hoc testing showed that MedAmyg stimulated sham lesion animals spent proportionally less time in the central squares of the open field than the MPOA stimulated ST lesion animals \( (p<.05) \). Also, MPOA stimulated animals in the ST lesion condition spent proportionally more time in the central squares of the open field than the MPOA not stimulated ST lesion animals \( (p<.05) \). There were no differences between groups for emergence testing.

Insert figure 24 about here

Histological analysis of the brain tissue indicated that the location of the electrode placements was fairly well restricted to the intended sites (figure 25).
Figure 24
Discussion

The results of this experiment are consistent with the previous investigations: MedAmyg stimulation in postpartum rats attenuated maternal behavior. However, MedAmyg stimulation did not as effectively attenuate maternal behavior in the lesion condition (compared to the sham lesion condition). MedAmyg stimulation only increased latencies to hover over or retrieve in the sham condition, although there was a trend toward this in the latencies to criteria (hover over and retrieve). This suggests that MedAmyg inhibition of maternal behavior is (at least, partially) dependent on its neural connections with the MPOA. It is possible that lesioning the ST eliminated the transmission of olfactory information from the medial amygdala to the MPOA. This may make pup odors unable to provide either reinforcing or aversive cues via
EXPERIMENT 4

SHAM LESION

Figure 25
the MedAmyg.

Further, ST lesions produced increased latencies (lower maternal responsiveness) in both the MPOA and MedAmyg not stimulated groups. The main effect of ST lesions may reflect a general attenuation of maternal responsiveness in the post-partum animal. Interestingly, there was a greater frequency of retrievals (during the last two days of induction testing) in the ST lesion groups. The animals were noted to move pups in a confused manner. This suggests that the attenuated maternal behavior displayed by ST lesion animals may be caused by a disorganized pattern of maternal behavior.

The difference between ST lesion and sham lesion conditions disappeared when considering the latencies to only retrieve or hover over pups. This again may be due to a disorganized approach. As a result these groups took longer to reach the outlined criteria of a coordinated maternal response.

Further analysis of behaviors performed during
maternal inductions revealed that MPOA stimulated animals initiated more pup sniffing in the first two days of induction, but only in the sham lesion condition. This is consistent with the previous two experiments using virgin animals, but surprisingly this effect was not found in the first experiment using postpartum animals. This is difficult to explain, but may reflect some of the effects of MPOA electrode tract lesioning mentioned in experiment one. The failure of MPOA stimulation to induce a quick onset of pup sniffing in the ST lesion condition may also be a result of electrode tract lesioning, but more likely reflects an attenuation of maternal responsiveness.

This attenuation of maternal responsiveness would not be consistent with Numan's findings (1974). Although, like Numan's study, there appeared to be a difference in the onset of nest building between sham and ST lesion animals, this experiment appears to have found a general reduction in maternal responsiveness. This apparent discrepancy may reflect the difference in species strain
used in the two studies (Numan used Wistar, whereas this study used Sprague-Dawley). Also, the time interval between lesion and testing may be important - Numan tested postpartum animals immediately after lesioning the ST whereas there was approximately a three week interval in this experiment. It is possible that ST lesions have an effect only detectable after a long period between parturition and test.

Consistent with previous investigations is the finding that MedAmyg stimulation attenuated attraction to pup odors in sham animals (as measured in the hole board). There were no differences between groups in the ST lesion condition. It appears that disconnecting the neural communication between the MPOA and the MedAmyg eliminated the pup-specific stimulation induced affective changes associated with maternal behavior. This suggests that motivation towards pup-related stimuli in the onset and maintenance of maternal behavior is dependant on communication between the MPOA and the MedAmyg.
Sham lesioned animals that were MedAmyg stimulated were also more 'anxious' in a novel environment. This effect has been replicated in a number of paradigms (Adamec & Morgan, 1994; Henke & Sullivan, 1985). This strong effect was absent in ST lesioned animals, suggesting that amygdala-related emotions are transmitted via the ST. Further, this suggests that the more generalized affective changes associated with maternal behavior are also dependant on communication between the MPOA and the MedAmyg. However, there was a trend toward MedAmyg stimulated anxiogenesis in ST lesioned animals. It is possible that this information is (partially) transmitted via the ventral amygdalofugal pathway to the hypothalamus.

Interestingly, the MPOA not stimulated animals in the ST lesion condition also appeared to be particularly anxious. This finding could reflect damage to the nucleus by the lowering of electrodes. It is possible that the combination of unintentional co-lateral damage and ST
lesions was anxiogenic.

**A Further Comparison of Experiment 1 and Experiment 4**

*(postpartum animals)*

Experiments one and four investigated the effects of stimulating the MPOA and the MedAmyg in postpartum animals. Further, the interaction of the MPOA and the MedAmyg was investigated using ST lesions (in experiment four).

Both experiments yielded similar facilitations and attenuations of MPOA stimulation and MedAmyg stimulation respectively (although the MPOA facilitation in experiment one was not significant). In experiment four, the addition of a ST lesion, severing the main connection between the MPOA and the MedAmyg, (at least partially) eliminated the attenuating effects of MedAmyg stimulation, but not the facilitating effect of MPOA. The effect was seen when comparing groups for latency to either crouch over pups or retrieve them and not the more
stringent criteria of crouch over and retrieve. There was a trend toward this effect for the latter criteria (this may reflect a more subtle change that is undetected by our strict criteria to become fully maternal). These findings suggest that MedAmyg inhibition of maternal behavior is (at least partially) dependent on its neural connections with the MPOA. It is possible that lesioning the ST eliminated the transmission (of amydaloid response to olfactory information) from the medial amygdala to the MPOA, making pup odors unable to provide either reinforcing or aversive cues via the MedAmyg.

We were unable to compare affective changes between animals in experiment one and four. However, it is interesting to note that there were similar changes, as a result of stimulation, between animals in experiment two and four (sham lesion animals). MedAmyg stimulation induced anxiety (as measured by the open field) in both experiments. Also, MedAmyg stimulation reduced the amount of pup odor sniffing (as measured by the hole board) in
both experiments. Interestingly, in experiment four, there was an attenuation of anxiety for MPOA stimulated animals that had ST lesions (compared to MPOA/not stimulated/ST lesion and MedAmyg/stimulated/sham lesion groups). This was not observed in the sham lesion control condition or in experiment two, and may indicate an interaction between MPOA stimulation and ST lesioning.

Finally, the effects of ST lesions shows an increase in latency to become maternal, a decrease in pup sniffing (during the first two days of induction), and an increase in retrieval behavior (compared to sham lesion animals). These results suggest that ST lesions have a detrimental effect on maternal responsiveness for multiparous animals.
General Discussion

The results of these experiments add important information to our understanding of the functional neurocircuitry of maternal behavior. In addition, the stimulation treatments used in this thesis introduce an intriguing new tool for investigating the functions of neural substrates important in maternal behavior.

A summary of results across experiments is provided in tables 5 and 6; table 5 shows the main effects of MPOA stimulation across experiments, whereas table 6 shows the main effects of MedAmyg stimulation.

Insert table 5 & 6 about here

This general discussion will be divided into three parts. First, an overview of the results will be given. Experiments will be compared with each other. Second, the mechanism of change seen in the stimulated groups will be
Table 5: A schematic comparative representation (and significance level) of significant MPOA stimulation results for a) maternal behavior tests (induction latencies and frequencies of behaviors for the first two [1st] and last two [fin] days) and b) emotional and motivational tests (hole board, open field, and CPP) from Experiments 1, 2, 3, and 4. Checkerboard cells indicate experiments in which particular tests were not performed. Results in brackets indicate chi-square analyses.

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<tr>
<th>Comparison of Experiments (MPOA stimulation)</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>a) MATERNAL BEHAVIOR</td>
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<tr>
<td></td>
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<tr>
<td>Latency to become maternal</td>
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<td>(more MPOA/S became maternal within 10 days than MA/S animals)</td>
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<tr>
<td>Latency to crouch or retrieve</td>
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<tr>
<td>Sniff air (1st)</td>
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<tr>
<td>Sniff air (fin)</td>
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<td></td>
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<tr>
<td>Sniff pup (1st)</td>
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<tr>
<td>MPOA/S&gt;MPOA/NS, MA/S, MA/NS (p&lt;.05)</td>
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<td></td>
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<td>Groom (1st)</td>
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<td>Open field (proportion of central squares crossed)</td>
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<td>CPP (% preference)</td>
<td>(MPOA/S spent more time in pup associated box; other groups showed no preferences)</td>
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MPOA-medial preoptic area
MA-medial amygdala
S-stimulated
NS-not stimulated
E-pup exposed
NE-not exposed
ST-stria terminalis lesion
SL-sham lesion
Table 6: A schematic comparative representation (and significance level) of significant MedAmyg stimulation results for a) maternal behavior tests (induction latencies and frequencies of behaviors for the first two [1st] and last two [fin] days) and b) emotional and motivational tests (hole board, open field, and CPP) from Experiments 1, 2, 3, and 4. Checkerboard cells indicate experiments in which particular tests were not performed. Results in brackets indicate chi-square analyses.

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<td>Open field (proportion of central squares crossed)</td>
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<td>CPP (% preference)</td>
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MPOA - medial preoptic area  
MA - medial amygdala  
S - stimulated  
NS - not stimulated  
E - pup exposed  
NE - not exposed  
ST - stria terminalis lesion  
SL - sham lesion
discussed. Finally, a section on what these results mean and what the future directions for research will be discussed.

Summary and Comparison of Results

The General Effect of Stimulation on Latencies to Become Maternal

First, kindling-like stimulation of the MedAmyg can attenuate the expression of maternal behavior (as measured in latency to become maternal in days). The cause of this attenuation is likely the result of neuronal activity in the MedAmyg caused by the kindling-like stimulation. This attenuation is more obvious in postpartum animals than nulliparous (virgin) animals. Hence, there is probably a pre-existing difference in the substrate being stimulated between parturitional conditions, possibly reflecting neural reorganization (or priming) in the postpartum animal in response to hormones and pup experience. The inhibitory role of the MedAmyg is
naturally suppressed in experienced females. However, it appears that kindling-like stimulation can re-induce this inhibitory role in postpartum animals. MedAmyg stimulation in virgin animals likely showed little effect in latency to become maternal because these animals have a pre-existing pup phobia. We may indeed have extended the latencies in many of these animals, but induction testing was discontinued at day 10.

Kindling-like stimulation of the MPOA appeared to facilitate maternal behavior, although this effect appeared to be weaker and less consistent (between studies). MPOA stimulation appeared to induce more virgin animals to become maternal (compared to not stimulated animals) within the induction testing period. The effect of MPOA stimulation on multiparous cycling animals seemed less clear. In experiment one, MPOA stimulation appeared to have a marginal effect, whereas there was a significant reduction in latencies to become maternal in experiment four (comparing sham lesion and ST lesion
groups). One explanation for this difference (between experiments for MPOA stimulation) is the possibility of co-lateral damage in the nucleus caused by lowering the electrode. An observational re-analysis of the placement of electrodes in the MPOA of experiments one and four show that the electrode tips were lowered further in experiment one, possibly causing more damage. Also, the difference between the effect of MPOA stimulation on maternal behavior and the effect of MedAmyg stimulation may reflect difference in responsiveness to the electrical stimulation. The MPOA 'kindles' more slowly than the MedAmyg and may require additional stimulation to bring it to the same afterdischarge level as the MedAmyg.

The results from experiments one and four also suggest an interaction of stimulation and parturitional condition. Postpartum (primed) but naturally less pup responsive MPOA stimulated animals showed a quicker reinduction of maternal behavior than not stimulated
animals and all nulliparous (virgin) animals regardless of stimulation condition. This suggests an additive effect of kindling-like stimulation and parturitional experience in the MPOA on the control of maternal behavior.

The General Effect of Stimulation on Motivational and Emotional Behaviors

The results also suggest that kindling-like stimulation induced motivational and emotional changes in these animals. MedAmyg stimulation in both nulliparous (virgin) and multiparous animals appeared to increase anxiety (as measured by the open field) and decrease the amount of exploration for pup odors (as measured by the hole board). However, it should also be pointed out that these effects were absent in experiment three. This apparently discrepant result may be explained by the slightly different experimental procedures in experiment three, which may have changed the animals' motivational
It is interesting to note that a preference for pup-associated stimuli developed in virgin animals that were stimulated in the MPOA (as measured by the CPP in experiment two). This suggests that the MPOA is also involved in motivational changes associated with maternal behavior. In experiments two, three and four there was an increase in pup sniffing during the first two days of induction testing, with MPOA stimulation. This suggests that MPOA stimulation facilitates the animals' motivation to approach and sniff pup stimuli. Also, hole board exploration for pup odors in experiment four (using post-partum animals) showed a trend toward MPOA stimulated pup-odor attraction (in the sham lesion condition), although this may only reflect a difference with MPOA not stimulated groups that have suffered electrode tract damage (see figure 23). This experiment also yielded similar results as experiment two for the open field, where MedAmyg stimulated animals were more anxious than
the other groups (sham lesion).

The role of the MPOA in motivational and emotional responses to pup stimuli is controversial. It is not clear whether the MPOA is simply involved in coordinating the effector response or changing the animal’s affective response (toward pup stimuli). However, there is some recent evidence to suggest that the MPOA is indeed involved in these responses. Lee & Fleming (in press) found that MPOA lesions reduced bar-pressing for pup rewards in postpartum female rats. There is also evidence that the MPOA is involved in motivating the animals toward reproductively relevant stimuli. Notably, the MPOA appears to be involved in sexual motivation in male rats; MPOA lesions reduce sexual motivation (Paredes et al., 1993), infusion of naloxone into the MPOA blocks sexual reinforcement (Agmo & Gomez, 1993), and dopamine antagonists infused into the MPOA decrease sexual motivation (Hull et al., 1995; Moses et al., 1995; Warner et al., 1991). The results of this thesis would tend to
support the hypothesis that the MPOA is involved in motivating the dam toward pup stimuli, although further experiments should be conducted using ‘motivational’ measures.

**Mechanisms of Behavioral Change in Stimulated Animals**

The enhanced function that kindling-like stimulation produces in this study may reflect (at the behavioral level) long-term neural changes that stimulation within these parameters usually produces. We assume that these changes are localized to the targeted substrates since there was a dissociation in the effect of stimulating the two structures (MPOA and MedAmyg) even though they are connected in a circuit. Further, we have evidence that this circuit is in series since pilot (calibration) testing showed a transfer of afterdischarge from the MedAmyg to the MPOA but not in the other direction. Hence, electrical stimulation applied to the MPOA did not activate the MedAmyg within these stimulation parameters.
Also, in the ST lesion study (experiment four) stimulating the MPOA after an ST lesion had the same effect as without, whereas stimulating the MedAmyg after an ST lesion had a reduced effect (compared to stimulating the MedAmyg in intact animals). This suggests the effects of MedAmyg activation on maternal behavior are dependent its connection with the MPOA, but not vice versa. It is possible that some of the detrimental effects on maternal behavior observed after MedAmyg stimulation might not have been the direct result of MedAmyg activity, but instead might have been the result of disorganized electrical activity within the MPOA. However, the effect of MPOA activation with MedAmyg stimulation needs to be investigated further.

There is evidence that kindling stimulation and maternal experience induce a similar neurological reorganization. First, kindling stimulation results in a hyperexcitable cells at the stimulation focus (Racine, 1991). This hyperexcitability of cells in the kindled
brain is thought to involve a modification of synapses at the kindling focus (McNamera, 1995). Modification of these synapses could involve structural rearrangements. Sutala and colleagues (Sutula et al., 1998; Sutula, He, Cavazos & Scott, 1988) and Nadler, Perry & Cotman (1980) have discovered that kindling is accompanied by sprouting of the mossy fiber axons of the dentate granule cells of the hippocampus. Also, Represa and Ben-Ari (1992) have found that kindling induced mossy-fiber sprouting is associated with the establishment of novel synapses (with basilar dendrites of CA3 pyramidal neurons). It is not clear whether these changes can occur as a result of kindling other parts of the brain, however, Nishizuka et al. (1991) have found that hippocampal kindling modified the number of dendritic synapses with amygdala.

There is also evidence that the brain of maternally behaving rats is altered as a result of the behavior of the animal towards her pups. Modney and Hatton (1990) have found morphological changes in the supraoptic
nucleus of lactating animals and virgin animals induced to become maternal. The supraoptic nuclei in these animals have a higher incidence of dendritic bundling relative to virgin animals that have not been induced to behave maternally (Modney & Hatton, 1994; Modney & Hatton, 1990). Further, electrical stimulation (10Hz for 10 minutes) of the lateral olfactory tract was found to elevate the coupling among supraoptic neurons of maternally behaving animals (Modney, Yang & Hatton, 1990). Hence, the cellular changes that result from kindling stimulation may involve similar neurological changes that occur as a result of maternal experience.

Although we believe the effects of electrical stimulation of MPOA and MedAmyg are due to the activation of behaviorally relevant neural substrates we cannot rule out the possibility that the endocrine system is also being activated and that changes in adrenal or ovarian steroids are mediating the behavioral effects. However, this seems unlikely for a number of reasons. First,
MedAmyg lesions decrease (Peters & Gala, 1980) and 100Hz 800 micro amp electrical stimulation for 30 minutes increases (Peters & Gala, 1975) the release of prolactin, a hormone important in the onset of maternal behavior. These findings suggest that MedAmyg stimulation could initially elicit maternal behavior by prolactin release. Our findings show the opposite effect, suggesting that we are inducing a long term change in the behavioral functioning of the medial amygdala, independent of hormonal mediation. MPOA stimulation has been found to decrease prolactin release. Colombo and Phelps (1981) found that 30 minutes electrical stimulation 50Hz, 200 micro amps resulted in a transient (4 hour) decrease in prolactin levels (compared to not stimulated controls). Similarly, Wiersma and Kastelijn (1990) found that 25 minutes at 100Hz, 300 micro amps resulted in a prolactin decrease that lasted a half day. Both of these studies required stimulation sessions much longer than used in this study, and the changes were transitory. Additional
studies of both Med Amyg and MPOA stimulation show that changes in estrogen and progesterone release and estrous cycling occur only with stimulation at much higher intensities and duration than those used in this study, and those changes were transitory, lasting six hours or less (Wiersma & Kastelijn, 1990; Everett, 1965; Fink & Aiyer, 1974; Kawakami et al., 1973; Terasawa & Sawyer, 1969). There also evidence that MPOA stimulation increases blood LH levels (Everett & Tyrey, 1983; Arendash & Gallo, 1979). This increase appears to parallel an increase in stimulation intensity (Arendash & Gallo, 1979). However, even with 45 minutes stimulation at 100 Hz, 400 micro amp, the surge only lasted a maximum of two hours. For these reasons we believe that the reported stimulation-induced behavioral results are not due to the effects of electrical stimulation on hormone release. It should be pointed out that we attempted to monitor hormonal changes in experiment four by taking daily vaginal smears during and after stimulation.
Unfortunately, this data was lost.

It is also possible that electrical stimulation results in the large release of neurotransmitter substance, which in turn contributes to behavioral change. Low levels of electrical stimulation applied to the MPOA results in the simultaneous release of noradrenaline, which is in facilitated by estrogen (Herbison, Heavens & Dyer, 1990) and attenuated by opioids (Diez-Guerra, Augood, Emson & Dyer, 1987). Moltz, Roland, Steele & Halaris (1975) found an increase in hypothalamic norepinephrine turnover at parturition. Hence, a MPOA stimulated increase in noradrenaline release may have facilitated the onset of maternal behavior. Kindling stimulation of the amygdala has been found to increase the release of corticotropin-releasing factor (CRF) (Smith et al., 1997). Pedersen, Caldwell, McGuire & Evans (1991) have reported that icv infusions of CRF into virgin rats resulted in increased incidences of pup killing and lower incidences of maternal care.
Amygdala stimulated release of CRF could contribute to the attenuation of maternal behavior.

Conclusions and Future Directions

The results of these studies confirm that the MPOA and the MedAmyg are involved in facilitating and attenuating maternal responsiveness and related (precursor) behaviors, respectively. It appears that chronic (kindling-like) stimulation of these neural substrates enhances their functions. MPOA stimulated facilitated function was further enhanced by pup experience. Also, the attenuation of maternal behavior appears to be (at least somewhat) dependent on the MedAmyg connections with the MPOA. This set of experiments clarifies our understanding of MPOA/MedAmyg functioning and their relationship with regards to maternal behavior, using a unique technique.

The results of this experiment have provided interesting answers the main questions that have
constituted the focus of this thesis. First, the role of the MPOA in maternal behavior has been further elucidated. The stimulation results herein suggest that the MPOA is involved in maternal motivation (results from CPP in experiment two and increase in pup sniffing during the first two days of induction testing in experiments two, three and four). Second, the medial amygdala appears to be involved in general motivational/emotional changes (increased anxiety and decreased head-dipping in pup-odored holes seen in experiments two and four). Third, the stimulation treatment appeared to result in the long term changes in a maternal responsivness. Finally, the MPOA and MedAmyg appear to be most strongly related in a serial processing arrangement from the MedAmyg to the MPOA. If the main pathway of this connection is severed, both maternal responsiveness and motivational/emotional aspects of stimulation induced change are discontinued in response to MedAmyg stimulation. However, MPOA stimulation continues to facilitate maternal behavior.
The result of this set of studies further elucidates the functional dissociation between the MPOA and MedAmyg, and provides support for the dual circuit system for the control of maternal expression postulated by Numan. Numan & Sheehan (1997) suggest that an olfactory bulb-to-medial amygdala-to-ventromedial hypothalamus (VMH) circuit may compose part of a neural circuit that regulates an olfactory based inhibition of maternal behavior. A separate neural circuit involving medial preoptic/ventral bed nucleus of the stria terminalis (VBNST) projections to the ventral tegmental area promotes maternal behavior by favoring approach to pups and regulating rewarding properties of behaving maternally (Numan & Sheehan, 1997). Our findings suggest that MedAmyg stimulation may have attenuated maternal behavior by increasing anxiety in the animal and by decreasing the attractiveness of pup odors. Further, MPOA stimulation appears to have facilitated maternal responsiveness, enhanced the rewarding properties of pup stimuli and promoted approach
to pups. The minimal overlap in associated behavioral change with MPOA and MedAmyg stimulation suggests that the two structures are involved in separate but interconnected neural circuits.

A further investigation of these circuits and their component structures might constitute one future focus of kindling-stimulation research. We speculate that kindling-like stimulation of the VBNST and/or the VTA would facilitate the same behavioral changes as MPOA stimulation. Also, olfactory bulb and/or VMH kindling-like stimulation would cause the same behavioral changes as MedAmyg stimulation.

Kindling-like stimulation may be used to answer a number of other questions raised in this paper. First, we found that pup experience can further enhance the facilitative effects MPOA stimulation. It is possible that hormones (estrogen, progesterone, prolactin, etc.) could have the same effect. We could study the interaction of exogenously administered hormones in
ovariectomized females that are being kindled. Second, we found that the MedAmyg is at least partially dependent on its connections with the MPOA through the ST for is influence on maternal behavior. This issue should be further explored using kindling-like stimulation in animals with knife-cuts around the MPOA or MedAmyg (severing ventral amygdalofugal as well as stria terminalis connections). Third, the issue of specificity of behavioral change should be further explored. It is not clear whether behavioral changes induced by MPOA stimulation are pup specific. This might be investigated using the CPP apparatus with some stimuli other than pups.

In addition, we might parametrically manipulate the length of pup experience, duration of kindling, number of pups, etc. This may further clarify the additive effects of stimulation and experience. Finally, c-fos studies show neural activation in a number of additional brain structures with maternal behavior, including: the
basolateral amygdala, parietal cortex, and prefrontal cortex (Fleming & Korsmit, 1996); the nucleus accumbens, lateral habenula, parventricular thalamic nucleus (Lonstein et al., 1998), and periaqueductal grey (Lonstein & Stern, 1997); the lateral septum and retrorubal field (Numan & Numan, 1997). These sites may be candidates for functional studies using the kindling-stimulation procedure.
References


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Ohkura, S. Fabre-Nys, C, Broad, KD & Kendrick, KM (1997). Sex hormones enhance the impact of male sensory cues on both primary association cortical components and visual and olfactory processing pathways as well as in limbic and hypothalamic regions in female sheep. *Neuroscience, 80(1),* 285-297.


Appendix 1: The Effects of 1, 5 & 10 Days Postpartum Pup Exposure on Subsequent Maternal Responsiveness 22 Days Later
Before examining the effects of (partial kindling) stimulation on target behaviors it was necessary to determine the optimal amount of experience dams should receive with their pups in order to show an increase or decrease in maternal responsiveness. From the final day of pup exposure to the beginning of induction testing an interim period of 22 days would result (with surgery, stimulation treatment and recovery periods forming the interim). It was not known how much exposure to the pups was sufficient for the dams to subsequently show maternal behavior after 22 days without maternal experience. Too much experience to pups may induce a quick onset of maternal behavior after the 22 day interim period, which would mask any facilitative effects that the stimulation may produce, whereas too little experience may have the opposite effect, masking any attenuating effect of stimulation. The purpose of the pilot testing was to determine if 1, 5 or 10 days of pup exposure were necessary to avoid ceiling or floor effects, and attempt to find an intermediate level of maternal responsiveness during maternal induction testing 22 days later. In the absence of stimulation treatment, it would be preferable to have the dam exhibit a maternal latency period of approximately 5 days (half way between day 1 and the day 10 discontinuation).

Method
SUBJECTS AND HOUSING
Subjects (N=15) were Sprague-Dawley females, (250-300g) selected from the same colony as the main experiments' rats. The females were divided into three groups: 1 day experience (n=5), 5 days experience (n=5), and 10 days experience (n=5).

Females were housed in plastic cages (22x45x15cm) with wood shavings serving as bedding. Purina rat chow and water was available ad lib throughout the study. Temperature of the room was controlled at 22 degrees Celsius with a 12:12 hr light cycle. Three day prior to maternal induction testing, the subjects were relocated to larger clear plastic cages (37x47x21cm), wood shavings served as bedding and two pieces of paper towel were added for nesting material.
PROCEDURES

Experienced males were placed into the female's cages for five days of mating. After five days the male was removed and females were housed individually until parturition. Day of parturition was labeled day 0. On days 1, 5 and 10 the pups were removed from the dams of the respective groups.

BEHAVIORAL TESTING

Maternal induction testing was conducted using the procedures outlined in the General Methods section of this thesis. Although all behaviors using the procedures were recorded, only the maternal criteria stated was examined as a measure of maternal responsiveness.

RESULTS

The comparison of the three groups was done using the median of the days to become maternal according to criteria. The 5 day group showed a longer latency to become maternal (Mdn=5 days) than did both the 1 day group (Mdn=3 days) and the 10 day group (Mdn=2 days).

DISCUSSION

These results suggest that females with 5 days of experience with their pups at parturition show the optimal maternal latency for this experimental paradigm. A 5 latency to become maternal permits ant experimental effects of (partial kindling) stimulation to produce detectable changes (whether inhibitory or facilitative) in maternal responsiveness. The day 10 and day 1 groups displayed a relatively small latency to become that would mask any facilitative stimulation effects. However, the differences in responsiveness were not as predicted. More exposure to pups was expected to produce a decrease in maternal latency. However, 1 day exposure was expected to have the opposite effect. As demonstrated, 1 and 10 days of exposure before the 22 day interim period had similar latencies at induction testing, whereas 5 days exposure produced a longer latency. These results may be explained by pup size/age during exposure. It is possible that small pups such as those for the 1 day group may produce lasting reinforcing properties. Hence, after the 22 day interval the female continues to be highly motivated toward pup stimuli. The 10 day group would have had
relatively larger pups, which generally produce a higher level of anxiety in the dam. The anxiety experienced by the dam was probably compensated for by the increased length of exposure, allowing the female to more quickly exhibit maternal behavior.

Regardless of the outcome (or speculated reasons for the outcome) for 1 day and 10 exposure latencies, the 5 day exposure period yielded the desired median latency period. This exposure period was utilized for experiments 1 and 4, where post partum, pup experienced but minimally maternally responsive dams were required.
Appendix 2: A schematic comparative representation (mean and standard error of the mean) for the results of experiment two. Experiment two was originally divided into eight groups (MPOA/stimulated/pup exposed, MPOA/not stimulated/pup exposed, MedAmyg/stimulated/pup exposed, MedAmyg/not stimulated/pup exposed, MPOA/stimulated/not exposed, MPOA/not stimulated/not exposed, MedAmyg/stimulated/not exposed, MedAmyg/not stimulated/not exposed. There were no differences, on any measures, between groups for the exposure condition. Subsequent analyses were conducted with the pup-exposed and not exposed conditions combined. Results are shown for a) maternal behavior tests (induction latencies and frequencies of behaviors for the first two [lst] and last two [fin] days) and b) emotional and motivational tests (hole board, open field, and CPP).
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Note: The data includes means and standard errors (in parentheses).
## Means and SEMs for groups in Experiment Two

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MP-medial preotic area  
MA-medial amygdala  
E-pup exposed  
NE-not exposed  
S-stimulated  
NS-not stimulated
Appendix 3: Lesion sites are shown for ST lesion animals in Experiment 4. Lesions are shown in grey.

MPOA/STIM = 3325
    44
    1035
    859
    857
    3246

MPOA/NOT STIM = 37
    70
    1058
    855
    830

MedAmyg/STIM = 40
    74
    3248
    3250
    1033
    867
    862

MedAmyg/NOT STIM = 30
    32
    61
    1061
    100
    835