Neural Control of Parental Behaviour in California Mice, *Peromyscus californicus*

by

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Abstract

The experiments described in this dissertation investigate the neural control of male and female parental behaviour in California mice, *Peromyscus californicus*. Chapter 1 describes maternal behaviour in rodents, reviews the literature on the neuroanatomy of maternal behaviour, discusses the importance of male parental care in biparental species, and reviews studies of the neural control of paternal behaviour in rodents. Chapter 1 concludes with a description of the ecology, reproduction, and parental behaviour of California mice. Chapter 2 (experiment 1) shows that medial preoptic area (MPOA) lesions disrupt parental behaviour in male and female California mice, suggesting that the MPOA is important for paternal and maternal behaviour in this species. Chapter 3 (experiment 2) shows that nucleus accumbens (NA) lesions have no effect on parental behaviour, while basolateral amygdala (BA) lesions disrupt male, but not female parental behaviour in this species. Chapter 3 also shows that MPOA lesions consistently produce deficits in parental behaviour in California mice. Chapter 4 (experiment 3) shows that parental behaviour induces c-Fos-immunoreactivity (ir) in the MPOA, MA, and piriform and somatosensory cortices. Parental behaviour, however, does not increase FosB or c-Jun-ir in California mice. Chapter five consists of a general discussion which addresses issues relevant to the three studies, including a comparison of male and female California mice and a description of their behavioural profiles.

List of Abbreviations

ac	anterior commissure
acp	posterior anterior commissure
Aco	anterior cortical amygdala
BA	basolateral amygdala
BLA	anterior basolateral amygdala
BMP	posterior basomedial amygdala
BNST	bed nucleus of the stria terminalis
BSTLD	bed nucleus of the stria terminalis - lateral division, dorsal
BSTLJ	bed nucleus of the stria terminalis - lateral division, juxtacapsular
BSTLP	bed nucleus of the stria terminalis - lateral division, posterior
BSTLV	bed nucleus of the stria terminalis - lateral division, ventral
BSTMA	bed nucleus of the stria terminalis - medial division, anterior
BSTMV	bed nucleus of the stria terminalis - medial division
CeC	central amygdala, caps division
CeL	central amygdala, lateral division
CA	central amygdala
DV	dorsal - ventral
IBI	inter-birth interval
ICC	immunocytochemistry

IEG	immediate early gene
ir	immunoreactivity
ITF	inducible transcription factor
LPA	lateral preoptic area
LSV	lateral septal nucleus, ventral division
MA	medial amygdala
MePD	posterodorsal medial amygdala
MePV	medial posteroventral medial amygdala
ML	medial - lateral
MPOA	medial preoptic area
MPOC	medial preoptic nucleus, central
MPOL	medial preoptic nucleus, lateral
MPOM	medial preoptic nucleus, medial
NA	nucleus accumbens
NAC	nucleus accumbens core
NAS	nucleus accumbens shell
NMDA	N-methyl-DL-aspartic acid
S1BF	somatosensory 1, barrel field
S2	secondary somatosensory cortex
SPSS	Statistical Package for Social Sciences
VDB	ventral diagonal band
VTA	ventral tegmental area

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1. Parental behaviour

This chapter reviews studies of parental behaviour in rodents with an emphasis on the rat model of maternal behaviour, as well as a model which involves both male and female parental behaviour. Parental behaviour refers to any action(s) of the parent that increases the likelihood of survival of the offspring. Parental behaviour may be direct, which includes physical interactions such as adopting a nursing posture, licking and retrieving pups, or indirect, which includes behaviours that may occur in the absence of the offspring such as nest building, providing resources and protection from predators (Kleiman & Malcolm, 1981). Parental behaviour in mammals has been studied most extensively in rodents, particularly in the female rat, and thus, the majority of the literature on the neural and endocrine control of parental behaviour is based on the female rat model of maternal behaviour. This chapter will begin by describing some landmark findings in the study of the physiological basis of maternal behaviour in rats. The neuroendocrine control of male parental behaviour has largely been ignored. Thus, parental behaviour of both males and females in other rodents such as mice, voles, gerbils, and hamsters will be discussed where literature exists.

1.1. The female rat model of parental behaviour

The neuroendocrine determinants of parental behaviour in mammals have been studied most extensively in rodents and the rat (*Rattus norvegicus*) is

commonly used as a model for maternal behaviour. In the rat model of maternal behaviour, changes in estrogen, progesterone and prolactin during pregnancy initiate maternal behaviour (Rosenblatt et al., 1988). The rise in estrogen, followed by the rise and fall of progesterone and the increase in prolactin plus the burst of oxytocin at birth trigger maternal behaviour in female rats (Rosenblatt et al., 1988), but sensory stimuli and pup stimuli are also important (Pryce, 1992).

Female rats express maternal behaviour for the first time during late pregnancy. Within hours of parturition, the new mother reconstructs the nest, retrieves pups into it, licks them, and adopts a nursing posture over them, allowing them to suckle. These behaviours are regulated by an integrated neural program, which is suppressed in virgin rats (Fleming et al., 1979). When virgin female rats are presented with newborn foster pups, they do not respond maternally to them, but instead, avoid, bury, or cannibalize them. However, if virgin female rats are provided with daily contact with 1-3-day-old pups, a process known as sensitization, they begin to stay near them, and after 5 - 7 days of continuous contact, begin to respond parentally to them (Rosenblatt, 1967).

1.1.1. Importance of sensory stimuli

Auditory, olfactory, visual, and tactile stimuli are all important for maternal behaviour in the rat, but none of these appear to be crucial, with the possible exception of tactile stimuli in the perioral region, which are important for pup retrieval (Beach & Jaynes, 1956; Benuck & Rowe, 1975; Herrenkohl &

Rosenberg, 1972; Kenyon et al., 1981). Olfactory stimulation from both the primary olfactory epithelium and vomeronasal organ may play a role in the induction of maternal behaviour. Virgin rats with severed vomeronasal nerves start showing maternal behaviour with shorter latencies than females without such lesions (Fleming et al., 1979). The same is true for virgin females with lesions of the olfactory bulbs and accessory olfactory bulbs, which receive vomeronasal input, and with lesions of the bed nucleus of the stria terminalis, which receives input from the accessory olfactory bulb (Del Cerro et al., 1991; Fleming et al., 1979). Thus, in virgin female rats, pup olfactory stimuli appear to inhibit maternal behaviour. However, the importance of olfactory and accessory olfactory input appears to change during pregnancy, as pup odours are attractive for lactating rats (Fleming et al., 1989).

1.1.2. Hormonal control of maternal behaviour

There is substantial evidence that the hormones associated with late pregnancy and parturition account for the rapid activation of maternal responsiveness seen at the end of pregnancy and following parturition. When virgin rats are presented with pups for the first time, they ignore or cannibalize them. During pregnancy, responsiveness towards pups increases gradually until about 1-1.5 days before parturition, when females show all aspects of maternal behaviour (Mayer & Rosenblatt, 1984). This increase in maternal responsiveness is caused by the rise in estrogen and prolactin in the later stages of pregnancy and the sudden drop in progesterone at the time of birth (Bridges,

1990; Rosenblatt et al., 1988). Once maternal behaviour has been induced, these hormones need not be present to maintain the behaviour (Rosenblatt, 1967), but appear to enhance the quality of behaviour (Bridges, 1990), suggesting that the regulation of maternal behaviour should be subdivided into at least two different phases, onset and maintenance.

The virgin female, in contrast, does not undergo these same hormonal changes, even when she begins to show maternal behaviour as a result of sensitization procedures (Terkel & Rosenblatt, 1972). Many sensitized virgin females show increased prolactin levels, but this occurs several days after the onset of maternal behaviour (Koranyi et al., 1978). Thus, the rise in prolactin does not play a role in the onset of maternal behaviour in virgin female rats, as it does in primiparous females. Nulliparous rats injected with plasma from a newly parturient animal show maternal behaviour in 2 days (Terkel & Rosenblatt, 1972). However, nulliparous rats receiving blood from females in late pregnancy and 24 h postpartum do not show the same rapid onset of maternal behaviour, further supporting a role of hormones at parturition for the initiation of maternal responsiveness.

1.1.3. Neural control of maternal behaviour

The study of the neuroanatomy of parental behaviour in mammals has been primarily the study of maternal behaviour in the rat (see Numan, 1994 for review). The medial preoptic area of the hypothalamus (MPOA) is one of the most important neural sites for the control of parental behaviour in the female rat

(Figure 1). Electrolytic, radiofrequency, and axon-sparing neurochemical lesions of the MPOA, as well as knife cuts that sever the lateral or anterior connections of the MPOA, severely disrupt maternal behaviour (Miceli et al., 1983; Numan, 1974; Numan & Callahan, 1980; Numan & Corodimas, 1985; Numan et al., 1988; Numan et al., 1977). The MPOA receives sensory input from the main olfactory bulb and the vomeronasal organ via the medial amygdala and the bed nucleus of the stria terminalis (BNST; Fleming, 1986). The MPOA has projections to the lateral preoptic area and the ventral tegmental area (VTA; Numan, 1994). The VTA has ascending projections to the nucleus accumbens, which has also been shown to be important for the retention of maternal behaviour in female rats (Lee, et al., 1999).

Several studies have used c-Fos immunocytochemistry (ICC) to identify neurons that are activated in female rats during maternal behaviour. *c-fos* is an immediate early gene in the *fos* family, which also includes *fosB, fra-1*, and *fra-2* (Sheng & Greenberg, 1990). c-Fos, the protein product of the *c-fos* gene, functions as a transcription factor and its production can be activated by a variety of extracellular stimuli, such as nociceptive stimulation (Hunt et al., 1987), light pulses (Rusak et al., 1990), and psychostimulants (Chase et al., 2003). Thus, c-Fos immunoreactivity has been useful in identifying brain sites activated by various physiological and pharmacological stimuli and can be used as a neuronal marker of activation during maternal behaviour.

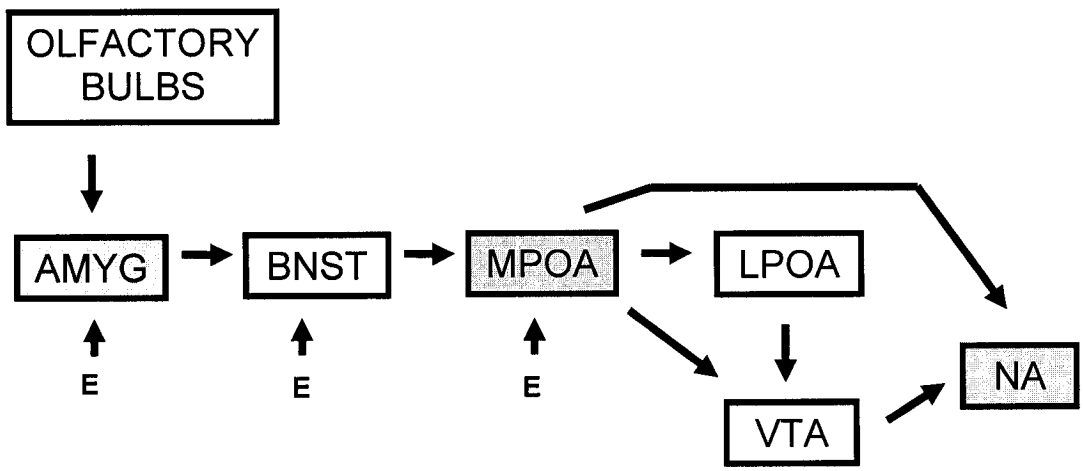


Figure 1. Neural circuitry of maternal behaviour. Olfactory input from the main olfactory bulbs and vomeronasal organ project to the medial preoptic area (MPOA) via the medial amygdala (AMYG) and bed nucleus of the stria terminalis (BNST). The MPOA has projections to the lateral preoptic area (LPOA) and then from the LPOA to the ventral tegmental area (VTA). The MPOA may also have lateral efferents to the NA, either directly or via the VTA. The amygdala, BNST, and MPOA contain estradiol receptors. Estrogen (E) is involved in the hormonal induction of maternal behaviour in rats.

Using Fos ICC, many brain sites have been implicated in maternal behaviour including the MPOA, ventral BNST, nucleus accumbens, and basolateral, central, medial and cortical amygdala (Fleming & Korsmit, 1996; Fleming & Walsh, 1994; Numan & Numan, 1994; Numan & Numan, 1995; Walsh et al., 1996). The Fos response seems to be specific to experience with pups, as the presentation of novel food or an adult conspecific fails to elicit the same degree of *c-fos* expression in the MPOA and ventral BNST (Fleming et al., 1994; Numan & Numan, 1994). Furthermore, the expression of *c-fos* in the MPOA and ventral BNST appears to be associated with the performance of maternal behaviour. For example, females with olfactory bulbectomies or thelectomies (nipple removal) behaving maternally show increased c-Fos immunoreactivity in these areas, suggesting that maternal interaction with pups, regardless of olfactory and suckling stimuli, elicits the Fos response (Numan & Numan, 1995; Walsh et al., 1996).

Although c-Fos is the most studied of the inducible transcription factors, Brown et al. (1996) showed that a transgenic mouse line that lacked the *fosB* gene had a disruption in maternal behaviour. This study stimulated other laboratories to examine the expression of *fosB* during maternal behaviour and they found increased FosB immunoreactivity in the MPOA and ventral BNST following maternal behaviour in rats (Lin et al., 1998; Numan et al., 1998).

In addition to immediate early genes (IEG) in the *fos* family, one other IEG, erg -1, also referred to as krox-24, zif-268, or ngfi-a, has been investigated in maternal behaviour in rats. Levels of Erg-1 were elevated above controls after 2

h of pup exposure, but decreased after 4 or 6 h of pup exposure in rats (Numan et al., 1998). The expression of the IEG, *c-jun*, during maternal behaviour has not been investigated.

1.1.3.1. Neural control of maternal behaviour in hamsters, voles, gerbils, and mice

The neural control of maternal behaviour has been studied less extensively in other rodents, including hamsters, voles, gerbils, and mice. In female hamsters, sagittal knife cuts severing the lateral connections of the MPOA disrupt maternal behaviour, consistent with studies in female rats (Miceli & Malsbury, 1982). Lesions in the septum disrupt maternal behaviour in CF-1 mice (Slotnick & Nigrosh, 1975), and olfactory bulbectomy eliminates maternal behaviour in both virgin and lactating albino Rockland-Swiss mice and C57BL/10J mice (Gandelman et al., 1971). However, in rats, olfactory bulbectomy induces a more rapid onset of maternal behaviour in both virgin and lactating females (Fleming & Rosenblatt, 1974; Fleming et al., 1979). Olfactory bulbectomy reduces affiliative behaviour but does not affect parental behaviour in female prairie voles, *Microtus ochrogaster* (Williams et al., 1992).

The expression of *c-fos* as a measure of the pattern of neural activation during maternal behaviour has been investigated in female mice and voles. Behavioural interaction with pups has been reported to increase Fos immunoreactivity in the MPOA in C57BL/6J and DBA/2J mice (Mathieson et al., 2002) and in the MPOA as well as in the olfactory areas in balb/c mice

(Calamandrei & Keverne, 1994). Similarly, Fos immunoreactivity increases immediately after parturition in the MPOA, hypothalamus, lateral septum, BNST, olfactory bulbs, and primary somatosensory area in female prairie voles (Katz et al., 1999).

1.1.4. Neuroendocrine control of maternal behaviour

The MPOA, which contains a high concentration of estrogen-binding neurons (Pfaff & Keiner, 1973), may be a site where estrogen acts to facilitate maternal responsiveness in female rats. Intracerebral injection of estradiol or prolactin into the MPOA facilitates maternal behaviour in female rats (Bridges et al., 1990; Numan et al., 1977). However, the neuroendocrine regulation of maternal behaviour is extremely complex and probably involves a large number of neurochemicals (Bridges, 1996; Keverne, 1988). For example, oxytocin, cholecystokinin, and dopamine seem to facilitate maternal behaviour, while corticotropin-releasing hormone inhibits maternal behaviour (Bridges, 1996). With respect to dopamine, on the day of parturition, levels are decreased in the preoptic area and increased in the dorsolateral striatum, suggesting that these changes in dopamine levels are important for maternal onset in rats (Lonstein et al., 2003). Extensive work continues to be done on the neurochemistry of maternal behaviour in the female rat.

1.2. An overview of male parental behaviour

Among the vertebrates, there are species that show no parental care,

uniparental male or female care, and biparental care. The existence of paternal behaviour is an interesting evolutionary occurrence, as males of most species maximize reproductive success by mating with several females. Any male investment in one female's offspring could reduce the male's chances of mating with other females. Therefore, time spent in parental care potentially decreases the male's reproductive success with other females.

Although male parental care occurs throughout the vertebrates, it is most common in bony fish, birds, and amphibians and rare in reptiles and mammals (Clutton-Brock, 1991). Male parental care occurs in 49% of bony fish species, biparental care in 13% and female only parental care in 7% (Gross & Sargent, 1985). Among the herptiles, 46% of amphibians studied and only 2% of reptiles show male parental behaviour (Gross & Shine, 1981). Over 90% of bird species have biparental care and less than 0.5% have male only parental care (Silver et al., 1985). Paternal behaviour is rare in mammals (about 3%), but is occasionally observed in monogamous rodents, carnivores and primates (Clutton-Brock, 1991). Numerous hypotheses have been put forward to explain the evolution of these different modes of parental care (Clutton-Brock, 1991; Crawford & Balon, 1996; Ketterson & Nolan, 1994; Ridley, 1978; Wesolowski, 1994), but will not be discussed in this thesis.

Male parental behaviour in mammals can be direct, with males huddling and sleeping with pups, licking, grooming and retrieving pups, or indirect, with males providing resources, and defending against predators, but showing no direct contact with pups (Kleiman & Malcolm, 1981). Very few male mammals

show direct paternal behaviour and those that do are mostly from monogamous species of rodents, carnivores or primates. However, even males of polygynous species may huddle with the young, groom and carry them, and retrieve them to the nest (Clutton-Brock, 1991). As discussed by Wynne-Edwards (1998), ecological conditions such as lack of food or water provide the proximal conditions for the evolution of paternal care in mammals, while the increases in pup survival and growth provide the ultimate causes.

Although males may exhibit behaviour similar to maternal behaviour, the underlying mechanisms of male and female parental behaviour may be different. The hormonal and neural basis of maternal behaviour in rats is well described (see Numan, 1994 for review). However, considerably less effort has been directed at describing paternal behaviour in rodents. With a few exceptions, studies of male parental behaviour are mainly descriptive and do not provide information on the underlying mechanisms. Furthermore, studies of male parental behaviour have generally used rats as subjects, which do not normally show paternal care.

The rest of this chapter describes male parental behaviour in rats, and discusses biparental behaviour in gerbils, voles, and hamsters, with a particular emphasis on California mice.

1.2.1. Male parental behaviour in rats

Although juvenile male rats show parental behaviour toward pups, adult males do not normally show paternal care. Once they are over 3 months of age,

most male rats fail to become parentally responsive even after prolonged exposure to pups, which induces parental responsiveness in virgin female rats (Brown, 1986a; Mayer et al., 1979). Some report that male rats show parental behaviour if repeatedly exposed to pups (Rosenblatt, 1967) or housed with females during pregnancy (Brown, 1986a), but the frequency of male rat parental behaviour is far below that of females. Furthermore, males which have not cohabitated with females are more likely to cannibalize pups than to show parental care (Brown, 1986b).

Male rats will show parental care if gonadectomized and primed with estrogen, progesterone and prolactin (Lubin, et al., 1972) and estradiol injections into the MPOA will also activate parental behaviour in male rats (Rosenblatt & Ceus, 1998). However, the male rat is not a good model for studying naturally occurring paternal behaviour. Brown (1993) has criticized the use of male rats in studying paternal behaviour and has suggested that gerbils or California mice, which show paternal care in the wild, would be more ideally suited for studies of paternal behaviour.

1.2.2. A model for male parental behaviour

Male parental behaviour has been studied far less extensively than female parental behaviour. This may be due to the minimal amount of male parental care observed in commonly used laboratory animals. Paternal care is displayed in approximately 6% of rodent genera, including Mongolian gerbils (*Meriones unguiculatus*; Elwood, 1975), California mice (*Peromyscus californicus*;

Gubernick & Alberts, 1987), Djungarian hamsters (*Phodopus campbelli*; Wynne-Edwards, 1995), and prairie voles (Wang & Insel, 1996). Males of monogamous species such as Mongolian gerbils and California mice are more likely to be parental than males of polygynous species such as rats and house mice. Although males of monogamous species such as prairie voles (Lonstein & De Vries, 1999) and California mice (Gubernick & Alberts, 1987) show levels of parental behaviour similar to those of females, paternal behaviour in male mammals is not the same as maternal behaviour in female mammals. Thus, it is not appropriate to study paternal behaviour as "male maternal behaviour".

The initiation of maternal behaviour in females is caused by hormonal changes during pregnancy and pup stimuli after parturition, whereas paternal behaviour in males results from neuroendocrine changes in response to environmental, female, and pup stimuli (see Brown, 1993 for review). In Brown's model of male parental behaviour, the expression of paternal behaviour is determined by the male's prenatal hormone environment, the mating system of the species, the type of parental care received, exposure to infants during development, the rearing environment, dominance status, recent mating experience, pair-bonding and cohabitation with a pregnant female, pup stimuli, and the ability of stimuli from the mate and pups to modulate neuroendocrine activity in the male. According to this model, there should be sex and species differences in the pattern of variables that are optimal for the expression of paternal behaviour in each species. The remainder of this chapter examines rodent species with male parental care, with particular emphasis on California

mice. Because the literature of the neural and endocrine basis of paternal behaviour in rodents is so sparse, gerbils, hamsters, voles, and mice will be discussed together at the end of the chapter.

1.2.3. Gerbils

Mongolian gerbils (*Meriones unguiculatus*) are small, plant-, root-, and seed-eating rodents, native to dry areas of eastern Mongolia and western Manchuria (Elwood, 1975). They live socially in cooperative pairs or extended family groups and form complex burrow systems (Hendrie & Starkey, 1998). Stable male-female pairs are formed, and only one female is normally in reproductive condition (Elwood, 1980). Therefore within a particular colony, reproduction is restricted to one dominant pair and the pair must remain in close contact for male infanticide to be inhibited (Elwood, 1980).

Male gerbils spend little time in the nest area on the day of birth because of the female's aggression towards the male (Elwood, 1975). Cases of "pup stealing" have been reported, in which the male approaches the nest where the female is giving birth and manages to sniff and lick the newborn before being chased away by the female (Elwood, 1975). There is a great reduction in female aggression one day after parturition, at which time, the male is allowed into the nest (Elwood, 1975). However, the male does not have complete access to the pups unless the female is absent from the nest (Elwood, 1975).

Male gerbils display all aspects of parental behaviour, but males generally spend more time in the nest area, while females show more sniffing and licking

pups (Elwood, 1975). When one of the parents is active outside the nest, the other parent is likely to be relatively inactive in the nest, and therefore, pups are seldom left alone (Waring & Perper, 1980).

1.2.4. Hamsters

The Siberian (*Phodopus sungorus*) and Djungarian (*Phodopus campbelli*) hamster both occupy a generalist niche in the forest-steppe zone for the palearctic (Wynne-Edwards, 1987). The Djungarian and the Siberian hamster are closely related species of dwarf hamsters; however, the species are behaviourally distinct. The Djungarian, but not the Siberian hamster, appears to be monogamous and show biparental care. Differences between the two species in their ability to tolerate and unload excess heat acquired while on the nest are suggested to account for differences in the need for biparental care in *P. campbelli* (Wynne-Edwards & Lisk, 1989). Both species of dwarf hamsters are small, well-insulated rodents, which have limited access to water and are intolerant of elevated temperatures (Wynne-Edwards,1995). While females of both species of dwarf hamsters become hyperthermic during lactation, Djungarian hamster mothers are more intolerant of hyperthermia and less able to remain in contact with pups than Siberian hamster mothers (Scribner & Wynne-Edwards, 1994b). The presence of the male thus allows the female to leave the pups and cool down without leaving the pups unprotected. Water restriction and extreme environmental temperatures decrease pup survival in both species and the presence of the male increases pup survival under environmental extremes

in the Djungarian but not Siberian hamster (Scribner & Wynne-Edwards, 1994a, b; Wynne-Edwards & Lisk, 1989; Wynne-Edwards, 1995). Thus, the reproductive success of the female Djungarian hamster depends on the presence of the male, while the reproductive success of the female Siberian hamster is independent of the presence of the male, even under harsh environmental conditions. Wynne-Edwards (1998) has developed the hypothesis that biparental behaviour is one mechanism that small rodents use to solve the problems associated with reproduction in harsh climates.

As with California mice (Lee & Brown, 2002), the male Djungarian hamster helps the female to give birth and shows paternal behaviour as soon as the pups are born (Jones & Wynne-Edwards, 2000). In the Djungarian hamster, the presence of the father facilitates pup survival and the removal of the father induces a high incidence of pregnancy block (Wynne-Edwards, 1987; Wynne-Edwards et al, 1984). When the father is present, Djungarian hamster pups spend less time alone, but Siberian hamster fathers are rarely alone with pups and show little parental care toward pups (Wynne-Edwards, 1995). Over 95% of Djungarian hamster pups reared by both parents survive to weaning, while less than 45% of pups reared by the mother alone survive to weaning. In Siberian hamsters, the presence of the male does not influence pup survival, with 74% of pups surviving when the male is absent and 64% surviving when the male is present (Wynne-Edwards, 1987; Wynne-Edwards & Lisk, 1989).

1.2.5. Voles

The mating systems of different species of voles range from monogamy to polygamy (see Wang & Insel, 1996). The prairie vole (*Microtus ochrogaster*) has a monogamous mating system, in which males and females show pair-bonding, share a nest, and live in an extended family, consisting of both parents and offspring of various ages (Oliveras & Novak, 1986). Both parents show parental care (Hofmann et al., 1984). Less is known about the social organization of pine voles (*M. pinetorum*), but field studies suggest that males and females establish a pair bond and live in colonies composed of family members (Oliveras & Novak, 1986). On the other hand, the meadow vole (*M. pennsylvanicus*) and the Montane vole (*M.montane*) have promiscuous mating systems (McGuire & Novak, 1986). These voles live in small, isolated habitats and the home ranges of males and females do not overlap. Males only visit the females' nest to mate and the basic social unit consists of a mother and young, with the mother as the sole parental caregiver (Madison, 1980; Webster & Brooks, 1981). These differences in mating systems make the voles ideal subjects for the study of species differences in paternal behaviour.

When mated pairs of meadow voles are housed in laboratory cages, males will display paternal care, likely due to confined laboratory conditions (Hartung & Dewsbury, 1979). Paternal responsiveness in meadow voles increases with exposure to pups, but copulation and cohabitation with females does not facilitate paternal behaviour in male meadow voles (Storey & Joyce, 1995). Postpartum exposure to odours and tactile stimuli from the female

reduces aggression in male meadow voles and tactile stimuli from the female and pups facilitate parental behaviour (Storey & Walsh, 1994). Once male meadow voles become sensitized to pups through repeated exposure, they remain responsive for at least 2 months after being separated from the pups. Thus, male meadow voles will show parental behaviour under the right conditions and it appears that the females prevent the males from contacting the pups (Storey et al., 1994). In the absence of mothers, meadow vole fathers remain in the nest area with the pups and prevent unfamiliar males from entering the nest. Thus, nest sharing and paternal behaviour in meadow voles appear to be flexible to social conditions.

Male prairie voles participate in nest building, retrieve pups, and remain in the nest with pups (Gruder-Adams & Getz, 1985), and the presence of the father facilitates pup development (Wang & Novak, 1992). Interestingly, virgin male prairie voles are more responsive to pups than virgin females (Lonstein & De Vries, 1999). Pine voles also exhibit high levels of paternal care, such as pup retrieval and grooming of pups (Oliveras & Novak, 1986). In contrast, male meadow voles build and use nests away from the female and rarely visit the female's nest. Meadow vole fathers do not exhibit parental care and mothers are aggressive toward males that approach the nest area. Meadow vole pups develop more slowly in the presence of the male (Wang & Novak, 1992).

1.2.6. California mice

California mice are the largest *Peromyscus* species in the United States,

with adults weighing between 40 - 70 g, and have a life expectancy of 9 - 18 months in the wild (Gubernick, 1988; Merritt, 1978). *P. californicus* are fairly sedentary and have low but stable population sizes. Their activity level peaks a few hours before nightfall and predawn (Marten, 1973). Their diet consists of seeds, vegetation, invertebrates, and various larvae (Merritt, 1978). California mice live in chaparral, sage scrub, and oak woodland habitats in coastal California, from the San Francisco Bay south to the Baja California Peninsula (Merritt, 1978). They often live in large surface dens of wood rats (*Neotoma fuscipes*) or build large nests made of sticks and grass in sheltered areas such as under fallen logs (Merritt, 1978).

Males have home ranges close to their natal area, whereas females disperse greater distances (Ribble & Salvioni, 1990). Ribble (1992b) suggests that intrasexual mate competition drives female dispersal and resource competition drives male dispersal. The male settles on a home range first and is later joined by a female. The home ranges of mated pairs of male and female *P. californicus* overlap extensively and are exclusive from home ranges of other pairs, suggesting that mated pairs defend territories (Ribble & Salvioni, 1990).

Males and females may associate together for several months before mating (Ribble, 1992a). Long-term pair bonds are formed between males and females and pairs remain together for life (Ribble, 1992a). Unlike other monogamous mammals, California mice are exclusively monogamous in the wild (Ribble, 1991). DNA fingerprinting and paternity exclusion analyses indicate that there are no extra-pair fertilizations or multiple paternities (Ribble, 1991).

In the laboratory, virgin female *P. californicus* develop a preference for a male within 24 h of introduction (Gubernick & Addington, 1994). However, a female's social preference for a male does not predict mating preference, suggesting that the pair bond needs to be well established before a female is willing to mate (Gubernick & Addington, 1994). Furthermore, females in postpartum estrous prefer to associate with their mate rather than an unfamiliar male, but 15-20% of females will mate with an unfamiliar male in the presence or absence of her mate (Gubernick & Nordby, 1993). Males prefer to associate with their partner rather than an unfamiliar female in estrous (Gubernick & Nordby, 1993). Surprisingly, males do not copulate with an unfamiliar estrous female even in the absence of their mate (Gubernick & Nordby, 1993). These results suggest that both male and female sexual fidelity is self-imposed, and not due to a lack of mating opportunities.

P. californicus breed throughout the year in the laboratory (Gubernick, 1988). In the wild, they breed primarily during the rainy season from November to May, but can breed throughout the year. The average age of first reproduction is 250 days (Ribble, 1992a). Females have small litters of 1 - 4 pups, with an average litter size of 1.7 in the wild (Ribble, 1992a). In the laboratory, females have a mean of 2.3 pups in the first litters and 2.6 pups in subsequent litters (Cantoni & Brown, 1997b). Pups are more altricial and heavier than other *Peromyscus* pups, each weighing 3 - 5 g. Juveniles emerge from their nest at about 35 days of age and leave the nest at about 75 - 80 days of age (Ribble, 1992b).

Females show postpartum estrous and mate on the day of birth or one day postpartum (Gubernick, 1988). There is no evidence of estrus or mating during lactation (Gilbert, 1984). Gestation lengths vary depending on the reproductive state of females. Nonlactating females have a mean gestation length of 31.6 ± 0.2 days with a range of 31 - 33 days, while lactating females have longer gestation lengths, with a mean of 34.2 days (Gubernick, 1988). Postpartum females without pup-suckling stimulation have a mean gestation length of 32.7. Thus, Gubernick (1988) reported that gestation length is extended from 2 to 5 days during lactation. Layne (1968) reported an extended gestation length of 2 - 7 days in *Peromyscus* due to delayed implantation. Rood (1966) also reported a longer gestation length of 30 to 39 days in lactating females.

In the laboratory, the removal of the male early or late in the female California mouse's gestation period influences the female's inter-birth interval (IBI). When parents are required to run in a wheel for food, females that had their mate removed 1 or 2 days after mating have a longer IBI (M = 53 days) than paired females (M = 37 days; Cantoni & Brown, 1997a). Cantoni and Brown (1997) hypothesized that delayed implantation occurred in females with offspring who had to forage for food with no mate to help them. Lee and Brown (2002) found that removal of the male near the end of the gestation period delayed parturition by one day. In the wild, paired females have an average IBI of 60 days (range = 28 - 92), while females that have lost their mate and remated with another male have an IBI of 88 days (Ribble, 1992a).

Male California mice show all aspects of parental behaviour and to the same extent as females, with the exception of lactation (Gubernick & Alberts, 1987). Although males do not lactate, they do display a "nursing posture" (Dudley, 1974b). The male aids the female during parturition by licking her anogenital region and they both eat the placenta (Lee & Brown, 2002). Fathers often lick and huddle over the first born pup(s) while the female gives birth to more pups (Gubernick & Alberts, 1987; Lee & Brown, 2002). Fathers display parental care as soon as the pups are born and continue until the pups are weaned on day 31 postpartum (Gubernick & Alberts, 1987). Both males and females increase the time in the nest with the pups when the mate leaves the nest or is removed from the nest (Dudley, 1974b).

Males initially exhibit parental responsiveness before females, during the last trimester of the female's pregnancy (Gubernick & Alberts, 1989). During the first 3 days postpartum, pup stimulation is necessary for the maintenance of maternal behaviour, but not paternal behaviour (Gubernick & Alberts, 1989). Pup stimulation helps to maintain paternal responsiveness by day 5 postpartum (Gubernick & Alberts, 1989). Interestingly, paternal behaviour in the male California mouse is maintained during the first 3 days postpartum by his mate's maternal excreta (Gubernick & Alberts, 1989). Paternal responsiveness is specific to the male's mate and may be maintained by the volatile fraction of maternal urine (Gubernick, 1990). The presence of the male does not affect maternal responsiveness (Gubernick & Alberts, 1989). Therefore, there appears

to be different mechanisms underlying male and female parental behaviour in California mice.

Dudley (1974a) reported that in California mice, pups raised by both parents until about 22 days of age are heavier than pups raised with the mother alone. He suggested that the presence of the father facilitated development of pups because the father allowed the pups to maintain higher body temperatures than if they were left alone (Dudley, 1974a). In contrast to Dudley's results, others report that male parental investment increases pup survival only under adverse housing conditions (Cantoni & Brown, 1997b; Gubernick et al., 1993; Wright & Brown, 2002). For example, when parents are housed at low temperatures or are required to run on a running wheel for food (mimicking foraging conditions in the wild), the presence of the male facilitates pup survival (Cantoni & Brown, 1997b; Gubernick et al., 1993).

1.3. Neural control of paternal behaviour

Although the neural basis of maternal behaviour is well established, considerably less is known about the neural mechanisms responsible for the expression of paternal behaviour. What is known suggests that the MPOA is important in the regulation of parental behaviour in male and female rodents. Bilateral radiofrequency lesions of the MPOA block the induction of parental behaviour in male rats (Rosenblatt et al., 1996) and N-methyl-DL-aspartic acid (NMDA) lesions of the MPOA disrupt ongoing paternal behaviour in male rats (Sturgis & Bridges, 1997). Furthermore, when fiber connections between the

preoptic area and the septal area are cut, male rats, like females, are less responsive to pups (Koranyi et al., 1988). These studies suggest that the MPOA is necessary for the onset and maintenance of parental behaviour in both male and female rats.

Studies on paternal behaviour in male prairie voles have implicated the MPOA, as well as several other brain areas, in the expression of paternal behaviour. Bilateral olfactory bulbectomy decreases parental behaviour in male prairie voles (Kirkpatrick et al., 1994c), and NMDA lesions of the corticomedial amygdala, an olfactory relay site, also result in decreased paternal behaviour in male prairie voles (Kirkpatrick et al., 1994a).

Kirkpatrick et al. (1994b) used c-Fos immunocytochemisty to study the areas of the brain that are activated during paternal behaviour in prairie voles and found that exposure to pups increased *c-fos* expression in the MA, accessory olfactory bulb, lateral septum, MPOA, medial BNST, nucleus reuniens, and paraventricular nucleus of the thalamus. Furthermore, males showed more *c-fos* expression to pups in the MPOA, medial amygdala, and accessory olfactory bulb than females, suggesting that these areas may be sexually dimorphic with respect to parental behaviour in these voles (Kirkpatrick et al., 1994b).

Almost nothing is known about the neural mechanisms responsible for the expression of paternal behaviour in *P. californicus*. To date, there has been one study examining the morphology of the MPOA in *P. californicus*. Gubernick et al. (1993) report a sexual dimorphism in the size of the MPOA in virgin California mice, with males having a larger MPOA than females due to a greater number of

neurons in the MPOA. When males and females become parents, there is no difference between sexes due to a volumetric increase in the MPOA of females (Gubernick et al., 1993a).

1.4. Hormonal control of paternal behaviour

Several hormones (estrogen, progesterone, prolactin, and oxytocin) have been implicated in the onset and/or maintenance of maternal responsiveness in rats (Bridges, 1990). The hormonal profile associated with paternal behaviour in mammals had been less explored. Brown et al. (1995) measured androgen and prolactin levels in male gerbils in response to cohabitation with a pregnant female and to pup stimuli. Androgen levels of paired males rose during pregnancy and dropped following parturition. Prolactin levels of paired males were significantly elevated above unmated males 20 days after pups were born. These hormonal changes indicate that male gerbils show a hormonal response similar to that of biparental species of birds, such as ring doves (*Streptopelia risoria*; Goldsmith et al., 1981).

Reburn and Wynne-Edwards (1999) measured hormonal changes in male hamsters housed with their mates during gestation and early lactation. Male Djungarian hamsters, which show paternal care, showed different patterns of hormonal changes than male Siberian hamsters, which showed little parental behaviour. Male Djungarian hamsters showed a drop in cortisol levels after pairing; a rise in testosterone throughout pregnancy and then a drop after parturition; and a rise in prolactin levels following parturition. Male Siberian

hamsters also showed a drop in cortisol after mating, but showed no change in testosterone levels over the reproductive cycle. And, although prolactin levels increase during pregnancy in male Siberian hamsters, the level of prolactin during lactation was lower than during pregnancy. These patterns of hormonal change indicate that male Djungarian hamsters show a hormonal response consistent with that of biparental species, while male Siberian hamsters do not.

In California mice, at 2 days postpartum, fathers have higher prolactin levels than do virgin males, suggesting a role of prolactin in paternal behaviour (Gubernick & Nelson, 1989). Plasma testosterone and oxytocin levels do not differ between fathers and virgin males, suggesting that these two hormones are not involved in the onset of paternal behaviour in California mice (Gubernick & Nelson, 1989; Gubernick et al., 1995).

1.5. Summary

This chapter described male and female parental behaviour in rodents. Most of what is known about the neural and endocrine control of parental behaviour stems from studies with female rats. The neural control of maternal behaviour in other species and the neural control of paternal behaviour in rodents in general, has been studied less extensively. California mice, which are naturally biparental, are an ideal species to study both male and female parental behaviour. The following three chapters describe three experiments that use two different approaches to investigate the neural control of male and female parental behaviour in California mice. The first two experiments use electrolytic lesions to

destroy areas in the brain previously implicated in the neural control of maternal behaviour in rats. The third experiment uses c-Fos, FosB and c-Jun immunocytochemistry to examine the pattern of neural activation during parental behaviour.

2. Experiment 1: Effects of medial preoptic area lesions on male and female parental behaviour in California mice

Although the paternal behaviour of males is similar to the maternal behaviour of females in *P. californicus*, the underlying mechanisms of male and female parental behaviour may be different. Examination of the MPOA, an area critical for the expression of maternal behaviour in rats, in a naturally biparental species will allow us to discover similarities and differences in the neural control of parental behaviour in males and females. The present study investigated the effects of electrolytic MPOA lesions on the expression of parental behaviour in male and female California mice. MPOA lesions were predicted to disrupt parental behaviour in both sexes.

2.1. Method

2.1.1. Subjects

Subjects were male and female *P. californicus* 60 – 130 days of age, randomly selected from a population of 4th- to 11th-generation offspring bred at Dalhousie University. Mice were obtained from a stock provided by C. Marler (University of Wisconsin, Madison) and were originally captured in the Santa Monica mountains, northeast of Los Angeles. Additional pairs of mice were used as donor parents, which provided test pups.

Each mouse was housed individually in a standard Plexiglas cage (45 cm long × 22 cm wide × 15 cm high) with wood shavings for bedding and a wire top. Rodent Laboratory Chow # 5001 (Agribrand Purina, St. Louis, MO) and water were provided ad libitum, and cages were cleaned once a week. The colony room was kept at ~ 22 °C, on a 16:8-h light–dark cycle, with the onset of lights at 11:00 a.m.

2.1.2. Procedure

At 60–130 days of age, each female was bred by pairing her with a male. In California mice, nonlactating females have a mean gestation length of 31.6 days (Gubernick, 1988) and give birth during the light phase of the light–dark cycle, usually within the first 4 h after the onset of lights (Lee, 1998). From 30 days after pairing and onwards, females were weighed once a week to determine if they were pregnant. Females that were gaining weight on a weekly basis were observed closely for parturition. The 1st day on which a female was discovered with a litter was designated as Day 0, and on that day, shredded paper towel was provided for nesting material. As California mice give birth to only 1 – 4 pups, the pairs' own pups were taken away, and parental behaviour tests were done with donor test pups on Day 1 and Day 2 postpartum. Mice that were not parental (defined as retrieving all three pups to the nest site during at least one of the 8-min tests; see the *Parental Tests* section) during Day 1 and Day 2 were not included in the experiment. On Day 3, either the male or female from each pair was given an MPOA or sham lesion, resulting in the following four groups:

females given MPOA lesions, females given sham lesions, males given MPOA lesions, and males given sham lesions. One day after the lesion (Day 4), parental behaviour tests with donor test pups commenced, lasting for 10 consecutive days or until the mouse reached the criteria for parental behaviour (see the *Parental Tests* section).

2.1.2.1. Parental tests

The parental behaviour test procedure was adapted from a study by Lee et al. (1999). First, each cage was checked for the presence of a nest (paper towel moved to one quadrant of the cage and organized into a nest). Between 10 a.m. and 12 p.m., the mate of the test subject and the pups were removed, and three 1–4-day-old freshly nourished donor pups were presented to the test subject (female or male) of each pair. On odd-numbered test days, pups were placed in the quadrant of the cage diagonally opposite the nest area or sleeping corner. On even-numbered test days, a pup was placed in each of the three quadrants outside the nest area. The occurrence of each of the following parental behaviours was recorded on a score sheet at 5-s intervals for 8 min in each test: (a) approach: the mouse moves toward a pup (or pups); (b) withdrawal: the mouse moves away from a pup (or pups); (c) retrieve pup: the mouse picks up a pup and carries it to the nest site; (d) mouth pup: the mouse picks up a pup but does not carry it to the nest site; (e) lick pup: the mouse licks any region of the pup, including the anogenital region; (f) crouch: the mouse adopts a nursing posture over the pups; and (g) build nest: the mouse carries or

moves nesting material toward the nest site. By recording the approach and withdrawal of the parent to and from pups, the time spent near pups was determined.

After the 8-min observation period, the mate was returned to the cage, and two spot-checks were made approximately 2 and 8 h after the test to record the position and behaviour of the female and male and the position of the three pups. The pups remained with the pair and were replaced with recently fed donor pups the next morning, before the parental test. Testing continued for 10 consecutive days, unless the mouse met the parental criteria or was cannibalistic for 2 consecutive days. The maternal criteria for females consisted of 2 consecutive days of retrieving pups into the nest area during the 8-min observation period and one crouch posture in either the 8-min observation period or during a spot-check. The criteria for males were similar to those for females; however, the crouch component was not part of the criteria for males, as females most often assumed that position over the pups

2.1.2.2. Surgery

Mice were anaesthetized with ketamine (120 mg/kg i.p., diluted 1:5 in sterile saline, Bimeda-MTC Pharmaceutical, Cambridge, Ontario, Canada) and xylazine (20 mg/kg i.p., Bayer, Toronto, Ontario, Canada). The fur on their head was shaved with an electric razor and they were securely placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the skull level between bregma and lambda. Because there is no brain atlas for *Peromyscus,*

the stereotaxic coordinates (AP + 0.5, DV - 7.3, ML ± 0.3) were determined by pilot lesions from a standard atlas of the mouse brain (Franklin & Paxinos, 1997). Electrolytic lesions were made by passing a direct current of 2.0 mA for 5 s through a stainless steel electrode (0.7 mm diameter), insulated with Epoxylite except for 0.5 mm at the tip. For sham lesions, the electrode was lowered to the same coordinates, but no current was passed through the electrode. For the first few animals, the incision was glued with krazy glue; however, the subjects' mate often chewed at the glue, opening up the incision. Thus, incisions were stitched with thread instead. Mice were loosely wrapped with paper towel and placed in a new cage, under a lamp to keep them warm.

2.1.2.3. Histology

At the completion of the experiment, all mice were killed with an overdose of sodium pentobarbital and perfused with 0.9% (wt/vol) saline followed by 10% (wt/vol) Formalin. The brains were removed and stored in 10% Formalin until they were ready to be sectioned, at which point brains were kept in 30% (wt/vol) sucrose overnight. They were then frozen and sectioned on a cyrostat microtome at 40-μm. The brains were stained with cresyl violet Nissl stain.

2.1.3. Statistical analyses

A two-way (Lesion × Sex) analysis of variance (ANOVA) was done to compare behaviours during the 2 days before the lesion. The latency in days to reach parental criteria and the latency to show each of the individual parental

behaviours were analyzed with nonparametric Mann–Whitney *U* tests for two-group comparisons. Mice that failed to reach parental criteria or to show individual parental behaviours were assigned latencies of 11 days. A two-way (Lesion × Sex) ANOVA was done to compare behaviours during the last 2 days of testing after the lesion. The means (± *SEM*) or medians are reported, and an alpha level of .05 was used for all statistical tests. All statistical analyses were conducted with the Statistical Package for Social Sciences (SPSS), version 6.1.

2.2. Results

2.2.1. Number of mice that died

Seventeen out of 50 mice (34%) were excluded from the study because they died (Table 1). Seven (14%) mice died shortly after they were anaesthetized, prior to being given a lesion. Six (12%) mice never recovered from the lesion. Four (8%) MPOA-lesioned mice died within 1 to 3 days after the lesion.

It was difficult in general to anaesthetize California mice, as they had a wide range of reactions to the anaesthetic. Fifteen out of the 17 mice that died were only given the initial dose of anaesthetic (20 mg/kg of xylazine and 120 mg/kg of ketamine). Two out of the 15 mice that died were given a boost 0.05 ml of ketamine because they were not sufficiently anaesthetized. Of the 33 mice that survived, 19 were given the standard dose, while 13 were boosted with 0.05 – 0.20 ml of ketamine because they were not sufficiently anaesthetized. One mouse was injected with the standard dose of xylazine and ketamine, plus 0.50

Table 1.

Mortality rates for mice in experiment 1.

	After Injection		After Lesion		1-3 Days After Lesion	
Lesion	Male	Female	Male	Female	Male	Female
Sham	0	1 (2%)	2 (4%)	1 (2%)	0	0
MPOA	2 (4%)	4 (8%)	1 (2%)	2 (4%)	1 (2%)	3 (6%)

n = 50

ml of ketamine (almost double the dose of ketamine in total) and lived. Thus, 30% of mice died with the standard dose of anaesthetic, while 28% of mice lived with an increased amount of ketamine. During pilot studies, similar reactions to Somnotol were observed.

2.2.2. Histology

Coronal sections were examined under magnification for signs of tissue damage, and the extent of damage was recorded for each mouse. A schematic representation of the greatest and least amount of tissue damage is shown in Figure 2. Only mice with bilateral MPOA damage were included in the study, resulting in the exclusion of 2 MPOA-lesioned females and 2 MPOA-lesioned males. The size or location of the lesion had no effect on parental behaviour.

2.2.3. Parental behaviour before lesion

Two males and one female cannibalized pups on the first day of testing, before the lesion. As soon as a mouse was observed cannibalizing a pup, the pups were removed and the mouse was excluded from the study. Twenty-six males and three females did not reach the parental criteria during the 2 days of testing before lesion, mostly because they did not satisfy the pup-retrieval component of the criteria. These mice did not continue with the study.

During the 2 days of parental testing before surgery, males spent significantly more time sniffing pups ($F_{1,25}$ = 11.54, p = .002), and less time hovering over pups ($F_{1,25}$ = 12.68, p = .002) than females (see Table 2). There

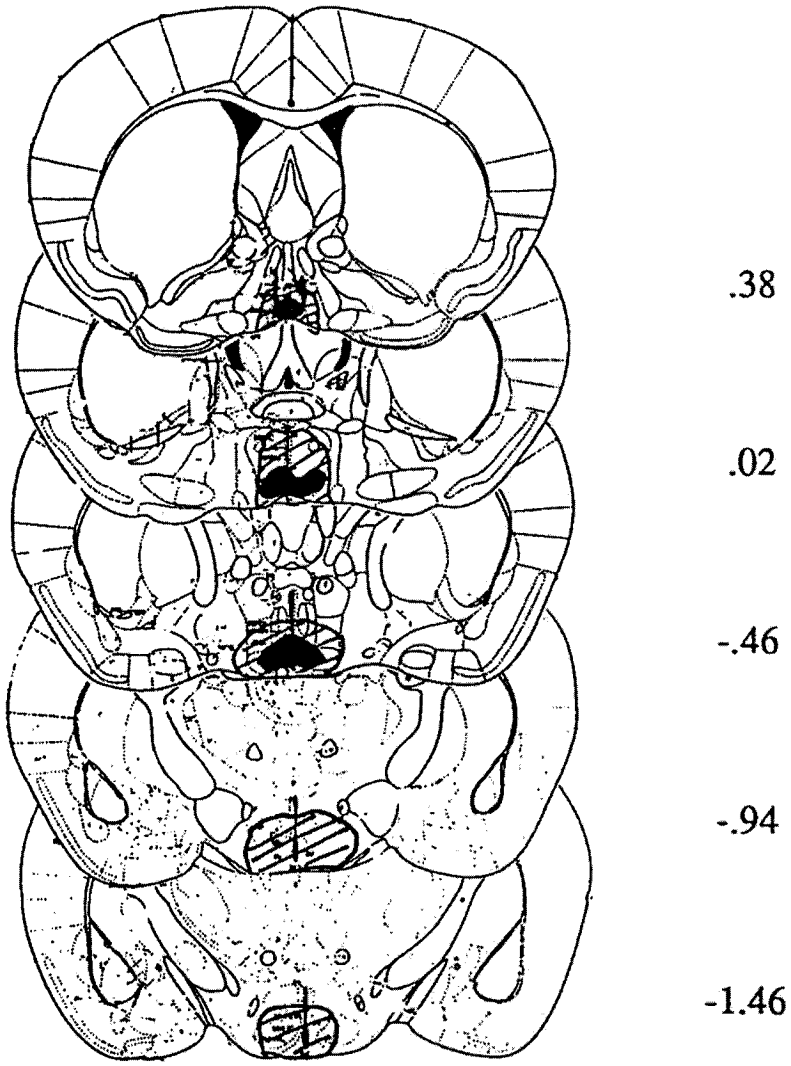


Figure 2. Schematic representations of the smallest (solid) and largest (striped) medial preoptic area lesions. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 28, 31, 35, 39, and 43), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

Table 2

Mean (± *SEM*) percentage of time showing each of four parental behaviours before and after MPOA lesions in female and male *P. californicus*

Behaviours	Female		Male	
	MPOA (n = 8)	Sham (n = 5)	MPOA (n = 8)	Sham (n = 8)
Parental Behaviours During the 2 Days Before MPOA Lesion				
Near Pup	98.70 (0.22)	98.96 (0.29)	97.85 (0.30)	98.37 (0.46)
Sniff Pup	31.25 (2.34)	30.42 (6.97)	42.64 (1.46)	45.25 (4.61)
Lick Pup	30.99 (3.47)	40.10 (7.52)	27.02 (3.94)	33.20 (6.84)
Crouch	17.51 (5.15)	20.83 (4.49)	1.56 (1.14)	9.38 (3.32)
Parental Behaviours During the Last 2 days of Testing After MPOA Lesion				
Near Pup	73.31 (11.25)	99.17 (0.71)	74.87 (12.40)	98.31 (0.61)
Sniff Pup	16.54 (2.71)	30.31 (4.35)	20.31 (3.42)	25.65 (1.96)
Lick Pup	20.44 (6.46)	39.90 (6.88)	15.69 (5.63)	34.70 (6.70)
Crouch	19.53 (5.98)	20.73 (15.83)	9.70 (6.66)	14.91 (4.50)

were no other significant sex differences, no significant differences between MPOA and sham-lesioned groups, and no significant Sex × Group interactions in the amount of parental behaviour shown before surgery (see Table 2).

2.2.4. Parental behaviour after lesion

Two females (1 MPOA- and 1 sham-lesioned) cannibalized pups during the 1st and 2nd day after the lesion. These females were excluded from the analyses. One day after the MPOA or sham lesion, mice appeared a little sluggish and took longer to approach pups (see Table 3), but by the 2nd day, they approached pups as quickly as they did before the lesion.

The majority of MPOA-lesioned mice did not reach the parental criteria and thus were tested for the full 10 days. The parental behaviour of MPOA-lesioned mice did not change over the 10 days of testing. As seen in Figure 3, the amount of time MPOA-lesioned males and females spent licking pups did not increase over days. Similarly, the amount of time MPOA-lesioned mice spent near pups, sniffing pups, and hovering over pups did not increase over the 10 days of parental testing.

The behaviour of MPOA- and sham-lesioned mice was compared during the last 2 days of testing after surgery. These were the 2 days in which the mice showed parental behaviour or Days 9 and 10 of testing for those that did not reach the parental criteria. On these 2 days, MPOA-lesioned mice spent less time near pups ($F_{1,25} = 6.72$, $p < .05$), sniffing pups ($F_{1,25} = 9.56$, $p = .005$), and licking pups ($F_{1,25} = 8.50$, $p = .007$) than sham-lesioned mice (see Table 3).

Table 3

Mean Latency (± *SEM*) in Seconds to Approach Pups During the 2 Days Before Lesion and the First 3 Days of Testing After Lesion in Female and Male *P. californicus*

Latency to Approach Pups During the 2 Days Before MPOA Lesion

	Female		Male	
Behaviours	MPOA (n = 8)	Sham (n = 5)	MPOA (n = 8)	Sham (n = 8)
Day 1	10.00 (1.64)	9.00 (1.87)	12.5 (2.11)	10.00 (1.64)
Day 2	10.63 (1.99)	10.00 (2.24)	8.13 (1.32)	8.13 (1.62)

Latency to Approach Pups During the First 3 days of Testing After MPOA Lesion

	Female		Male	
Behaviours	MPOA	Sham	MPOA	Sham
Day 1	28.13 (7.13)	44.00 (6.21)	62.50 (41.52)	78.75 (65.93)
Day 2	8.13 (1.88)	7.00 (2.00)	9.38 (1.99)	7.50 (0.95)
Day 3	13.75 (3.98)	6.00 (1.00)	12.50 (4.23)	6.88 (0.92)

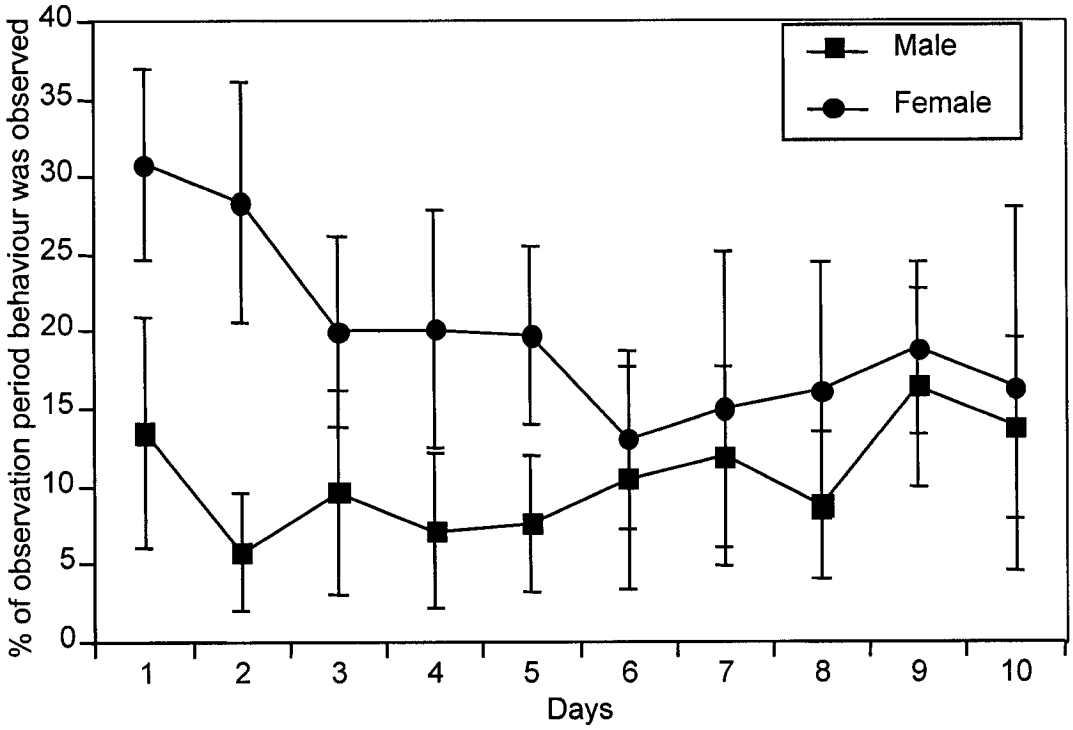


Figure 3. Mean (± *SEM*) percentage of time male (n = 8) and female (n = 8) *P. californicus* were observed licking pups during the 10 days after medial preoptic area (MPOA) lesions.

There was no significant effect of MPOA lesion on the time spent crouching over pups and no sex difference in the time spent showing any parental behaviour.

2.2.5. Latencies to show parental behaviour

As seen in Figure 4, MPOA-lesioned males and females had longer latencies to reach the parental criteria (2 consecutive days of retrieving pups to the nest for both males and females and hovering over pups at least once during those 2 days for females only) than sham-lesioned males and females ($U = 9$, $n_1 = 8$, $n_2 = 8$, $p < .05$ for males; $U = 3$, $n_1 = 8$, $n_2 = 5$, $p < .05$ for females). Only 12.5% of MPOA-lesioned males and 25% of MPOA-lesioned females reached the parental criteria, whereas 75% of sham-lesioned males and 100% of sham-lesioned females reached the parental criteria.

MPOA-lesioned males had longer latencies to retrieve pups ($U = 10$, $n_1 = 8$, $n_2 = 8$, $p < .05$), lick pups ($U = 14$, $n_1 = 8$, $n_2 = 8$, $p < .05$), and hover over pups ($U = 11$, $n_1 = 8$, $n_2 = 8$, $p < .05$) than sham-lesioned males (Figure 5). MPOA-lesioned females had longer latencies to retrieve pups ($U = 2.5$, $n_1 = 8$, $n_2 = 5$, $p < .01$) and build nests ($U = 5$, $n_1 = 8$, $n_2 = 5$, $p < .05$) than sham-lesioned females (see Figure 4). MPOA-lesioned males showed longer latencies to hover over pups ($U = 10.5$, $n_1 = 8$, $n_2 = 8$, $p < .05$) than MPOA-lesioned females.

2.3. Discussion

The results show that the MPOA is important for the maintenance of parental behaviour in both male and female California mice. Although the effect

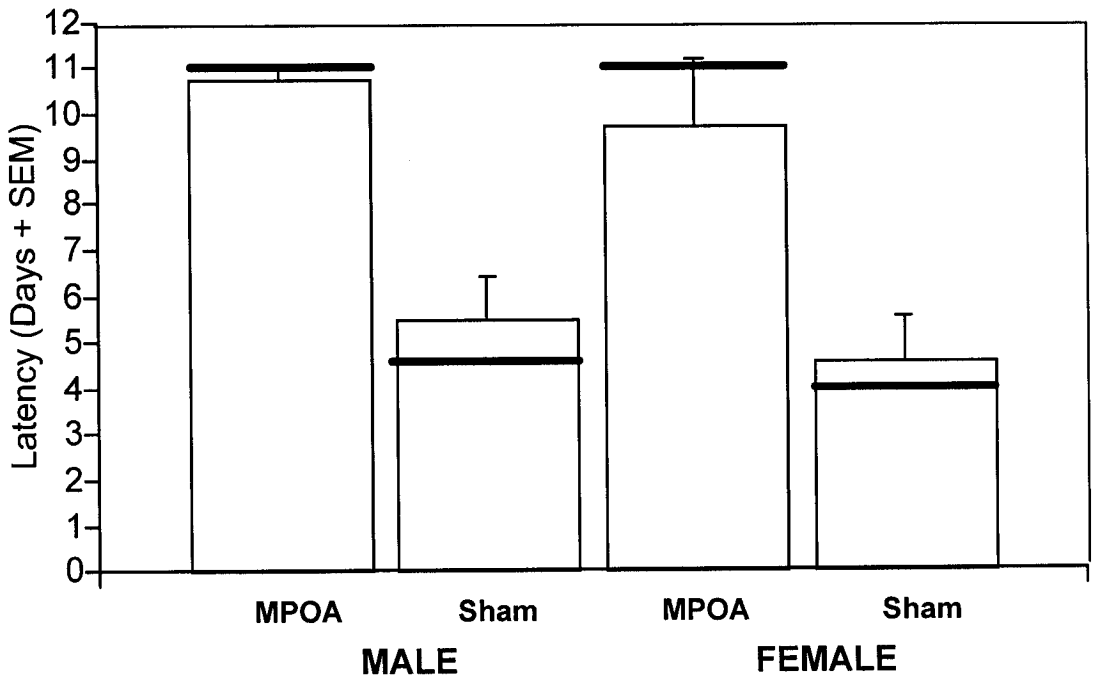


Figure 4. Mean (+ *SEM*) and median (dark bars) latencies in days for male and female *P. californicus* in the medial preoptic area (MPOA)- and sham-lesioned groups to reach the parental criteria (2 consecutive days of retrieving pups to the nest for both males and females and hovering over pups at least once during those 2 days for females only). MPOA – male, n = 8; Sham – male, n = 8; MPOA – female, n = 8; Sham – female, n = 5

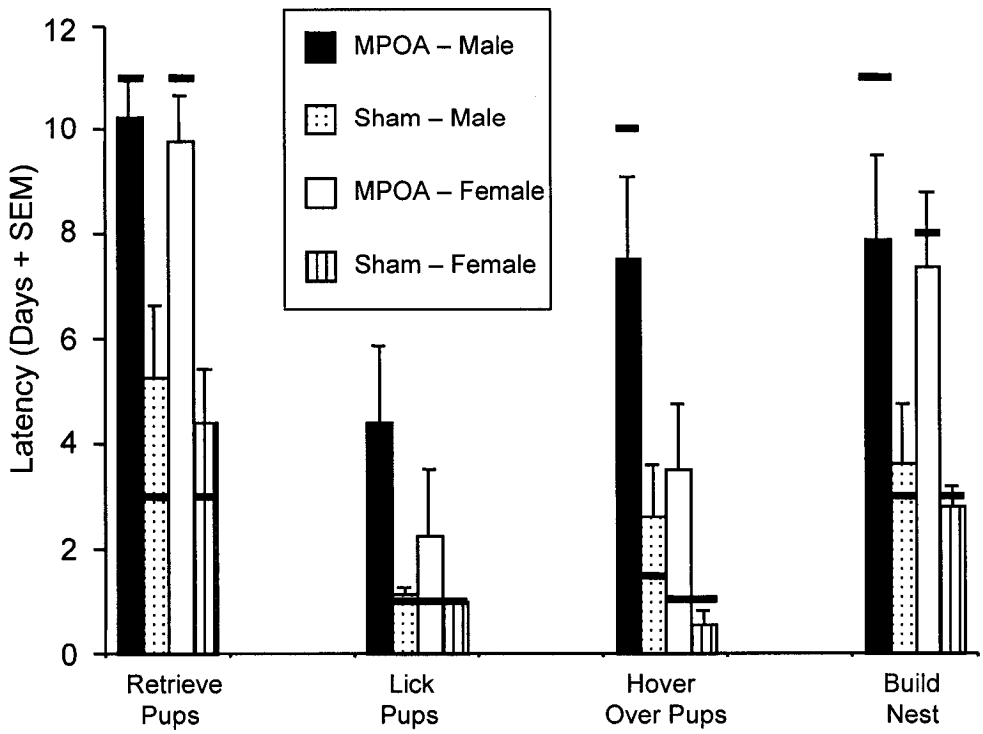


Figure 5. Mean (+ *SEM*) and median (dark bars) latencies in days to retrieve pups, lick pups, hover over pups, and build a nest by medial preoptic area (MPOA)- and sham-lesioned male and female *P. californicus*. MPOA – male, n = 8; Sham – male, n = 8; MPOA – female, n = 8; Sham – female, n = 5

of MPOA lesions on parental behaviour of male and female rats has been studied extensively (Numan, 1974; Numan & Corodimas, 1985; Numan et al., 1977, 1988; Rosenblatt et al., 1996; Sturgis & Bridges, 1997), to our knowledge, this is the first study of MPOA lesions in a naturally biparental species. Our results are consistent with previously published studies on the effect of MPOA lesions on parental behaviour in female and male rats and suggest that the neural substrates that mediate paternal behaviour may be similar to those that mediate maternal behaviour in other rodents.

2.3.1. MPOA lesions produce clear deficits in pup-retrieval

Although MPOA-lesioned males (but not females) showed a longer latency to hover over pups than sham-lesioned males in our study, there were no significant differences in the proportion of time spent hovering over pups between groups during the last 2 days of testing. However, MPOA-lesioned males and females both showed a significantly longer latency to retrieve pups than sham-lesioned mice. The clear deficit in pup retrieval and the partial deficit in hovering behaviour is consistent with studies of MPOA lesions' effects on maternal behaviour in rats, with some studies reporting that MPOA lesions inhibit pup retrieving, nest building, and nursing (Numan, 1974; Numan et al., 1988), and others reporting that pup retrieving and nest building, but not nursing, are disrupted by MPOA lesions (Jacobson et al., 1980; Numan, 1990; Numan & Callahan, 1980; Terkel et al., 1979). Thus, the pattern of maternal deficits produced by MPOA lesions remains unclear. Factors such as the size of the

lesion, the species, and the methods used to evaluate parental behaviour may contribute to the variability in behavioural disruption reported.

Studies on the long-term effects of MPOA damage, however, emphasize that pup retrieval is disrupted, whereas nursing remains intact (Numan, 1994). Thus, although MPOA lesions disrupt all aspects of parental behaviour to some degree, the oral components seem to be disrupted the most, including sniffing and licking pups, but particularly pup retrieval. Furthermore, the effect of MPOA lesions on pup retrieval is not due to a general oral motor deficit, as females with lateral MPOA cuts can retrieve candy of size and weight similar to those of rat pups (Numan & Corodimas, 1985). Numan (1994) has suggested that the long-term effects of MPOA damage are to disrupt the execution of particular maternal responses, such as pup retrieval, but not maternal motivation. Lee and colleagues (2000) addressed this issue with a study examining the effects of MPOA lesions on the expression of maternal behaviour and operant responding for pup reinforcement. MPOA-lesioned female rats showed deficits in maternal behaviour and a reduction in operant responding for pups, suggesting that pups were not reinforcing to them. Therefore, MPOA lesions may disrupt all aspects of parental behaviour, including maternal motivation, to some degree and produce clear deficits in pup-retrieval.

2.3.2. Methodological issues

2.3.2.1. Problems with anaesthetic

The most significant issue in this study was the mortality rate. California

mice appear to be highly reactive to various stimuli and often died from an initial dose of ketamine and xylazine.

2.3.2.2. Many California mice have difficulty retrieving 3 pups in 8 min

It was also apparent that 8 min was not long enough for the parental behaviour tests. The 8-min test was adapted from Lee et al.'s (1999) work on the effects of various lesions on maternal behaviour in female rats. In their study, 8 min was more than sufficient for rats with sham lesions to retrieve four rat pups. In our tests, 8 min was sufficient to show parental behaviours such as sniffing and licking pups, but for many subjects, it was not sufficient to retrieve all 3 pups. Many subjects were excluded after the initial parental testing before surgery because they did not reach the parental criteria (almost always due to the retrieval component). Thus, because of the strict parental criteria, only successful pup-retrievers were selected to be included in the study. Furthermore, during many occasions, a sham-lesioned mouse was able to retrieve 1 or 2 pups within the 8 min, but not all 3 pups. Thus, it was sometimes difficult for sham-lesioned mice to reach the parental criteria because it was difficult to meet the pup-retrieval criterion.

California mice normally give birth to one to four pups, whereas rats often given birth to more than a dozen pups. Perhaps the fact that rats are able to retrieve pups faster than California mice has to do with the large size of their litters. A longer test, such as a 15-min test, would have allowed for more

subjects to be included in the present study and shortened the latencies to reach parental criteria for the sham-lesioned mice. MPOA-lesioned mice would not likely have been affected by the parental tests being too short, as pup retrieval is usually an all-or-nothing event, and MPOA-lesioned mice normally do not retrieve any pups. In our study, MPOA-lesioned mice rarely showed any pup retrieval. Thus, a longer test would facilitate inclusion of sham-lesioned subjects without affecting latencies for MPOA-lesioned subjects, thereby showing a greater difference in the latency to show parental behaviour between groups.

2.3.2.3. One day of recovery from lesion may be insufficient

Mice that had sham lesions did not reach the parental criteria until they had about 5 days of pup exposure. Their latency to show parental behaviour was significantly shorter than MPOA-lesioned mice, but not as short as expected. There was only one day of recovery after the lesion, which may not have been enough time. However, we wanted to keep the mice in male–female pairs, consistent with their natural mating system, as they are a naturally biparental species. Furthermore, during the first 3 days postpartum, it is important to keep the mice in a family unit, as pup stimulation is necessary for the maintenance of maternal behaviour, and the maternal female's excreta are important for the maintenance of paternal behaviour in California mice (Gubernick, 1990; Gubernick & Alberts, 1989). Thus, separating the experimental subject from its mate and pups for a week to allow them to recover from the surgery did not appear to be a better alternative. Some of the mice seemed a little sluggish on

the first day of testing after surgery and took longer to approach pups. However, the lesioned mice seemed to recover from the surgery remarkably quickly and, by the second day, were as active as their nonlesioned mates.

3. Experiment 2: Effects of basolateral amygdala, nucleus accumbens, and medial preoptic area lesions on male and female parental behaviour in California mice

Experiment 1 showed that the MPOA is important for parental behaviour in both male and female California mice. The purpose of experiment 2 was to investigate the effects of two other brain regions, the basolateral amygdala and nucleus accumbens, which have been shown to be important for the expression of maternal behaviour in rats. Effects of MPOA lesions were investigated again in order to compare across groups, as the methods for this experiment differed from those of experiment 1 in the following six ways:

1. In experiment 1, subjects were mice in primiparous pairs. *P. californicus* have small litters and can take several months before producing the first litter. Furthermore, California mice often have only one pup in their first litter and the incidence of cannibalism is highest when there is only one pup born. Therefore, for pragmatic reasons, in experiment 2, the subjects were mice in multiparous pairs. One advantage of using multiparous pairs was that we could predict the day of birth for the second and subsequent litters, as females show postpartum estrous. Another advantage of using multiparous pairs was that we could include more subjects in the study, as pups of multiparous pairs are more likely to survive than those of primiparous pairs. Lastly, while there is only one opportunity to use primiparous pairs, there are four opportunities (2^{nd} to 5^{th} litters)

to use multiparous pairs, resulting in more subjects in our colony being included in the study.

2. In experiment 1, only mice that met the parental criteria on the 2 days of testing before the lesion were included in the study. However, many mice were unable to reach the parental criteria in the 8-min test and these subjects were not included in the study. In experiment 2, the criteria for inclusion were more lenient. Mice that showed parental behaviours (retrieving, sniffing, licking, hover over pups) at least 90% of time were included in the study. The more lenient parental criteria and the use of multiparous mice resulted in the exclusion of fewer pairs than in experiment 1.

3. In experiment 1, mice were tested for parental responsiveness for 10 days or until they reached the parental criteria. Thus, most MPOA-lesioned mice were tested for the full 10 days, while sham-lesioned mice were tested for an average of 5 days. In experiment 2, all mice were tested for 10 consecutive days so that groups could be compared across the full 10 days.

4. In experiment 2, pups were weighed daily for 30 consecutive days to determine whether lesions to the mother or father affected the development of pups. Pups were not weighed in experiment 1.

5. In experiment 1, subjects were tested with three 1-to-4-day-old freshly nourished pups. For each test pair, we needed 3 – 6 donor parents to provide pups. In a species with a long gestation period (34 days) and small litters (1 - 4 pups), we had to keep a large breeding colony just for donor pups. Again for pragmatic reasons, in experiment 2, each test pair was matched with one donor

pair that gave birth on the same day. Subjects were tested with their own pups on even-numbered days and with the same set of donor pups on odd-numbered days. In other words, the test subjects' pups remained with their biological parents on even-numbered days, and remained with donor parents on odd-numbered days.

6. In experiment 1, 34% of subjects died, a third of which died while anaesthetized, and two thirds died after the lesion. In experiment 2, several precautions were taken in an attempt to decrease the mortality rate. Mice were brought into the surgery room at 10 am and allowed to acclimatize to the new room for at least 4 h. Mice were anaesthetized with Somnotol injected s.c. instead of ketamine/xylazine i.p. Mice were covered with a cotton cloth to keep them warm as soon as they were anaesthetized and the temperature in the surgery room was maintained at 25 °C. The fur on their head was not shaved to prevent them from hearing the loud motor of the shaver. All mice were provided with a slice of apple after the lesion.

3.1. Method

3.1.1. Subjects

Seventy-seven pairs of *P. californicus* were mated and served as subjects. Mice were offspring bred at Dalhousie University, obtained from a stock provided by Dr. C. Marler (University of Wisconsin, Madison) and were originally captured in the Santa Monica mountains, northeast of Los Angeles. Additional pairs of mice were used as donor parents, which provided test pups.

Mice were mated and housed in standard Plexiglas cages (45 cm long × 22 cm wide × 15 cm high) with wood shavings for bedding and wire tops. Rodent Laboratory Chow # 5001 (Agribrand Purina, St. Louis, MO) and water were provided ad libitum, and cages were cleaned once a week. The colony room was kept at ~ 22 °C, on a 16:8-h light–dark cycle, with the onset of lights at 11:00 a.m.

3.1.2. Procedure

Females were mated and gave birth to at least one litter before the experimental manipulations began. Female California mice show postpartum estrus, and therefore, the day of birth for second and subsequent litters can be determined. On day 32 of gestation of the 2nd to 5th litters, females were checked daily for parturition. The first day on which a female was discovered with a litter was designated as postnatal Day 0, and on that day, shredded paper towel was provided for nesting material. Each test pair was matched with another pair that gave birth on the same day, which provided donor pups and were foster parents to pups of the test pair. Each test and foster pair reared 3 pups. As California mice normally give birth to 1 – 4 pups, several pairs did not give birth to enough pups. Pairs that gave birth to only one pup were excluded from the study. Pairs that gave birth to 2 pups were only included in the study if there was another pair that gave birth on the same day to provide an extra pup for the test pair.

The pairs' own pups were taken away, and parental behaviour tests were done with donor test pups on Day 1 postpartum and with their own pups on Day

2 postpartum. Mice that did not show parental behaviours (retrieving, sniffing, licking, hovering over pups) for at least 90% of the observation period during Day 1 and Day 2 were excluded from the experiment.

On Day 3, either the male or female from test each pair was given an NA, BA, MPOA, or sham lesion. After histological analyses, mice that did not have good NA, BA, or MPOA lesions were combined in a separate "missed lesion" group, resulting in the following 10 groups: females given NA lesions (n = 7), males given NA lesions (n = 8), females given BA lesions (n = 8), males given BA lesions (*n* = 6), females given MPOA lesions (n = 5), males given MPOA lesions (n = 4), females given either NA, BA, or MPOA sham lesions (n = 14), males given either NA, BA, or MPOA sham lesions (n = 13), female with missed lesions (n = 7), male with missed lesions (n = 5). Starting one day after the lesion (Day 4), mice were tested for parental behaviour for 10 consecutive days. The test pairs' pups were weighed daily from Day 1 postpartum until the end of parental behaviour testing (Day 13).

About halfway into the experiment, we decided to continue taking litter weights until pups were 30 days of age, which is when pups are weaned in our laboratory. After the 10 days of parental behaviour testing (Day 13), the test pair's pups were given to the foster pair, which reared these pups for another 17 days (until Day 30). Thus, for the first 13 days, pups were reared by two sets of parents (natural and foster), which alternated daily. For the next 17 days, pups were reared only by foster parents. The first day in which the eyes of at least 2 of the 3 pups in a litter opened, and the sex of the pups, were also recorded.

3.1.2.1. Parental behaviour tests

The parental behaviour tests were conducted in the same manner as in experiment 1, but donor pups were alternated with the pairs' own pups throughout the study, beginning with donor pups on Day 1 postpartum. First, each cage was checked for the presence of a nest (paper towel moved to one quadrant of the cage and organized into a nest). Between 10 a.m. and 12 p.m., the mate of the test subject and the pups were removed, and three freshly nourished pups were presented to the test subject (female or male) of each pair. On odd-numbered test days, pups were placed in the quadrant of the cage diagonally opposite the nest area or sleeping corner. On even-numbered test days, a pup was placed in each of the three quadrants outside the nest area. The occurrence of each of the following behaviours was recorded on a score sheet at 5-s intervals for 8 min in each test: (a) approach: the mouse moves toward a pup (or pups); (b) withdrawal: the mouse moves away from a pup (or pups); (c) retrieve pup: the mouse picks up a pup and carries it to the nest site; (d) mouth pup: the mouse picks up a pup but does not carry it to the nest site; (e) lick pup: the mouse licks any region of the pup, including the anogenital region; (f) crouch: the mouse adopts a nursing posture over the pups; (g) build nest: the mouse carries or moves nesting material toward the nest site; (h) groom; the mouse licks any region of its own face or body. By recording the approach and withdrawal of the parent to and from pups, the time spent near pups was determined. The time spent in parental behaviours was defined as the time

spent in retrieving pups, mouthing pups, licking pups, and/or crouching over pups.

After the 8-min observation period, the mate was returned to the cage, and two spot-checks were made approximately 2 and 8 h after the test to record the position and behaviour of the female and male and the position of the three pups. The pups remained with the pair and were replaced with recently fed pups the next morning, before the parental test. Testing continued for 10 consecutive days unless the mouse was cannibalistic for two consecutive days. When mice were observed cannibalizing pups during a parental behaviour test, testing stopped immediately and pups were removed from the cage. Mice that cannibalized pups for 2 consecutive days were removed from the study.

3.1.2.2. Surgery

Mice were brought into the surgery room at 10 am and allowed to acclimatize to the new room for at least 4 h. Mice were anaesthetized with Somnotol (65 mg/kg sc, diluted 1:4 in sterile saline) and were covered with a cotton cloth to keep them warm as soon as they were anaesthetized. The temperature in the surgery room was maintained at 25 °C. The fur on their head was not shaved to prevent them from hearing the loud motor of the shaver. Mice were securely placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the skull level between bregma and lambda. Because there is no brain atlas for *Peromyscus,* the stereotaxic coordinates were determined by pilot lesions from a standard atlas of the mouse brain (Franklin & Paxinos, 1997),

NA = AP + 2.4, DV 5.5, ML ± 1.0; BA = AP – 0.5, DV 7.2, ML ± 4.2; MPOA = AP + 1.0, DV 6.8, ML ± 0.3. Electrolytic lesions were made by passing a direct current of 2.0 mA through a stainless steel electrode (0.7 mm diameter), insulated with Epoxylite except for 0.5 mm at the tip. Current was passed for 10 s for NA and MPOA lesions and 8 s for BA lesions. For sham lesions, the electrode was lowered to the same coordinates, but no current was passed through the electrode. Incisions were stitched with thread. Following surgery, mice were loosely wrapped with paper towel and placed in a new cage, under a lamp to keep them warm and were provided with a slice of apple.

3.1.2.3. Histology

At the completion of the experiment, mice were killed with an overdose of sodium pentobarbital and perfused with 0.9% saline followed by 10% Formalin. The brains were removed and stored in 10% Formalin until they were ready to be cut, at which point brains were kept in 30% sucrose overnight. They were then frozen and sectioned on a cyrostat microtone at 40 μm. The brains were stained with cresyl violet Nissl stain.

3.1.3. Statistical analyses

Behaviours across successive 2 – day periods were averaged to form 6 blocks of 2- day periods (before lesion days 1 - 2, after lesion days 1 – 2, 3 – 4, 5 – 6, 7 – 8, and 9 – 10). A two-way (Lesion x Sex) ANOVA and t – tests for two group comparisons were done to test whether there were any baseline

differences in parental behaviours during the 2 days of testing before lesion.

Nonparametric tests were conducted on each of the behaviours during the periods after lesion because the sample sizes were unequal and the data violated the normality, homogeneity of variance, and sphericity assumptions for ANOVA. Kruskal – Wallis tests for multi – group comparisons and Mann – Whitney *U* tests for two group comparisons were conducted to test whether there were any differences between groups and between sexes for each of the behaviours during each of the 2 – day periods.

The latency in days to show each of the individual parental behaviours was analyzed with nonparametric Kruskal – Wallis tests for multi-group comparisons and Mann – Whitney *U* tests for two-group comparisons. Mice that did not show individual parental behaviours were assigned latencies of 11 days.

Litter weights across successive 3 - day periods were averaged to form 10 blocks of 3 - day periods (postpartum days 1 - 3, 4 - 6, 7 - 9, 10 - 12, 13 - 15, 16 - 18, 19 - 21, 22 - 24, 25 - 28, 29 - 30). Litter weights over the 30 days were compared with a two-way (group x sex) repeated measures (day) ANOVA. A 5 x 2 ANOVA was conducted to determine whether there were any differences between groups on the day in which pups first opened their eyes. Pearson product moment correlations were conducted to examine whether male:female ratio in a litter affected litter weight. The means (± *SEM*) are reported, and an alpha level of .05 was used for all statistical tests. All statistical analyses were conducted with the Statistical Package for Social Sciences (SPSS), version 6.1.

3.2. Results

3.2.1. Response to Somnotol

Several changes were made to the surgical practice and extra precautions were taken to reduce the rate of death observed in experiment 1 (see above). Eighteen out of 103 (17.5%) mice died after the lesion (Table 4). Fourteen (13.6%) mice did not recover from the lesion and four (3.9%) died 1 or 2 days after the lesion. No mice died while anaesthetized, before they were given a lesion.

With subcutaneous injections of Somnotol, no animals died before they were lesioned. Of the 18 mice that died, 12 were given the standard dose of Somnotol (65 mg/kg) and six were boosted with 0.05 - 0.15 ml of Somnotol. Of the 85 mice that lived, 43 of them were sufficiently anaesthetized with the standard dose of Somnotol, while 42 of them had to be given an extra 0.05 – 0.15 ml of Somnotol.

3.2.2. Histology

Coronal sections were examined under magnification for signs of tissue damage, and the extent of damage was recorded for each mouse. A schematic representation of the greatest and least amount of tissue damage for each lesion group is shown in Figures 6 - 8. Only mice with bilateral damage were included in the study. Mice that were excluded from the NA, BA, or MPOA groups due to partial or missed lesions were combined as a missed lesion group (Figures 9 - 11).

Table 4

Mortality rates for mice in experiment 2

	After Injection		After Lesion		1-2 Days After Lesion	
Lesion	Male	Female	Male	Female	Male	Female
Sham	0	0	0	1 (1%)	0	0
NA	0	0	6 (6%)	0	0	0
BA	0	0	0	1 (1%)	0	0
MPOA	0	0	4	2 (2%)	2 (2%)	2 (2%)
Total	0	0	10	4	2	2

n = 103

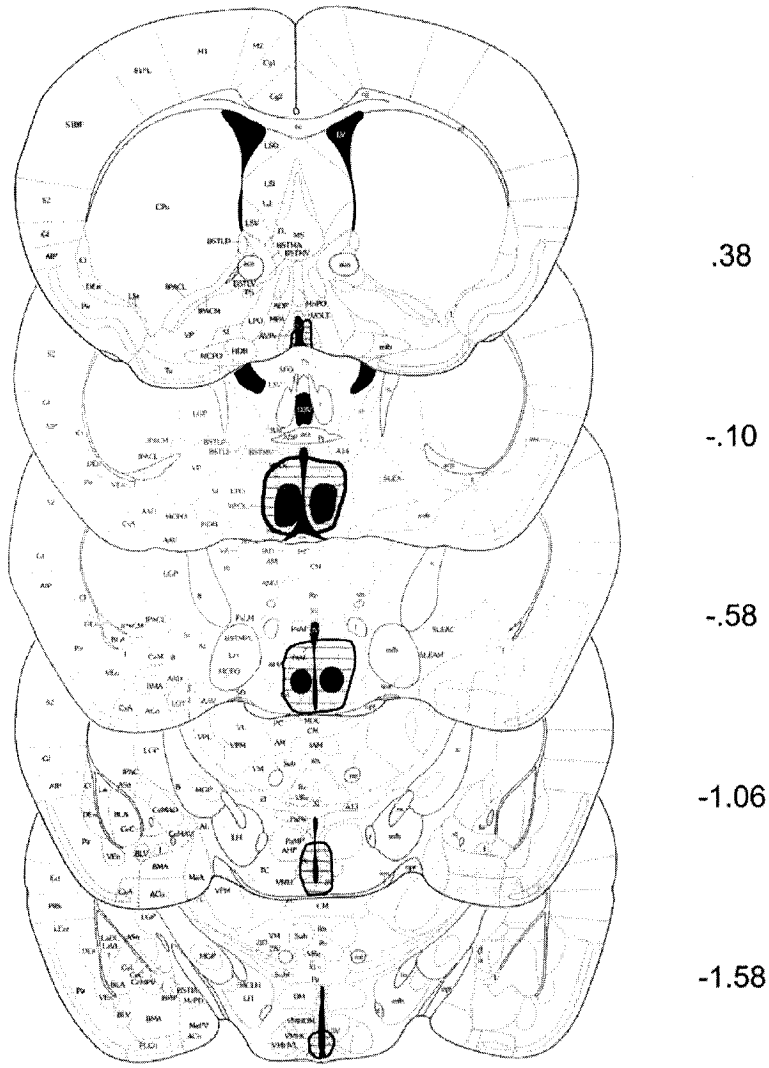


Figure 6. Schematic representations of the smallest (solid) and largest (striped) medial preoptic area lesions. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 28, 32, 36, 40, and 44), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

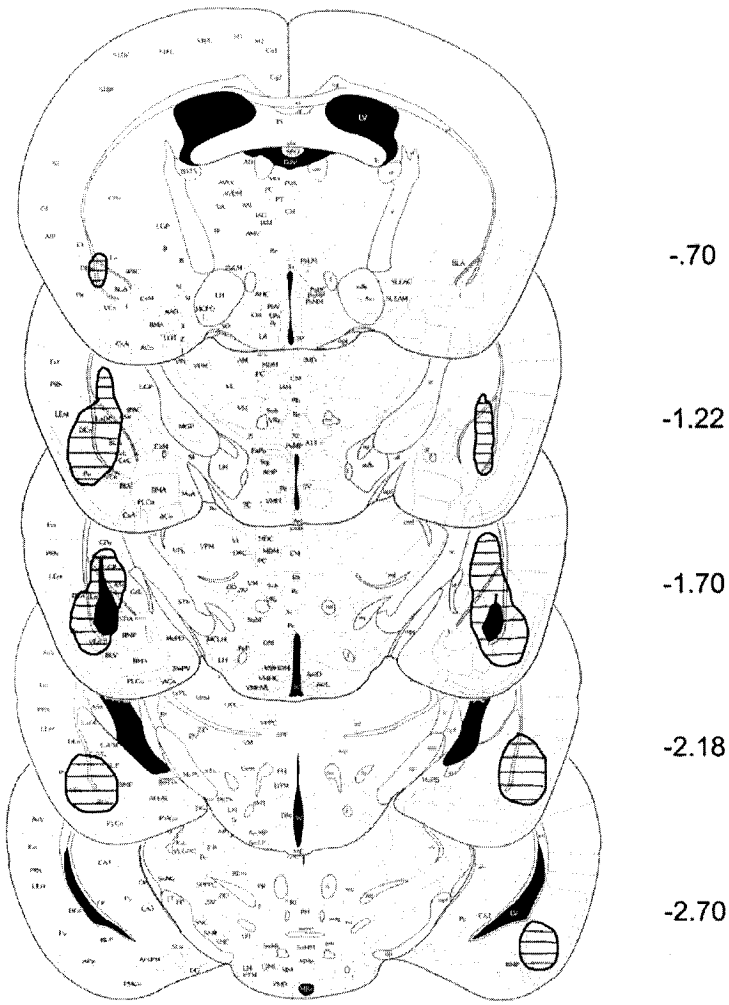


Figure 7. Schematic representations of the smallest (solid) and largest (striped) basolateral amygdala lesions. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 37, 41, 45, 49, and 53), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

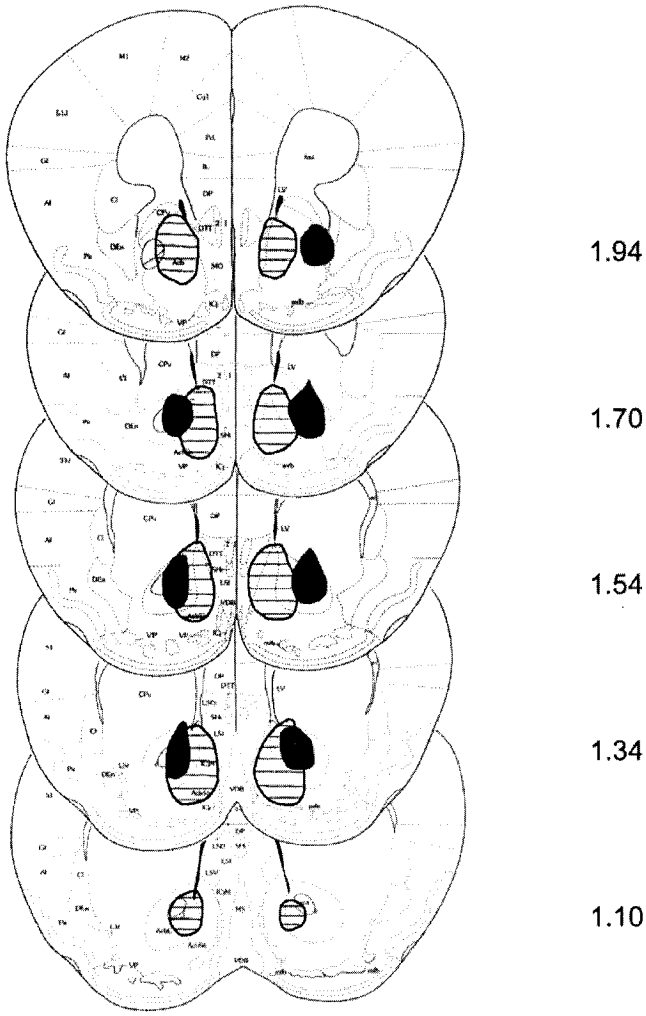


Figure 8. Schematic representations of the smallest (solid) and largest (striped) nucleus accumbens lesions. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 16, 17, 18, 20, and 22), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

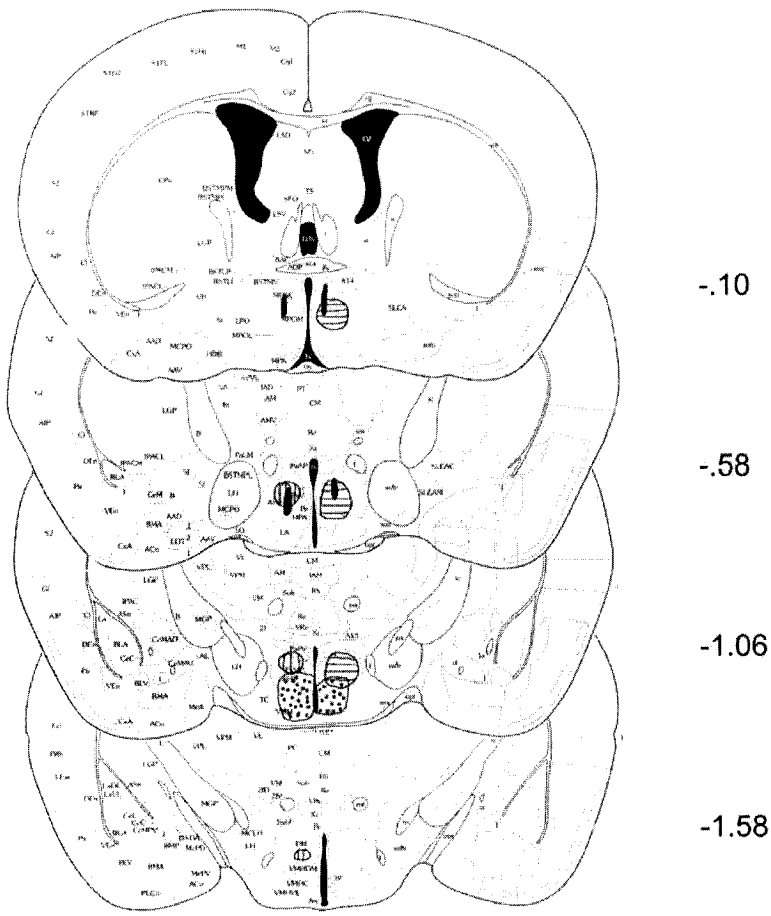


Figure 9. Schematic representations of four MPOA lesions that missed the intended area. The solid, vertical striped, and horizontal striped areas are lesions in three males. The dotted lesion is one of a female. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 28, 32, 36, 40, and 44), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

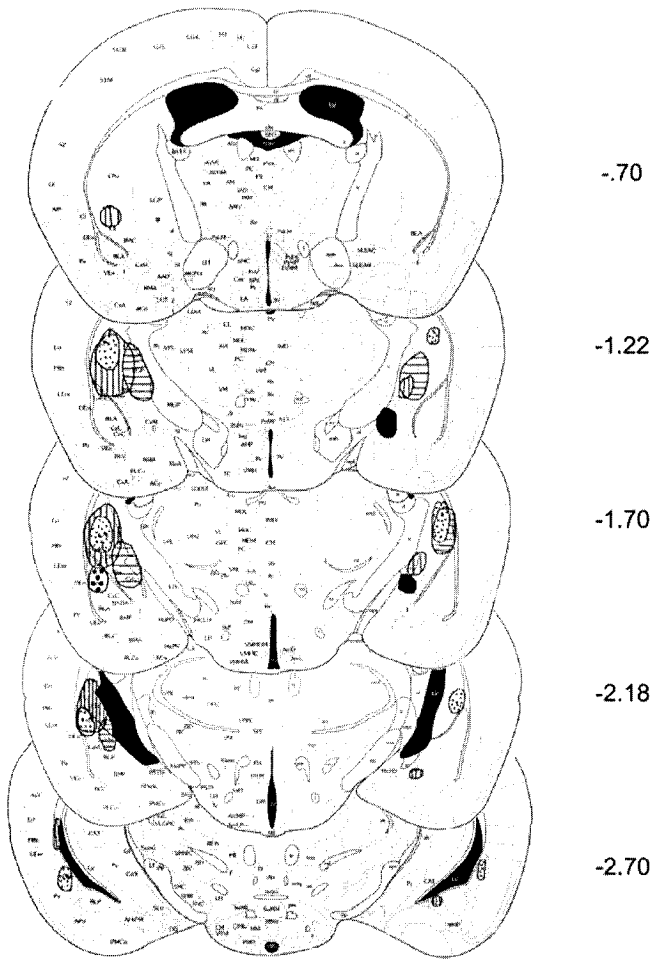


Figure 10. Schematic representations of five basolateral amygdala lesions that missed the intended area. The vertical and horizontal striped areas are lesions in two males. The solid, small dotted, and large dotted areas are lesions in three females. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 16, 17, 18, 20, and 22), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

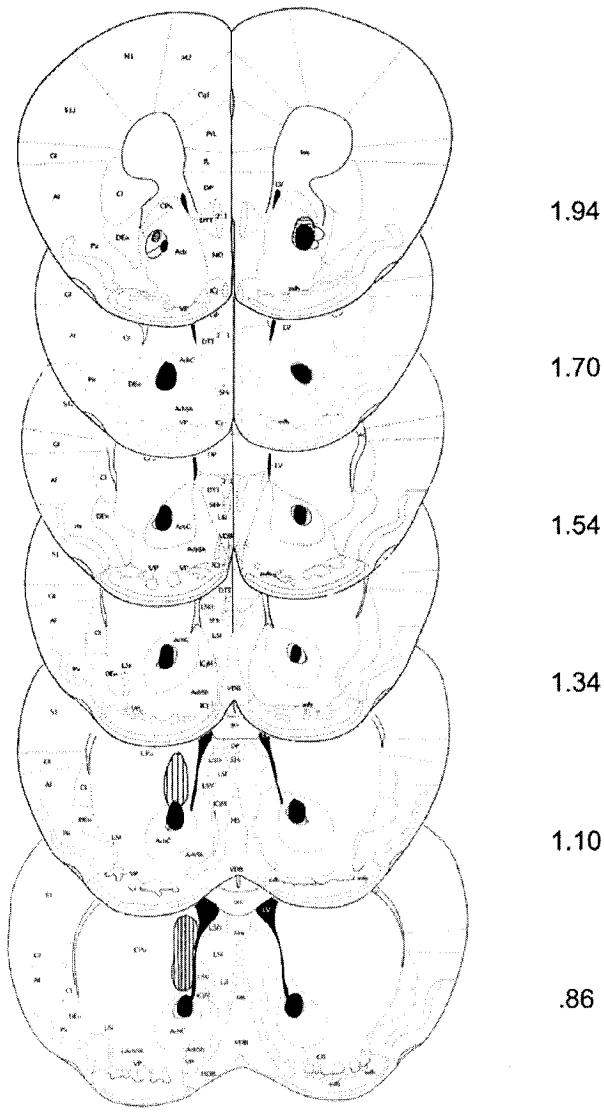


Figure 11. Schematic representations of three nucleus accumbens lesions that missed the intended area. All three lesions are in females. Numbers to the right of the sections indicate distance (in millimeters) from bregma. From *The Mouse Brain in Stereotaxic Coordinates* (Figures 16, 17, 18, 20, 22, and 24), by K.B.J. Franklin and G. Paxinos, 1997, San Diego, CA: Academic Press.

3.2.3. Parental behaviour before lesion

One male and one female cannibalized pups on the first day of testing, before the lesion. Pups were removed as soon as the mouse was observed cannibalizing a pup, and these mice were removed from the study. Four males and three females were not parental during the 2 days of testing before the lesion and these mice were also excluded from the study.

During the 2 days of parental testing before the lesion, there were significant group differences on the time spent licking pups ($F_{4,67} = 4.17$, $p < .005$) and sniffing pups ($F_{4,67} = 4.41$, $p < .004$). When groups were compared to each other, results showed that mice in the NA group spent significantly more time sniffing pups than mice in the MPOA ($t_{22} = 2.87$, $p < .01$), sham lesion ($t_{40} = 3.94$, $p < .001$), and missed lesion groups ($t_{25} = 2.25$, $p < .04$). Mice in the MPOA group spent significantly more time licking pups than mice in the missed lesion group ($t_{19} = 2.15$, $p < .05$). There were no significant sex differences and no group by sex differences in parental behaviour before the lesion (see Figures 12 - 25).

3.2.4. Parental behaviour after lesion

There were no significant differences between mice given sham lesions in the MPOA, BA, or NA on parental behaviour. Thus, all sham lesioned mice were combined for males and for females.

For males, when parental behaviours across successive 2 – day periods during the 10 days of testing after lesion were compared between groups, there

were significant group differences on the latency to approach pups on days 1 – 2 (X^2 = 16.82, p < .003) and 3 – 4 (X^2 = 9.78. p < .05; Figure 12) and on the percentage of time spent in parental behaviours on test days 1 – 2, 7 – 8, and 9 - 10 (X^2 = 10.33 – 16.72, p = .002 – .035). There were also significant differences between groups on the percentage of time spent licking pups (X^2 = 9.97 – 18.74, p = .0009 – .041) in each of the 2 – day periods, near pups on days 1 – 2, 3 – 4, 7 – 8, and 9 - 10 (X^2 = 9.99 – 17.06, p = .002 – .041) and sniffing pups on days 1 – 2 (X^2= 12.96, p < .02). Figures 14, 16, 18, 20, 22, and 24 show the mean percentage of time males were observed in total parental behaviour, licking pups, sniffing pups, near pups, crouching over pups, and self-grooming, respectively.

For females, when parental behaviours across successive 2 – day periods were compared between groups, there were significant group differences on the percentage of time spent in parental behaviours in each of the 2 – day periods (X^2 = 12.88 – 15.04, p = .005 – .012), licking pups on days 5 – 6, 7 - 8, and 9 -10 (X^2 = 12.48 - 16.28, p = .003 – .014), and near pups on days 3 – 4, 5 – 6, and 7 – 8 (X^2 = 12.84 – 16.28, p < .003 – .012). There were also significant differences between groups in the percentage of time spent crouching over pups on days 1 – 2, 5 -6, and 7 - 8 (X^2 = 9.58 – 13.41, p = .009 – .048) and self-grooming on days 1 – 2 (X^2 = 12.75, p < .02). Figures 13, 15, 17, 19, 21, 23, and 25 show the latency for females to approach pups and the mean percentage of time males were observed in total parental behaviour, licking pups, sniffing pups, near pups, crouching over pups, and self-grooming, respectively.

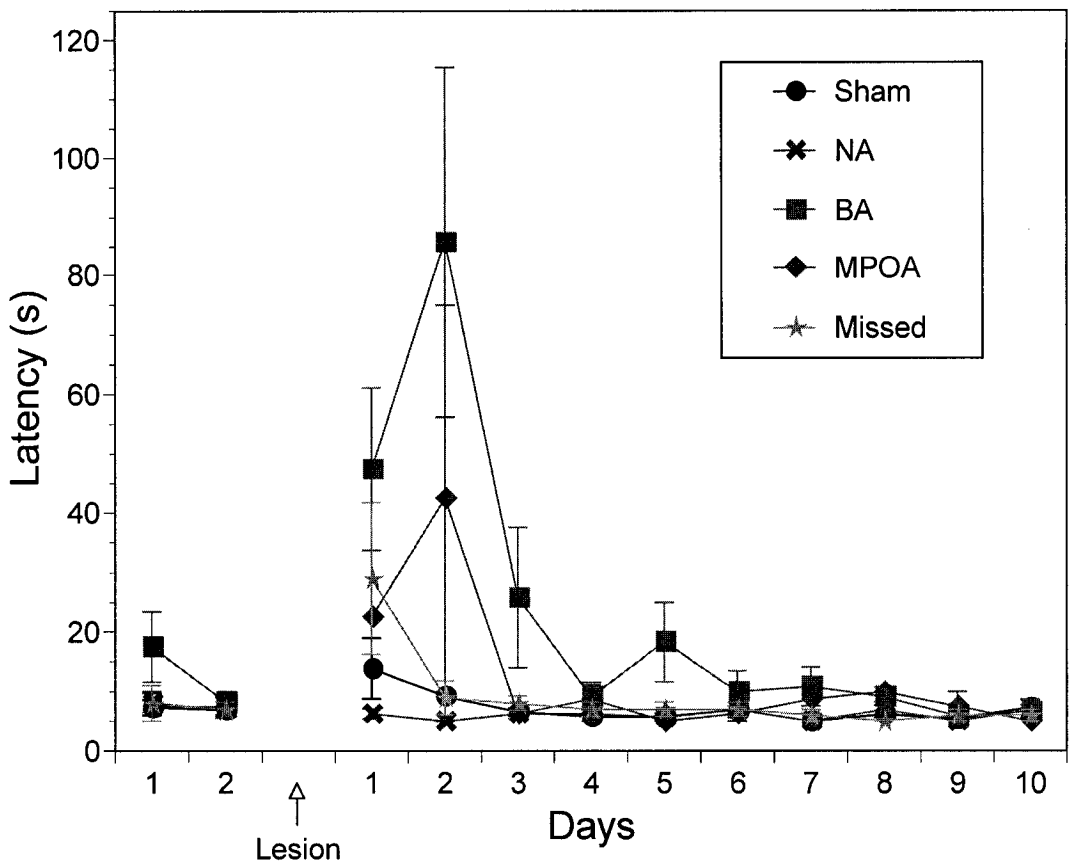


Figure 12. Mean (± *SEM*) latency in seconds for males to approach pups during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

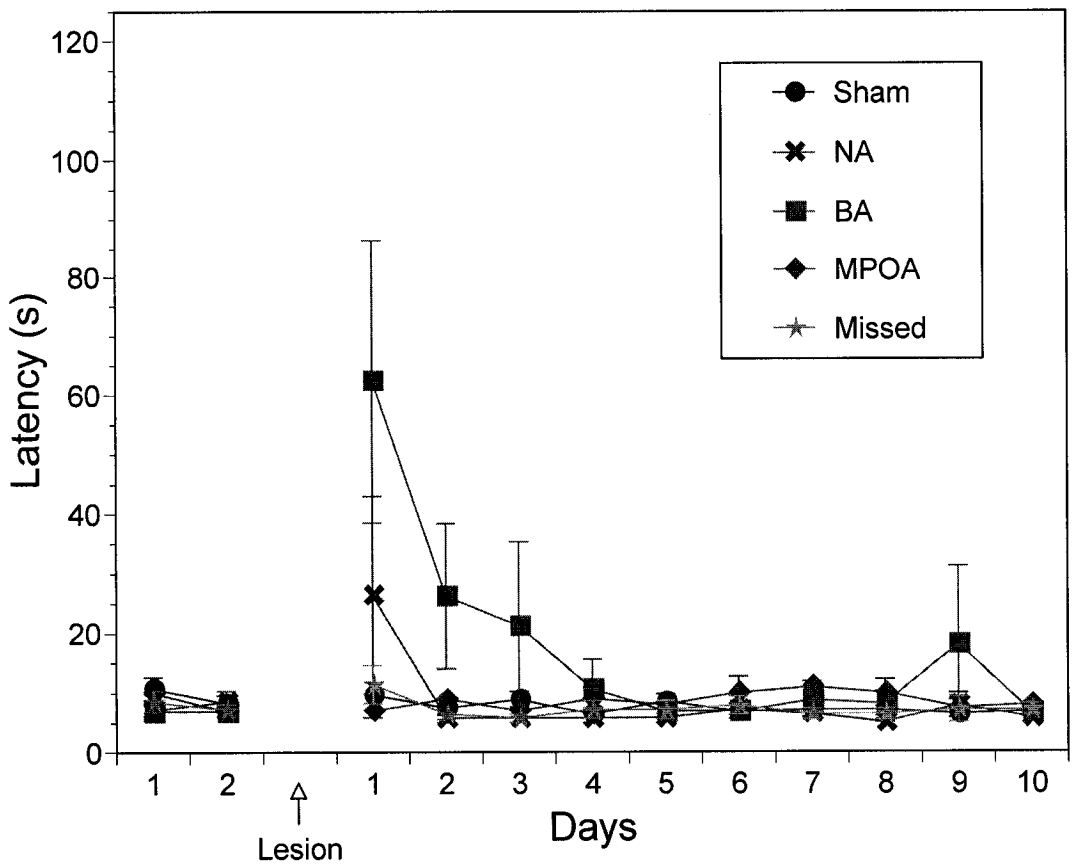


Figure 13. Mean (± *SEM*) latency in seconds for females to approach pups during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

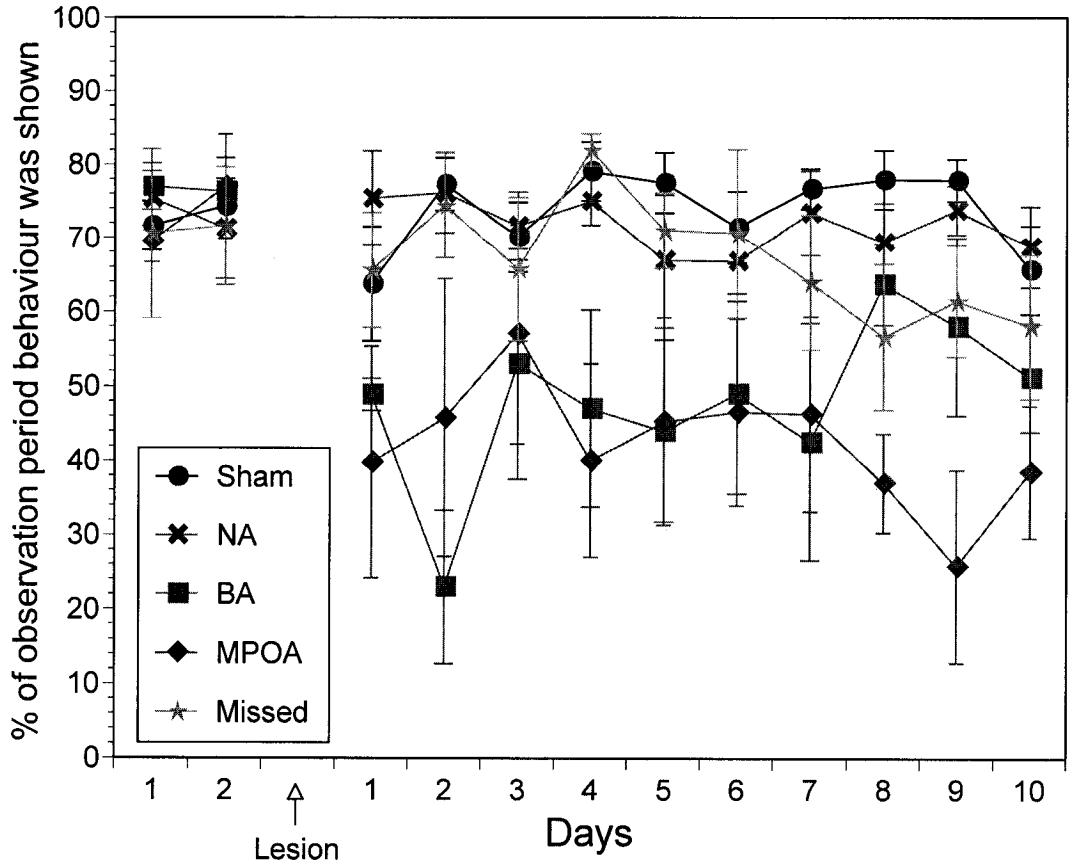


Figure 14. Mean (± *SEM*) percentage of time males were observed in parental behaviours during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

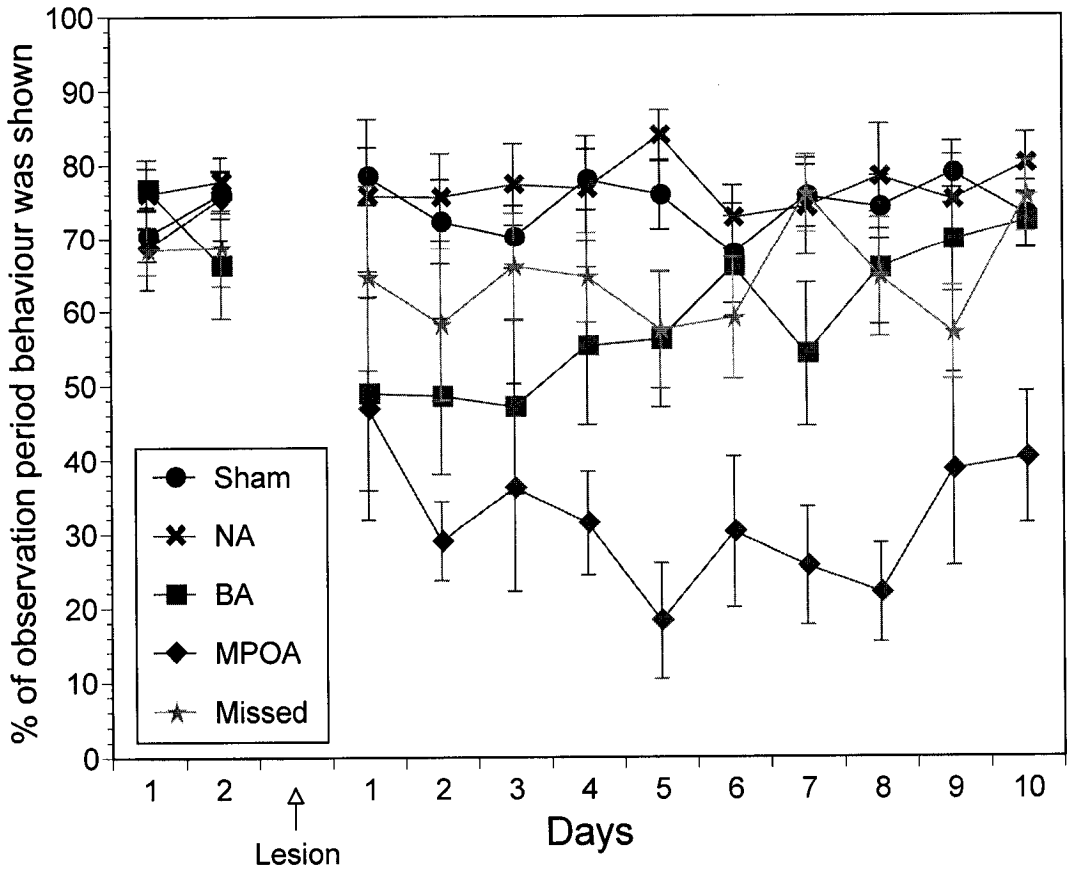


Figure 15. Mean (± *SEM*) percentage of time females were observed in parental behaviours during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

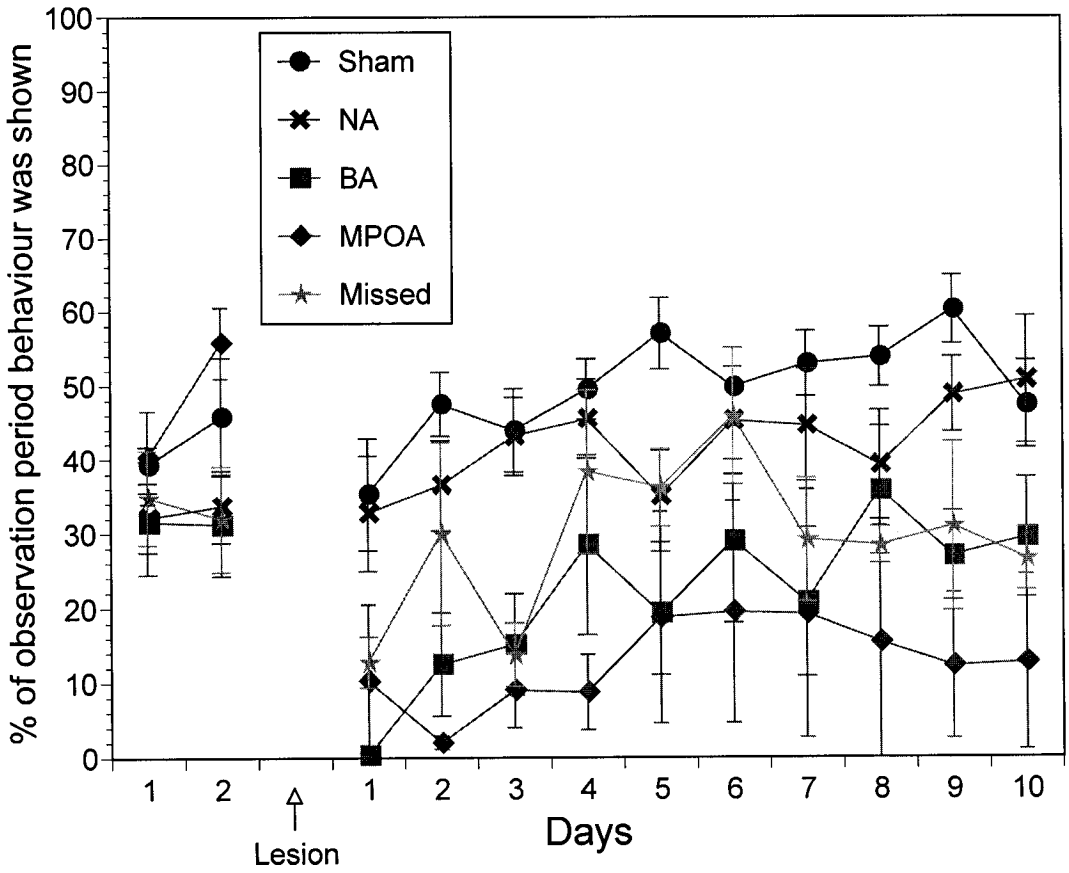


Figure 16. Mean (± *SEM*) percentage of time males were observed licking pups during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

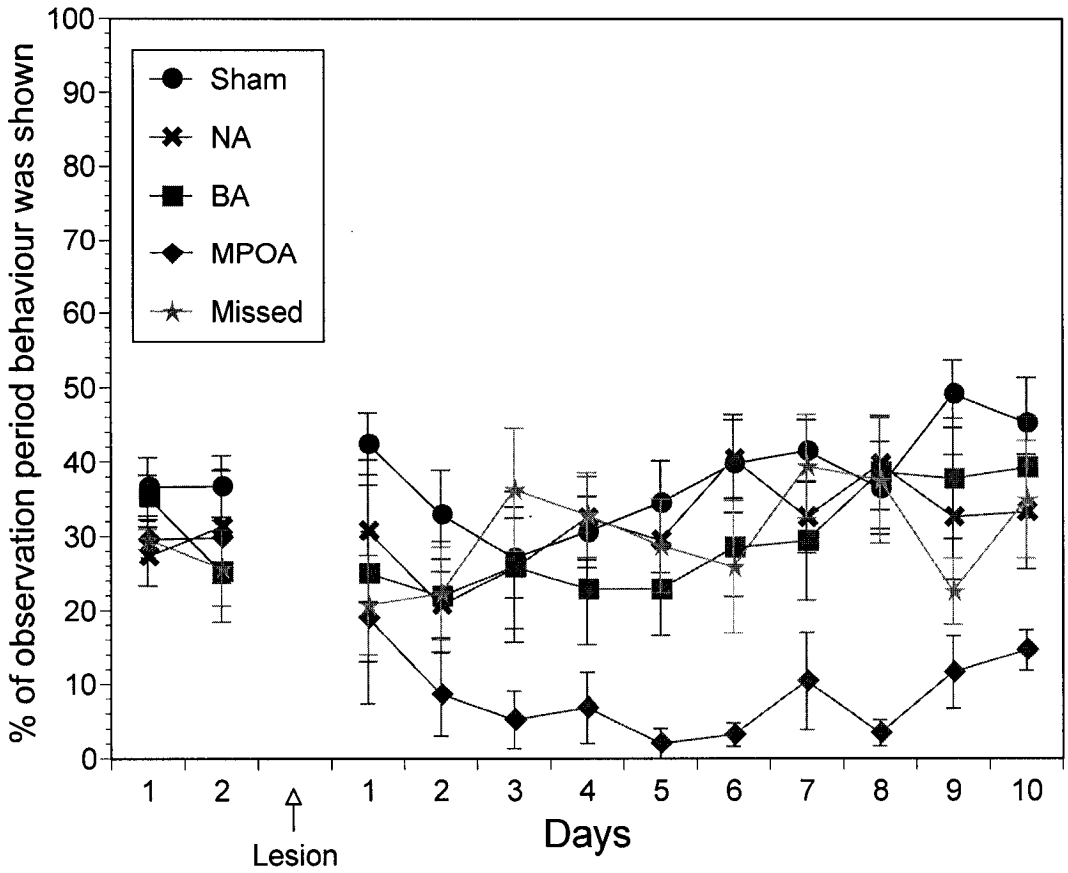


Figure 17. Mean (± *SEM*) percentage of time females were observed licking pups during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

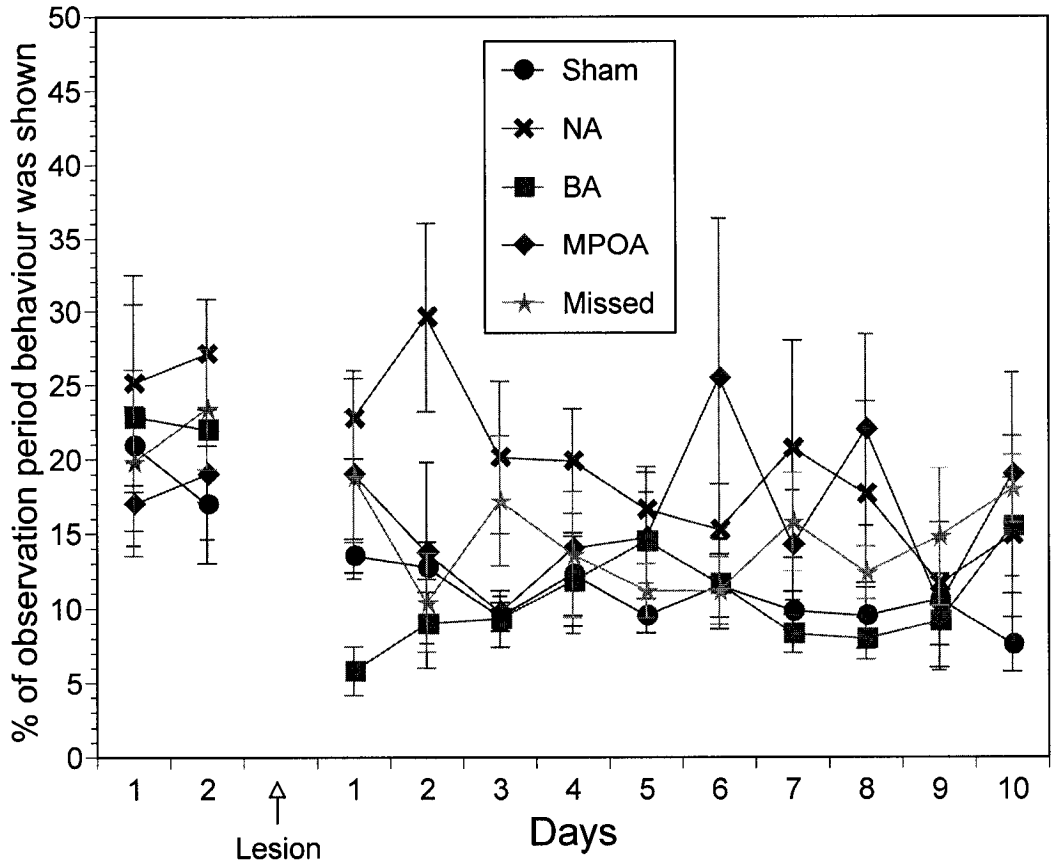


Figure 18. Mean (± *SEM*) percentage of time males were observed sniffing pups during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

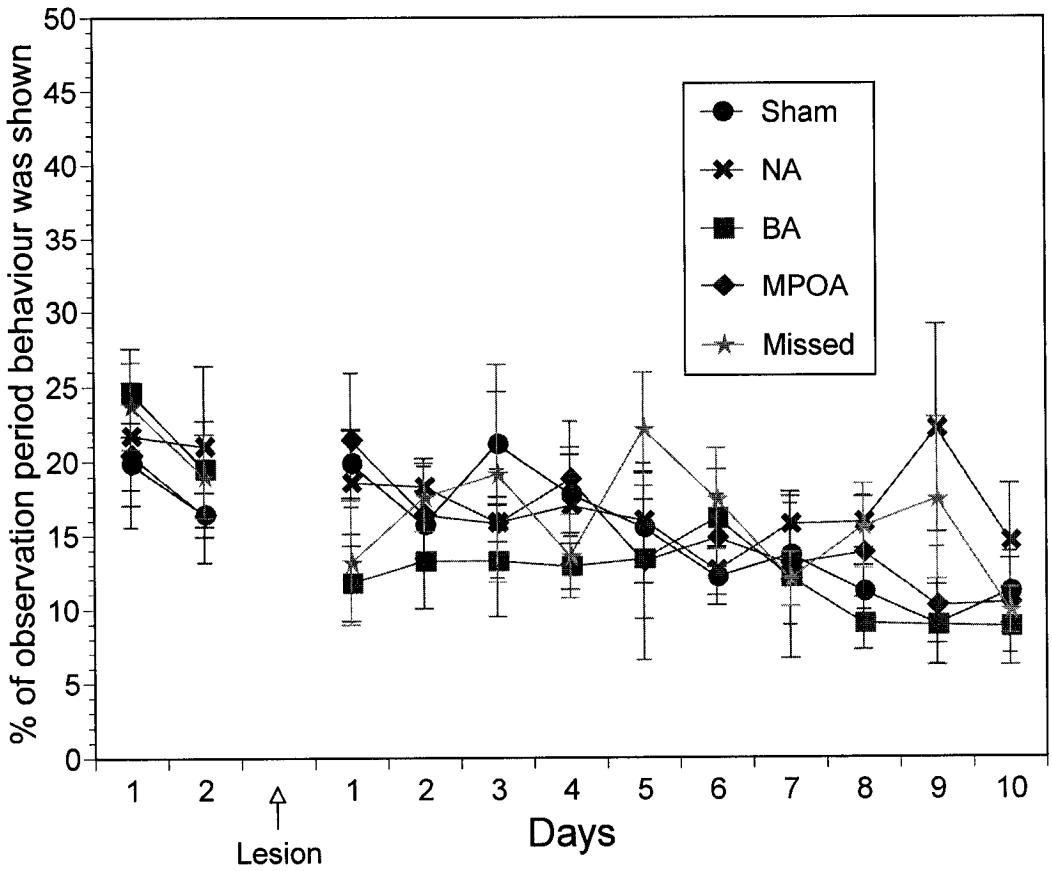


Figure 19. Mean (± *SEM*) percentage of time females were observed sniffing pups during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

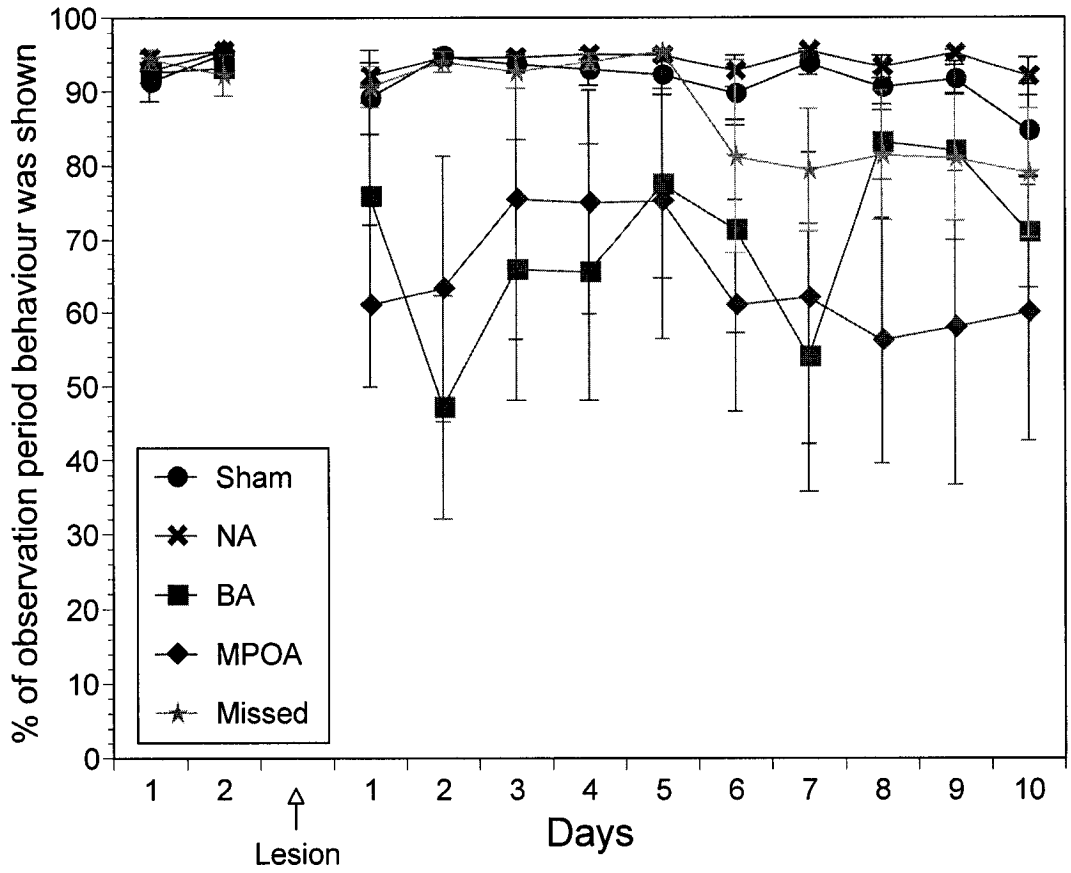


Figure 20. Mean (± *SEM*) percentage of time males were observed near pups during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

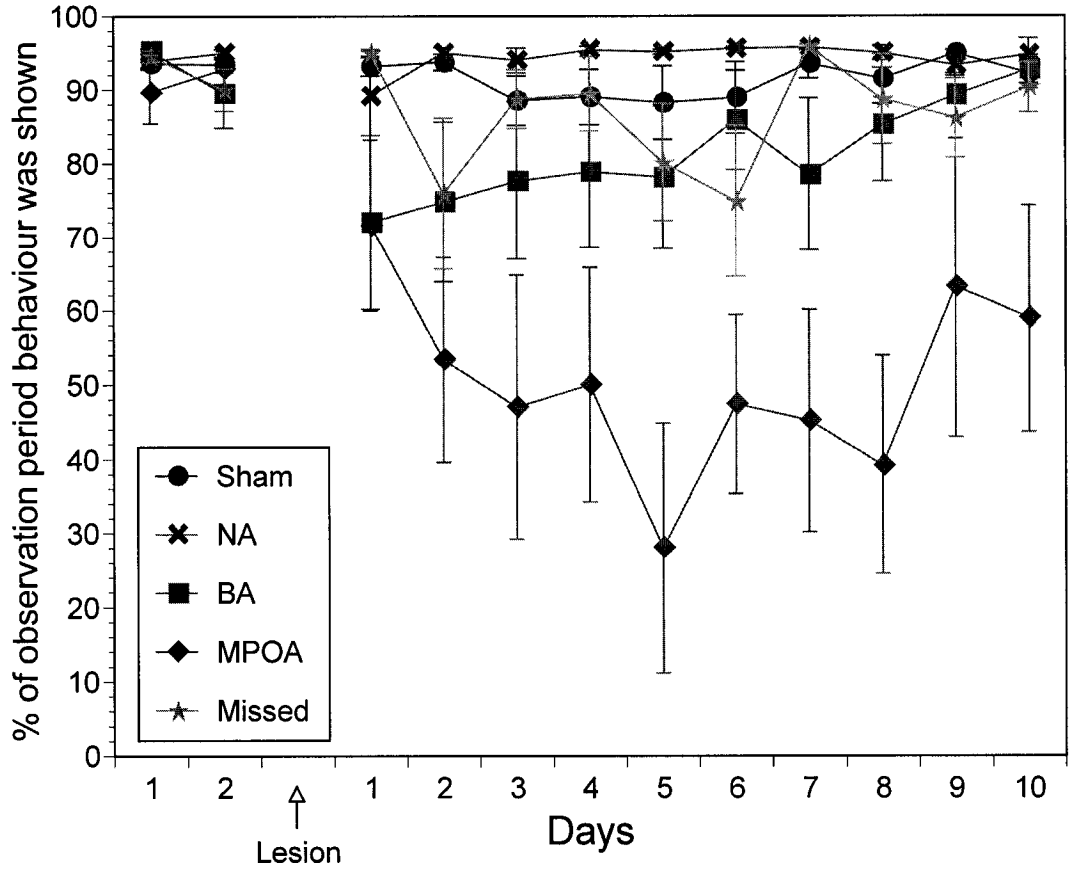


Figure 21. Mean (± *SEM*) percentage of time females were observed near pups during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

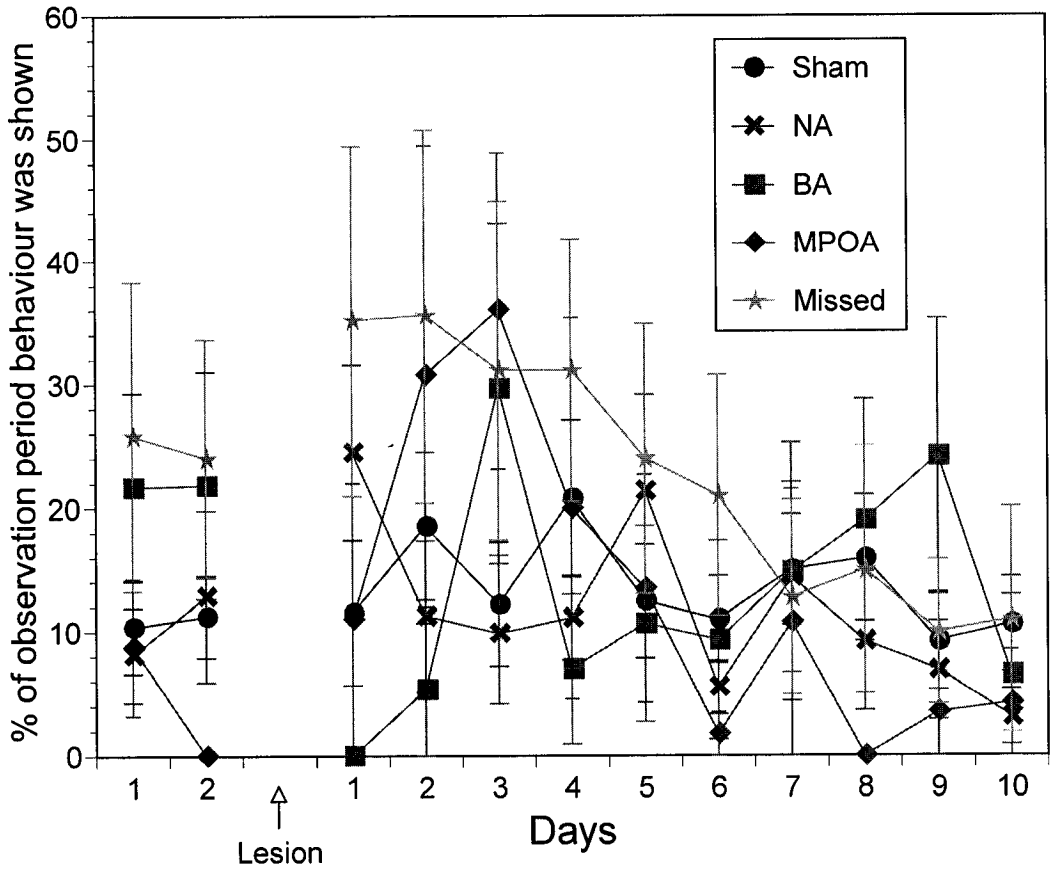


Figure 22. Mean (± *SEM*) percentage of time males were observed crouching over pups during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

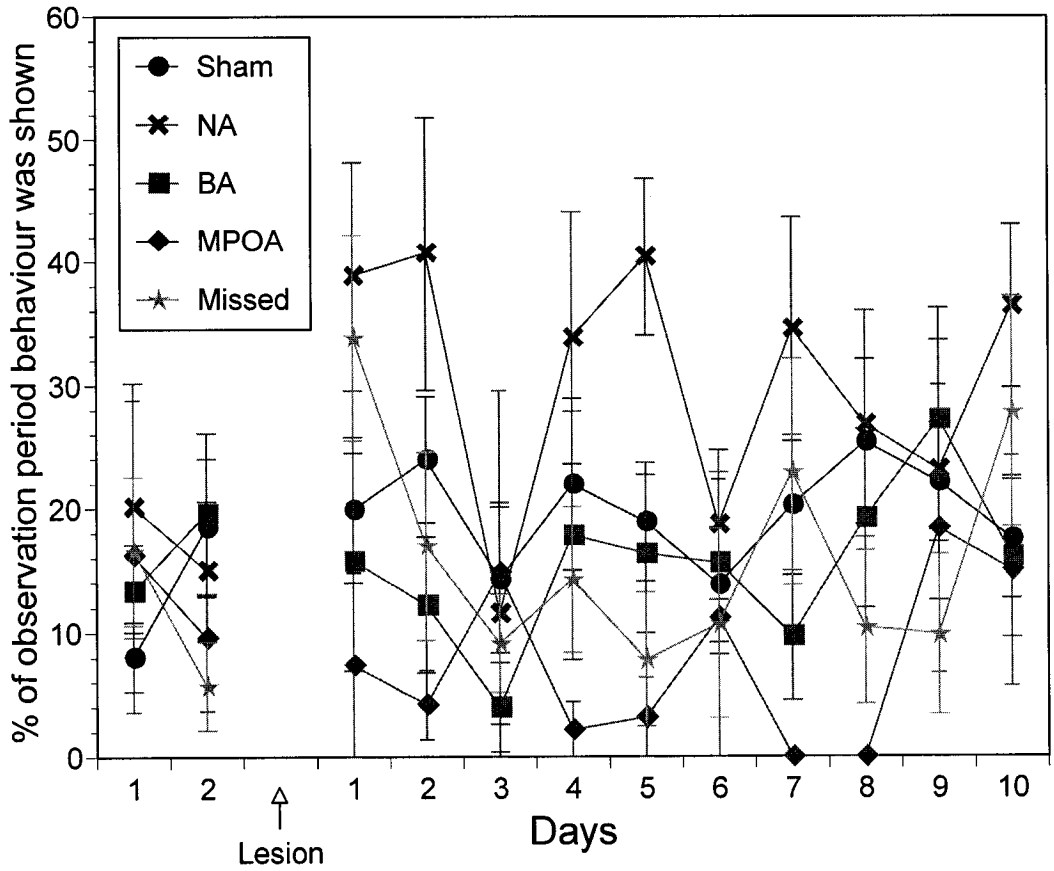


Figure 23. Mean (± *SEM*) percentage of time females were observed crouching over pups during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

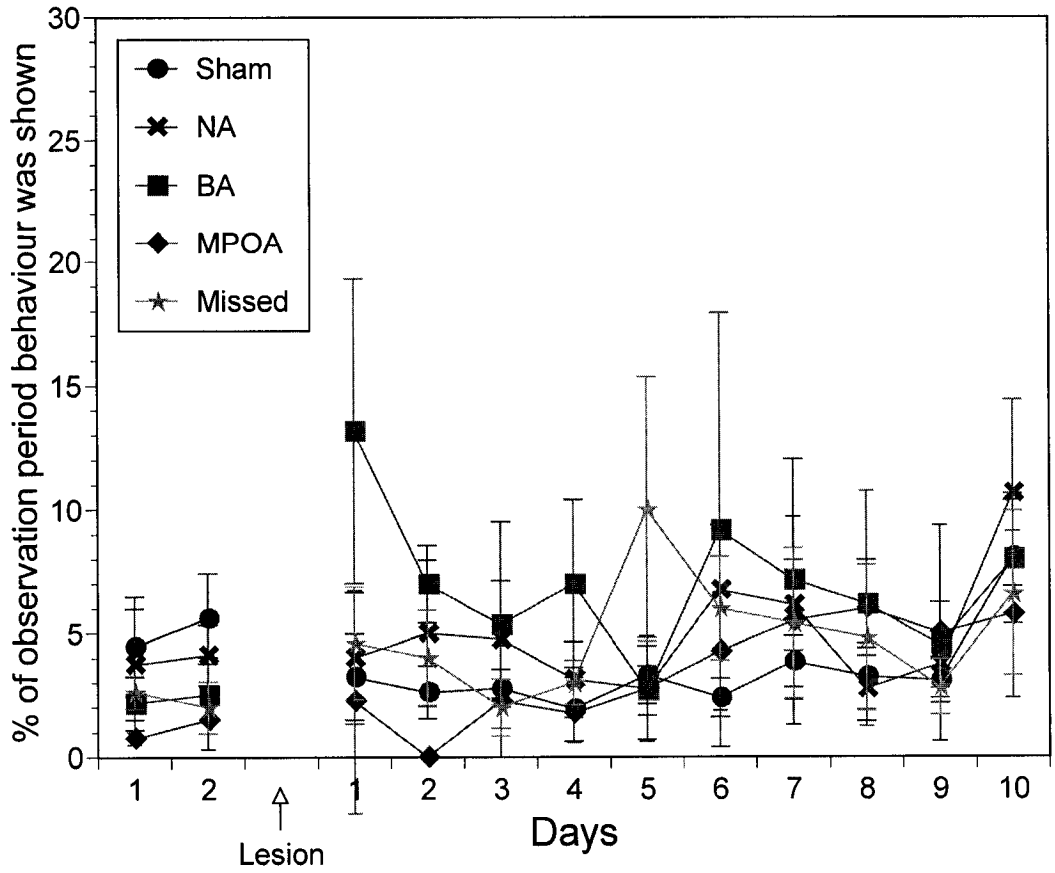


Figure 24. Mean (± *SEM*) percentage of time males were grooming themselves during the 2 days before and 10 days after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4, or missed (n = 5) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

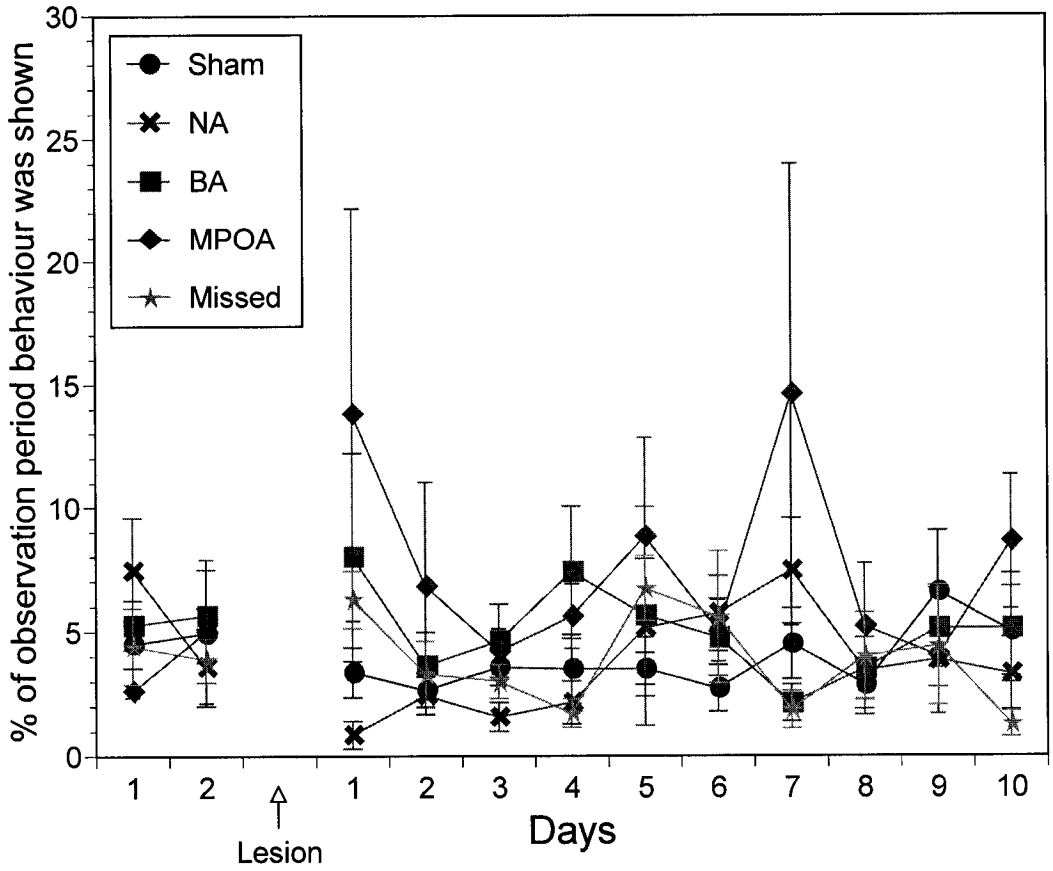


Figure 25. Mean (± *SEM*) percentage of time females were grooming themselves during the 2 days before and 10 days after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions. The missed lesion group consists of mice with lesions that missed one of the intended (NA, BA, MPOA) sites.

Males spent more time licking pups on test days 3 – 4 (X^2 = 3.98, p < .05) and 5 - 6 (X^2 = 6.65, p < .01) and less time crouching over pups on days 9 – 10 (X^2 = 11.98, p < .0006) than females. There were no other significant differences between males and females during the 10 days of testing after lesion.

3.2.4.1. Comparison between MPOA and sham lesioned mice

MPOA lesioned males showed significantly fewer parental behaviours during days 3 – 4 (U = 3, n_1 = 4, n_2 = 13, p < .006) and 5 – 6 (U = 1, p < .002) than sham lesioned males (Figure 14). MPOA-lesioned males also spent significantly less time licking pups on days 1 – 2, 3 – 4, and 9 - 10 (U = 0 – 4, p = .0008 – .0101; Figure 16) and less time near pups on days 1 - 2 (U = 4, p < .02) and 7 – 8 (U = 7, p < .04; Figure 20). There were no other significant differences between sham and MPOA lesioned males during the 10 days after lesion.

MPOA lesioned females showed significantly fewer parental behaviours on each of the 2 – day periods than sham-lesioned females (U = 0 - 5, n_1 = 5, n_2 = 14, p = .0002 – .0033; Figure 15). MPOA-lesioned females spent significantly less time licking pups on Days 5 – 6, 7 – 8, and 9 - 10 (U = 0 – 1, p = .0002 – .003; Figure 17), near pups on days 3 – 4, 5 – 6, and 7 - 8 (U = 2 – 6, p = .0007 – .005; Figure 21), and crouching over pups on days 1 – 2 (U = 12.5, p < .04) and 7 – 8 (U = 2.5, p < .0008; Figure 23) than sham-lesioned females. MPOA lesioned females also spent significantly more time grooming themselves on day 1 – 2 (U = 10.5, p < .02; Figure 25). There were no other significant differences between sham and MPOA lesioned females during the 10 days after lesion.

3.2.4.2. Comparison between BA and sham lesioned mice

Amygdala lesioned males took significantly longer to approach pups than sham lesioned males on days 1 – 2, ($U = 5$, $n_1 = 6$, $n_2 = 13$, $p < .002$) and 3 - 4 ($U = 9$, $p < .007$; Figure 12) and spent significantly less time in parental behaviours on days 1 – 2 ($U = 0$, $p < .0002$). Amygdala lesioned males spent significantly less time near pups on days 1 - 2, 3 – 4, and 7 – 8 ($U = 5 – 16$, $p = .001 – .046$; Figure 20) and licking pups on each of the 2 – day periods ($U = 2.5 – 14.5$, $p = .003 – .029$; Figure 16) than sham lesioned males. There were no other significant differences between sham and BA lesioned males during the 10 days after lesion.

Amygdala lesioned females spent significantly less time in parental behaviours on days 1 – 2 ($U = 24$, $n_1 = 8$, $n_2 = 14$, $p < .03$) and 3 – 4 ($U = 27.5$, $p = .05$; Figure 15) and less time near pups on days 7 - 8 ($U = 26$, $p < .05$; Figure 19) than sham lesioned females. There were no other significant differences between sham and BA lesioned females during the 10 days after lesion.

3.2.4.3. Comparison between NA and sham lesioned mice

NA lesioned males spent significantly more time sniffing pups on days 1 - 2 ($U = 13$, $n_1 = 8$, $n_2 = 13$, $p < .004$; Figure16) than sham lesioned males. There were no other significant differences between NA and sham lesioned males during the 10 days after lesion.

NA lesioned females spent significantly more time near pups ($U = 16.5$, $n_1 = 7$, $n_2 = 14$, $p < .02$) and crouching over pups on days 5 - 6 ($U = 20$, $p < .04$;

Figure 23) than sham lesioned females. There were no other significant differences between sham and NA lesioned females during the 10 days after lesion.

3.2.4.4. Comparison between missed lesion and sham lesion controls

Males in the missed lesion group spent significantly less time near pups on days 7 – 8 ($U = 11.5$, $n_1 = 13$, $n_2 = 5$, $p < .04$) and licking pups on days 1 – 2, 3 - 4 , 7 – 8, and 9 - 10 ($U = 3 – 16$, $p = .002 – .046$; Figure 16) than sham lesioned males. There were no other significant differences between missed lesion and sham lesioned males during the 10 days after lesion.

Females in the missed lesion group also spent significantly less time licking pups on days 9 - 10 ($U = 15$, $n_1 = 14$, $n_2 = 7$, $p < .01$; Figure 17) than sham lesioned females. There were no other significant differences between females in the missed lesion and sham lesion groups during the 10 days after lesion.

3.2.5. Latencies to show parental behaviour

There were significant differences between groups on the latency to show pup retrieval ($X^2 = 21.6384$, $n = 79$, $p = .0002$), pup-licking ($X^2 = 13.11$, $p < .05$), and crouching over pups ($X^2 = 18.76$, $p = .009$; Figures 26 and 27). Males had longer latencies to retrieve pups ($X^2 = 5.63$, n 70, $p < .05$) than females. In the sham lesioned groups, the latency to build nests was longer when sham lesions

were given to females than males ($U = 46.5$, $n_1 = 13$, $n_2 = 14$, $p < .03$). There were no differences between groups or sexes on the latency to sniff pups.

3.2.5.1. Comparison between MPOA and sham lesioned mice

MPOA lesioned males had longer latencies to retrieve pups ($U = 4.5$, $n_1 = 4$, $n_2 = 12$, $p < .02$) than sham lesioned males (Figure 26). MPOA lesioned males took longer to lick pups, but this difference was not statistically significant ($U = 9.5$, $p = .06$). Females given MPOA lesions showed longer latencies to retrieve pups ($U = 9.5$, $n_1 = 5$, $n_2 = 15$, $p < .02$) than sham lesioned females (Figure 27). MPOA lesioned females also took longer to crouch over pups, but this difference was not statistically marginally significant ($U = 15$, $p = .07$). There were no differences between MPOA lesioned males and females on the latency to show parental behaviours.

3.2.5.2. Comparison between BA and sham lesioned mice

BA lesioned males showed longer later latencies to retrieve pups ($U = 16$, $n_1 = 7$, $n_2 = 12$, $p < .02$), lick pups ($U = 18$, $p < .03$), and crouch over pups ($U = 18.0$, $p < .03$; Figure 26) than sham lesioned males. BA lesioned females had longer latencies to retrieve pups ($U = 15$, $n_1 = 9$, $n_2 = 14$, $p < .002$; Figure 27) than sham lesioned females. BA lesioned males and females did not differ in their latencies to show parental behaviours.

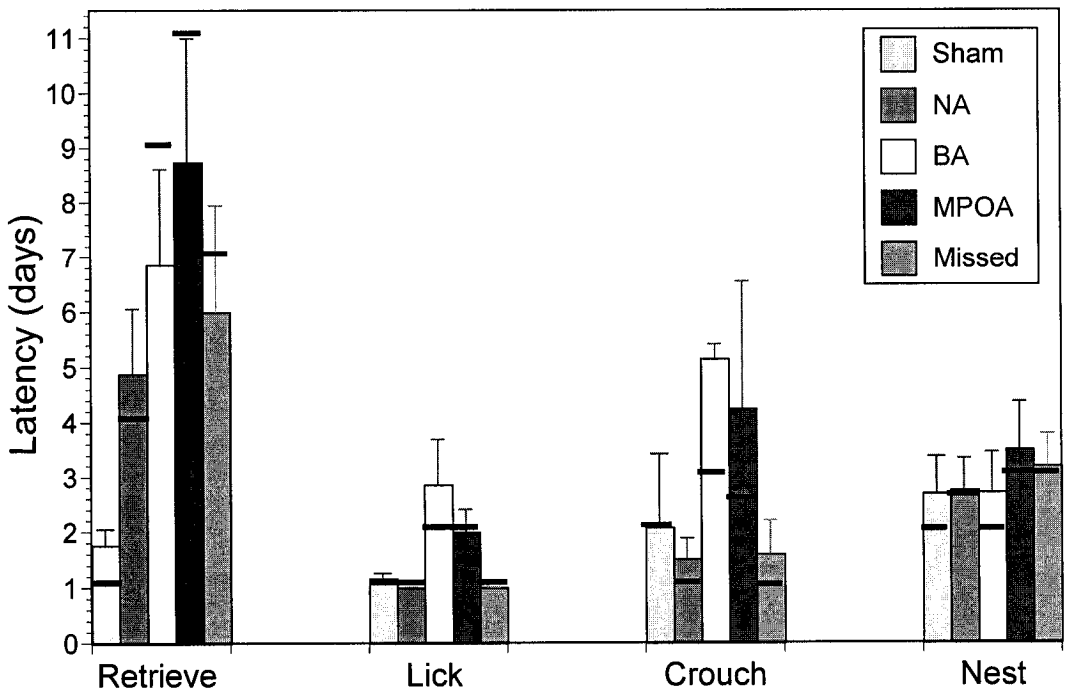


Figure 26. Mean (+ *SEM*) and median (dark bars) latencies in days to retrieve pups, lick pups, hover over pups, and build a nest after sham (n = 13), nucleus accumbens (NA, n = 8), basolateral amygdala (BA, n = 6), medial preoptic area (MPOA, n = 4), or missed (n = 5) lesions in male *P. californicus.*

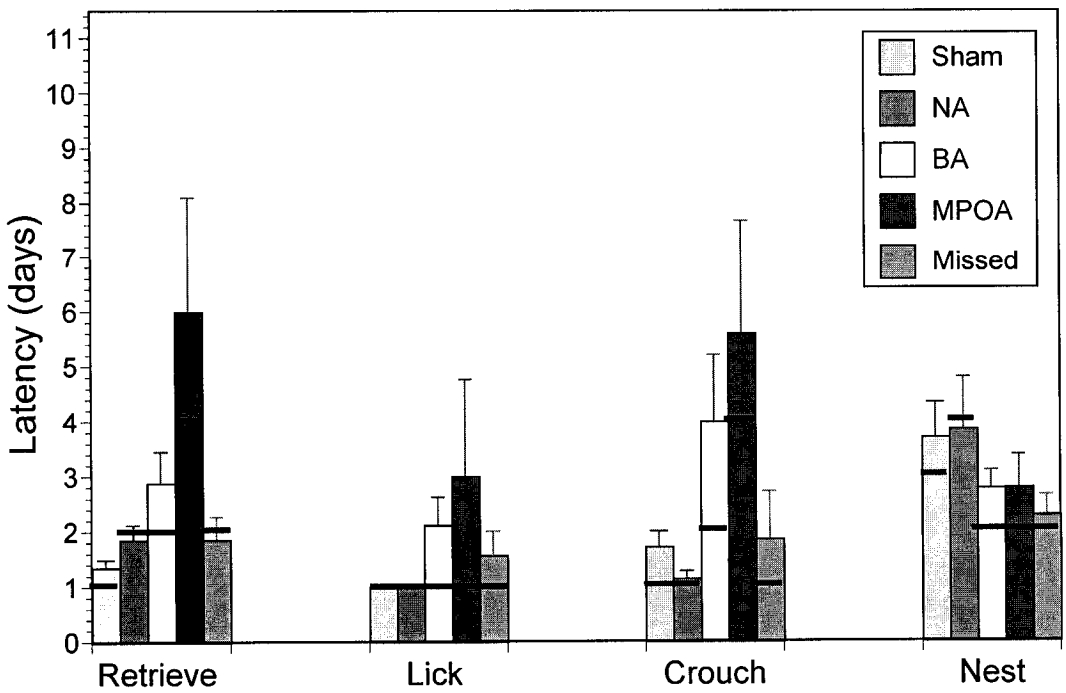


Figure 27. Mean (+ *SEM*) and median (dark bars) latencies in days to retrieve pups, lick pups, hover over pups, and build a nest after sham (n = 14), nucleus accumbens (NA, n = 7), basolateral amygdala (BA, n = 8), medial preoptic area (MPOA, n = 5), or missed (n = 7) lesions in female *P. californicus*.

3.2.5.3. Comparison between NA and sham lesioned mice

NA lesioned males showed longer latencies to retrieve pups than sham lesioned males ($U = 18$, $n_1 = 8$, $n_2 = 12$, $p < .02$). NA and sham lesioned females did not differ on the latency to show any parental behaviours. NA lesioned males showed significantly longer latencies to retrieve pups than NA lesioned females ($U = 10.0$, $p < .05$).

3.2.5.4. Comparison between missed lesion and sham lesion controls

There were no differences between the sham lesion and missed lesion groups in the latency to show parental behaviours.

3.2.6. Effect of lesions on litter weight

Litter weight increased significantly over days ($F_{9,37} = 648.52$, $p < .001$; Figure 28 – 29), indicating that pups were gaining weight. There was a significant main effect of the sex of the parent given the lesion, such that litters in which the mother was given a lesion weighed less than those in which the father was given a lesion ($F_{1,45} = 5.32$, $p < .05$; Figure 28 - 29). Finally, there was a significant main effect of group ($F_{4,45} = 2.90$, $p < .05$). Comparing between groups showed that litters in which parents had a lesion in the MPOA weighed less than litters in which parents had NA lesions ($F_{1,8} = 8.01$, $p < .02$). Litters in which the parent had MPOA lesions also weighed less than litters in which the parent had sham lesions, but this difference was not statistically significant ($F_{1,20}$

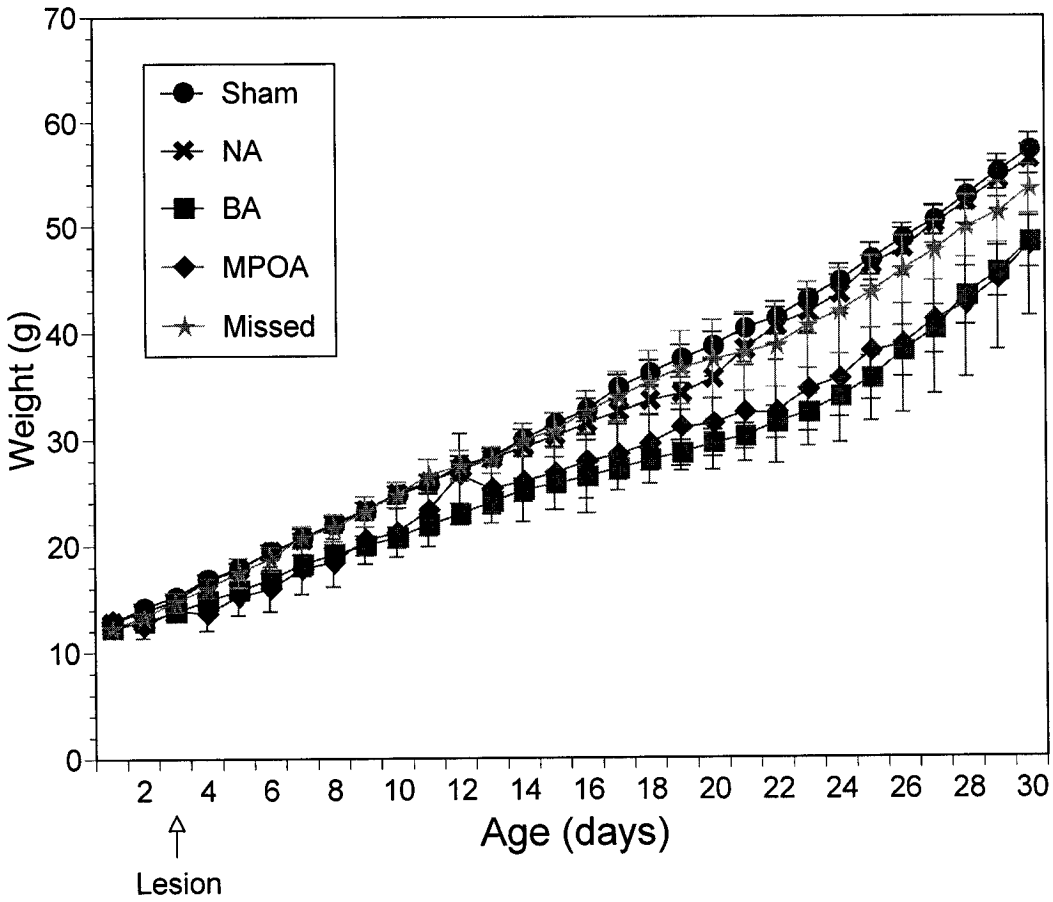


Figure 28. Mean (+ *SEM*) litter weight of pups, in which the father was given a sham (n = 9), nucleus accumbens, (NA, n = 7), basolateral amygdala (BA, n = 5), medial preoptic area (MPOA, n = 3), or missed (n = 3) lesion. From day 1 – 13, pups were given to foster parents on odd numbered days and to their natural parents on even numbered days. From day 14 and on, pups remained with foster parents.

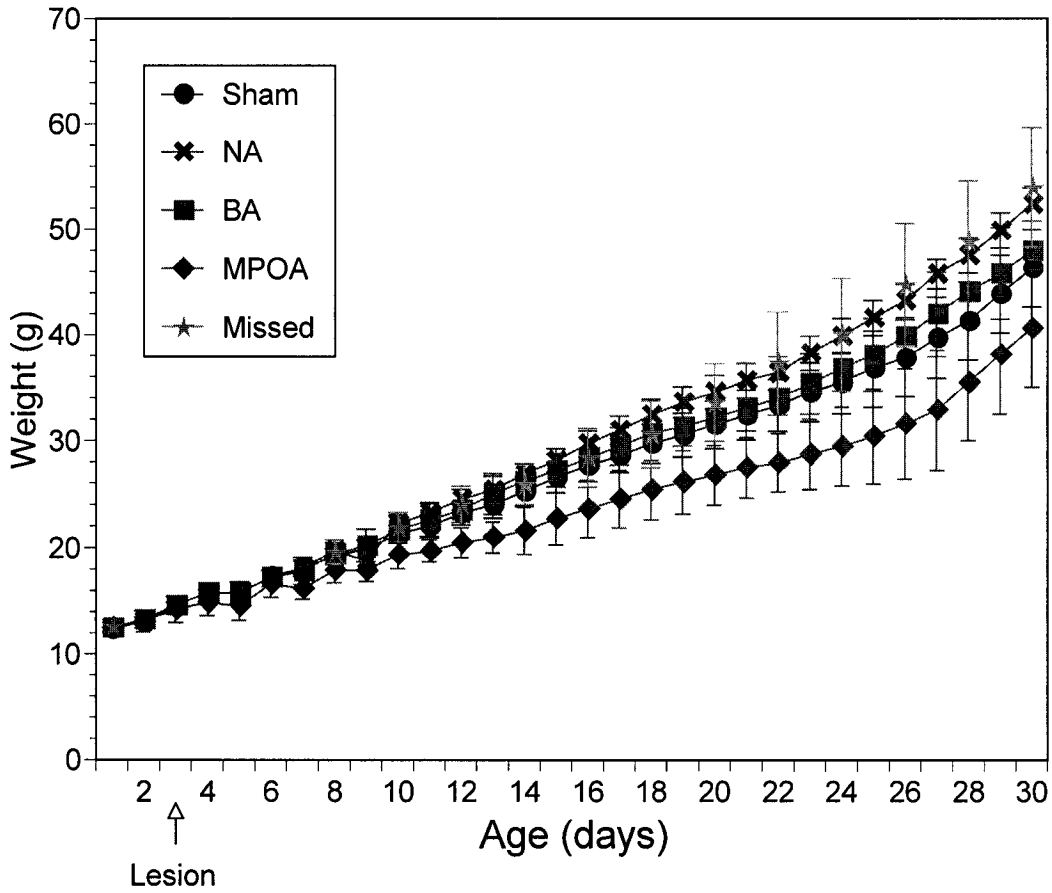


Figure 29. Mean (+ *SEM*) litter weight of pups, in which the mother was given a sham (n = 7), nucleus accumbens, (NA, n = 7), basolateral amygdala (BA, n = 7), medial preoptic area (MPOA, n = 3), or missed (n = 4) lesion. From day 1 – 13, pups were given to foster parents on odd numbered days and to their natural parents on even numbered days. From day 14 and on, pups remained with foster parents.

= 3.63, p = .07). Lastly, litters in which parent had BA lesions weighed less than those in which parents had NA lesions ($F_{1,24}$ = 6.92, p < .02; Figures 28 – 29). There were no other significant differences in litter weight between groups and there were no significant interactions.

There were no significant effects of lesion or sex of the parent given the lesion on the first day in which 2 of 3 pups opened their eyes (M = 15.64, SEM = 0.17). The ratio of males to females in a litter was not correlated with litter weight at any point during the 30 days postpartum (r = 0.064 – 0.098, p ≥ .50).

3.3. Discussion

The results of experiment 2 show that the MPOA is important for the maintenance of parental behaviour in both male and female California mice. These results are consistent with those from experiment 1, as well as with numerous published studies that have implicated the MPOA in both the onset and maintenance of maternal behaviour in rats (Miceli et al., 1983; Numan, 1974; Numan & Callahan, 1980; Numan & Corodimas, 1985; Numan et al., 1977; Numan et al., 1988). The results also suggest that the BA is important for the maintenance of parental behaviour in male and, to a lesser extent, in female California mice. With the exception of one parental measure, the latency to retrieve pups in NA-lesioned males, NA lesions do not appear to have a role in the maintenance of parental behaviour in *P. californicus* of either sex.

3.3.1. MPOA lesions disrupt both male and female parental behaviour

MPOA lesioned females consistently spent significantly less time in total parental behaviours (sniffing, licking, crouching, and retrieving pups) than sham lesioned females throughout the 10 days of parental behaviour testing. MPOA lesioned males showed decreased total parental behaviour only during days 3 – 6, suggesting that MPOA lesions disrupt parental behaviour to a greater extent in female than in male California mice.

MPOA lesioned males and females spent less time near pups, licking pups, and hovering over pups, which is consistent with the results of experiment 1. However, the specific days in which males and females showed less time near pups and less time licking pups varied. MPOA lesioned males spent less time near pups during the first 2 days and days 7 – 8, while MPOA lesioned females spent less time near pups during the middle 6 days of parental behaviour testing. MPOA lesioned males showed decreased pup – licking during the first 4 days and last 2 days, while females showed decreased pup – licking during the latter 6 days of parental testing. MPOA lesions, however, did not disrupt pup-sniffing, which is in contrast to results of experiment 1.

MPOA lesions produced slight deficits in crouching (or hovering) over pups in females, such that females spent less time crouching over pups during the first 2 days of parental behaviour testing compared to sham lesioned controls. MPOA lesions had no effect on crouching in males. In experiment 1, MPOA lesioned males, but not females showed longer latencies to hover over

pups. The contradictory results of the two experiments on the effect of MPOA lesions on hovering over pups is not surprising as this inconsistency appears throughout the maternal behaviour literature, often within the same laboratories (Jacobson et al., 1980; Numan, 1974; Numan, 1990; Numan & Callahan, 1980; Numan et al., 1988; Terkel et al., 1979). Perhaps the difference in size of the lesion contributed to the variability of the effects on hovering over pups in experiments 1 and 2. MPOA lesions in experiment 1 tended to be larger, extending more laterally and caudally than lesions in experiment 2.

Another possibility for the discrepancy of the effects of MPOA lesions on crouching may involve the temperature of the testing room or the female's body. Croskerry et al. (1978) suggest that when the female's ventral temperature reaches a critical level, she may terminate nursing. In support of this hypothesis, female rats show shorter nursing bouts when environmental temperatures are high (Croskerry et al., 1978). Thus, the temperature of the room and the number of pups the female must nurse may affect the female's ventral temperature, which in turn may affect the length of nursing bouts. To further complicate this picture, the MPOA is suggested to be the area where temperature factors influence maternal behaviour, as a large proportion of MPOA neurons are thermosensitive (Boulant, 1980). Direct heating of the MPOA terminates nursing (Woodside et al, 1980), while MPOA lesions produce impairments in thermoregulation in rats (Szymusiak & Satinoff, 1984; Van Zoeren & Stricker, 1977).

In summary, MPOA lesions produce clear deficits in retrieval and licking in

both male and female California mice. MPOA lesions may also produce deficits in crouching over pups and sniffing pups, but these results have not been consistently produced, both in this thesis as well as in other laboratories.

3.3.2. BA lesions disrupt male parental behaviour

Males given BA lesions took significantly longer to approach pups during the first few days of parental behaviour testing after the lesion. Although not statistically significant, females given BA lesions also tended to take longer to approach pups during the initial 3 days after lesion. This suggests that BA lesioned mice, particularly male mice, took longer to recover from the lesion. These mice often appeared sluggish and showed little interaction with pups during the first day after lesion.

A role for the BA in maternal behaviour in rats was first demonstrated by Lee et al. (1999), who showed that lesions in the BA reduced maternal licking and crouching. BA lesions have also been shown to reduce maternal motivation for pups, as BA lesioned female rats exhibit fewer bar-presses for pups in an operant responding paradigm (Lee et al., 2000). The effect of BA lesions on male parental behaviour has never been studied before.

Interestingly, the results from this experiment suggest that BA lesions produce more profound deficits in male than in female California mice. BA lesions increased the latency to retrieve pups in both males and females, consistent with the effect of BA lesions on maternal behaviour in rats (Lee et al., 1999; Lee et al., 2000). BA lesioned males, but not females, also took longer to

show pup - licking and crouching over pups. Finally, BA lesioned males clearly showed a reduction in licking pups throughout the 10 days of parental behaviour, also consistent with the previous studies on rats (Lee et al., 1999, 2000).

3.3.3. NA lesions do not disrupt parental behaviour

For the most part, NA lesioned males and females did not differ in the latency or the percentage of time spent in parental behaviours from sham lesioned controls. NA lesioned males, did however, show longer latencies to retrieve pups compared to sham lesioned males and to NA lesioned females. This was the only deficit in parental behaviour observed in NA lesioned mice. For two of the parental behaviours, licking, and crouching, NA lesioned mice actually spent more time than sham lesioned mice in exhibiting these behaviours.

Lesions of the NA, a region normally associated with the reinforcement system, have been shown to produce deficits in maternal behaviour in rats (Lee et al., 1999, 2000). The effects of NA lesions on male parental behaviour have never been studied before. In contrast to the results reported for female rats, NA lesions had no effect on parental behaviour in male and female California mice. These results suggest that the NA is not important for parental behaviour in California mice.

3.3.4. Missed lesions produce some deficits in parental behaviour

Males in the missed lesion group showed deficits in only one of the parental behaviours, licking. Three of the males in the missed lesion group were

mice with lesions that were intended in the MPOA, but were too caudal. The other two males in the missed lesion group were mice with lesions that were intended in the BA, but destroyed a portion of the caudate putamen, one unilaterally and the other bilaterally.

No deficits were observed in females in the missed lesion group. One female in the missed lesion group had a lesion that was too caudal from the MPOA. Three females in the missed lesion group were mice with lesions intended for the BA; one was a unilateral central amygdala lesion, one was a small unilateral BA lesion, the other was a small unilateral caudate putamen lesion. Three females in the missed lesion group had lesions intended for the NA; one was a lesion in the anterior commisure, one was lesion in the caudate putamen, the other was a unilateral lesion around the anterior commisure. The sample sizes were too small to determine whether a specific location of the lesion disrupted licking in these males.

3.3.5. Litter weight is affected by lesions in parents

Litter weight increased over days, indicating that pups were being fed and gaining weight. However, litters in which the mother was given a lesion, independent of lesion site, weighed less than litters in which the father was given a lesion. Furthermore, litters in which one of the parents were given a MPOA or BA lesion weighed less than pups in which a parent was given a NA lesion. There was also a marginally significant trend for litters in the MPOA group to

weigh less than those in the sham lesion group. With a larger sample size, this trend would likely be statistically significant.

Decreases in litter weight have been reported in rats with lesions in the caudal periaqueductal gray, an area important for the upright nursing posture (Lonstein & Stern, 1998) and in the paraventricular nucleus of the hypothalamus, an area involved in the mild-ejection pathway (Olazabal et al., 1997). Decreases in litter weight has also been reported in rats and mice with septal – forebrain lesions (Flannelly et al., 1986; Slotnick & Nigrosh, 1975). To our knowledge, no one has investigated the effects of MPOA, BA, or NA lesions in females on litter weight, survival and development, and no one has investigated the effects of lesions in any brain site in males on litter weight, survival and development. If brain lesions affect parental behavior significantly, this should be shown in the developmental parameters of the pups.

Denenberg and colleagues (1976) reported decreases in litter weight in rat litters that were given to foster mothers at one-hr of age for 12 hr. All litters were raised by foster mothers, but some remained with their natural mothers for the first 12 hrs of life, while others were given to foster mothers at one-hr of age. Litters that were fostered at one-hr of age weighed less than litters fostered at 12-hrs of age throughout the weaning period, including 21 days of age. Behavioral observations during the second hr of life showed that fostered mothers, which were females that had nursed their own pups for 10-14 days, spent less time nursing, licked the pups less, and left the pups alone more than natural mothers. Thus, in rats, the quality of maternal behavior during the first 12

hrs of life can affect litter weight.

The effects of a variety of factors, such as presence of the father, cold temperatures, and conditions mimicking foraging conditions in the wild, on pup survival and development has been studied in *P. californicus*. In ideal laboratory conditions with ad lib food and water, warm ambient temperatures, and no predators, there is little effect of the presence of the male on the survival and development of offspring (Gubernick et al., 1993). Only in conditions where a single mom has to run in a running wheel for food and raise 4 pups, do pups fail to develop normally (Cantoni & Brown, 1997b).

All mice in the present study were housed as a family unit consisting of a male – female pair and three pups from Day 1 postpartum until the end of the experiment. Pups were raised and fed by foster parents on alternating days beginning on Day 1 until Day 13 postpartum. From Day 14 and onwards, pups were raised by their foster parents only. Thus, pups spent only 5 out of 30 days with their natural parents while the one parent had a lesion. Twenty-five out of the 30 days, litters were with two "normal" parents, with ad lib food and water under warm temperatures. Thus, the data suggest that a lack of "normal" parental care for even 5 days during the first 2 weeks of life can inhibit pup development. The differences in litter weight existed even at 30 days of age, the age at which pups are weaned in our laboratory, and after being with foster parents for 17 days. Having litters raised by parents in which one parent had a lesion in the MPOA and possibly in the BA for the entire 30 days would likely

have a profound effect on pup development. It is not known whether these pups would show any behavioural deficits as juveniles or as adults.

3.3.6. Low survival rates

Although the rate of mortality was lower in experiment 2 than in experiment 1, it was still too high. There was, however, considerable improvement in the second experiment, in that none of the mice died prior to being given a lesion. Furthermore, mice seemed to recover more quickly in experiment 2. Mice that were still sluggish on the first day(s) of parental testing after the lesion were inactive, often sleeping for most of the 8-min test and took longer to approach pups. Therefore, the latency to approach pups can be used as a crude measure of how well the mouse recovered from the lesion. As shown in Figures 12 and 13, with the exception of BA lesioned mice, there were no differences between groups in the latency to approach pups during the initial few days of parental behaviour testing.

In addition to using a different anaesthetic, there were several other things that were different between experiments 1 and 2. For example, anaesthetic was administered subcutaneously in the second experiment rather than intraperitoneally, as in the first experiment. Also, mice were allowed to acclimatize to the surgery room for several hours before they were anaesthetized and were kept warm as soon as they were anaesthetized. Therefore, it is not clear which of the factors played a role in reducing the mortality rate in the second experiment.

As stated previously in this thesis, the difficulty in anesthetizing *P. californicus* was likely due to their highly reactive nature. Since completing the two lesion experiments, I met Dr Mark Lewis at the International Behavioral Neuroscience Society conference in June 2003. Dr Lewis stated that his laboratory has observed similar reactions to ketamine and acepromazine (0.4 ketamine and 0.1 ml acepromazine in 2.8 ml saline, 0.015 ml/g body weight i.p.) in wild deer mice, *Peromyscus maniculatus*. Dr Lewis has also reported that they now use isofluourane with good success (personal communication via E-mail, May 19, 2003).

4. Experiment 3: Expression of *c-fos, fosB,* and *c-jun* during parental behaviour in California mice

Immediate early genes (IEG) code for the production of nuclear proteins that serve as transcription factors. These proteins can either activate or repress additional target genes, which causes long-term changes in cellular functioning (Morgan & Curran, 1989). The IEGs *c-fos* and *fosB*, which have protein products of the same name (c-Fos and FosB respectively), are members of the *fos* family, which also includes *fra 1* and *fra 2* (Sheng & Greenberg, 1990). *c-jun* is another IEG which encodes the protein c-Jun. The Fos and Jun proteins function as transcription factors and their production can be activated by a variety of extracellular signals (Hughes & Draganow, 1995; Morgan & Curran, 1991). Hence, they are known as inducible transcription factors (ITF).

c-Fos is the most studied of all the ITFs. Basal levels of Fos are normally low, but once activated, the Fos protein binds to DNA in behaviourally activated neurons (Sagar et al., 1988). c-Fos and FosB can dimerize with proteins of the Jun family, producing heterodimers that can bind to AP-1 sites in the promoter regions of various genes, and thereby affect transcriptional regulation (Morgan & Curran, 1991).

c-Fos can be detected within neurons by immunohistochemical techniques 20 – 90 minutes after neuronal excitation and disappears 4 – 16 h later (Morgan et al., 1987). Thus, the expression of c-fos-like immunoreactivity (ir)

can be used as a specific marker of neuronal activity, permitting the mapping of functional pathways in the brain.

Using c-Fos immunocytochemistry, several laboratories have shown the involvement of the MPOA, ventral BNST, amygdala, and NA in maternal behaviour in rats (Fleming et al., 1995; Fleming & Walsh, 1994; Numan & Numan, 1994; Numan & Numan, 1995; Walsh et al., 1996). The MPOA and the MA have also been implicated in paternal behaviour, as parental male prairie voles show increased *c-fos* expression in these brain areas following pup exposure (Kirkpatrick et al., 1994b).

FosB ICC has been used less frequently and c-Jun ICC has not been used at all to investigate the pattern of neural activation during parental behaviour in rodents. *fosB* expression is elevated in the MPOA and ventral BNST in maternally behaving rats (Lin et al., 1998; Numan et al., 1998). Furthermore, mice with a knockout mutation for the *fosB* gene lack maternal responsiveness (Brown et al., 1996).

The purpose of experiment 3 was to examine the expression of the protein products of the immediate early genes *c-fos, fosB,* and *c-jun* during parental behaviour in male and female California mice. We hypothesized that parental behaviour would activate c-Fos and FosB-ir in several brain sites implicated in maternal behaviour, including the MPOA, amygdala, NA, BNST, and lateral septum. There were no predictions on the pattern of *c-jun* activation, as this gene has never been studied in parental behaviour studies.

4.1. Method

4.1.1. Subjects

Eight multiparous male–female breeding pairs of *P. californicus* served as subjects. All mice were born at Dalhousie University from a stock originally captured in the Santa Monica Mountains, northeast of Los Angeles, and provided by Dr. C. Marler (University of Wisconsin, Madison, WI).

Pups were weaned at 30 days of age and each mouse was housed individually in standard plexiglas cages (45 X 22 X 15 cm) with wood shavings for bedding and wire tops. Rodent Laboratory Chow # 5001 (Agribrand Purina, St. Louis, MO) and tap water were provided ad libitum and cages were cleaned once a week. The colony room was kept at ~ 22 °C, on a 16:8 h light:dark cycle with the onset of lights at 11:00 h. Additional pairs of mice were used as donor parents who provided test pups.

4.1.2. Procedure

Pairs were allowed to give birth and raise at least one litter. Males were vasectomized on day 22-25 of the females' gestation period and allowed to recover alone in a clean cage for 2 days before being returned to their mates. California mice show postpartum estrous and mate on the day of or one day after parturition. Thus, males were vasectomized so that females would not become pregnant. Females were allowed to give birth (Day 0) and the pairs remained with the pups for one day. The next morning (Day 1), pups were removed and given to foster parents and the male-female pairs were individually housed in

clean cages in a room without pups. On Day 2 at 12:00 pm, males and females were individually given 1 h of experience with pups. Three 1-4 day old pups were presented to the female or male. Pups were placed in the quadrant of the cage diagonally opposite the nest area or sleeping corner. Behaviours were recorded at 5-s intervals for 15 min and spot-checks were made every 15 min until the end of the 1 h observation period. The spot checks recorded the position and behaviour of the female or male and the position of the three pups. At the end of the 1-h pup exposure, when female subjects were picked up to be anaesthetized, the number of pups suckling was recorded.

The following behaviours were recorded during the 15 min test: (a) approach; the mouse moves towards a pup(s), (b) withdrawal; the mouse moves away from a pup(s), (c) retrieve pup; the mouse picks up a pup and carries it to the nest site, (d) mouth pup; the mouse picks up a pup but does not carry it to the nest site, (e) lick pup; the mouse licks any region of the pup, except the anogenital area, (f) anogenital lick pup; the mouse licks the anogenital region of the pup, (g) crouch; the mouse adopts a nursing posture over the pups, (h) build nest; the mouse carries or moves nesting material towards the nest site. At the end of the 1 h experience with pups, the parents were sacrificed and their brains prepared for immunohistochemistry.

4.1.2.1. Vasectomies

Mice were anaesthetized with i.p. injections of Somnotol (65 mg/kg, diluted 1:4 in sterile saline) until a toe pinch failed to elicit a reflex reaction. The

ventral side of the mouse was shaved and a small incision below the ribcage was made. The vas deferens was ligated with surgical thread in two spots, about 5 mm apart and about 5 mm from the testes and cut between the ligated spots. The muscle layer and skin was then sutured.

4.1.2.2. Immunohistochemistry

After the 1-h parental behavioural test, mice were deeply anaesthetized with an overdose of Somnotol (0.03ml/10g body weight, i.p.) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Brains were removed from the skull and post-fixed in 4% PFA until they were ready to be cut, at which time they were cryoprotected with 30% sucrose for at least one day before sectioning. Brains were frozen and embedded in O.C.T. and cut coronally at 40 μm with a freezing cryostat microtome. Every fourth section (160 μm intervals) was selected beginning with the nucleus accumbens and ending with the amygdala.

Floating sections were rinsed three times in 0.2% Triton X-100 in 0.1 M phosphate buffer (PBS-Tx) and rinsed with 0.4% hydrogen peroxide in PBS-Tx for 20 min. After rinsing in PBS-Tx, sections were incubated with 3% goat serum in PBS-Tx for 1 h and then incubated with rabbit polyclonal antibodies against either c-Fos (Santa Cruz Biotechnology, Santa Cruz, CA, cat # 52) at 1:40,000 or FosB (Santa Cruz Biotechnology, cat # 7203) at 1:2000 diluted in 3% goat serum/PBS-Tx for 4-5 days at 4 °C. Sections were rinsed in PBS-Tx and incubated in biotinylated goat anti-rabbit secondary antibody (Vector

Laboratories, Burlington, Ontario) for 60-90 min at 1:500 diluted in 3% goat serum/PBS-Tx. Sections were rinsed with PBS-Tx and incubated in avidin-biotin solution for 60-90 min (Vectastain ABC kits, Vector Laboratories; 4 μl/ml PBS). Sections were rinsed with PBS-Tx and immunoreactivity was revealed using 0.02% 3.3'-diaminobenzidine (DAB), 0.6% nickel(II) ammonium sulphate and 0.006% H_2O_2 in Tris-HCL (pH 7.4). Each rinse consisted of three 10-min washes in PBS-Tx. Sections were rinsed with PBS-Tx and mounted onto gelatin-coated slides and air-dried overnight. Sections were then dehydrated and coverslipped. Immunohistochemistry was carried out in two batches for c-Fos and one batch for FosB. Equal numbers of males and females and those with and without pup exposure were run in the two c-Fos batches.

The protocol for c-Jun immunocytochemistry differed from the Fos protocol in the following ways. All rinses were done with 0.01 M PBS. Sections were rinsed in 0.3% H_2O_2 in PBS for 10 min. Sections were incubated in rabbit polyclonal antibody against c-Jun (Santa Cruz Biotechnology, cat # 1694) at 1:2000 for 2 days. Sections were incubated in biotinylated goat anti-rabbit serum at 1:200. Immunoreactivity was revealed using 0.02% DAB and 0.00035% H_2O_2 in 0.1 M PB. All sections were run in one batch.

4.1.2.3. Analysis of staining

A microscope – video camera – computer image analysis system (courtesy of Dr. Mike Wilkinson) was used for quantification. Images of the sections were taken using a Leaf Microlumina digital camera or a Q Imaging

Retiga 1300 digital camera mounted on a Leitz Laborlux-S microscope. Greyscale images were transferred to an Apple G4 computer. Grayscale images were adjusted using Adobe Photoshop 6.0 so that the black level was increased and the background was consistent across images. Images were stored as an uncompressed TIFF (Tag Image File Format) file. Immunopositive cell nuclei were identified by the medium gray-to-black reaction products in cell nuclei. The number of stained cells within each area was counted using NIH (National Institutes of Health) Image 1.60 (for Macintosh) or Scion Image 4.0.2 Beta (NIH Image for PC, Scion Corporation, Frederick, MD) software.

All cell counts were performed blind to group and anatomical criteria were specified for each brain region to ensure that the same locations were examined in all animals. Immunopositive cells were quantified in the MPOA, NAC, NAS, BA, MA, CA, BNST, lateral septum, and piriform and somatosensory cortices, brain sites that have been implicated in parental behaviour in rodents. With the exception of the MPOA and NAS, which are at the midline of the brain, all areas were counted in both the left and right hemispheres. Images of the MPOA and NAS were taken centered at the midline. Brain nuclei were identified according to the mouse brain atlas of Franklin and Paxinos (1997). The anterior-posterior coordinates at which the sites were counted were chosen based on the levels at which the greatest number of positive cells were identified by eye.

For the MPOA, three rostral-to-caudal sections, with the most rostral section beginning at the level of the crossing of the anterior commissure (Figure 30 – 32 of Franklin & Paxinos, 1997) were chosen. The camera's field of view

was positioned so that it was centered along the third ventricle in the medial-lateral (ML) axis and centered between the ventral aspect of the anterior commissure (ac) and the base of the brain (see Figures 30 A and B). Images of the MPOA consisted of the lateral preoptic area (LPA), MPOA, medial preoptic nucleus, central (MPOC), medial preoptic nucleus, lateral (MPOL), and medial preoptic nucleus, medial (MPOM). Because the MPOA and its surrounding regions were larger than the camera's field of view, levels of immunoreactivity were quantified in the entire image.

For the basolateral, medial, and central amygdala, two rostral-to-caudal sections were chosen corresponding to Figures 45 and 46 of Franklin and Paxinos (1997). The chosen sections were the sections where the optic tract still reaches the base of the brain. The amygdala nuclei were easily located and levels of immunoreactivity were counted within its boundaries. For the left BA, the left bottom corner of the field of view was aligned with the left ventral tip of the external capsule (see Figures 38 A and B). Images of the right BA were taken in the same manner. Images of the BA consisted mainly of the anterior basolateral amygdala (BLA) and posterior basomedial amygdala (BMP) and the entire area of the image was quantified for immunoreactivity. For the central amygdala, the camera's field of view was positioned so that it was centered over the central amygdala (see Figures 39 A and B). A circular area around the CA, consisting of the central amygdala, caps division (CeC) and central amygdala, lateral division (CeL), was selected in each image and this area was quantified for levels of immunoreactivity. For the left medial amygdala, the bottom right

corner of the field of view was positioned so that it aligned with the rounded corner of the left medial posteroventral medial amygdala (MePV; see Figures 31 A and B). Images of the right MA were taken in the same manner. The captured images of the MA consisted of the MePV and the posterodorsal medial amygdala (MePD), as well as some of the basolateral medial amygdala and anterior cortical amygdala (Aco). Levels of immunoreactivity were quantified only in the MePV and MePD.

For the core and shell of the NA, two rostral-to-caudal sections corresponding to Figures 22 and 23 of Franklin and Paxinos (1997), which were the two sections where the major Island of Calleja was the longest, were chosen. For the core of the NA, the field of view was positioned so that it was centered over the ac (see Figures 32 A and B). An elliptical area was selected around the core of the NA and levels of immunoreactivity were quantified in this area. For the shell of the NA, the field of view was centered at the midline and positioned so that the bottom of the field of view was aligned with the most ventral part of the brain section (see Figures 33 A and B). First, the levels of immunoreactivity were quantified in the total area of the image. The level of immunoreactivity in a triangular area, which consisted of the ventral diagonal band (VDB) was then subtracted from the total count.

For the BNST, two rostral-to-caudal sections corresponding to Figures 29 and 30 of Franklin and Paxinos (1997), at which the crossing of the anterior commissure occurs, were chosen. The field of view was positioned so that as much of the BNST was shown as possible, and centered right below the lateral

ventricle (see Figures 34 A and B). A diamond shaped area that was centered over the lateral ventricle in the ML axis and centered over the posterior anterior commissure (acp) in the dorsal - ventral (DV) axis was quantified for immunoreactivity. This diamond shaped area consisted of the BNST - lateral division, dorsal (BSTLD), BNST - lateral division, juxtacapsular (BSTLJ), BNST - lateral division, posterior (BSTLP), BNST - lateral division, ventral (BSTLV), BNST - medial division, anterior (BSTMA), and BNST - medial division, (BSTMV).

For the lateral septum, two rostral-to-caudal sections, corresponding to Figures 26 and 27 of Franklin and Paxinos (1997) were chosen. The medial septal nucleus is in the shape of an arrow pointing upwards in Figure 26 and is the shape of a diamond in Figure 27. The field of view was positioned so that the ventral base of the view was centered over the ac in the DV plane and centered over the lateral ventricle n the ML plane (see Figures 35 A and B). Images consisted of the anterior ac, BNST, and ventral division of the lateral septal nucleus (LSV). Only the LSV was quantified for immunoreactivity.

For the piriform and somatosensory cortices, two rostral-to-caudal sections, corresponding to Figures 30 and 31 of Franklin and Paxinos (1997) were chosen. The crossing of the ac occurs in these two sections. For the piriform cortex, the field of view was centered over the part of the piriform cortex that curves in towards the brain (see Figures 36 A and B). The piriform was easily located and quantified for immunoreactivity. For the left somatosensory cortex, the top of the field of view was positioned so that it was aligned with the

top of the left cingulum and then moved to the left until it reached the most lateral part of the brain (see Figures 37 A and B). Images of the right somatosensory cortex were taken in the same manner. The entire captured images, consisting of the somatosensory 1, barrel field (S1BF) and secondary somatosensory cortex (S2) were quantified for immunoreactivity.

The MPOA, BA, and CA were examined at 100 x magnification (10x objective). The MA, BNST, lateral septum, core and shell of the NA, and the piriform and somatosensory cortices were examined at 63 x magnification (6.3x objective).

4.1.3. Statistical analysis

c-Fos immunocytochemistry was conducted first with four mice per group. When c-Fos immunoreactivity was quantified, we surprisingly did not find a statistically significant difference between mice exposed to pups and those not exposed to pups in the MPOA, BA, NAC, and NAS. Thus, we decided to look at the two other IEGs, *fosB* and *c-jun*. FosB and c-Jun immunohistochemistry were performed on the same tissue 3 months after c-Fos was examined. However, brains of three mice had dried up and were discarded. Thus, the sample size of males with pup experience remained at four and the other three groups had sample sizes of three per group.

Results for the cell counts are presented as the mean (± SEM) number of c-Fos, FosB, or c-Jun positive cells observed per section for each brain site. For all sites, data were analyzed with a two-way analysis of variance (ANOVA)

comparing pup-experience and sex. Significant effects were followed up with planned t tests. Levels of significance were set at $p \leq 0.05$. All statistical analyses were conducted with the Statistical Package for Social Sciences (SPSS), version 6.1.

4.2. Results

4.2.1. Parental behaviour in home cage

The mean percent of time spent by males and females in parental behaviour during the first 15 min of the 1-h pup exposure is shown in Table 5. During the first 15 min of the 1-h experience with pups, male and female California mice spent almost all of their time near pups and more than 75% of their time in a nursing posture. The 15-min interval spot-checks showed that mice continued to be parentally responsive throughout the 1-h experience. Every mouse was observed in a nursing posture in each of the three spot-checks of the remaining 45 min. By the end of the 1-h pup exposure, 3 of the 4 female subjects had all three pups suckling. One female had 2 of 3 pups suckling. Thus, all mice showed high levels of parental behaviour during the 1-h experience with pups on day 2 postpartum.

4.2.3. *c-fos* expression

Table 6 shows the mean (± SEM) number of c-Fos-immunoreactive cells per section. Males and females with pup exposure had higher levels of c-Fos than those without pup exposure, but this difference was not statistically

Table 5

Mean Percent of Time (± *SEM*) Showing Each of Four Parental Behaviours During the First 15 min of the 1-h Experience with Pups in Female and Male *P. californicus*

Behaviours	Female	Male
Near Pup	96.25 (3.20)	99.31 (0.42)
Sniff Pup	10.56 (1.38)	27.08 (9.50)
Lick Pup	41.11 (7.37)	26.53 (2.92)
Crouch	76.53 (3.74)	76.81 (3.78)
Total Parental	96.67 (1.59)	96.39 (1.66)

Table 6

Mean (± SEM) number of c-Fos-immunoreactive cells per section

	Female		Male	
Brain Region	Pups	No Pups	Pups	No Pups
MPOA	289 ± 70^{+}	201 ± 20	236 ± 30^{+}	149 ± 33
BA	77 ± 17	78 ± 13	83 ± 7	43 ± 4
CA	111 ± 20	98 ± 25	111 ± 7	81 ± 7
MA	234 ± 57*	196 ± 24	209 ± 25*	96 ± 40
NAC	113 ± 21$^{\#}$	167 ± 3$^{\#}$	100 ± 22	82 ± 4
NAS	259 ± 55*$^{\#}$	306 ± 47$^{\#}$	228 ± 40	145 ± 21
BNST	267 ± 65$^{\#}$	337 ± 13$^{\#}$	206 ± 32	195 ± 38
LS	89 ± 23	120 ± 14$^{\#}$	122 ± 14*	64 ± 15
Piriform	279 ± 90*	144 ± 33$^{\#}$	262 ± 47*	106 ± 57
Somatosen	181 ± 65*	85 ± 33	208 ± 64*	53 ± 20

Abbreviations: BA = basolateral amygdala; BNST = bed nucleus of the stria terminalis; CA = central amygdala; LS = lateral septum; MA = medial amygdala; MPOA = medial preoptic area; NAC = nucleus accumbens core; NAS = nucleus accumbens shell; Somatosen = somatosensory cortex.

n = 4 per group

* $p < .05$, significantly different from no pups group.

$^{+}$ $p < .10$, marginally significant from no pups group.

$^{\#}$ $p < .05$, significantly different from males.

significant ($F_{1,12}$ = 5.19, p = .06; Figure 30). Males and females did not differ in the level of c-Fos in the MPOA, nor was there a sex by group interaction.

In the BA and CA, there were no significant differences between mice exposed to pups and those with no pup exposure, no difference between males and females, and no pup exposure by sex interaction on the expression of *c-fos.* In the MA, c-Fos levels in the pup exposed groups were significantly elevated above the no pup groups ($F_{1,12}$ = 7.65, p < .02; Figure 31). There were no differences between males and females, nor a sex by group interaction of the expression of *c-fos* in the MA.

In the NAC and NAS, there were no differences between pup exposed and no pup groups on the expression of *c-fos.* There was, however, a significant effect of sex, as females had higher levels of c-Fos than males in both the core ($F_{1,12}$ = 10.21, p = .008; Figure 32) and shell ($F_{1,12}$ = 5.06, p < .05; Figure 33) of the NA. There was also a significant group by sex interaction on the expression of *c-fos* in the NAC ($F_{1,12}$ = 5.12, p < .04), as females, but not males, in the no pup group had more *c-fos* expression than females with pup exposure (t_4 = 2.56, p < .05).

In the BNST, there were no differences between the pup and no pup groups, or a pup exposure by sex interaction on the expression of *c-fos.* Females showed significantly higher c-Fos levels than males in BNST ($F_{1,12}$ = 5.93, p < .04; Figure 34).

In the lateral septum, there were no significant main effects of pup exposure or sex on the expression of *c-fos.* There was a significant pup

Figure 30. Distribution of c-Fos labeled cells in the medial preoptic area (MPOA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying c-Fos immunoreactivity in the MPOA. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the MPOA. Note there were no significant differences in c-Fos immunoreactivity between groups. Scale bar is 100 µm. Abbreviations: f = female; LPO = lateral preoptic area; m = male; MPA = medial preoptic area; MPOC = medial preoptic nucleus, central; MPOL = medial preoptic nucleus, lateral; MPOM = medial preoptic nucleus, medial. 100 x magnification.

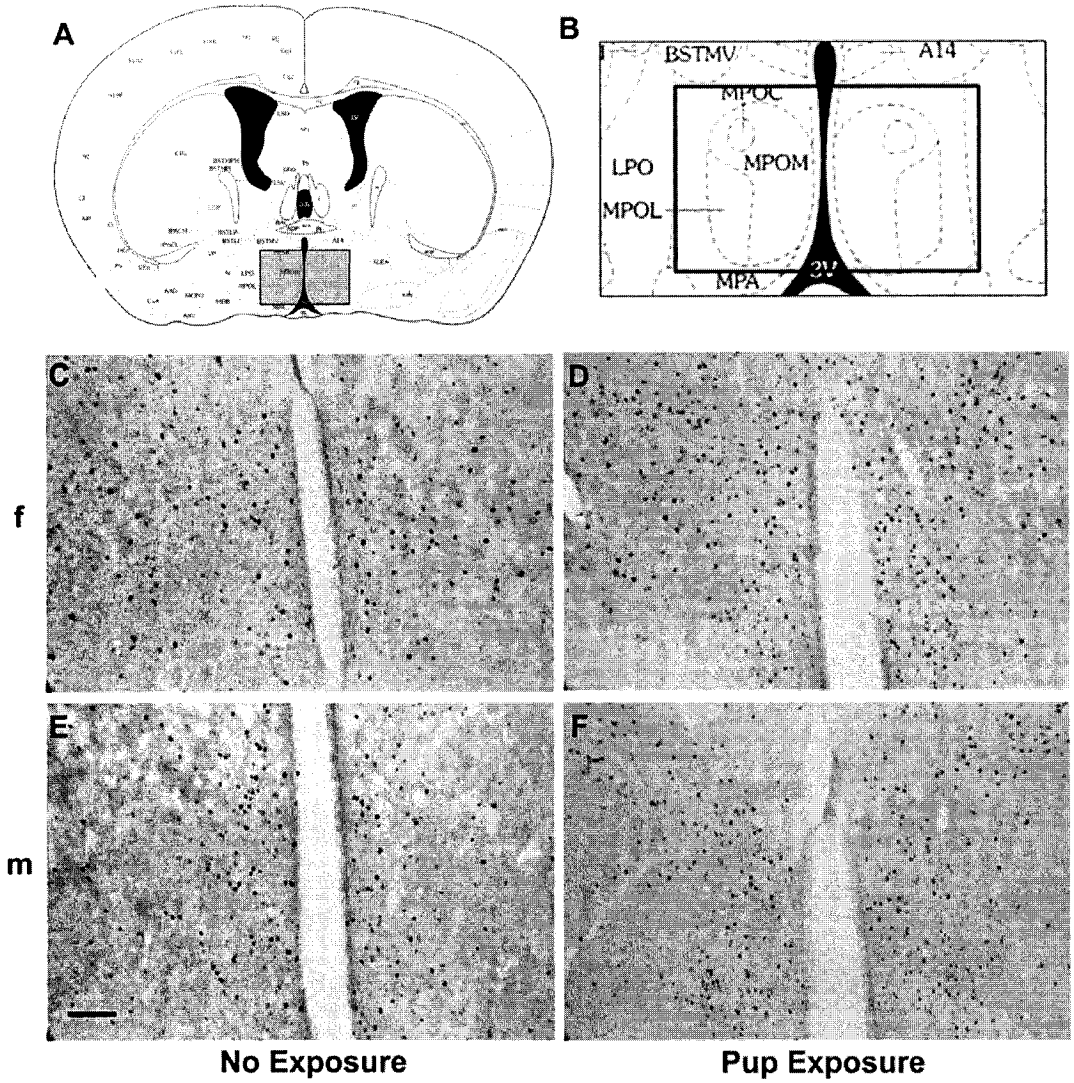

A
B
BSTMV
A14
MPOC
LPO
MPOM
MPOL
MPA
3V
C
D
f
E
F
m
No Exposure
Pup Exposure

Figure 30.

Figure 31. Distribution of c-Fos labeled cells in the medial amygdala (MA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying c-Fos immunoreactivity in the MA. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the MA. c-Fos immunoreactivity was quantified only in the MePD and MePV. Scale bar is 200 μm. Abbreviations: BLA = basolateral amygdaloid nucleus, anterior; BLV = basolateral amygdaloid nucleus, lateral; BMA = basolateral amygdaloid nucleus, anterior; BMP = basolateral amygdaloid nucleus, posterior; BSTIA = bed nucleus of the stria terminalis, intraamygdala; CeC = central amygdaloid nucleus, caps division; f = female; LaVL =lateral amygdaloid nucleus, ventrolateral; m = male; MePD = medial amygdaloid nucleus, posterodorsal; MePV = medial amygdaloid nucleus, posteroventral. 63 x magnification.

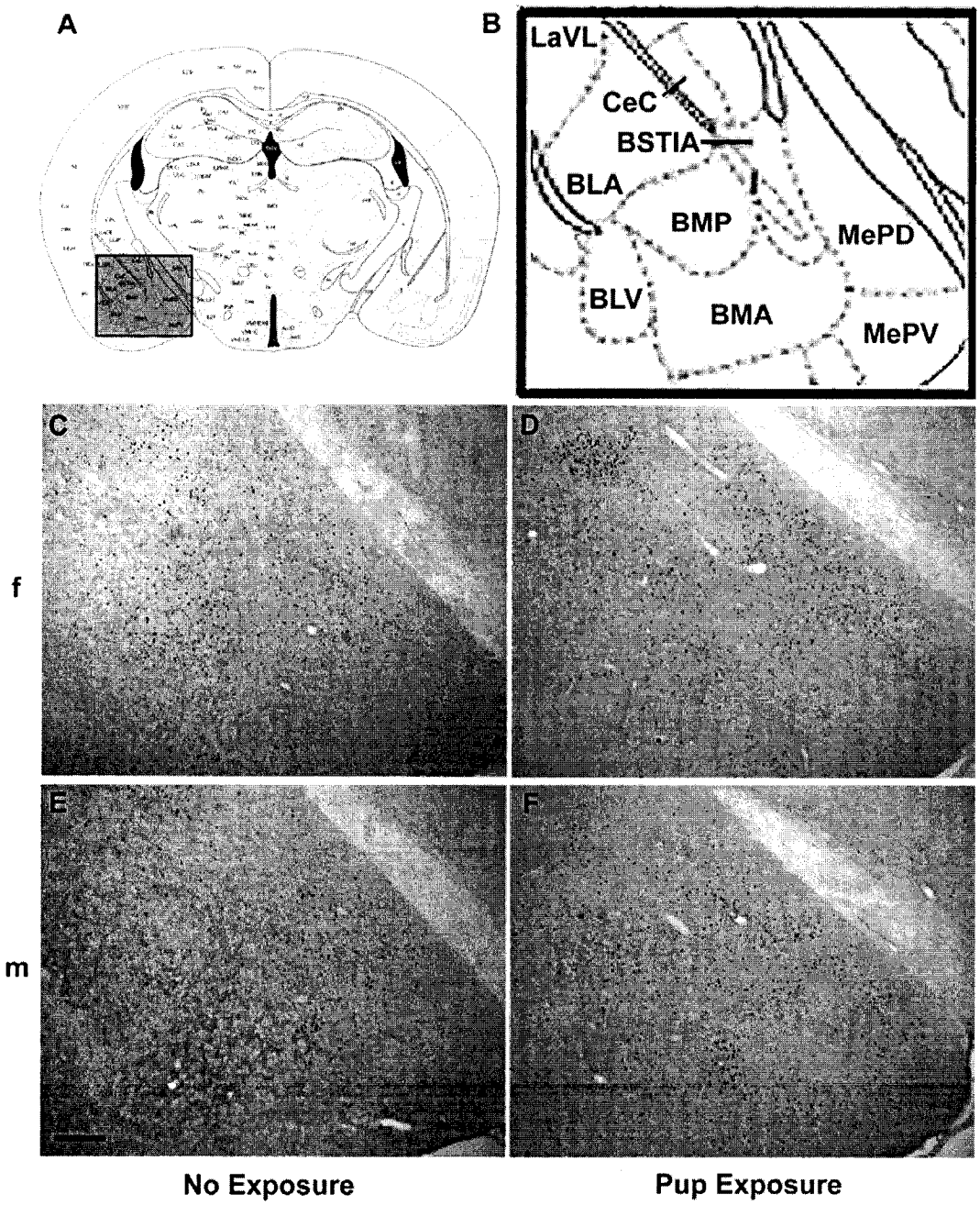

A
B
LaVL
CeC
BSTIA
BLA
BMP
MePD
BLV
BMA
MePV
C
D
f
E
F
m
No Exposure
Pup Exposure

Figure 31.

Figure 32. Distribution of c-Fos labeled cells in the core of the nucleus accumbens (NAC) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin and Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Fos immunoreactivity in the NAC. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the NAC. c-Fos immunoreactivity was quantified only in the NAC. Scale bar is 200 μm. Abbreviations: aca = anterior commissure, anterior; f = female; m = male; NAS = nucleus accumbens shell. 63 x magnification.

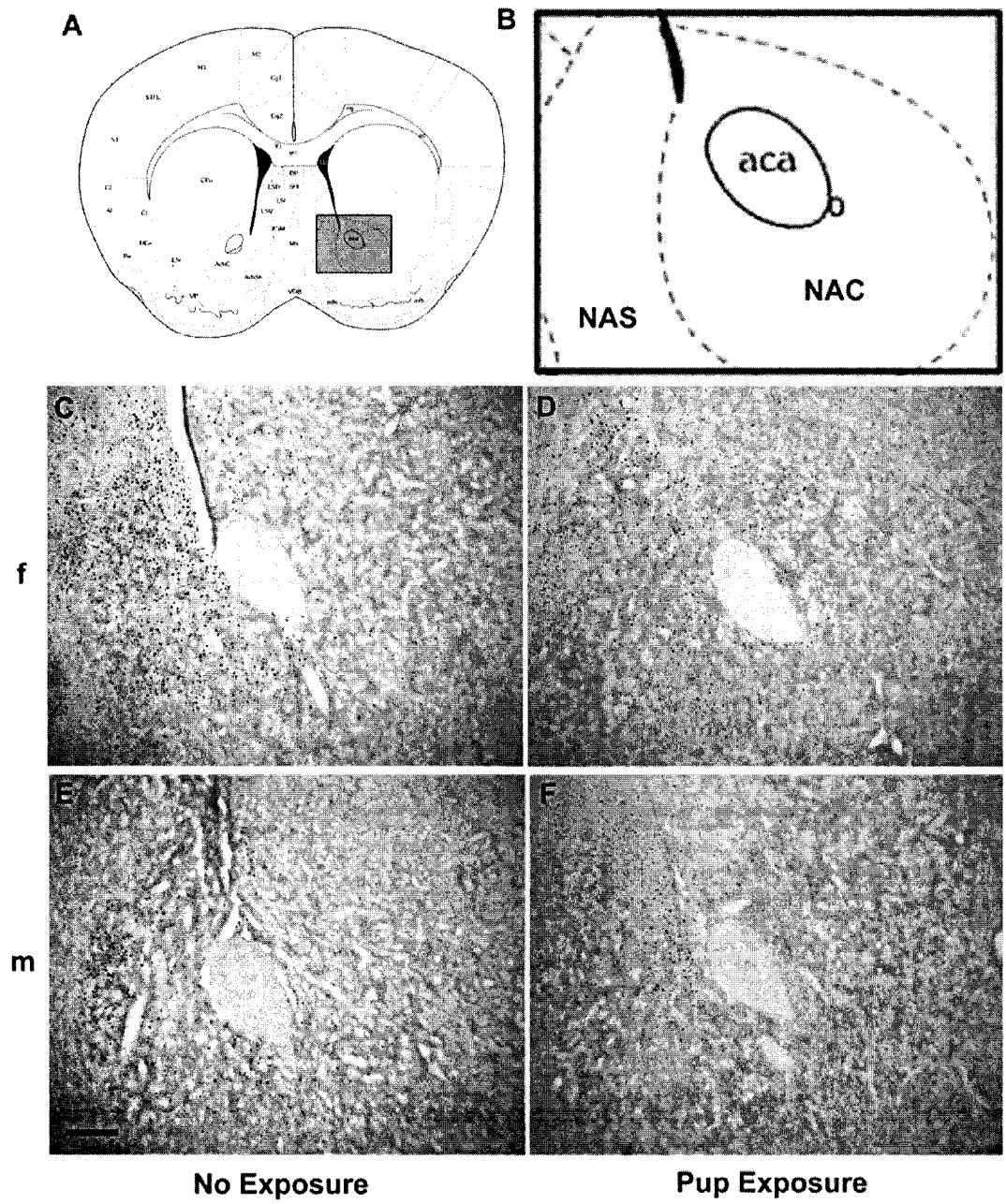


Figure 32.

Figure 33. Distribution of c-Fos labeled cells in the shell of the nucleus accumbens (NAS) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying c-Fos immunoreactivity in the NAS. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the NAS. c-Fos immunoreactivity was quantified only in the NAS. Scale bar is 200 μm. Abbreviations: mfb = medial forebrain bundle; f = female; m = male; VDB = ventral diagonal band nucleus. 63 x magnification.

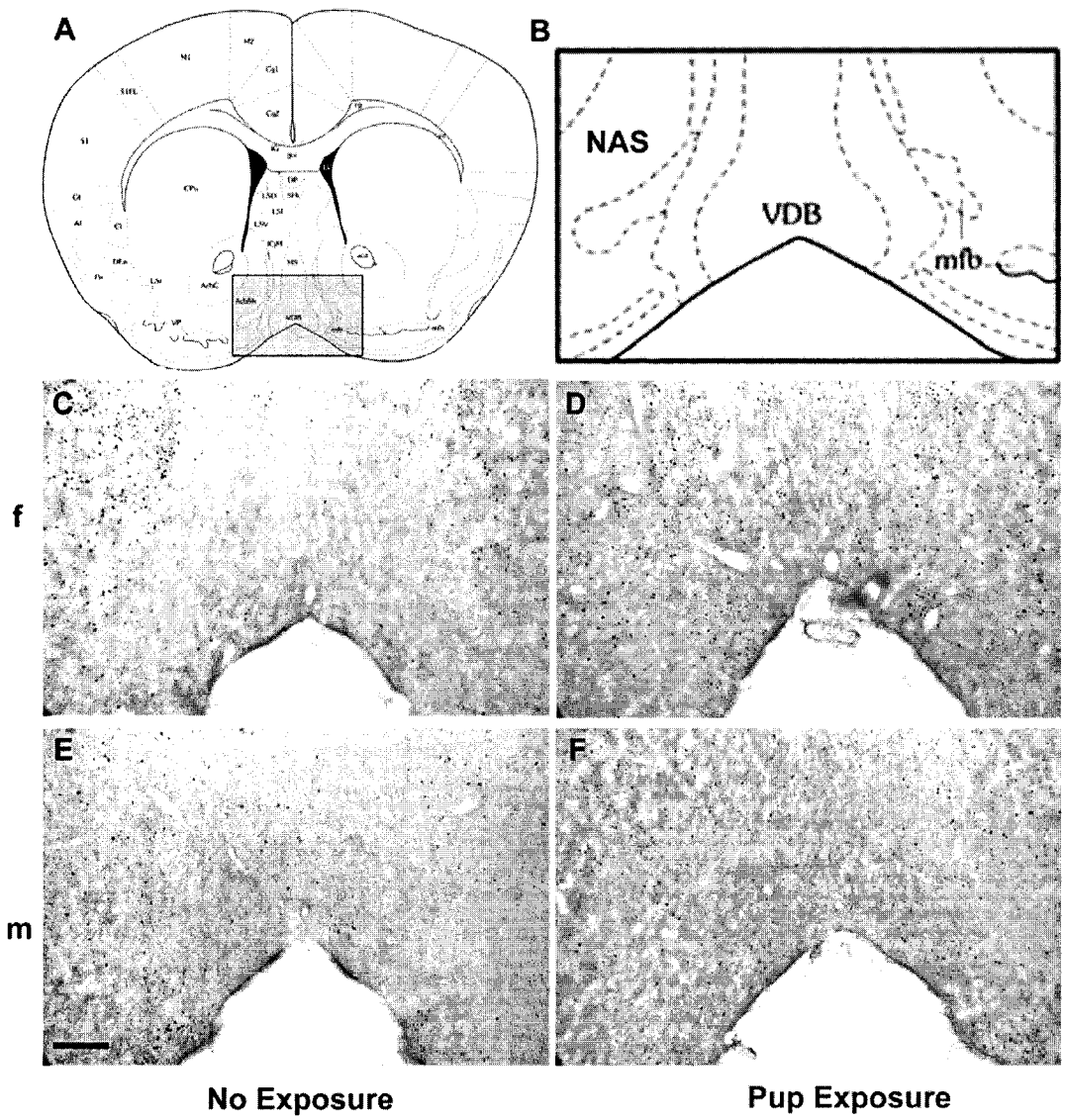


Figure 33.

Figure 34. Distribution of c-Fos labeled cells in the core of the bed nucleus of the stria terminalis (BNST) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Fos immunoreactivity in the BNST. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the BNST. c-Fos immunoreactivity was quantified in all BNST divisions. Scale bar is 200 μm. Abbreviations: acp = anterior commissure, posterior; BSTLD = bed nucleus of the stria terminalis, lateral division, dorsal; BSTLJ = bed nucleus of the stria terminalis, lateral division, juxtacapsular; BSTLP = bed nucleus of the stria terminalis, lateral division, posterior; BSTLV = bed nucleus of the stria terminalis, lateral division, ventral; BSTMA = bed nucleus of the stria terminalis, medial division, anterior; BSTMV = bed nucleus of the stria terminalis, medial division, ventral; f = female; m = male. 63 x magnification.

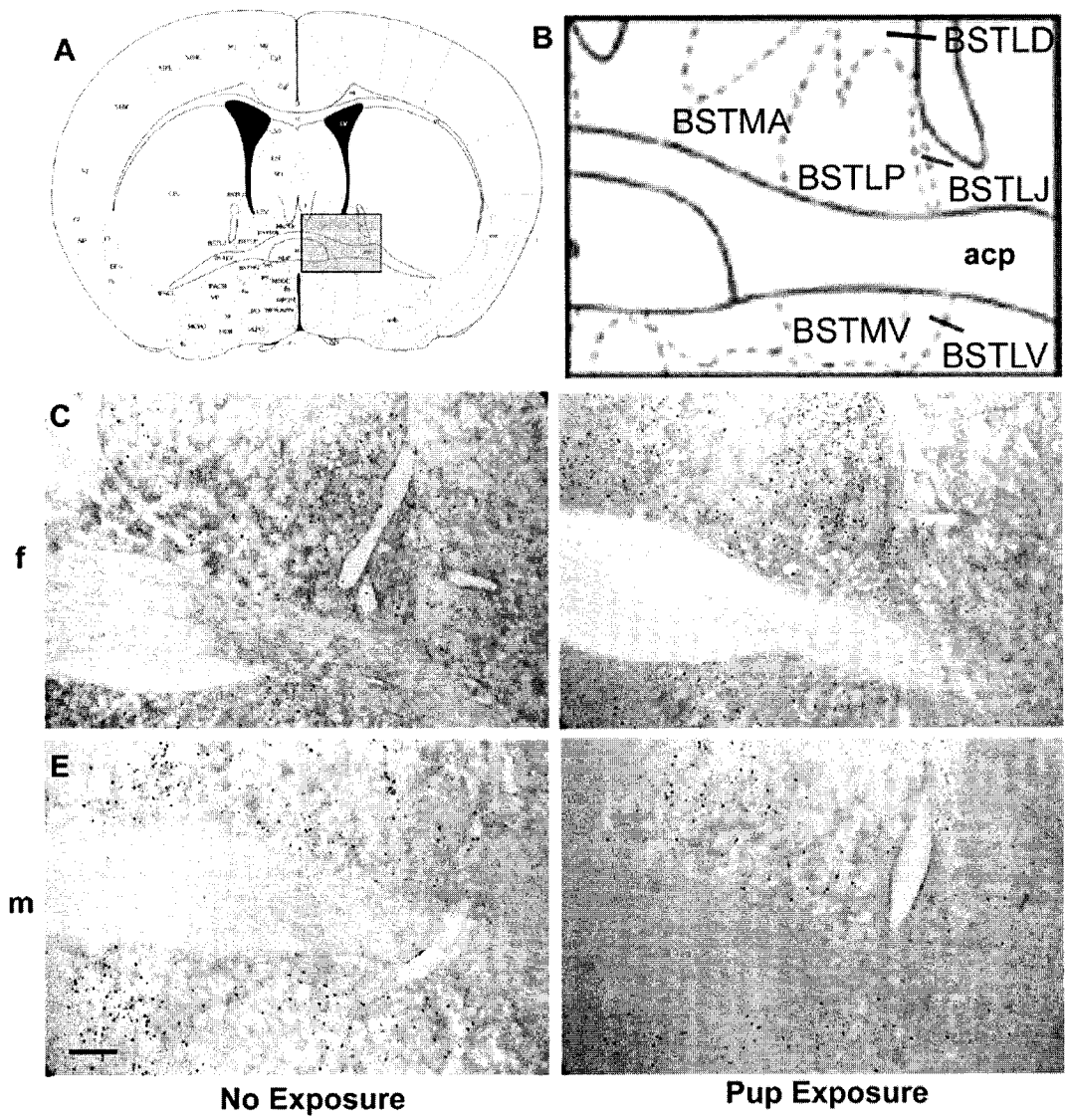

A
B
BSTLD
BSTMA
BSTLP
BSTLJ
acp
BSTMV
BSTLV
C
f
E
m
No Exposure
Pup Exposure

Figure 34

exposure by sex interaction ($F_{1,12}$ = 7.20, p = .02; Figure 35), as males, but not females with pup exposure had more c-Fos in the lateral septum than males without pup exposure (t_6 = 2.88, p < .03). Also, within the no pups groups, females had higher c-Fos levels in the lateral septum than males (t_6 = 2.75, p < .04). There were no differences in *c-fos* expression between males and females within the pup exposure groups.

In the piriform and somatosensory cortices, males and females with pup exposure showed significantly more c-Fos immunoreactivity than did those without pup exposure ($F_{1,12}$ = 5.72, p < .04; Figure 36 and $F_{1,12}$ = 6.45, p < .03; Figure 37, respectively). There was no main effect of sex, or pup exposure by sex interaction on the expression of *c-fos* in the piriform or somatosensory cortices.

4.2.4. *fosB* expression

Table 7 shows the mean (± SEM) number of FosB-immunoreactive cells per section. In the MPOA, there was no significant main effect of pup exposure or sex, and no pup exposure by sex interaction on FosB immunoreactivity.

In the BA, there was no significant main effect of pup exposure or pup exposure by sex interaction on the expression of *fosB*. Females showed significantly higher levels of FosB than males ($F_{1,9}$ = 17.14, p = .003; Figure 38).

In the CA, there were no main effects of pup exposure or sex on the expression of *fosB*. There was, however, a significant pup exposure by sex interaction in the CA ($F_{1,9}$ = 8.93, p < .02), as males, but not females, with pup

Figure 35. Distribution of c-Fos labeled cells in the lateral septum (LS) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Fos immunoreactivity in the LS. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the LS. c-Fos immunoreactivity was quantified in the ventral division of the LS (LSV). Scale bar is 200 μm. Abbreviations: aca = anterior commissure, anterior; BST = bed nucleus of the stria terminalis; CPu = caudate putamen; f = female; LSI = lateral septal nucleus, intermediate; LSV = lateral septal nucleus, ventral; m = male; MS = medial septal nucleus. 63 x magnification.

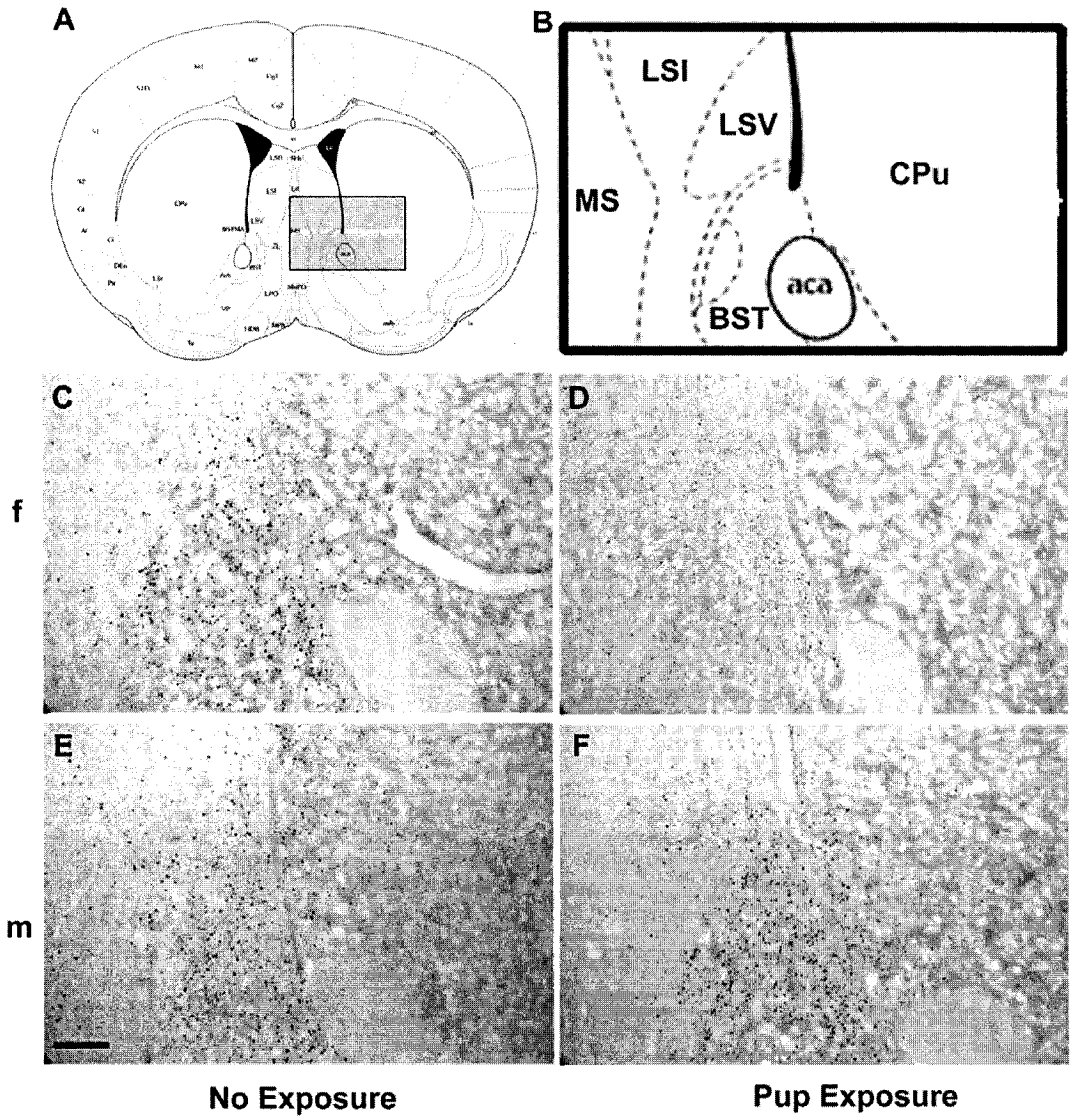


Figure 35

Figure 36. Distribution of c- Fos labeled cells in the piriform cortex of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying c-Fos immunoreactivity in the piriform. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the piriform. Scale bar is 200 μm. Abbreviations: f = female; m = male; pir = piriform cortex. 63 x magnification.

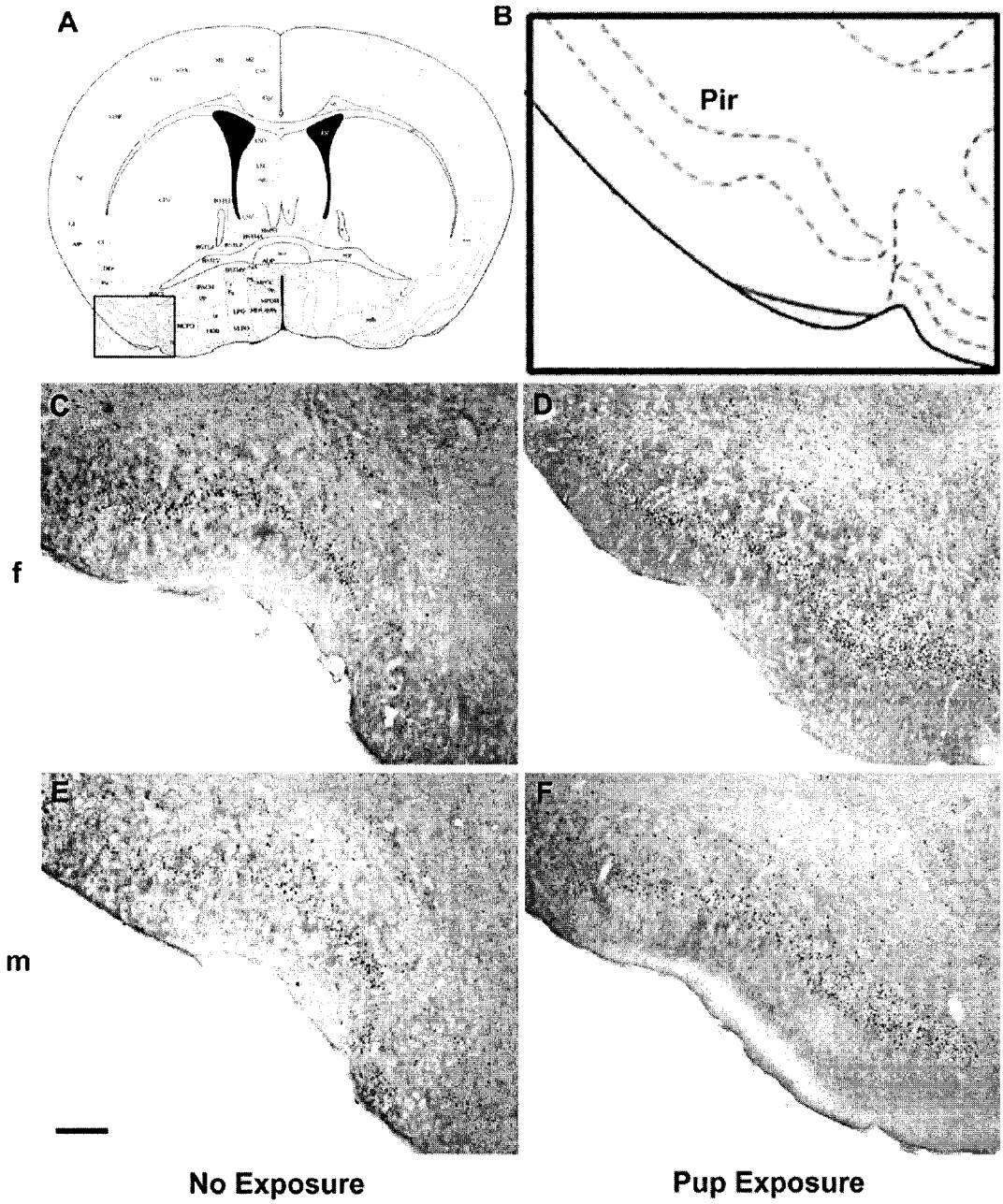


Figure 36.

Figure 37. Distribution of c- Fos labeled cells in the somatosensory cortex of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying c-Fos immunoreactivity in the somatosensory cortex. Panels C – F are representative photomicrographs illustrating c-Fos immunoreactivity in the somatosensory cortex. Scale bar is 200 μm. Abbreviations: f = female; m = male; S1BF = somatosensory 1, barrel field; S2 = secondary somatosensory cortex. 63 x magnification.

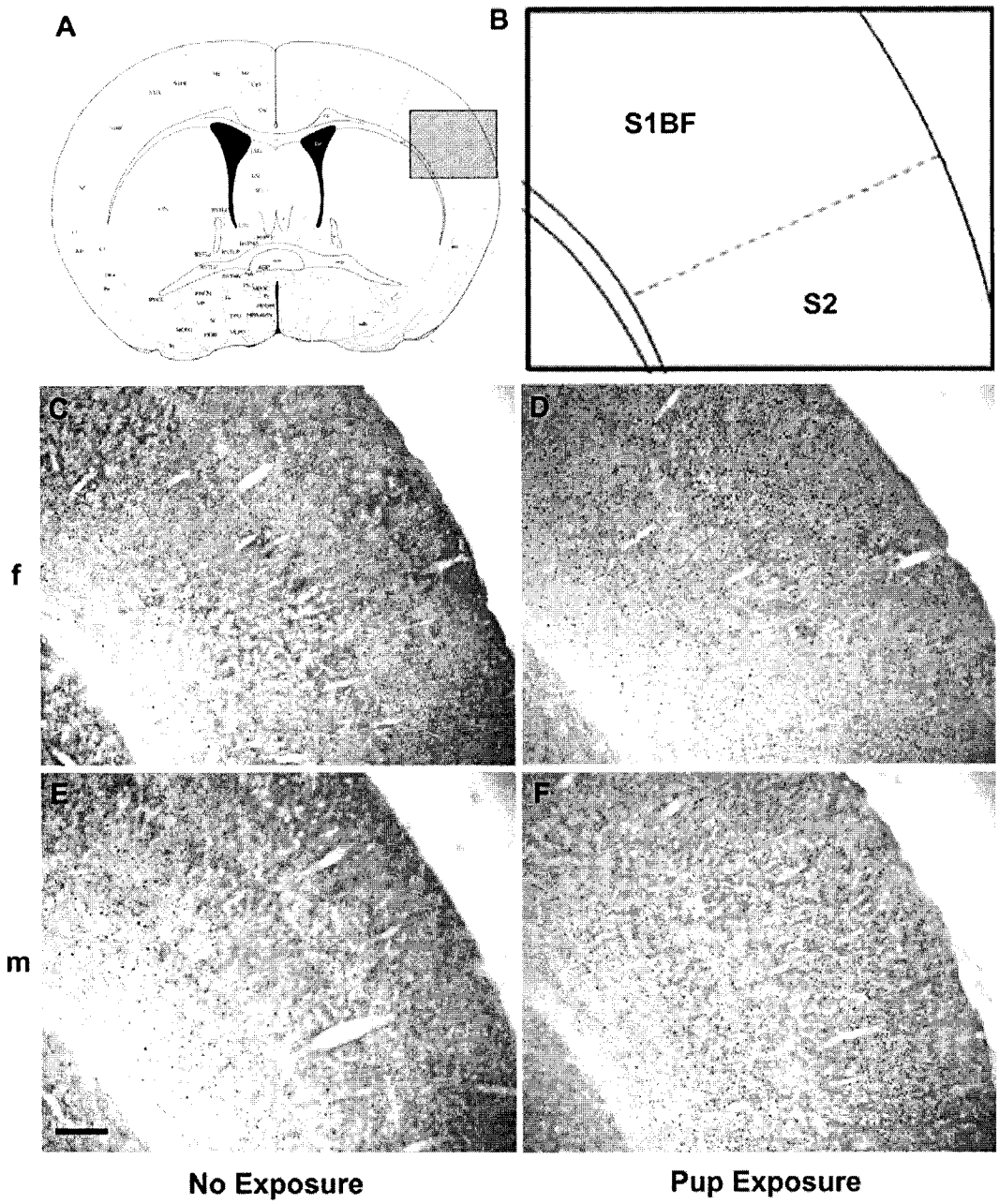


Figure 37.

Table 7

Mean (± SEM) number of FosB-immunoreactive cells per section

	Female		Male	
Brain Region	Pups	No Pups	Pups	No Pups
MPOA	52 ± 3	64 ± 9	40 ± 8	48 ± 13
BA	260 ± 15*#	186 ± 22#	144 ± 16	148 ± 20
CA	5 ± 1	11 ± 4	16 ± 2*	6 ± 3
MA	128 ± 17#	139 ± 19#	83 ± 14	64 ± 7
NAC	268 ± 27+#	306 ± 51#	126 ± 9+	215 ± 37
NAS	209 ± 34*#	101 ± 12#	77 ± 6	72 ± 39
BNST	195 ± 24*#	109 ± 9#	126 ± 3*	67 ± 21
LS	136 ± 22	104 ± 8	90 ± 29	68 ± 26
Piriform	78 ± 7+#	122 ± 33#	36 ± 2+	53 ± 3
Somatosen	67 ± 6	79 ± 21	51 ± 4	65 ± 18

Abbreviations: BA = basolateral amygdala; BNST = bed nucleus of the stria terminalis; CA = central amygdala; LS = lateral septum; MA = medial amygdala; MPOA = medial preoptic area; NAC = nucleus accumbens core; NAS = nucleus accumbens shell; Somatosen = somatosensory cortex.

n = 4 for males with pups group; n = 3 for all other groups

* $p < .05$, significantly different from no pups group.

+ $p < .10$, marginally significant from no pups group.

# $p < .05$, significantly different from males.

Figure 38. Distribution of FosB labeled cells in the basolateral amygdala (BA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying FosB immunoreactivity in the BA. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the BA. Scale bar is 100 µm. Abbreviations: BLA = basolateral amygdaloid nucleus, anterior; BLV = basolateral amygdaloid nucleus, lateral; BMA = basomedial amygdaloid nucleus, anterior; BMP = basomedial amygdaloid nucleus, posterior; f = female; LaVL =lateral amygdaloid nucleus, ventrolateral; m = male. 100 x magnification.

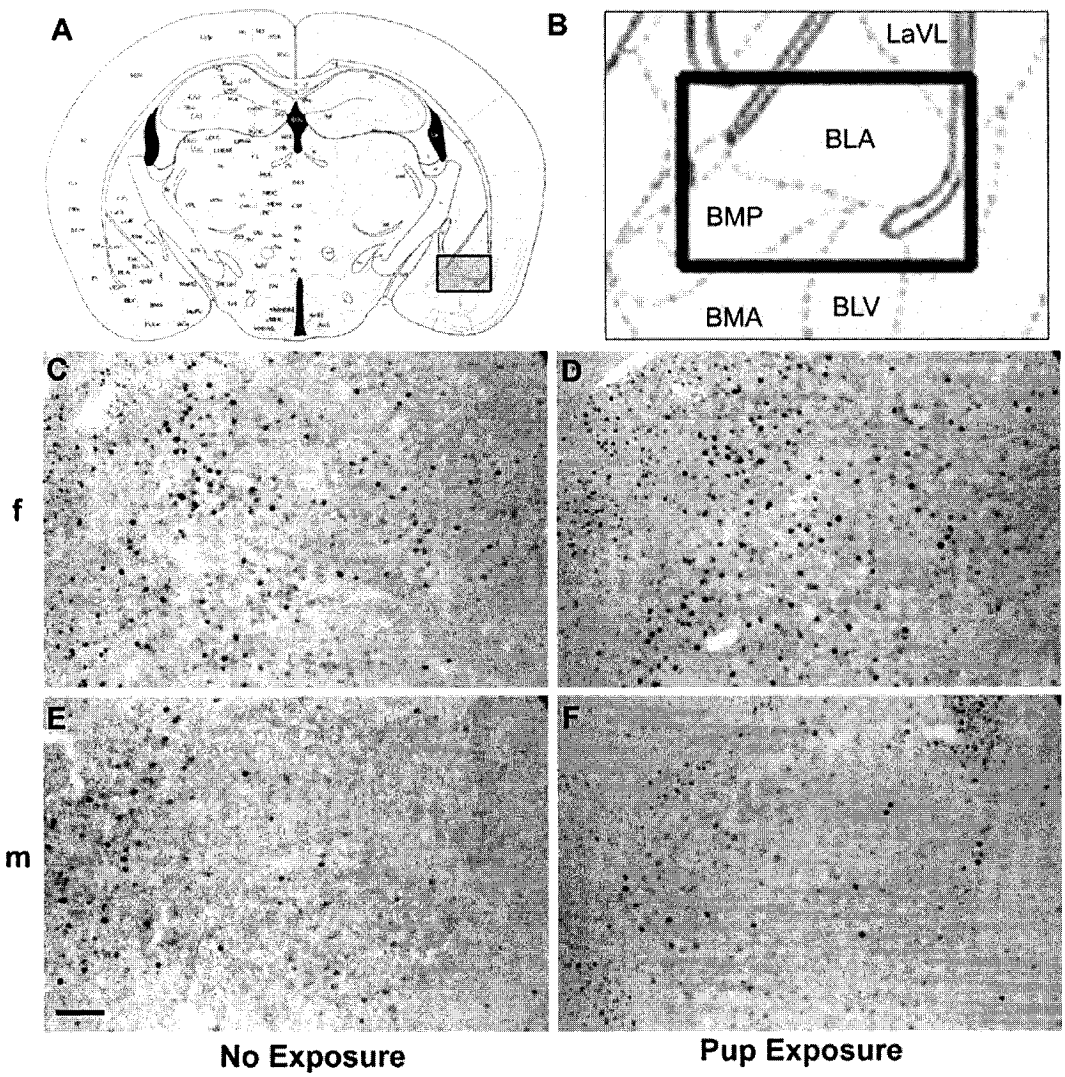


Figure 38.

exposure showed elevated FosB levels over those with no pup exposure (t_5 = 3.07, $p < .05$; Figure 39).

In the MA, there was no main effect of pup exposure and no pup exposure by sex interaction on the expression of *fosB*. Females showed higher FosB levels than males ($F_{1,9}$ = 16.02, p = .003; Figure 40) in the MA.

In the NAC, females had higher FosB levels than males in the NAC ($F_{1,9}$ = 13.30, p = .005; Figure 41). There was no effect of pup exposure or pup exposure by sex interaction on FosB immunoreactivity in the NAC.

In the NAS, males and females with pups exposure had higher levels of FosB than those without pup exposure ($F_{1,9}$ = 5.33, $p < .05$; Figure 42) and females showed higher *fosB* expression than males ($F_{1,9}$ = 10.93, $p < .01$). There was no pup exposure by sex interaction on FosB immunoreactivity in the NAS.

In the BNST, mice with pup exposure showed higher FosB levels than those without pup exposure ($F_{1,9}$ = 22.82, p = .001; Figure 43) and females showed elevated FosB levels over males ($F_{1,9}$ = 13.46, p = .005). There was no significant pup exposure by sex interaction on the expression of *fosB* in the BNST.

In the lateral septum, there were no significant main effects of pup exposure or sex and no pup exposure by sex interaction on the expression of *fosB*.

In the piriform cortex, females showed significantly higher *fosB* expression than males ($F_{1,9}$ = 13.16, p = .006; Figure 44). There was no effect of pup exposure and no pup exposure by sex interaction on *fosB* expression in the

Figure 39. Distribution of FosB labeled cells in the central amygdala (CA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying FosB immunoreactivity in the CA. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the CA. FosB immunoreactivity was quantified in the CeC and CeL only. Scale bar is 100 µm. Abbreviations: AStr = amygdalostriatal transition area; BLA = basolateral amygdaloid nucleus, anterior; BSTIA = bed nucleus of the stria terminalis, intraamygdala; CeC = central amygdaloid nucleus, capsular division; CeL = central amygdaloid nucleus, lateral division; f = female; ic = internal capsule; m = male; opt = optic tract; st = stria terminalis. 100 x magnification.

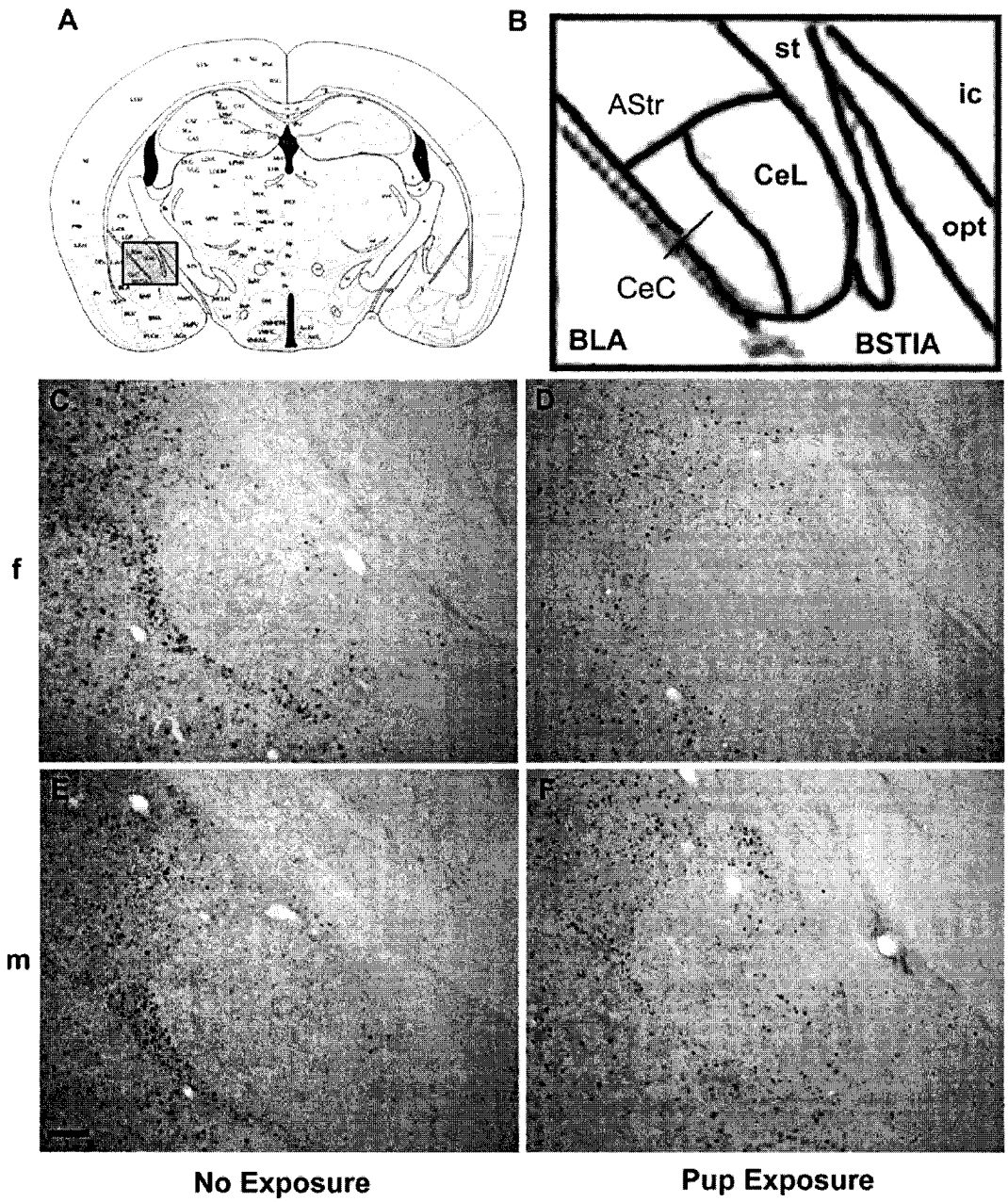

A
B
st
AStr
ic
CeL
opt
CeC
BLA
BSTIA
C
D
f
E
F
m
No Exposure
Pup Exposure

Figure 39.

Figure 40. Distribution of FosB labeled cells in the medial amygdala (MA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying FosB immunoreactivity in the MA. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the MA. FosB immunoreactivity was quantified in the MePD and MePV only. Scale bar is 200 μm. Abbreviations: BLA = basolateral amygdaloid nucleus, anterior; BLV = basolateral amygdaloid nucleus, lateral; BMA = basomedial amygdaloid nucleus, anterior; BMP = basomedial amygdaloid nucleus, posterior; BSTIA = bed nucleus of the stria terminalis, intraamygdala; CeC = central amygdaloid nucleus, caps division; f = female; LaVL =lateral amygdaloid nucleus, ventrolateral; m = male; MePD = medial amygdaloid nucleus, posterodorsal; MePV = medial amygdaloid nucleus, posteroventral. 63 x magnification.

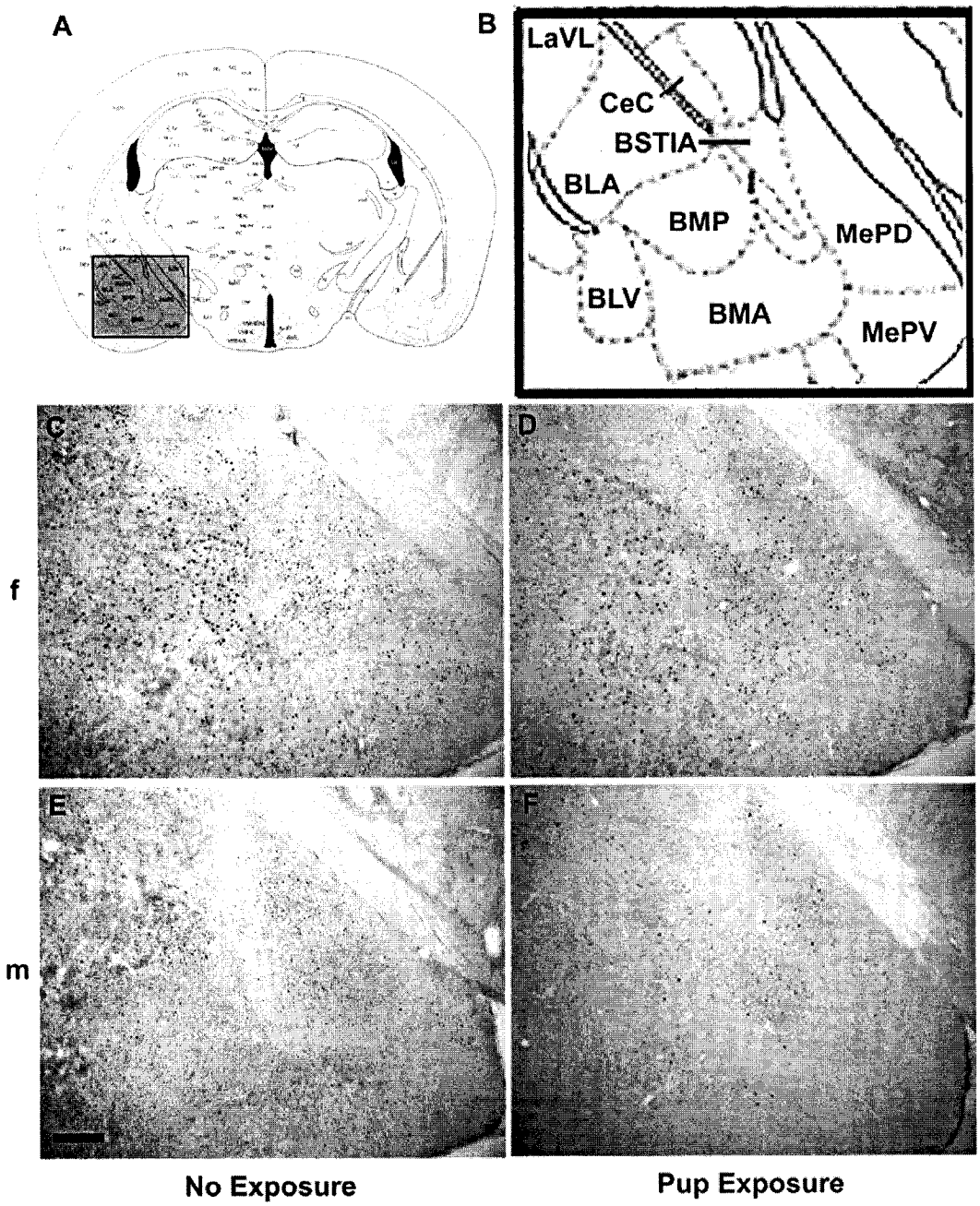

A
B
LaVL
CeC
BSTIA
BLA
BMP
MePD
BLV
BMA
MePV
C
D
f
E
F
m
No Exposure
Pup Exposure

Figure 40.

Figure 41. Distribution of FosB labeled cells in the core of the nucleus accumbens (NAC) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying FosB immunoreactivity in the NAC. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the NAC. FosB immunoreactivity was quantified in the NAC only. Scale bar is 200 µm. Abbreviations: aca = anterior commissure, anterior; f = female; m = male; NAS = nucleus accumbens shell. 63 x magnification.

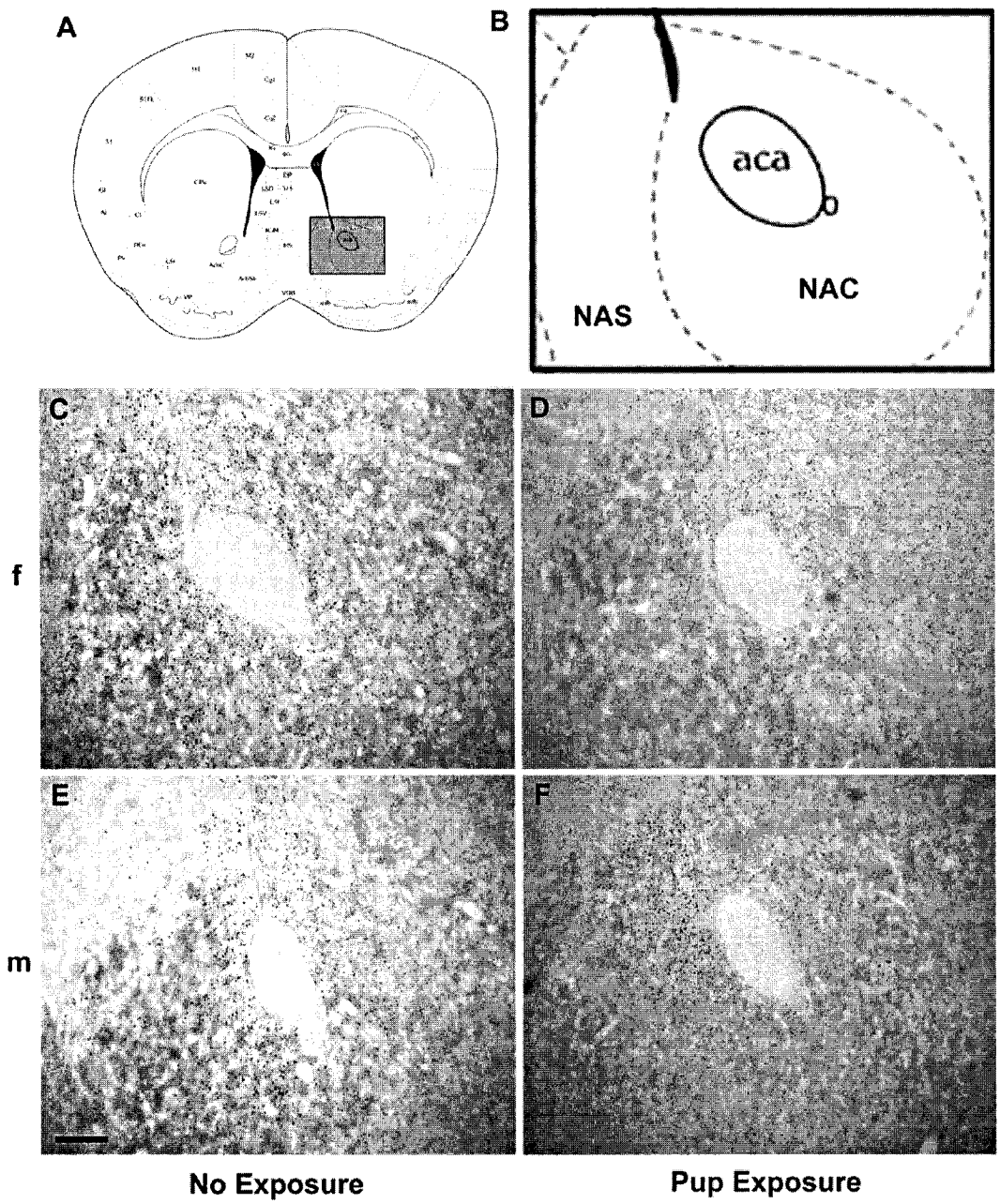


Figure 41.

Figure 42. Distribution of FosB labeled cells in the shell of the nucleus accumbens (NAS) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying FosB immunoreactivity in the NAS. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the NAS. FosB immunoreactivity was quantified in the NAS only. Scale bar is 200 μm. Abbreviations: mfb = medial forebrain bundle; f = female; m = male; VDB = ventral diagonal band nucleus. 63 x magnification.

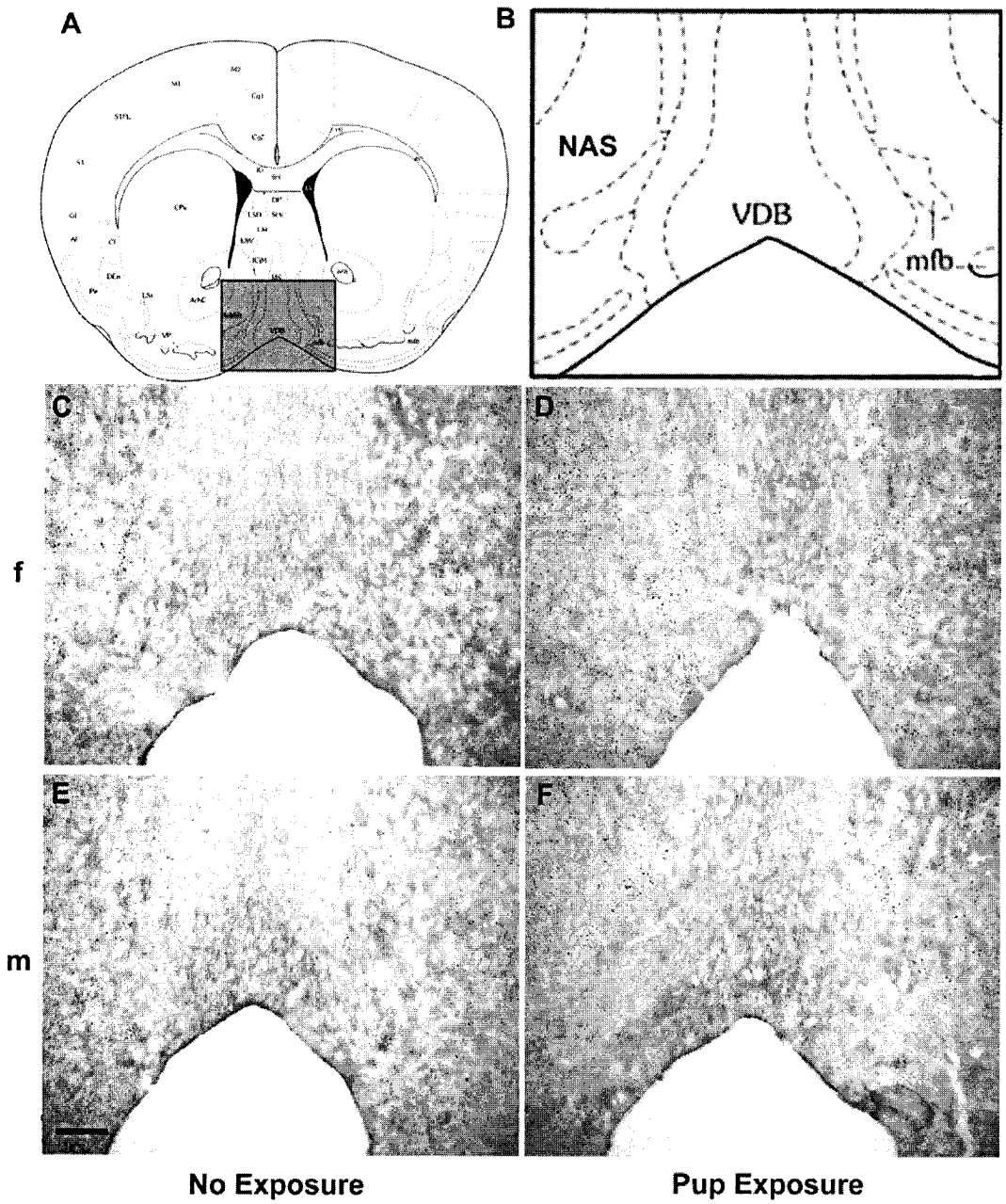


Figure 42.

Figure 43. Distribution of FosB labeled cells in the core of the bed nucleus of the stria terminalis (BNST) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying FosB immunoreactivity in the BNST. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the BNST. FosB immunoreactivity was quantified in all BNST divisions. Scale bar is 200 μm. Abbreviations: acp = anterior commissure, posterior; BSTLD = bed nucleus of the stria terminalis, lateral division, dorsal; BSTLJ = bed nucleus of the stria terminalis, lateral division, juxtacapsular; BSTLP = bed nucleus of the stria terminalis, lateral division, posterior; BSTLV = bed nucleus of the stria terminalis, lateral division, ventral; BSTMA = bed nucleus of the stria terminalis, medial division, anterior; BSTMV = bed nucleus of the stria terminalis, medial division, ventral; f = female; m = male. 63 x magnification.

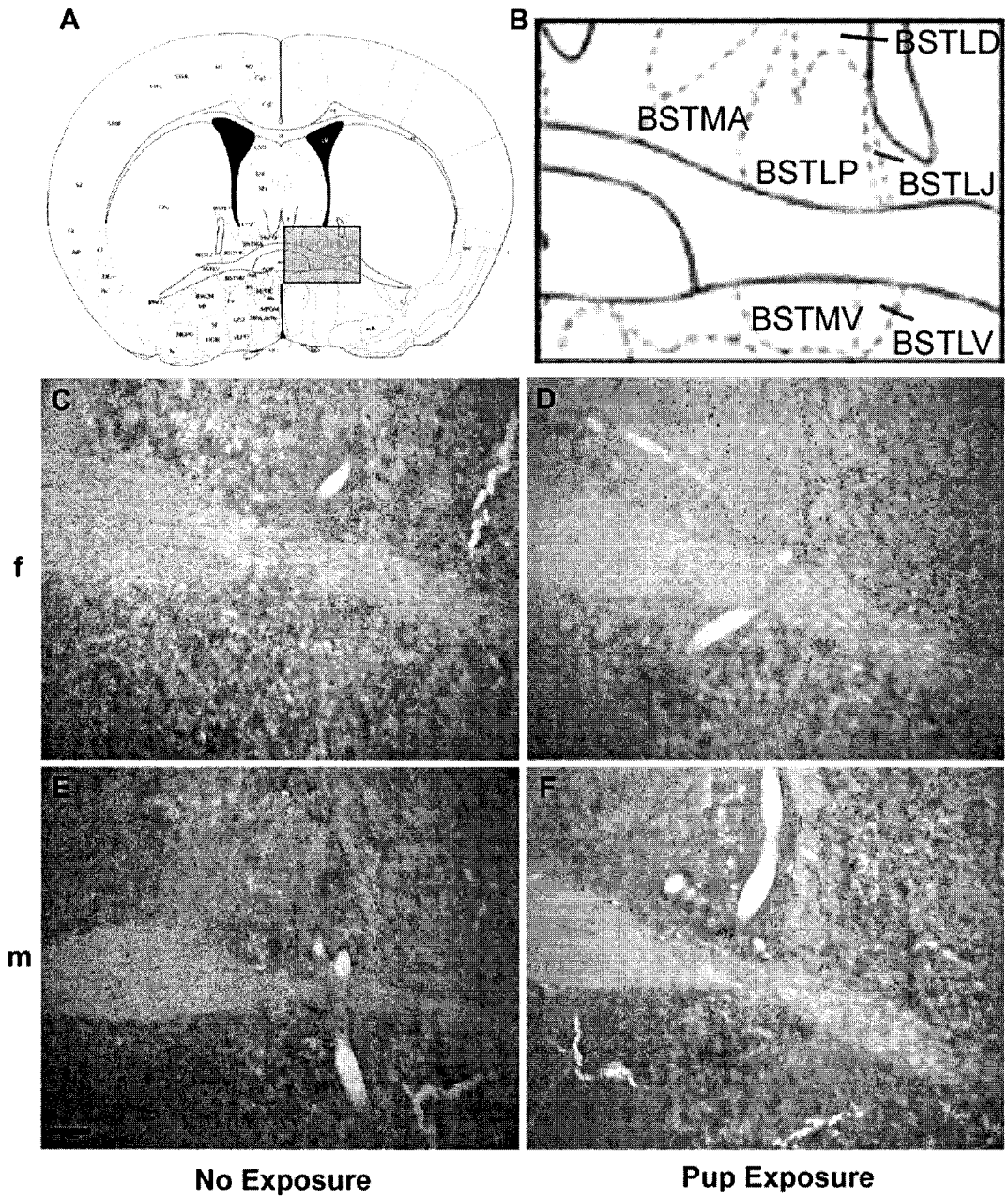

A
B
BSTLD
BSTMA
BSTLP
BSTLJ
BSTMV
BSTLV
C
D
f
E
F
m
No Exposure
Pup Exposure

Figure 43.

Figure 44. Distribution of FosB labeled cells in the piriform cortex of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying FosB immunoreactivity in the piriform. Panels C – F are representative photomicrographs illustrating FosB immunoreactivity in the piriform. Scale bar is 200 μm. Abbreviations: f = female; m = male. 63 x magnification.

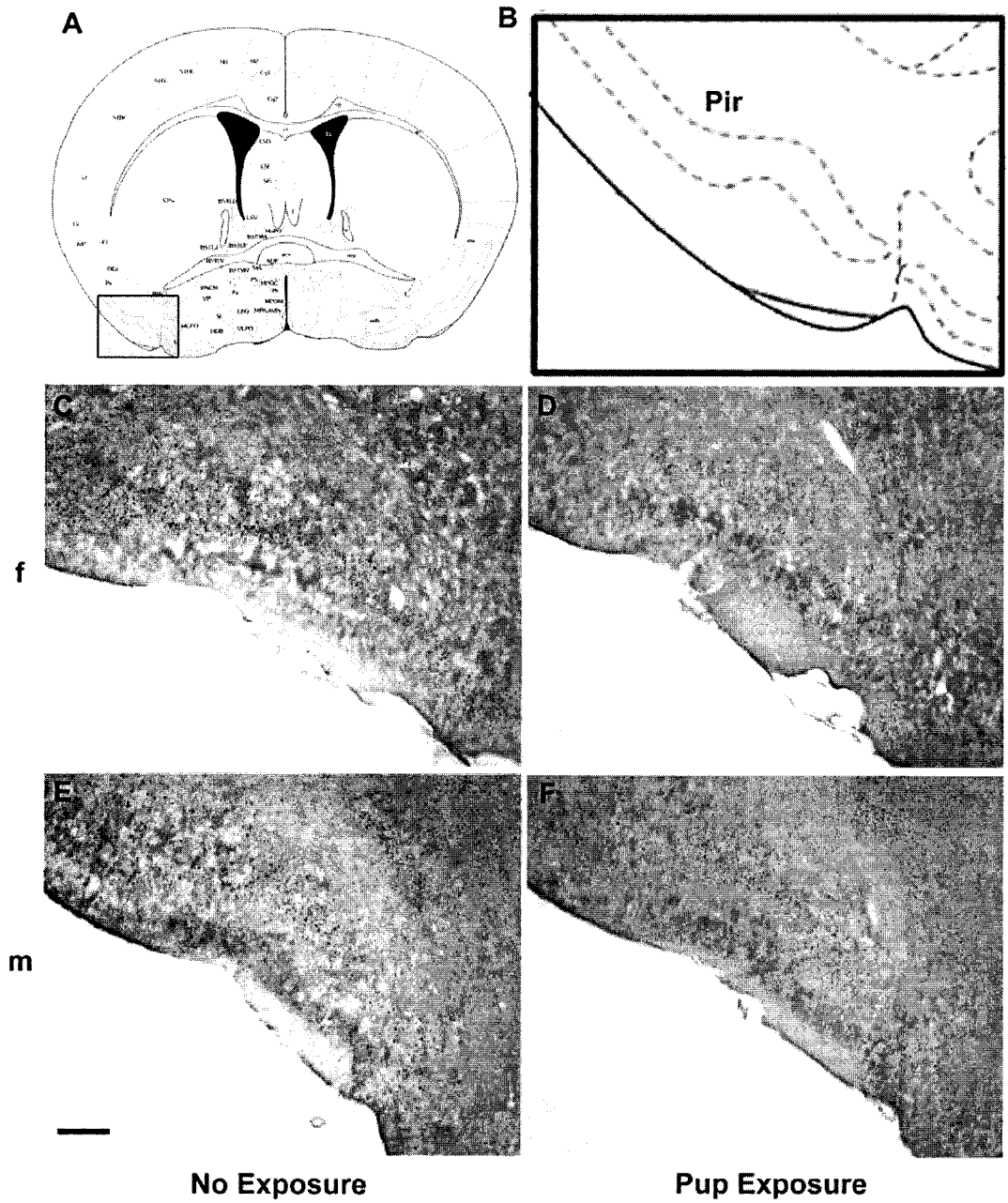


Figure 44.

piriform cortex.

In the somatosensory cortex, there were no significant main effects of pup exposure or sex, and no pup exposure by sex interaction on *fosB* expression.

4.2.5. c-jun expression

Table 8 shows the mean (± SEM) number of c-Jun-labeled cells per section. In the MPOA, there were no significant differences between pup exposed and non exposed groups, between sexes, and no pup exposure by sex interaction in the level of c-Jun.

In the BA, there were no significant effects of sex, pup exposure, or pup exposure by sex interaction on c-Jun immunoreactivity in the BA.

In the CA and MA, females showed significantly higher c-Jun levels than males ($F_{1,9}$ = 19.19, p = .002; Figure 45; $F_{1,9}$ = 14.95, p = .004; Figure 46, respectively). There was no significant effect of pup exposure or pup exposure by sex interaction in *c-jun* expression in these two amygdala regions.

In the NAC, there were no significant differences in c-Jun levels between mice with and without pup exposure. Females had higher levels of c-Jun than males ($F_{1,9}$ = 34.24, p < .001; Figure 47). There was a significant pup exposure by sex interaction on the expression of *c-jun* ($F_{1,9}$ = 5.57, p < .05), as females, but not males in the no pup group showed more c-Jun in the core of the NA than females in the pup exposure group (t_4 = 2.68, p = .055).

In the BNST, there was no main effect of pup exposure or pup exposure by sex interaction on the expression of *c-jun*. Females showed elevated c-Jun

Table 8

Mean (± SEM) number of c-Jun-immunoreactive cells per section

	Female		Male	
Brain Region	Pups	No Pups	Pups	No Pups
MPOA	35 ± 4	31± 5	26 ± 5	23 ± 7
BA	433 ± 67	511± 29	452 ± 19	568 ± 82
CA	27 ± 4#	29 ± 4#	10 ± 2	16 ± 4
MA	206 ± 17#	269 ± 62#	98 ± 10	136 ± 18
NAC	291 ± 53#	515 ± 64#	133 ± 32	144 ± 28
NAS	468 ± 76	417 ± 102	267 ± 79	287 ± 145
BNST	332 ± 95#	274 ± 59#	64 ± 10	104 ± 67
LS	86 ± 14*#	145 ± 6#	31 ± 3*	80 ± 16
Piriform	586 ± 68*	1358 ± 134	627 ± 23*	1102 ± 187
Somatosen	221 ± 5	290 ± 52	129 ± 16	424 ± 235

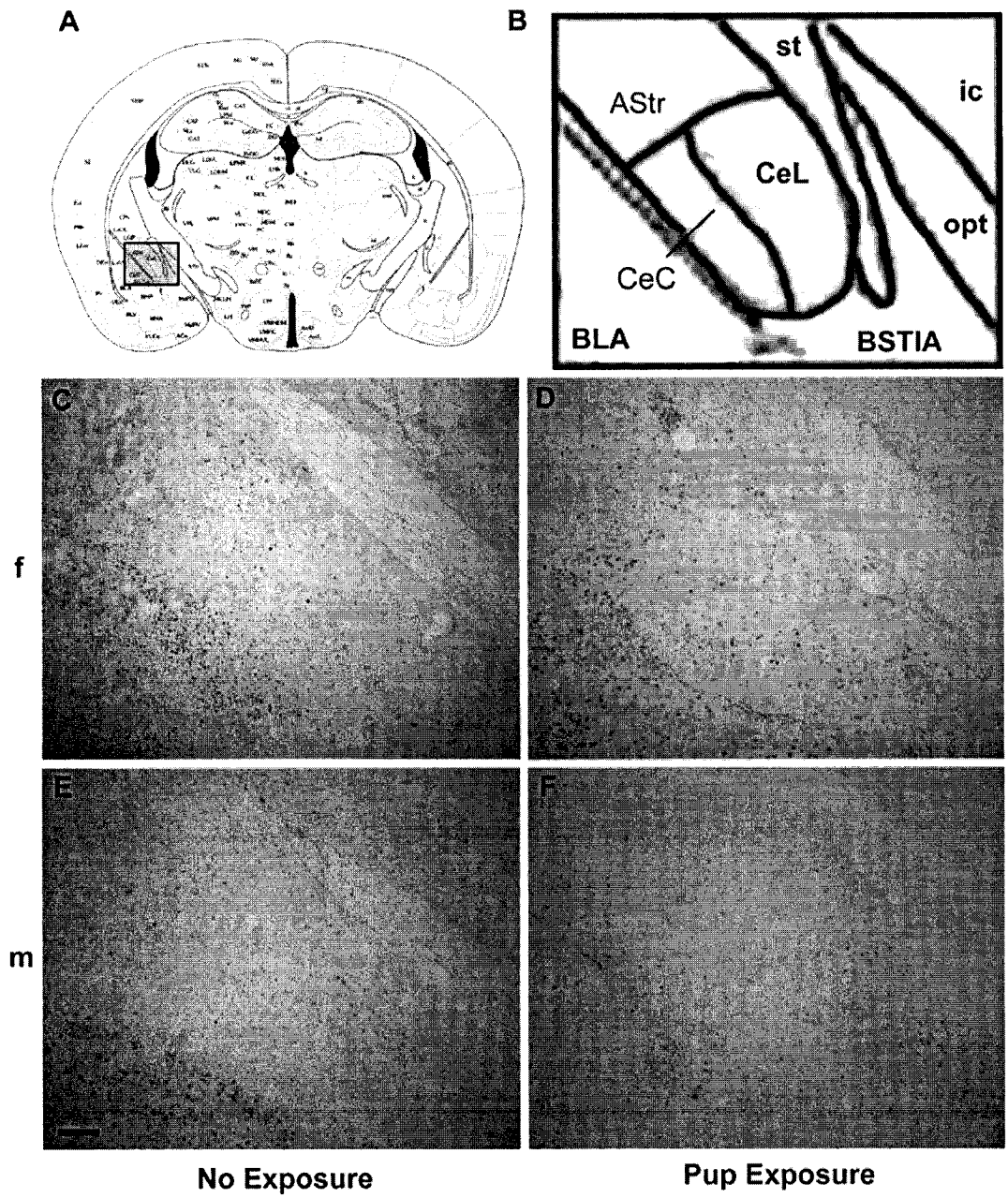
Abbreviations: BA = basolateral amygdala; BNST = bed nucleus of the stria terminalis; CA = central amygdala; LS = lateral septum; MA = medial amygdala; MPOA = medial preoptic area; NAC = nucleus accumbens core; NAS = nucleus accumbens shell; Somatosen = somatosensory cortex.

n = 4 for males with pups group; n = 3 for all other groups

* $p < .05$, significantly different from no pups group.

# $p < .05$, significantly different from males.

Figure 45. Distribution of c-Jun labeled cells in the central amygdala (CA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Jun immunoreactivity in the CA. Panels C – F are representative photomicrographs illustrating c-Jun immunoreactivity in the CA. c-Jun immunoreactivity was quantified in the CeC and CeL only. Scale bar is 100 µm. Abbreviations: AStr = amygdalostriatal transition area; BLA = basolateral amygdaloid nucleus, anterior; BSTIA = bed nucleus of the stria terminalis, intraamygdala; CeC = central amygdaloid nucleus, capsular division; CeL = central amygdaloid nucleus, lateral division; f = female; ic = internal capsule; m = male; opt = optic tract; st = stria terminalis. 100 x magnification.


A
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BSTIA
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f
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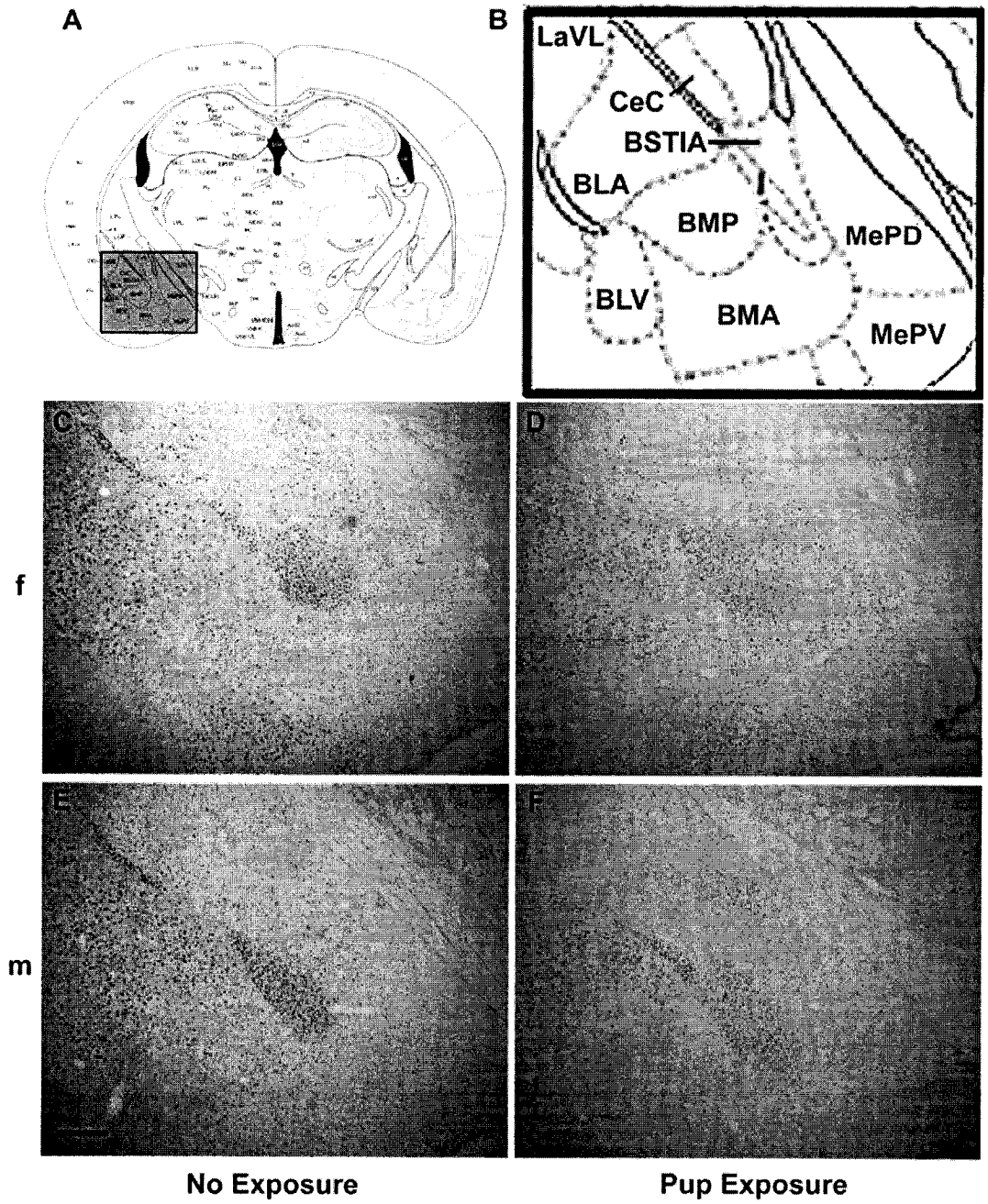
F
m
No Exposure
Pup Exposure

Figure 45.

Figure 46. Distribution of c-Jun labeled cells in the medial amygdala (MA) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Jun immunoreactivity in the MA. Panels C – F are representative photomicrographs illustrating c-Jun immunoreactivity in the MA. c-Jun immunoreactivity was quantified in the MePD and MePV only. Scale bar is 200 µm. Abbreviations: BLA = basolateral amygdaloid nucleus, anterior; BLV = basolateral amygdaloid nucleus, lateral; BMA = basomedial amygdaloid nucleus, anterior; BMP = basomedial amygdaloid nucleus, posterior; BSTIA = bed nucleus of the stria terminalis, intraamygdala; CeC = central amygdaloid nucleus, caps division; f = female; LaVL =lateral amygdaloid nucleus, ventrolateral; m = male; MePD = medial amygdaloid nucleus, posterodorsal; MePV = medial amygdaloid nucleus, posteroventral. 63 x magnification.


A
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MePV
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D
E
F
f
m
No Exposure
Pup Exposure

Figure 46

Figure 47. Distribution of c-Jun labeled cells in the core of the nucleus accumbens (NAC) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Jun immunoreactivity in the NAC. Panels C – F are representative photomicrographs illustrating c-Jun immunoreactivity in the NAC. c-Jun immunoreactivity was quantified in the NAC only. Scale bar is 200 μm. Abbreviations: aca = anterior commissure, anterior; f = female; m = male; NAS = nucleus accumbens shell. 63 x magnification.

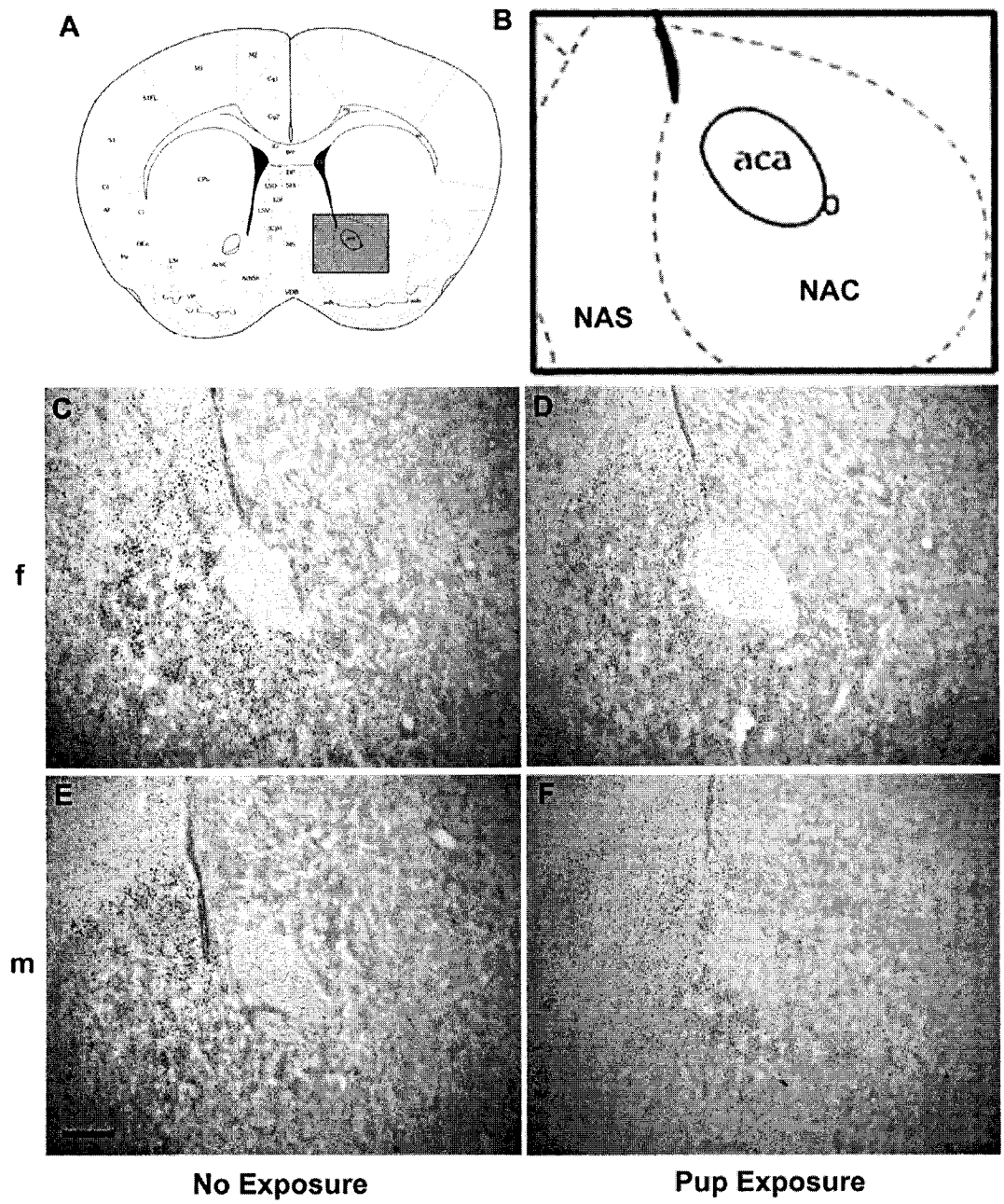

Figure 47.

over males in the BNST ($F_{1,9} = 13.39$, $p = .005$; Figure 48).

In the lateral septum, males and females without pup exposure had higher c-Jun levels than those with pup exposure ($F_{1,9} = 28.71$, $p < .001$; Figure 49) and females had higher c-Jun levels than males ($F_{1,9} = 35.43$, $p < .001$). There was no pup exposure by sex interaction on the expression of *c-jun* in the lateral septum.

In the piriform cortex, mice without pup exposure showed significantly more c-Jun immunoreactivity than those with pup exposure ($F_{1,9} = 44.57$, $p < .001$; Figure 50). There were no differences between sexes and no pup exposure by sex interaction on the expression of *c-jun* in the piriform cortex.

In the somatosensory cortex, there were no main effects of pup exposure, sex, or a pup exposure by sex interaction on the expression of *c-jun.*

4.3 Discussion

The present study examined the expression of the three IEGs, *c-fos, fosB*, and *c-jun*, following parental behaviour in male and female California mice. We measured the number of cells that contained c-Fos, FosB, and c-Jun-ir within the MPOA, extended amygdala (BA, CA, MA), core and shell of the NA, and BNST of male and female California mice with or without pup exposure.

Previous studies have shown that c-Fos-ir is induced in several brain sites during maternal behaviour in rats, mice, and voles, including the MPOA, BA, CA, MA, NA, and BNST (Calamandrei & Keverne, 1994; Fleming & Korsmit, 1996; Fleming & Walsh, 1995; Katz et al., 1999; Mathieson et al., 2002; Numan &

Figure 48. Distribution of c-Jun labeled cells in the core of the bed nucleus of the stria terminalis (BNST) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Jun immunoreactivity in the BNST. Panels C – F are representative photomicrographs illustrating c-Jun immunoreactivity in the BNST. c-Jun immunoreactivity was quantified in all BNST divisions. Scale bar is 200 μm. Abbreviations: acp = anterior commissure, posterior; BSTLD = bed nucleus of the stria terminalis, lateral division, dorsal; BSTLJ = bed nucleus of the stria terminalis, lateral division, juxtacapsular; BSTLP = bed nucleus of the stria terminalis, lateral division, posterior; BSTLV = bed nucleus of the stria terminalis, lateral division, ventral; BSTMA = bed nucleus of the stria terminalis, medial division, anterior; BSTMV = bed nucleus of the stria terminalis, medial division, ventral; f = female; m = male. 63 x magnification.

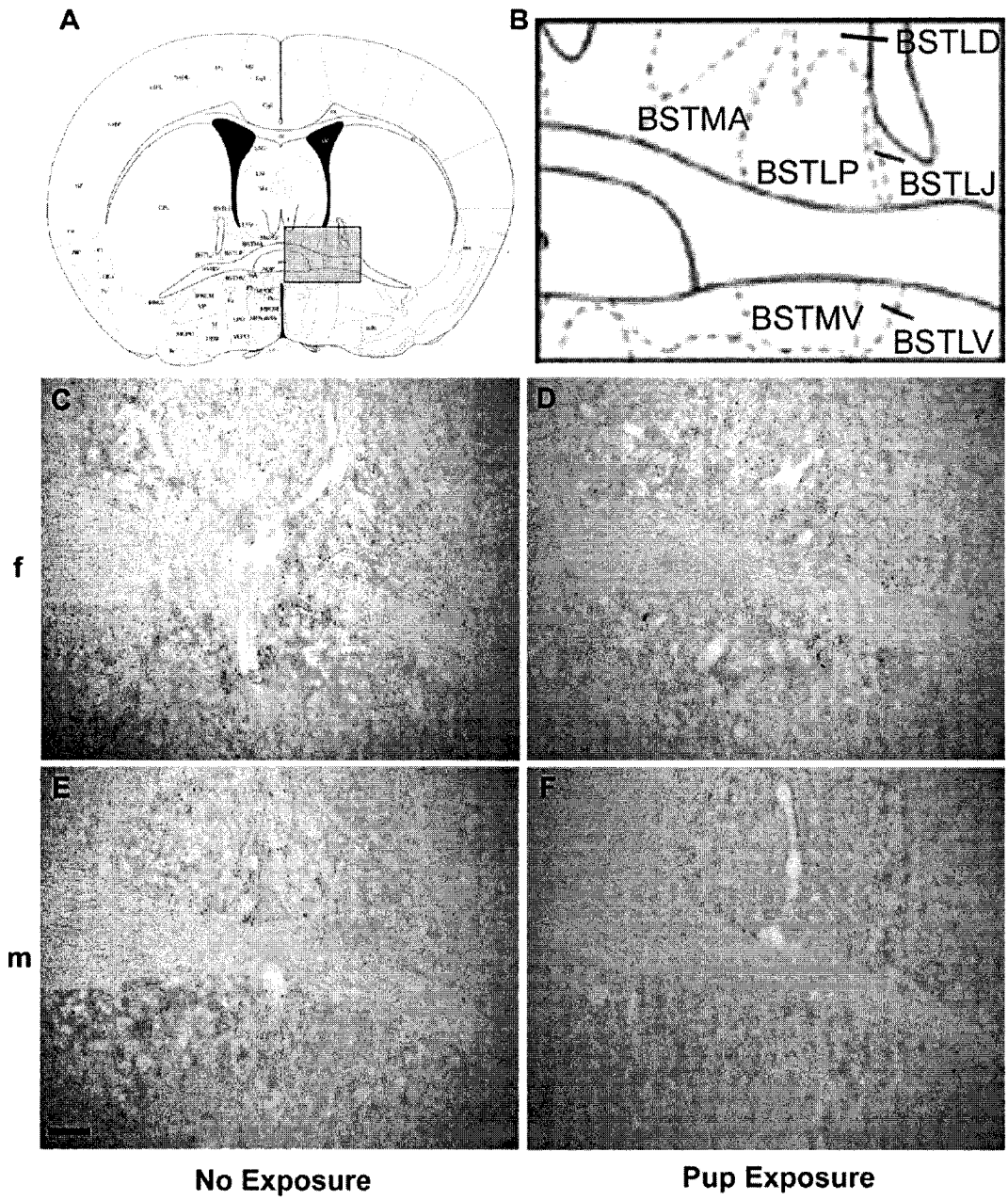
A
B
BSTLD
BSTMA
BSTLP
BSTLJ
BSTMV
BSTLV
C
D
f
E
F
m
No Exposure
Pup Exposure

Figure 48.

Figure 49. Distribution of c-Jun labeled cells in the lateral septum (LS) of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey) selected for quantifying c-Jun immunoreactivity in the LS. Panels C – F are representative photomicrographs illustrating c-Jun immunoreactivity in the LS. c-Jun immunoreactivity was quantified in the ventral division of the LS (LSV). Scale bar is 200 μm. Abbreviations: aca = anterior commissure, anterior; BST = bed nucleus of the stria terminalis; CPu = caudate putamen; f = female; LSI = lateral septal nucleus, intermediate; LSV = lateral septal nucleus, ventral; m = male; MS = medial septal nucleus. 63 x magnification.

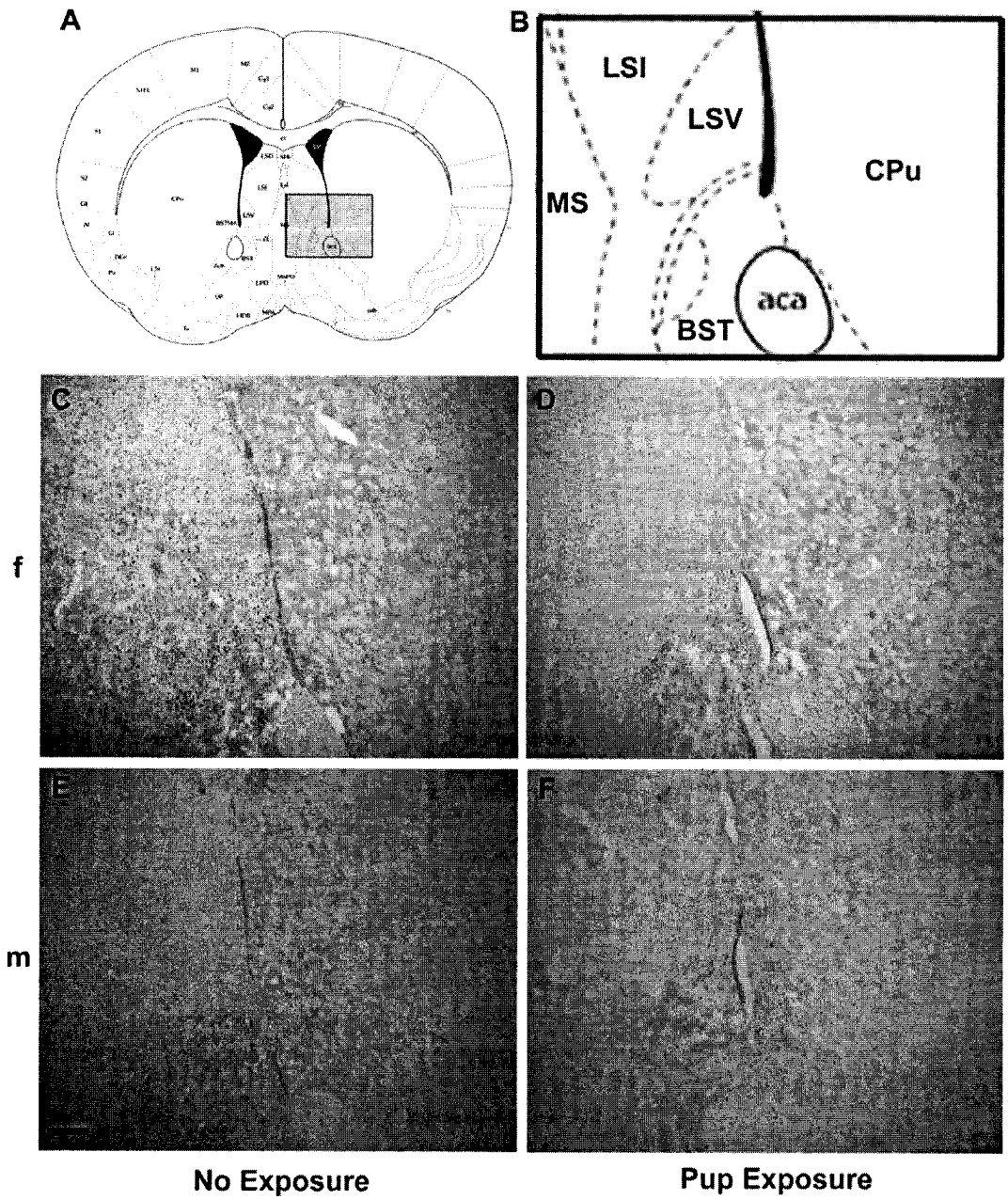

Figure 49.

Figure 50. Distribution of c - Jun labeled cells in the piriform cortex of male and female *P. californicus* with pup exposure and no pup exposure. Panel A and B show schematic diagrams (from Franklin & Paxinos, 1997) illustrating the area (in grey in panel A) selected for quantifying c-Jun immunoreactivity in the piriform. Panels C – F are representative photomicrographs illustrating c-Jun immunoreactivity in the piriform. Scale bar is 200 μm. Abbreviations: f = female; m = male; Pir = piriform cortex. 63 x magnification.

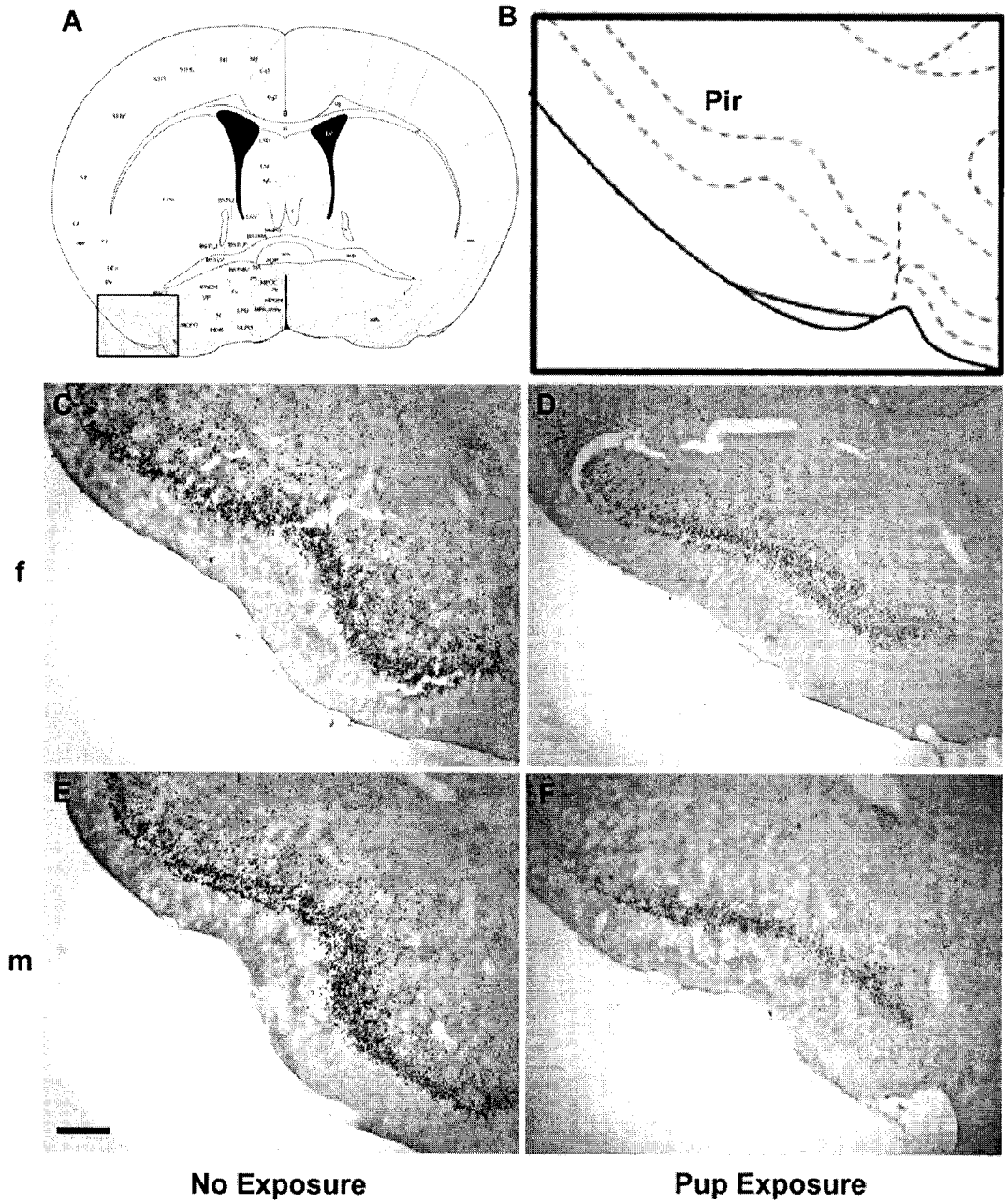

Figure 50.

Numan, 1994; Numan et al., 1998). c-Fos-ir is also elevated in the MPOA and MA during paternal behaviour in voles (Kirkpatrick et al., 1994b). In every one of these studies which have used c-Fos ICC to investigate brain areas that are activated during parental behaviour, the MPOA is consistently reported to have increased *c-fos* expression. Likewise, FosB-ir has also been shown to be elevated in the MPOA and ventral BNST during maternal behaviour in rats (Stack & Numan, 2000).

In the present study, there was an increase in c-Fos-ir in the MPOA, MA, and piriform and somatosensory cortices and increased FosB-ir in the BNST following parental behaviour. In contrast, with the exception of the CA in males, there were no increases of *c-jun* in any brain sites following parental behaviour. In fact, mice with pup exposure showed reduced c-Jun-ir in the lateral septum and piriform cortex compared to those without pup exposure, perhaps indicating a decrease in neuronal activity in these neurons.

4.3.1. Parental behaviour activates *c-fos*, but not *fosB* or *c-jun* expression in the medial preoptic area

Numerous studies have emphasized the critical role of the MPOA for maternal responsiveness in rats. For example, lesions to the MPOA disrupt maternal behaviour (Jacobson et al, 1980; Numan, 1974; Numan et al., 1977), hormone treatment into this area stimulates maternal behaviour (Bridges et al., 1990), and c-Fos and FosB immunoreactivity is increased in the MPOA during maternal behaviour in rats (Fleming & Korsmit, 1996; Fleming & Walsh, 1995;

Kalinichev et al., 2000; Numan & Numan, 1994; Numan et al., 1998; Stack & Numan, 2000). Paternally behaving male and female prairie voles also show increased *c-fos* expression in the MPOA (Katz et al., 1999; Kirkpatrick et al., 1994b). These reports, along with the a study showing that mice lacking the *fosB* gene exhibit a deficit in maternal responsiveness (Brown et al., 1996), make a case for *fos* regulation of neurons associated with maternal behaviour within the MPOA.

In the present study, although males and females with pup exposure had higher levels of c-Fos in the MPOA, this difference only approached statistical significance. This lack of difference may be due to the small samples or that mice without pup exposure tended to have relatively high levels of c-Fos, suggesting the California mice may have high basal levels of c-Fos in the MPOA. There were also no differences between pup-exposed and non-exposed mice on the expression of *fosB* and *c-jun* in the MPOA in California mice. Unlike the c-Fos results, in which all groups showed considerable *c-fos* expression, mice in all four groups showed little FosB and c-Jun immunoreactivity in the MPOA.

The results of the present study suggest that 1) male and female California mice have high basal levels of c-Fos in the MPOA, and 2) the expression of *c-fos,* but not *fosB*, and *c-jun* within the MPOA may be related to parental behaviour in male and female California mice.

4.3.2. Parental behaviour activates different IEG patterns in the amygdala

The expression of IEGs in the amygdala, as well as in other brain regions relevant to parental behaviour, has been investigated less frequently than in the MPOA. Elevated levels of c-Fos have been reported in the medial, central, basolateral, and cortical amygdala in rats (Fleming & Walsh, 1994; Kalinichev et al., 2000; Walsh et al., 1996) and in the corticomedial amygdala in balb/c mice (Calamandrei & Keverne, 1994) following maternal behaviour. In prairie voles, increased *c-fos* expression is observed in the medial amygdala of both male and females with pup exposure (Kirkpatrick et al., 1994b).

In the present experiment, the expression of *c-fos, fosB*, and *c-jun* during parental behaviour in the basolateral, medial, and central amygdala during parental behaviour showed varying results. In the BA, *fosB*, but neither c-fos nor *c-jun* expression, was induced by parental behaviour in females only. This result is consistent with those reported for maternal rats (Fleming & Walsh, 1994). Females had higher levels of fosB expression in the BA than males.

In the MA, c-Fos-ir, but not FosB-ir nor c-Jun-ir, was increased by parental behaviour in male and female California mice. This is consistent with the results of male and female prairie voles, which are also monogamous and biparental. In prairie voles, however, males show a greater c-Fos response to pups in the medial amygdala than females (Kirkpatrick et al., 1994b), which was not the case in the present study. Finally, female California mice showed more *fosB* and *c-jun* expression in the medial amygdala than males, but parental behaviour did not

increase levels of immunoreactivity of these proteins in the MA. Thus, in California mice, females appear to have higher basal levels of FosB and c-Jun in the MA than males.

Finally, in the CA, *fosB*, but not *c-fos* nor *c-jun* expression was increased by parental behaviour in males only. For the CA results, it is worth noting that although the effects were statistically significant, the actual number of FosB labeled cells in the CA were very low. Interestingly, there was dense *c-jun* expression in the BA. However, high levels of c-Jun were observed across all four groups, suggesting California mice have high basal levels of c-Jun in the BA.

4.3.3. Parental behaviour does not activate *c-fos, fosB,* or *c-jun* expression in the nucleus accumbens

Several studies have reported increased *c-fos* expression in the NA during maternal behaviour in rats (Fleming & Walsh, 1994; Lonstein et al., 1998; Stack et al., 2002). Two of these studies did not distinguish between the core and shell of the NA (Fleming & Walsh, 1994; Lonstein et al., 1998). Stack et al. (2002) examined c-*fos* expression in the core and shell of the NA separately and found that maternally behaving rats had increased *c-fos* expression in the NAS but not the NAC. They did not find increased *fosB* expression in either area.

In the present study, there was a decrease in *c-jun* expression in the NAS in females only, following parental behaviour. There were no other differences in IEG expression between pup exposed and nonexposed groups. Females showed higher levels of immunoreactivity than males for all three IEGs in the

core of the nucleus accumbens. Females also showed elevated levels of c-Fos and FosB in the shell of the NA over males. These results are contrary to that reported of maternally behaving rats (Fleming & Walsh, 1994; Lonstein et al., 1998; Stack et al., 2002) and do not support a role for the NA in parental behaviour in California mice.

4.3.4. Parental behaviour activates *fosB*, but not *c-fos* or *c-jun* expression in the bed nucleus of the stria terminalis

Studies using Fos ICC to examine the neural pathways important for parental behaviour suggests the BNST is important. For example, female rats show elevated c-Fos and FosB in the ventral BNST following parental behaviour (Kalinichev et al., 2000; Numan & Numan, 1995; Numan et al., 1998; Stack & Numan, 2000). Both male and female prairie voles show elevated *c-fos* expression in the medial BNST following pup exposure (Katz et al., 1999; Kirkpatrick et al., 1994b). In the present study, male and female California mice showed increased FosB, but not c-Fos or c-Jun-ir in the BNST following pup exposure. The two studies that examined *fos* expression in female rats looked at the ventral portion of the BNST, while the study that used prairie voles as subjects looked at the medial portion of the BNST. In the present study, Fos and Jun-ir was quantified in several divisions of the BNST, including the BSTMA, BSTLP, BNTLJ, BSTMV, BSTLV, and BSTLD. However, the data shown combines all divisions of the BNST. It is possible that examining the individual divisions of the BNST separately may yield different results.

4.3.5. Parental behaviour activates *c-fos*, but not *fosB* or *c-jun* expression in the lateral septum of males

Previous Fos activation studies implicated the lateral septum in parental behaviour. Female rats show elevated c-Fos, but not FosB, in the intermediate and ventral divisions of the lateral septum following maternal behaviour (Stack & Numan, 2000). In male and female prairie voles, exposure to a pup increases c-fos expression in the lateral septum (Katz et al., 1999; Kirkpatrick et al., 1994b). In the present study, parental behaviour increased c-Fos-ir in the ventral lateral septum of males only. Thus, the data suggest that the lateral septum may be involved in paternal, but not maternal behaviour in California mice. However, as previous studies found activation of Fos in the intermediate division of the lateral septum, further examination Fos in this area should also be conducted.

Males and females with pup exposure had decreased c-Jun-ir in the lateral septum than mice without pup exposure, suggesting an inhibition in this area. The lateral septum receives input from the medial amygdala (Canteras et al., 1995), an area which is suggested to be involved in the inhibition of maternal behaviour in virgin rats (Fleming et al., 1980). However, the hormonal changes associated with pregnancy and parturition is suggested to reduce the activity of the neural circuit which inhibits maternal behaviour in virgin rats. The subjects in experiment 3 were multiparous pairs. Thus, it is unclear why there was an inhibition c-Jun-ir activity in the lateral septum.

4.3.6. Parental behaviour activates *c-fos,* but not *fosB* or *c-jun* expression in the piriform and somatosensory cortices

Studies suggest that the piriform cortex, which is a recipient of primary olfactory input (Scalia & Winas, 1975) and the somatosensory cortex show increased Fos-ir following maternal behaviour in rats (Walsh et al., 1996) and balb/c mice (Calamandrei & Keverne, 1994). In contrast, exposure to a pup does not increase c-Fos-ir in the piriform cortex of male and female prairie voles (Kirkpatrick et al., 1994b).

In the present study, parental behaviour increased c-Fos-ir in both the piriform and somatosensory cortices. Activation of Fos in the piriform likely reflects olfactory pup stimuli, while activation of Fos in the somatosensory area reflects stimuli involved in adopting a nursing posture. It should be noted that all mice in the pup exposure were observed hovering over pups.

Males and females with pup exposure had decreased c-Jun-ir in the piriform cortex. It is unclear why there was an inhibition of the piriform cortex in pup exposed groups.

4.3.7. Limitations of the study

It is possible that the paradigm used in the present study was not appropriate to detect differences in c-Fos, FosB, and/or c-Jun-ir between groups. For example, 1 h of pup exposure, which is what mice in the present study had, may have been insufficient to produce significant changes in *c-fos, fosB* or *c-jun* expression. The majority of studies that have used c-Fos ICC to study parental

behaviour allow at least 2 h of pup experience (Kalinichev et al., 2000; Kirkpatrick et al., 1994b; Numan et al., 1998; Stack & Numan, 2000). However, many studies have given subjects 1 h of pup interaction before being sacrificed (Fleming & Korsmit, 1996; Walsh et al., 1996) and in one study, 30 min of pup interaction was sufficient to increase Fos-ir in balb/c mice (Calamandrei & Keverne, 1994). Furthermore, parental behaviour increased Fos-ir and decreased Jun-ir in the piriform and somatosensory cortices, indicating that sensory stimuli from the pups activated *c-fos* and *c-jun* expression.

Another consideration is that levels of c-Fos may have been elevated in mice in all groups because they were "stressed" during the hours prior to being perfused, due to the separation from their mate. California mice form persistent pair bonds and mate for life (Ribble, 1992a). Thus, altering the social environment by separating the pairs may have been a stressor. In squirrel monkeys for example, social separation can induce long-lasting increases in cortisol (Levine et al., 1997). To investigate this possibility, *c-fos* expression was examined in the paraventricular nucleus of the hypothalamus (PVN), as a variety of stressors, such as restraint and water-avoidance stress, have been shown to produce dense Fos-ir in this region (Bonaz & Tache, 1994; Chowdhury et al., 2000; Covenas et al., 1993). The nuclei in the PVN of California mice in the present study did not have the dense Fos-ir normally observed following acute or chronic stress, suggesting that California mice were not stressed. However, it is not known whether stress would produce an increase in Fos-ir in the PVN of California mice.

While c-Fos can be activated by an acute exposure to a stimulus, FosB may require prolonged activation by the same stimulus. For example, *c-fos* expression increases in the MPOA of females with 1 or 2 h of pup exposure (Fleming & Korsmit, 1996; Kalinichev et al., 2000; Kirkpatrick et al., 1994b; Numan et al., 1998; Stack & Numan, 2000; Walsh et al., 1996), but *fosB* expression increases in the MPOA and ventral BNST only after 4 h from the start of maternal behaviour (Numan et al., 1998; Stack & Numan, 2000). Stack & Numan (2002) suggest that assuming the downstream target gene of *c-fos* and *fosB* is the same and important for maternal behaviour, the expression of *c-fos* in the early stages would shift to *fosB* after long durations of mother-pup interactions. It is possible that 1 h was not long enough to alter *fosB* expression in the present study. Future studies should examine the temporal pattern of fosB expression by varying the duration of pup exposure.

One possible flaw in the study is with respect to the small sample sizes, with an n of 3 or 4 per group. Studies that examine IEG activation often have similar sample sizes and produce significant results. California mice appear to have a lot of variability in their data and thus, it would have been more appropriate to include more subjects per group.

Finally, it should be noted that studies involving immediate early genes have potential for false positives and negatives. Also, it is not possible to determine the specific stimuli (olfactory, visual, or tactile) which elicited Fos activation in the present study. Therefore, the results of the present study suggest that several brain sites including the MPOA, BA, MA, NAS, BNST, LS,

and piriform and somatosensory cortices *may* be important for parental behaviour in California mice, but these results should be corroborated with other methods.

5. General discussion

The neural control of maternal behaviour in the female rat has been studied extensively. Several brain regions have been implicated in the expression of maternal behaviour in rodents and among these, the MPOA is the most integrative (Fleming et al., 1994; Numan, 1974; Numan et al., 1977; Numan & Numan, 1994). Other areas, which have extensive anatomical connections to the MPOA, including the amygdala, and NA may also be important for the expression of maternal behaviour (Fleming & Walsh, 1994; Lee et al., 1999, 2000; Lonstein et al., 1998; Walsh et al., 1996).

On the other hand, the neural control of paternal behaviour has been studied far less frequently. This is not surprising given the minimal amount of paternal behaviour normally observed in laboratory rats. There are some species, however, such as California mice and prairie voles which exhibit biparental care. The MPOA and amygdala appear to be relevant for paternal behaviour in voles (Kirkpatrick et al., 1994a, b), but the literature on the neural control of paternal behaviour in rodents is sparse.

The purpose of the three experiments described in this thesis was to investigate the neural control of male and female parental behaviour in California mice. In doing so, this thesis has added significantly to the sparse literature on the neuroanatomy of paternal behaviour and addresses whether 1) the neural control of maternal behaviour in California mice is similar to that of rats, and 2)

whether the neural control of parental behaviour in males is similar to that of females in California mice. Experiments 1 and 2 used a conventional technique, electrolytic lesions, to investigate whether three brain regions, the MPOA, BA, and NA, are important for male and female parental behaviour. Results from the first two experiments showed that the MPOA is important for both male and female parental behaviour, that the BA is important for male parental behaviour, and that the NA is not important for ongoing parental behaviour in either male or female California mice. Experiment 3 used Fos and Jun immunocytochemistry to map activated brain sites following parental behaviour in male and female California mice. Data from the third experiment showed that parental behaviour increases c-Fos-ir in the MPOA, MA, and piriform and somatosensory cortices. The data for FosB-ir and c-Jun-ir was more dificult to interpret. For the most part, there was no evidence of *fosB* or *c-jun* activation in the brain following parental behaviour.

It has been suggested that *c-fos* mediates the transcription effects of acute stimulation while *fosB* mediates the effects of chronic stimulation (Doucet et al., 1996). Thus, one can speculate that *fosB* expression may increase with a longer period of pup exposure. For example, Numan et al. (1998) found that the temporal pattern of *c-fos* and *fosB* expression differed during maternal behaviour in rats. While the number of cells containing c-Fos increased at 2 h and remained high through 6 h of pup exposure, the number of cells expressing *fosB* increased more slowly, increasing above controls at 4 h and peaking at 6 h. One possible explanation of our results is that FosB-ir, and perhaps c-Jun-ir within the

brain take longer than one hr to increase over controls in California mice. Further examination of these IEGs in studies that vary the duration of pup exposure is necessary.

5.1. The medial preoptic area is important for male and female parental behaviour in California mice

The role of the MPOA in maternal behaviour has been studied through a variety of approaches, including lesioning the MPOA, severing its lateral efferents, injecting estrogen into the MPOA, and examining the pattern of Fos immunocytochemistry. Research from several laboratories has consistently shown that this region is critical for the expression of maternal behaviour in rats.

Results from experiments 1 and 2 provide further evidence for the importance of the MPOA for both male and female California mice. Experiment 1 showed that electrolytic lesions in the MPOA led to deficits in parental behaviour in primiparous male and female California mice. Experiment 2 replicated these findings in parentally experienced male and female California mice who had raised at least one litter previously. Thus, these results indicate that MPOA lesions disrupt the maintenance of both male and female parental behaviour in California mice.

Because electrolytic lesions are nonselective, destroying all tissue around the electrode, it is impossible to tell from our study whether the observed parental deficits were due to damage to MPOA neurons or to axons passing through the MPOA. NMDA lesions, which selectively destroy neuronal cell bodies while

sparing fibers of passage, disrupt parental behaviour in female and male rats, demonstrating that at least for rats, it is the MPOA neurons that are important for parental behaviour (Numan et al., 1988; Sturgis & Bridges, 1997).

To examine the neural activation during parental behaviour, experiment 3 investigated the expression of *c-fos, fosB*, and *c-jun*, measured by immunocytochemistry. Although not statistically significant, there was a trend towards higher levels of c-Fos in the MPOA in males and females with pup exposure. FosB-ir and c-Jun-ir, however, did not increase with pup exposure. One possible explanation is that parental behaviour in California mice does not alter the expression of *c-fos, fosB* or *c-jun* in the MPOA. However, one cannot draw such a conclusion at this time, particularly with the vast amount of published studies that report otherwise for *c-fos* and *fosB* (Fleming & Korsmit, 1996; Fleming & Walsh, 1995; Kalinichev et al., 2000; Katz et al., 1999; Kirkpatrick et al., 1994b Numan & Numan, 1994; Numan et al., 1998; Stack & Numan, 2000). A more likely explanation may be that the sample sizes were too small or that 1 h of pup exposure was too short to activate *fosB*. The fact that Fos-ir was elevated in the sensory-receiving areas suggests that 1 h of pup exposure was sufficient for the activation of *c-fos*.

MPOA neurons contain prolactin and oxytocin receptors (Kremarik et al., 1995). Since prolactin and oxytocin may be involved in maternal behaviour and these genes contain AP-1 sites within their regulatory regions (Hu et al., 1996; Rozen et al., 1995), they may be putative candidates that may be regulated by *c-fos/fos B* during maternal behaviour.

5.2. Different divisions of the amygdala may be important for male and female parental behaviour in California mice

The previous literature on the role of the amygdala in parental behaviour was initially based on Fleming et al.'s (1980) work, showing that electrolytic lesions in the medial or corticomedial amygdala, which receive projections from the olfactory system, reduce the latency to show maternal behaviour in virgin rats. Similar results have been shown with NMDA lesions in the amygdala (Numan et al., 1993). Fleming et al. (1980) proposed that olfactory input to the amygdala inhibits maternal behaviour in virgin rats by depressing the activity of the MPOA.

For many years, an intact amygdala was not thought to be essential for the expression of maternal behaviour in rodents, as lesions in the medial amygdala do not disrupt maternal behaviour in mice (Slotnick & Nigrosh, 1975) or rats (Fleming et al., 1980; Numan et al., 1993). MA lesions do, however, disrupt paternal behaviour in voles (Kirkpatrick et al., 1994a). Also, several laboratories reported increases in *c-fos* expression in the amygdala of parentally behaving female rats and mice and both male and female prairie voles (Calamandrei & Keverne, 1994; Fleming & Walsh, 1994; Kirkpatrick et al., 1994b). More recently, studies have shown that electrolytic lesions of the BA disrupt maternal behaviour in rats, further supporting a role for the amygdala in maternal behaviour (Lee et al., 1999, 2000).

Experiment 2 showed that lesions in the BA disrupt parental behaviour in male California mice. Males with BA lesions showed significantly less licking than sham controls and this deficit in pup-licking continued throughout the 10

days of testing with pups. Males with BA lesions also spent less time near pups than sham controls. Females with BA lesions did not show a reduction in parental behaviour compared to sham controls. These results suggest that in California mice, the importance of the BA in parental behaviour is specific to males.

With respect to the activation of IEGs in the amygdala, *c-fos, fosB*, and *c-jun* expression during parental behaviour varied across the different amygdala regions. There was an increase in c-Fos-ir following parental behaviour in both male and female California mice in the MA. Males with pup exposure showed elevated *fosB* expression in the CA, while females showed elevated *fosB* expression in the BA. Thus, the two approaches used in this thesis, electrolytic lesions and Fos and Jun immunocytochemistry produced conflicting results. One conclusion that can be drawn is that an intact BA is important for the expression of paternal behaviour in male California mice. The same does not hold true for female California mice. As stated already for the MPOA data, *fos* and *jun* expression needs to be further studied with longer durations of parental behaviour in California mice.

5.3. The nucleus accumbens is not important for parental behaviour in California mice

Mother rats find pups reinforcing, as they will bar press for them in an operant paradigm (Lee et al., 2000). The dopamine system, which is involved in general motivational processes, has a component which originates in the VTA

and terminates in the NA (Ikemoto & Panksepp, 1999). A circuit involving MPOA/ventral BNST lateral efferents to the NA either directly or indirectly via the VTA fits nicely with the view that pups are reinforcing to postpartum rats. There is some evidence that lends support to this circuit. For example, electrolytic and 6-hydroxydopamine (a dopaminergic neurotoxin) lesions of the VTA or NA disrupt maternal behaviour in rats (Hansen, 1994; Lee et al., 2000; Li & Fleming, 2003; Numan & Smith, 1984); microdialysis techniques indicate that maternal interaction increases dopamine release in the NA of mother rats (Hansen et al., 1993); microinjection of cis-flupenthixol (a mixed D1 and D2 dopamine antagonist) into the NA of female rats disrupts maternal behaviour (Keer & Stern, 1999); cycloheximide (a protein synthesis inhibitor) injected into the NAS disrupts maternal behaviour in rats (Li & Fleming, 2003); and finally, *c-fos* expression is increased in the NA in maternally behaving rats (Fleming et al., 1994; Lonstein et al., 1998; Stack et al., 2002). Together, these data provide strong evidence that the NA is important for maternal behaviour in rats.

In the present set of experiments, neither NA lesions, nor the IEG activation patterns showed much evidence of an involvement of the NA in parental behaviour in male and female California mice. The only deficit in parental behaviour was that NA lesioned males had longer latencies to retrieve pups than sham lesioned controls. NA lesioned males and females showed no deficits in the proportion of time spent in any of the parental behaviours compared to sham lesioned controls. In fact, during a few of the 10 days of

parental testing, NA lesioned mice actually spent more time engaged in some parental behaviours than sham lesioned controls.

The nucleus accumbens, consisting of a core and shell, is a large region. The NA lesions produced in experiment 3 were relatively small and did not fully destroy the NA. The smallest lesion destroyed only part of the core of the NA and the largest lesion destroyed part of the core and part of the shell of the NA. Li and Fleming (2003) recently reported that lesions in the NAS, but not NAC, disrupted maternal behaviour rats. Thus, it is possible that larger lesions or lesions specific to the NAS would produce deficits in parental behaviour in California mice. During pilot studies, when larger NA lesions were attempted by increasing the duration of current from 10 to 15 s, the survival rate fell drastically from the already poor rate. The role of the NA in parental behaviour in California mice should be considered again with further studies using the dopaminergic neurotoxin, 6-hydroxydopamine or with larger lesions, using gaseous anaesthetic.

Consistent with the lesion data, Fos and Jun ICC did not support a role of the NA during parental behaviour in male and female California mice. Females with pup exposure had increased FosB-ir in the NAS than females without pup exposure. However, females with pup exposure also had decreased c-Fos-ir in the NAS than females with no pup exposure. It is unclear why there was an activation of FosB and inhibition of c-Jun in the NAS in females with pup exposure. *c-fos* expression did differ between groups with pup exposure and no pup exposure. These data are in contrast to those reported in maternal rats, which have elevated *c-fos* expression in the NA (Fleming et al., 1994; Lonstein et

al., 1998; Stack et al., 2002). There are no published reports of *fos* or *jun* expression in the NA during parental behaviour in a biparental species. Although the NA is involved in maternal behaviour in rats, it is possible that this area may not play a role in parental behaviour in California mice. Before drawing such conclusions, however, two further studies need to be done, both of which have already been mentioned. One of these studies involves lesioning the NA more fully than that of experiment 3. More importantly, the second study involves re-examining Fos and Jun-ir with longer durations of pup exposure. Ideally, this study would include several durations of pup exposure, testing the temporal pattern of *fos* and *jun* expression in California mice.

5.4. Basal levels of c-Fos, FosB, and c-Jun in California mice

Our findings suggest that California mice have high basal levels of Fos and FosB in several areas of the brain in the absence of parental behaviour. For example, California mice appear to have high basal levels of Fos in the MPOA, NAS, BNST, and piriform cortex. However, basal levels of Fos and FosB proteins are normally low in the adult rat brain (Herdegen et al., 1995). In experiment 3, although mice in the no exposure groups did not interact with pups, the male and female of each pair were housed individually in the same room. Therefore, it is likely that they could hear and see each other, which may explain the high levels of Fos proteins in the absence of pup exposure.

Besides a decrease in c-Jun-ir in the piriform cortex of mice with pup exposure, *c-jun* expression did not change following parental behaviour in

California mice. There was dense *c-jun* expression in the dentate gyrus and piriform cortex of mice in all groups. Likewise dense basal c-Jun expression has been reported in the rat visual cortical layer V (Herdegen et al., 1993) and dentate gyrus (Harlan & Garcia, 1995). It is not known why there are such high basal levels of c-Jun in these areas.

5.4. Neurocircuitry of maternal behaviour revisited

The MPOA, the most relevant brain region for maternal behaviour, receives input from a variety of sources, the most important being olfactory input. Olfactory input from the primary and accessory olfactory bulbs project to the amygdala, and are possibly integrated within the medial amygdala (Numan, 1994), the region where lesions facilitate maternal behaviour in virgin rats (Fleming et al., 1980; Numan et al., 1993). The corticomedial amygdala projects to the MPOA via the stria terminalis (Raisman, 1972). Another route that the amygdala can project to the MPOA is by the medial aspect of the BNST, which in turn projects to the MPOA (Krettek & Price, 1978; Simerly & Swanson, 1986).

The lateral efferents of the MPOA and ventral BNST region innervate the LPOA, amygdala, nucleus accumbens, lateral septum, lateral hypothalamus, medial hypothalamus, posterior to the MPOA, lateral habenula, ventral tegmental area of the midbrain, periaqueductal gray, peripeduncular region of the midbrain, pedunculopontine region at the midbrain – pontine border, and the raphe nuclei (Numan, 1994; Stack et al., 2002). Of these neural connections, the MPOA to VTA projections appear to be important for maternal responsiveness (Numan &

Smith, 1984). The lateral efferents of the MPOA can reach the VTA by a direct route or by projections to the LPOA and then to the VTA (Conrad & Pfaff, 1976; Swanson , 1976). Of these two routes, there is some evidence that the MPOA – to – LPOA – to – VTA circuit is important for maternal behaviour rats (Numan et al., 1985).

It is also possible that MPOA efferents pass through the VTA to more caudal brainstem sites (Numan & Numan, 1991). The VTA has rich ascending and descending projections, including projections that reach the nucleus accumbens (Swanson, 1982). It is unclear where the MPOA efferents terminate and what processes they influence. One suggested circuit consists of the MPOA/ventral BNST – to – VTA – to – shell of the NA and lateral septum (Stack et al., 2002).

The NA receives inputs from the hypothalamus and cortical and subcortical limbic structures (de Olmos & Heimer, 1999) and projects to the ventral pallidum, which projects to the cortical and brainstem systems involved in movement control (Heimer et al., 1991). The NA is suggested to serve as a link between the limbic system and the motor system, functioning to translate motivational and emotional states into action (Mogenson, 1987). Furthermore, mesolimbic DA input to the NA modulates the transfer of information from the limbic system to the ventral pallidum via the NA (Mogenson, 1987).

The results from this thesis indicate that the MPOA is critical for the expression of male and female parental behaviour in California mice. With the exception of the MPOA data, the results from the three experiments do not

provide any additional clarification to what is known about the maternal circuit in rats. The results of this thesis call for further studies with larger and more specific lesions in the NA and a reexamination of Fos and Jun-ir with a longer pup exposure.

5.5. Comparison between male and female California mice

The high variability in the pattern of paternal care across rodent species suggests that there may be neural mechanisms of parental care that differ from the female rat model, particularly in biparental species. It is, therefore, important to examine sex differences in parental responsiveness in biparental species such as California mice and continue doing cross-species comparisons. This comparative method has been used to compare the mechanisms underlying parental care in the biparental male Djungarian hamster (*Phodopus campbelli*) with those of the nonparental male Siberian hamster (*P. sungorus*; Wynne-Edwards, 1995) and the parental male prairie vole with the nonparental male meadow vole (*M. pennsylvanicus*; McGuire & Novak, 1986; Wang & Insel, 1996), and this method may also provide insight into the neural basis of parental care in other species such as humans and nonhuman primates.

In California mice, virgin males have a larger MPOA than females as a result of a greater number of neurons in the MPOA (Gubernick et al., 1993). When males and females become parents, this sexual dimorphism disappears because of a volumetric increase in the size of MPOA neurons in females, which may be related to an estrogen-dependent activational effect (Gubernick et al.,

1993). In the monogamous prairie vole, there is reduced sexual dimorphism in the sexually dimorphic nucleus of the preoptic area (SDN-POA), whereas in polygamous montane voles (*M. montanus*), which show little paternal care, males have a much larger SDN-POA than females (Shapiro et al., 1991). These comparative studies suggest that in biparental species, there is less sexual dimorphism in the MPOA than in species in which only the female is parental.

With a few exceptions, the results from the three experiments are generally similar in male and female California mice. For both male and female California mice, the MPOA, but not NA lesions disrupted parental behaviour. BA lesions, on the other hand, produced deficits in parental behaviour in males only.

5.6. Behavioural profile of California mice

Our data suggest that parental behaviour does not alter gene expression in the MPOA. It is not clear whether *c-fos, fos B,* and *c-jun* activation is unimportant for parental behaviour in California mice, as the results of experiment 3 would suggest. With a vast amount of literature showing that elevated *c-fos* expression occurs following maternal behaviour in rats (Fleming & Korsmit, 1996; Fleming & Walsh, 1994; Kalinichev et al., 2000; Numan & Numan, 1994; Numan & Numan, 1995; Walsh et al., 1996), voles (Katz et al., 1999), and mice (Calamandrei & Keverne, 1994), and following paternal behaviour in voles (Kirkpatrick et al., 1994b), it is difficult to entertain the view that activation of *c-fos* is not important during parental behaviour in California mice. This would suggest that there was a flaw in the study.

The other possibility to consider is that in California mice, the activation of these IEGs is not important for the expression of parental behaviour. Although this view is in stark contrast to published reports in the literature, it parallels many other observations of unusual behaviour in *P. californicus*. Over the past several years, a variety of experiments have been conducted using California mice as subjects in our laboratory. In almost every study, there have been difficulties or unexpected results, making interpretation of data often impossible. For example, when California mice were tested in the elevated plus maze, a test commonly used to measure anxiety in rodents, 65 % of the mice did not complete the 5 – min test because they either had seizures, jumped off the maze, or fell off the maze. Of the 35 % of mice that did complete the test, many showed behaviours not reported in the literature, such as hanging upside down by their hind paws at the end of the open arms or walking on the upper ledge of the enclosed arms. These mice showed high levels of activity and did not show a preference for the enclosed arms of the plus maze. Thus, we suggest that the behavioural profile of California mice is one of high reactivity and escape motivation.

Our challenges with anaesthetizing mice in experiments 1 and 2 are consistent with this view. In experiment 1, mice were anesthetized with i.p. injections of ketamine and xylazine, an anaesthetic regime that normally works well in other rodents. Fourteen percent of mice died soon after they were injected, prior to being given a lesion. The reason for their deaths was unclear. As discussed in chapters 2 and 3, California mice appear to have a wide range of reactions to injectible anaesthetics, as mice often die with just an initial dose of

ketamine and xylazine, while others survive with several additional supplements of ketamine. Other laboratories have observed similar reactions in *P. californicus* (Ms. Marleen DeGroot, personal communication, Nov 2002) and in *P. maniculatus* (Dr. Mark Lewis, personal communication, April 2003). Thus, the unpredictible and variable nature of their response to injectible anaesthetics is consistent with the notion that California mice are highly reactive.

5.7. Future studies

The results of this thesis suggest that further studies need to be conducted to determine whether *fosB* and *c-jun* are important for parental behaviour in California mice. Such a study would involve allowing mice to interact with pups for varying lengths of time to examine the temporal pattern of activation of these genes. In an attempt to further examine whether Fos proteins in the MPOA are important for maternal behaviour, future studies may involve injecting antisense oligonucleotides targeting c-fos and fosB mRNA into the MPOA. If Fos expression in the MPOA is determined to be essential for maternal behaviour, then future studies should determine which extracellular signals activate Fos expression and which late-responding genes respond to the transcriptional effects of Fos proteins.

5.7. Summary

The results of experiments 1 and 2 show that 1) the MPOA is important for the maintenance of male and female parental behaviour, and that 2) the BA is

important for the expression of parental behaviour in male, but not in female California mice. There was no evidence that the NA is important for either male or female parental behaviour. However, it is unclear whether larger NA lesions would produce deficits in parental behaviour. Results of experiment 3, showed that parental behaviour increased *c-fos* expression in the MPOA, MA, and piriform and somatosensory cortices. For the most part, parental behaviour did not lead to *fosB* and *c-jun* activation. It is not clear whether these results are due to a design flaw in the study, such as small sample sizes, an insufficient length of pup stimuli or whether *fos B* and *c-jun* activation really is not important for parental behaviour in California mice. This thesis adds to the sparse literature on the neural control of paternal behaviour in a biparental species. However, the findings also call for further investigation of the pattern of activation of IEGs following parental behaviour and can be a basis for future studies in the neural control of parental behaviour in biparental species.

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