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Abstract

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Morphine disrupts full parental behavior (FPB) in adult rats when administered into the preoptic area; effects are reversed with naloxone. The current study emphasizes central administration of morphine into the preoptic area and effects on parental behavior in juveniles and possible naloxone blockade. In Experiment 1, juveniles outfitted with cannulae assemblies aimed at the preoptic region were administered a regimen of morphine, naloxone, or saline, and 30 minutes later were exposed to neonates. Behavior was scored for 1 hour and animals were considered parental if they responded with FPB for 2 consecutive days. Saline and naloxone groups responded to the pups; the morphine groups did not. Experiment 2 treated already parentally behaving juveniles with the same regimen. Again, saline and naloxone groups responded to the pups, whereas morphine groups did not. These data suggest that parental behavior in juveniles is regulated by endogenous opioid systems acting on the preoptic region.

I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Arts/Science.

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CENTRAL OPIOID REGULATION OF PARENTAL BEHAVIOR IN JUVENILE RATS

By

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Central Opioid Regulation of Parental Behavior in Juvenile Rats

Research with various species has focused on the neural, sensory, neuroendocrine, and neurochemical events surrounding the expression of parental behavior in adults. Few studies, however, have considered the development of parental behavior. The mechanisms which underlie parental behavior in the rat can be dichotomized as those controlling onset and those controlling maintenance (Numan, Rosenblatt, & Komisaruk, 1977). The onset of parental behavior at parturition has been shown to be mediated through hormonal events of late pregnancy and delivery. Maintenance depends on the interactive behavioral bond between the mother and the young which begins developing during parturition and is believed to be non-hormonally regulated (Mayer, Freeman, & Rosenblatt, 1979). A postpartum female rat normally engages in a pattern of functionally related behaviors that promote the survival and development of her young. Numerous features that shape the interactions between lactating females and pups from birth through weaning are also evident in the interactions between sensitized virgin females and foster pups (Mayer, Freeman, & Rosenblatt, 1979). This process of pup-induced parental behavior is called sensitization or concaveation.

Prepubertal rats will exhibit parental behavior to pups in the absence of the conditions of pregnancy and/or parturition, similar to the sensitized virgins discussed above (Mayer & Rosenblatt, 1979). Behaviors elicited from these sensitized juveniles include nest-building, retrieval and grouping of pups, licking of the anogenital region to

stimulate urination and defecation, lying close to or on top of the pups, and the adoption of a crouching posture over the pups (Mayer & Rosenblatt, 1979; Mayer, Freeman, & Rosenblatt, 1979). Juvenile rats (20-30 days of age) of both sexes show a rapid onset (2-3 day latency) of full parental behavior (FPB) which includes the retrieval and grouping of, and crouching over pups (Bridges, Zarrow, Goldman, & Denenberg, 1974; Brunelli, Shindledecker, & Hofer, 1985; Gray and Chesley, 1984; Mayer & Rosenblatt, 1979). In these particular behavioral aspects, therefore, juvenile rats resemble adult postpartum females. The rapid onset of parental behavior at this age stands in marked contrast to the adult expression of the behavior, where in nulliparous females the latency to respond to pups approximates 4-6 days and in males, 6-8 days (Rosenblatt, 1967; Rosenblatt, Mayer, & Siegel, 1985; Samuels & Bridges, 1983). Furthermore, within juveniles there is an apparent reversal of the sex difference that normally accompanies pup-induced parental behavior in adults: Juvenile males respond more rapidly to foster young than juvenile females, whereas adult males take longer to respond to pups than adult females (Bridges et al., 1974; Gray & Chesley, 1984).

In an attempt to localize where in the brain parental behavior is mediated, Numan (1974) showed that the medial preoptic area (MPOA) and its lateral connections are essential for the normal display of parental behavior in postpartum lactating female rats. Furthermore, he found that the MPOA was involved in both the onset and maintenance of

parental behavior. Numan, Rosenblatt, and Komisaruk (1977) discovered a disruption of parental behavior when they caused lesions in the MPOA, whereas estrogen implants at this same site facilitated the onset of the behavior. These results strengthen the position that the MPOA is directly involved in the mediation of this particular behavior. Again in 1980, Numan and Callahan found that by severing the lateral connections of the MPOA in lactating rats as well as in virgin females which had displayed parental behavior as a result of pup exposure, parental behavior was disrupted. More severe deficits in parental behavior were found by cutting the anterior connections of the MPOA. Based on their findings, they concluded that the MPOA and its connections are important for all components of parental behavior.

Knowing that morphine disrupted, and naloxone restored, parental behavior in postpartum female rats and that the POA served as the site of control for parental behavior, Rubin and Bridges (1984) examined the ability of central morphine administration to disrupt parental responsiveness in both lactating mothers and pup-induced nulliparous females that had displayed parental behavior for two consecutive days. Upon administration of morphine directly to the POA, parental behavior was disrupted. They concluded that the behavioral effects of central morphine administration are site-specific since no disruption of parental behavior occurred with a central administration of morphine to the ventromedial hypothalamus (VMH). Furthermore, they found that a concurrent administration of naloxone with morphine into the MPOA reversed the

disruptive effects of morphine and normal levels of parental responsiveness were exhibited. These studies show that morphine affects parental behavior by way of an opiate receptor mechanism and that morphine's effect on parental behavior is indicative of endogenous opioid systems mediating parental behavior by acting on the preoptic region.

Laboratory and field research has shown that the juveniles of many species perform various parental-like behaviors including food provision, defense, and retrieval of young (Brunelli & Hofer, 1990). Recent studies have suggested that endogenous opioid systems may play an inhibitory role in the control of parental behavior in the rat. Bridges and Grimm (1982) found that a systemic injection of morphine sulfate in pregnant rats significantly disrupted the onset of parental behavior and that concurrent administration of naloxone, an opiate antagonist, with morphine reversed the disruptive effects of morphine on parental behavior. Given the behavioral similarities between the parental responsiveness of lactating females and juvenile animals, would morphine decrease parental behavior in the pre-pubertal, and obviously, non-lactating animal? Juvenile male and female animals (25 days of age) treated with morphine (5.0 mg/kg) and saline failed to respond to 1- to 6-day-old neonates over a 10 day period (Kinsley, Wellman, Graham, & Carr, 1993). Animals which had been injected with morphine and naloxone (0.5 mg/kg) or saline and saline responded in just a few days. In a second experiment, juvenile male and female rats (25 days of age) which had been sensitized to foster neonates and then treated with morphine (5.0 mg/kg) and saline failed to respond to pups with FPB. On the

other hand, sensitized juveniles that had been treated with morphine and naloxone (0.5 mg/kg) or saline and saline responded quickly to pups. In keeping with findings from past research, juvenile male rats, regardless of treatment, responded to pups more rapidly with FPB than juvenile females (Kinsley et al., 1993). These data suggest that the onset and maintenance of parental behavior in juvenile animals appear to be regulated by opiates. The current work attempts to determine if central administration of the opiate morphine in the preoptic area disrupts parental behavior in juvenile rats. Furthermore, will blockade of morphine's effects with the narcotic antagonist naloxone restore the behavior within the juveniles? These proposed studies are an extension of our earlier findings on systemic injections. Together, this work will shed light on the developmental mechanisms underlying the regulation of parental behavior.

Method

Animals

Female Sprague-Dawley rats originally purchased from Charles River Laboratories, Inc. were timed-mated with stud males. Following positive sperm identification in the vaginal lavage, the females were isolated until parturition. At 21 days of age (day of birth = Day 0), offspring of both sexes were housed individually in 20 x 45 x 25 polypropylene cages, the floors of which were covered with pine shavings. Food (Purina rat chow) and water were available ad libitum and all animals were housed in light - (on from 0500-1900h) and temperature - (21-24°C) controlled testing rooms. Animals

used in this study were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee (I.A.C.U.C.) of the University of Richmond and the I.A.C.U.C. on Care and Use of Laboratory Animals Resources, National Research Council (DHHS Publication No. [NIH] 85-23: Revised, 1985).

Stereotaxic surgery

At 21 days of age, each animal was outfitted with bilateral, double-barreled cannulae assemblies aimed at the preoptic area consisting of 23-gauge outer guide cannulae and 28-gauge inserts. Animals were anesthetized with sodium pentobarbital and guide cannulae were implanted using standard stereotaxic procedures and a Kopf stereotaxic instrument. Implant coordinates were chosen according to the atlas of Sherwood and Timiras (1970). Since the animals do not start testing until 25 days of age, 4 days after stereotaxic surgery, implant coordinates were chosen taking into account any maturation by averaging the anterior-posterior, medial-lateral, and dorsal-ventral measurements of rats 21 and 39 days of age. Using the auditory meatus as the zero or reference point, the preoptic area for rats 21 days of age is 6.2 mm anterior-posterior, 2 mm medial-lateral, and 3.2 mm dorsal-ventral. For rats 39 days of age, the anterior-posterior, the medial-lateral, and the dorsal-ventral measurements are 6.5 mm, 3 mm, and 3 mm, respectively. Two millimeters were added to the dorsal-ventral measurement, taking into account the thickness of the skull and the meninges. Guide cannulae were cut to 5 mm in length and lowered into the brain through two small holes drilled into the skull

with a dental drill. The implant was secured to the skull with dental cement and the skin sutured around it. Dummy insert cannulae cut to just the same length as the guide cannulae were placed in the guide cannulae between testing periods. Removable insert cannulae with empty lumen were cut 1 mm longer than the guide cannulae to reach the specific brain sites.

Drug administration

Drugs were administered to the CNS via infusion of a solution containing the drug directly into the POA. Drugs were dissolved in sterile saline and a total volume of 0.5 µl of the solution was infused bilaterally through the insert cannulae at a rate of 1.0 µl/min. using an automatic syringe infusion pump (Harvard). A period of 30 seconds followed each infusion to allow for diffusion and to prevent backflow.

Procedure

Experiment 1 - Initiation:

All testing began at 0800h at 25 days of age. In this first phase of the study, the juvenile males and females were administered one of three regimens (N = 6/group): Morphine (0.5 µg/0.25 µl saline) + saline; morphine + naloxone (0.25 µg/0.25 µl saline); or saline (0.25 µl) + saline (0.25 µl). To initiate testing, thirty minutes after treatment three 1- to 6-day-old freshly suckled pups obtained from donor mothers were proffered to the subjects and their behavior was scored continuously for 15 minutes and then spot

checked again at 30, 45, and 60 minutes. The behaviors scored (and hereafter designated as FPB) consisted of retrieving, grouping, and crouching over young during the test session. The treatment of the animal was unknown to the observer when the behaviors were scored. The animals were scored as fully parental if they retrieved the three test young, grouped them, and crouched over them within the 60 minute test period for two consecutive testing days. The latency of the animal (in days) to exhibit a parental response was based on the first day of two consecutive test sessions on which FPB was observed. For example, if an animal displayed FPB on day 3 and then again on day 4, a latency of 3 days was assigned. Each animal was exposed to rat pups continuously for the duration of testing. On each day at the time of testing, the position of the juvenile and the pups which had remained in the cage overnight was recorded. Subsequent to this pretest, regular testing occurred 30 minutes later.

Experiment 2 - Maintenance

During this portion of the study, juvenile male and female rats (N = 6/group) were exposed to three freshly suckled 1- to 6-day-old neonates for a period of days until they displayed two consecutive days of FPB. On each day, in the absence of any treatments, each animal's behavior was scored continuously for 15 minutes and then checked again at 30, 45, and 60 minutes. On the day following the second day of FPB, three different groups were treated with the same three regimens as in the initiation phase of the experiment: Morphine (0.5 μg/0.25 μl saline) + saline; morphine + naloxone (0.25

μg/0.25 μl saline); or saline (0.25 μl) + saline (0.25 μl). Thirty minutes later, animals were exposed to three recently fed 1- to 6-day-old neonates and behavior was scored over a 60 minute period at 15 minute intervals. Central administration to the POA took place for two consecutive days with parental behavior being scored as before on both Days 1 and 2. Each animal was exposed to pups continuously for the duration of testing. On each day at the time of testing, the position of the juvenile and pups which had remained in the cage overnight was recorded. Subsequent to this pretest, regular testing occurred 30 minutes later.

Histological analysis

Verification of cannulae placement sites occurred no later than 7 days post-testing. Following completion of all behavioral testing, all animals were sacrificed with an overdose of sodium pentobarbital and then perfused intracardially with saline followed by 10% formalin. The brain was sectioned at 80 μm intervals in the area of the cannulae and stained with thionin. Cannulae placement was correlated with parental responsiveness.

Statistical analysis

In the first experiment, to determine the overall significant difference in the latencies to the onset of FPB among the 3 groups, the Kruskal-Wallis H statistic was used. The Mann-Whitney U statistic was used to analyze the differences when comparing the latencies between 2 groups. To analyze the results of the maintenance phase of the

experiment, the percentage of animals displaying FPB after treatment was calculated and the Fishers' exact probability test was used to evaluate the number of subjects per group that responded with the various components of maternal behavior during testing.

Results

It was predicted that those animals administered morphine into the POA would show little or no parental responsiveness to pups, as reflected by either a failure to respond with full or partial FPB, or by displaying a significantly longer latency to do so. Those receiving a concurrent infusion of morphine and naloxone and those treated with only saline were predicted to exhibit normal levels of parental responsiveness toward the foster young.

Experiment 1 - Initiation:

Figure 1 displays the median latencies for the three treatment groups (MOR + SAL, MOR + NAL, or SAL + SAL) to exhibit full parental behavior for Experiment 1. It is evident that morphine had a significant effect on the parental behavior displayed by juvenile rats. For the males, the overall Kruskal-Wallis revealed a significant effect of morphine treatment on the latency to display 2 consecutive days of FPB, ($H = 7.94$, $p < 0.05$). Further, latencies to display 1 day of FPB ($H = 12.03$, $p < 0.01$), to retrieve one ($H = 7.80$, $p < 0.05$), two ($H = 8.54$, $p < 0.05$), and three pups ($H = 8.17$, $p < 0.05$), to group ($H = 11.94$, $p < 0.01$), and to crouch over pups ($H = 9.77$, $p < 0.01$) were significantly

extended by morphine treatment in juvenile males.

The follow up with the Mann-Whitney U showed that, in males, latencies to display 2 consecutive days of FPB were significantly protracted in the MOR + SAL group vs. the SAL + SAL group ($U = 6.0$, $p < 0.05$) (see figure 1), as were latencies to exhibit 1 day of FPB ($U = 0$, $p < 0.001$), to retrieve one ($U = 2$, $p < 0.01$), two ($U = 2$, $p < 0.01$), and three pups ($U = 3.5$, $p < 0.01$) (see Table 1). Further, latencies to group pups ($U = 0$, $p < 0.01$), and to crouch over the pups ($U = 1$, $p < 0.01$) were significantly longer for the MOR + SAL group relative to the SAL + SAL group. Moreover, the MOR + SAL group required significantly longer than the MOR + NAL group to display 2 consecutive days of FPB ($U = 0$, $p < 0.001$) (see figure 1), to display 1 day of FPB ($U = 0$, $p < 0.001$), to retrieve one ($U = 5$, $p < 0.05$), and three pups ($U = 3$, $p < 0.01$), to group pups ($U = 0$, $p < 0.001$), and to crouch over the pups ($U = 8$, $p < 0.05$) (see Table 1). The only significant difference in any behavioral measure between the MOR + NAL and the SAL + SAL groups was the latency to retrieve 2 pups ($U = 6.5$, $p < 0.05$), and the latency to crouch over pups ($U = 4.5$, $p < 0.05$), demonstrating that naloxone was mostly capable of antagonizing the disruptive effects of morphine on the parental behavior displayed by juveniles.

For the juvenile females in experiment 1, sample size for both the MOR + SAL and SAL + SAL groups was only 5 instead of the intended 6. Unfortunately, data was discarded after the histological analysis due to either dehydration of the brain tissue or

because cannulae placement could not be accurately determined. The Kruskal-Wallis revealed that morphine treatment was similarly disruptive on the latencies to retrieve one ($H = 8.11$, $p < 0.05$), two ($H = 8.42$, $p < 0.05$), and three pups ($H = 7.71$, $p < 0.05$), to group pups ($H = 8.22$, $p < 0.05$), and to crouch over pups ($H = 9.12$, $p < 0.05$).

Individual comparisons within the female groups showed that the MOR + SAL group took significantly longer than the SAL + SAL group to display two consecutive days of FPB ($U = 3.0$, $p< 0.05$) (see figure 1), to display one day of FPB ($U = 2.5$, $p < 0.05$), to retrieve one ($U = 2.0$, $p < 0.01$), two ($U = 2.5$, $p < 0.05$), and three pups ($U = 3.5$, $p < 0.05$), to group pups ($U = 2.5$, $p < 0.05$), and to crouch over pups ($U = 0$, $p < 0.001$) (see Table 1). Furthermore, MOR + SAL females took significantly longer than MOR + NAL females to display one day FPB ($U = 4.5$, $p < 0.05$), to retrieve one ($U = 0.5$, $p < 0.01$), two ($U = 0.5$, $p < 0.01$), and three pups ($U = 0.5$, $p < 0.01$), to group ($U = 0.5$, $p< 0.01$), and to crouch over the pups ($U = 1$, $p < 0.01$) (see Table 1). There were no significant differences between MOR + NAL animals and SAL + SAL animals.

There were few individual sex differences in behavior. The Mann-Whitney U statistic revealed that there were no significant differences in any behaviors between male and female MOR + SAL or MOR + NAL groups. However, there were significant differences between male and female SAL + SAL groups for retrieving two pups ($U = 4.5$, $p < 0.05$) and for crouching over pups ($U = 5.5$, $p < 0.05$) (see Table 1).

Experiment 2 - Maintenance:

Experiment one demonstrated that morphine disrupted, and naloxone could reverse, the initiation of parental behavior displayed by male and female juvenile rats. Experiment 2 sought to examine how morphine would affect the established behavior in sensitized juvenile males and females (fig. 2, Table 2). For males, following two consecutive days of FPB in the absence of treatment, significantly fewer of the MOR + SAL group displayed FPB on the first day of injection compared to either the MOR + NAL or SAL + SAL groups (MOR + SAL vs. MOR + NAL, $P = 0.001$, $p < 0.05$; vs. SAL + SAL, $P = 0.001$, $p < 0.05$). There were no significant differences between MOR + NAL and SAL + SAL males.

For females, sample size for all three groups was 5 instead of 6 due to loss of data after the histogical analysis. Significantly fewer MOR + SAL treated females displayed FPB compared to both MOR + NAL and SAL + SAL animals (MOR + SAL vs. MOR + NAL, $P = 0.024$, $p < 0.05$; vs. SAL + SAL, $P = 0.004$, $p < 0.05$). There were no significant differences between MOR + NAL and SAL + SAL females.

For males, additional analyses revealed that fewer MOR + SAL than MOR + NAL animals retrieved one ($P = 0.001$, $p < 0.05$), two ($P = 0.001$, $p < 0.05$), and three pups ($P = 0.001$, $p < 0.05$), grouped pups ($P = 0.001$, $p < 0.05$), and crouched over pups ($P = 0.001$, $p < 0.05$) on this first day of injection. For MOR + SAL vs. SAL + SAL, there were significant differences for retrieving one ($P = 0.001$, $p < 0.05$), two ($P = 0.001$, $p < 0.05$), and three pups ($P = 0.001$, $p < 0.05$), grouping pups ($P = 0.001$, $p < 0.05$), and

crouching over pups ($P = 0.001$, $p < 0.05$). There were no significant differences between MOR + NAL and SAL + SAL.

For females, significantly fewer animals from the MOR + SAL group than from the MOR + NAL group retrieved one ($P = 0.024$, $p < 0.05$), two ($P = 0.024$, $p < 0.05$), and three pups ($P = 0.024$, $p < 0.05$), grouped pups ($P = 0.024$, $p < 0.05$), or crouched over pups ($P = 0.024$, $p < 0.05$). For MOR + SAL vs. SAL + SAL, there were significant differences in retrieving one ($P = 0.004$, $p < 0.05$), two ($P = 0.004$, $p < 0.05$), and three pups ($P = 0.004$, $p < 0.05$), grouping pups ($P = 0.004$, $p < 0.05$), and crouching over pups ($P = 0.004$, $p < 0.05$). There were no significant differences between MOR + NAL and SAL + SAL.

There were no significant sex differences between MOR + SAL males and females; in both groups parental behavior was equally disrupted. There were no significant sex differences between MOR + NAL and SAL+ SAL males and females.

Discussion

Previous research suggests that endogenous opioids act to depress parental responsiveness. The data in the present study demonstrate that the display of parental behavior in the juvenile animal is under the influence of the endogenous opioid system. Central administration of the opiate morphine into the preoptic area of the juvenile rat disrupted parental behavior and similar treatment with naloxone, a narcotic antagonist, restored the behavior in both males and females. These effects were seen during the initial

exposure to neonates (Experiment 1, Initiation), as well as in juveniles that had been sensitized to young and in which parental behavior had been established (Experiment 2, Maintenance). In both experiments, when the juveniles were treated with morphine plus saline, the animals made little or no attempt to be parental. This disruption in behavior seemed specific; most of the morphine-treated animals approached the pups and made contact by either licking or sniffing the pups, while others ignored the neonates and spent most of the time either eating or jumping around the cage. The dose of morphine used in this study had not previously been shown to result in a loss of motor capabilities (Kinsley & Bridges, 1988a). In either the saline plus saline or the combination of morphine plus naloxone condition, there was no effect on the display of parental behavior; males and females exhibited retrieving, grouping, and crouching toward the three neonates. Interestingly, for the females that received morphine plus saline in the first experiment, the latency to display full parental behavior for two consecutive days was significantly longer than for those receiving saline plus saline but was not significantly different from those receiving morphine plus naloxone. These results were not as expected; the morphine plus saline group was predicted to have a significantly longer latency to display the behavior than the morphine plus naloxone group. However, small sample size within each group and variability in sample size between the groups could possibly explain why the results were not as expected and did not follow the same trend as in the previous experiment with systemic injections of morphine and naloxone (Kinsley et al., 1993). Collectively,

however, the data support the view that opioids appear to regulate maternal-like behavior in juveniles as they do in adult female rats (Bridges & Grimm, 1982; Grimm & Bridges, 1983). Other studies also support the view that an endogenous opioid system is developing at this time in juveniles (Johnston & Negro-Vilar, 1986). Therefore, as Zais et al. (1996) point out, it is possible that increasing endogenous opiate activity during this prepubertal period from approximately 24-30 days of age is responsible for the decreased responsiveness of juveniles to foster young by 30 days of age.

As previously discussed, within juveniles there is a reversal of the sex difference that normally accompanies pup-induced parental behavior in adults; juvenile males respond more quickly to foster young that do juvenile females. In the present experiment, however, there were few significant sex differences in the display of parental behavior. In the first experiment, there was a significant sex difference among the saline plus saline groups in the latency to retrieve two pups and to crouch over the pups. There were no significant sex differences in Experiment 2. Once again, small group numbers and variability in sample size between the groups could account for these unexpected results.

If endogenous opioids do inhibit maternal-like behavior, the question becomes on what particular area of the brain, important for the control of the behavior, are the opioids acting. The data from the present experiment demonstrates that the preoptic area is essential for the display of parental behavior in juveniles as well as in adult female rats (Numan, 1974; Numan, Rosenblatt, & Komisaruk, 1977; Numan & Callahan, 1980; Rubin

& Bridges, 1984). The medial preoptic area is part of the basal forebrain; this area lies just rostral to anterior hypothalamus and just caudal to the diagonal band-septal area. Lateral to the medial preoptic area is the lateral preoptic area and dorsal are the medial aspect of the bed nucleus of the stria terminalis and the anterior commissure (Numan, 1994). The projections of the medial preoptic area are extensive and include regions thought to be important in the control of reproductive and maternal behaviors (Sachs & Meisel, 1994; Pfaff, Schwartz-Giblin, McCarthy, & Kow,1994; Numan, 1992).

Neural control of maternal behavior in the female rat has been extensively investigated (Numan, 1994). Early studies focused on the establishment of the medial preoptic area as a structure in the brain that is important for the occurrence of parental behavior (Jacobson, Terkel, Gorski, & Sawyer, 1980; Numan, 1974; Numan, Corodimas, Numan, Factor, & Piers, 1988). More recent studies emphasize the medial preoptic area as a part of a larger neural circuitry and attempt to define the parentally relevant afferents and efferents of the medial preoptic area. Evidence as to which particular medial preoptic efferents may be important for parental behavior has been provided by studies that used the knife-cut technique; these studies demonstrated that it is the lateral efferent projections from the medial preoptic area that are critical for maternal behavior (Numan, 1988; Franz, Leo, Steuer, & Kristal, 1986; Miceli, Fleming, & Malsbury, 1983; Numan, 1974; Numan & Callahan, 1980; Numan & Corodimas, 1985; Terkel, Bridges, & Sawyer, 1979).

Studies have attempted to discover to what structures the efferents of the medial

preoptic area project to exert its influence. Numan and his colleagues have explored the possibility that the medial preoptic area influences the ventral tegmental area of the ventromedial midbrain and that this projection is important for parental behavior. Two routes by which the medial preoptic area projects to the ventral tegmental area were discovered: The medial preoptic area can reach the ventral tegmental area directly or indirectly through the lateral preoptic area. Evidence suggests that it is the indirect route that is most important for the control of parental behavior; the critical medial preoptic area lateral efferents first synapse in the lateral preoptic area and these efferents then descend via the lateral hypothalamus to the ventral tegmental area (Numan, Morrel, & Pfaff, 1985; Numan & Smith, 1984). There is also evidence that the medial preoptic area projects to the paraventricular nucleus of the hypothalamus influencing the oxytocinergic pathways; this projection of the medial preoptic area is important because several studies have indicated that these pathways play a positive role in promoting parental responsiveness (Pedersen & Prange, 1987).

Preoptic neurons not only terminate in the ventral tegmental area but as evidence has shown, actually pass through the ventral tegmental area to reach more caudal brainstem regions (Numan & Numan, 1991; Conrad & Pfaff, 1976; Swanson, 1976; Swanson, Mogenson, Gerfen, & Robinson, 1984; Swanson, Mogenson, Simerly, & Wu, 1987). Numan attempted to examine the role of the ventral tegmental neurons in the

control of parental behavior by injecting an axon-sparing excitotoxic amino acid into the ventral tegmental area of postpartum rats and he found that parental behavior was not disrupted; thus, it appears that the fibers which pass through the ventral tegmental area may be important for parental behavior. It is unknown as to where preoptic efferents important for parental behavior traveling through the ventral tegmental area to other regions of the brainstem are terminating and what processes they are influencing; however, Numan (1994) outlines two possibilities. Warranting further investigation into their role in parental behavior are two structures caudal to the ventral tegmental area that receive substantial input from preoptic efferents: the caudal periaqueductal gray and the central tegmental field which includes the pedunculopontine region. The periaqueductal gray influences sensory and motor functions within the trigeminal system which may be important since the main long-term effect of preoptic damage on parental behavior is interference with retrieval (Corter & Fleming, 1990; Panskepp, 1991). Thus, it is likely that preoptic efferents to the brainstem are ultimately influencing neural sites involved in oral and perioral sensorimotor integration (Numan, 1994). Although the exact brainstem termination sites of preoptic efferents important in the regulation of parental behavior are as yet undetermined, Numan provides a working model which will guide future research.

Most of the research so far on the medial preoptic area has focused on the efferent projections important for parental behavior to the brainstem; the preoptic area, however, also projects to areas of the diencephalon and the telencephalon, both of which may

contribute to the regulation of parental responsiveness. Evidence suggests that preoptic projections to the lateral subdivisions of the habenular complex may influence certain aspects of parental behavior (Corodimas, Rosenblatt, & Morrell, 1992; Corodimas, Rosenblatt, Canfield, & Morrell, 1993; Corodimas, Rosenblatt, Matthews-Felton, & Morrell, 1995). Radiofrequency lesions of the habenular complex disrupted pup retrieval, nursing, and nest building. Previous work has shown that radiofrequency lesions on the lateral habenular complex produced deficits in the hormonally mediated onset of parental behavior and not on postpartum maintenance of the behavior (Corodimas, Rosenblatt, & Morrell, 1992; Corodimas et al., 1993). Knowing that estrogen acts on the medial preoptic area (Numan, 1994), Matthews-Felton, Corodimas, and Rosenblatt (1995) hypothesize that estrogen receptor-containing neurons of the medial preoptic area project to the lateral habenular complex to influence its role in the regulation of parental behavior. Future studies will focus on the neurochemistry and neural circuits of the lateral habenular complex and their role in the hormone-dependent display of parental behavior.

Although most research has emphasized the efferent projections of the medial preoptic area, sources of afferent input to the medial preoptic area are also necessary for the occurrence of the parental behavior. Olfactory input reaches the medial preoptic area via the medial amygdala and bed nucleus of the stria terminalis and is probably the most important neural input to the medial preoptic area (Numan, 1994). The medial amygdala, which receives input from both the accessory olfactory bulb and the main olfactory bulb,

projects to the medial preoptic area both directly and indirectly via a synapse in the bed nucleus of the stria terminalis. Fleming, Vaccarino, & Luebke (1980) hypothesized that the medial amygdala may be a part of the neural circuit underlying olfactory inhibition of parental behavior in nulliparous rats. These researchers found that lesions of the bed nucleus of the stria terminalis also facilitated parental behavior in virgin rats thus further supporting the hypothesis that olfactory input may inhibit parental behavior by depressing the activity of the medial preoptic area.

Studies have found that the median raphe projects to and through the medial preoptic area to enter the diagonal band-septal region (Azmitia & Segal, 1978). Simerly, Swanson, & Gorski (1984) provide evidence that both serotonergic and non-serotonergic neurons from the raphe nuclei project to the medial preoptic area; studies suggest that the serotonin neurons may play a role in regulating parental behavior (Copenhaver, Schalock, & Carver, 1978; Moore & Hampton, 1974). Damage to the median raphe serotonin neurons caused a partial disruption of several of the components of parental behavior and an increase in the incidence of infanticide (Barofsky, Taylor, Tizabi, Kumar, & Jones-Qaurtey, 1983). Important to note, however, is that the destruction of these neurons depletes serotonin from several regions in the brain, not just from the medial preoptic area; future research, therefore, should explore the role of serotonergic input to the medial preoptic area in parental behavior control (Numan, 1990).

Other studies have shown a projection from the locus coerulus (Berk &

Finkelstein, 1981; Vertes, 1988) and from the caudal nucleus of the solitary tract (Berk & Finkelstein, 1981; Day, Blessing, & Willoughby, 1980; Ricardo & Koh, 1978; Simerly & Swanson, 1986; Sofroniew, 1983; Vertes, 1988) to the medial preoptic area. The caudal nucleus of the solitary tract is a source of norepinephrine input to the medial preoptic area and there is the possibility that this input is involved in temperature-regulatory mechanisms (Day, Willoughby, & Geffen, 1979; Millan, Millan, & Hertz, 1983). An important relationship does exist between body-temperature regulation and parental behavior, particularly nursing behavior (Leon, Croskerry, & Smith, 1978); Numan (1994) suggests that the preoptic area may be a site where temperature relevant input acts to influence parental behavior.

Pedersen and Prange (1979) were the first to provide evidence for oxytocin's role in parental behavior. High levels of parental responsiveness were induced in virgin rats that received a subcutaneous injection of estradiol followed by administration of oxytocin into the lateral ventricle. Research has not shown that oxytocinergic pathways are necessary for the continuance of parental behavior that is already established in postpartum rats (Numan & Corodimas, 1985), although more recent studies have indicated that oxytocin's role in parental behavior is important for the onset of the behavior at parturition (Fahrbach, Morrell, & Pfaff, 1985; Van Leengoed, Kerker, & Swanson, 1987). Evidence that the medial preoptic area projects to the paraventricular nucleus of the hypothalamus is important for parental behavior because this nucleus is the

main source of oxytocinergic neural pathways within the brain and several studies have indicated that these pathways play a positive role in promoting parental responsiveness (Pedersen & Prange, 1987). Perhaps the medial preoptic area efferents to the paraventricular nucleus activate oxytocinergic neural pathways in order to promote parental behavior (Numan, 1990). Oxytocin projections and receptors are found in or near the ventral tegmental area and the medial preoptic area (De Kloet, Voorhuis, Boschma, & Elands, 1986; Insel, 1986, Jirikowski, Caldwell, Pedersen, & Stumpf, 1988; Kozlowski & Nilaver, 1986; Nieuwenhuys, 1985).

It has been suggested that changes in the levels of estrogen and progesterone in late pregnancy may influence the postpartum onset of parental behavior (Bridges, 1990; Numan, 1988; Rosenblatt, Mayer, & Giordano, 1988) by stimulating a rise in oxytocin binding sites in the ventral tegmental area and in the medial preoptic area. One study found that at parturition, there is an increase in the number of oxytocin binding sites in the bed nucleus of the stria terminalis (Insel, 1992; Insel, 1990) and that this increase is associated with a rise in the release of oxytocin into the bed nucleus of the stria terminalis region (Caldwell, Greer, Johnson, Prange, & Pedersen, 1987; Landgraf, Neumann, & Pittman, 1991). It is believed that the source of this oxytocin is the oxytocinergic pathways originating in the paraventricular nucleus and terminating in the bed nucleus of the stria terminalis. Numan hypothesizes that since the bed nucleus of the stria terminalis receives olfactory input from the amygdala, perhaps oxytocinergic input to the bed nucleus

of the stria terminalis prevents the inhibitory effects of olfactory input on parental behavior (Numan, 1994). Clearly, more work needs to be done on oxytocin's role in the expression of parental behavior in rats.

When neurons are influenced by extracellular and intracellular signals, a class of genes referred to as immediate early genes may be activated. These genes code for proteins that serve as transcriptional factors that either activate or repress other target genes: c-Fos is an immediate early gene which codes for the protein Fos (Morgan & Curran, 1991; Sheng & Greenberg, 1990). Located within the nucleus of neurons, Fos can thus serve as a marker for individual cells that are modified under certain conditions. Detection of Fos gives a picture of the neural circuits that are activated as a result of particular forms of neural stimulation. Fos-labeling studies support the findings of lesion and hormone implant studies that the medial preoptic area occupies a central role in promoting maternal behavior. For instance, Numan & Numan (1994) found that sensitized virgin females had more fos-labeled cells in the lateral preoptic area and in the bed nucleus of the stria terminalis than non-parentally responding females. Future studies on the expression of parental behavior in juveniles using fos-labeling are warranted. The mapping of neural circuits with fos-labeling could provide further information on the larger neural circuitry within which the medial preoptic area operates to influence parental behavior.

Opiates have been shown to inhibit parental behavior in adult rats (Bridges &

Grimm, 1982; Grimm & Bridges, 1983; Kinsley & Bridges, 1988b) and in juvenile animals (Kinsley et al., 1993). These studies support the view that parental behavior is under an inhibitory influence by opioids. Endogenous opioid peptides and highly selective opioid receptors in the brain were discovered after observations of the potent behavioral effects of opiates. Neurons expressing three different opioid precursors, pro-enkephalin, pro-dynorphin, and pro-opiomelanocortin are distributed throughout the central nervous system. Each of these precursors are enzymatically cleaved to yield opioid peptides that serve as neuromodulators and neurotransmitters. These peptides bind to one of three major classes of opioid receptors, μ, δ, κ, which are also widely distributed throughout the central and peripheral nervous systems (Loughlin, Leslie, & Fallon, 1995). The activation of these receptors usually results in an inhibition of activity (Schoffelmeer, Van Vliet, De Vries, Heijna, & Mulder, 1992). The μ receptors have high affinity for morphine-like drugs and for several endogenous opioid peptides including β–endorphin which is cleaved from pro-opiomelanocortin. β–endorphin producing neurons have widespread projections to several brain regions including the preoptic area (Kimball, 1979; Mezey, Kiss, Mueller, Eskay, O'Donohue, & Palkovits, 1985; Finley, Lindstrom, & Petrusz, 1981), an area which is rich in μ opiate receptors (Desjardins, Brawer, & Beaudet, 1990; Mateo, Higazi, & Hammer, 1992). Other areas of the brain thought to be important for parental behavior also contain μ opiate receptors including certain amygdaloid nuclei, the periaqueductal

gray, several raphe nuclei, and the ventral tegmental area (Loughlin, Leslie, & Fallon, 1995). Several studies have provided evidence that it is the β–endorphin system, acting at the level of the medial preoptic area, that is inhibitory for parental behavior and that this inhibition is the result of β–endorphin binding to μ opioid receptor sites (Mann, Kinsley, & Bridges, 1991; Mann & Bridges, 1992).

Additional findings support the view that β–endorphin is inhibitory to the parental behavior regulatory systems in the medial preoptic area; β–endorphin concentrations increase in the medial preoptic area during pregnancy but decline at parturition and remain low during the postpartum period (Hammer & Bridges, 1987; Bridges & Ronsheim, 1987). Importantly, these changes in opiate levels can be induced by treatment with estradiol and progesterone in a manner similar to the conditions of normal pregnancy (Bridges & Ronsheim, 1987). Throughout most of pregnancy in the rat, peripheral plasma levels of progesterone are high, reaching peak levels on days 14 and 15 of a 22 day pregnancy, but a large fall in progesterone levels precedes parturition. Towards the end of pregnancy, estradiol levels begin to rise. Over the final two days of pregnancy there is also a peak in prolactin levels (Amenomori, Chen, & Meites, 1970; Linkie & Niswender, 1972; Morishige, Pepe, & Rothchild, 1973). Prolactin concentration is high at parturition making it an important hormone in the facilitation of the occurrence of parental behavior. The rise in β–endorphin levels during pregnancy are presumably due to the elevation in

estradiol as it has been shown that estradiol can decrease both levels of opioid in the hypothalamus (Wardlaw, Thoron, & Frantz,1982) and hypothalamic mRNA for the production of pro-opiomelanocortin (Wilcox & Roberts, 1985). Thus, it may be that these changes in opioid levels during pregnancy may underlie the alteration in parental responsiveness in the female (Kinsley, Morse, Zoumas, Corl, & Billack, 1995).

Moreover, research shows that there are changes in opiate receptor levels during the various stages of pregnancy. Hammer and Bridges (1987) have reported that μ opiate receptors increase in females that are either pregnant or that have been treated with pregnancy-like levels of estradiol and progesterone. These data suggest that a reduction of μ opiate receptors, and thus a decline in opiate level, in the medial preoptic area could facilitate parental behavior. Future studies on the concentration of opiate receptors within the juvenile rat brain could elucidate the reason for enhanced parental responsiveness; it may be that the short latency to respond to pups is due to the lack of development of opiate receptors in the medial preoptic area. Preliminary data gathered by Bridges and his colleagues suggest that in juveniles, the level of β–endorphin in the preoptic area differ between the males and females with the females having greater levels. These levels increase between the ages of 20 to 36 (Kinsley, Graham, Billack, & Bridges, unpublished observations). Thus, the data in juveniles strongly implicate a developmental role for endogenous opioid in certain regions of the brain, specifically the medial preoptic area, in

the expression of parental behavior.

Also important for the promotion of parental responsiveness in juvenile male and female rats is the prepubertal hormonal contribution as examined by Kinsley & Bridges (1988b). They discovered that males have significantly higher levels of prolactin than females and that when juvenile males were treated with bromocriptine, a prolactin-release inhibitor, parental behavior was significantly disrupted. Given prolactin's facilitatory role in parental behavior, it seems that there is an association between the levels of prolactin and responsiveness to pups in males at this age.

A large body of evidence exists that the preoptic area is involved in the control of parental responsiveness in a number of species (Miceli & Malsbury, 1982; Komisaruk, 1967; Demski & Knigge, 1971). The present work has provided significant information regarding the development of maternal behavior within the rat. In summary, parental behavior in the adult as well as in the juvenile rat appears to be under mediation by the endogenous opioid systems, especially those regulated by the μ opiate receptor (Mann, Kinsley, & Bridges, 1989); these parental behaviors develop in conjunction with the opioid systems that are influenced by the steroid (estradiol and progesterone) and the peptide (prolactin) hormones of pregnancy and lactation.

As in many previous studies, the juvenile rats in the present study displayed adequate parental behavior when the behavior was not disrupted by morphine treatment. Why are these juvenile animals so motivated to be parentally responsive? In other words,

what is the adaptive value of such behavior? Many reasons exist as to why these prepubertal animals behave in such a way towards neonates. First, these animals, just as their adult counterparts, have a genetic investment to protect. By ensuring the reproductive success of their siblings, they are also ensuring their own genetic fitness. Also, when a female rat has overlapping litters, the benefits are many to the juveniles of the first litter to help provide care to the second litter. By delaying their own breeding period, these juveniles increase their foraging efficiency and may even inherit their mother's territorial rights, thus maximizing the breeding conditions. In exchange for providing care to their siblings, these animals also benefit from their mother's protection, and their own nursing period may be extended. Another obvious advantage to early parental experience is that these animals have the opportunity to learn parenting skills that can be used later in adulthood (Gray & Chesley, 1984). Studies have shown that when rats with such experience later produce offspring, they behave parentally more readily and more efficiently. All of these benefits help protect their genetic investment. Finally, it has been shown that within both the adult female rat and the male and female juvenile rats that the site of control for parental behavior is in one area, the preoptic area, and that one neurochemical system is acting in this region. Thus, it is rational to expect that ultimately these animals are behaving parentally for the same reasons.

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Table 1.

Median Latencies in Days for Juvenile Male and Female Rats to Exhibit Full Parental Behavior (FPB) and its Various Components for Experiment 1, Initiation.

	JUVENILE MALES			JUVENILE FEMALES		
	MOR+ SAL	MOR+ NAL	SAL+ SAL	MOR+ SAL	MOR+ NAL	SAL+ SAL
FPB (2 consecutive days)	10^{a**b*}	6	3	10^{b*}	4	4
1 day FPB	10^{a**b**}	4	2	10^{a*b*}	4	4
Retrieve 1 pup	7^{a*b**}	2	1	6^{a**b**}	3	2
Retrieve 2 pups	7^{b**}	3^{b*}	1^{c}	6^{a**b*}	3	4
Retrieve 3 pups	10^{a**b**}	4	1	10^{a**b*}	3	4
Group	10^{a**b**}	4	2	10^{a**b*}	3	4
Crouch	8^{a*b**}	3^{b*}	1^{c}	6^{a**b**}	1	2
N's	6	6	6	5	6	5

Note: Overall data were analyzed with the Kruskal-Wallis H, with individual comparisons performed with the Mann-Whitney U.

*: $p < 0.05$; **: $p < 0.01$ at least.

a: Significantly different from MOR + NAL.

b: Significantly different from SAL + SAL.

c: Significant sex difference, same treatment group, $p < 0.05$.

Table 2.

Percentages of Juvenile Male and Female Rats that Exhibited the Various Components of Full Parental Behavior (FPB) for Experiment 2, Maintenance.

	JUVENILE MALES			JUVENILE FEMALES		
	MOR+ SAL	MOR+ NAL	SAL+ SAL	MOR+ SAL	MOR+ NAL	SAL+ SAL
1 Day FPB	0^{a**b**}	100	100	0^{a*b**}	80	100
Retrieve 1 pup	0^{a**b**}	100	100	0^{a*b**}	80	100
Retrieve 2 pups	0^{a**b**}	100	100	0^{a*b**}	80	100
Retrieve 3 pups	0^{a**b**}	100	100	0^{a*b**}	80	100
Group	0^{a**b**}	100	100	0^{a*b**}	80	100
Crouch	0^{a**b**}	100	100	0^{a*b**}	80	100
N's	6	6	6	5	5	5

Note: Data analyzed using the Fisher's exact probability test.

*: $p < 0.05$; **: $p < 0.01$ at least.

[a]: Significantly different from MOR + NAL.

[b]: Significantly different from SAL + SAL.

Figure Caption

Figure 1. Median latency (in days) to display full parental behavior for morphine, morphine-naloxone, and saline treated males and females. Latency to display FPB is significantly longer for morphine treated males than for morphine-naloxone and saline treated males. Latency to display FPB is significantly longer for morphine treated females than for morphine-naloxone treated females.

Figure 1: Initiation
Median Latency (in days) To Display FPB

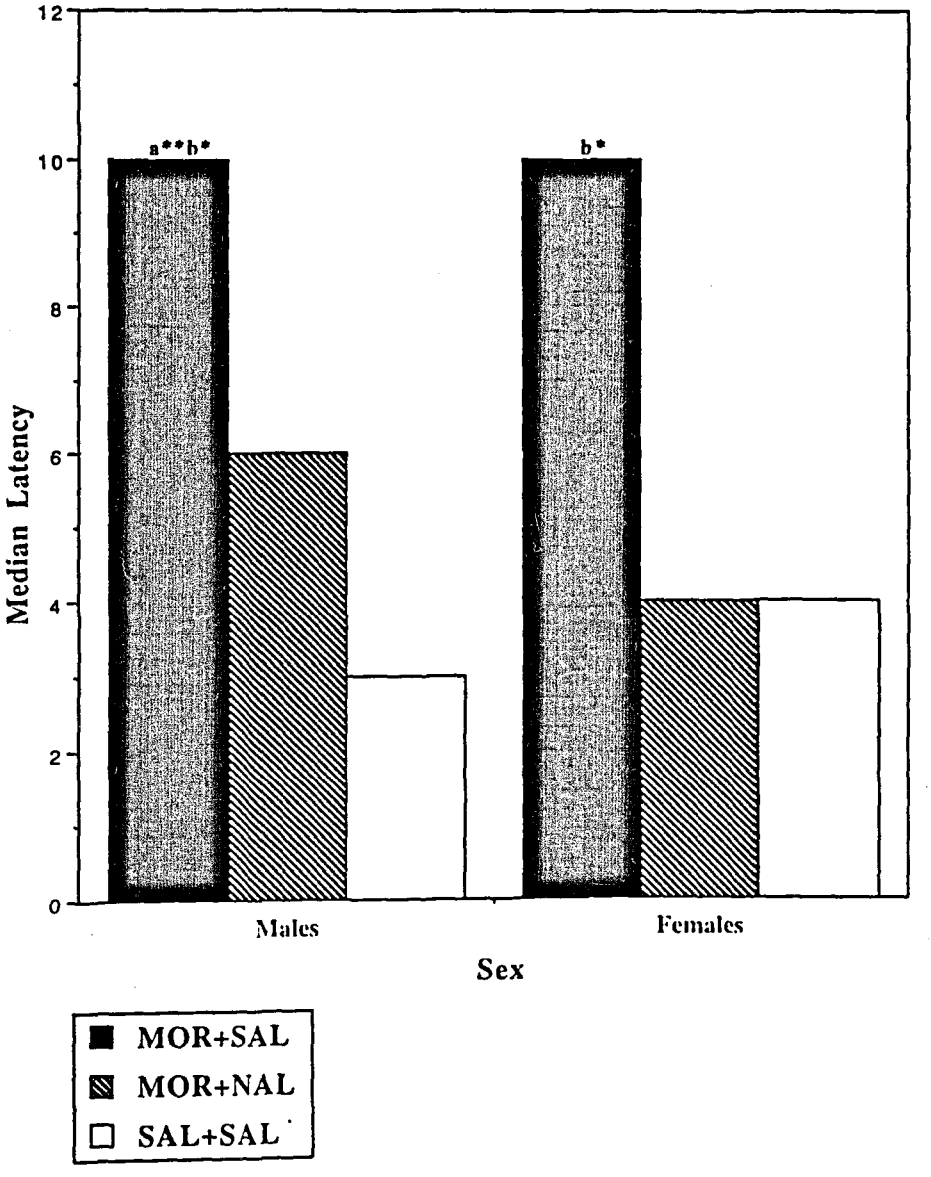
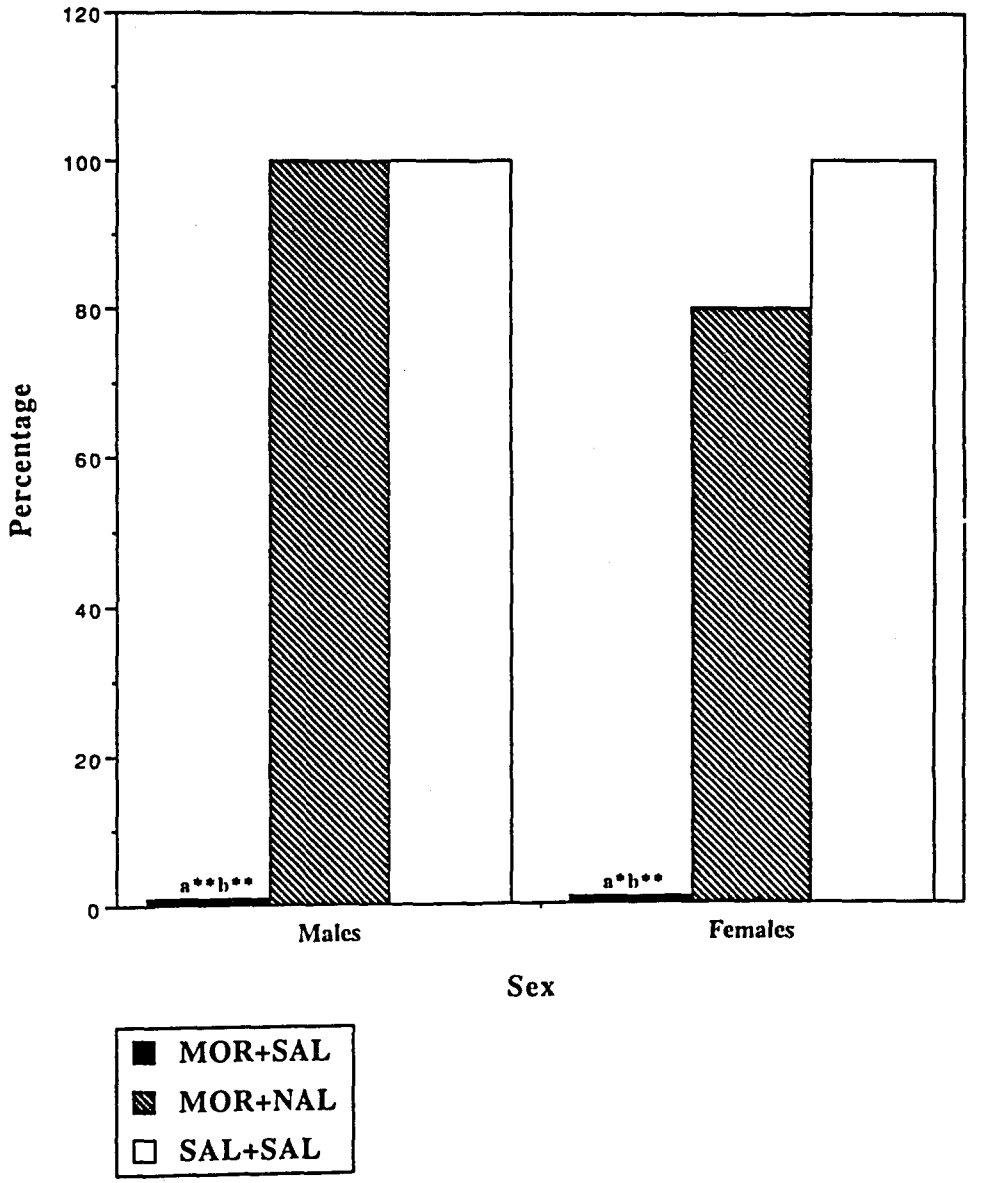


Figure Caption

Figure 2. Percentage of males and females which responded with FPB after treatment with morphine, morphine-naloxone, and saline. Significantly fewer males and females treated with morphine responded than those juveniles treated with morphine-naloxone or saline.

Figure 2: Maintenance
Percentage of Juveniles which Responded with FPB



Vita

Jacquelyn Cassandra Wellman was born on February 28, 1966 in Raleigh, North Carolina. She graduated from the University of North Carolina-Chapel Hill in 1988 where she received her Bachelor of Science degree in Biology and the equivalent to a Bachelor of Science degree in Psychology. After marrying James Labus in 1992, she moved to Charlottesville, Virginia where she currently works as a research specialist in the Cell Biology Department at the University of Virginia.