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Opiate Disruption of Maternal Behavior: Morphine Reduces, and Naloxone Restores, c-*fos* Activity in the Medial Preoptic Area of Lactating Rats

GRACIELA STAFISSO-SANDOZ,* DANIEL POLLEY,* ELIZABETH HOLT,*
KELLY G. LAMBERT† AND CRAIG HOWARD KINSLEY*[1]

*Department of Psychology, University of Richmond, Richmond VA, USA;
†Department of Psychology, Randolph-Macon College, Ashland, VA, USA

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ABSTRACT: Morphine significantly impairs maternal behavior; naloxone, an opiate antagonist, restores it. Maternal behavior is associated with c-*fos* expression, an immediate early gene product, in the medial preoptic area (mPOA) of females. In two experiments, the effects of morphine-alone and morphine plus naloxone on the expression of c-*fos* were examined. On postpartum day 5, females were injected with morphine or saline (experiment 1), and morphine + naloxone or morphine + saline (experiment 2) and placed back in the home-cage, separated from their pups by a wire-mesh partition. A separate group in experiment 1 was injected but not exposed to pups. Processing for c-*fos* immunohistochemistry commenced, and c-*fos* positive cells in a proscribed portion of mPOA were counted. Morphine-treated females had fewer c-*fos* cells in mPOA compared to saline-treated females, and the presence of pups accounted for a significant increase in c-*fos*-expressing neurons, whereas in females not exposed to pups, morphine treatment did not significantly reduce baseline c-*fos* expression (experiment 1). Furthermore, naloxone mitigated the effect as morphine + naloxone-treated females expressed more c-*fos* cells compared to morphine + saline females (experiment 2). Morphine-treated females, therefore, may exhibit reductions in maternal behavior because of relative opiate-induced inactivation of areas of the brain devoted to the regulation of maternal behavior. © 1998 Elsevier Science Inc.

KEY WORDS: Opiate receptors, fosB, Immunocytochemistry, Parental behavior, Pro oncogenes, Image analysis, Immediate early genes.

INTRODUCTION

Maternal behavior, arguably the most motivated response in which a female engages, involves two major classes of behavior, pup-directed patterns such as retrieving, grouping, licking, and nursing, and nonpup-directed patterns such as nest building, maternal aggression, foraging, etc.-all of which are important for the survival of the young [24,28]. Following a 21–22 day pregnancy, females display an immediate onset of maternal behavior toward their own or foster young that remains intense throughout the postpartum period, particularly the early half of lactation [2].

The medial preoptic area (mPOA) regulates maternal behavior in females [14,32,33,36]. For instance, lesions of the mPOA disrupt many components of maternal behavior in the rat, including retrieving, nest building, nursing behaviors, and other pup-directed responses [23,32,36]. The mPOA contains a high concentration of estrogen-binding and progesterone-binding neurons, and may be the primary central site wherein gonadal steroids such as estradiol influence the behavior [40].

Though highly motivated, maternal behavior is subject to disruption and interference. Bridges and Grimm [3] demonstrated an inhibitory influence of morphine on parental behavior in studies using pregnancy-terminated females (who normally display a rapid onset of the behavior). When the effect was blocked with the opiate antagonist naloxone, the females' parental behavior was completely restored. Grimm and Bridges [19] replicated and extended these data, showing additional disruptions in parental behavior following morphine treatment. Furthermore, Rubin and Bridges [41] demonstrated central mediation of the opioid effect by specific administration of morphine sulfate. When lactating or pup-sensitized females were infused with morphine into the preoptic area (POA), 100% of the females failed to engage in the behavior and, when naloxone was infused concurrent with the morphine, the effect was prevented. Therefore, opiates, in particular mu-receptor ligands [29], disrupt maternal behavior in a very selective, naloxone-reversible fashion.

When neurons and other cells receive extracellular and intracellular signals, a class of genes, referred to as immediate early genes (IEG), becomes active [42]. The proto-oncogene c-*fos*, a nuclear protein that serves as a transcriptional factor, is one of a class of IEG that is expressed in response to a variety of stimulus conditions and coacts with other IEG products to express other genes [31,42]. The immunohistochemical detection of c-*fos* activity can provide a glimpse of neurons that are activated as a result of particular forms of neural stimulation [31,43]. Though fos protein does not appear indiscriminately in neurons when they are

[1] Address for correspondence: Craig H. Kinsley, Ph.D., Department of Psychology, University of Richmond, Richmond, VA 23173, USA. Tel: (804) 289-8132; Fax: (804) 289-8943; *E*-mail: ckinsley@richmond.edu

active, it has been shown to be expressed in a variety of neurons responding to sensory, hormonal, neurochemical, and behavioral events [1,7,9,13,22,43]. Therefore, c-*fos*—it is believed—is a marker for behaviorally important neural activation.

When female rats and mice are exposed to young, and respond with maternal behavior, there is increased c-*fos* activity in mPOA compared to nonresponding females [8,16,36]. Fos activity, then, may be a critical marker for the neurobiological activity underlying maternal responsiveness. Garcia et al. [17] reported that acute morphine administration had a complex effect in the forebrain of rats, activating or inhibiting c-*fos* activity in a site-dependent manner. Chang and Harlan [10] reported that morphine increased c-*fos* activation in ventromedial nucleus, but not in arcuate nucleus. Therefore, given: (1) that the mPOA and neural activation therein are necessary for the display of maternal behavior; (2) that morphine may have biphasic effects on c-*fos* in the forebrain; (3) that morphine administration can disrupt maternal behavior; and (4) that naloxone can restore it, the current investigation explored whether, and to what extent, morphine and morphine in conjunction with naloxone administration affects c-*fos* activation in the mPOA. If indeed reductions occur, the data will suggest that effects on c-*fos* activation may, in part, regulate morphine disruption of maternal behavior.

MATERIALS AND METHODS

Animals

Adult nulliparous female Sprague-Dawley rats purchased from Charles River Laboratories, Inc. (Wilmington, MA) and bred in our laboratory served as subjects in the two experiments. All rats were mature adults, approximately 90 days of age, weighing 275–300 g at the beginning of the study. The females were randomly assigned to one of six treatment groups: either pup-exposed saline or morphine, or nonpup-exposed saline or morphine (experiment 1); or a separate group of females injected with morphine + saline or morphine + naloxone (experiment 2). All animals were housed in 20 × 45 × 25 cm polypropylene cages filled with loosely packed wood shavings for bedding. The animals were maintained on a 14:10 light/dark schedule (lights on: 0500), in rooms kept at approximately 24° ± 3°C. Purina rat chow and water were available ad lib. All animals used in this study were maintained according to the standards set forth by the University of Richmond Institutional Animal Care and Use Committee and the National Institutes of Health.

Procedure

All animals were timed-mated, and litters culled to five to six pups on the day of parturition. On the afternoon of postpartum day 4, a wire-mesh partition was placed in each female's cage with mother and pups on the same side. Because morphine-treated females are less likely to engage in maternal behavior [2,3,19], and would thus be expected to have little or no contact with pups relative to saline-treated control females, the partition was used in order to avoid potential confounding differences between the morphine and saline groups, and was placed in the cage 24-h prior to testing to allow the animals to accommodate to it. As described later, both groups could see, hear, and smell, but have no tactile contact with the pups on the day of testing (day 5).

On postpartum day 5, the six pups were removed from the cage, and the female was injected subcutaneously with 5.0 mg/kg morphine sulfate ($n = 6$), (a dose previously shown to have maternal behavioral, but no motoric, effects [25]), or saline ($n = 5$) (for experiment 1). Animals were observed for their reactions to the partition. Separate groups of morphine-treated ($n = 3$) and saline-treated ($n = 3$) females not exposed to pups were included for experiment 1 to compare baseline levels of c-*fos* expression in the absence of pup stimulation.

On postpartum day 5, the pups were removed from the cage, and females were injected with 5.0 mg/kg morphine + saline ($n = 3$) or morphine + naloxone (0.5 mg/kg; $n = 4$) (for experiment 2). For the pup-exposed groups in both experiments, the pups were removed for 30 min, at the end of which they were placed back in the home cage separated from the mother by the partition for 60 min. The pups were placed in a corner of the cage, approximately 20 cm from the partition. The test female could not gain access to the pups, nor was she able to contact them through the partition. Observations were made of the females' attempts to circumvent the partition to gain access to the pups, and we recorded latencies to approach and contact the partition. We wanted to ensure that all animals eventually responded by moving toward and contacting the wire-mesh partition; any animal that did not eventually respond was not included in either experiment. The nonpup-exposed females in experiment 1 were merely injected with either morphine or saline and left in the home cage with the partition. Because pups were not involved, this latter group was not tested for their behavior toward the partition. This nonpup-exposed group was then killed 1.5 h following injection with either the saline or the morphine.

c-*fos Protocol*

At the end of the 60 min of pup exposure, or 1.5 h after injection in the nonpup-exposed group (experiment 1 only), all females were given a lethal injection of sodium pentobarbital and perfused for 3 min with heparinized PBS, followed by 20 min with cold 4% paraformaldehyde. The brains were removed and post-fixed in paraformaldehyde for 60 min, followed by overnight immersion in 10% sweetened phosphate buffered saline (PBS). Brains were blocked in the coronal plane, approximately 1 mm anterior to 2 mm posterior to optic chiasm, using a brain matrix that follows the plane-of-angle provided in the atlas by Paxinos and Watson [37] (Kopf Instruments, Tujunga, CA). Blocks were attached to chucks and frozen sectioned (at −17°C) at 60 μm on a Zeiss Microm cryostat and washed 6 × 10 min in PBS. Eight sections were taken per brain, which included control sections (described later). Immediately after being cut, the sections were incubated for 15 min in 1% H_2O_2, followed by 6 × 10 min washes in PBS. The sections were processed for c-*fos* immunohistochemistry in 24-well μl plates where they were exposed to c-*fos* primary antibody (1:20,000 in PBS + 0.4% Triton-X) (Oncogene Science, Union Dale, NY), for 1 h at room temperature and then for 48 h at 4°C. This incubation was followed by 10 × 6 min rinses in PBS, then incubation in biotin-goat antirabbit IgG (1:600 in PBS + 0.4% Triton-X) (Vector Labs, Burlingame, CA) for 1 h. The sections were rinsed 5 × 10 min in PBS, and incubated in ABC (ELITE) (Vector Labs, Burlingame, CA) solution for 1 h. After this incubation they were rinsed 3 × 5 min in PBS, followed by 3 × 5 min rinses in sodium acetate. The sections were then incubated in nickel sulfate-DAB chromogen solution for 6 min. Next, the tissues were rinsed again in sodium acetate 3 × 5 min, to stop the reaction. To finalize the procedure, the sections were rinsed 3 × 5 min in PBS and then mounted onto chrome-alum-coated slides. They were left under a hood overnight, dehydrated in alcohol (70%, 90%, 100%, 1 min each), cleared in xylene, and cover slipped using Permount or Cytoseal. One section per series was saved for later verification using thionin, and one was used as a negative control for specificity of binding (which included the complete procedure described earlier, with the exception of exposure to primary antibody).

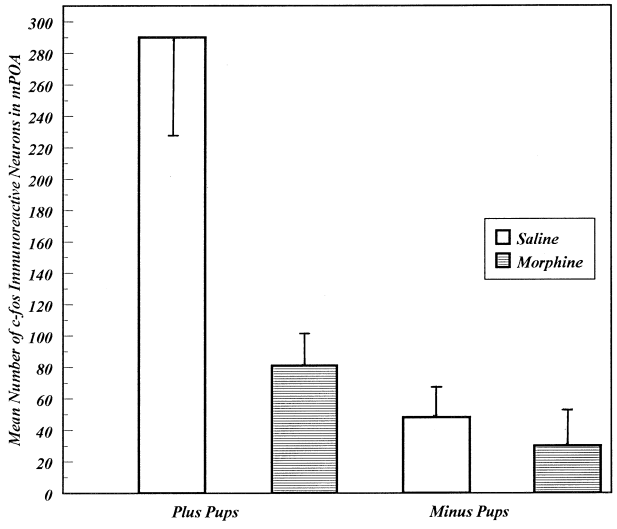

FIG. 1. Mean numbers of c-*fos* immunoreactive (IR) neurons in mPOA following morphine or saline treatment. Postpartum females were injected with 5.0 mg/kg morphine sulfate ($n = 6$) or an equal volume of saline ($n = 5$), and 30 min later allowed all but tactile contact with their own young, who were separated from the dams by a wire-mesh partition. Two groups (minus pups; $n = 3$/group) were treated the same way (i.e., injected with either morphine or saline), but not exposed to pups across the wire mesh partition. One hour later, the brains were processed for c-*fos* immunohistochemistry. Saline + pups had significantly more c-*fos* immunoreactive neurons compared to morphine + pups and saline-minus pups. There were no significant differences among morphine-minus pups, saline-minus pups, and morphine + pups. See Materials and Methods and Results for more detailed information.

Image Analysis and Quantification

c-*fos* cells were counted in the mPOA by two investigators blind to the treatment groups. We began counting cells beginning with the first section in which the anterior commissure was continuous [36]. The area selected for counting was the one with the highest concentration of well defined cells, including the black or blue–black reaction product confined to the cell's nucleus, in an area 300 μm by 300 μm from the ventral portion of the third ventricle. For accuracy, the number of cells was counted in each section of a slide, bilaterally, by each investigator, and a mean was drawn from both numbers. The number of c-*fos*-immunoreactive cells was summed and thus represents the total number of c-*fos*-expressing neurons per entire mPOA (i.e., approximately 360 μm). The cells were counted, at $\times$ 200, on a Zeiss Axioplan microscope fitted with an Optronics color camera. The camera was interfaced to a computer with a Bravado Truevision frame-grabber board, and the cells were counted using Neurolucida (Microbrightfield, Burlington, VT).

Statistics

For experiment 1, statistical analysis was performed using a two-way-analysis of variance (ANOVA) with treatment (saline vs. morphine) and pups (present vs. absent) as the independent variables, and the number of c-*fos* immunoreactive cells as the dependent variable. Post hoc tests used were the Tukey test. In experiment 2, a one-way ANOVA was used with treatment (morphine + saline vs. morphine + naloxone) as the independent variable, and the number of c-*fos* immunoreactive cells as the dependent variable. Latencies to approach and touch the partition (as measures of maternal motivation and motor activation) were analyzed using one-way ANOVA.

TABLE 1

THE EFFECTS OF MORPHINE AND NALOXONE ON TEST FEMALES' (NS) RESPONSES TO PUPS POSITIONED ON THE FAR SIDE OF A WIRE-MESH PARTITION. LATENCIES ARE IN SECONDS (± S.E.M.) AND ARE IN REFERENCE TO THE PARTITION. (THE GROUP OF NONPUP-EXPOSED FEMALES IN EXPERIMENT 1 WAS NOT TESTED FOR THEIR BEHAVIOR TOWARD THE PARTITION AND THEREFORE ARE NOT INCLUDED IN THIS TABLE)

	Latency to Approach (within 10 cm)	Latency to Contact
Experiment 1		
Morphine (6)	39.2 (21.0)	41.2 (22.5)
Saline (5)	8.0 (2.6)	9.6 (3.9)
Experiment 2		
Morphine + Saline (3)	17.6 (1.2)	25.3 (1.2)
Morphine + Naloxone (4)	13.2 (2.9)	19.0 (5.4)

RESULTS

Experiment 1

Morphine administration, at a dose (5.0 mg/kg) capable of significantly disrupting ongoing maternal behavior was found to depress c-*fos* activation in the mPOA compared to saline alone (Fig. 1). Furthermore, the presence of pups had a significant effect on, and interacted with, the activation of c-*fos*. There were main effects for treatment, $F(1, 20) = 12.2$, $p < 0.003$, and pups, $F(1, 20) = 7.3$, $p < 0.015$. More importantly, there was an interaction between treatment and pups, $F(1, 20) = 5.2$, $p < 0.036$. Post hoc analyses showed that the number of c-*fos* immunoreactive neurons

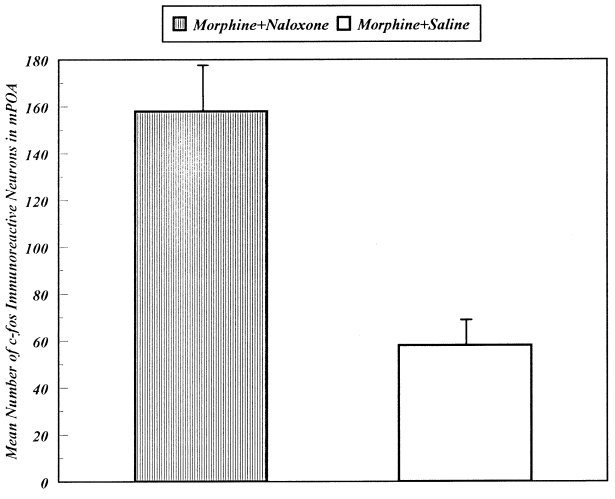

FIG. 2. Mean numbers of c-*fos* immunoreactive (IR) neurons in mPOA following morphine + naloxone or morphine + saline treatment. Postpartum females were injected with 5.0 mg/kg morphine sulfate and an equal volume of saline ($n = 3$), or morphine + 0.5 mg/kg naloxone ($n = 4$) and 30 min later allowed all but tactile contact with their own young, who were separated from the dams by a wire-mesh partition. One hour later, the brains were processed for c-*fos* immunohistochemistry. Morphine + naloxone had significantly more c-*fos* immunoreactive neurons compared to morphine + saline. See Materials and Methods and Results for more details.

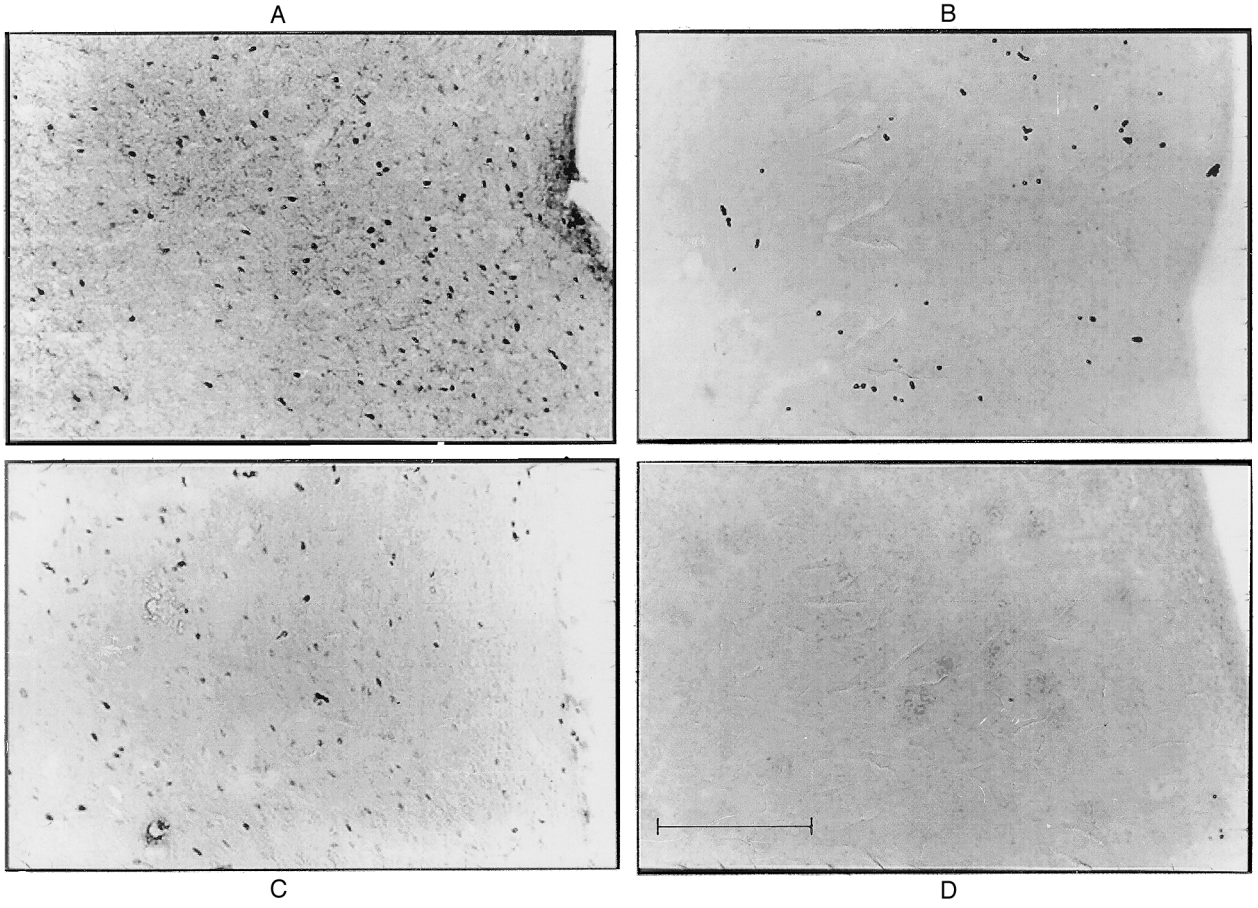

FIG. 3. Representative coronal brain sections of mPOA taken from (A) saline-treated female and (B) morphine-treated female (experiment 1) (both pup-exposed); (C) morphine + naloxone-treated female, and (D) morphine + saline-treated female (experiment 2). Dorsal is up, and the third ventricle is to the right in each section. Scale bar = approximately 50 μ.

was significantly different between the morphine + pups and saline + pups groups, ($p < 0.026$). Moreover, the number of c-*fos* immunoreactive neurons was significantly different between saline + pups and saline-minus pups, ($p < 0.02$). Morphine-minus pups was not significantly different from saline-minus pups, $F(1, 8) = 1.5$, $p > 0.3$, and morphine + plus pups was not significantly different from morphine-minus pups, ($p > 0.15$). Therefore, morphine suppresses c-*fos* activation as does absence of pup stimulation, but fails to alter baseline levels of c-*fos* activation.

With regard to behavior displayed toward the partition (in pup-exposed females only), there were trends, but no significant differences observed, between morphine and saline-treated animals for approach, $F(1, 6) = 1.14$, $p > 0.3$ or touch, $F(1, 6) = 0.6$, $p > 0.5$. Table 1 displays these data.

The c-*fos* data follow from what one would expect based on the data from studies that found that morphine disrupts maternal behavior [3,19]. In the next experiment we administered naloxone, an opioid antagonist, which has been shown to reverse morphine disruption of maternal behavior [3,19].

Experiment 2

Simultaneous administration of morphine plus the opiate antagonist naloxone, which should block the effect of morphine, revealed that morphine + naloxone-treated females expressed significantly higher numbers of c-*fos* neurons, compared to morphine + saline-treated females, $F(1, 5) = 23.084$, $p < .005$) (Fig. 2). As predicted, naloxone treatment reversed the effect of morphine and increased the number of c-*fos* cells being expressed. Therefore, naloxone antagonism of morphine disruption of maternal behavior may reflect increased c-*fos* activation.

In neither experiment did morphine appear to have nonspecific effects on the females' motor activity as all females were active (Table 1). In experiment 2, as in experiment 1, latencies were again longer, with morphine + saline taking longer to approach and contact than morphine + naloxone (17.6 s and 25.3 s vs. 13.3 s and 19.0 s, respectively), but the differences were not significant, $F(1, 5) = 1.5$, $p > 0.28$, and $F(1, 5) = 0.93$, $p > 0.38$, respectively. It is important to note, however, that all morphine-treated animals in both experiments were active within seconds, moving toward and contacting the partition, though controls were more likely to grapple with the partition in an effort to get past it. Therefore, we believe that morphine-induced torpor as an explanation for the behavioral disruption is unlikely.

Fig. 3 A–D displays representative sections from morphine/saline and morphine + saline/morphine + naloxone animals depicting the relative amounts of c-*fos* activated in mPOA. Only data for those animals exposed to pups are shown. Fig. 3A and

B display representative sections from saline-treated and morphine-treated females, respectively, from experiment 1; and Fig. 3C D display representative sections from morphine + naloxone and morphine + saline-treated females, respectively, from experiment 2.

DISCUSSION

Our data suggest a possible mechanism for morphine disruption of maternal behavior. Morphine administration, at a dosage that disrupts maternal behavior without motoric side effects [25] involves the suppression of c-*fos* activity in an area of the brain, the mPOA, that regulates the behavior. As previously demonstrated [36], the presence of the pups appears to be required for the stimulation of c-*fos* as females without pups—though treated only with saline—expressed less c-*fos* than those with pups; morphine, which suppressed c-*fos* in the pups-plus group, did not decrease c-*fos* significantly in nonpup-exposed animals (experiment 1). Naloxone, given at a dose known to restore maternal behavior following morphine administration, significantly increases c-*fos* expression compared to animals treated with morphine alone (experiment 2). Together, the data show that the effects of morphine and naloxone administration on c-*fos* expression in the mPOA parallel the effects of the two substances reported for maternal behavior [3,19]. Therefore, opiate regulation of maternal behavior in the rat, and reversal of morphine disruption of maternal behavior by concurrent treatment with the opiate antagonist naloxone, may be regulated by the relative activation of c-*fos* expression in mPOA.

Morphine administration has many effects on brain activity as measured by c-*fos*. Garcia et al. [17] reported that acute administration of morphine initiated high expression of c-*fos* in dorsomedial caudate putamen, nucleus accumbens, and specific nuclei of the thalamus; activation declined by 3 h postinjection. Chang et al. [11] found that naloxone could abolish the morphine-induced increase in c-*fos* in caudate-putamen; Chang and Harlan [10] showed c-*fos* expression in ventromedial nucleus of the hypothalamus, but not in arcuate nucleus. The picture that emerges is of multiple regulatory processes induced by morphine, but it is a cloudy picture nevertheless. We have found that morphine inhibits c-*fos* in mPOA, but we must caution the reader that both the mediation of maternal behavior, and of morphine's effects in other brain regions, are complex and composed of multiple interactions and reciprocal relationships. Given the latter intricacy, discrete placement of morphine into the mPOA, followed by processing for c-*fos* immunohistochemistry, would help to define more precisely the way in which morphine affects c-*fos* and maternal behavior.

The mPOA plays a crucial role in the regulation of maternal behavior, and the present data add to what is known about mPOA-maternal behavior relationships. Estradiol implants into mPOA facilitate, whereas lesions disrupt, maternal behavior [14,23,33]. Morphine administration directly into the POA suppresses the behavior in previously maternal females whereas naloxone, a selective mu-opioid antagonist, blocks morphine's actions, restoring the female's maternal behavior [41]. Medial preoptic area neurons express c-*fos* upon exposure to pups and engagement in maternal behavior [15,35]. Our data demonstrate that morphine suppresses c-*fos* activation in the mPOA, and that naloxone is capable of restoring it. Morphine disruption of maternal behavior, one of the most striking behavioral effects given the high degree of maternal motivation characteristic of a lactating female, appears to be correlated with the relative inactivity of c-*fos* neurons in the brain, especially within mPOA.

In the pregnant female, there are numerous changes in, and linkages among hormonal patterns, opiate activity, and parental behavior. Gintzler [18] showed that pregnancy was accompanied by significant increases in analgesia, which were subject to blockade with naltrexone, and subsequent reduction just before parturition and during lactation, thereby establishing a link. Beta endorphin concentrations in hypothalamus and mPOA are elevated during pregnancy, but relatively low during the prepartum and postpartum periods [4,5,12,21,39,44,45]. These modifications in endogenous opioids can be mimicked by administration of E_2 and P in a pregnancy-like sequence [5]. The ligand levels among the various stages of pregnancy are also accompanied by changes in opiate receptors. For example, Hammer and Bridges [21] reported that mu-opiate receptors increase in pregnant females and those treated with pregnancy-mimicking levels of E_2 and P. It would be of interest to examine c-*fos* activity in mPOA across the stages of pregnancy for which so-called opiate ''tone'' or baseline opioid activity has been reported. Are there inverse relationships between opioid and c-*fos* activity, for example?

The actions of morphine, at least in part, are related to the alteration of the perception of pup stimuli, in particular, odors. The perception of olfactory signals, upon which the female is dependent to properly care for her young [15], are themselves dependent on the internal opiatergic milieu [26,27] as hitherto maternal females treated with morphine will display an aversion to pup odors; furthermore, treatment with the morphine antagonist, naloxone, will reverse the effect of olfactory aversion [27]. It is important to point out that the above experiments were designed to examine the effects of morphine on aversion to, vs. reduced olfactory capacity for, the odor of pups. Whereas the morphine made the pup odors aversive, it did not make general olfactory perception less acute. That the initiation of a bout of maternal behavior begins with the sensory perception of the pups, primarily through olfactory stimulation, the further examination of morphine/opioid–olfactory relationships seems warranted, particularly as they relate to neuroanatomical sites.

Though we have observed morphine reduction of c-*fos* activation in mPOA, the effects may be due to morphine's actions in other brain regions, perhaps ''upstream'' sites to which morphine is binding. In the present work, it should be noted, we did not find any relationship between rostral vs. caudal mPOA and c-*fos* expression, though, it may be argued, we examined mainly periventricular sites through the mPOA. The mPOA receives input from a variety of sources that may be relevant to maternal behavior control. Of these sources of neural input, probably the most important arises from olfactory/vomeronasal sites, which are capable of reaching the mPOA via projections from the medial amygdala and bed nucleus of the stria terminalis [34]. The main and accessory olfactory bulbs, together with other forebrain structures, contain high concentrations of mu-opiate receptors [20,30,38]. If morphine is acting at the level of the olfactory bulb, the afferent information to the mPOA may be decreased or otherwise modified, thereby altering the perception or preference for the young and diminishing the input for the stimulus-induced activation of the immediate early gene, c-*fos*. The net result may be disruption of maternal behavior.

We found high expression of c-*fos* in mPOA of saline-treated, pup-exposed females compared to morphine-treated animals. One possible alternative explanation for the high c-*fos* is that there is some type of ''frustration'' response-that is, an inability to reach the pups, an artifact-in a female faced with a barrier to access to her offspring. Therefore, c-*fos* ''turning-on'' may be a response to thwarted attempts to breach the barrier. We do not believe this is a tenable explanation for several reasons. First, the Fleming and Walsh [16] and Numan and Numan [36] data demonstrate that c-*fos* expression occurs when females are engaging in maternal behavior. Second, c-*fos* expression, an immediate early gene, does

not occur indiscriminately in response to random stimuli [31,42]. Pup stimuli emanating from the other side of the partition, then, would be more likely to elicit c-*fos* activity from mPOA neurons compared to behavior directed at the partition itself. Lastly, the saline-pups-minus group from experiment 1, who displayed little c-*fos*, would be expected to express some c-*fos* in response to separation from their litter if stress was a key variable.

In experiment 2, the morphine + naloxone group displayed an increase in the number of activated c-*fos* cells relative to the morphine + saline group. Though significantly higher, the numbers do not approach those from the saline-alone group in experiment 1. Yet, the dosage of naloxone has been shown to restore maternal behavior in morphine-treated females. The dosage of morphine, which was absent from the saline-alone group in experiment 1, appears to be capable of suppressing c-*fos* in mPOA but the concurrent naloxone, based on behavioral data [3,19,25] can reinstate it. A larger question is what minimum number of active cells is required to bring about maternal behavior. In other words, why should there be more cells active than necessary? Perhaps the *fos*-expressing neurons in mPOA in the morphine + naloxone group are a necessary subset of neurons to which the naloxone binds and which turn-on owing to a cascade of afferent inputs. With systemic morphine having widespread effects in brain, perhaps otherwise important but redundant information is suppressed. What is left—and what was observed—is c-*fos* activity needed to display the behavior.

Recently, the importance of *fos*-activation for the display of maternal behavior has been reported. Brown et al. [6], using a strain of knock-out mice lacking the gene for *fos*B, demonstrated a profound defect in maternal behavior on the part of mothers lacking the gene. Though normal in all other respects (e.g., pregnancy hormone levels, act of parturition, lactation capacity), the females displayed virtually no interest in nor behavior toward their own offspring. Furthermore, these deficient females evinced little *fos* activity in the POA in the presence of their offspring. The similarity between the behavior of these *fos*B-knock-out mice and females treated with morphine is striking. The common link, then, may be the lack of *fos* activity in the mPOA, one induced by a genetic defect, the other through morphine administration.

We are arguing here that morphine administration leads to decreased *fos* expression and then to disrupted maternal behavior. It should be kept in mind, however, that morphine may lead to decreases in the females' motivation to approach the pups or engage in behavior toward them, thereby, also reducing *fos*-activation [36]. Additional work parceling-out the respective roles of the three variables may prove interesting in helping to understand the regulation—and the disruption—of maternal behavior.

In summary, the present investigation has demonstrated that in morphine-treated females, the number of c-*fos* cells expressed in mPOA is reduced in comparison with saline-treated females. Furthermore, in morphine + naloxone-treated females the number of c-*fos* neurons was greater than that in females treated with morphine + saline. Morphine disrupts maternal behavior, and naloxone can restore the behavior. The extent to which *fos* activation—either c-*fos*, which was examined in the present work, or *fos*B [6]—is a necessary and sufficient condition for maternal behavior remains to be elucidated. It is clear, however, that reduced *fos* activity has dramatic consequences for the display of one of a female's most motivated responses, maternal behavior.

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