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Opiate regulation of behavioral selection during lactation

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Abstract

Treatment of postpartum female rats with morphine inhibits maternal behavior. The same type of treatment stimulates foraging in adult animals. The aim of the present study was to investigate, in lactating rats, the functional role of opioid systems in the choice between caring for pups versus hunting insects. Experiment 1 was designed to test how acute morphine treatment with 3.0 mg/kg interferes with choosing between caring for pups versus predatory behavior. Morphine-treated dams decreased maternal behavior while increasing efficiency in hunting insects. The next step was to test the opioid antagonist naloxone in the same context of maternal versus predatory behavior. Naloxone restored maternal care and reduced hunting in morphine-treated rats. Finally, in order to test the role of endogenous opioidergic stimulation in this scenario, lactating rats were treated with the opioid antagonist naloxone alone. Consistently, naloxone treatment induced a decrease in number of insects captured and an increase in the percentage of animals displaying nursing behavior. These results provide important insight into the role of opioidergic transmission in the regulation of behavioral selection during lactation. The present results suggest that endogenous opioids may stimulate hunting by replacing maternal behavior during lactation.

Keywords: Opioid; Morphine; Drug abuse; Periaqueductal gray; Lactation

1. Introduction

It has been widely shown that morphine disrupts, while naloxone restores, maternal responsiveness in female rats (Bridges and Grimm, 1982). Central infusions of the endogenous opioid β-endorphin, dose-dependently block the expression of maternal behavior, suggesting that changes in endogenous opiate levels alter maternal responsiveness in rats (Felicio et al., 1991; Mann et al., 1995).

In animals subject to a morphine-induced sensitization-like mechanism, referring to an increase in response with repetition of the same of the drug, we have demonstrated that maternal behavior may be inhibited with low doses of morphine otherwise ineffective for inducing such inhibition in morphine-naïve lactating rats (Felicio et al., 2001; Miranda-Paiva et al., 2001, 2002; Slamberová et al., 2001; Yim et al., 2006). Accordingly, acute morphine injection of 5.0 mg/kg inhibits maternal behavior, while 3.0 mg/kg does not inhibit it, unless the dam is submitted to opioidergic stimulation during late pregnancy (Bridges and Grimm, 1982; Miranda-Paiva et al., 2001, 2003, 2007; Yim et al., 2006).

Examining the neural basis underlying maternal behavior inhibited by low doses of morphine in morphine-experienced dams, we found that morphine treatment is likely to induce a behavioral switch from maternal to predatory behavior. Hence, morphine-challenged dams, tested in an environment containing both pups and roaches (which served as prey), clearly preferred hunting instead of nursing (Sukikara et al., 2006).

The aim of the present study is to investigate, in lactating rats, the functional role of opioid systems in the choice between caring for pups versus hunting insects. We first studied the behavioral effects of subcutaneous injections of low doses of morphine sulphate (3.0 mg/kg) on morphine-naïve dams exposed simultaneously to insects and pups, and observed that low doses of morphine alone were able to increase the motivational drive to hunt insects. To evaluate the role of opioid receptor in this behavioral modulation, we next tested the opioid receptor antagonist naloxone for its capacity to reverse the morphine-induced shift from maternal to predatory hunting behavior.

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Finally, we ran a third experiment, and tested whether an endogenous opioid tone influenced this behavioral shift under more natural conditions without morphine challenge. We observed in morphine-naïve dams that a single injection of the opioid receptor antagonist naloxone alone was able to decrease predatory hunting and increase maternal care. These findings support an important feature of opiates in the context of maternal behavior, which is modulating the dam's motivational drive to forage and hunt.

2. Materials and methods

2.1. Animals and housing

Subjects were adult Wistar nulliparous female rats, weighing 190–220 g and approximately 90 days of age at the beginning of the experiments. In all experiments, females were mated by placing them with sexually experienced males. The day when sperm was observed in the vaginal lavage was designated day 1 of pregnancy. Pregnant females were individually housed in opaque polypropylene cages (41 × 34 × 16 cm) containing approximately 1.0 L of medium-grade pine flakes. Food and water were available ad-libitum in light – (06:00–18:00 h) and temperature – (23–25 °C) controlled testing rooms. After giving birth (day 0 of lactation), females were left with their litters (culled to 8 pups on day 1 of lactation — four males and four females) until testing on day 5 of lactation. On postpartum days 3 and 4, dams were placed into the experimental cage for 30 min/day for adaptation. The size of the experimental cage was identical to the home-cage but without wood flakes and with a plexi glass top to permit the animal behavior to be video taped. Animals were maintained in accordance with the guidelines of the Committee on Animals of the Colégio Brasileiro de Experimentação Animal (COBEA) and the Committee on care and use of Laboratory Animal Resources, National Research Council, USA. In all experiments, animals were tested a single time.

2.2. Behavioral observations

On the morning of postpartum day 5, dams were tested simultaneously for maternal care towards the pups and predatory-hunting behaviors. The dams were placed into the experimental cage, thirty-minutes later, they received an acute injection of saline, morphine, or naloxone, according to the experimental design. Thirty-minutes later, eight pups and five

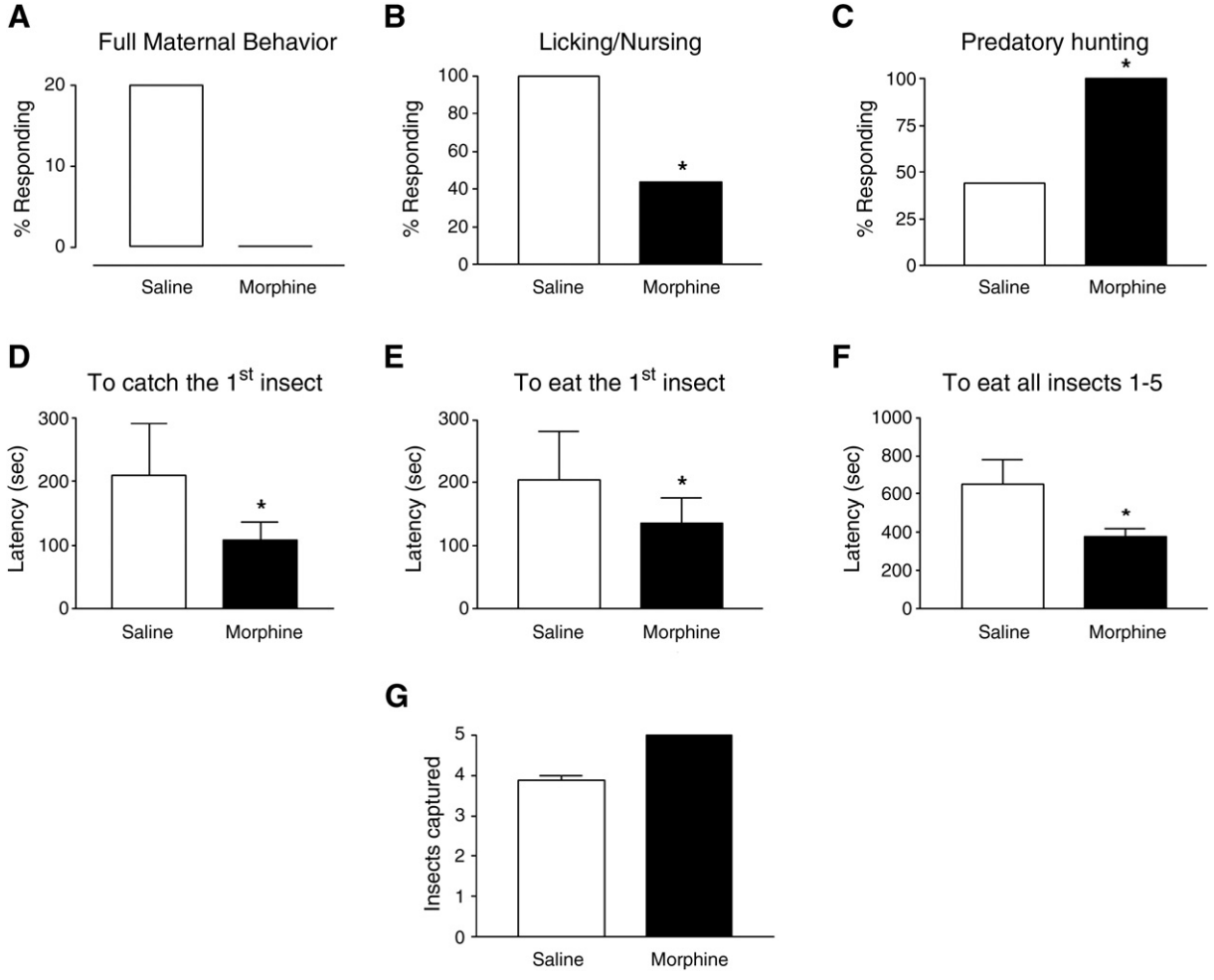

Fig. 1. Effects of morphine sulphate (3.0 mg/kg; group Morphine; $n=9$) on the percentage of animals expressing full maternal behavior (A), licking and nursing (B), predatory hunting (C). Latencies in seconds to catch the first insect (D), to eat the first insect (E), the time interval between eat all 5 insects (F). Number of insects captured for each rat since group Morphine has no variability t test does not apply (G). Data of latencies are expressed as mean ± SEM. *$p<0.05$ compared with the saline group ($n=10$).

Table 1
Effects of morphine on parameters of maternal and hunting behavior

	Saline (10)	Morphine (9)
% licking the pups before hunting	100	11* (n=1)
% grouping the pups before hunting	90	22* (n=2)
% nursing the pups before hunting	80	0*
% animals hunting	80	100
Insects captured	4±0.4	5±0.0

Number of animals is in parenthesis. *p<0.05 (Fisher test).

live mature cockroaches (*Periplaneta americana*) were introduced into the cage for behavioral testing. During the 30-min trial, dams were observed for maternal and insect-hunting behaviors. Predatory hunting behavior was evaluated by percent of animals hunting; latencies to capture the first insect; time to eat the first and all insects; and number of insects captured. The following parameters were recorded for maternal behavior: percent of animals expressing different aspects of maternal behavior such as grouping, licking or nursing pups before hunting. Once the dam allowed any pup to attach to her nipple, it was considered nursing, which generally occurred before the display of full maternal behavior. Full maternal behavior was characterized when a dam, after retrieving and grouping all pups, with her legs splayed, arched over the pups, attached to her nipples, remained like this at least for two minutes. In all cases, latencies were considered only for responders.

2.3. Drugs

According to the experimental design, dams were treated with saline, morphine sulfate (3.0 mg/kg, Cristália Laboratory, São Paulo, Brazil), and naloxone (1.0 mg/kg; Sigma-Aldrich, Brazil). All injections were subcutaneous in a volume of 1 ml/kg of body weight.

Experiment 1. Effects of morphine sulfate on lactating rat choice: care for pups or predatory insect-hunting. Thirty minutes before the behavioral testing, lactating rats received an

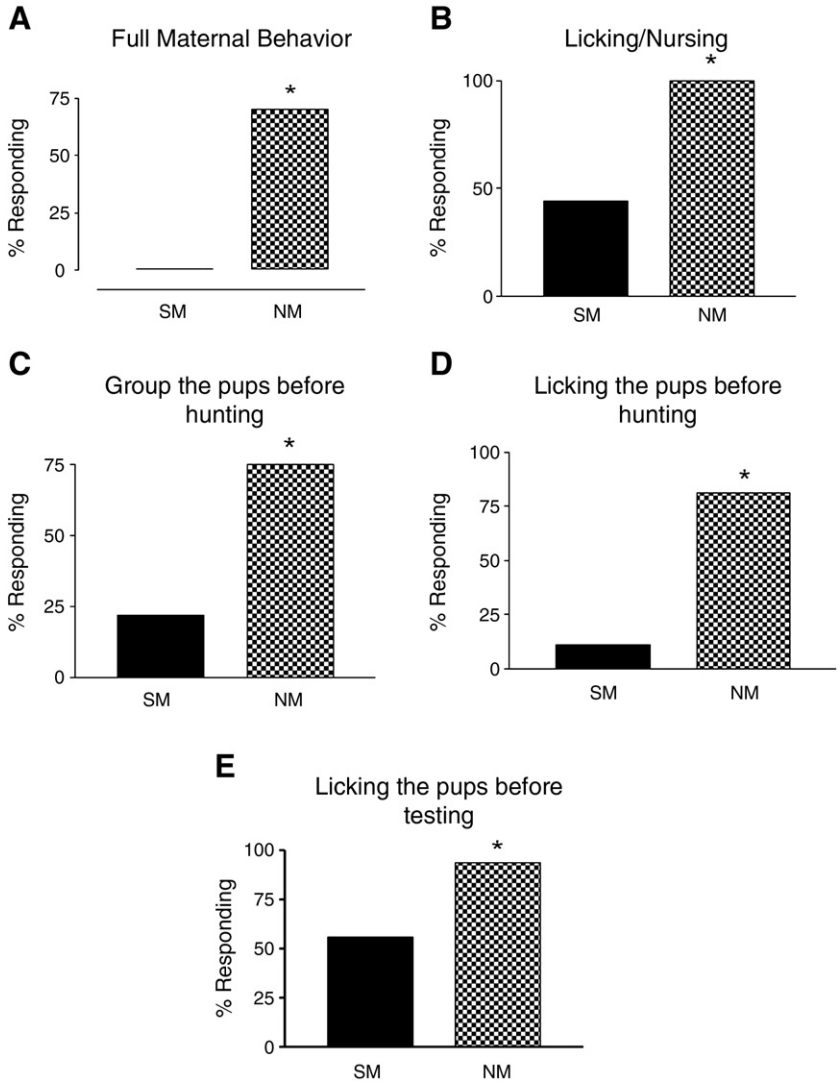

Fig. 2. Effects of naloxone treatment (0.1 mg/kg; group NM; n=16) on maternal behavior of morphine (3.0 mg/kg) treated lactating rats (SM; n=16). Percentage of dams showing full maternal behavior (A), licking and nursing (B), grouping (C) and licking (D) the pups before hunting, licking the pups during testing (E). *p<0.05 compared with the group treated with saline and morphine (group SM).

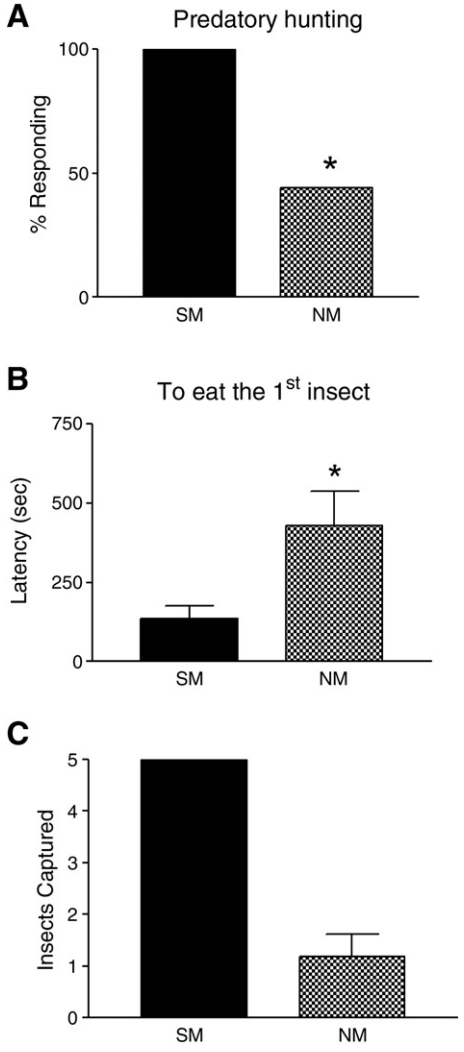

Fig. 3. Effects of naloxone treatment (0.1 mg/kg; group NM; $n=16$) on predatory behavior of morphine (3.0 mg/kg) treated lactating rats (SM; $n=16$). Percentage of animals hunting (A) and latency to eat the 1st insect (B), number of insects captured for each rat: since group SM has no variability, Student's t test does not apply (C). Latencies to eat the first insect and number if insects captured by each rat expressed as mean±SEM. *$p<0.05$ compared with the group treated with saline and morphine (group SM).

acute injection of morphine sulfate (3.0 mg/kg s.c.; $n=9$) or saline ($n=10$). The behavioral test was performed as described above. In all experiments the cage test was washed with a water-alcohol (5%) solution before behavioral testing to eliminate possible bias due to odors left by previous subjects. To minimize possible circadian influences on rat behavior, experimental and control observations were inter-mixed.

Experiment 2. Role of opioid receptor blockade on the effects of morphine-induced replacement of maternal care by predatory hunting behavior. In this experiment the following groups were tested: group SM ($n=16$), lactating rats treated with saline 3 min before morphine sulphate injection (3.0 mg/kg, sc.); and group NM ($n=16$), rats treated with naloxone (1.0 mg/kg, sc.) 3 min before an injection of morphine sulphate (3.0 mg/kg, sc.). During the 30-min behavioral test, dams' maternal and predatory behaviors were observed. The behavioral test was performed as described above.

Experiment 3. Effects of opioid receptor blockade by naloxone on spontaneous maternal and predatory behavior. Lactating rats were treated with naloxone (1.0 mg/kg, sc.; $n=10$) or saline ($n=11$) 30 min before the behavioral test. The behavioral test was performed as described above.

2.4. Statistical analysis

Comparisons among the groups for full maternal behavior, time to catch and to eat the insects were analyzed using the Student's t test. Since number of insects captured showed no variability in animals treated with morphine (experiment 1 and 2 all of then captured all 5 insects), this parameter was compared statistically only in Experiment 3. The Fisher Exact Probability

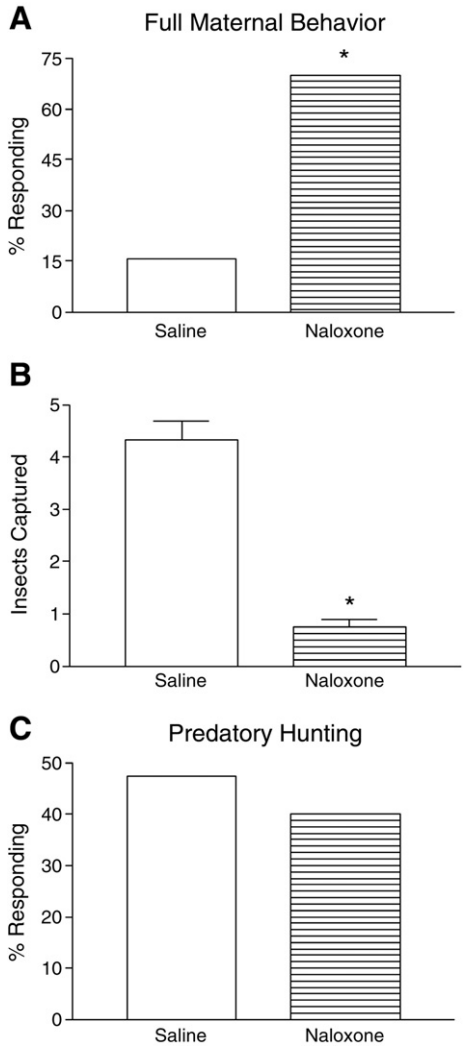

Fig. 4. Effects of naloxone treatment (0.1 mg/kg; group Naloxone; $n=10$) on percentage of dams showing full maternal behavior (A), number of insects captured by each group (B), percentage of dams displaying predatory hunting (C). Number of insects captured by each group expressed as mean±SEM. *$p<0.05$ compared with the saline group ($n=11$).

Test was used to compare the number of animals displaying licking the pups, grouping the pups, active nursing, and predatory hunting. A probability of $P<0.05$ was considered to be significant for all comparisons made.

3. Results

3.1. Experiment 1

The percent of dams displaying licking or nursing was significantly reduced in morphine-treated group ($p<0.05$; Fisher test; Fig. 1). Morphine-treated animals showed shorter latencies in catching and eating the first insect, as well as total time spent for eating all five insects ($t_{(19)}=1.084$; $p<0.05$; Student's t test; Fig. 1). Although, no significant difference was found for percent of animals showing full maternal behavior (Fig. 1), the percents of dams displaying licking, grouping or nursing pups before hunting were significantly reduced in the morphine-treated group ($p<0.05$; Fisher test; Table 1). There were no significant differences in percent of animals hunting and number of insects captured by each animal (Fig. 1 and Table 1). Interestingly, although most dams captured all the insects, saline treated animals usually groomed the pups during time intervals in-between hunting each insect, while morphine treated rats ignored the pups and remained actively searching for the insects during those intervals.

3.2. Experiment 2

Naloxone treatment restored maternal behavior to the original levels in morphine-treated animals. Percent of dams showing full maternal behavior, licking or nursing, grouping the pups before hunting, licking the pups before hunting and licking the pups during testing were significantly increased in NM group as compared to SM group ($p<0.05$; Fisher test; Fig. 2). In addition, naloxone treatment reduced hunting behavior. Thus, percent of dams displaying hunting was significantly decreased ($p<0.05$; Fisher test; Fig. 3), latency to eat the first insect was increased ($t_{(15)}=2.908$; $p<0.05$; Student's t test; Fig. 3) and number of insects captured was decreased in NM as compared to SM group (Fig. 3).

Table 2
Effects of naloxone on parameters of maternal and hunting behavior

	Saline (11)	Naloxone (10)
% licking/nursing	100	100
% licking the pups before hunting	100	100#
% nursing the pups before hunting	80	100#
Latency to catch the 1st insect	209±80	867±242*($n=4$)
Latency to eat the 1st insect	205±76	1010±439*($n=2$)
Time interval between to eating the 1st and 5th insect	648±130	NE

Number of animals in parenthesis. #: since animals did not hunt, the expression of these parameters before hunting is the same as total licking and nursing. NE: no event.
*$p<0.05$ (Student's t test).

3.3. Experiment 3

Naloxone treatment increased the percent of dams showing full maternal behavior as compared to saline treated animals ($p<0.05$; Fisher test; Fig. 4). Although, percent of dams displaying licking or nursing was approximately the same in saline and naloxone treated rats, the number of insects captured by each dam was significantly reduced by naloxone treatment ($t_{(11)}=6.177$; $p<0.05$; Student's t test; Fig. 4). There was no significant difference in percent of animals showing licking or nursing before hunting either during the whole test or before hunting. Only four animals treated with naloxone hunted insects: two of them captured and ate one insect each, and the other two captured one insect each and ate half an insect each. Their latencies both to capture and to eat the first insect were significantly longer then those displayed by saline treated mothers ($t_{(11)}=6.177$; $p<0.05$; Student's t test; Table 2).

4. Discussion

The present study evaluated how morphine influenced motivation, when lactating rats were allowed a choice between maternal and predatory behavior. Clearly, morphine diminished maternal behavior but elevated predatory behavior when dams were simultaneously exposed to pups and roaches. Next, we showed, in morphine-naïve dams, that naloxone alone was able to increase the expression of full maternal behavior and reduce predatory activity.

Importantly, in previous works, the morphine doses herein employed were found insufficient to inhibit maternal behavior in morphine-naïve dams tested only with pups (Miranda-Paiva et al., 2001, 2003; Yim et al., 2006). In the present study, by exposing dams simultaneously to pups and insects, we have demonstrated that a decrease in maternal behavior occurs in dams without previous morphine experience acutely challenged with low doses of morphine. These dams prefer to hunt when tested in an environment containing pups and roaches. Moreover, we were able to show that naloxone pre-treatment restored maternal behavior to its original levels and decreased predatory hunting in morphine-treated animals. This result suggests that morphine effect on this behavioral shift is due to its actions on opioid receptors.

The third experiment was designed to test whether an endogenous opioidergic tone influenced a shift from maternal to predatory behavior in non-treated dams. In order to test this hypothesis, lactating animals received a single injection of naloxone before the behavioral test. Although naloxone alone did not change the percentage of animals exhibiting hunting behavior, the number of roaches captured by each dam was significantly reduced, and the percentage of rats displaying full maternal behavior was increased by naloxone treatment. In line with this view, previous works showed that the amount of time the lactating females spend with pups in the nest and nursing pups is increased by naloxone injections (Byrnes et al., 2000). Collectively, the evidence suggests that there is an endogenous opioidergic tone promoting predatory activity.

The morphine-induced inhibition of maternal behavior seems to be part of a more general effect that morphine has on social

interactions. In studies of specific social behaviors in rodents, low doses of morphine decreases proximity maintenance time in socially housed animals, decrease maternal aggression and offspring/maternal proximity maintenance time (Panksepp et al., 1980; Nelson and Panksepp, 1998). Although it has been suggested that brain opioids modulate social emotions and behaviors, an alternative explanation to this view is that opiods are indeed likely to increase motivational drive to behaviors other than social ones. In fact, this seems to be the case for the maternal behavior inhibition seen with low doses of morphine, when the motivational drive is supposedly switched toward predatory or foraging behaviors. In line with this view, it has been shown that opioids have been involved in neural circuits that integrate food hedonic values, influencing searching and consumption of palatable food (Pomonis et al., 2000).

Previous works on the neural basis of the morphine-induced disruption of maternal behavior in response to low doses of morphine revealed that there is direct opioidergic activation of the rostal lateral pariaqueductal gray (PAG), a critical site to modulate the motivational drive to predate or possibly forage (Miranda-Paiva et al., 2003; Sukikara et al., 2006). In contrast to the ventrolateral part of the PAG, which has been directly involved in controlling a number of maternal responses (Lonstein and Stern, 1997 and 1998.), the rostral lateral PAG does not seem to project to any element of the neural circuit controlling maternal responses (N.S. Canteras and S.R. Mota-Ortiz, personal observation). In fact, one of the main targets of the rostral lateral PAG is the orexinergic cell group in the lateral hypothalamus (N.S. Canteras and S.R. Mota-Ortiz, personal observation), which has been suggested by a wealth of experimental data as associated with enhanced arousal and locomotor activity, thus potentially increasing the prey-chasing motivation, as well as the likelihood to encounter food during foraging activity (Saper et al., 2002).

Overall, the switching mechanism discussed here is related to the survival issue that mothers must contend with in balancing subsistence and reproduction. Thus, the present data relate highly adaptive mechanisms that increase the lactating animal survival chances in food-lacking environments. Since a lactating female has to nurse and gather food, she has to use her time more efficiently than a non-lactating female in addressing both demands. Consistently, it has been suggested that pregnancy and motherhood induce an improved predatory behavioral repertoire which is accompanied by neural enhancements in the rat (Kinsley et al., 2006). Of particular relevance, our results further suggest that endogenous opioidergic transmission may normally promote predatory or foraging activity.

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