ELSEVIER

Brain Research 743 (1996) 184–201

BRAIN RESEARCH

Research report

Opioid stimulation in the ventral tegmental area facilitates the onset of maternal behavior in rats

Alexis C. Thompson *, Mark B. Kristal

Behavioral Neuroscience Program, Department of Psychology, State University of New York at Buffalo, Buffalo, NY 14260, USA

Accepted 20 August 1996

Abstract

This research investigated the effect of an increase or decrease in opioid activity in the ventral tegmental area (VTA) on the onset of maternal behavior in rats. In Experiment 1, the latency to show maternal behavior toward foster rat pups (sensitization latency) was determined in maternally naive female rats given either nothing or a unilateral intra-VTA injection of morphine sulfate (MS) (0.0, 0.01, 0.03, 0.1 or 0.3 μg), on the first three days of a 10-day period of constant exposure to pups. Rats treated with 0.03 μg MS had significantly shorter sensitization latencies than did rats treated with 0.0 μg MS, 0.01 μg MS, or receiving no treatment (higher doses of morphine produced intermediate results). The facilitating effect of intra-VTA MS on the onset of maternal behavior was blocked by pretreatment with naltrexone hydrochloride and was found to have a specific site of action in the VTA (MS injections dorsal to the VTA were ineffective). In Experiment 2, sensitization latencies were determined in periparturitional rats given a bilateral intra-VTA injection of either the opioid antagonist naltrexone methobromide (quaternary naltrexone), its vehicle, a sham injection, or left untreated 40 min after delivery of the last pup. The mothers' own pups were removed at delivery; mothers were nonmaternal at the time of testing. Quaternary naltrexone treatment produced significantly slower sensitization to foster pups than did control conditions. Total activity and pup-directed activity did not differ significantly with treatment. The results demonstrate that increased opioid activity in the VTA facilitates the onset of maternal behavior in inexperienced nonpregnant female rats, and decreased opioid activity in the VTA disrupts the rapid onset of maternal behavior at parturition.

Keywords: Maternal behavior; Opioid; Mesolimbic; Ventral tegmental area; Naltrexone methobromide; Morphine; Rat

1. Introduction

The onset of maternal behavior in the rat, in the natural context of parturition, is abrupt, rapid, and easily quantifiable by the appearance of pup retrieval, pup grooming, and crouching over pups [13,61,82]. Maternal behavior can also be elicited by the presence of rat pups independent of the circumstances of parturition in, for instance, virgin females, males, and juveniles [60,82]. In these nonparturitional cases, the maternal behavior exhibited is similar to postpartum maternal behavior; pup retrieval, pup licking, and crouching over pups is present, differing mainly in the duration of pup exposure required to elicit the behavior (h or days vs. min) [15]. These observations regarding the onset of maternal behavior have led to the idea that maternal behavior is induced primarily by stimuli associated with parturition and emanating from the pups, and is facilitated by the endocrine and neurochemical changes associated with pregnancy and parturition [52,68,70].

Endogenous opioids have been considered as one possible candidate mediating the rapid onset of maternal behavior at parturition [38]. Plasma and brain levels of endogenous opioids increase over the course of pregnancy, peak during parturition, and then decline to pre-pregnancy levels during lactation [58,80]. These changes in endogenous opioids are consistent with at least one behavioral change during parturition: pain threshold is greatly elevated in the periparturitional period by an opioid-mediated mechanism [43]. Only a few studies have assessed the role of opioids in the onset and maintenance of maternal behavior, and the findings are mixed. Given systemically, both opioid agonists and antagonists have been shown to reduce or delay maternal responding [5,20,40,50,63]. Given centrally, opioid agonists injected i.c.v. have been shown to facilitate the onset of maternal behavior in sheep [35,37] whereas

* Corresponding author. Present address: NIH/NIDA/IRP, Neuroscience Branch, Neuroimaging Section, PO Box 5180, Baltimore, MD 21224, USA. Fax: +1 (410) 550-1441; E-mail: athompso@irp.nida.nih.gov

PII S0006-8993(96)01041-4

injections directly into the medial preoptic area have been shown to block ongoing maternal behavior in the rat [45,46,62]. Finally, Kristal et al. [43] have found some measures of maternal behavior to be highly correlated with increased opioid activity during parturition.

One explanation for these apparently contradictory results may lie in the distribution and action of endogenous opioids. Endogenous opioids are found at many levels of the nervous system and the responses elicited by opiate administration at different brain sites frequently oppose each other [30,31]. Systemically administered opioid agonists and antagonists may produce a variety of effects depending upon where in the nervous system they act, or which opioid receptor field contributes most to the final behavior. A better approach to studying opioid effects on maternal behavior would be to apply opioid agonists and antagonists to specific brain areas thought to be part of the maternal behavior neural substrate and also known to contain an opioid receptor field. Bridges [45,46,62] has shown that increased opioids in the medial preoptic area inhibit maternal responsiveness; the medial preoptic is an area widely accepted to be crucial to the mediation of maternal behavior [52]. The experiments presented here investigate the actions of endogenous opioids in the ventral tegmental area (VTA) on maternal responsiveness [76]. The VTA, like the medial preoptic area, is a crucial component of the 'maternal neural substrate' [18,22–25,54], but in contrast to the medial preoptic area, the VTA is also a principal site at which opioids act to stimulate motivated behavior [2,30,31].

Experiments 1A and 1B were designed to test the hypothesis that increased opioid activity in the VTA facilitates the onset of maternal responsiveness in a dose-dependent fashion. First, the effect of injecting several doses of the opioid agonist morphine sulfate (MS) into the VTA on the latency to onset of maternal behavior was determined (Expt. 1A). Second, the pharmacological specificity of the action of morphine on opioid receptors was directly assessed by treating some rats with the opioid-receptor antagonist naltrexone hydrochloride, systemically, prior to the intra-VTA injection of morphine (Expt. 1B). If the action of morphine on maternal behavior in Expt. 1A is through the opioid receptor, then administering an opioid-receptor antagonist should block the facilitating effect of morphine on the onset of maternal responsiveness. Finally, the anatomical specificity of the observed results was assessed in some rats by injecting morphine dorsal to the VTA. If the effect of morphine on maternal behavior observed in Expt. 1A is specific to its action in the VTA, then morphine injected dorsal to the VTA should have no effect on the latency to onset of maternal responsiveness (Expt. 1B).

Experiment 2 was designed to determine if the findings of Expt. 1 could be applied to the induction of maternal behavior in a more natural context. Bilateral intra-VTA injections of the quaternary opioid antagonist naltrexone methobromide, or its vehicle, were administered to newly parturient rats that had been prevented from developing maternal responsiveness during parturition, and the consequent latency to show full maternal behavior was assessed. Control rats were expected to show rapid development of maternal responsiveness because the periparturitional period provides the natural context for that rapid development [4,55]. We hypothesized that VTA opioid activity is necessary for the development of maternal behavior; therefore we would expect the experimental dams, those with blocked VTA opioid activity, to show a delay in the development of full maternal behavior (i.e., longer latencies to onset of maternal responsiveness).

2. Materials and methods: General

2.1. Subjects

Subjects were nulliparous female Long-Evans (hooded) rats, 3 to 5 months old, raised in our laboratory from Harlan Sprague-Dawley (Blue Spruce Farms) stock.

Rats were maintained in a controlled environment with an ambient temperature of 22 ± 1°C, a relative humidity of about 50%, and a 14 h on/10 h off light cycle (lights on at 05.00 h). During lights off, the room was illuminated by a red 60-W bulb. Rats were housed individually in 24.5 × 18 × 18-cm suspended wire-mesh cages except during the testing period when they were housed in specialized observation cages (see below). Food (Agway Prolab Rat/Mouse/Hamster Formula 3000) and water were available ad libitum throughout all experiments.

Each potential subject was screened for spontaneous retrieval of pups or cannibalism by observing her initial response to two foster pups [70]. Rats observed to retrieve or bite the pups in a single 15-min period (approximately 5% of those tested) were removed from the pool of subjects. (Pup-naive nulliparae that show spontaneous retrieval or cannibalism are rare enough not to be distributed evenly across groups, and acquire different experience with pups than do the more common pup-avoiders [27,57].)

2.2. Stereotaxic surgery

Subjects, except those assigned to no-treatment control groups, were implanted with permanent indwelling guide cannulae through which drugs could be injected directly into the VTA. Guide cannulae consisting of either a 22-gauge stainless steel tube (Plastic Products, Expts. 1A and 1B) or a 23-gauge stainless steel tube (Small Parts, Inc., Expt. 2), were aimed at an area 1 mm dorsal to the posterior aspect of the VTA (A–P: −3.8; L: ±0.6; and D–V: −9.5 [56]). It was expected that these coordinates would place the tip of the microinjection cannula into the area of the VTA previously shown to yield the best behavioral response to opioid microinjections [2,59]. In

Expts. 1A and 1B, guide cannulae were implanted unilaterally, alternating between left and right placements. In Expt. 2, cannulae were implanted bilaterally approximately 1 mm apart. During surgery, rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) after pretreatment with atropine sulfate (4 mg/kg, s.c.) to suppress mucus secretion. At the end of surgery, the cannulae were closed by inserting stylets, and the wound was covered with a sulfa-urea antiseptic powder (Sulf-U-Dex). A systemic antibiotic (Combiotic, 0.5 ml, i.m.) was given immediately after surgery and on postoperative Day 3 as a prophylactic measure. Rats were given an 8- to 14-day recovery period before testing.

2.3. Stimulus pups

In all experiments, stimulus pups were foster rat pups obtained from the litters of donors maintained in a separate colony room. Exposing maternally naive adults to foster pups for a prolonged period (concaveation leading to maternal sensitization) necessitates replacing the pups at regular intervals to insure continued stimulus quality and survival of the pups because the subjects neither lactate nor necessarily behave maternally [60,70]. In these experiments, pups were exchanged approximately every 12 h with pups that had been fed and cared for by their natural mother (donor) over the preceding 12 h (at minimum) period. Pup exchange occurred at the start of each 12 h observation period.

2.4. Apparatus

Observation cages, in which experimental rats were housed during the testing period, were 10-gallon glass or plastic aquaria. Mirrors were positioned behind each cage to facilitate observation by the experimenter. Rats were prevented from seeing one another by cardboard partitions placed between the cages.

All behavior observations were made over closed-circuit television equipped with a remotely controlled zoom lens and pan-tilt gimbal. The zoom and pan-tilt controls, a recorder, and a monitor were housed in a room adjacent to the observation room.

A computer (DOS-based 286 micro-processor, 16 MHz) was used as a continuous recorder (I/O sampling every 8 s) with the aid of a program (MONITOR2) designed to record and then extract frequency, onset, and duration data for each of several behaviors during a testing period [73,74]. Observations were limited to 1–2 rats at a time to maximize visibility.

2.5. Drug injections

Injection of drug into the VTA was accomplished by inserting a 28-gauge internal cannula into the guide cannula and injecting into brain tissue a 0.5 μl solution containing the appropriate dose of drug. A micro-infusion pump (Harvard, Model 944) was used to inject at a rate of 1 μl/min. The internal cannula extended 1 mm beyond the guide cannula and was left in place for 30 s after the drug injection was completed. The experimenter was blind to the drug condition until the end of the sensitization/testing period.

2.6. Behavior observations

During the testing period, two 15-min behavior observations were made each day: once during the lights-on phase (between 09.00 h and 11.00 h) and once during the lights-off phase (between 21.00 h and 24.00 h). In Expt. 2, additional behavior observations were made during the first 12 h of the sensitization period.

2.6.1. Maternal-behavior tests

During the behavior observations, the presence or absence of full maternal behavior was noted. At the start of each observation period, four freshly fed and cared-for pups were scattered about the observation cage and the presence of pup retrieval to the nest, pup licking, and crouching over the pups was scored. When a rat showed all three of these pup-directed behaviors consistently, in two consecutive observation periods, she was considered fully maternal. The dependent measure derived from the maternal-behavior test was latency, in days (Expts. 1A and 1B) or hours (Expt. 2), to show full maternal behavior. Each rat was assigned the latency that corresponded to the first observation period of the two consecutive observation periods in which she showed full maternal behavior. The protocol used to score full maternal behavior corresponds to the standard maternal-behavior test protocol used during sensitization to rat pups [60,70,82]. The latency to show full maternal behavior (sensitization latency) was used to test the primary hypothesis that an increase in opioid activity in the VTA facilitates the onset of maternal responsiveness.

In addition to measuring sensitization latency, the latency to engage just in retrieving, which is highly correlated with the other maternal behaviors, was indirectly assessed by noting whether or not the test rat had gathered her foster pups into her nest between behavioral observations (latency to show pup-grouping). Such pup grouping would be an indication of some maternal responsiveness even in rats that failed to meet the criteria for full maternal behavior.

2.6.2. Other behavioral measures

Other behaviors were also measured during each 15-min observation period to test secondary hypotheses about drug-induced changes in pup-directed behavior and motor activity. These behavior measures assessed duration of (a) pup-directed behavior (sniffing, licking, retrieving, and crouching over the pups); (b) object-directed behavior

(sniffing, licking, carrying, and gnawing the other stimulus objects; Expts. 1A and 1B only); (c) self-directed behavior (grooming and eating); (d) duration of nest-related activity (nest building and nest rearrangement within the nest quadrant); (e) forward locomotion; and (f) resting.

2.7. Statistical analysis

2.7.1. Latency data

The sensitization/testing period was terminated after 9.5 days, thereby truncating the latency data for nonresponders (Expts. 1A and 1B). Therefore, medians were used to express group latencies (sensitization latency and latency to show pup-grouping), and nonparametric statistical tests were used to assess the significance of group differences. The test statistic was the Kruskal-Wallis one-way analysis of variance by ranks (KW test) corrected for tied ranks [64]. A multiple-comparisons test [64] was used to probe significant group differences identified by the KW test. The number of pairwise comparisons necessary to identify differences among experimental and control groups was minimized by first comparing control groups, and, when found not to differ from each other (as expected), using only the single most appropriate control group.

2.7.2. Duration data

The durations of self-directed, pup-directed, object-directed, and nest-directed behaviors, forward locomotion, and resting were determined during each 15-min observation. However, only a sample of the observation periods obtained for each rat was used to assess, statistically, the effect of intra-VTA drug treatment on responsiveness to pups and on general level of activity. Observation periods were chosen so as to maximize the chance of detecting either acute (immediately after treatment) or enduring (long after treatment) differences in the behavior of rats before, during, and after becoming maternal. We hoped that such information would complement the interpretation of treatment effects on the onset of maternal behavior, for example, that specific treatments appear to enhance the onset of maternal behavior because they result in increased contact with pups. The initial inspection of the data revealed that rats tended to spend most of the observation period engaged in only one or two of the behavior categories (resting, nest-directed, forward locomotion, or self-directed behavior). Duration scores within each behavior category were not normally distributed, but clustered at each end of the duration interval, thereby compromising a multivariate statistical analysis on all dependent variables [72]. Therefore, a more limited data analysis was performed to assess whether significant differences in (a) the amount of pup contact (total time spent in pup-directed behavior) and (b) amount of total activity (total time spent in any active behavior), existed between treatment groups. The effects of treatment on pup contact and total activity were assessed separately using one-way ANOVAs and adjusting α to control for multiple comparisons.

2.8. Histological examination

Each cannulated rat was injected with pentobarbital sodium (1 ml, i.p.) and its brain removed, frozen ($-20°C$), and cut in 40-μm sections. Methyl blue dye (0.5 μl) was injected through an internal cannula just before the pentobarbital injection to facilitate visual localization of the cannula tip during sectioning. Every fourth section cut from the area around the cannula site was saved and stained with cresyl violet. Cannula placement was verified by inspection and comparison with the stereotaxic atlas of Pellegrino et al. [56].

3. Experiment 1A

The effect of each of five doses of intra-VTA morphine sulfate on the sensitization latency was examined. Intra-VTA morphine was expected to decrease, in a dose-dependent fashion, the latency for nulliparous, maternally naive female rats to show maternal behavior (i.e., expected to facilitate the onset of maternal behavior). The morphine dose ranged from 0.01 μg to 0.3 μg per injection and was intended to be less than that needed to produce large increases in locomotor activity [29]. In a pilot study in which rats were given the highest dose of morphine (0.3 μg) for 3 consecutive days, we observed no marked increase in locomotor activity and no overt behavioral/physiological symptoms of withdrawal upon drug cessation.

3.1. Method

3.1.1. Subjects

Sixty nulliparous nonpregnant (virgin) rats were randomly and evenly distributed among six groups: four doses of morphine (0.01, 0.03, 0.1, and 0.3 μg); a vehicle-injected control group (0.0 μg morphine); and a no-surgery control group (no treatment).

3.1.2. Apparatus

In these experiments, four stimulus objects other than rat pups were also presented to the experimental rats at the same time as the stimulus pups, to provide alternative novel stimuli. The four stimulus objects were an eyedropper bulb (1.25-cm long $\times$ 1.25-cm diameter at its widest point); an acrylic pompom (2.5 cm, Darice); a hamster chew stick (5 $\times$ 1.25 $\times$ 0.75 cm, V.I.P.); and a plastic ring (2.5-cm diameter, Dritz). Eyedropper bulbs have been used by other experimenters for roughly the same purpose [27].

The observation cage was 51 $\times$ 25 $\times$ 31 cm, covered by a wire-mesh top. The cage floor was subdivided into quadrants by 3-cm-high Plexiglas dividers. The height of

the dividers was sufficient to prevent pups from crawling out of the quadrant, and thereby initiating contact with the adult, without impeding the movement of the adult rat around the cage [53]. The cage floor was covered with 1–2 cm of standard wood shavings (Aspen laboratory-grade shavings).

3.1.3. Drugs

Morphine sulfate was mixed in 0.9% sterile saline and injected once each day during the first 3 days of the sensitization period (Day 0, 1, and 2).

3.1.4. Experimental procedures

Each rat was moved into an observation cage one day before the start of sensitization, to habituate to the cage environment. One hour before the morning observation on each day of sensitization, stimulus pups and other stimulus objects were removed, stage of estrous cycle of the test rat was determined by vaginal smear, and on the first three days of the testing period, drug was administered. For each rat, these procedures took about 3–5 min. The vaginal smears were taken and drug was injected in a room adjacent to the observation room. Immediately after drug injection, rats were returned to their observation cages; food, water and fresh bedding were added if necessary.

One hour later, fresh stimulus pups and stimulus objects were placed in the test cage, and the observation period was begun. Stimulus pups and objects were 'scattered' around the observation cage by placing two pups and two objects in the quadrant diagonal to the rat's resting quadrant and one pup and one object in each of the quadrants adjacent to the resting quadrant. For the dark-phase test, pups were switched but other manipulations, and the 1-h delay, were omitted.

In addition to testing for maternal behavior, as described above, we assessed the durations of pup-directed behavior and general activity during the morning observation on Day 2 (1 h after the last daily drug injection), the morning observation on Day 5 (72 h after the last daily drug injection), and during the first observation in which full maternal behavior was observed.

3.2. Results and discussion

During the experiment, four rats failed to complete the entire testing sequence (e.g., had unusable indwelling cannulae) and were dropped from all subsequent analyses. At the end of the experiment, histological examination showed that cannula placement in 35 of the remaining 47 cannulated rats fell within the VTA (Fig. 1). In these rats, the cannula placement fell within the area bounded anteriorly by the appearance of the interpeduncular nucleus, posteriorly by the appearance of the pons, medially by the interpeduncular nucleus, laterally by the lateral lemniscus, ventrally by the base of the brain and dorsally by the dorsal extent of the interpeduncular nucleus. In 7 of the 12 rats in which cannulae were judged to have missed the VTA, placement was ventral to the VTA, piercing through the base of the brain (2 treated with 0.3 μg morphine, 1 treated with 0.1 μg morphine, 1 treated with 0.03 μg morphine, 2 treated with 0.01 μg morphine, and 1 treated with 0.0 μg morphine). The five remaining 'misses' were anterior (1 treated with 0.3 μg morphine and 1 treated with 0.03 μg morphine) or dorsal (1 treated with 0.1 μg morphine, 1 treated with 0.03 μg morphine, and 1 treated with 0.01 μg morphine). Data from these 'missed' cannula placements were excluded from group statistical analyses. During this experiment, all rats continued to show normal estrous cyclicity, regardless of drug treatment; no significant correlation between estrous cycle stage and the onset of maternal behavior was observed.

Table 1
Median sensitization latency to show full maternal behavior and median latency to show pup grouping in Expt. 1A

Treatment	n	Median sensitization latency (days)	Median latency to show pup grouping [c] (days)
Intra-VTA morphine			
0.3 μg	7	10.0	9.0
0.1 μg	8	8.5	8.0
0.03 μg	7	5.5 [b]	4.5
0.01 μg	5	10.0	10.0
0.00 μg	8	10.0	10.0
No treatment [a]	9	10.0	8.0

[a] Not used in the statistical analysis of the differences between specific drug doses. No treatment and 0.0 μg control groups did not differ significantly from each other in either sensitization latency or latency to show pup grouping ($P > 0.05$).
[b] Significantly different from 0.0 μg and 0.01 μg MS groups ($P < 0.05$).
[c] The overall comparison of the drug-treated groups showed a statistically significant difference ($P < 0.05$).

3.2.1. Latency to the onset of full maternal behavior (sensitization latency)

The median sensitization latencies of each group are reported in Table 1. A KW test comparing the sensitization latencies in each group was statistically significant (KW(5) = 17.81, $P = 0.003$). A subsequent multiple pairwise comparison probe of drug-treated rats (excluding the no-treatment group) revealed that rats treated with 0.03 μg MS had significantly shorter sensitization latencies (median = 5.5 days) than did rats treated either with 0.01 μg MS or 0.0 μg MS (median = 10 days for each group; $P < 0.05$). The sensitization latencies in rats treated either with 0.1 μg MS (median = 8.5 days) or 0.3 μg MS (median = 10 days) were not significantly different from any other group ($P > 0.10$). As expected, no statistically significant differences were found between vehicle-injected rats (0.0 μg MS) and those in the no-treatment group (KW(1) = 0.07, $P = 0.80$).

The intermediate effectiveness of MS at the higher doses is suggested by the proportion of rats showing full

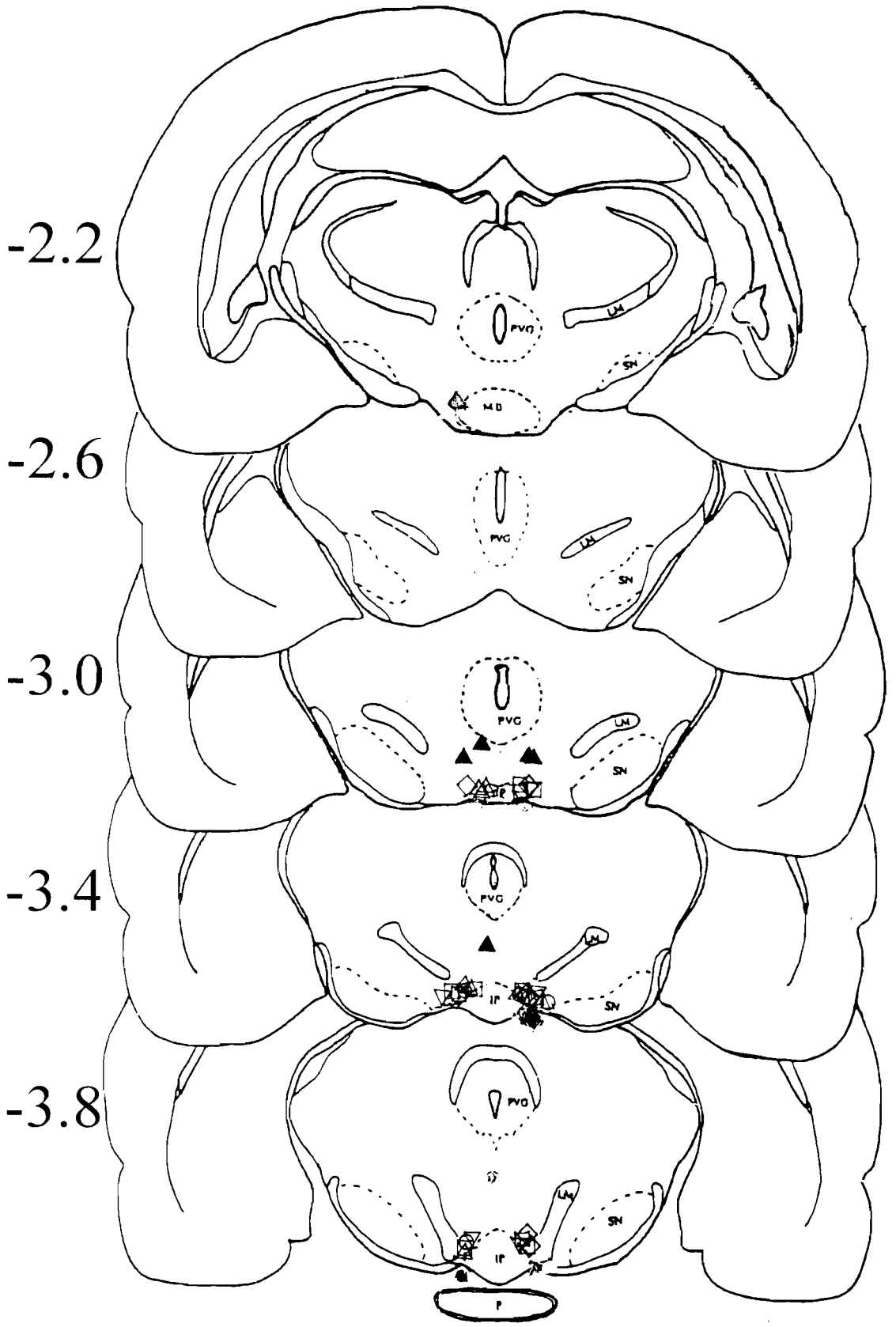

Fig. 1. Schematic representation of the location of cannula tips in Expts. 1A and 1B. Open symbol, 'hits' Expt. 1A, data used in the group statistics; gray symbol, 'misses' Expt. 1A, data excluded from the group statistics. □ 0.0 μg morphine, ○ 0.01 μg morphine, △ 0.03 μg morphine, ▽ 0.1 μg morphine, ◇ 0.3 μg morphine, ▲ dorsal placements in Expt. 1B (0.03 μg morphine). IP, interpeduncular nucleus; LM, medial lemniscus; MB, mammillary bodies; PVG, periventricular gray substance; P, pons; SN, substantia nigra. Diagram after Pellegrino et al. [56].

maternal behavior at different points during the testing period (Fig. 2). Whereas only 22–25% of the control rats (no-treatment and 0.0 μg MS) showed full maternal behavior during the 10-day test period, 100% of the rats treated with 0.03 μg MS, 75% of the rats treated with 0.1 μg MS, and 43% of the rats treated with 0.3 μg MS showed full maternal behavior during the sensitization period. Nonparametric analyses [14] revealed that the proportions of the 0.03 and 0.1 μg MS groups showing full maternal behavior during the testing period were significantly greater than that of the 0.0 μg MS group (overall $\chi^2(4) = 13.7$; $P < 0.01$; 0.03 μg vs. 0.0 μg: $\chi^2(1) = 8.7$, $P < 0.005$; 0.1 μg vs. 0.0 μg: $\chi^2(1) = 4.0$, $P < 0.05$; 0.3 μg vs. 0.0 μg: $\chi^2(1) = 0.5$, $P > 0.05$).

The sensitization latencies in rats with cannula placements outside the VTA were similar to those of controls. Three rats injected with 0.03 μg MS into areas dorsal ($n = 1$), anterior ($n = 1$), and ventral ($n = 1$) to the VTA, showed sensitization latencies similar to control values (10 days each) rather than similar to those of rats treated with 0.03 μg MS in the VTA (median = 5.5 days).

3.2.2. Latency to show pup grouping

A test comparing the median latencies to show pup grouping in each treatment group (Table 1) revealed an overall statistically significant difference (KW(5) = 11.75, $P = 0.04$). However, pairwise comparisons of the five drug-treated groups did not find any reliable group differences at $P < 0.05$. Although the extraneous objects (chew stick, pompom, eyedropper bulb, plastic ring) were occasionally manipulated and mouthed by the subjects, no reliable patterns of behavior (e.g., relocation, retrieving, grouping) directed toward these objects was ever detected.

3.2.3. Behavior during the observation period

One-way ANOVA was used to test group differences in pup-directed behaviors and total active behavior at each observation (Day 2, Day 5, and during full maternal behavior), yielding a total of six 1-way ANOVAs. A modified α level was used so that statistical significance in any one test was achieved if α was less than 0.008 (overall for 6 ANOVAs, total $\alpha = 0.048$).

Day 2 morning observations. The time spent (mean ± S.E.M.) in each behavior category during the 15-min observation on the morning of Day 2 (1 h after drug treatment) is depicted in Fig. 3. Overall, rats in all groups spent most of their time engaged in an active behavior, with mean total activity scores ranging from 560.6 ± 120.5 s (in the 0.03 μg MS group) to 883.4 ± 9.6 s (in the 0.1 μg MS group). Very little time was spent in pup-directed behavior, typically less than 30 s. No significant differences in pup-directed behavior or total active behavior were found among groups by 1-way ANOVA (pup-directed behavior: $F_{5,38} < 1$; total active behavior: $F_{5,38} =$

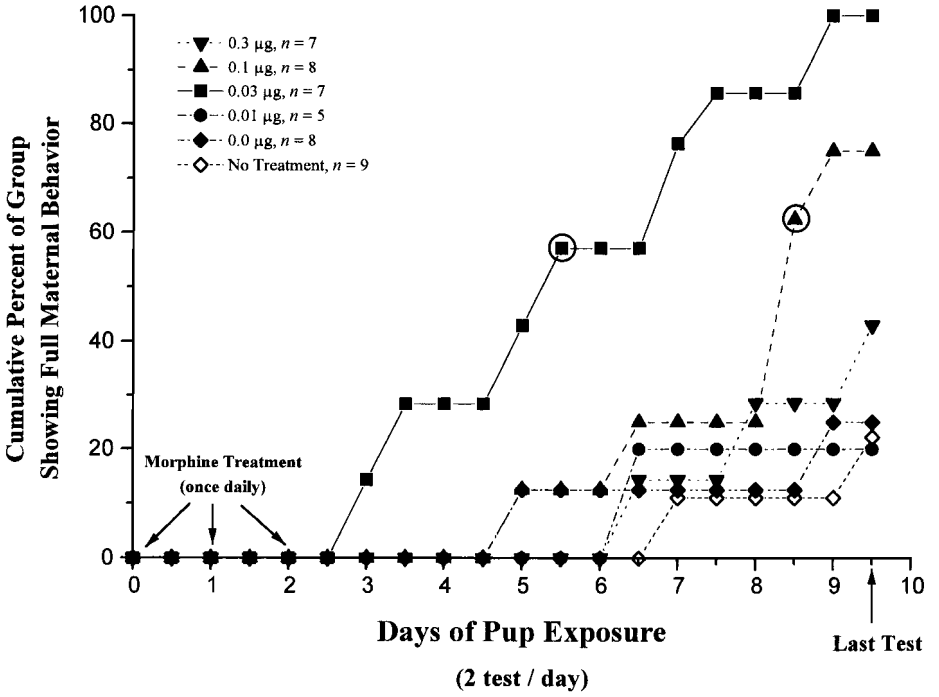

Fig. 2. Cumulative percent of group showing full maternal behavior at each test over the 9.5-day sensitization/testing period. Nonmaternal nulliparae were given unilateral intra-VTA injections of morphine (0.0, 0.01 0.03, 0.1, or 0.3 μg) or no treatment 1 h before the morning test on Days 0, 1, and 2. The median latency of the 0.03 μg and 0.1 μg morphine groups is indicated by an open circle. Remaining groups were assigned a median latency of 10 (0.5 day longer than the longest possible median) because fewer than half of the rats in the these groups showed full maternal behavior during the sensitization period. The proportion of rats showing full maternal behavior during the pup-exposure period was significantly greater in the 0.03 μg and 0.1 μg morphine groups than in the 0.0 μg morphine group ($P < 0.05$).

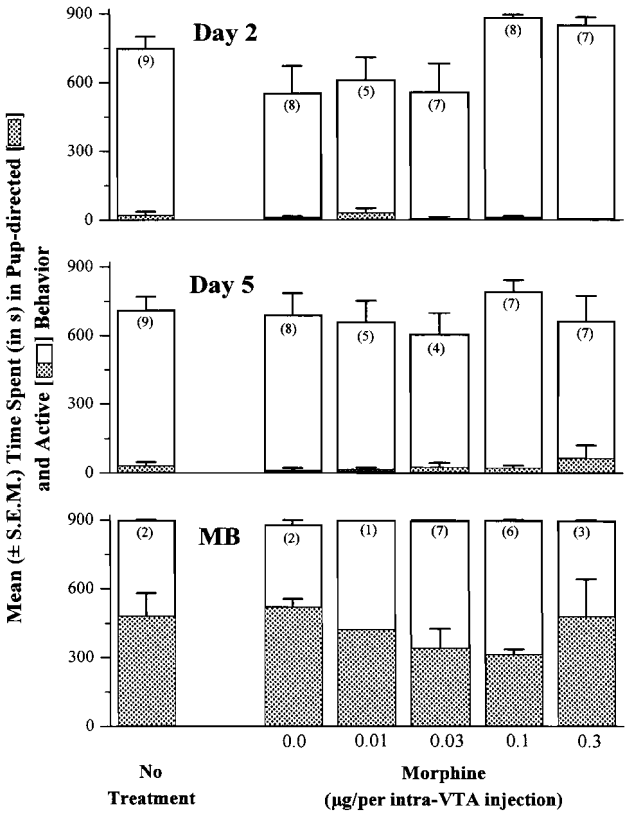

Fig. 3. Mean ± S.E.M. time spent (in s) in pup-directed or active behavior during the 15-min test on the morning of Days 2 and 5, and during the test in which full maternal behavior was observed (MB). Nonmaternal nulliparae were treated with either intra-VTA morphine (0.0, 0.01, 0.03, 0.1 or 0.3 μg) or no treatment 1 h before the morning test on Days 0, 1, and 2. The number in parentheses represents the number of rats observed on that test day (rats showing maternal behavior before Day 5 were not among those tested on Day 5; rats not ever showing full maternal behavior were not tested on Day MB). No significant differences among groups in pup-directed behavior or active behavior were found on any day.

3.45, $P > 0.008$). A more limited comparison between the group that showed the most rapid onset of maternal behavior (0.03 μg morphine) and the controls also revealed no differences in either pup-directed or total active behavior (Ps > 0.05).

Day 5 morning observations. The time (mean ± S.E.M.) spent in each behavior category during the 15-min observation on the morning of Day 5 (3 days after the last drug injection) is reported in Fig. 3. The pattern of data is similar to that obtained in the Day 2 observation. No differences in either pup-directed behavior ($F_{5,33} < 1$) or total active behavior ($F_{5,33} < 1$) were found. Note that five rats (three from the 0.03 μg MS group, one from the 0.1 μg MS group, and one from the 0.0 μg group) were not observed during this observation as they had already shown full maternal behavior by Day 5. None of the remaining rats was showing full maternal behavior during the Day 5 observation, although approximately 55% (21/39) of these rats would show full maternal behavior over the next 4 days.

Observation after the onset of full maternal behavior. The time (mean ± S.E.M.) spent in each behavior category during the 15-min observation in which each rat showed full maternal behavior for the first time is reported in Fig. 3. In contrast to the rats on the previous observation days (Day 2 and Day 5), those showing full maternal behavior spent a much greater proportion of time in pup-directed behavior (approximately 360–480 of 900 s). No significant differences were found in the 1-way ANOVA that compared pup-directed behavior among the rats receiving the three highest drug doses (0.3, 0.1, and 0.03 μg MS) and controls (no treatment and saline combined) ($F_{3,16} = 1.51$, $P = 0.25$).

The results of Expt. 1A support the hypothesis that opioid activity in the VTA facilitates the onset of maternal responsiveness, by showing that increased opiate activity in the VTA (by injection of morphine) decreased the maternal sensitization latency in a dose-dependent fashion. An inverted U-shaped pattern of dose-responsiveness was obtained in which the effectiveness of MS was reduced as the dose increased, presumably as a result of an increase in a competitive response(s), reduced receptor selectivity, or both. The first observation period was intended to evaluate the possibility of the appearance of a competitive response. This observation was made 1 h after the third MS administration, that period when the effect of MS should have been maximal: Acute microinjection of MS into the VTA increases activity over several hours, peaking at about the end of the first hour [26,29], and repeated MS administration enhances this effect [79]. No unusual behaviors were observed by casual inspection and no significant group differences in total activity were found during the first observation period. Although the activity level of the two highest drug dose groups may have been maximal in that the rats remained active over the entire 15-min sampling period (Fig. 2). It is possible that had the observation period been longer, it would have revealed significantly higher total activity levels in these two highest dose groups relative to the remaining treatment groups. Such a finding would then suggest that an increase in general activity may have competed with the development of maternal behavior. Changes in receptor selectivity seem less likely. Certainly the doses used here were not so high as to induce non-opioid receptor-mediated effects. The doses we used were well below those typically used in studies of opioid-induced locomotor activation [26,29]. However, the VTA contains multiple opioid receptor types [47] and so it is possible that the proportional stimulation of these receptor types varied over the range of MS doses used here. In the absence of additional data (i.e. longer observation periods or more selective opioid-receptor agonists), we can only speculate about the reduced efficacy of the higher drug doses.

No rat showed full maternal behavior (or pup grouping)

during the 3-day period of drug injections, therefore our results do not reflect an acute drug-bound change in the expression of behavior, but rather a drug-induced increase in the induction of maternal behavior toward pups.

Fewer than half of the rats in control groups developed full maternal behavior during the 10 day testing period. Large variations in sensitization latencies between laboratories have been reported and ascribed to differences in strain [28,67] and housing environment [75]. In this case, it seems probable that the steps we took to reduce pup-initiated contact (the addition of floor dividers) increased the co-habitation time needed to induce full maternal behavior rather than blocked the development of maternal behavior. Whereas it is possible that these rats may have never shown maternal behavior, the overwhelming preponderance of data in this field suggests that they would eventually do so.

Finally, the ancillary behaviors of rats in that dose group with the shortest sensitization latencies (0.03 μg MS) did not differ from those of control rats. In addition, the reduced efficacy of higher MS doses would seem to rule out the possibility that drug-induced increases in the period of activation increased incidental pup contact and reduced sensitization latencies; higher doses of MS treatment would in fact be predicted to produced *faster* development of maternal behavior. Therefore, no specific differences in general behavior or pup-directed behavior were observed that might account for the facilitated onset of maternal responsiveness among the rats receiving 0.03 μg MS.

4. Experiment 1B

The conclusion reached in Expt. 1A, that morphine sulfate injected into the VTA facilitates the onset of maternal behavior, rests on either or both of two key assumptions about the action of the MS once it is injected into the VTA. Specifically that (a) the effect of intra-VTA morphine on maternal responsiveness is the result of the action on opioid receptors (receptor specificity), and (b) the effect of intra-VTA morphine on maternal responsiveness is the result of the action of morphine within the VTA (anatomical specificity). Experiment 1B tested these assumptions by determining the sensitization latency in two groups of rats pretreated systemically with the opioid antagonist naltrexone hydrochloride or vehicle and receiving the most effective intra-VTA dose of morphine found in Expt. 1A (0.03 μg), and one group of rats pretreated systemically with vehicle and receiving the most effective dose of morphine in a neuroanatomical site dorsal to the VTA. We expected that either pretreatment with the antagonist or injection into a site other than the VTA would prevent the facilitating effects of morphine on the onset of maternal behavior, therefore reflecting the receptor-specific and location-specific actions of intra-VTA morphine injection.

4.1. Method

4.1.1. Subjects

Twenty-five virgin rats were used. In 20, cannula placement was aimed at the VTA; in the remaining five, the cannula placement was aimed at a point 2 mm dorsal to the VTA. Rats with VTA placements were randomly assigned to one of two pretreatment conditions (naltrexone hydrochloride: $n = 10$, or vehicle: $n = 10$); rats with dorsal-to-VTA placement were pretreated only with vehicle (for comparison to VTA/vehicle-pretreated rats).

4.1.2. Drugs

The naltrexone hydrochloride (1 mg/kg, s.c.) was mixed in 0.9% sterile saline and injected 10 min before the intra-VTA injection of morphine sulfate (0.03 μg). The dose of naltrexone hydrochloride (1 mg/kg) was found in a pilot study to be effective in blocking the action of intra-VTA MS (1 μg in 0.5 ml) on morphine-induced forward locomotor activity.

4.2. Results and discussion

One rat from the dorsal VTA placement group was lost as the result of surgery and two rats from the vehicle-pretreatment intra-VTA group did not complete the testing sequence after the integrity of their indwelling cannula was compromised. Histological examination revealed that cannula placements in 16 of the remaining 22 rats fell in the expected area of the brain (Fig. 1). In six rats, the cannula placement that was intended to hit the VTA fell outside the area of the VTA (4 from the naltrexone-pretreatment group and 2 from the vehicle-pretreatment group). The missed cannula placements were dorsal ($n = 4$), anterior ($n = 1$), or ventral ($n = 1$) to the VTA. Data from the missed cannula placements were excluded from group statistical analyses, except for one rat with a dorsal placement that had also received pretreatment with vehicle. Her treatment was identical to that of rats in the planned dorsal-to-VTA group and so her data were added to that group.

4.2.1. Maternal behavior

Median sensitization latencies for each group in Expt. 1B are reported in Table 2. As expected, pretreatment with 1 mg/kg naltrexone hydrochloride blocked the facilitating effect of 0.03 μg MS in the VTA on sensitization latencies. Vehicle-pretreated rats with cannulae in the VTA had significantly shorter sensitization latencies (median = 6.5 days) than did naltrexone-pretreated rats (median = 10 days) ($KW(1) = 5.32$, $P = 0.02$). The same differences were found in the comparison of the latency to show grouping into a nest ($KW(1) = 4.68$, $P = 0.03$; vehicle pretreatment latencies (median = 5.5 days) < naltrexone pretreatment latencies (median = 10.0 days)).

The vehicle-pretreated rats receiving non-VTA morphine (5 dorsal, 1 anterior) showed long sensitization

Table 2
Median sensitization latency to show full maternal behavior and proportion of rats showing full maternal behavior during the 10-day period of cohabitation with pups in Expt. 1B

Pretreatment	*n*	Median sensitization latency (in days)	Proportion of rats showing full maternal behavior within 10 days
Rats injected with 0.03 μg MS intra-VTA			
Naltrexone HCl (1 mg/kg, s.c.)	6	10.0 [b]	0.17
Vehicle (1 ml/kg, s.c.)	6	6.5	0.83
Rats injected with 0.03 μg MS dorsal to the VTA			
Vehicle [a] (1 ml/kg, s.c.)	5	10.0 [b]	0.20

[a] Rats received vehicle pretreatment for the purpose of comparison to the intra-VTA MS-injected group receiving vehicle.
[b] Significantly different from vehicle pretreated rats receiving intra-VTA morphine ($P < 0.05$).

latencies (median = 10 days) relative to those vehicle-pretreated rats receiving VTA morphine (median = 6.5 days; KW(1) = 4.44, $P = 0.035$, dorsal vs. VTA site). Therefore, the onset of full maternal behavior and of pup-grouping were facilitated only in rats with cannula placements in the VTA.

The results of Expt. 1B strengthened the conclusion drawn from Expt. 1A that an increase in opioid activity in the VTA during the first few days of pup exposure hastens the onset of maternal responsiveness. In Expts. 1A and 1B, rats treated with a single injection of MS on each of the first three days of sensitization showed full maternal behavior in little more than half the time of controls (5.5 to 6.5 days vs. > 10 days). Blockade of opioid receptors by pretreatment with systemic naltrexone prevented the facilitating effect of MS on maternal responsiveness, supporting an opioid-receptor specific site of action for the MS. An alternative interpretation is that systemic naltrexone interfered with the onset of maternal behavior through a mechanism independent of the blockade of opioid receptors in the VTA. However, this interpretation is not supported in previous studies by Mayer et al. [50] which showed that systemic naltrexone and naloxone alone do not prevent the onset of maternal behavior in the rat. Finally, the long sensitization latencies in rats with cannula placements lying outside the VTA argue that the site of the opioid-receptor field in which the intra-VTA morphine is acting to facilitate sensitization latency is within the VTA.

5. Experiment 2

In Expt. 2, the effect of blocking VTA opioid receptors on the onset of maternal behavior was tested during the time when maternal behavior normally develops very rapidly – the periparturitional period. Forty minutes after parturition, mothers whose pups had been removed at delivery were injected with the quaternary opioid antagonist naltrexone methobromide (QN) bilaterally into the VTA. QN has been shown to diffuse slowly from the site of injection in the CNS, and to be resistant to crossing the blood-brain barrier [6,11,78]. Thirty minutes after the test rat was injected, foster stimulus pups were introduced into the cage and the development of maternal behavior was observed.

5.1. Method

5.1.1. Subjects

Forty-seven postpartum primiparous rats were divided into four groups: a QN-injected group; a vehicle-injected group; a surgery-control group (sham treatment); and a no-treatment group.

5.1.2. Drugs

QN (MRZ 2663 BR, Batch H) was mixed in 0.9% sterile saline. Bilateral injections consisted of 0.5 μg QN in 0.5 μl, injected at a rate of 1 μl/min. The vehicle-injected rats received 0.5 μl saline, injected at a rate of 1 μl/min. This QN dose is within the range used previously to block other opioid-mediated behaviors effectively when applied centrally [7,8,77].

5.1.3. Pretesting procedures

After the recovery period, accuracy of intra-VTA cannula placement was assessed indirectly in 27 rats by determining if a unilateral injection of MS (1 μg in 0.5 μl; 1 μl/min) induced contralateral circling [26] during a 1-h period after the injection. Two days after the first contralateral-circling test, the rat was again tested, this time by injecting MS into the other cannula. Any rat that failed to show a net increase in contralateral circling after each MS injection was not tested further, since it was unlikely that her cannulae placements fell in the VTA.

Approximately 1 week after the two contralateral-circling tests, rats in proestrus were mated by co-housing them with males overnight. Mating was considered successful if sperm was found in the female's vaginal smear the next morning (Day 1 of pregnancy).

5.1.4. Testing procedures

On Day 21 of pregnancy, rats were moved to delivery cages so they could habituate to them for at least 24 h. The characteristics of the delivery cage were identical to those

of the observation cages described above, except that the bottom was constructed from a coarse wire mesh, so that pups would fall through the bottom of the cage onto a soft mat (cotton batting) as they were delivered, thereby allowing easy and unobtrusive removal by the experimenter. In addition, a small, opaque, square of Plexiglas ($10 \times 10 \times 0.3$ cm) was placed in the cage to provide a platform on which the female could easily adopt an anogenital-grooming/birthing posture. The pad was not large enough to be occupied by both the female and the pups. Plexiglas dividers were not used to partition the aquaria into quadrants as in Expts. 1A and 1B. Finally, great care was taken to remove the pups as soon as possible, since it seemed in a prior test of this apparatus that the mother became agitated by the presence of an out-of-reach pup on the floor beneath the delivery cage. The parturient behavior of control rats in response to these experimental procedures was similar to the parturient behavior described by Dollinger et al. [12] and by Kristal et al. [43]; for instance, delivery times fell within an expected range and females remained relatively active during the immediate postpartum period.

Dams were observed continuously starting on Day 22 of pregnancy for the appearance of labor and delivery. Forty minutes postpartum, each dam received her assigned drug treatment and was returned to her delivery cage, which was now equipped with a solid Plexiglas floor. Bilateral injections were given consecutively using the same microinfusion pump used in Expts. 1A and 1B.

Pups were reintroduced to the female's cage 30 min after the drug injection. To provide a stimulus similar to that existing during parturition, pups were introduced with birth fluids and tissues. The four foster pups were covered with a mixture of 1 ml amniotic fluid and four placentas (minced and heated to 37°C) immediately before placing them in the test rat's cage for the first time. Amniotic fluid and placenta from Day 21 pregnant females is regularly collected and stored in our laboratory [42]. Fifteen-minute behavior observations were conducted at 0, 2, 6, 12 h, and subsequently every 12 h during the postpartum testing period. Pups were replaced every 12 h with clean well-fed pups. During each behavior-observation period, the presence or absence of full maternal behavior and pup grouping was noted and the duration of pup-directed behavior, self-directed behavior, other activity, and resting were determined as in Expt. 1A. In addition, the duration of parturition, total number of pups, number of pups that were cleaned off during parturition, and the number of placentas eaten were recorded. The latter information was used to assure that the parturitional experiences of rats in each group were similar. Group statistical comparisons were made on latency data and on duration data obtained during the 0 and 2 h observation periods and also during the first observation period in which full maternal behavior was observed.

5.2. Results and discussion

The contralateral-circling test indicated that 18 of the 27 rats tested had bilateral cannulae terminating in the VTA. In the nine remaining rats, eight showed contralateral circling after one of the two circling tests, indicating that they were unilateral 'hits' only; one rat failed to show contralateral circling after either test, indicating that both cannulae missed the VTA. Only rats shown to have bilateral 'hits' were used for the remainder of the experiment. The remaining 18 rats were combined with another 10 rats that had bilateral implants aimed at the VTA but had not been screened in the contralateral-circling test, and then randomly assigned to treatment conditions: 12 were assigned to the experimental treatment group (QN), 8 to the vehicle-injection group, and 8 to the sham-injection group. Because all control groups were expected to perform similarly and because some rats were expected to be lost over the course of pregnancy (see below), a larger number of rats was assigned to the QN treatment group, so that at minimum, a statistical comparison between rats in the experimental condition and rats in the combined control conditions would be possible.

Thirty-eight rats were bred (18 contralateral circlers, 10 non-screened VTA-implanted, and 10 no-treatment controls). Of these, 24 carried their pregnancies to term and

Table 3
Mean ± S.E.M. of several measures of labor and delivery in Expt. 2

Treatment	n	Duration of parturition (min)	Proportion of whole placenta eaten [a]	Proportion of total number of pups cleaned [b]	Litter size
Naltrexone methobromide	6	147.5 ± 12.7	0.63 ± 0.03	0.76 ± 0.06	10.5 ± 0.8
Vehicle	5	127.0 ± 27.2	0.86 ± 0.10	0.88 ± 0.09	7.6 ± 1.7
Sham injection	4	133.8 ± 38.6	0.80 ± 0.06	0.80 ± 0.08	10.0 ± 1.2
No treatment	5	158.0 ± 38.9	0.66 ± 0.10	0.71 ± 0.06	12.0 ± 0.5
$F_{3,16}$		< 1	2.05	1.10	2.61
P			0.15	0.38	0.09

[a] Number of whole placenta eaten/number delivered.
[b] Number of pups with birth membranes removed during delivery/number delivered.

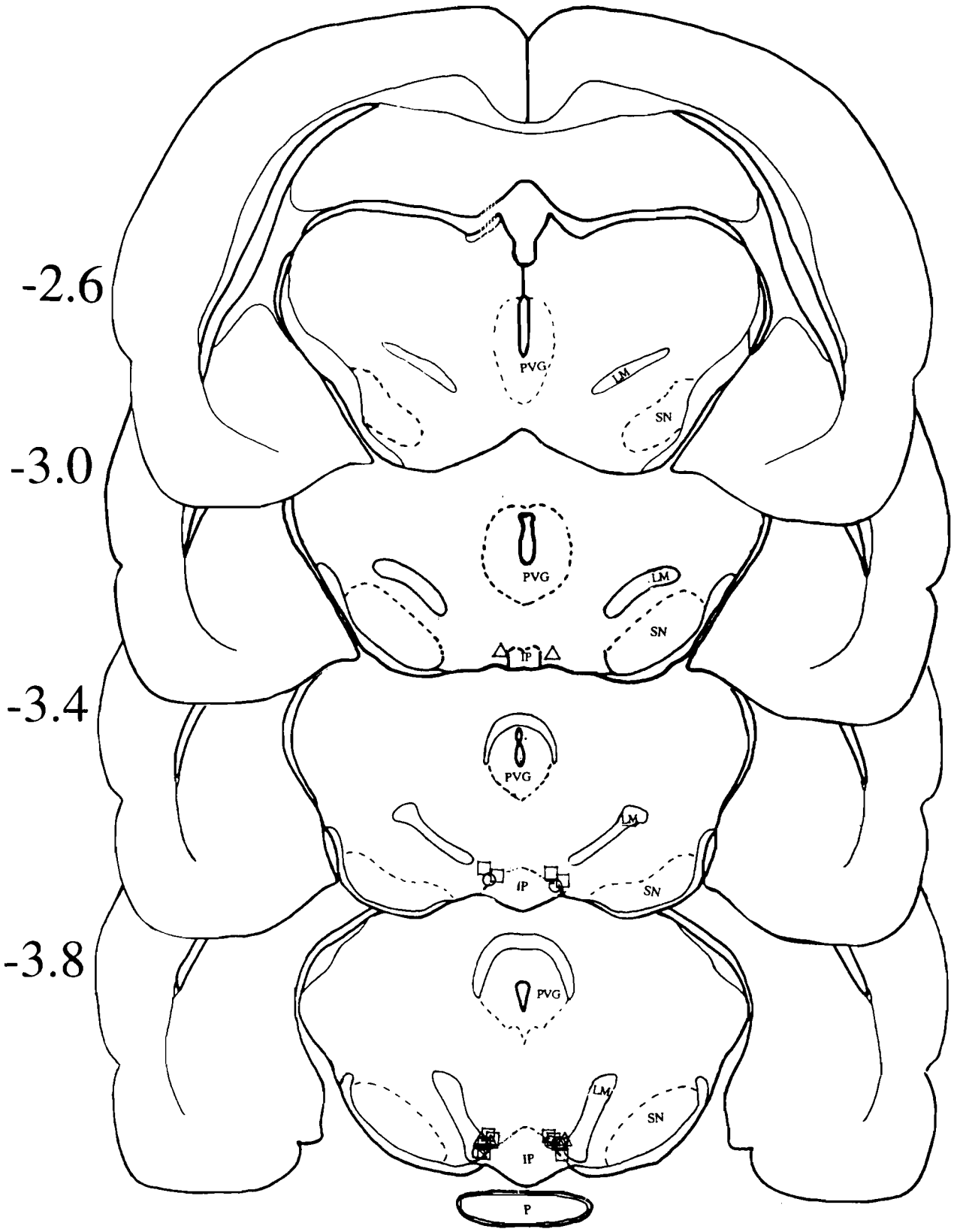

Fig. 4. Schematic representation of the location of cannula tips in Expt. 2. □ QN treatment; ○ sham injection; △ no injection. Missed placements not presented. IP, interpeduncular nucleus; LM, medial lemniscus; MB, mammillary bodies; PVG, periventricular gray substance; P, pons; SN, substantia nigra. Diagram after Pellegrino et al. [56].

could be tested (the remainder either failed to become pregnant, resorbed the fetuses, or sustained damage to the cannula assembly; no trends relating treatment (surgery) and the ability to initiate or maintain pregnancy were detected). Four of the 24 were found upon histological examination to have unilateral or bilateral cannula placement outside the VTA (none of the four had been screened), leaving a total of 20 rats in which complete data were collected and cannula placement was verified to lie within the VTA (Fig. 4). The results obtained from these 20 rats (5 no-treatment rats, 6 QN-injection rats, 4 no-injection rats, and 5 vehicle-injection rats) are reported here.

Because early pilot results indicated that the extent to which the female was 'distressed' during delivery would affect her subsequent behavior toward the pups, several measures of delivery were taken including day, time and duration of birth, total number of pups, total number of pups with birth membranes removed ('clean pups'), and total number of placentas eaten. The latter two measures also assessed indirectly the amount of pup contact the female had during delivery. Regarding the day and time of birth, 45% of the rats (9/20) gave birth on Day 22 of pregnancy, 55% (11/20) gave birth on Day 23, and all gave birth during the lights-on phase of the light/dark cycle (as is usual). Table 3 presents the statistics on the remaining birthing variables, by group.

Overall, no significant group differences in the birthing variables were found. Therefore, it seemed that the groups had a similar parturition experience prior to any manipulation. In addition, the periparturitional behavior of the 20 rats seemed comparable to the periparturitional behavior of undisturbed and unmanipulated dams observed in an another ongoing experiment in our laboratory, at least in terms of the expected time of birth (during lights on), expected day of birth (evenly split between Day 22 and Day 23 of pregnancy), and duration of birth (1 to 3 h with no periods of cessation greater than 30 min). Once the experimental manipulation had been performed and foster pups introduced into the cages of the postpartum females, maternal responsiveness was tested at 0, 2, 6, 12 h, and then every 12 h from the initial pup presentation until the rats showed full maternal behavior. Maternal sensitization latency was the primary dependent variable. Latency to show pup grouping was also recorded, however it did not differ from latency to show full maternal behavior and so was not analyzed statistically. In addition, as in Expts. 1A and 1B, the time spent in various behaviors during each observation period was measured.

5.2.1. Maternal responsiveness

The median sensitization latencies and cumulative percent of rats showing full maternal behavior at each test are presented in Fig. 5. QN-treated rats took significantly longer to show maternal behavior than did controls (QN: median = 30 h; Vehicle: median = 2 h; No injection: median = 4 h; No treatment: median = 6 h) ($KW(3) = 14.3$, $P = 0.0025$). As predicted, blockade of opioid receptors in the VTA during the natural onset of maternal behavior significantly delayed the onset of maternal responsiveness.

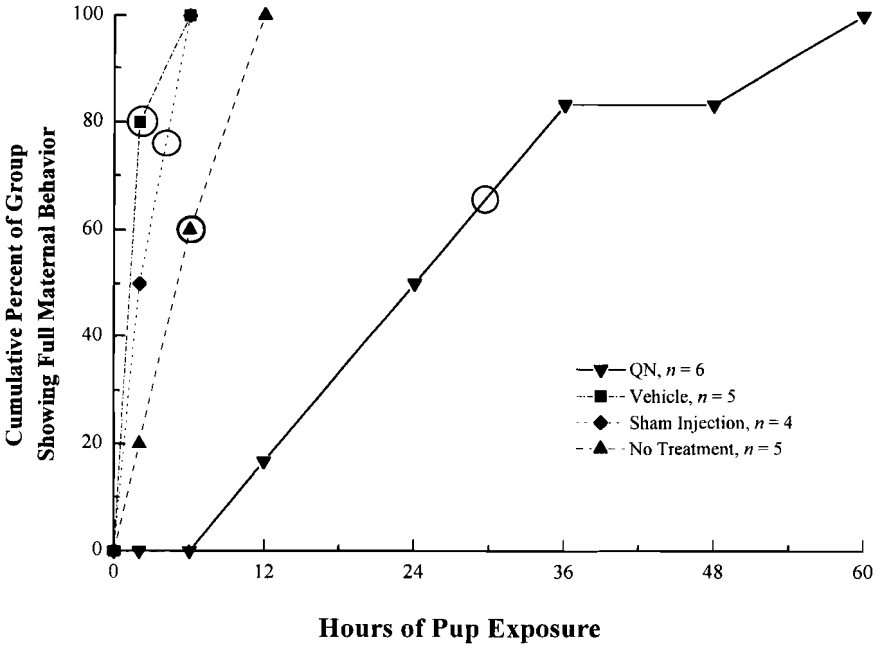

Fig. 5. Cumulative percent of group showing full maternal behavior after 0, 2, 6, 12, 24, 36, 48, and 60 h of pup exposure. Rats were given bilateral intra-VTA injections of either naltrexone methobromide (QN), vehicle, sham injection, or no treatment, at 40 min postpartum (30 min before pup presentation). The dams' own pups had been removed during parturition to slow or prevent the development of maternal behavior. No rat showed full maternal behavior during the first postpartum test. The median latency of each group is indicated by an open circle. The QN group showed a significantly slower onset of full maternal behavior than did the control groups ($P < 0.05$). Control groups did not differ significantly from each other ($P > 0.05$).

5.2.2. Other behavioral measures

The data from the 0 h observation period, the 2 h observation period, and the observation period in which full maternal behavior was observed were chosen for analysis, and are presented in Fig. 6. During the first observation (0 h), 30 min after the drug infusion and immediately after the first re-exposure to rat pups, no differences in either pup-directed behavior or in total active behavior were found (pup-directed behavior: $F_{3,16} < 1$; total active behavior: $F_{3,16} = 1.47$, $P = 0.25$).

At the second observation (2 h), 2.5 h after drug injection, a trend toward lower total active behavior was observed in QN-treated rats, however, the number of rats not yet showing full maternal behavior in control groups at this time was too small to assess statistically. Instead, data from the control groups were combined and then compared to the QN group. In these analyses, no differences in pup-directed behavior were found between QN-treated (37.6 ± 29.3 s) and control-treated (121.3 ± 80 s) rats ($F_{1,11} < 1$), and a significant decrease in total active behavior was found between QN-injected (212.3 ± 106.1 s) and control-treated (707.8 ± 102.8 s) rats ($F_{1,11} = 11.16$, $P = 0.006$).

No group differences in the duration of the ancillary behaviors were apparent during the observation period in which full maternal behavior was first observed (pup-directed behavior: $F_{3,16} = 2.52$, $P = 0.09$; total active behavior: $F_{3,16} < 1$).

These results strongly support the hypothesis that increased opioid activity in the VTA is necessary for the almost immediate onset of maternal behavior during the periparturitional period, as blockade of opioid activity in the VTA at that time impedes the natural onset of maternal responsiveness. No significant differences between treatment groups were found in the duration of various ancillary behaviors observed during the first observation (0 h) and, therefore, there are no obvious changes in behavior that are correlated with the difference in maternal responsiveness. At this time, all rats were observed to approach, sniff, and clean the placenta-covered pups, and no rat showed marked lethargy or aberrant stereotypic behavior. The significant decrease in total active behavior observed during the 2-h observation period was not correlated with sensitization latency; control-treated rats showing the least total active behavior (similar to the QN-injected rats) were nearest to showing full maternal behavior. In this observation period, any rat, regardless of drug treatment, not engaged in some pup-directed behavior was observed to be resting in a typical sleep posture. Overall, these data argue against the possibility that a decrease in pup contact (passive or active) delayed the sensitization process in QN-injected rats.

The delay in the onset of maternal behavior among QN-treated rats was longer than expected given previous reports on the time course of QN drug action. Peak effects of QN on other opioid-mediated behaviors have been

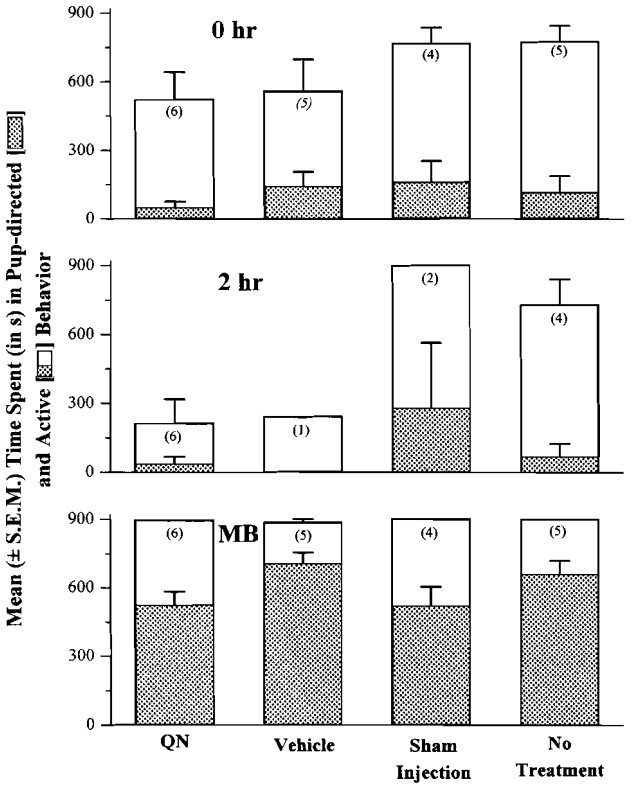

Fig. 6. Mean ± S.E.M. time spent (in s) in pup-directed or active behavior during the 15-min tests at 0 h and 2 h after pup presentation, and during the test in which full maternal behavior was observed (MB). Rats were treated with either naltrexone methobromide (QN), vehicle, sham injection, or no treatment at 40 min postpartum (30 min before pup presentation). The number in parentheses represents the number of rats observed on that test (rats showing maternal behavior before the 2-h test were not among those tested at 2 h). No significant differences among groups in either pup-directed behavior or active behavior were found at 0 h or MB. At 2 h, the QN-treated rats showed significantly less active behavior than did the combined controls ($P < 0.006$) but did not differ in pup-directed behavior.

obtained within 15 to 30 min of drug administration [7,8,44,77]. We reintroduced pups 30 min after the QN or control injection, and found that more than 90% of the control dams exhibited full maternal behavior within 6 h, whereas the fastest QN-treated dam showed full maternal behavior only after 12 h. If the time course for action of QN is similar to that reported for other opioid-mediated responses, then the appearance of maternal behavior among QN-treated rats required considerably more time than can be explained by the acute action of QN. This suggests that the effect of QN treatment was on the development of maternal behavior and not simply its expression. Although maternal behavior appears rapidly during parturition because of the facilitation effect of the parturitional, hormonal, and neurochemical milieu, a number of studies have demonstrated that in the absence of this facilitating milieu, as in the virgin rat, the induction of maternal behavior involves a gradual development of sensitivity toward pup stimuli [52,68]. In the natural context, this

begins during parturition and the immediate postpartum period when the dam has extensive contact with the pups in the processes of delivery and of cleaning and ingesting afterbirth materials. The importance of this contact is apparent when pup contact is reduced during parturition (as in this experiment) and the capacity of dams to respond to pup stimuli at a later time is hampered. By reducing pup contact during parturition, the appearance of full maternal behavior required several hours of subsequent exposure to pups. Recently, Fleming and Sarker [17] have shown that the first 2 h of postpartum pup exposure are critical for subsequent rapid induction of full maternal behavior. Disruption of mother–pup contact during this period increases significantly the sensitization latencies to pups at a later time. Perhaps a similar phenomenon occurred in this experiment. The advantage conferred to dams by pup contact during the immediate postpartum period was blocked by opioid reduction due to QN treatment, and so a longer period of development was required before full maternal behavior was exhibited in this group.

Observations made during the initial 15 min of postpartum pup exposure in Expt. 2 revealed no significant differences in the amount time spent with pups, and all dams were observed to lick and ingest the afterbirth materials. Casual notes taken during this period, however, suggested that QN-treated dams were less responsive to pup stimuli. These dams did not avoid stepping on pups, and showed no response to the pups when they did step on them. In contrast, dams in other treatment groups typically 'walked around' pups and 'jumped back' if they did step on one. However, once full maternal behavior was expressed, no differences in the quantity and quality of maternal behavior were found. In summary, QN treatment interfered with the development of maternal behavior during the immediately postpartum period, but did not produce a long-term change in the rat's capacity to develop this behavior or the quality and quantity of maternal behavior once it was established.

6. General discussion

The results of Expts. 1A and 1B show that increased opioid activity in the VTA is sufficient to reduce the amount of pup exposure needed to induce maternal behavior in virgin rats. Among rats treated with the most effective dose of morphine, the median sensitization latency was half that of control rats receiving no treatment, vehicle treatment, or an ineffective (low) dose of morphine. No evidence was obtained to suggest that the facilitating effect of morphine on maternal responsiveness was limited to the acute actions of the drug: no rat showed maternal behavior within the drug-injection period and grouping behavior was not dissociated from the onset of maternal responsiveness among drug-treated rats. Nor was any evidence obtained to suggest that the effect of morphine on maternal responsiveness was the result of the formation of a conditioned association between the presence of pups and the application of morphine: no group differences were obtained in the amount of time spent with pups, and no change in the females' responsiveness to novel objects, other than pups that were paired with the drug application, were observed at any time. Finally, no significant differences in activity level were observed among groups after three days of drug treatment, so incidental increases in pup contact due to an increase in general activity seem unlikely. Therefore, increased opioid activity in the VTA facilitated the onset of maternal responsiveness in virgin rats without overtly increasing pup contact.

The facilitating effect of intra-VTA MS on maternal responsiveness was shown to be specific to VTA and not the result of activation in areas immediately dorsal, anterior, or lateral to the injection site (Expts. 1A and 1B). Furthermore, the facilitating effect of intra-VTA MS appeared to be opioid-receptor mediated as the effect was blocked by pretreatment with systemically administered naltrexone.

In Expt. 2, blockade of opioid receptors in the VTA blocked maternal responsiveness during the periparturitional period without changing the amount of initial total activity or pup contact. This strongly suggests that opioid activity in the VTA is necessary for the rapid onset of maternal behavior associated with the periparturitional period.

Together, these results provide evidence for a stimulatory role for endogenous opioids in the development of maternal behavior. This would be in agreement with several papers on the initiation of maternal behavior in the sheep [35,37] and the expression of some maternal behaviors in the primate [48,63] and the rat [41,43,50]. These research findings seem to be in contrast with those of Bridges who showed that morphine produced an inhibitory effect on maternal behavior through its action on μ-opioid receptors in the medial preoptic area [5,20,45,46,62]. These seemingly conflicting results may reflect the difference in neuromechanism mediating the development and expression of maternal behavior. Another possibility is that facilitating or inhibiting effects of opioids are mediated by different neural substrates under different physiological conditions [66].

The dissociation between drug treatment and the eventual appearance of maternal behavior suggests that the results obtained in these experiments do not reflect an acute drug effect on the expression of maternal behavior. No virgin rat displayed full maternal behavior or any component of maternal behavior during the several-hour period following morphine microinjection into the VTA. Furthermore, in postpartum dams the appearance of maternal behavior after blockade of VTA opioid receptors occurred many hours later. Given the short action of the antagonist treatment, it seems that blockade of VTA opioid receptors did not simply prevent the expression of mater-

nal behavior; instead it apparently prevented the natural development of maternal behavior.

It is well established that microinjection of opioid agonists or antagonists into the VTA change A10 dopamine neurochemistry, increasing or decreasing dopamine levels respectively in the n. accumbens [9,10,21,33,49]. Furthermore, repeated opioid stimulation produces long term changes in mesolimbic dopaminergic activity [34]. Therefore, one possible mechanism of action for intra-VTA opioid stimulation or inhibition would be to increase or decrease dopamine neurotransmission.

Manipulations that increase or decrease mesolimbic dopamine activity affect a wide variety of motivated behaviors, including maternal behavior [3,24,34,51]. Disruption of the DA terminals in the n. accumbens by electrolytic lesion [65] or by 6-OHDA lesion [22–25] decreases the expression and initiation of maternal behavior. Dopamine receptor blockade by haloperidol disrupts some aspects of maternal behavior [19], whereas stimulation of dopamine, indirectly by tail pinch [1], facilitates the development of maternal behavior [71]. In contrast, cocaine, which dramatically increases dopamine in the n. accumbens and elsewhere, does not facilitate the onset or expression of maternal behavior and, in fact, has a negative effect on the development and expression of maternal behavior [32,39]. In these latter studies, however, cocaine was administered systemically, so it may have produced competitive effects at different neuroanatomical or neurochemical sites. Overall, the available data on dopaminergic manipulations within the mesolimbic system support the idea that the development and expression of maternal behavior are mediated in part by this dopaminergic system. Our data would lend some support in that it is well established that endogenous opioids regulate dopaminergic neurotransmission at the level of the VTA.

Manipulation of the mesolimbic dopamine system affect a broad range of behaviors; consequently, several theories have been developed to provide a unifying conceptual framework in which to view the role of the mesolimbic dopamine system in behavior, all of which emphasize the importance of this substrate in mediating sensory-motor responses [3,34,51,81]. A fundamental characteristic of maternal behavior is that it is stimulus-induced and stimulus-dependent [60,61,82]. Considerable research has suggested that among nulliparous females, olfactory stimuli inhibit the onset of maternal responsiveness [16], whereas somatosensory stimuli facilitate the onset of maternal responsiveness [36,69]. Interestingly, West and Michael [81] have shown that increases in nucleus accumbens dopamine selectively modify the electrophysiological response to somatosensory and olfactory stimuli. Perhaps in the experiments presented here, opioid manipulation modified the sensory quality of pup contact. Therefore, although total pup contact did not change, differences in the effect of pup contact may have existed. In Expts. 1A and 1B, intra-VTA morphine may have increased mesolimbic dopaminergic activity and enhanced the tactile sensory stimuli emitted by the pups, thereby facilitating the development of maternal behavior. In Expt. 2, blockade of VTA opioid receptors may have disturbed the initiation of maternal behavior by blocking the stimulating actions of endogenous opioids on mesolimbic dopamine, which in turn may have reduced the effects of pup contact.

No evidence is available to show whether endogenous opioid levels in the VTA are changed during the immediate postpartum period. However, endogenous opioid activity is greatly affected by gonadal steroids [58,80]. Wardlaw and Frantz [80] suggested that dynamic changes in midbrain opioid activity do occur around parturition, reporting that increases in opioid levels in the midbrain occur during later pregnancy, peak near parturition, and decrease in the first 24–48 h postpartum. Additional research will be necessary to determine if similar changes occur in the VTA and if so, whether these changes reflect an increase in opioid release that is correlated with the period when maternal behavior develops.

In conclusion, the data presented here provide further support for the importance of the VTA in the underlying neuroanatomic substrate mediating maternal behavior. Furthermore, these results add to a growing body of evidence that suggest that endogenous opioids participate in significant ways in periparturitional behavior, in this case by facilitating the onset of maternal behavior through action in the VTA.

Acknowledgements

This research was supported by NSF Grants BNS 88-19837 and IBN 91-23748 awarded to M.B.K., and a Mark Diamond Fund Award to A.C.T. The authors thank Michael Bozarth for his helpful advice; Herbert Merz and Boehringer Ingelheim, A.G., for generously donating quaternary naltrexone; and Jean Di Pirro and Patricia Abbott for their assistance. The protocols used herein were approved by the IACUC of the State University of New York at Buffalo.

References

[1] Boutelle, M.G., Zetterström, T., Pei, Q., Svensson, L. and Fillenz, M., In vivo neurochemical effects of tail pinch, *J. Neurosci. Methods*, 34 (1990) 151–157.

[2] Bozarth, M.A., Neuroanatomical boundaries of the reward-relevant opiate receptor field in the ventral tegmental area as mapped by conditioned place preference, *Brain Res.*, 414 (1987) 77–84.

[3] Bozarth, M.A., The mesolimbic dopamine system as a model reward system. In P. Willner and J. Scheel-Kruger (Eds.), *The Mesolimbic Dopamine System: From Motivation to Action*, John Wiley and Sons, New York, 1991, 299–328.

[4] Bridges, R.S., Parturition: Its role in the long term retention of maternal behavior in the rat, *Physiol. Behav.*, 18 (1977) 487–490.

[5] Bridges, R.S. and Grimm, C.T., Reversal of morphine disruption of

maternal behavior by concurrent treatment with the opiate antagonist naloxone, *Science*, 218 (1982) 166–168.

[6] Brown, D.R. and Goldberg, L.I. The use of quaternary narcotic antagonists in opiate research, *Neuropharmacology*, 24 (1985) 181–191.

[7] Brown, D.R. and Holtzman, S.G., Opiate antagonists: central sites of action in suppressing water intake of the rat, *Brain Res.*, 221 (1981) 432–436.

[8] Brown, D.R., Robertson, M.J. and Goldberg L.I., Reversal of morphine-induced catalepsy in the rat by narcotic antagonists and their quaternary derivatives, *Neuropharmacology*, 22 (1983) 317–321.

[9] Cador, M., Rivet, J.-M., Kelly, A.E., Le Moal, M. and Stinus, L., Substance P, neurotensin and enkephalin injections into the ventral tegmental area: Comparative study on dopamine turnover in several forebrain structures, *Brain Res.*, 486 (1989) 357–363.

[10] Dilts, R.P. and Kalivas, P.W., Autoradiographic localization of μ-opioid and neurotensin receptors within the mesolimbic dopamine system, *Brain Res.*, 488 (1989) 311–327.

[11] Di Pirro, J.M., Thompson, A.C. and Kristal, M.B., Amniotic fluid (POEF) ingestion enhances morphine analgesia centrally but not peripherally, *Brain Res. Bull.*, 26 (1991) 851–855.

[12] Dollinger, M.J., Holloway, W.R. Jr. and Denenberg, V.H., Parturition in the rat (*Rattus norvegicus*): Normative aspects and the temporal patterning of behaviors, *Behav. Proc.*, 5 (1980) 21–37.

[13] Fahrbach, S.E. and Pfaff, D.W., Hormonal and neural mechanisms underlying maternal behavior in the rat. In D.W. Pfaff (Ed.), *The Physiological Mechanism of Motivation*, Springer-Verlag, New York, 1982, pp. 253–285.

[14] Fleiss, J.L., *Statistical Methods for Rates and Proportions*, Wiley, New York, 1981.

[15] Fleming, A.S. and Rosenblatt, J.S., Maternal behavior in the virgin and lactating rat, *J. Comp. Physiol. Psychol.*, 86 (1974) 957–972.

[16] Fleming, A.S. and Rosenblatt, J.S., Olfactory regulaton of maternal behavior in rats: effects of peripherally induced anosmia and lesions of the lateral olfactory tract in pup-induced virgins, *J. Comp. Physiol. Psychol.*, 86 (1974) 233–246.

[17] Fleming, A.S. and Sarker, J., Experience-hormone interactions and maternal behavior in rats, *Physiol. Behav.*, 47 (1990) 1165–1173.

[18] Gaffori, O. and Le Moal, M., Disruption of maternal behavior and appearance of cannibalism after ventral mesencephalic tegmentum lesions, *Physiol. Behav.*, 23 (1979) 317–323.

[19] Giordano, A.L., Johnson, A.E. and Rosenblatt, J.S., Haliperidol-induced disruption of retrieval behavior and reversal with apomorphine in lactational rats, *Physiol. Behav.*, 48 (1990) 211–214.

[20] Grimm, C.T. and Bridges, R.S., Opiate regulation of maternal behavior in the rat, *Pharmacol. Biochem. Behav.*, 19 (1983) 609–616.

[21] Gysling, K. and Wang, R.Y., Morphine-induced activation of A10 dopamine neurons in the rat, *Brain Res.*, 277 (1983) 119–127.

[22] Hansen, S., Maternal behavior of female rats with 6-OHDA lesions in the ventral striatum: characterization of the pup retrieval deficit, *Physiol. Behav.*, 55 (1991) 6125–620.

[23] Hansen, S., Bergvall, Å.H. and Nyiredi, S. Interaction with pups enhances dopamine release in the ventral striatum of maternal rats: A microdialysis study, *Pharmacol. Biochem. Behav.*, 45 (1993) 673–676.

[24] Hansen, S., Harthon, C., Wallin, E., Loftberg, L. and Svensson, K., The effect of 6-OHDA-induced dopamine depletions in the ventral or dorsal striatum on maternal and sexual behavior in the female rat, *Pharmacol. Biochem. Behav.*, 39 (1991) 71–77.

[25] Hansen, S., Harthon, C., Wallin, E., Loftberg, L. and Svensson, K., Mesotelencephalic dopamine system and reproductive behavior in the female rat: Effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness, *Behav. Neurosci.*, 105 (1991) 588–598.

[26] Holmes, L.J. and Wise, R.A., Contralateral circling induced by tegmental morphine: Anatomical localization, pharmacological specificity, and phenomenology, *Brain Res.*, 326 (1985) 19–26.

[27] Jakubowski, M. and Terkel, J., Pup and object carrying by maternally and nonmaternally behaving female albino rats (*Rattus norvegicus*), *J. Comp. Psychol.*, 98 (1984) 331–317.

[28] Jakubowski, M. and Terkel, J., Incidence of pup killing and parental behavior in virgin female and male rats (*Rattus norvegicus*): Differences between Wistar and Sprague-Dawley stocks, *J. Comp. Psychol.*, 99 (1985) 93–97.

[29] Jenck, F., Bozarth, M. and Wise, R.A., Contraversive circling induced by ventral tegmental microinjections of moderate doses of morphine and [D-Pen^2,D-Pen^5]enkephalin, *Brain Res.*, 450 (1988) 382–386.

[30] Jenck, F., Gratton, M.A. and Wise, R.A., Opposite effects of ventral tegmental and periaqueductal gray morphine injections on lateral hypothalamic stimulation-induced feeding, *Brain Res.*, 399 (1986) 24–32.

[31] Jenck, F., Gratton, A. and Wise, R.A., Opioid receptor subtypes associated with ventral tegmental facilitation and periaqueductal gray inhibition of feeding, *Brain Res.*, 423 (1987) 39–44.

[32] Johns, J.M., Noonan, L.R., Zimmerman, L.I., Li, L. and Pedersen, C.A., Effects of chronic and acute cocaine treatment on the onset of maternal behavior and aggression in Sprague-Dawley rats, *Behav. Neurosci.*, 108 (1994) 107–112.

[33] Kalivas, P.W., Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon, *Neurosci. Biobehav. Rev.*, 9, (1985) 573–578.

[34] Kalivas, P.W. and Stewart, J., Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity, *Brain Res. Rev.*, 16 (1991) 223–244.

[35] Kendrick, M.K. and Keverne, E.B., Effects of intracerebroventricular infusions of naltrexone and phentolamine on central and peripheral oxytocin release and on maternal behaviour induced by vaginocervical stimulation in the ewe, *Brain Res.*, 505 (1989) 329–332.

[36] Keynon, P., Cronin, P. and Keebles, S., Disruption of maternal retrieving by perioaral anesthesia, *Physiol. Behav.*, 27 (1981) 313–321.

[37] Keverne, E.B. and Kendrick, M.K., Morphine and corticotrophin-releasing factor potentiate maternal acceptance in multiparous ewes after vaginocervical stimulation, *Brain Res.*, 540 (1991) 55–62.

[38] Kimball, C.D., Do endorphin residues of β-lipotropin hormone reinforce reproductive function? *Am. J. Obstet. Gynecol.*, 143 (1979) 127–132.

[39] Kinsley, C.H., Turco, D., Bauer, A., Beverly, M., Wellman, J. and Graham, A.L., Cocaine alters the onset and maintenance of maternal behavior in lactating rats, *Pharmacol. Biochem. Behav.*, 47 (1994) 857–864.

[40] Kinsley, C.H., Wellman, J.C., Carr, D.B. and Graham, A., Opioid regulation of parental behavior in juvenile rats, *Pharmacol. Biochem. Behav.*, 44 (1993) 763–768.

[41] Kristal, M.B., Enhancement of opioid-mediated analgesia: A solution to the enigma of placentophagia, *Neurosci. Biobehav. Rev.*, 15 (1991) 425–435.

[42] Kristal, M.B., Abbott, P. and Thompson, A.C., Dose-dependent enhancement of morphine-induced analgesia by ingestion of amniotic fluid and placenta, *Pharmacol. Biochem. Behav.*, 31 (1988) 351–356.

[43] Kristal, M.B., Thompson, A.C., Abbott, P., Di Pirro, J.M., Ferguson, E.J. and Doerr, J.D., Amniotic fluid ingestion by parturient rats enhances pregnancy-mediated analgesia, *Life Sci.*, 46 (1990) 693–698.

[44] Locke, K.W. and Holtzman, S.G., Characterization of the discriminative stimulus effects of centrally administered morphine in the rat, *Psychopharmacology*, 87 (1985) 1–6.

[45] Mann, P.E., Kinsley, C.H. and Bridges, R.S., Opioid receptor sub-

type involvement in maternal behavior in lactating rats, *Neuroendocrinology*, 53 (1991) 487–492.
[46] Mann, P.E., Pasternak, G.W. and Bridges, R.S., Mu opioid receptor involvement in maternal behavior in lactating rats, *Physiol. Behav.*, 47 (1990) 133–138.
[47] Mansour, A., Fox, C.A., Akil, H. and Watson, S.J., Opioid-receptor mRNA expression in the rat CNS: anatomical and function implications, *Trends Neurosci.*, 18 (1995) 22–29.
[48] Martel, F.L., Nevison, C.M., Rayment, F.D., Simpson, M.J.A. and Keverne, E.B., Opioid receptor blockade reduces maternal affect and social grooming in rhesus monkeys, *Psychoneuroendocrinology*, 18 (1993) 307–321.
[49] Matthews, R.T. and German, D.C., Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine, *Neuroscience*, 11 (1984) 617–625.
[50] Mayer, A.D., Faris, P.L., Komisaruk, B.R. and Rosenblatt, J.S., Opiate antagonism reduces placentophagia and pup cleaning by parturient rats, *Pharmacol. Biochem. Behav.*, 22 (1985) 1035–1044.
[51] Mogenson, G.J., Jones, D.L. and Yim, C.Y., From motivation to action: functional interface between the limbic system and the motor system, *Prog. Neurobiol.*, 14 (1980) 69–97.
[52] Numan, M., Maternal behavior. In E. Knobil and J. Neill, et al. (Eds.), *The Physiology of Reproduction, 2nd edn.*, Raven Press, New York, 1994, pp. 221–302.
[53] Numan, M. and Corodimas, K.P., The effects of paraventricular hypothalamic lesions on maternal behavior in rats, *Physiol. Behav.*, 35 (1985) 417–425.
[54] Numan, M. and Smith, H.G., Maternal behavior in rats: Evidence for the involvement of preoptic projections to the ventral tegmental area, *Behav. Neurosci.*, 98 (1984) 712–727.
[55] Orpen, B.G. and Fleming A.S., Experience with pups sustains maternal responding in postpartum rats, *Physiol. Behav.*, 40 (1987) 47–54.
[56] Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J., *A Stereotaxic Atlas of the Rat Brain*, Plenum Press, New York, 1979.
[57] Peters, L.C. and Kristal, M.B., Suppression of infanticide in mother rats, *J. Comp. Psychol.*, 97 (1983) 167–177.
[58] Petraglia, F., Baraldi, M., Giarre, G., Facchinetti, F., Santi, M., Volpe, A. and Genazzani, A.R., Opioid peptides of the pituitary and hypothalamus: Changes in pregnant and lactating rats, *J. Endocrinol.*, 105 (1985) 239–245.
[59] Phillips, A.G. and LePiane, F.G., Reinforcing effects of morphine microinjection into the ventral tegmental area, *Pharmacol. Biochem. Behav.*, 12 (1980) 965–968.
[60] Rosenblatt, J.S., Nonhormonal basis of maternal behavior in the rat, *Science*, 156 (1967) 1512–1514.
[61] Rosenblatt, J.S. and Lehrman, D.S., Maternal behavior of the laboratory rat. In H.L. Rheingold (Ed.), *Maternal Behavior in Mammals*, John Wiley and Sons, New York, 1963, pp. 8–57.
[62] Rubin, B.S. and Bridges, R.S., Disruption of ongoing maternal responsiveness in rats by central administration of morphine sulfate, *Brain Res.*, 307 (1984) 91–97.
[63] Schino, G. and Troisi, A., Opiate receptor blockade in juvenile macaques: Effect on affiliative interactions with their mothers and group companions, *Brain Res.*, 576 (1992) 125–130.
[64] Siegel, S. and Castellan, N.J., Jr., *Nonparametric Statistics for the Behavioral Sciences, 2nd edn.*, McGraw-Hill, New York, 1978.
[65] Smith, M.O. and Holland, R.C., Effects of lesions of the nucleus accumbens on lactation and postpartum behavior, *Physiol. Psych.*, 3 (1985) 331–336.
[66] Spanagel, R., Herz, A. and Shippenberg, T.S., Opposing tonically active endogenous opioid systems modulate the mesolimbic dopamine pathway, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 2046–2055.
[67] Stern, J.M., Maternal behavior priming in virgin and ceasarean-delivered Long-Evans rats: Effects of brief contact or continuous exteroceptive pup stimulation, *Physiol. Behav.*, 31 (1983) 757–764.
[68] Stern, J.M., Maternal behavior: Sensory, hormonal, and neural determinants. In S. Levine and F.R. Brush (Eds.), *Psychoendocrinology*, Academic Press, New York, 1988, pp. 1–104.
[69] Stern, J.M. and Kolunie, J.M., Perioral anesthesia disrupts maternal behavior during early lactation, *Behav. Neural Biol.*, 52 (1989) 20–38.
[70] Steuer, M.A., Thompson, A.C., Doerr, J.C., Youakim, M. and Kristal, M.B., Induction of maternal behavior in rats: Effects of pseudopregnancy termination and placenta-smeared pups, *Behav. Neurosci.*, 101 (1987) 219–227.
[71] Szechtman, H. Siegel, H.I., Rosenblatt, J.S. and Komisaruk, B.R., Tail-pinch facilitates onset of maternal behavior in rats, *Physiol. Behav.*, 19 (1977) 807–809.
[72] Tabachnick, B.G. and Fidell, L.S., *Using Multivariate Statistics, 2nd edn.*, Harper and Row, Cambridge, 1989.
[73] Tarapacki, J.A. and Kristal, M.B., An adaptable microcomputer program for recording behavior durations, *Physiol. Behav.*, 47 (1990) 381–384.
[74] Tarapacki, J.A. and Kristal, M.B., An adaptable microcomputer program for monitoring behavior, *Physiol. Behav.*, 53 (1993) 621–622.
[75] Terkel, J. and Rosenblatt, J.S., Aspects of non-hormonal maternal behavior in the rat, *Horm. Behav.*, 2 (1971) 161–171.
[76] Thompson, A.C. and Kristal, M.B., Opioids in the ventral tegmental area facilitate the onset of maternal behavior in the rat, *Soc. Neurosci. Abstr.*, 18 Part 1 (1992) 539.
[77] Ukai, M. and Holtzman, S.G., Suppression of deprivation-induced water intake in the rat by opioid antagonists: central sites of action, *Psychopharmacology*, 91 (1987) 279–284.
[78] Valentino, R.J., Herling, S., Woods, J.H., Medzihradsky, F. and Merz, H., Quaternary naltrexone: Evidence for the central mediation of discriminative stimulus effects of narcotic agonists and antagonists, *J. Pharmacol. Exp. Ther.*, 217 (1981) 652–659.
[79] Vezina, P. and Stewart, J., Conditioning and place-specific sensitization of increases in activity induced by morphine in VTA, *Pharmacol. Biochem. Behav.*, 20 (1984) 925–934.
[80] Wardlaw, S.L. and Frantz, A.G., Brain β-endorphin during pregnancy, parturition, and the postpartum period, *Endocrinology*, 113 (1983) 1664–1668.
[81] West, C.H.K. and Michael, R.P., Responses of units in the mesolimbic system to olfactory and somatosensory stimuli: modulation of sensory input by ventral tegmental stimulation, *Brain Res.*, 532 (1990) 307–316.
[82] Wiesner, B.P. and Sheard, N.M., *Maternal Behavior in the Rat*, Oliver and Boyd, Edinburgh, 1933.