parasitoids were reared at 27°C and 80 percent relative humidity, except when the pupae were kept at 16°C. Adults used in the tests emerged soon after the temperature was changed to 27°C.

9. The preparation of the artificial eggs has been described (*4, 5*). The resulting artificial egg had a diameter of about 2.5 mm. Each glass slide held three wax eggs, and a slide for each solution tested was placed inside a petri dish (10 by 1.5 cm) that was modified by removal of the lid tabs to prevent the escape of the minute parasitoids. The sex ratio was 1:1 and we usually used about 300 to 600 of the adult female parasitoids in each dish. The number varied because counting these small insects precisely was difficult. The petri dishes containing the artificial eggs and *Trichogramma* were rotated at 1 rev/min for 16 hours. Half of each test group was held at 27°C and 80 percent relative humidity and the other half was exposed to 25°C and 40 percent relative humidity. The artificial eggs were broken open, and visual counts of the *Trichogramma* eggs were made. Because of the variable number of parasitoids in each dish, we expressed the results as percentages of the total number of eggs collected in the artificial eggs inside each petri dish. Within the range used there was no effect from the number of parasitoids on the percentage distribution of eggs between test solutions. Holding conditions (temperature and relative humidity) had no effect on percentage distribution of eggs, but the number of eggs deposited was reduced at the lower temperature and relative humidity.
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Reversal of Morphine Disruption of Maternal Behavior by Concurrent Treatment with the Opiate Antagonist Naloxone

Abstract. *Rats whose pregnancies were surgically terminated on day 17 of gestation were injected with morphine, morphine plus naloxone hydrochloride, or saline, and then tested for maternal responsiveness toward foster young. Morphine treatment alone significantly disrupted the rate of onset and quality of maternal responsiveness. Concurrent administration of naloxone to morphine-injected rats reinstated the rapid onset of behavioral responsiveness toward foster young, such that the responsiveness of the rats treated with both morphine and naloxone was indistinguishable from that shown by saline-injected controls. The disruptive effects of morphine did not appear to result from a general reduction in activity levels as measured in an open-field apparatus. These findings suggest that the normal onset and maintenance of maternal behavior in the rat may be regulated by endogenous opiates.*

Endogenous opiate concentrations have recently been shown to change during the course of pregnancy and lactation. The immunoreactivity of β-endorphin increases during the second half of pregnancy in the hypothalamus of rats (*1*) and in the last trimester in the plasma of women (*2*). Changes in peptide concentrations during this reproductive period appear to be correlated with changes in sensory and behavioral processes. In rats, pain thresholds rise gradually during the second half of pregnancy, increase sharply about 2 days before parturition, and return to prepregnancy levels postpartum. A further decline in pain thresholds below that of control levels is found during lactation in the rat (*3*). These changes in pain thresholds appear to be opiate-mediated, since treatment with the opiate antagonist, naloxone hydrochloride, lowers pain thresholds during this reproductive period (*3*).

We have investigated the possibility that changes in opiate levels during pregnancy and parturition may reflect changes in central nervous system function and influence the expression of maternal behavior at the time of birth and during lactation. The onset of maternal behavior is hormonally (estradiol, progesterone) regulated in rats (*4*). Recent findings indicate that estradiol, the predominant sex steroid present in the mother at parturition (*5*), lowers brain β-endorphin concentrations in female rats (*1*). We hypothesized that the low levels of opiate activity during the postpartum period (*3*) may be involved in the regulation of maternal responsiveness in the rat. We therefore examined the effects of the opiate morphine and the opiate antagonist, naloxone hydrochloride, on the onset and quality of maternal responsiveness in rats after termination of pregnancy. This procedure stimulates the fast onset of maternal responsiveness as a result of endogenous changes in hormone secretion (*4, 6*). We focused on the period after pregnancy termination, reasoning that if lower opiate concentrations at the time of parturition are essential for maternal behavior, increasing the concentration of opiates after pregnancy termination would disrupt the behavior. Indeed, we found that morphine treatment delayed the onset and diminished the quality of maternal behavior after pregnancy-termination, and that these effects could be reversed in morphine-treated rats by the concurrent administration of the opiate antagonist naloxone hydrochloride.

Virgin female rats (Sprague-Dawley strain, Charles River Breeding Laboratories) weighing 225 to 250 g were mated in our laboratory. The morning after mating (day 1 of pregnancy) females were individually housed in polypropylene cages (25 by 45 by 20 cm). Food (Purina Rat Chow) and water were constantly available and room temperature was maintained from 21° to 24°C. In the first experiment 25 rats were ovariectomized and hysterectomized on day 17 of pregnancy as described (*6*). This procedure results in a high incidence of maternal responsiveness 22 to 24 hours later.

At the completion of surgery the females were assigned to one of three treatment groups, and received a subcutaneous injection of morphine sulfate (5 mg/kg), morphine sulfate (5 mg/kg) plus naloxone hydrochloride (0.5 mg/kg), or 0.9 percent saline (0.5 ml). All animals received the same dosages again 19 to 22 hours later, 1 hour before they were tested for maternal behavior.

To test for maternal behavior, we placed three recently fed rat pups (2 to 8 days old) in the home cage of the female and monitored her behavior continuously for 15 minutes. We checked her behavior again at 30, 45, and 60 minutes. Latencies to carry a pup, retrieve pups to the nest site, and crouch over the young were recorded. The pups and the females remained together until the following morning when their positions were again recorded; this checkpoint was referred to as the post-test rating. Pups were then removed from the home cages and the females were injected with freshly prepared solutions of morphine sulfate, morphine sulfate plus naloxone, or saline, at the same dosages as before. An hour later the test for maternal behavior was repeated with another set of recently fed pups. Each female was tested for 11 days or until she responded fully (that is, retrieved, grouped, and crouched over all three pups) during the 1-hour test on two consecutive test days. Animals that responded fully in the home cage were then tested for the intensity of maternal responsiveness in a T-maze apparatus for 4 days (*7*).

Morphine treatment significantly dis-

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rupted the rapid onset of maternal behavior induced by pregnancy-termination (Fig. 1). Saline-treated females responded maternally within 1 day, whereas morphine-treated rats required exposure to pups for 5 days before they displayed maternal behavior. The morphine disruption of maternal behavior was reversed by concurrent administration of naloxone hydrochloride (*8*). Rats treated with morphine and naloxone showed full maternal behavior in less than 1 day, as did the saline-treated controls. The disruptive effect of morphine treatment was not complete or long-lasting. Prior to the morphine injection on the second day of testing, five of the eight morphine-treated animals were crouching over the young in the nest site, whereas all the rats treated with both morphine and naloxone or with saline exhibited this response on the second test day. The apparent similarities among the three groups in post-test maternal ratings may have resulted from either the metabolism or the inactivation of the morphine, since the morphine-treated rats did not retrieve pups and were not maternal in the test following the next morphine injection.

Morphine also affected the quality of maternal responsiveness. Although six of eight morphine-injected animals eventually responded during the 1-hour test session, they responded more slowly than the rats in the other two groups on both the first and second days of home cage responsiveness (Table 1). The T-maze results revealed further deficits in the morphine-treated rats (Fig. 1). The six morphine-treated rats that responded in the home cage test were not as responsive to pups in the T-maze test, since only one such rat retrieved a pup from the T-maze, and that occurred on day 4 of testing. Females injected with morphine plus naloxone or with saline retrieved pups from the T-maze on each test day. The failure of the morphine-treated animals to retrieve did not result from lack of exploration of the T-maze, because there were no differences among the three groups in the number of rats contacting the pups in the arms of the T-maze over the four days of testing (see Fig. 1). Thus retrieval of the young appears to be more affected by morphine than the other components of maternal behavior, such as the crouching response, which was either unaffected or less affected by the morphine injections.

We also examined the possibility that the deficits in maternal behavior of the morphine-treated rats were a result of reduced activity. The performance of ten morphine-treated (5 mg/kg-day) and ten saline-treated rats (with pregnancies terminated on day 17 of gestation) were compared in an open field activity test which measures activity and timidity in a novel environment (*9*). As in the first experiment, rats were injected with morphine or saline after surgery and again 19 to 22 hours later, 1 hour before the testing. Each rat was placed in a designated square in a wooden enclosure (3 feet by 3 feet) on which a grid of 6-inch squares was demarcated. The number of times the animal reared on its hind legs and the number of squares entered during a 5-minute observation period were then recorded. Injections and testing were repeated daily for 4 days. There were no differences in the number of rears between the two groups on any test day (*10*). Rather than suppressing open field activity, the morphine (5 mg/kg) slightly enhanced the animals' activity. Animals injected with morphine over the 4 days entered more squares (473 ± 46, mean ± standard error) than did the saline-injected animals (268 ± 60, $P < .03$). This effect was primarily the result of differences present on day 4 of testing (morphine, 125 ± 24; saline, 44 ± 11, $P < .05$).

These results show that morphine disrupts maternal behavior after pregnancy-termination and that this effect is reversed by treatment with the opiate re-

Table 1. Effect of morphine on maternal responsiveness in rats. The data show latencies, in minutes, to respond to test pups on days in which full maternal responsiveness was observed in the home cage. Full maternal responsiveness includes retrieval, grouping, and crouching over three pups during the 1-hour test session.

Behavior	Treatment groups		
	Saline ($N = 8$)	Morphine ($N = 6$)	Morphine and naloxone ($N = 9$)
Day 1 of full maternal responsiveness			
Retrieval of first pup	1.4 ± 0.7	27.7 ± 5.4*	1.0 ± 0.4†
Full maternal responsiveness	12.6 ± 5.8	41.3 ± 6.7*	3.9 ± 1.2†
Day 2 of two consecutive days of full maternal responsiveness			
Retrieval of first pup	0.3 ± 0.1	2.0 ± 0.5‡	0.3 ± 0.1§
Full maternal responsiveness	5.2 ± 3.6	30.3 ± 11.4‖	1.2 ± 0.3§

*$P < .001$ compared to saline-treated controls. †$P < .001$ compared to morphine-treated animals. ‡$P < .05$ compared to saline-treated controls. §$P < .05$ compared to morphine-treated animals. ‖$P < .01$ compared to saline-treated controls.

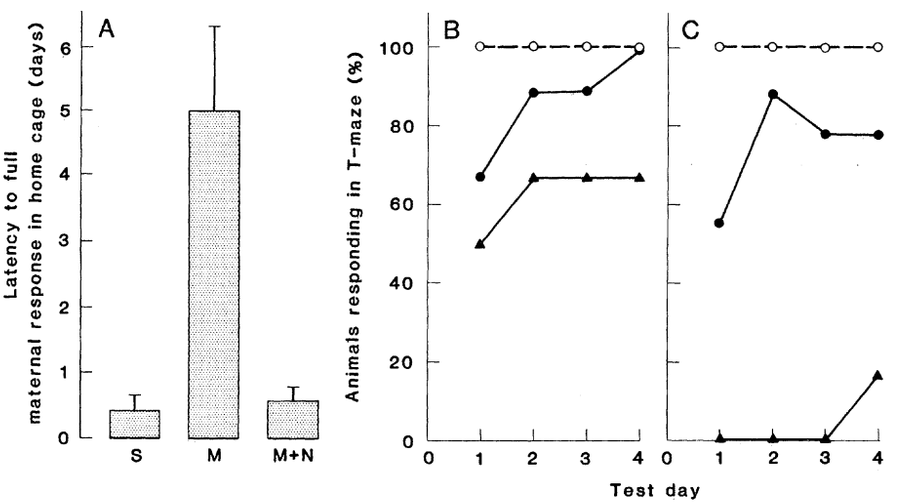

Fig. 1. (A) Maternal responsiveness in rats injected with morphine (*M*), morphine plus naloxone hydrochloride (*M* + *N*), or saline (*S*). The rats were tested daily for 1 hour in their home cages. Animals that responded fully during the first day (that is, retrieved, grouped, and crouched over the three test pups) were assigned a latency of 0 days. The latency of the morphine-treated rats ($N = 8$) to respond maternally during the test session was significantly (ANOVA, $P < .01$) longer than the latency of the saline-treated rats ($N = 8$) or rats treated with morphine plus naloxone ($N = 9$). The last two groups did not differ from each other. (B and C) Percentages of rats treated with morphine (▲) ($N = 6$), morphine plus naloxone hydrochloride (●) ($N = 9$), and saline (○) ($N = 8$) that contacted at least one pup (B) and retrieved at least one pup (C) on each day of T-maze testing. The day before the start of T-maze testing, each of these rats had responded fully for two consecutive days in the home cage. There were no statistical differences among the groups in the percentages of rats contacting pups on each test day. However, on each day significantly fewer morphine-treated rats retrieved pups than did rats treated with morphine plus naloxone or with saline (Fisher exact probability, all $P < .05$ to $P < .005$).

ceptor blocking agent naloxone hydrochloride. The disruption of the onset and quality of maternal responsiveness in the morphine-treated animals appeared to be somewhat specific, since activity in the open field test was enhanced rather than depressed in these animals. Taken together, these findings indicate that morphine affects maternal behavior by way of an opiate receptor mechanism, and lend support to the hypothesis that the normal expression of pup-oriented behaviors is opiate mediated.

That the intensity of behavioral responsiveness rather than the absolute incidence of the responses to pups was affected by morphine indicates that the action of the opiate system on maternal behavior is modulatory. One neural site of morphine's disruptive action on maternal behavior appears to be the medial preoptic area (*11*). Direct application of morphine to the medial preoptic area, an area involved in the estrogen-stimulated onset (*12*) and the nonhormonally regulated maintenance (*13*) of maternal behavior in the rat, disrupts pup-oriented responses in previously maternal rats. The site of morphine's action appears to occur with some degree of neural specificity, since application of morphine to the ventromedial nucleus of the hypothalamus fails to disrupt ongoing maternal behavior (*11*). It is not known whether morphine disrupts hormonal events responsible for the onset of maternal behavior or whether it has an independent nonhormonal mode of action.

The effect of morphine on maternal behavior does not appear to be simply a performance effect, since maternal behavior appeared in the morphine-treated rats after about 5 days of exposure to pups, at about the same time that morphine treatment affected open-field activity. Furthermore, the appearance of the behavior at this time does not appear to result from habituation or tolerance to morphine, since retrieval of young from the arms of the T-maze was relatively absent in the morphine-treated females that showed full home cage maternal responsiveness. Given these disruptive effects of morphine on maternal behavior in rats, the possible effects of opiate administration and opiate addiction on maternal responsiveness in other animals, including humans, warrant examination.

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Male Lek Formation and Female Calling in a Population of the Arctiid Moth *Estigmene acrea*

Abstract. *Abdominal coremata in male* Estigmene acrea *(Lepidoptera: Arctiidae) are inflated and displayed in aggregations to which females and males are attracted and where mating occurs (leks). Female* E. acrea *also release a sex pheromone which attracts males. These two mating behaviors occur in the same populations at different peak times on the same nights. Thus male lek formation and female calling occur in the same species, and the male coremata, or related structures, appear to be integrally associated with lek behavior.*

Males of the North American salt marsh moth, *Estigmene acrea* (Drury), aggregate and inflate paired abdominal coremata (air-filled tubes covered with hairs) (Fig. 1). Male brushes, hair-pencils, and analogous organs are found throughout the Lepidoptera, in both butterflies and moths, where precopulatory eversion by males is associated with the release of scent (pheromone) (*1*). Large coremata are inflated by males of the Asian arctiid moths, *Creatonotos gangis* and *C. transiens* (*2*), but their biological role has not yet been determined. Only in one species of arctiid moth, *Utetheisa ornatrix*, has the biological function of the coremata been defined (*3*) and, in this species, males that inflate the coremata and release pheromone just before making genital contact are more likely to succeed in mating. Males of many other arctiid moths also have a variety of morphologically different coremata and hair-bearing structures whose behavioral roles are not known (*4*). The few reports of live male *E. acrea* with inflated coremata are of isolated males and not in the context of natural behavior (*5*).

As is typical of moths, female *E. acrea* release a sex attractant pheromone (*6*) which stimulates upwind flight and orientation to the female by males. In this case, copulation occurs without any display of the coremata. In addition, however, male *E. acrea* form aggregations in which they adopt a specific display posture with their coremata fully extended and inflated (Fig. 1). We observed aggregations on alfalfa plants or other low vegetation, and some males would display extended coremata while on fence posts or barbed wire adjacent to plants. Males adopted a display posture from almost ground level up to about 1 m above ground and did so on the tips of vegetation (Fig. 1) or other structures where they were clearly visible and accessible. Females and males oriented upwind to these male aggregations; males would join the aggregations, and females would fly directly to them and mate with males in them. Females were

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