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Effects of Morphine and Naloxone on Separation Distress and Approach Attachment: Evidence for Opiate Mediation of Social Affect[1]

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HERMAN, B. H. AND J. PANKSEPP. *Effects of morphine and naloxone on separation distress and approach attachment: evidence for opiate mediation of social affect.* PHARMAC. BIOCHEM. BEHAV. 9(2) 213–220, 1978.—In order to determine the relationship between endorphins and social attachment, the effects of morphine (an opiate agonist) and naloxone (an opiate antagonist) on various indices of attachment in guinea pigs were studied. In infants, crying or separation-induced distress vocalizations were significantly decreased by single injections of low morphine doses (0.25, .050 and 0.75 mg/kg) in a dose-dependent manner. Naloxone (1 mg/kg) reliably increased separation distress vocalizations in both juvenile and adult guinea pigs. Therefore, similar to opiate withdrawal symptoms, separation distress appeared to be alleviated by morphine and potentiated by naloxone. As for approach attachment, offspring/maternal proximity-maintenance time was significantly decreased by morphine (1.0, 2.5 and 5.0 mg/kg), suggesting that opiates may be capable of replacing a function normally subserved by endorphins in reinforcing attachments. These data support the hypothesis that an endorphin-based addiction-like process may underlie the maintenance of social attachments, and that separation distress may reflect a state of endogenous "endorphin withdrawal".

Morphine Naloxone Separation distress Distress vocalizations Attachment Endorphins Guinea pigs

ONE OF the most ubiquitous social phenomena existing in nature is the attachement an infant displays towards its mother. While several behavioral theories of social attachment have been proposed [4, 6, 35], physiological hypotheses about the nature of attachment have yet to be formulated. Recently, several investigators have theorized and provided data indicating that changes in brain opiate peptide systems may underlie narcotic addiction [11, 23, 39, 41, 47]. Based upon several lines of evidence suggesting parallels between social attachment and opiate addiction, we have proposed that brain endorphins may play a critical role in the mediation of intraspecies social bonds [25]. In the present context, we are using the term endorphins as a generic descriptor for endogenous opiate-like substances as suggested by Goldstein [11].

A striking measure of social attachment is the crying or distress vocalizations observed in the young of a wide variety of species when involuntarily separated from the mother [2, 10, 21, 31, 37]. The abstinence agony experienced by humans during narcotic withdrawal would seem to parallel many of the symptoms of separation distress including lacrimination, anxiety and depression. Since blocking brain endorphins has been shown to enhance anxiety [12], it is possible that brain opiate peptide circuits also mediate other types of affective states such as separation distress. Our hypothesis assumes that a type of endogenous endorphin addiction underlies social cohesiveness.

The purpose of the present investigation was to evaluate the relationship between brain endorphins and attachment using a psychopharmacological approach and guinea pigs as subjects. In the major test of our hypothesis, we studied the effects of morphine and naloxone on separation-induced distress vocalizations in young guinea pigs. As in children [30], infant monkeys [16], puppies [10] and kittens [31], infant guinea pigs have been reported to distress vocalize when placed in social isolation [22, 27, 44]. If the emotional distress an infant experiences during acute social isolation resembles opiate withdrawal, then distress vocalizations should be reduced by opiate agonists and potentiated by narcotic antagonists. Indeed, we have found that opiates dramatically decrease distress vocalizations in young dogs [25].

Behaviors which facilitate the maintenance of proximity by the infant to its primary caretaker have also been utilized to measure attachment strength in guinea pigs [14, 29, 40] as well as in monkeys [13] and humans [4]. In order to determine if endorphins play a role in approach attachment as well as in separation distress, we also investigated the effects of morphine and naloxone on infant/maternal proximity-maintenance time in guinea pigs. We predicted that morphine would decrease approach attachment by replacing the

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function normally subserved by endorphins in the reinforcement of social bonds.

It should be noted that one advantage of using guinea pigs for exploring biological correlates of attachment is their adult-like neurological status at birth [9,43], thereby eliminating potentially complex postnatal ontogenetic changes that could confound any pharmacological study using infants.

EXPERIMENT 1

The purpose of this experiment was to determine if the distress vocalizations (DVs) exhibited by guinea pigs tested in social isolation would be decreased by morphine and increased by naloxone, as would be expected if separation distress reflects an endogenous state of endorphin withdrawal. Infant as well as adult guinea pigs were used, since it became apparent that the DV rate of infants was so high that a ceiling effect might obscure potential naloxone-induced increases in DVs. A quantitative measure of locomotor activity was also obtained to clarify the specificity of drug effects on separation distress as opposed to arousal. However, activity could also be employed as a gross secondary index of separation distress in infants, since following acute isolation the young of several species demonstrate marked increases in activity [7, 10, 33].

METHOD

Animals

A total of 43 male and female guinea pigs were obtained from 12 pregnant Dunkin Hartley albino guinea pigs (Dutchland Lab Animals). Upon arrival at Bowling Green, pregnant females were housed individually in 24×17×16 cm wire-mesh cages with woodchips covering the floor. With the exception of test sessions, all animals remained with their mother and littermates and were on ad lib food and water.

Apparatus

The apparatus consisted of a 69×69×57 cm opaque-walled cubicle box with sheet metal walls and floor that was sound-insulated and equipped with a fan for ventilation and masking of outside sounds. The chamber was also temperature regulated and maintained at 70 to 75°F. A small one-way mirror on the door of the box enabled the experimenter to view an animal's behavior. Two microphones were installed on top of the wire mesh ceiling of the cubicle. One microphone, connected to an amplifier and a set of earphones, provided a method for the experimenter to hear and record DVs on a hand tally counter. A second microphone, connected to a voice relay indicator and a print-out counter, automatically recorded vocalization frequency at 5 min intervals. For quantifying activity, the floor of the chamber was marked off into 9 equal 23 cm squares, and the number of squares crossed were registered on a hand tally counter and recorded at 5 min intervals.

Procedure

All drugs were injected SC in the nape of the neck in a volume of 0.1 ml/100 g body weight using 0.9% saline as the drug vehicle, and were administered 30 min prior to the onset of each test session.

In our first drug trials, using a Latin square to determine drug sequence, 23 infant guinea pigs (Group 1) derived from six of the litters were injected with saline, morphine sulfate (1.0, 2.5 and 5.0 mg/kg) or naloxone hydrochloride (1.0 mg/kg) at 8, 10, 12, 14, or 16 days of age. These animals were also tested under saline on intervening drug days (7, 9, 11, 13, 15 and 17 days of age). Since no drug carry-over effects were indicated, a mean saline baseline was obtained by averaging performance across all seven saline days. Since results of our first trials revealed powerful effects of the three morphine doses studied, we tested an additional group of animals with lower morphine doses. From the remaining six litters, a total of 20 infants (Group 2) were administered saline or morphine (0.125, 0.25, 0.50 and 0.75 mg/kg) in a counterbalance Latin square design at 10, 11, 12, 13 or 14 days of age. Group 2 infants were also tested under saline at 9 days of age, and a mean control baseline value was computed by averaging performance across both saline test days. For both Group 1 and 2 infants, animals were given saline trials in the apparatus for 3 consecutive days prior to the drug regime to ensure habituation to the novel testing situation.

To further clarify naloxones' effects on DVs, nine adult guinea pigs (110 to 116 days of age) derived from two of the Group 1 litters, were administered saline and naloxone (1.0 mg/kg) in a counterbalanced fashion over 2 consecutive test days.

Each subject was tested individually in complete social isolation. Each test session had a duration of 15 min for infants and 10 min for adults, and began about 30 sec after an animal was placed in the center square of the cubicle floor. At 5 min intervals, the total number of DVs produced by a subject as well as the number of squares crossed were recorded. Crossing a square was defined as an animal placing at least the two front paws into a new square. For Group 1 infants, righting times were obtained at the end of each test session primarily to assess morphines' effects on motoric reflexes. DV data described in this report refers to data recorded on the tally counter, and in every case parallel resuts were obtained with the automatically recorded DV data. Hand-tallied data is presented since it includes both low and high amplitude DVs, whereas automatically recorded data just consists of high amplitude sounds. In addition, the hand-tallied data is more accurate, since the relay was occasionally triggered by extraneous motor sounds made by the animal.

For both experiments, computer-run one-way analyses of variance (repeated measures for the drug factor) and Student's t tests (dependent within groups, independent between groups) were used to analyze data.

RESULTS

The data depicted in Fig. 1 illustrate the effects of morphine on DVs in infant guinea pigs tested in social isolation. Clearly, morphine induced dramatic dose-dependent decreases in separation DV frequency. The reliability of the DV dependent measure is indicated by the practically identical saline baseline DV rates displayed by the two groups of guinea pigs studied. Overall analyses revealed that in comparison with saline, morphine produced highly significant decreases in DVs for infants receiving the three highest morphine doses (Group 1), $F(3,66)=87.05$, and the four lowest morphine doses (Group 2), $F(4,76)=37.28$ (all p's<0.001). Each of the three high morphine doses significantly reduced DVs as compared with saline, t's≥ 11.30, p's<0.001, with the 5 mg/kg morphine dose decreasing DVs to about 6% of

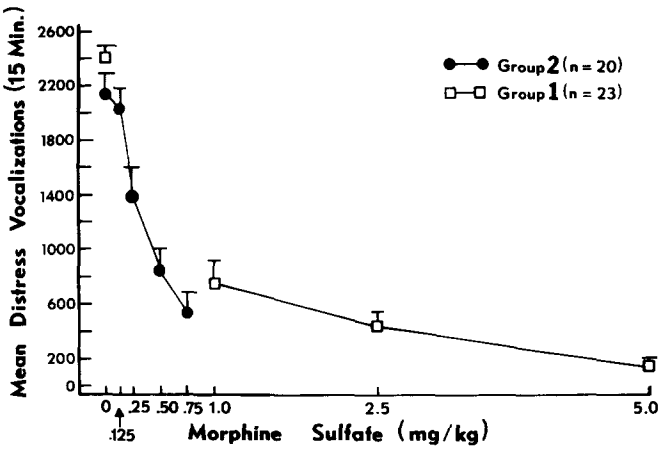

FIG. 1. Effect of morphine on mean distress vocalization frequency (+ SEM) displayed by infant guinea pigs tested in social isolation. Group 1 infants received the three highest morphine doses, whereas Group 2 infants were tested under the four lowest morphine doses.

baseline levels. For Group 2 animal's, individual comparisons between saline and morphine suggested that 0.25 mg/kg was the lowest morphine dose capable of producing reliable DV decreases, $t(19)=4.20$, $p<0.001$, and even further reductions were obtained using either a 0.50 or 0.75 mg/kg morphine dose, t's≥ 9.04, p's<0.001. In comparison with saline baseline levels, the 0.25, 0.50 and 0.75 mg/kg morphine doses decreased separation DV rate by 35, 61 and 75%, respectively. Overall, these data suggest that systemic administration of low morphine doses results in clear dose-dependent decreases in separation-induced DVs in infant guinea pigs.

Analyses of concomitantly obtained activity data suggested that at doses of morphine 0.75 mg/kg and below, opiate-induced decreases in DVs probably could not be attributed to decreased arousal. Individual comparisons between saline (M=2 ± 1) and the four lowest morphine doses (0.125 mg/kg, M=6 ± 3; 0.25 mg/kg, M=2 ± 1; 0.50 mg/kg, M=3 ± 2; 0.75 mg/kg, M=3 ± 2) failed to reveal any reliable activity effects, t's$(19)\leq 1.18$. In comparison, each of the three higher morphine doses (1 mg/kg, M=5 ± 1; 2.5 mg/kg, M=8 ± 4; 5 mg/kg, M=3 ± 1) did significantly reduce activity, t's≥ 2.95, p's<0.01, from 18 to 47% of control levels (M=17 ± 3). At the 1.0 mg/kg dose, morphine-induced decreases in activity were not correlated with opiate DV reductions (r=+.03), although evidence for such a correlation was found with the 2.5 mg/kg morphine doses ($r=+.44$; $p<0.05$). Righting time was unaffected by morphine. Under all morphine doses, as soon as the door of the testing apparatus was opened, each animal scurried around attempting to avoid human capture like an undrugged animal. In general, these data suggest a dissociation between morphines' effects on DVs as opposed to activity at doses 0.75 mg/kg and below, whereas at doses 2.5 mg/kg and above decreases in DVs might have been functionally related to activity reductions. However, insofar as the baseline open-field activity levels for Group 2 animals were so low, a replication with morphine doses below 2 mg/kg using a more sensitive activity index would be desirable.

Since morphine reduced separation DVs, we reasoned that administration of an opiate antagonist should increase separation distress. In infants, though DV frequency was higher under 1 mg/kg of naloxone (M=165 per min) than saline (M=157 per min), this effect failed to reach statistical significance, probably because infants distress vocalized almost at maximal frequencies. In adults, whose saline DV baseline rats (M=50 per min) were considerably below that of infants, reliable 52% increases in DV frequency were produced by 1 mg/kg naloxone (M=76 per min), $t(8)=2.85$, $p<0.05$.

Activity also appeared to be differentially affected by naloxone in infants vs. adults tested in social isolation. In infants, naloxone (M=45 ± 9) produced a significant 164% increase in activity in comparison with saline (M=17 ± 3), $t(22)=3.38$, $p<0.01$, whereas naloxone (M=33 ± 11) had no reliable effects on the activity of adults (saline, M=26 ± 8), $t(8)=0.48$. Observationally, infants treated with naloxone looked highly aroused and stressed, whereas naloxone-adults were indistinguishable from undrugged animals. Overall, these data suggest that naloxone potentiates separation distress. Naloxone-induced increases in DVs did not appear to be simply due to increased arousal, since a dissociation between the effects of naloxone on DVs as opposed to activity was indicated for adults.

DISCUSSION

As predicted by our hypothesis, these data suggest that separation distress is alleviated by opiate agonists and potentiated by opiate antagonists. In infants tested in social isolation, low morphine doses (0.25 to 0.75 mg/kg) induced highly significant dose-dependent decreases in DVs without reliably influencing activity. Although higher morphine doses (1.0 to 5.0 mg/kg) reduced DVs even further, these latter effects may have reflected either decreased arousal or reduced separation distress [7, 10, 33]. Naloxone reliably increased DV rates in adults without influencing activity, and we believe that our failure to obtain significant naloxone-induced DV increases in infants simply reflected a ceiling effect.

EXPERIMENT 2

If endorphins do play an important role in reinforcing social bonds, then these bonds should be influenced by drugs which act upon brain opiate peptide receptor systems. The primary purpose of this study was to determine if morphine and/or naloxone would influence the behavioral interaction of a young guinea pig towards its mother. Drugs were administered to juvenile guinea pigs tested in the presence of the mother and in social isolation, and drug effects were evaluating using several measures that have been shown to be reliable attachment indices in guinea pigs including proximity-maintenance time [14, 29, 40], DVs [21, 27, 44] and activity [28].

METHODS

Animals

Animals consisted of 20 juvenile guinea pigs (22 to 30 days of age at onset, and 39 to 47 days of age at termination of study) derived from five locally-bred females of the Group 1 litters employed in Experiment 1. Animals were housed with the mother and littermates at all times with the exception of test sessions.

Apparatus

All animals were tested in the chamber described in Experiment 1, and an additional 23×23×20 cm cage having wire mesh sides and a sheet metal floor and ceiling was placed in a diagonal and square in a corner of the test cubicle. This small cage was used to restrain the mother.

Procedure

Guinea pigs were divided into two experimental groups. Half of the animals constituted a *Mother Present Group* and were tested under both drug and no-drug conditions with the mother housed in a small wire-mesh cage. The remaining animals comprised a *Mother Absent Group* and were tested in social isolation with an empty cage. At the beginning of a session, each animal was placed in the corner square at the diagonal end of the square containing the cage. Proximity-maintenance time was measured with a stopwatch for 10 min, and the time a juvenile remained in one of the two proximity squares closest to the cage was recorded. If the attachment bond is intact, then *Mother present* animals should spend a greater amount of time in the proximity squares than *Mother Absent* animals. In addition, DV frequency and number of squares crossed were also recorded as described in Experiment 1. Behavioral indices were recorded at 5 min intervals.

On the first baseline test day, animals were injected with saline and tested in social isolation with an empty cage, and proximity-maintenance times were used to match subjects into the *Mother Absent* and *Mother Present Groups*. An attempt was also made to match animals on DVs and activity as well. On the second test day, saline data were obtained for the two groups. On the third, 4th and 5th Test Days, animals were tested 30 min following an SC injection of physiological saline, 1.0 mg/kg morphine and 1.0 mg/kg naloxone according to a Latin square design. On the seventh, 8th and 9th Test Days animals were tested under saline, 2.5 mg/kg morphine and 5.0 mg/kg morphine according to a second Latin square. Starting on the 3rd Test Day, 48 hr intervened between successive test sessions. Since no drug carryover effects were indicated, a mean saline basline value was obtained by averaging performance across the saline test days.

RESULTS

Figure 2 depicts the effects of morphine sulfate (1.0, 2.5 and 5.0 mg/kg) on proximity-maintenance time in juvenile guinea pigs tested in the presence vs. the absence of the mother. Results of a two-way ANOVA suggested a highly significant main effect for Groups, $F(1,18)=12.68$; $p<0.005$, reflecting the fact that juveniles spent a greater amount of time near the cage containing the mother as opposed to an empty cage. Under saline, experimental animals spent 71% of available time (i.e., 600 sec) in the two squares next to the mother's cage whereas controls remained in the same area next to an empty cage for a chance time period of 23%. The data illustrated in Fig. 2 suggest that the two groups were differentially affected by morphine. On the one hand, isolation controls showed no change in proximity time as a function of morphine. In contrast, experimental displayed morphine-induced decreases (about 44 to 66% of saline control levels) that approached significance with the 1 mg/kg, dose, $t(9)=2.10$, $p<0.10$ and was reliable with the next highest morphine dose (2.5 mg/kg), $t(9)=2.63$, $p<0.05$. Naloxone failed to effect proximity time in the *Mother Present Group*

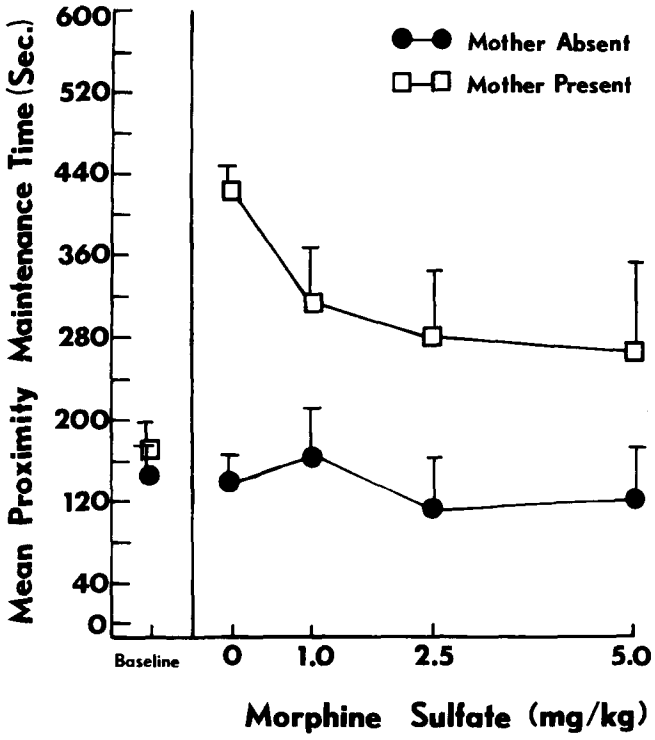

FIG. 2. Effect of morphine on mean proximity-maintenance time (+ SEM) in juvenile guinea pigs tested with the mother housed in a cage (Mother Present Group, N=10) or with an empty cage (Mother Absent Group, N=10). Left-hand panel represents baseline group matching data in which all animals were tested with just an empty cage.

in comparison with saline, $t(9)=1.12$, and no consistent drug effects were evident. Overall, these data suggest that morphine decreased (but, did not entirely block) the motivation of a juvenile guinea pig to obtain close proximity with its mother.

The effects of morphine on DVs in juvenile guinea pigs tested in the presence and absence of the mother are illustrated in Fig. 3. Morphine produced dramatic dose-dependent decreases in DVs in juveniles as it did in infants (Experiment 1), and this was reflected in a highly significant main effect for Drugs, $F(3,54)=56.19$; $p<0.001$. It is apparent from Fig. 3 that the ordering of groups varied as a function of drug treatment, and this effect resulted in a reliable Drug×Groups interaction, $F(3,54)=5.66$; $p<0.005$. Under saline, animals tested in the presence of the mother emitted significantly fewer DVs than isolation controls, $t(18)=2.55$, $p<0.05$. Thus, even without contact comfort, the mother was capable of reducing DVs in her offspring by about 28%. In contrast, under all morphine doses no reliable group differences were detected, t's$(18)\leq 1.72$. Isolation controls showed significant reductions in Dvs under each of the three morphine doses in comparison with saline, t's$(9)=6.23$, $p<0.001$. Rather surprisingly, in *Mother Present* animals, 1 mg/kg of morphine failed to produce reliable DV decreases, $t(9)=1.73$, although under the two higher morphine doses significant reductions in DVs were obtained, t's$(9)\geq 4.72$, p's<0.01. In general, these data suggest that morphine as well as the mother's presence decreased DVs in juvenile

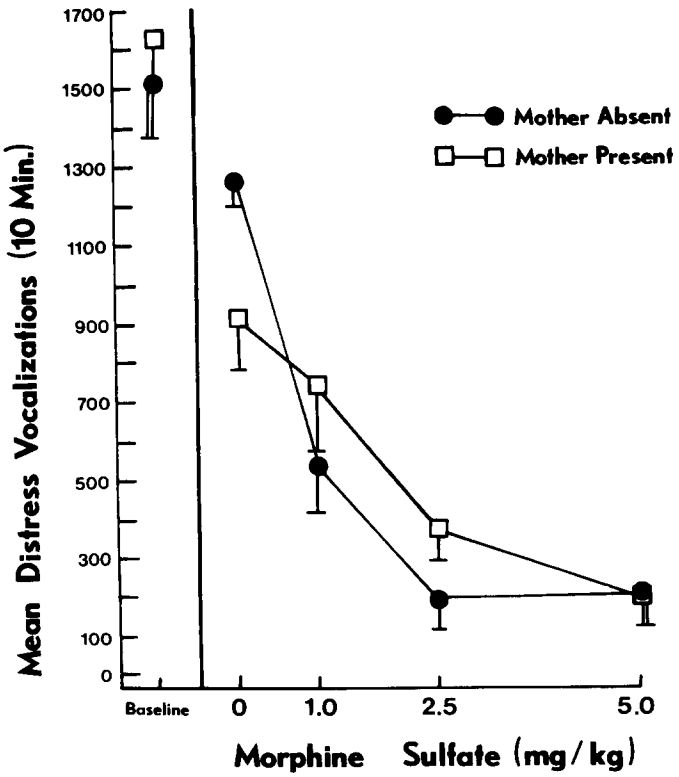

FIG. 3. Effect of morphine on distress vocalizations (M – SEM) in juvenile guinea pigs tested in the presence of the mother housed in a cage (Mother Present Group, N=10) or in social isolation with an empty cage (Mother Absent Group, N=10). Left-hand panel represents baseline group matching data in which all animals were tested in social isolation.

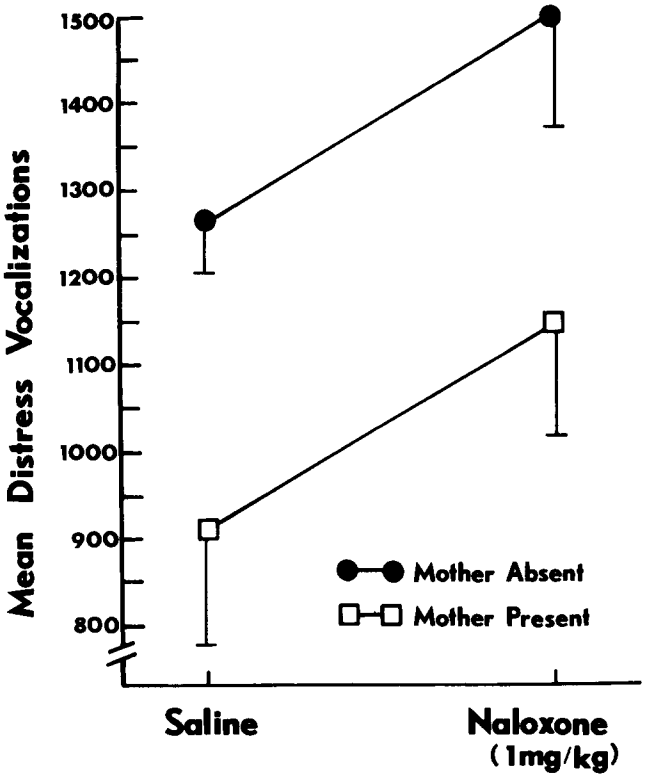

FIG. 4.Effect of naloxone on distress vocalizations (M – SEM) emitted by juvenile guinea pigs tested in the presence of a caged mother (Mother Present Group, N=10) or in isolation (Mother Absent Group, N=10). Left-hand panel represents baseline group matching data in which all animals were tested in social isolation.

guinea pigs, though morphine effects were less striking in animals tested in the presence of the mother.

Naloxone's (1.0 mg/kg) influence on DVs in juveniles tested in the presence of the mother and in social isolation are depicted in Fig. 4. In comparison with saline, naloxone produced about a 26% increase in DVs which resulted in a significant main effect for Drugs, $F(1,18)=8.03$; $p<0.05$. Although naloxone yielded reliable DV increases for Mother Present animals, $t(9)=2.96$, $p<0.02$, this effect failed to reach significance for isolation controls, $t(9)=1.61$. However, nine out of 10 isolation controls displayed naloxone-induced increases in DVs, a frequency that was significantly greater than chance, $\chi^2(1)=6.40$; $p<0.025$. If one aberrant animal is eliminated from the statistical analysis, naloxone yields a very reliable increase in DVs in comparison with saline, $t(8)=8.08$, $p<0.001$. Under both saline and naloxone, juveniles tested in the presence of the mother emitted fewer DVs than isolation controls, Group $F(1,18)=6.43$; $p<0.05$. Overall, these data suggest that naloxone potentiated DVs in juvenile guinea pigs, and strengthen our findings in infants and adults (Experiment 1).

The effects of morphine on locomotor activity in juveniles tested in the presence and absence of the mother are presented in Fig. 5. In general, morphine decreased activity in comparison with saline, Drugs $f(3,54)=20.33$; $p<0.001$, and this effect was reliable under all doses for isolation controls, t's$(9)\geq 3.13$, p's<0.02. In Mother Present animals, 1 mg/kg of morphine did not reliably depress activity, $t(9)=1.79$, although the two higher doses did significantly reduce activity, t's$(9)=2.93$, p's<0.02. For both groups tested under the 1 mg/kg dose, morphine-induced decreases in activity were not correlated with opiate DV reductions (r's$\leq +.14$). Under all morphine doses, juveniles appeared vigilant and exhibited normal motoric movement, although under the 5 mg/kg dose the quantitative decrease in locomotion was observationally apparent. Viewed as a whole, these data suggest that morphine decreased activity. However, under the lowest morphine dose studied, reductions in DVs and activity were unrelated.

The data depicted in Fig. 5 suggest that Mother Present animals were more active than isolation controls, although this effect only approached significance as a main effect, $F(1,18)=4.12$; $p<0.10$. Observationally, undrugged animals tested with the mother housed in a cage appeared to be more active than subjects tested with an empty cage only in the perimeter surrounding the cage. It was as if these former subjects were frantically attempting to gain physical access to the mother. In an attempt to clarify these observations, we reanalyzed the data by counting the squares crossed near the cage (three squares) as opposed to the remaining space of five squares defined as the outside perimeter. Within the Mother Present Group, 64% of total activity occurred in the mother's cage perimeter as compared with 36% in the outside perimeter. These percentages were practically reversed

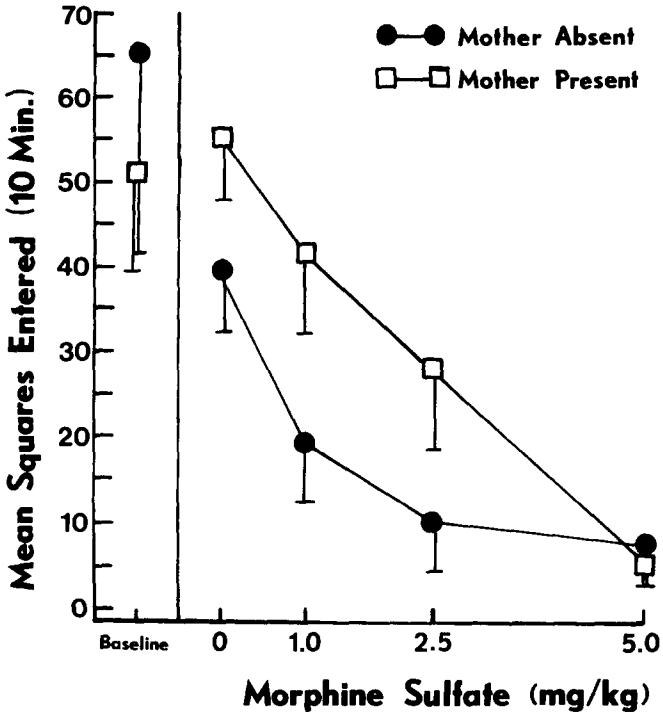

FIG. 5. Effect of morphine on mean number of squares (- SEM) crossed by juvenile guinea pigs tested in a closed-field chamber either in the presence of the mother (Mother Present Group, N=10) or in isolation (Mother Absent Group, N=10). Left-hand panel depicts baseline group matching data in which all animals were tested in social isolation.

in isolation controls, with 33% of total activity occuring in the empty cage perimeter in contrast with 67% in the outside perimeter.

In other words, as compared with isolation controls, experimental were proportionately more active in the area closest to the mother, whereas they were less active in an area away from the mother. If the increased activity displayed near the mother's cage reflected separation distress, then we reasoned that morphine should specifically decrease this activity. As predicted, Mother Present animals showed significant decreases in activity under morphine (1 mg/kg) as compared with saline only in the area near the mother's cage, $t(9)=3.94$, $p<0.01$, while no reliable differences were obtained in the outside perimeter activity, $t(9)=0.54$. In isolation controls, the same dose of morphine induced equivalent decreases in activity in the two perimeters. In short, a low dose of morphine appeared to specifically decrease activity near the mother while activity away from the mother remained unaffected.

In contrast to the effects of naloxone on DVs in juveniles (Fig. 4), naloxone did not appear to have an effect on the activity of either Mother Present animals (saline, M=55 ± 7; naloxone, M=65 ± 11) or Mother Absent animals (saline, M=40 ± 7; naloxone, M=44 ± 11), t's≤1.04. These data suggest a dissociation between the effects of naloxone on DVs as opposed to activity in juveniles, and parallel our findings in adults (Experiment 1).

DISCUSSION

Our findings that morphine decreased approach attachment (offspring/maternal proximity time) in juvenile guinea pigs, suggest that opiate drugs may replace the function normally subserved by endorphins in the maintenance of social bonds. The results of Experiment 2 also suggested that separation distress (as measured by DVs) was alleviated by morphine and potentiated by naloxone in juvenile guinea pigs, whether tested in social isolation or in the presence of the mother. These data are consistent with our hypothesis that separation distress may reflect an endogenous state of endorphin withdrawal.

One seemingly puzzling finding was that juveniles tested with a caged mother were more active than isolation controls. Similar results have been obtained by other researchers also using guinea pigs and practically the same testing situation [29], and were interpreted as indicating that the mother's presence increases an infant's exploratory behavior by providing a familiar security base. We disagree with this interpretation, since our guinea pigs exhibited a frantic hyperactivity only in the area near the mother's cage which appeared to reflect the desire of these animals to be reunited with the mother, and not to passively explore the environment.

GENERAL DISCUSSION

The results of these experiments provide consistent support for our hypothesis that brain endorphins may play an important role in mediating social attachment. Our primary measure of attachement was isolation-induced DVs, an index that has proven to be a reliable and convincing measure of separation distress in young guinea pigs [21, 27, 44], kittens [31,32], puppies [10, 28, 29], infant monkeys [13, 16, 33, 37 42] and children [2, 4, 30]. If separation distress represents an endogenous endorphin withdrawal process, we reasoned that it should be alleviated by opiate agonists and potentiated by opiate antagonists. Confirming our prediction, low doses of morphine were found to produce powerful dose-dependent decreases in DVs emitted by both infant and juvenile guinea pigs tested in social isolation. A 0.25 mg/kg morphine dose reliably reduced separation DVs in infant guinea pigs, and to our knowledge this is the lowest dose of an opiate ever reported to produce significant behavioral effects in a rodent. Conversely, our findings that naloxone reliably increased separation DVs in juvenile and adult guinea pigs further argues for a specific relationship between brain endorphins and separation distress. Infant/maternal proximity-maintenance time was utilized as a secondary index of attachment, and this measure has been shown to reliably reflect approach attachment in humans [1, 4, 34] and monkeys [13,15] as well as guinea pigs [14, 29, 40]. We found that morphine decreased the amount of time a juvenile guinea pig chose to remain in close proximity with its mother, suggesting that opiate agonists may be capable of replacing a function normally subserved by endorphins in the maintenance of social attachments.

Naloxone represents an ideal tool for exploring endorphin/behavioral relationships, since it is a pure antagonist practically devoid of agonist properties [20]. Moreover, naloxone selectively binds with brain opiate receptors [41]. The capacity of naloxone to potentiate DVs in the present study appeared to reflect a specific enhancement of separation distress rather than a nonspecific arousal ef-

fect. Naloxone produced significant increases in DVs in both juvenile and adult guinea pigs without influencing activity. As in guinea pigs, naloxone has not been found to influence activity of adult rats [17,18]. The failure of naloxone to significantly increase DVs in infant guinea pigs appeared to reflect a ceiling effect in the dependent measure, since in both juveniles and adults, whose baseline DV rates were lower (about 20 and 50%, respectively) than infants, naloxone reliably increased DVs. The naloxone-induced activity potentiation observed in isolated infants may have reflected enhanced separation distress, since increases in activity upon acute isolation have been reported to occur in other species such as rats [7], dogs [10] and monkeys [33].

The data presented in this report also indicate that the mother was an effective social object in alleviating separation distress in her offspring, and this capacity was reliably reduced by naloxone. However, even under naloxone, juvenile guinea pigs tested in the presence of the mother still emitted significantly fewer DVs than isolation of controls, indicating that the mother's capacity to decrease separation distress was not entirely blocked by naloxone. It is possible that prior conditioned associations of maternally-induced endorphin reward may have partially superseded the effects of central opiate blockade. Indeed, a parallel exists in narcotic addiction where conditioned reinforcers to self-administration behavior as well as to the environment represent obstacles in the effectiveness of opiate antagonist withdrawal programs [8,48].

Morphine's capacity to decrease DVs in guinea pigs appeared to reflect a reduction in separation distress rather than decreased arousal with the possible exception of the highest morphine dose studied. Low morphine doses (0.25 to 0.75 mg/kg) reliably decreased DVs by as much as 75% in infants without influencing activity. In young dogs, we have also found that similar low doses of morphine produce striking decreases in separation DVs [25]. While higher morphine doses (1.0 to 5.0 mg/kg) did reliably reduce activity as well as DVs in young guinea pigs, these activity effects may have reflected a decrease in isolation-induced distress [7, 10, 33]. At the 1 mg/kg dose, morphine-induced DV reductions were not correlated with activity decreases, further suggesting that DV opiate effects were not simply a concomitant response to activity changes. At each of the three higher morphine doses, animals displayed normal motor coordination and normal motor reflexes. The capacity of animals to exhibit normal activity levels was also present, since under all morphine doses animals scurried away from the experimenter when attempting capture. It is noteworthy that the higher morphine doses used in this study have traditionally been found to be free of sedative effects in rats [24] and mice [5]. Combining these lines of evidence, we conclude that morphine is specifically capable of alleviating separation distress in young animals.

It is conceivable that brain circuits for separation distress represent an evolutionary elaboration of an endorphin-based pain network. Part of the distribution of opiate receptors in the mammalian brain overlap with traditional pain pathways [41], and the extension of opiate receptors into the limbic system [22,26] suggests an additional affective role for endorphins. Although it is likely that a variety of affective processes are controlled by these limbic endorphin circuits, our data suggest that at least one function of these systems is to modulate emotions arising from social variables.

If endorphins do mediate social attachment, then how might this process be elaborated? First, attachment may simply represent an endogenous cellular addiction process in which an infant becomes physiologically dependent upon its mother for endorphin stimulation. Indeed, there is in vitro evidence that cells exposed chronically to enkephalin or morphine show biological tolerance and withdrawal which appear to parallel symptoms of opiate dependence [23,38].

It is also likely that psychological variables play experiential roles in mediating social cohesion. For example, Scott [35] has proposed that separation distress is the basic mechanism underlying social attachment. Physiologically, this process may mean that separation from the caretaker triggers a state of endogenous endorphin withdrawal, which would be negatively reinforcing to the infant. Returning to the caretaker would reward the infant by terminating this state of internal punishment. Administration of naloxone may simulate this withdrawal state, whereas opiate agonists may act as a physiological substitute for the caretaker.

Alternatively, it is possible that the capacity of opiates to reduce separation distress may be uniquely dependent upon the well known rewarding properties of opiates [3,19]. Within our theoretical framework, it is conceivable that maternal contact comfort stimuates a brain endorphin reward system in the infant which reinforces attachment formation. Of course, brain endorphins may not be specific to social affect, but may be a generalized process which is activated by a variety of incentives.

There is no question that additional research is required to determine the specificity of endorphins' role in attachment. Endorphins may well have a general modulatory influence on blunting many aversive affective states in addition to separation distress. However, since non-opiate behavioral depressants such as sodium pentobarbitol, major tranquilizers, and anti-anxiety agents have not been found to decrease separation DV in young dogs except at toxic doses [36], it seems possible that opiate drugs may be uniquely efficacious in alleviating separation distress.

The proposed hypothesis of social attachment as an endorphin-based addiction-like process may clarify the physiological nature of the affective concomitants of separation distress (e.g., panic during acute social isolation, anaclytic depression during prolonged separation), as well as some of the psychodynamic causes of opiate abuse in humans. For many addicts, opioid self-administration appears to be a way to attenuate persistent feelings of personal isolation [45]. Opiate drugs may replace the "endorphin reward" normally obtained from socialization, and it is this pharmacological substitution that may underlie a common trait of addicts to fail to enjoy and nurture relationships with others [45,46].

It is clear that much remains to be learned about the relationship between brain opiate peptides and social behavior, but we are hopeful that this line of research will enlarge our physiological and psychological conceptualization of opiate addiction as well as clarify the nature of psychiatric disorders characterized by aberrations in attachment.

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