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Opiate Effects on Social Behavior of Juvenile Dogs as a Function of Social Deprivation

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KNOWLES, P. A., R. L. CONNER AND J. PANKSEPP. *Opiate effects on social behavior of juvenile dogs as a function of social deprivation*. PHARMACOL BIOCHEM BEHAV **33**(3) 533–537, 1989.—The relationship between opioids and social behavior was examined by administering morphine (an opioid agonist) and naloxone (an opioid antagonist) to juvenile dogs and measuring various social behaviors (e.g., tail wagging) in a large room. Drugs were administered following social deprivation and nondeprivation. It was hypothesized that morphine would ease effects of social deprivation while naloxone would result in behavior typical of untreated socially-deprived dogs. Social deprivation (24 hr) resulted in more contact with the experimenter and increased tail wagging relative to nondeprivation. Morphine (0.25 mg/kg) resulted in more contacts with the experimenter and entrances into the "experimenter's area" relative to vehicle injections. Further, morphine decreased and naloxone increased tail wagging in the dog's area and there was a significant social condition × drug interaction for that measure. Naloxone (0.25 mg/kg) increased wagging following nondeprivation while morphine decreased wagging following deprivation. These data suport the hypotheses that social deprivation can increase social behaviors, and that social behavior is regulated by activity in brain opioid systems.

Social behavior Endorphins Opioids Tail wagging Dogs Morphine Naloxone Attachment Social deprivation

WHILE social attachment is an important dimension of the behavioral spectrum of all mammalian species, little is known about its neurochemical basis. According to one model (11), brain opioid systems play an important role in the elaboration of social emotions which contribute to bonding. It has been hypothesized that exposure to objects of attachment (conspecifics or familiar environments) evokes activity in, and separation from objects of attachment can reduce activity in certain brain opioid systems (15,16).

Evidence for a role of endogenous opioids in the control of social emotions has been provided by studies of several species, including guinea pigs (8), rats (14), dogs (5, 13, 15), and chicks (12,17). In those studies, morphine, an opioid agonist, was found to decrease isolation-induced distress vocalizations (DVs) in guinea pigs, chicks, and young dogs (8, 12, 15), tail wagging in juvenile dogs (5), and approach behavior of guinea pigs and juvenile dogs (5,8) while naloxone, an opioid antagonist, increased those behaviors. Further, it has been reported that morphine increased and naloxone decreased obedience in dogs suffering from severe separation-syndrome (13) [also labeled the "kennel-dog syndrome" (20)].

Since self-administration and self-stimulation studies have shown that activity of endogenous opioid systems is reinforcing (3, 4, 10), such reinforcement, evoked by social contact, is assumed to provide a biological basis for the emergence of social attachment. Several lines of evidence, aside from the ability of opioids to modulate social processes (16), support the assertion that physical contact results in central release of opioids. First, there are high levels of opioids in the dorsal horns of the spinal cord and in sensory cell groups of the medulla which receive relevant auditory, vestibular, and visceral inputs (1,2). Second, acupuncture analgesia can be blocked by naloxone, an opioid antagonist (9). Third, naloxone decreases the soothing effect of contact in chicks (12). Fourth, isolated mice are more tolerant of the effects of morphine (an opioid agonist) than socially-housed animals suggesting higher opioid activity has transpired in socially-housed mice (6,7). Finally, naloxone is apparently more aversive to rats in a social crowding situation than in normal living conditions suggesting higher endogenous opioid activity in animals living in social contact (18).

The accompanying negative affect arising from social isolation is hypothesized to be mediated by reduced activity of brain opioid systems. Arguing by analogy, Panksepp and colleagues (15,16) note that withdrawal of opiates from addicts produces behavioral signs of emotional distress which are similar to those seen when an organism is separated from an object of attachment (e.g., vocalizations, escape attempts). Those similarities suggest that attachment might be viewed as a kind of addiction to endogenous

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opioids which can be sustained by proximity to objects of attachment.

Pursuant to the opioid theory of attachment, the present study investigated the effects of modest social deprivation and pharmacological modification of opioid systems upon social behavior of juvenile dogs. In order to measure those effects, a "behavioral assay" was developed to assess social attachment in juvenile dogs that had become attached to the experimenter as well as to their littermates. Contact-seeking and care-soliciting behaviors (e.g., latency to approach, duration of contact) studied by other investigators (15, 19, 21) were employed in the present procedure. It was hypothesized that administration of morphine would ease behavioral manifestations of social deprivation while naloxone administered to socially nondeprived dogs would cause behavior similar to that of untreated socially-deprived dogs.

METHOD

Subjects

Twenty-two juvenile 6–8-month-old dogs from 5 litters (10 Beagles, 7 males, 3 females; 6 Telomians, 5 males, 1 female; 6 Beagle × Telomian hybrids, 2 males, 4 females) served as subjects. They were bred, born,and reared at the Bowling Green State University and weaned at 11 weeks of age. Animals were housed with littermates except during social deprivation and testing periods. The dogs were maintained on a normal daylight/night cycle with wet mash provided once daily and tap water and dry kibble (Wayne Promix) freely available. All testing was done in the afternoon.

All purebred animals (n = 16) had served in experiments prior to the present study. Prior studies ranged from an observational study of the ontogeny of play behavior and bone dominance (3 Beagles, 3 Telomians) to chronic naloxone treatment during several weeks in the puppies' early development (7 Beagles, 3 Telomians). A minimum of 8 weeks had elapsed since prior experimentation before the present study was initiated. The use of experimentally sophisticated animals was premised only on economic concerns.

Apparatus

At weaning, animals were maintained in 3.7 × 3.0 × 3.7 m group kennels with their littermates. For testing, each animal was introduced into a 4.3 × 3.0 × 3.7 m testing room from a 0.9 × 0.8 × 1.0 m holding cage placed just inside the entrance to the large room. Standard counters and stopwatches were used to measure the latency, duration, and frequency of various behaviors of interest. The rectangular testing room was divided into 3 areas by paint marked on the floor (see Fig. 1). Two of those areas were designated as the *Experimenter's Area* and one the *Dog's Area*. The experimenter's area consisted of one-half circle (just skirting the experimenter's knees when seated cross-legged in the corner) within another larger half circle (the distance to where the experimenter could reach when seated in the corner) painted on the floor in one corner of the room while the dog's area consisted of the remainder of the room. The holding cage was placed in the dog's area in the corner diagonally opposite to the experimenter's area. The experimenter sat in the half circle furthest from the holding cage.

Procedure

In order for attachment between the experimenter and each animal to occur, the experimenter (P.A.K.) participated in the normal cleaning, feeding, and handling of the dogs from birth.

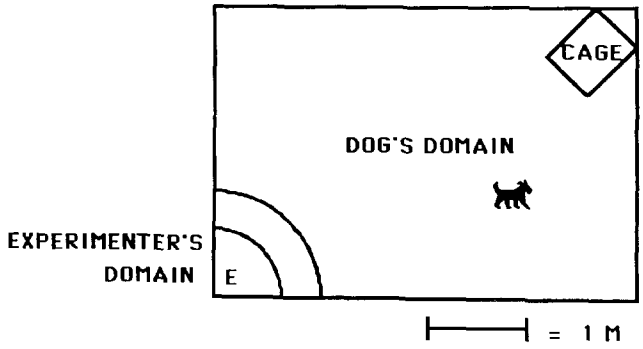

FIG. 1. Sketch of the testing room. The experimenter sat at "E" and the dog was placed in the holding cage prior to testing.

Each animal was tested for 7 twenty-min sessions. Prior to testing, animals were either deprived of social contact (other dogs or humans) for 24 hr in a familiar room with food and water freely available (deprivation condition) or left with littermates and exposed to humans as normally occurred during the regular feeding and cleaning regimen of the laboratory (nondeprivation condition). In order that social deprivation would not be confounded with room novelty, familiarization with the deprivation room was effected by housing animals with littermates in the deprivation room for at least 24 hr two weeks or more before the experiment began. Further, the first testing session for all dogs was a nondeprivation/vehicle condition and served to familiarize the animals with the testing procedure. Nondeprivation and deprivation days were then alternated and half the animals began testing after a deprivation period and half began after a nondeprivation period.

Prior to a testing session, the experimenter entered the deprivation or colony room, captured and carried a dog to another room to be weighed and administered either 0.25 mg/kg morphine sulfate (Lilly), 0.25 mg/kg naloxone hydrochloride (Narcan®, Endo), or equal volumes of the distilled water vehicle subcutaneously in the nape of the neck. Blind procedures were used throughout the experiment. The dog was then returned to the deprivation or colony room for 20 min. Doses of morphine were selected on the basis of data obtained in an earlier study (13) with a view toward using a very low dose causing behavioral effects without causing sedation or motor disturbance. Since this study was primarily a behavioral study which sought to induce neurochemical changes which might be deemed to be within a "physiological" range, a dose-response strategy was not pursued. After the first test session, during which all dogs received the vehicle, the experimenter remained blind as to the drugs injected until testing was complete. Animals received each drug and social condition combination once (at 24-hr intervals) over the next 6 consecutive days. To the extent possible, drug order was counterbalanced across animals.

Each dog was recaptured 20 min after injection and confined in a holding cage in one corner of the testing room. After 1 min, the experimenter raised the door via a pulley and rope apparatus and allowed the dog free access to the testing room for the 20-min session. The experimenter remained quietly in the corner of the room and recorded behaviors of the dog as they occurred. Dependent measures included: 1) latency to emerge from the holding cage; 2) latency to enter the experimenter's area; 3) number of entrances into the experimenter's area (number of times a dog stepped over the outer half circle of the experimenter's area); 4) number of contacts with the experimenter (number of times a

dog stepped over the inner half circle of the experimenter's area); 5) total time spent in the experimenter's area; 6) tail wags in the dog's area; and 7) tail wags in the experimenter's area. In addition, observations of any unusual or conspicuous behaviors shown by each dog were recorded.

RESULTS

Generally, the experimenter could not determine which drug had been administered to any dog based on general behavioral observations or the ease of capturing dogs either before or after each session. Nor could two independent observers distinguish between dogs given morphine, the vehicle, or naloxone prior to testing.

Breed effects were not included in the present analysis because of the low numbers of each breed in the study. In any case, there were no clearly evident breed effects on any of the dependent measures. Further, careful inspection of the data did not suggest any effects of sex on any dependent measures.

Given that data from experimentally-naive dogs were essentially identical to that from dogs which had been used in prior experiments, preexperimental history apparently had no effect on the present results. One-way ANOVAs revealed no significant differences between juvenile dogs exposed to earlier chronic naloxone treatment, those serving as subjects in an earlier observational study of play behavior, and/or naive juvenile dogs for any dependent measure. Furthermore, given that previous research found that rats exposed to 1 mg/kg naltrexone during early development weighed about 11% less than controls at Day 21 of age (23), body weights of dogs in the different preexperimental groups were analyzed. A one-way between subjects ANOVA revealed no significant differences in body weights between groups.

A logarithmic transformation was performed on the latency to emerge data in order to effect a more normal distribution as well as homogeneity of variance. Data were analyzed by two-way (2 social conditions × 3 injection conditions) ANOVAs with repeated measures on both factors adopted from Winer's model for repeated measures (22). All the means reported reflect standard error of the mean (SEM). Individual comparisons were made using the Newman-Keuls post hoc test. Chi-square analyses were computed for frequency comparisons.

As predicted, several measures of social attachment were increased by the social deprivation treatment relative to the social nondeprivation treatment. Specifically, the total time spent in the experimenter's area and both tail wagging measures were modestly albeit significantly increased following social deprivation, $Fs(1,21) \geq 4.77$, $ps < 0.05$. Following deprivation, the juvenile dogs, on the average, spent approximately 60% (± 2.48) of the session in the experimenter's area compared to approximately 55% (± 2.38) following nondeprivation. Further, 15 of 22 dogs (68%) spent more time in the experimenter's area following social deprivation relative to nondeprivation but this trend was not reliable, $\chi^2(1,N=22)=2.91$.

In the experimenter's area (per minute spent in that area), dogs wagged their tails an average of 84 (± 4.81) times per minute following deprivation compared to 72 (± 4.14) times per minute following nondeprivation. Further, 18 of 22 dogs (82%) wagged their tails more following social deprivation relative to nondeprivation, $\chi^2(1,N=22)=8.91$, $p<0.005$.

In the "dog's area," dogs wagged their tails an average of 42 (± 3.93) times per minute following deprivation relative to only 33 (± 3.93) times per minute following nondeprivation. Also, 21 of 22 dogs (95%) wagged their tails more following social deprivation relative to nondeprivation, $\chi^2(1,N=22)=18.18$, $p<0.001$.

Morphine and naloxone significantly affected 3 measures of social behavior; namely the number of entrances into the experimenter's area, the number of contacts with the experimenter, and tail wagging in the dog's area (per minute spent in the dog's area), $Fs(2,42) \geq 4.83$, $ps < 0.05$. Specifically, compared to vehicle injections, the number of entrances were significantly increased following morphine injections (37 ± 2.27 vs. 31 ± 4.62 entrances, respectively) with 17 of 22 dogs (77%) entering and leaving the experimenter's area more often following morphine injections. Further, morphine injections also resulted in more contacts with the experimenter relative to vehicle injections (41 ± 2.66 vs. 35 ± 1.90 contacts, respectively) with, again, 17 of 22 dogs (77%) making more contacts following morphine injections. Finally, morphine significantly decreased tail wagging in the dog's area (mean $= 23 \pm 3.34$ wags per minute) while naloxone significantly increased tail wagging (mean $= 52 \pm 5.11$ wags per minute) relative to vehicle (mean $= 37 \pm 4.86$ wags per minute) ($ps < 0.05$). Eighteen of 22 dogs (82%) wagged less following morphine injections relative to vehicle, $\chi^2(1,N=22)=8.91$, $p<0.005$, while 19 of 22 dogs (86%) wagged more following naloxone injections relative to vehicle, $\chi^2(1,N=22)=11.64$, $p<0.001$.

More important for the tail wagging measure, however, was a social condition × injection condition interaction, $F(2,42)=4.77$, $p<0.05$ (see Fig. 2). Post hoc analyses revealed that relative to vehicle, naloxone significantly increased wagging in the dog's area following social nondeprivation ($p<0.01$) but had no statistically significant effect following deprivation. Morphine, on the other hand, decreased wagging relative to vehicle following social deprivation ($p<0.01$) but had no statistically significant effect following nondeprivation. Further, there was no significant difference between morphine-injected deprived dogs and vehicle-injected nondeprived ones nor was there a significant difference between naloxone-injected nondeprived dogs and vehicle-injected deprived ones.

Without prior social deprivation, all dogs (100%) wagged more following naloxone injections relative to vehicle, $\chi^2(1,N=22)=22.0$, $p<0.001$, while following social deprivation, 18 of 22 dogs (82%) wagged less following morphine injections relative to vehicle, $\chi^2(1,N=22)=8.91$, $p<0.005$. In addition, during the nondeprivation condition, 16 of 22 dogs (73%) wagged less following morphine injections relative to vehicle, $\chi^2(1,N=22)=4.55$, $p<0.05$. Following social deprivation, 13 of 22 dogs (59%) wagged more following naloxone injections relative to vehicle but the trend was not reliable, $\chi^2(1,N=22)=0.73$.

DISCUSSION

The present results indicate that modest social deprivation increases certain social behaviors of juvenile dogs. Therefore, it appears that juvenile dogs are similarly affected by social deprivation as are young dogs and other organisms (15). Since one aim of the present study was to develop a "behavioral assay" of the effects of social deprivation on juvenile dogs, not all the dependent measures were necessarily expected to reflect social deprivation and, indeed, the 4 proximity seeking measures (latency to emerge from the holding cage, latency to approach the experimenter, number of entrances into the experimenter's area, and number of contacts with the experimenter) were not significantly affected by social deprivation. Although other explanations are possible, including a resistance of those proximity seeking behaviors to deprivation, one reason for a lack of effect of modest social deprivation on proximity seeking may be the type of subjects used. Juvenile beagles, Telomians, and the hybrids of those breeds are fairly large (mean weight = 11.0 kg, ±0.4 SEM) and mobile

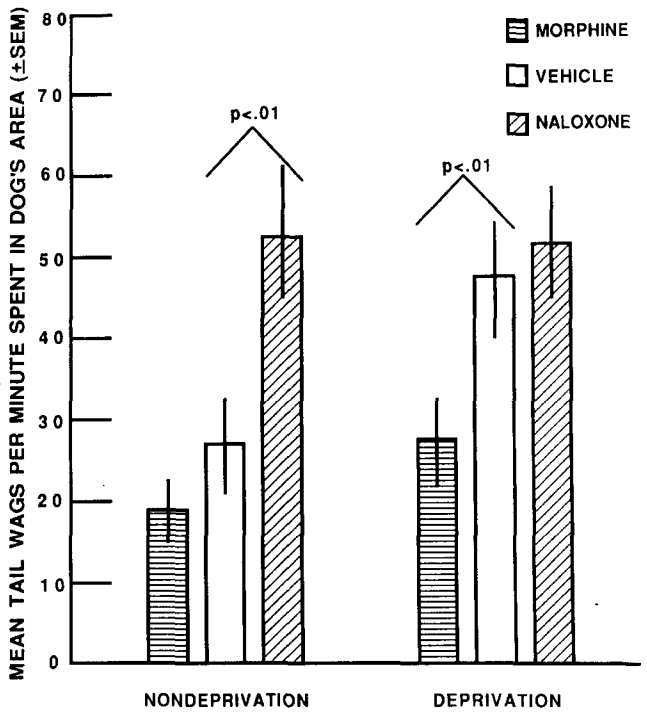

FIG. 2. The mean tail wags per minute spent in the dog's area as a function of injection condition and social condition.

animals and the testing room may not have been large enough to tease out deprivation effects on proximity seeking measures. Further, the dogs were able to run the distance of about 4 m from the holding cages to the experimenter's area in an average time of 1.4 sec and therefore, may have perceived some degree of nearness to the experimenter even when they were across the room. Therefore, for juvenile dogs, the proximity seeking measures may not be ideal measures of attachment but simply indicators of activity or exploration. The lack of a significant effect of social deprivation on either of the latency measures may be due to a "ceiling" effect of running speeds. The animals may not have been able to run any faster following deprivation than following nondeprivation. Perhaps other latency measures such as the latency to make the *second* approach to the experimenter could be used in future studies.

Despite the lack of effect of modest social deprivation on proximity seeking behavior, there were clear increases in proximity maintainance (duration of contact with the experimenter) and contact-soliciting behavior (tail wagging) following social deprivation relative to nondeprivation. Most pertinent to a study of social motivation is the increase in tail wagging by social deprivation. Davis (5) demonstrated that high frequency wagging with the tail in the out or downward position which was observed in the present study rarely occurs in a nonsocial situation. Further, social tail wagging is easily differentiated from tail waving or erect tail wagging associated with aggression. Other behaviors such as proximity maintenance do not similarly occur only to social stimuli but may include an exploratory or investigatory component as well.

The second purpose of the present study was to assess the effects of morphine and naloxone on social behavior. The observed effects are consistent with the opioid theory of social attachment (16). Pursuant to the opioid theory, one might expect that deprivation would increase social behavior, perhaps because of discomfort caused by decreased opioid activity during the deprivation period. The tail wagging data provide support for that expectation and are consistent with studies reporting altered analgesic responses to morphine in mice and rats following social isolation or social housing conditions (6, 7, 18). Further, the present results extend the findings reported previously from our laboratory (5, 8, 13, 15, 16). Consistent with previous studies of care-soliciting behavior (5,8), and as predicted by the opioid theory of social attachment, morphine decreased tail wagging in the dog's area after deprivation while naloxone increased that behavior following nondeprivation (see Fig. 2). Further, as expected, deprived morphine-treated dogs behaved like untreated socially nondeprived dogs (means $= 27.4 \pm 5.73$ vs. 26.9 ± 5.91, respectively) and nondeprived naloxone-treated dogs behaved like untreated socially deprived dogs (means $= 52.7 \pm 7.62$ vs. 47.3 ± 7.85, respectively). Thus, the present study extends the findings of earlier studies and establishes that care-soliciting behavior other than DVs are modulated by exogenous opiates in juvenile dogs.

Sedation by morphine was probably not a factor in these results since overall locomotor behavior was not decreased by morphine and opiate-drugged animals could not be distinguished from the other treatments. It should be noted, however, that there is some evidence of long-term changes in opiate receptors following naltrexone injections (24). Despite the very low doses of morphine and naloxone used in the present study, it is possible that naloxone injections resulted in some long-term supersensitivity to morphine. Therefore, present results must be tempered by a need for further investigation which more thoroughly manipulates naloxone-blockade of morphine effects and morphine-inhibition of naloxone effects. However, it would be hypothesized that care-soliciting behavior would be more markedly decreased by morphine injections and less markedly increased by naloxone injections in morphine-tolerant dogs compared to naive dogs.

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