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Behavioral Effects of Morphine in Mice: Role of Experimental Housing

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D'AMATO, F. R. AND C. CASTELLANO. *Behavioral effects of morphine in mice: Role of experimental housing.* PHARMACOL BIOCHEM BEHAV **34**(2) 361–365, 1989.—Behavioral effects of morphine were assessed in isolated-timid Swiss mice, and were compared with those observed following morphine administration in nonaggressive-grouped subjects. For this purpose saline- and morphine- (0.5, 1.0, 2.5, and 5.0 mg/kg, IP) injected isolated-timid and nonaggressive-grouped mice interacting with a social partner were observed during a 4-min test. Three main points emerged from the results: a) in basal conditions, compared with social mice, in timid mice the offensive ambivalent behaviors were significantly less pronounced, while the defensive ambivalent behaviors (and all flight behaviors) were significantly more evident; b) 2.5 mg/kg of morphine increased offensive and decreased defensive ambivalent behaviors in timid mice; c) in social mice morphine (2.5 mg/kg) treatment increased defensive ambivalent behaviors and time spent in crouch. The results, which show that the behavioral effects of morphine depend on the state of the individual, are interpreted on the basis of the antiemotional properties of this opiate.

Morphine Isolation-induced timidity Ambivalent behaviors Emotionality Mice

A number of researchers have shown that individual housing can result in aggressive behavior in mice in presence of a strange male intruder (15,18). It has, however, been demonstrated that in some male mice, housed singly for several weeks, isolation-induced timidity may also develop. This is characterized by alert and defensive postures, escape responses and squeaking instead of aggression in the presence of nonaggressive male congeners (7). In particular, isolation-induced timidity has been used as a measure of the anxiety-relieving effects of some drugs, such as diazepam and barbitone (7).

Further studies have recently suggested that morphine can exert an anxiolytic action in animals tested in fear-motivated tasks, and, in general, that opiates may attenuate emotional response in stressful situations (5). In addition, attenuation of emotionality has been suggested to account for the memory impairing effects of morphine and of other mu-opioid receptor agonists in rats and mice tested in one-trial inhibitory avoidance tasks (2,6). Finally, recent experiments have shown that morphine administration increases defensive behavior and decreases sociability in individually-housed aggressive mice (16) and that endogenous opioids have an important role in the nociceptive sequelae of submission and defeat (13).

In the present studies the behavioral effects of morphine were assessed in individually-housed timid mice, and were compared with those observed following morphine administration in social nonaggressive subjects. In addition to the defense-escape behaviors already mentioned as characteristic of isolated-timid mice, a special emphasis has been devoted to ambivalent behaviors, according to Mackintosh's definition (9). In fact, elements belonging to this subcategory of the agonistic behavior category show a graduation in their affinity compared with flight and aggressive behaviors (9). These ambivalent behaviors and the balance between their offensive and defensive components, might thus represent indicators that are more sensitive to the emotional state of the subject than behaviors falling into the flight or aggressive behavioral categories.

METHOD

Subjects

The subjects were random-bred male albino mice of the Swiss-Webster strain (Plaisant, Rome, Italy). When they weighed about 18–20 g (40–50 days old) the mice were randomly assigned to two different experimental housing conditions: part of them ($n = 105$) were housed singly in $30 \times 13 \times 13$ (H) cm Plexiglas cages (isolated subjects), while the remainder ($n = 144$) was housed in groups of eight in $27 \times 21 \times 14$ (H) cm cages. Social mice were randomly assigned to two different experimental groups: 18 served as social subjects (grouped subjects), while the others were used as partners in the social test of both isolated and grouped subjects. All animals were kept on a 12-hr light/12-hr dark cycle, with lighting switched on at 2000 hr and off at 0800 hr. Room temperature ranged from 21 to 24°C. Food and water were available ad lib.

Screening of Timidity

After a 3-week period of experimental housing, isolated and

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grouped subjects were exposed for a 4-min period to an unfamiliar social living mouse. These subjects were given 15 min of adaptation in a transparent observational cage (40 × 23 × 15 (H) cm) with wood shavings on the floor and open top, before the group-housed partner (marked with fur dye) was introduced. Behavioral observations were performed in a sound-proof cabin, under dim red lights, between 0900 and 1130 hr. The test cages were cleaned and their floors were covered with new wood shavings after each interaction. Each mouse was reintroduced in its home cage, thus continuing to experience the previous experimental housing.

The behavior of the dyad was recorded using a video-recording system. Tapes were then analyzed and behaviors quantified by an observer who did not know what kind of treatment the test animal had been subjected to. Decodification of behavior was performed by means of a key board connected to an Apple IIe P.C.

The three behavioral categories described by Mackintosh (9) were considered: (a) aggressive behavior, (b) ambivalent behavior, and (c) flight behavior.

In the case of isolated subjects, nonaggressive "timid" mice, i.e., those characterized by defense and escape behaviors (9), were selected. The social partners of the aggressive-isolated mice, that were discarded from the experiment, were left in their groups in order to maintain stable social environments.

In the case of grouped subjects, nonaggressive mice were selected, only two subjects being discarded from the experiment as a result of the instances of aggression.

Experimental Procedure

Each timid-isolated subject was tested one week later with its social partner 15 min after morphine (0.5, 1.0, 2.5, and 5.0 mg/kg) or saline injection. The experimental procedure was identical to that used in screening. Each nonaggressive-grouped subject was injected with 2.5 mg/kg of morphine or saline.

Morphine (HCl) (Carlo Erba, Milano) was dissolved in saline (0.9% NaCl) and injected 15 min before testing. Saline (0.9% NaCl) was used for control treatments. All injections were given intraperitoneally (IP) in a volume of 0.1 ml/10 g.

Behavioral Measures

The following behavioral elements belonging to two out of the three subcategories of agonistic behavior were recorded, in accordance with Mackintosh (9):

Ambivalent Behavior: offensive sideways, offensive upright, sideways posture, upright posture, defensive sideways and defensive upright.

Flight Behavior: evade, retreat, flee, on back, oblique posture, kick, crouch and straight legs.

All these behaviors were recorded as frequency measures, except crouch, for which the duration was considered (% time).

Possible morphine effects on locomotor activity were evaluated by dividing the test cage into 6 squares (displayed on the monitor when reviewing the recorded tapes), and measuring the number of lines crossed by the mouse. In addition, the number of approaches and withdrawals was compared by counting the entrances and exits of the square occupied by the partner. The percentage of time spent in the same square with the partner was taken as a measure of sociability (proximity measure).

Statistical Analysis

Timid-isolated subjects injected with saline (n = 9) were compared with nonaggressive-grouped subjects injected with saline (n = 8) using the Mann-Whitney U-test. The effect of morphine in timid-isolated mice was analyzed using the Kruskal-Wallis nonparametric analysis of variance; in the case of significant difference among the groups, Mann-Whitney U-tests were performed in order to evaluate the effects of each morphine dose (0.5 mg/kg: n = 7; 1.0 mg/kg: n = 9; 2.5 mg/kg: n = 10; 5.0 mg/kg: n = 9) with saline. The effect of morphine 2.5 mg/kg on nonaggressive-grouped mice (n = 8) was evaluated using Mann-Whitney U-test.

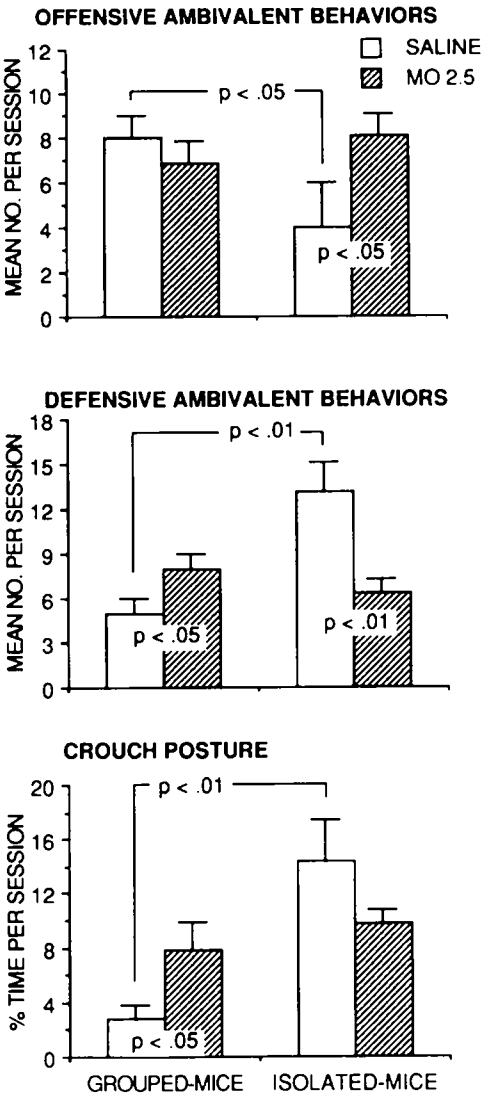

FIG. 1. Effects of morphine treatment in nonaggressive-grouped and timid-isolated mice (means+S.E.).

RESULTS

Screening of Timidity

In 44 out of the 105 dyads (42%) involving an isolated subject aggressive behavior was absent. These mice were characterized by defense and escape behavior, each of them was randomly assigned to one of the four treatment groups one week later. No between-groups difference was evident in baseline level of frequency of

TABLE 1

CHARACTERIZATION OF TIMID-ISOLATED MICE IN COMPARISON WITH NONAGGRESSIVE-GROUPED MICE INJECTED WITH SALINE

	Nonaggressive-Grouped n=8	Timid-Isolated n=9	U*	p
Flight	1.5 (0–2.5)	7.0 (6.0–10.0)	10.0	<0.05
Crouch	2.7 (1.2–4.4)	11.5 (8.6–24.9)	5.0	<0.01
Neutral Ambivalent	6.5 (5.5–9.0)	10.0 (6.5–12.0)	21.5	ns
Offensive Ambivalent	7.5 (7.0–8.5)	3.0 (1.5–7.0)	15.0	<0.05
Defensive Ambivalent	5.0 (3.5–6.5)	12.0 (10.0–16.0)	1.5	<0.01
Locomotion	56.5 (43.5–77.5)	54.0 (38.5–58.5)	25.5	ns
Approaches	14.0 (9.5–18.0)	10.0 (7.0–15.0)	21.5	ns
Leavings	10.5 (7.0–14.0)	7.0 (3.5–9.5)	15.5	0.05<p<0.10
Proximity	38.7 (32.1–42.3)	31.7 (28.3–48.7)	35.0	ns

Results are medians with interquartile ranges in parentheses.
*Two-tailed Mann-Whitney U-test.

ambivalent or flight behaviors, and in time spent in crouch posture.

Experimental Groups

Saline treatment. As far as the saline-injected mice are concerned, the isolated mice proved to be "timid" according to Krsiak definition (7); in fact, these mice differed from nonaggressive-grouped subjects (Table 1). In particular, isolated subjects displayed more flight behaviors and remained for a longer time in crouch posture. In addition, in the isolated subjects, defensive

TABLE 2

EFFECTS OF MORPHINE IN TIMID-ISOLATED MICE

	Saline n=9	Mo 0.5 mg/kg n=7	Mo 1.0 mg/kg n=9	Mo 2.5 mg/kg n=10	Mo 5.0 mg/kg n=9	H*	p
Flight	7.0 (6.0–8.5)	5.0 (5.0–5.5)	6.0 (4.0–14.0)	5.0 (3.0–8.0)	6.0 (2.0–8.5)	3.45	ns
Crouch	11.5 (9.4–17.7)	20.4 (9.2–32.7)	12.2 (6.1–21.7)	9.2 (4.9–13.1)	11.0 (6.5–13.2)	2.33	ns
Neutral Amb.	10.0 (6.5–12.0)	6.5 (5.0–8.0)	8.0 (6.0–11.5)	10.0 (6.0–11.0)	12.0 (9.0–13.0)	4.97	ns
Off. Amb.	3.0 (1.5–7.0)	3.0 (2.5–4.0)	10.0‡ (6.5–13.5)	8.0* (5.0–10.0)	6.0‡ (5.0–10.5)	13.97	<0.01
Def. Amb.	12.0 (10.0–16.0)	10.0 (9.5–12.0)	10.0 (6.0–17.5)	5.0† (5.0–7.0)	8.0‡ (5.5–12.5)	13.52	<0.01
Locomotion	54.0 (38.5–58.5)	54.5 (46.5–77.0)	54.0 (14.5–66.0)	58.0 (36.0–72.0)	53.0 (31.0–68.5)	1.80	ns
Approaches	10.0 (7.0–15.0)	12.0 (9.5–13.0)	6.0 (1.5–11.5)	12.0 (5.0–16.0)	9.0 (5.5–13.5)	3.47	ns
Leavings	7.0 (3.5–9.5)	11.0 (5.5–12.0)	6.0 (3.5–12.5)	9.0 (9.0–9.0)	6.0 (5.0–12.5)	1.87	ns
Proximity	31.7 (28.3–48.9)	31.7 (21.0–45.4)	35.0 (29.2–50.2)	32.1 (25.4–35.4)	30.8 (27.0–36.5)	3.01	ns

Results are medians with interquartile ranges in parentheses.
*Kruskal-Wallis analysis of variance.
*$p<0.05$ and †$p<0.01$ in comparison with saline (two-tailed Mann-Whitney U-test).
‡$0.05<p<0.10$ in comparison with saline (two-tailed Mann-Whitney U-test).

TABLE 3

EFFECTS OF MORPHINE IN NONAGGRESSIVE-GROUPED MICE

	Saline n = 8	Mo 2.5 mg/kg n = 8	U*	p
Flight	1.5 (0–2.5)	3.5 (2.5–4.0)	17.57	ns
Crouch	2.7 (1.2–4.4)	7.1 (3.3–12.7)	11.50	<0.05
Neutral Ambivalent	6.5 (5.5–9.0)	6.0 (4.5–8.5)	28.50	ns
Offensive Ambivalent	7.5 (7.0–8.5)	6.5 (4.5–9.0)	21.66	ns
Defensive Ambivalent	5.0 (3.5–6.5)	8.0 (6.0–10.5)	12.50	<0.05
Locomotion	56.5 (43.5–77.5)	44.0 (25.0–57.5)	21.00	ns
Approaches	14.0 (9.5–18.0)	8.5 (7.0–12.5)	14.50	$0.05<p<0.10$
Leavings	10.5 (7.0–14.0)	7.5 (5.5–12.0)	24.50	ns
Proximity	38.7 (32.1–42.3)	38.2 (31.7–43.9)	30.50	ns

Results are medians with interquartile ranges in parentheses.
*Two-tailed Mann-Whitney U-test.

ambivalent behaviors were more frequent, while the offensive counterpart of ambivalent behavior was extremely rare. No difference between isolated and grouped subjects was found in locomotion, or in the frequency of approaches; isolated mice tended to leave their social partner less frequently than grouped subjects. Finally, no difference was found between isolated and grouped mice as regards time spent in proximity of the partner.

Morphine treatment in timid-isolated mice. Morphine treatment did not affect flight, neutral ambivalent behaviors, or time spent in crouch posture (Table 2). On the contrary, the opiate modified the frequency of offensive and defensive ambivalent behaviors. In particular, 2.5 mg/kg of morphine increased the offensive ambivalent behaviors ($U = 20.0$, $p<0.05$), and decreased the number of defensive ambivalent behaviors ($U = 8.0$, $p<0.01$). The lowest dose (0.5 mg/kg) never significantly modified the behavior; 1.0 mg/kg of morphine showed a tendency to increase offensive ambivalent behaviors only ($U = 17.5$, $0.05<p<0.10$). The highest dose of the opiate (5 mg/kg) slightly modified the behavior too, but the pharmacological effect did not reach a statistically significant level (Off. Amb.: $U = 20.5$, $0.05<p<0.10$; Def. Amb.: $U = 19.5$, $0.05<p<0.10$). Locomotion, frequency of approaches and leavings, and time spent in proximity were not affected by morphine treatment.

Morphine treatment in nonaggressive-grouped mice. As far as the effects of morphine (2.5 mg/kg) on grouped mice are concerned, flight, neutral and offensive behaviors were not affected by the treatment (Table 3). On the contrary, these mice showed an increase in time spent in crouch posture, as well as an increase in defensive ambivalent behaviors. No effect on locomotion was evident, but a tendency towards a decrease in the number of approaches was observed.

Experimental housing and morphine treatment. The experimental paradigm of isolation-induced timidity is based on the hyperdefensiveness of isolated, compared with grouped, subjects (saline treatment, Table 1); morphine modified some of these behaviors, but the effects of the opiate were opposite in the two groups. Isolated-timid mice responded to the treatment differently than nonaggressive-grouped mice, all subjects being injected with 2.5 mg/kg of morphine (Fig. 1). As shown in Tables 2 and 3, morphine affected the amount of offensive and defensive ambivalent behaviors in timid-isolated mice (Table 2) while it increased time spent in crouch posture and defensive ambivalent behaviors only in nonaggressive-grouped males (Table 3). Furthermore, while experimental housing was responsible for differences in social behaviors in untreated animals, morphine-treated groups did not differ from each other, i.e., nonaggressive-grouped mice injected with morphine were similar to isolated-timid mice injected with morphine.

DISCUSSION

A number of points emerge from the results of the present research.

a) In basal conditions, compared with nonaggressive-grouped mice, in timid mice the offensive ambivalent behaviors were significantly less pronounced, while the defensive ambivalent behaviors (and all flight behaviors) were significantly more evident.

b) Morphine increased offensive ambivalent behaviors and decreased defensive ambivalent behaviors in timid-isolated mice. These effects were evident at doses not affecting flight behaviors and general activity. Not all doses were comparable in their behavioral effects. The lowest dose (0.5 mg/kg) was completely ineffective. The dose of 2.5 mg/kg increased offensive ambivalent behaviors and reduced defensive ambivalent behaviors. A tendency to modify these behaviors was evident following the administration of 1.0 and 5.0 mg/kg.

c) In nonaggressive-grouped mice, morphine treatment increased defensive ambivalent behaviors, slightly decreased the number of approaches and increased time spent in crouching.

These results clearly show that the effects of morphine administration depend on the state of the individual, different effects being evident in isolated and grouped mice. This emphasizes that the pharmacological effects of a drug can be drastically altered by the prior behavioral history of the animals and the resultant physiological and biochemical changes associated with that behavioral history (12).

In this context a number of pharmacological and biochemical studies (19,20) demonstrate the existence of several changes in the mechanisms of the central nervous transmission functioning, specially in noradrenergic, dopaminergic, serotonergic and gabaergic systems, as a result of social isolation. In addition, social isolation, like other types of stressors (14), has been shown to influence the functioning of the opioid system. In fact, the influence of morphine on pain sensitivity, as well as the analgesic effect of immobilization stress, vary according to the experimental housing (4,16). Finally, it has been demonstrated that the opioid system influences behavior through interactions with neurotransmitter systems in the brain (8). Thus, the different effects of

morphine dependent on experimental housing (isolated-timid vs. nonaggressive-grouped subjects) observed in the present study might at least in part be explained in terms of state-dependent differences (a) in the basal levels of neuromediators and/or (b) in the interaction between opioid and neurotransmitter systems (14).

The present results also suggest that morphine influences the emotional state of isolated-timid mice in such a way that their behavior becomes similar to that of nonaggressive-grouped mice. This study reports no difference between morphine-injected isolated mice and saline-injected grouped mice as far as offensive and defensive ambivalent behaviors were considered. However, it must be pointed out that, as far as the defensive-escape behaviors are concerned, differences between timid and social mice injected with morphine were still evident. Morphine treatment did not affect flight behaviors in timid-isolated mice while it increased defensive behaviors in nonaggressive-grouped subjects. It is interesting to note that the latter effect resembles the one recently observed in isolated-aggressive mice (17). As far as timid mice are concerned, in the present experiments, morphine mainly affected ambivalent behaviors, reestablishing an equilibrium between the offensive and the defensive components. These results can be interpreted on the basis of the antiemotional property of morphine. In fact, it has previously been demonstrated that morphine and related drugs can influence behavior through a decrease of emotional levels (2, 5, 6). In particular, morphine may attenuate emotional response in stressful situations (5), and a decrease in emotionality can account for morphine-induced memory impairment in rodents tested in one-trial inhibitory avoidance tasks (2,6).

Finally, it seems interesting to underline that only the dose of 2.5 mg/kg of morphine significantly reduced defensive and increased offensive ambivalent behaviors. With regard to this point it must be considered that in the recent years a number of studies have demonstrated that opiates, as well as other drugs, can influence animal behavior in a dose-dependent way (1). In particular, the fact that larger doses of a drug can produce less effect than some optimal dose (or no effect at all) has been interpreted in terms of differences in the population and distribution of receptors occupied by the drug at different doses (11), or of tachyphylaxis or fatigue at the level of a receptor occupied by the agonist drug (greater activity should lead to greater fatigue) (3). Our results can be interpreted in the light of this hypothesis, even if it must be emphasized that, as stated by Martinez and Kesner (10), dose-dependent effects "might also be the manifestation of an unknown biological process or of an interaction of known processes that is waiting to be elucidated."

In conclusion, taken as a whole, the present results show that the behavioral effects of morphine depend on the emotional state of the individual; they show, moreover, that ambivalent behaviors can be a suitable tool for evidencing such effects at doses that do not influence other behavioral categories, and that isolation-induced timidity could represent a behavioral model for testing emotionality.

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