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Social Behaviour in Pairs of C57BL/6 Mice of Both Sexes in the Open Field: Effects of Saline Drinking and of Naloxone

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VICKERS, C. AND A. T. PATERSON. *Social behaviour in pairs of C57BL/6 mice of both sexes in the open field: Effects of saline drinking and of naloxone.* PHARMACOL BIOCHEM BEHAV **23**(6) 905–909, 1985.–We have previously found that saline drinking increases fighting in male pairs and decided to test this treatment (0.9% NaCl for 24 hours before test day 1; SAL) on social behaviour of both males and females. Paired C57BL/6 mice (same-sex pairs) were observed in the open field in daily sessions for three days. One member of each pair (test mouse) was given either SAL treatment, a control injection of saline (SI), an injection of naloxone (1 mg/kg IP; NLX) or a combination of both treatments (NLX+SAL). NLX alone had previously been found to increase aggression in resident/intruder tests at the dose used. Open field testing is not associated with aggressive encounters in our experience. SAL had little effect on (unaggressive) social behaviour in males, but increased social contact seeking in females. The NLX and NLX+SAL treatments had essentially the same effects, irrespective of sex; the treated animals showed behavioural inhibition (reduced social and ambulatory behaviour), while their untreated partners showed significantly more than normal interest in the naloxone treated mice. The results are discussed in terms of opioid involvement in social behaviour.

Social behaviour Open field Pair behaviour Naloxone Sodium chloride Mice

WE have previously tested the behavioural effects of 0.9% sodium chloride drinking on male mice of the TO (albino) strain, alone [10] and in combination with naloxone [11]. In the resident/intruder test, fighting is reliably seen in the no-treatment condition, and aggressive behaviour is significantly increased by both saline drinking prior to the test (SAL) and by a naloxone injection (1 mg/kg; NLX). The combination of both treatments (NLX+SAL) caused a reduction overall in active behaviour, but increased fighting, i.e., the proportion of time spent fighting (as opposed to other behaviours) increased very sharply.

In an initial series of experiments we noted [10] that mildly hypertonic solutions of NaCl (2%, 5%) also stimulated aggressiveness in albino male mice, but that the effect appeared unrelated to the concentration and to the number of days of saline drinking (1–5 days). There were no correlations between plasma sodium concentration and plasma osmotic pressure, and the behavioural effects.

In the absence of any clues as to the mechanism by which modest NaCl intake alters behaviour patterns, we decided to examine the hypothesis that NaCl intake interacts with the opioid system. The choice of naloxone as a parallel treatment to saline drinking was based on two considerations: one, that this drug, presumably interacting with opioid pathways concerned in social [1, 8, 9] and in particular, aggressive behaviour [6, 15, 17, 18], can change aspects of such behaviours. Two, the *in vitro* action of sodium ions on opioid receptors is suggestive, in that sodium promotes increased dissociation rates of agonists and enhanced binding of antagonists (e.g., naloxone; [12,13]). We have found that in TO males, our usual treatments with naloxone and saline drinking counteract the inhibition of fighting seen after injections of met-enkephalin (Vickers and Paterson, unpublished).

In order to extend the study of these questions, we decided to look at the SAL and NLX treatments in the context of unaggressive social interactions. In previous tests, pair encounters in the open field were only very rarely associated with any components of fighting behaviour, even in the case of the aggressive TO males. As confirmed in other reports [16,18], we have found the pigmented mouse strain C57BL/6 unaggressive in most test situations. This strain differs in many aspects of behaviour, and endogenous opioid activity from TO mice [2, 3, 15, 16, 19].

We used the open field to examine social interaction in pairs of C57BL/6 mice of both sexes, and also noted locomotor (ambulation, rearing) activity. Treatments were given to one partner in the pair (SAL, NLX or NLX+SAL) at fixed time-points before each daily test session. Testing was carried out on three consecutive days, in order to establish whether familiarity with the test situation interacted with the treatment effects.

METHOD

Animals and Housing Conditions

Male and female mice of the strain C57BL/6 were taken from our own colony of outbred animals. At weaning, the mice were placed in large same-sex groups (15–20 mice/group) in opaque plastic cages. Members of the same

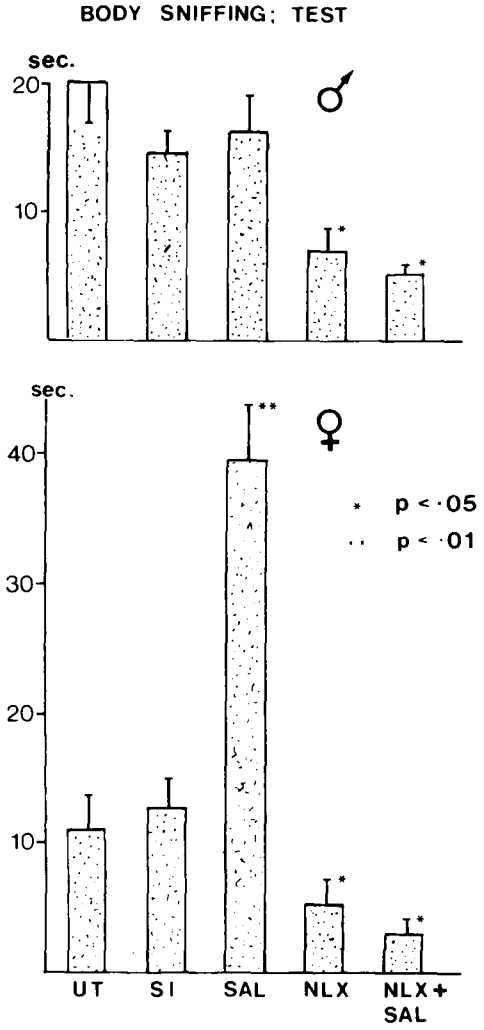

FIG. 1. Mean (±S.E.M.) duration of body sniffing by the test (treated) mouse of its pair partner (in seconds). There were 6 pairs per treatment group. Abbreviations of treatments (of test mouse): UT—untreated; SI—saline injection IP; SAL—saline drinking over the 3 days of testing, starting 24 hours before Test Day 1; NLX—1 mg/kg naloxone IP each Test Day; NLX+SAL—combined NLX and SAL treatment. Comparisons to the appropriate control group were carried out, using Dunnetts test. $*p<0.05$; $**p<0.01$

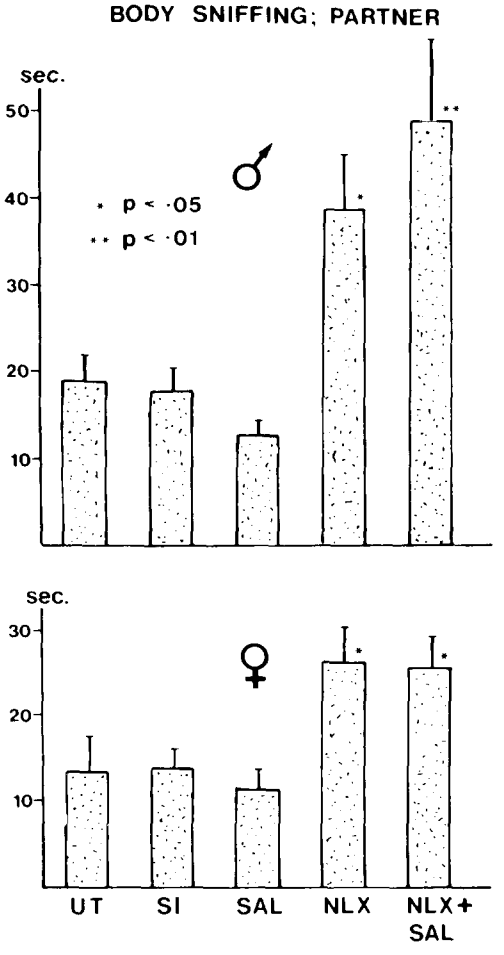

FIG. 2. Mean (±S.E.M.) of body sniffing by the partner (untreated) mouse of the treated member of the pair (in seconds). The treatments shown under each column refer to the treated animal. For other information, see caption to Fig. 1.

litter were kept together. At 4–5 weeks after weaning, 6 same-sex pairs were selected on the basis of compatible weights and healthy appearance. The paired mice were taken from different stock cages, so that, after cross matching (see Testing procedure below), the test pairs could not be siblings. The pairs were left undisturbed in their cages (29×12×11 cm) for 7 days. Conditions were controlled throughout (21±2°C; 12L:12D light cycle, lights on at 0500).

"Open Field" Testing Procedure

The first day of testing was on day 7 of the pair co-housing. The test pairs were formed by marking one of the co-housed pairs (tail marking with indelible pencil) and matching one marked and one unmarked animal from different housing cages. The same test pairs were observed at the same time of day (between 1600 and 1700, i.e., within one hour of lights off) for three consecutive days. The tests took place in the room where the housing cages were kept. The housing pairs showed no sign of conflict throughout the period.

The open field was made of black Perspex, with a surrounding wall (20 cm) and a white grid pattern (6×6 squares, 9 cm sides). Experienced observers recorded the behaviour of the animals, using a 6-channel event recorder (Campden Instruments Ltd., London). The following behaviours were recorded: Body sniffing—recorded for both members of the pair. This category includes several aspects of social behaviour; ano-genital and facial, as well as body sniffing, and following with nasal contact; Rearing—recorded for the treated member of the pair only. It includes both free-standing and wall rearing; Ambulation—recorded for the treated member of the pair only. Ambulation was measured in floor squares crossed/5 minute session.

Self-grooming was also recorded, but the data showed this behaviour to be erratic, occupy very short periods of the animals' time and not affected by treatment in any cases.

Treatments

Each treatment group consisted of six pairs, with the

TABLE 1

MEAN TIME (±S.E.M.) SPENT ON REARING (SECONDS) BY SEX AND TREATMENT

	Treatments				
Sex	UT	SI	SAL	NLX	NLX+ SAL
Males	36.9 ±2.4	36.8 ±2.4	32.1 ±2.9	22.1† ±2.1	22.8† ±1.9
Females	41.5 ±3.4	37.5 ±3.4	32.4 ±2.1	29.2* ±1.5	25.9† ±1.9

*Just failed to be significant at $p<0.05$.
†$p<0.01$ (compared to SI group; Dunnets t-test).

treatment being administered to one member of each pair. The treated mouse was called the "test mouse" and the untreated one the "partner mouse." Saline drinking treatment (SAL) meant substitution of the drinking water with 0.9% NaCl, 24 hours before Day 1 of the test period. Saline continued to be given for the remaining days of testing. The SAL animals were also given an IP injection of 0.2 ml physiological saline, 30 minutes before the start of each test session. NLX treatment consisted of IP injections of naloxone. HCl in saline (about 0.2 ml, to give a dose of 1 mg/kg), administered before each test as described. The mice in the combined treatment group (NLX+SAL) were given both saline to drink, and naloxone injections.

Two additional groups were assigned to control treatments: in one, both pair members were untreated (UT), and in the other one, saline injection, as described above, were given to one of the pair members.

Body Weight and Plasma Ion Concentrations

Immediately after the test session on Day 3, the animals were weighed and then killed by decapitation. The trunk blood was collected in heparinised tubes and centrifuged. Plasma samples were diluted 1:100 with de-ionised water, and kept at −20°C until analysed for ion content. Sodium, calcium and magnesium ion concentrations were determined in all samples, using an ICP (Inductively Coupled Plasma source; Phillips) spectrometer, courtesy of Dr. J. Walsh, Department of Geology, King's College.

Statistics

The time spent by the animals during each test session on each of the observed behaviours was calculated from the event recorder charts. The effect of Days on behaviour times was analysed separately using one-way analysis of variance. Two-way ANOVA was used to analyse the data for effects of Sex and Treatment, and multiple comparisons between means were carried out using the Dunnett test.

RESULTS

Body Sniffing

The data on body sniffing are shown in Figs. 1 and 2. Each test pair consisted of one treated and one untreated mouse, and the data from treated (test) animals, males and females, are displayed in Fig. 1, and the data from untreated (partner) animals of both sexes in Fig. 2. The amount of body sniffing did not vary significantly over days, so the mean (±S.E.M.)

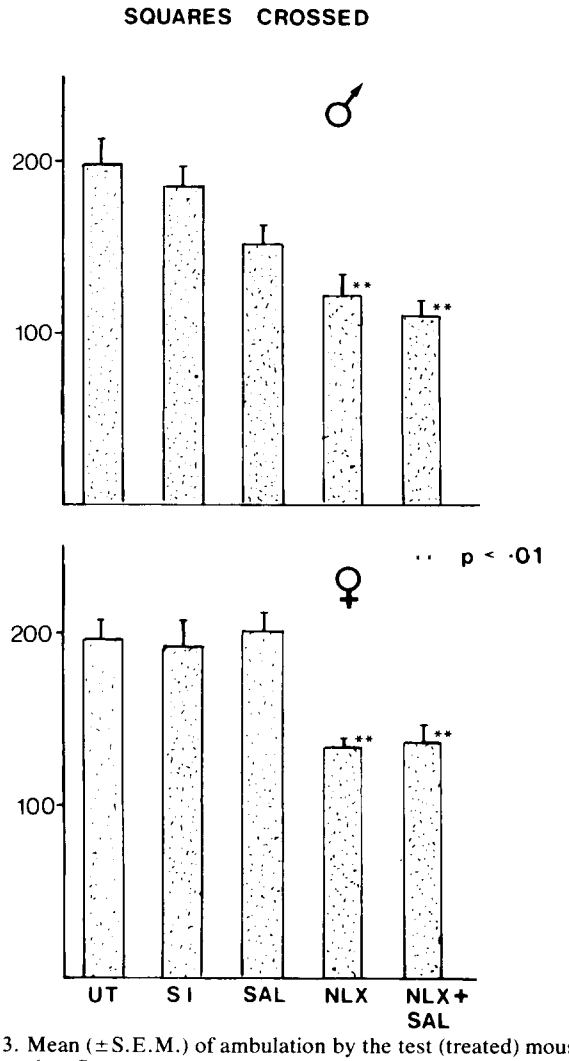

FIG. 3. Mean (±S.E.M.) of ambulation by the test (treated) mouse, measured as floor squares crossed. For other information, see caption to Fig. 1.

values of the pooled (over days) data are shown. The other observed behaviours also did not vary over days, and will be displayed in the same manner.

There was no significant effect of sex in untreated mice (untreated "test" mice and their partners) on body sniffing (social contact) behaviour. Saline injected (SI) test mice and their partners also showed no differences in social contact between the sexes. A slight tendency for the females to body sniff less than the males can be seen in all cases.

However, there is significant interaction between sex and treatment in other groups, $F=53.1$, $p<0.001$, for all groups. The test animals showed a marked sex difference in their social contact seeking after saline drinking (SAL) treatment: the females, but not the males, body sniffed their partners much more intensively ($p<0.01$). Treatments had significant effects overall $F=44.8$, $p<0.001$. Treatment with naloxone injections alone, and in combination with saline drinking (NLX and NLX+SAL) significantly reduced social contact seeking, in both males and females (for p-values in group comparisons with the appropriate control group, see Fig. 1). Interestingly, NLX and NLX+SAL treatments were also

associated with significant changes in the amount of body sniffing initiated by the partner mice ($F=11.7$, $p<0.001$, for treatment effects, and $F=11.2$, $p<0.001$, for effects of sex). The response to being paired with a NLX treated (alone or in combination with SAL) test animal was for the partner of either sex to increase social contact seeking (for p-values, see Fig. 2)

Ambulation and Rearing

Table 1 shows the means (±S.E.M.) of rearing times by the test animals in the different treatment groups. The values are pooled over days, since there were no significant differences between rearing times on different days. There was no significant effect of sex, nor treatment/sex interaction, but treatment was associated with a significant effect, $F=14.6$, $p<0.001$. NLX treatment of males, and NLX+SAL treatment of both males and females, caused significant reductions in rearing (for p-values, see Table 1).

The ambulation scores (floor squares crossed/5 minute session; pooled over days) are shown in Fig. 3. Again, only treatment gave any significant effects, $F=13.3$, $p<0.001$, and again, the NLX and NLX+SAL treatments caused reductions in both males and females (p-values, see Fig. 3).

Body Weights and Plasma Ion Determinations

There were no significant differences in body weights of animals of the same sex, irrespective of experimental conditions and treatment. The mean (±S.E.M.) male weight for test mice was 26.0±0.6 g (n=30), and for their partners 25.5±0.5 g. For the female test mice, the weight was 21.7±0.3 g (n=30) and for their partners 21.4±0.4 g.

There were no significant differences between the sexes, or between any other groups, with respect to plasma concentrations of sodium, calcium and magnesium. Values (mg/100 ml) were, for male test mice: sodium—373±5: calcium—10.7±0.4; magnesium—2.8±0.14, and for female test mice: sodium—357±6; calcium—11.8±0.4; magnesium—2.9±0.17. Male partner mice had values of: sodium—381±4; calcium—12.0±0.8; magnesium—2.7±0.10, and female ones: sodium—356±6; calcium—11.7±0.3; magnesium—2.9±0.09. The number of samples examined was in all cases n=30.

DISCUSSION

The present series of experiments did not support the starting hypothesis that SAL (saline drinking) and NLX (naloxone injection) have similar and additive effects on behaviour. While certain forms of aggression in mice do support this contention (see the introduction, and below), the treatments affected peaceful pair interaction in the open field in different ways. NLX, alone or in combination with SAL, reduced social contact seeking in both males and females. Non-social locomotor behaviour was also reduced. The untreated partners of the NLX animals however showed more social activity. SAL had no significant effects, except that social contact behaviour of the treated females increased.

The behavioural depression seen after NLX and NLX+SAL is in agreement with other reports of naloxone effects on single C57 mice in the open field [2, 3, 19, 20]. There is no evidence of any contribution of SAL to the effect of NLX+SAL treatment, which is comparable to that of NLX alone. There are no reports known to us of naloxone effects on pair behaviour in the open field (see however [1, 8, 9] for results from rats). Other studies deal with naloxone-induced changes in aggressive encounters [6, 11, 15, 17], and in sexually motivated exploratory behaviour [5]. Generally, the data suggest that NLX treatment depresses social contact seeking, and it has been suggested that opioids released serve as an "internal incentive" in social behaviour [8,9].

Our data does not contradict the "incentive hypothesis" of social contact, but the behaviour of naloxone treated animals was strikingly inactive overall, with significant declines in rearing and running behaviour, as well as in body sniffing. Our previous observations on the stimulating effects of the same dose of naloxone on aggressive behaviour [10,11] are not supported by several studies [3, 6, 15, 18]. Since the effect is very reliable, at midpoint of the light phase of the light cycle (but not in the dark), we can only conclude that the increased irritability seen after NLX is a highly situation-dependent phenomenon.

The behaviour of the untreated partners of NLX and NLX+SAL treated mice was very striking: in both male and female pairs, the partners sniffed their treated companions more intensively than in the control groups or after SAL treatment. A pheromonal mechanism may be involved, possibly related to response of the treated mice to the stress of testing. Although that stress is considerably milder than the stressors employed in studies of stress odours in rats [7], it is possible that our naloxone treated mice produce odour signals which attract social investigation. In rats, the social responses to stress odours produced both increases and decreases in social interest. Alternatively, it may be that the inertia shown by the naloxone animals is in itself sufficient stimulus to investigation. Stress of investigation alone cannot be a sufficient cause, since no similar responses by partner animals were seen in the control groups. Pilot experiments with mildly sedated mice do however not support this notion (Paterson and Vickers, unpublished), nor does naloxone treatment appear to cause sedation. The movements of the NLX and NLX+SAL test animals were instead rapid and jerky, when locomotor response were seen.

Treatment with SAL was without effect in all investigated behaviours, except one: females responded with very significantly increased levels of social contact seeking. The same response to SAL in females was seen in tests with female mice in the resident/intruder test, where the females establish dominant-subordinate relationships without any fighting. It seems that SAL, dependent on conditions can stimulate aggression in males and body sniffing in females, but we are no closer to an explanation of these effects.

REFERENCES

1. File, S. E. Naloxone reduces social and exploratory activity in the rat. *Psychopharmacology (Berlin)* **71:** 41–44, 1980.
2. Filibeck, U., C. Castellano and A. Oliverio. Differential effects of opiate agonists-antagonists on morphine-induced hyperexcitability and analgesia in mice. *Psychopharmacology (Berlin)* **74:** 134–136, 1981.
3. Gorris, L. G. M. and J. H. F. van Abeleen. Behavioural effects of (−)-naloxone in mice from four inbred strains. *Psychopharmacology (Berlin)* **74:** 355–359, 1981.
4. Katz, R. J., B. J. Carrol and G. Baldrighi. Behavioural activation by enkephalins in mice. *Pharmacol Biochem Behav* **8:** 493–496, 1981.

5. Landauer, M. R. and R. L. Balster. Opiate effects on social investigatory behaviour of male mice. *Pharmacol Biochem Behav* **17:** 1181–1186, 1982.
6. Lynch, W. C., L. Libby and H. F. Johnson. Naloxone inhibits intermale aggression in isolated mice. *Psychopharmacology (Berlin)* **79:** 370–371, 1983.
7. Mackay-Sim, A. and D. G. Laing. Discrimination of odors from stressed rats by non-stressed rats. *Physiol Behav* **24:** 699–704, 1980.
8. Panksepp, J., N. Najam and F. Soares. Morphine reduces social cohesion in rats. *Pharmacol Biochem Behav* **11:** 131–134, 1979.
9. Panksepp, J., B. H. Herman, T. Vilberg, P. Bishop and F. G. DeEskinazi. Endogenous opioids and social behaviour. *Neurosci Biobehav Rev* **4:** 473–487, 1980.
10. Paterson, A. T. and C. Vickers. Sodium chloride, aggression and time of day. *J Physiol* **326:** 33P, 1982.
11. Paterson, A. T. and C. Vickers. Social contact in pairs of male and female mice: Effects of saline and naloxone. *J Physiol* **345:** 111P, 1983.
12. Paterson, A. T. and C. Vickers. Saline drinking and naloxone: Light cycle dependent effects on social behaviour in male mice. *Pharmacol Biochem Behav* **21:** 495–499, 1984.
13. Pert, C. B. and S. H. Snyder. Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol Pharmacol* **10:** 868–879, 1974.
14. Pfeiffer, A., W. Sadee and A. Hertz. Differential regulation of the μ-, δ- and κ-opiate receptor subtypes by guanyl nucleotides and metal ions. *J Neurosci* **2:** 912–917, 1982.
15. Puglisi-Allegra, S., A. Oliverio and P. Mandel. Effects of opiate antagonists on social and aggressive behaviour of isolated mice. *Pharmacol Biochem Behav* **17:** 1829–1832, 1982.
16. Reggiani, A., F. Battaini, H. Kobayashi, P. Spano and M. Trabucci. Genotype-dependent sensitivity to morphine: Role of different receptor populations. *Brain Res* **189:** 289–294, 1980.
17. Rodgers, R. J. and C. A. Hendrie. Agonistic behaviour in rats: Evidence for non-involvement of opioid mechanisms. *Physiol Behav* **29:** 85–90, 1982.
18. Siegfried, B., E. Alleva, A. Oliverio and S. Puglisi-Allegra. Effects of isolation on activity, reactivity, excitability and aggressive behaviour in two inbred strain of mice. *Behav Brain Res* **2:** 211–218, 1981.
19. Van Abeleen, J. H. F. and C. M. van den heuvel. Behavioural responses to novelty in two inbred mouse strains after intrahippocampal naloxone and morphine. *Behav Brain Res* **5:** 199–207, 1982.
20. Van Abeleen, J. H. F. Genotype and cholinergic control of exploratory behaviour in mice. In: *The Genetics of Behaviour*, edited by J. H. F. van Abeleen. Amsterdam: North-Holland, 1974, pp. 347–374.