Pharmacology Biochemistry & Behavior, Vol. 21, pp. 495–499, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Saline Drinking and Naloxone: Lightcycle Dependent Effects on Social Behaviour in Male Mice

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Received 14 November 1983

PATERSON, A. T. AND C. VICKERS. *Saline drinking and naloxone: Lightcycle dependent effects on social behaviour in male mice.* PHARMACOL BIOCHEM BEHAV 21(4) 495–499, 1984.—Male mice (TO strain) were observed in the resident-intruder test and several behaviours monitored. Treatments were: isotonic saline drinking ad lib for 48 hours before testing (SAL), IP injections of 1 mg/kg naloxone 30 minutes before testing (NLX) or both treatments combined (NLX+SAL). The same sequence of tests were carried out both in the light and the dark phase of the 24-hour light cycle. The single treatments (SAL and NLX) both increased fighting (SAL, $p<0.02$) in the light phase. The combined treatment (NLX+SAL), which reduced behavioural activity overall, caused a marked increase in the proportion of behaviour time spent on aggression ($p<0.002$). These effects were either reversed (significant reductions in aggression with SAL and NLX, $p<0.05$) or cancelled (minor reduction, NLX+SAL) in the dark phase. Partner body sniffing showed a trend towards lower levels following treatments in the light, and higher levels in the dark. No other behaviour changed systematically with treatments. Plasma sodium levels were monitored: there was a trend of treatment group mean sodium levels changing in proportion to the aggressogenic effect of the treatment, but it was not confirmed by correlation tests on the plasma sodium and aggression levels of individuals. The data are discussed in terms of a possible interaction between opioid receptors and physiological sodium chloride loads.

Saline drinking Naloxone Aggression Social behaviour Lightcycle Mice

THE present study was planned to follow up our preliminary findings on the behavioural effects of small oral sodium loads [8, 9, 10]. Territorial aggression between groups of male albino mice is markedly and consistently stimulated by allowing the animals to drink saline solutions prior to the test, an effect which is not related to saline concentration in the range 0.9–2%, or time of access in the range 2–5 days [8]. The amount of sodium chloride intake required for a highly significant increase seemed very modest.

In a follow-up study, drinking of physiological saline (0.9% NaCL) for two days proved sufficient to increase the residents aggressiveness in the isolation-induced aggression test (Vickers, in preparation). Trials with naloxone or met-enkephalin treated residents in the same study gave an increase in aggression after naloxone and a decrease after met-enkephalin, both agents administered as single injections half an hour before the test. Treatment with naloxone has been previously shown to both potentiate [13] and inhibit shock-induced aggression in mice [5], and also to inhibit isolation-induced (resident/intruder) aggression [14]. Naloxone tends to reduce related social behaviour, e.g., play fighting [1] and contact seeking behaviour [7], at least in the light phase of the 24 hour light cycle.

The relationship between behavioural effects of saline drinking and those of treatment with opioid-receptor active agents is hypothetical. The opioid receptors form one group in a population of sodium-sensitive receptors; it has been shown that sodium chloride, in physiological concentrations, speeds agonist dissociation rate, and potentiates inhibitor binding at opioid receptors *in vitro* [11,16]. Both naloxone sensitive responses [3, 12, 15, 17] and behaviour, including aggressive behaviour [2, 4, 15, 18], show evidence of light-/dark rhythmicity, which indicates that experimental evidence concerning these functions should be obtained from at least one time-point in each phase of illumination.

In the present study, we administered 0.9% saline, instead of drinking water, for 48 hours prior to the test, to a group of single-housed animals. Other groups of mice, drinking either water or saline, were also given a pretest injection of naloxone. Aggression testing was carried out at the approximate midpoint of the light and the dark phase of the light cycle. We also determined plasma sodium levels in all tested animals.

METHOD

Animals and Housing Conditions

We used an outbred mouse strain, albino male TO mice (supplied by Tuck Ltd.). Mice transferred to the experimental rooms at 4–5 weeks of age (17–20 g body weight) and housed either singly (cages 29×12×11 cm) or in groups of 6–8 (cages 40×24×11 cm). The experimental rooms were kept in the same conditions (20±2°C; 12L:12D light-cycle). In one room, lights-on time was 0500 hours, and in the other, 2000 hours.

RESULTS

"Total behaviour time" excludes non-recorded behaviour, e.g., episodes of ambulation and sitting still, and takes up, on average, slightly less than half of the 10 minute observation time. The duration of fighting, partner sniffing, rearing and other behaviours have been expressed in percent of total behaviour time. The advantage of using a relative measure is that it permits comparison of shifts in the proportion of different behaviours across treatments, irrespective of overall changes in activity. When the "total behaviour time" is similar for different experimental groups, the percentage figures are equivalent to the absolute values to purposes of comparison.

Behaviour Test

The animals were kept in the experimental housing conditions for six weeks, undisturbed except for routine maintenance. During the week prior to testing, the sawdust bedding in the single cages were left unchanged. On Day 42, testing was carried out 3–5 hours after either lights-on or lights-off. One randomly selected intruder animal from the group-housed batch was transferred into the cage of a single-housed animal (resident) and the encounter was observed for 10 minutes.

Only the residents behaviour was recorded, using observer-operated event recorders (Campden Electronics Ltd., London). The duration, frequency and number of incidents of the recorded behaviours, as well as the sequences of different behaviours, could be determined from our event-recorder charts. The following six behaviours were noted: fighting (physical attack, with biting and wrestling); tail-rattling (characteristic flicking of the tail, often seen in dominant attacking mice); partner body sniffing (sniffing, sometimes combined with pawing, of various parts of the intruder); digging (in the saw-dust bedding); self-grooming; rearing (including all cases of two-legged stance, both free and against the wall of the cage).

Notes were kept on intruder behavior. Cases in which the resident submitted to an aggressive intruder (5.5% of all tested pairs) were excluded from the records. The animals were weighed and killed by stunning followed by decapitation immediately after the confrontation.

Treatments

Treatments were administered to residents only. Treated groups were tested in mixed batches, not as blocks. One control group received no treatment (UT group), and another an intraperitoneal (IP) injection of 0.2 ml 0.9% saline 30 minutes before the start of the test, with the injections spaced at 10 minute intervals, to allow for testing time (SI group).

Physiological saline solution (0.9% NaCL) was administered in the drinking bottles (no additional water provided) for 48 hours prior to the test to one group (SAL group). Naloxone injections (1 mg/kg in 0.2 ml 0.9% saline IP) were given as described above for saline injections, both to a water drinking (NLX group) and a saline drinking group of mice (NLX×SAL group).

Plasma Sodium Determinations

Trunk blood was collected after decapitation and aliqouts of plasma diluted with double-distilled water (1:100) to meet the minimum volume requirements of the instrument. The aliqouts were frozen and stored at −20°C. Large batches of samples were then analysed in the ICP (inductively coupled plasma source) spectrometer (Philips) courtesy of the Department of Geology, King's College.

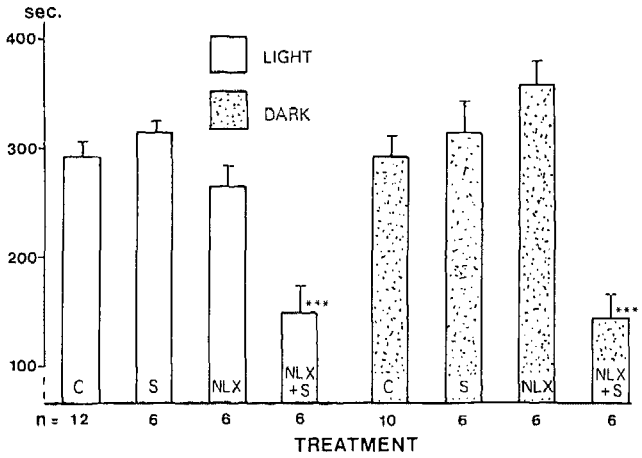

FIG. 1. Total behaviour time. Means and standard errors of total recorded behaviour time (seconds) for the different experimental groups (n=number of resident-intruder pairs in each group). ***$p<0.002$.

Experimental Groups and Statistics

Six resident/intruder pairs were tested for each treatment, apart from the UT and SI groups in the dark phase where five pairs were used for each. Groups were compared using the Mann-Whitney U-test (two tailed) on all behavioural measures and Student's t-test on plasma sodium levels.

Control Groups

The behaviours of the untreated (UT) and saline injected (SI) did not differ significantly on any measure, nor was there any difference in the plasma sodium level. These groups were therefore pooled, giving one control group (C group) for the light and one for the dark phase of the light cycle.

Total Behaviour Time

Total behaviour times for the different treatment conditions are shown in Fig. 1. Single treatments (SAL and NLX) did not cause any significant change in the total time spent on recorded behaviours. The combined treatment (NLX+SAL) reduced total behaviour time in both the dark and light phase ($p<0.002$, in all cases).

Aggressive Behaviour

The changes in aggressive behaviour are shown in Fig. 2. The relative amount of time spent fighting by animals in the control groups differed between the light and the dark phase: the dark phase proportion was almost twice that of the light phase ($p<0.05$). The single treatments caused comparably increases in fighting in the light phase, although the NLX effect just failed to be significant to a single low value (SAL, $p<0.02$). The combined treatment caused a highly significant increase in fighting ($p<0.002$), relative to the C group. Fighting was also increased over level of the SAL group ($p<0.05$) and just failed to be significantly greater than that of the

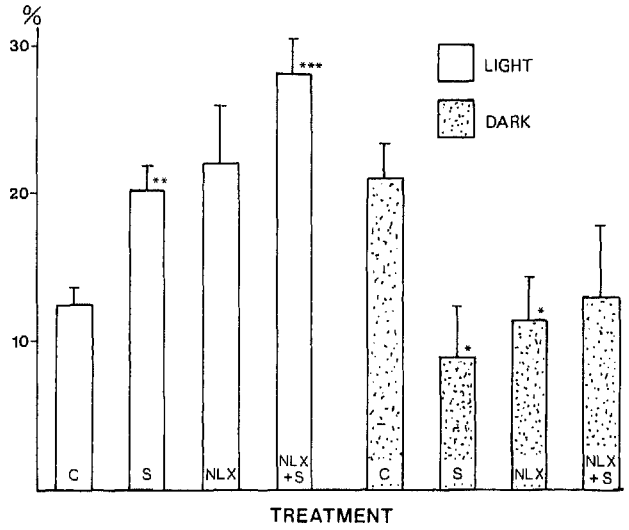

FIG. 2. Aggression. Means and standard errors of the percent of total behaviour time spent by resident attacking the intruder. $*p<0.05$ $**p<0.02$ $***p<0.002$.

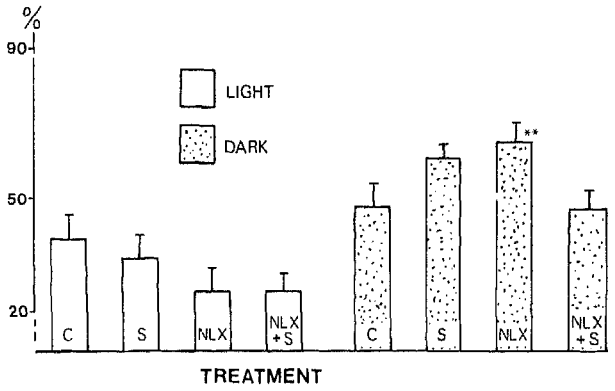

FIG. 3. Partner sniffing. Means and standard errors of the percent of total behaviour time spent by resident sniffing the intruder. $*p<0.05$ $**p<0.02$.

TABLE 1

MEAN (±S.E.M.) TIME SPENT ON REARING AND TAIL-RATTLING BEHAVIOUR, EXPRESSED IN PERCENT OF TOTAL BEHAVIOUR TIME

	C	SAL	NLX	NLX+SAL
a) Light phase (n=12)				
Rearing	35.0 ± 5.0	30.6 ± 3.0	34.8 ± 4.2	28.8 ± 3.6
Tail-rattling	5.3 ± 1.3	4.7 ± 1.21	11.7 ± 4.7*	7.7 ± 1.9
b) Dark phase (n=11)				
Rearing	25.2 ± 4.0	27.4 ± 1.4	21.0 ± 4.4	20.2 ± 5.4
Tail-rattling	2.6 ± 0.6	1.0 ± 0.6	0.7 ± 0.4	1.1 ± 0.7

$*p<0.02$.

Light/dark differences: Rearing: No significant differences; Tail-rattling: C group, N.S.; SAL, NLX and NLX+SAL groups, $p<0.02$.

NLX group. In the dark, single treatments reduced the proportion of fighting time ($p<0.05$ in each case), i.e., the effects of NLX and SAL were both opposite those seen in the light. The NLX+SAL group had an insignificant reduction in the level of fighting.

Partner Sniffing

Partner sniffing results are displayed in Fig. 3. Generally, there was more social investigation in the dark than in the light; this holds true for all the tested groups. Treatments tended to cause a decline in the proportion of partner sniffing in the light, and an increase, compared to the C group, in the dark (NLX, $p<0.02$).

Rearing

Rearing showed no consistent differences either with light cycle or treatment. Somewhat less rearing took place in the dark. None of the treatments caused a significant change. The data are shown in Table 1.

Tailrattling

Tail-rattling behaviour was highly associated with overt aggression and occurred both before and after attacks. It occured erratically, and showed no consistent pattern of treatment effects (see Table 1). Tailrattling behaviour was consistently (and often significantly, see Table 1) higher in the dark than in the light.

Self-Grooming

Self-grooming was carried out by some, but not all, of the residents, and varied considerably between individual animals. There was no lightcycle- or treatment-related pattern characterising this behaviour. Subjectively, it seemed that residents and intruders spent similar amounts of time grooming.

Digging

This behaviour rarely occurred in residents. Intruders spent considerable amounts of time on this behaviour.

Plasma Sodium Concentrations

The results of plasma sodium determinations are shown in Table 2. In the light phase, only one treatment led to a significant change: NLX+SAL caused a marked increase in the sodium level ($p<0.002$). In the dark phase, both SAL and NLX caused a significant decline (SAL, $p<0.02$; NLX, $p<0.02$). The combined treatments did not change the sodium level.

TABLE 2

MEAN (±S.E.M.) PLASMA SODIUM CONCENTRATIONS (mEq./l) IN TREATED MICE

Groups	C	SAL	NLX	NLX+SAL
a) Light phase	143.8 ±1.1	139.8 ±2.1	148.3 ±4.5	173.5† ±6.7
b) Dark phase	142.7 ±2.4	133.3* ±2.9	137.0* ±2.0	145.2 ±2.5

*$p<0.02$.
†$p<0.002$.

DISCUSSION

In this series of experiments, the animals responded to both saline drinking (SAL) and naloxone injection (NLX) treatments with increased fighting in the light phase, and decreased fighting in the dark. The combined treatment (NLX+SAL) led to a further increase in aggressiveness in the light, suggesting a potentiating interaction between SAL and NLX. In the dark phase, the single treatments had the opposite effect, i.e., reduced fighting, and the combined treatment no significant effect.

The changes in the proportion of aggressive behaviour, should be seen in context of the treatment effects on total behaviour time. While the single treatments did not change overall activity time, the combination of saline and naloxone caused highly significant decreases, both in the light and the dark phase. The increased level of fighting in the NLX+SAL (light phase) group indicates a marked shift in the likelyhood of this behaviour occurring.

Aggression was the only behaviour examined which responded to treatments with marked changes. Rearing and self-grooming showed no systematic change with treatment. In contrast, partner sniffing followed a consistent pattern, although most of the changes failed to be significant. Generally, the treatments caused less time to be spent on partner sniffing in the light, and more time to be spent in the dark. The data obtained for the control groups agree well with what is known of dark/light changes in rodent behaviour [2, 4, 18], i.e., all forms of social behaviour tend to increase in the dark. The main effect of the treatments could be summarised as a light/dark dependent change in the characteristics of social investigation in the dark. The subjective impression given by the animals in the low general activity, high aggression category (the NLX+SAL group, light), was of lack of interest in the surroundings, adequate motor capacity and high level of irritability. The dark-phase group was similarly uninterested, but less irritable.

Light-cycle dependent effects of NLX were expected. Bio-rhythmicity in brain opioid-sensitive (as well as in other) systems have been observed in several instances [6,15]. There are reports of light cycle dependent effects of naloxone in pain and aversive taste conditioning tests [3a, 12, 15, 17]. Maximal naloxone potency may occur twice in the 24-hour cycle, at the beginning (within 2–4 hours) of lights on and off [16].

Sodium ions has been shown to increase dissociation rates for opioid receptor agonists, and to potentiate opioid antagonist binding *in vitro* [11,16]. Sodium is however only one example of endogenous populations of metal ions able to change the binding characteristics of opioid receptors [16]. The *in vitro* studies show that the ionic effects vary in potency between opiate receptor types. If isotonic saline intake indeed leads to local shifts in the concentration of sodium (and possibly other) ions, the overt effects are at present impossible to predict. The possibility that the sets of receptors mediating respectively endorphin- and MSH-type responses [6] may be involved is currently under investigation.

We did not expect isotonic saline drinking for 2 days to cause drastic changes in the plasma sodium level. The relatively minor reductions seen in SAL and NLX treated mice after dark phase testing may be fortuitous. The highly significant increases seen in NLX+SAL (light phase tested) mice are inexplicable. At no stage in our work on saline effects has any correlation between behaviour and plasma sodium levels of individual animals been found [8, 9, 10], although marked changes in sodium level have occurred in some treatment groups. We have no reason to suspect our methodology, which involves repeated standard calibration of an extremely reliable and sensitive instrument. We can only assume that certain treatment conditions are associated with changes in function of one or more of the systems controlling sodium homeostasis.

The effects of naloxone are notoriously difficult to interpret. Apart from the light-cycle and housing dependent shifts in naloxone potency, strain differences and dose levels can be of critical importance. In a recent study, also examining naloxone effects on isolation-induced aggression in mice [14], naloxone, in the same dose range as in this investigation, caused a decline in aggression. This result, directly opposite to our findings, may be related to the strain used (DBA/2 mice), which had very low social behaviour times, compared to our animals. Time of testing in relation to the light-cycle may also have played a role (not enough information available).

To conclude, it seems justified to pursue the "saline effect" on social behaviour in terms of opioid receptor function, although the nature of the receptors and their relationship to opioid modification of social behaviour [7] are far from clear.

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