

The effect of naloxone on object exploration, object recognition and other types of spontaneous behavior

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Abstract. Object exploration was examined in naloxone injected (1 mg/kg or 4 mg/kg) and saline control rats. Naloxone rats explored an object for a shorter time than did controls, thus indicating a lower investigatory motivation. This effect was dose dependent. Higher drug dose (4 mg/kg) decreased the number of contacts with an object. Both doses increased the mean duration of contacts with an object. The naloxone groups showed intact recognition of a familiar object paired with a new one in two sessions - 4 h and 24 h after the injections. The higher drug dose depressed the locomotor activity and wall leaning. Grooming was not influenced by naloxone. The normal daily fluctuations in the level of grooming and locomotion were distorted following the injection of the higher dose of naloxone. The lower dose (1 mg/kg) did not affect the rats' performance in some tests. The results could be viewed as a naloxone-related depression of the behavior containing motor elements like locomotion, wall leaning and object approaching. The prolonged contact time with an object could be the result of a lowered flexibility of movement. However, the decrease of rewarding value of exploration could not be ruled out. Possibly, naloxone exerts several different interacting behavioral effects.

Key words: rat, object exploration, memory, grooming, locomotion, naloxone

INTRODUCTION

Opioid peptides have been found to be important in mediating a number of behavioral phenomena including the locomotor activity (Rodgers and Deacon 1979, Koek and Slangen 1984), learning and memory (Gallagher and Kapp 1978, Izquierdo 1979, Flood 1987) and exploratory behavior (Arnsten and Segal 1979, Katz and Gelbart 1978, File 1980). Some investigators reported that a blockade of the opiate receptors by naloxone produced changes in the rats' interaction with environmental stimuli (Arnsten and Segal 1979). The lower number of contacts may reflect a decrease of exploratory motivation by naloxone. However, the stimuli used by those authors were little bulbs made of wire mesh which hardly could hold the rats' attention. More conspicuous objects might have a different effect. To examine further the problem of exploratory motivation after naloxone treatment large objects like milk bottles or cans were used. The trial was terminated when the rat did not approach an object during the 2 min period. Assuming that such a long break of exploration reflects a diminution or even loss of motivation to explore, we adopted the period of object interaction till to 2 min break as a criterion of duration of the exploratory motivation. One of the purposes of this experiment was to compare this measure in the naloxone treated and control rats.

In the experiments dealing with exploration both the stimuli and their surroundings were usually new for the animal. Because our previous study (in preparation) showed that the degree of novelty linked to the training situation was an important factor for object exploration and the course of habituation, in the present experiment rats were exposed to a novel object in a familiar surrounding. The retention of information about an object acquired under the naloxone was also investigated. Recognition of the familiar object and its discrimination from a new one was tested in control rats in two 3 min sessions performed 4 h and 24 h after the initial (Session I) object presentation.

Additionally, the locomotor activity, grooming and wall leaning were also recorded in all three sessions. Two doses of naloxone i.e. 1.0 mg/kg and 4.0 mg/kg were applied to obtain different levels of blockade of endogenous opiates. This will further elucidate the role of opioid peptides in behavioral phenomena.

METHODS

Subjects

Fourty seven naive male Wistar rats, 7 months old were used. They were born in the Animal Breeding Laboratory of the Nencki Institute. Rats were housed in groups of 6 in semi-transparent plastic cages in the colony room. They were maintained on 12-h cycle light-dark (light on; 7 a.m.). Food pellets and water were available ad lib. during the entire experiment.

Apparatus

The apparatus was a circular arena 75 cm in diameter with the wall 38 cm high, painted uniformly gray. A camera fixed above the arena was connected to a video recorder and a TV screen. Thus an experimenter could observe the rats without disturbing their behavior. Three different objects were used (1) a rectangular glass bottle (6 cm x 8 cm base, 25 cm in height) filled with dark colored water, (2) a white opaque plastic milk bottle (8 cm dia., 22 cm in height), (3) a plastic dark bottle (7 cm dia., 21 cm in height). The objects existed in duplicate. Their weight was such that they could not be displaced by rats. Rats were introduced into the apparatus always in the same place. The apparatus was cleaned between subjects.

Procedure

Prior to the experiment rats were handled individually for several days. They were then familiarized with plastic bucket in which they were carried to the experimental room. Rats were permitted a 2 min preliminary exploration of an empty arena for 3-4 days.

Three sessions were performed. The first session (Session I) - around midday (between 10 a.m. and 12 noon); the second one (Session II) - four hours later (between 14 p.m. and 16 p.m.) and the third session (Session III) on the next morning (between 10 a.m. and 12 noon).

The Session I, following naloxone or saline injections consisted of two trials separated by a 1 min interval, which the rats spent in the plastic buckets in the same room. During the trial I an object was placed in the middle of the arena. Rats were confronted with one of the three objects used in the experiment. A duplicate object was used during trial II to avoid scent marks of a visually

familiar object. The length of each trial varied among the rats because the trial was interrupted when the rat did not explore the object during two consecutive minutes.

Four hours after the Session I rats were given one-trial Session II (under no drug conditions) in which two objects were presented: (1) the same object as that in the two trials of the Session I (visually familiar object) and (2) a new one. Different rats were exposed to different pairs of objects. The session lasted three minutes. On the next day, 24 h after the Session I, the third three-min Session III was applied, also under no drug conditions. Again two objects were presented - the familiar one, seen on the first and the second session, and a new one, never seen before. Locomotor activity was evaluated in all three sessions. For this purpose a picture of the arena was drawn on a translucent sheet and placed over the TV screen. The picture of the arena was divided into nine equal sectors. The number of sector crossings provided an index of the rats' locomotor activity.

Drug administration

Naloxone was administered i.p. before the first session, whereas the second and the third sessions were performed without drug. Rats were randomly assigned to three groups.

Group NAL-1 received 1mg/kg of naloxone hydrochloride (Sigma), Group NAL-4: - naloxone dose was 4.0 mg/kg. The control group (CONT) was given a matched volume of saline. All injections were performed 20 min before the start of the trial.

Data collection

The data from the videotape were computer-processed. The data consisted of the number and duration of contacts with an object. A contact was defined as a direct touching of an object with the rats snout or looking at an object from a distance up to 10 cm (visual contact). Using these data the overall exploration time was calculated.

RESULTS

Effect of naloxone upon object exploration (Session I)

In the trial I of Session I, which followed drug injections, naloxone rats explored an object for a shorter time

than did control rats before reaching criterion, i.e. no exploratory activity for two consecutive minutes. Thus, in the NAL-4 group exploration till that criterion lasted 3.8 min, in the NAL-1 group - 7.6 min while in the CONT group - 8.4 min. Due to habituation, time of object exploration till criterion was much shorter in trial II i.e. 1.6 min in the NAL-4 group, 1.7 min in the NAL-1 group and 2.4 min in the CONT group. Two way analysis of variance (Groups x Trials) revealed significant effect of groups [$F(2,44) = 3.200$, $P < 0.05$], and significant effect of Trials [$F(1,44) = 63.940$, $P < 0.001$]. The interaction Groups x Trials $F(2,93) = 4.640$, was significant at $P < 0.02$. Each group differed significantly from the other by Duncan test ($P < 0.001$).

Termination of trials I and II by the behavior of particular rats and, consequently, different trial duration in different rats precluded the comparison of the whole trials in different groups; statistical analyses were performed only for the initial three-minute period of trials when all rats were still engaged in object exploration. Figure 1 presents the course of object exploration of the control and the two naloxone groups in the initial period of trial I and trial II. For comparison Fig 2. presents the number of crossings of arena segments in the same periods. All groups showed the intra-trial and between-trial habituations in both types of behavior.

The influence of naloxone on object exploration could be satisfactorily evaluated only in trial I, because in trial II considerable number of rats stopped exploration before 3 min elapsed whereas in trial I they stopped between 3 and 8.4 min. Overall exploration time in the initial 3 min of trial I did not differ between the groups (Kruskal-Wallis Anova). The number of contacts was higher, and contact duration lower in control group than in naloxone groups as could be seen in Table IB. Although the number of contacts decreased with increased drug dose, the level of significance was missed [$F(2,44) = 3.104$, $P < 0.0548$], a significant difference between the groups was found by χ^2 test ($\chi^2 = 6.35$, $df = 2$, $P < 0.05$). When the number of contacts was considered not in the initial 3 min but in the very first minute of trial I (Table IA) Anova indicated significant differences between the groups [$F(2,44) = 4.819$, $P = 0.02$]. Tukey-Kramer test showed a significant difference between the CONT and NAL-4 group ($P < 0.05$) the difference between the CONT and the NAL-1 group was not significant.

Mean duration of contacts in the initial 3 min of trial I was lower in the control than in the naloxone groups

Object exploration (Session I)

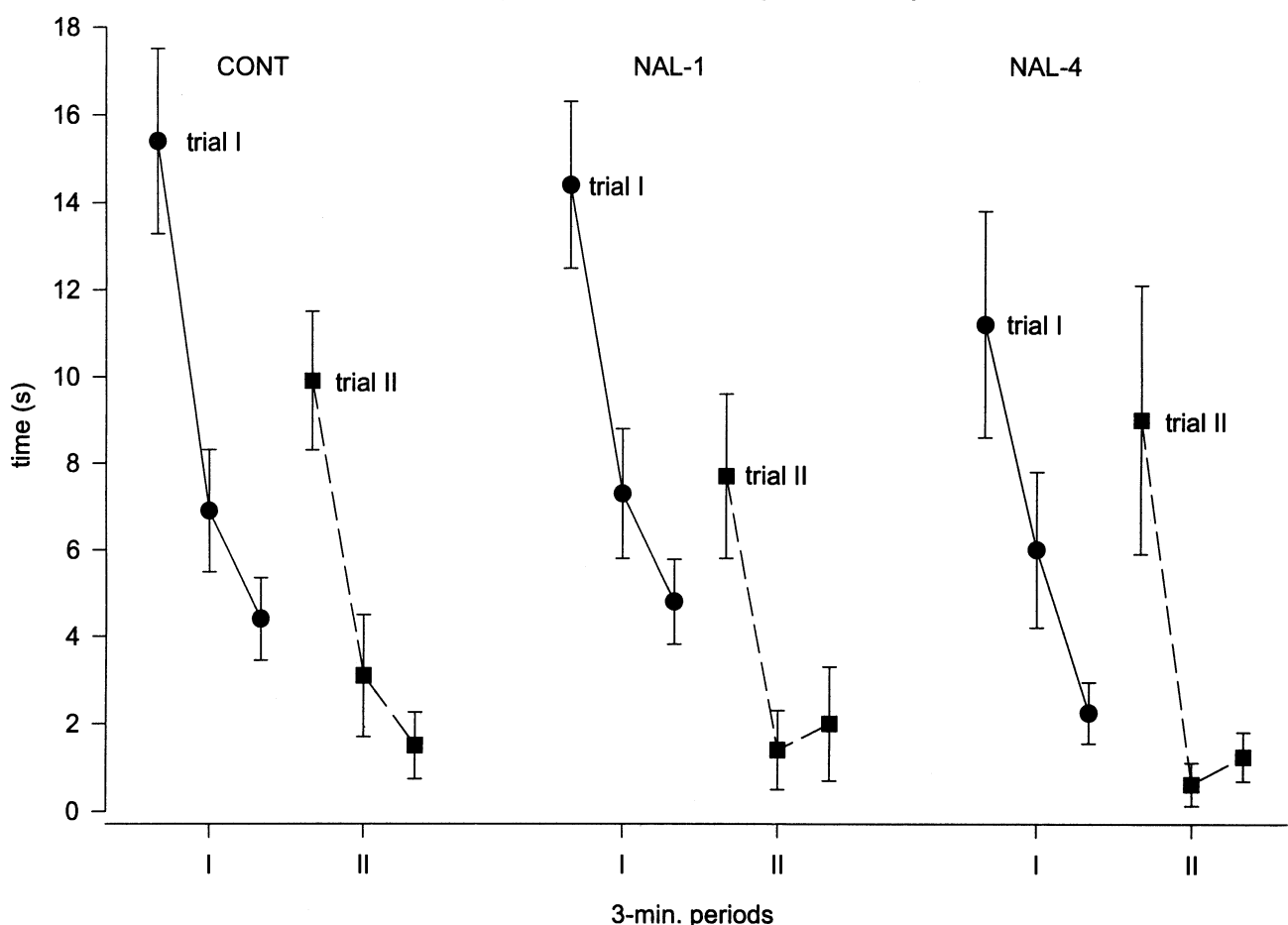


Fig. 1. Habituation of the object exploration by control and naloxone-treated rats in the initial 3 min periods of trial I and II. Session I. Means and SEM.

(Table IB). Analysis of variance did not demonstrate significant differences between the groups but the non-parametric test χ^2 which was performed following the Bartlett's statistic showed significant differences between the groups ($\chi^2 = 6.975$, $df = 2$, $P < 0.03$). The mean contact duration within the first minute (Table IA) also differed significantly between the groups ($\chi^2 = 9.63$, $df = 2$, $P < 0.01$). The mean contact duration within the initial 3 min of the trial was significantly lower than during the first minute in the CONT group ($P < 0.0002$) and in the NAL-1 group ($P < 0.0005$) by Wilcoxon test, indicating a within-trial habituation. In the NAL-4 group this difference was not significant.

The effect of naloxone on recognition of the familiar object after 1 min retention interval, when the animals were still under the influence of the drug could not be satisfactorily established because considerable numbers of

rats (4 in the NAL-1, 8 in the NAL-4 and 3 in the CONT group) refused to explore the object in trial II.

The remaining rats displayed a strong habituation of exploration (see Fig. 1), decreasing the number of contacts and mean contact duration in trial II, as compared to trial I (see Table I). Analysis of variance of the number of contacts in the initial 3 min period of the trials I and II only in rats exploring in trial II revealed a significant effect of trials [$F(2,28) = 48.180$, $P < 0.001$]. The effect of groups missed the level of significance. However, when the data of the very first minute were considered, Anova indicated a significant decrease of the number of contacts between trial I and II [$F(1,28) = 4.800$, $P < 0.05$] and also a significant group effect [$F(2,28) = 3.700$, $P < 0.05$].

Mean time per contact was also significantly lower in the first minute of trial II than in trial I [$F(1,28) = 13.610$, $P < 0.003$]. Group effect was significant [$F(2,28) = 5.060$,

Locomotion (Session I)

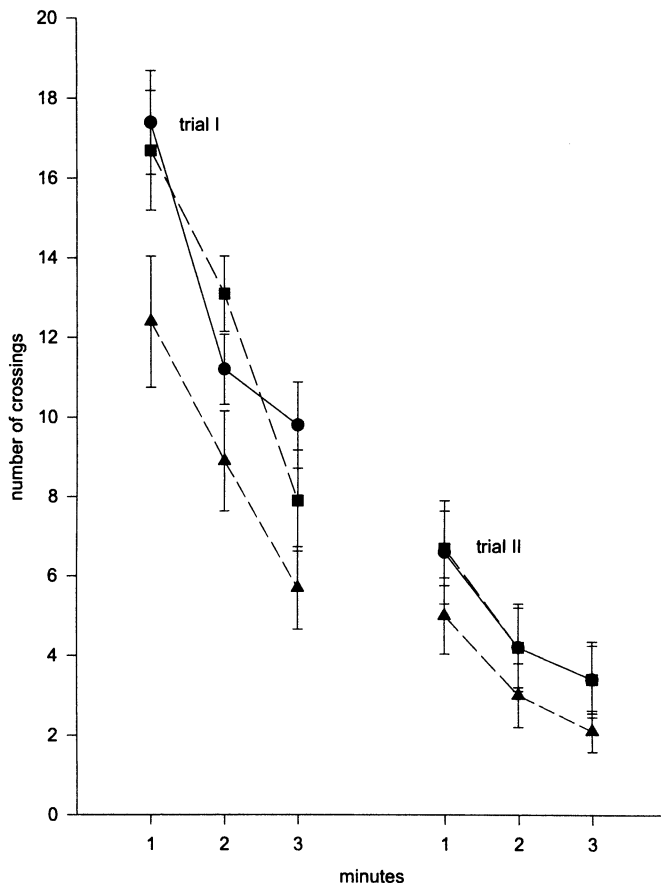


Fig. 2. Decrease of the number of crossings concurrent to object exploration. Means and SEM. Solid line and circles, CONT group. Dashed lines, naloxone-treated groups. Squares, NAL-1 group. Triangles, NAL-4 group.

$P < 0.02$]. Mean time per contact evaluated for 3 min period did not differ significantly between trial I and trial II.

Retention tests in Session II and Session III

In these two no-drug sessions, the retention of information acquired under naloxone in Session I was examined. The rats which explored for only a few seconds during Session I were discarded because they probably did not acquire an adequate amount of information about the object presented. The numbers of rats remaining in groups were 12 in the CONT, 11 in the NAL-1 group and 8 in the NAL-4 group.

In Sessions II and III rats were presented for 3 min two objects simultaneously; one of them was the familiar object explored by a given rat in Session I, the other one was

TABLE I

Object exploration (s) during the initial periods of trial I and trial II in Session I. (Means and SEM)

	A. The first minute		B. The 3 min period	
<hr/>				
	Number of contacts			
	Trial I	Trial II	Trial I	Trial II
CONT	4.4 ± 0.68	4.0 ± 0.62	9.75 ± 1.4	7.3 ± 1.05
NAL-1	2.6 ± 0.29	2.5 ± 0.51	7.6 ± 0.88	6.1 ± 1.35
NAL-4	2.2 ± 0.55	2.8 ± 0.87	5.4 ± 1.3	8.3 ± 3.27
	Duration of contacts			
	Trial I	Trial II	Trial I	Trial II
CONT	3.4 ± 0.48	2.8 ± 0.46	2.5 ± 0.33	2.7 ± 0.39
NAL-1	5.8 ± 0.71	3.4 ± 0.55	3.4 ± 0.22	3.3 ± 0.51
NAL-4	4.5 ± 0.94	2.5 ± 0.67	3.6 ± 0.82	2.9 ± 0.55

Scores of all rats were considered in these analyses.

novel. When Session III was conducted, the familiar object was the object presented to the rat during the two preceding sessions. The novel object was different from that presented to a given rat during Session II. As shown in Table II each group of rats paid more attention to the new object than to the familiar one in both sessions, either 4 h or 24 h after naloxone or saline injections.

SESSION II

In the early afternoon session the new object was explored for a significantly longer time than the familiar one [$F(1,28) = 18.880, P < 0.001$]. No difference between groups was found. The numbers of contacts with the new and familiar objects did not differ significantly in either group but the recognition of new object was reflected by a longer mean duration of contact with the new object than with the familiar one [$F(1,28) = 6.870, P < 0.02$]. No significant group effect and no significant interaction (objects × groups) was found.

The data shown in Table II suggest that the number of contacts in the afternoon (Session II) was higher in the groups injected previously with naloxone, especially with its 4 mg/kg dose (NAL-4 group) than in the saline control rats; this suggestion, however was not confirmed by a Kruskal-Wallis analysis of variance.

TABLE II

Object recognition 4 h (Session II) and 24 h (Session III) after naloxone injection (Means and SEM)

	Session II					
	Overall exploration (s)		No of contacts		Time/contact (s)	
	New	Familiar	New	Familiar	New	Familiar
CONT	21.75 ± 2.89	13.00 ± 2.15	7.42 ± 0.87	5.83 ± 0.98	3.04 ± 0.24	2.23 ± 0.30
NAL-1	24.27 ± 2.72	16.45 ± 2.19	8.09 ± 0.97	7.54 ± 0.97	3.05 ± 0.25	2.63 ± 0.35
NAL-4	25.00 ± 3.61	17.75 ± 3.21	9.12 ± 1.02	8.12 ± 1.44	2.72 ± 0.29	1.98 ± 0.42

	Session III					
	Overall exploration (s)		No of contacts		Time/contact (s)	
	New	Familiar	New	Familiar	New	Familiar
CONT	28.50 ± 3.11	19.75 ± 1.88	9.92 ± 1.16	9.50 ± 0.73	2.98 ± 0.31	2.07 ± 0.15
NAL-1	30.82 ± 2.59	21.45 ± 2.76	11.18 ± 0.99	8.81 ± 0.79	2.81 ± 0.23	2.45 ± 0.25
NAL-4	28.75 ± 1.89	19.12 ± 5.16	9.25 ± 1.29	9.12 ± 0.91	2.96 ± 0.32	2.07 ± 0.07

SESSION III

The rats also showed recognition memory when confronted with the new and the familiar objects 24 h after Session I. A possible effect of repetition should be considered, however, because the familiar object was seen three times before this session. An Analysis of variance has indicated that the difference in exploration time of the two objects was highly significant [$F(1,28) = 18.630$, $P < 0.001$]. As was the case in the previous session, the number of contacts did not differ between the objects and between the groups, but the duration of contacts with a new object was significantly longer than with the familiar object [$F(1,28) = 11.040$, $P < 0.003$]. The groups did not differ from each other in this respect.

Interestingly, in both sessions and all groups, the very first contact in the trial with the new object was significantly longer (mean 5.6 ± 0.5) than with familiar object (mean 3.3 ± 0.3), so the rats were able to recognize the new object at first glance [Anova for Session II, $F(1,28) = 9.870$, $P < 0.004$ for Session III $F(1,28) = 6.640$, $P < 0.05$].

One might notice from Table II that overall exploration time was higher in Session III than in Session II. Analysis of variance confirmed this impression [$F(1,28) = 17.810$, $P < 0.001$]. The groups did not differ significantly in this respect. The elevated exploration in Session III (next morning) was due to higher number of contacts with the objects either new or familiar than in Session II. ANOVA indicated significant difference be-

tween the session [$F(1,28) = 21.20$, $P < 0.001$]. The time per contact did not changed markedly between sessions.

Other behavioral measures

Locomotion, grooming and wall leaning in all three sessions are presented in Fig. 3. For Session I only the initial 3 min of trial I were considered to match the 3 min duration of Sessions II and III. The animals' behavior in Session I is of primary interest because in this session two groups of rats were under the influence of naloxone.

Kruskal-Wallis Anova indicated significant differences between the groups in locomotion (KW = 8.074, $P < 0.02$) but Dunns' test showed that the only significant difference was found between the CONT and NAL-4 group ($P < 0.05$). The lower drug dose did not affect locomotion. The wall-leaning time was shorter in the Naloxone-treated groups than in the Control group (see Fig 3). Kruskal-Wallis Anova indicated a significant inter-group difference (KW = 6.45, $P < 0.05$). A significant difference appeared between the CONT and NAL-4 group ($P < 0.05$). Again, 1mg/kg naloxone did not affect this measure whose value did not differ significantly from the control rats. The grooming time did not differ significantly between the groups (Kruskal-Wallis Anova).

It could be seen from Fig. 3 that most conspicuous behavioral differences between the three sessions appeared in Session II, in which locomotion showed the lowest values (similarly to wall leaning), whereas in grooming the values were higher in Session II than in the other two

Behavior in the three successive sessions

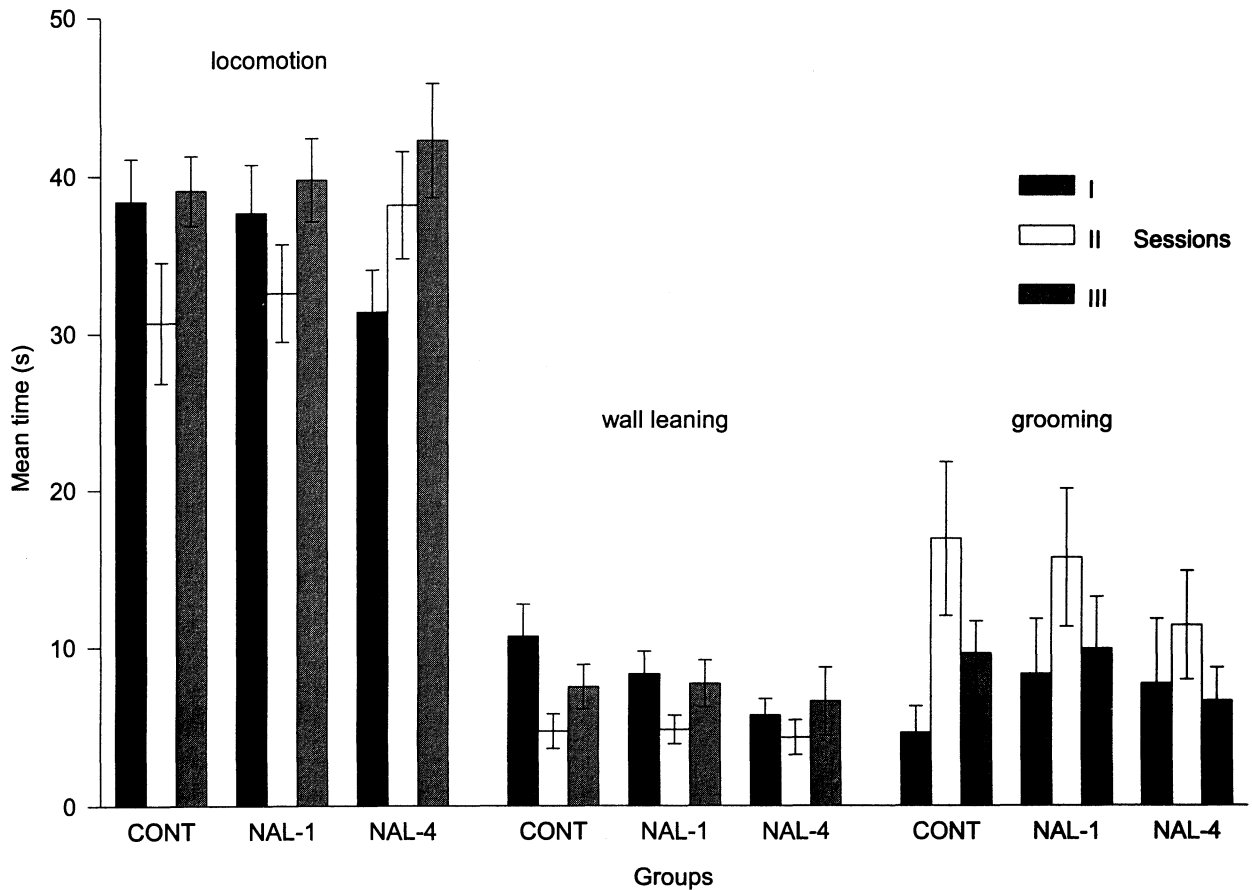


Fig. 3. Locomotion, wall leaning and grooming by the control and naloxone-treated groups in the three sessions. Session I, under the action of naloxone. Session II, 4 h later. Session III, 24 h after injections. Means and SEM.

sessions. This was true for the CONT and NAL-1 group. In the NAL-4 group the relationships between sessions were different. Separate analyses of variance indicated significant differences between the three sessions for the data of locomotion [$F(2,78) = 8.22$, $P < 0.001$] wall leaning [$F(2,78) = 9.28$, $P < 0.001$] and grooming [$F(2,78) = 5.160$, $P = 0.005$]. Group effect did not attain the level of significance in any analyses.

The differences of the groups in successive sessions were revealed by Wilcoxon tests, performed separately for different behavioral measures.

In locomotion, in the CONT group the changes between Sessions I and II as well as between Sessions II and III were significant (both $P < 0.05$). In the NAL-1 group a significant difference appeared between Sessions II and III. In both groups the locomotion values in Session III did not differ from those in Session I. In contrast, in the NAL-4 group the changes in locomotion be-

tween Sessions I-II and Sessions II-III were not significant but the difference between Session I and Session III appeared highly significant ($P < 0.005$).

The CONT group and NAL-1 group showed similar changes in wall leaning. In both groups time of wall leaning decreased significantly between Sessions I and II, ($P < 0.01$ and $P < 0.05$ for the CONT group and NAL-1 group, respectively). The increase observed between the Session I and II also was significant in both groups ($P < 0.05$). In the NAL-4 group the changes between Sessions I and II as well as between Sessions II and III missed the level of significance. The data of Session III did not differ significantly from those of Session I in any group.

The grooming time increased significantly in Session II in the CONT and NAL-1 group ($P < 0.05$) by Wilcoxon test for each group. The decrease between the Session II and III (see Fig. 3) appeared non-significant in each

group, probably due to a small number of rats performing. The same reason precluded the analysis of data of the NAL-4 group.

The outline presented in Fig. 3 and the above analyses suggest no difference between the CONT and NAL-1 group. The character of changes of behavioral data in the successive sessions suggests that the observed differences could be ascribed to the daily fluctuations. The Session I and Session III, both performed before noon, indicated similar values, in the CONT and NAL-1 group in each behavior. In the NAL-4 group, behavioral changes caused by daily fluctuations were distorted probably due to the maintained effect of naloxone treatment. Locomotion and wall leaning had the common feature - the lowest value in Session II in contrast to grooming, which showed the highest value in Session II.

It seems that this feature is due to the motor element. The supposition that grooming and wall leaning are two different behavioral types could be supported by the differences in duration of their single episodes in the three sessions. Figure 4 clearly shows that the pattern of changes of grooming differs from that of wall leaning. The former roughly parallels the changes of total duration in successive sessions (compare Figs. 3 and 4) whereas the latter shows no changes. The mean duration of wall leaning fluctuated around 2 s in each group and session.

DISCUSSION

Rats explore an object with brief intervals between consecutive contacts. Supposing that long intervals be-

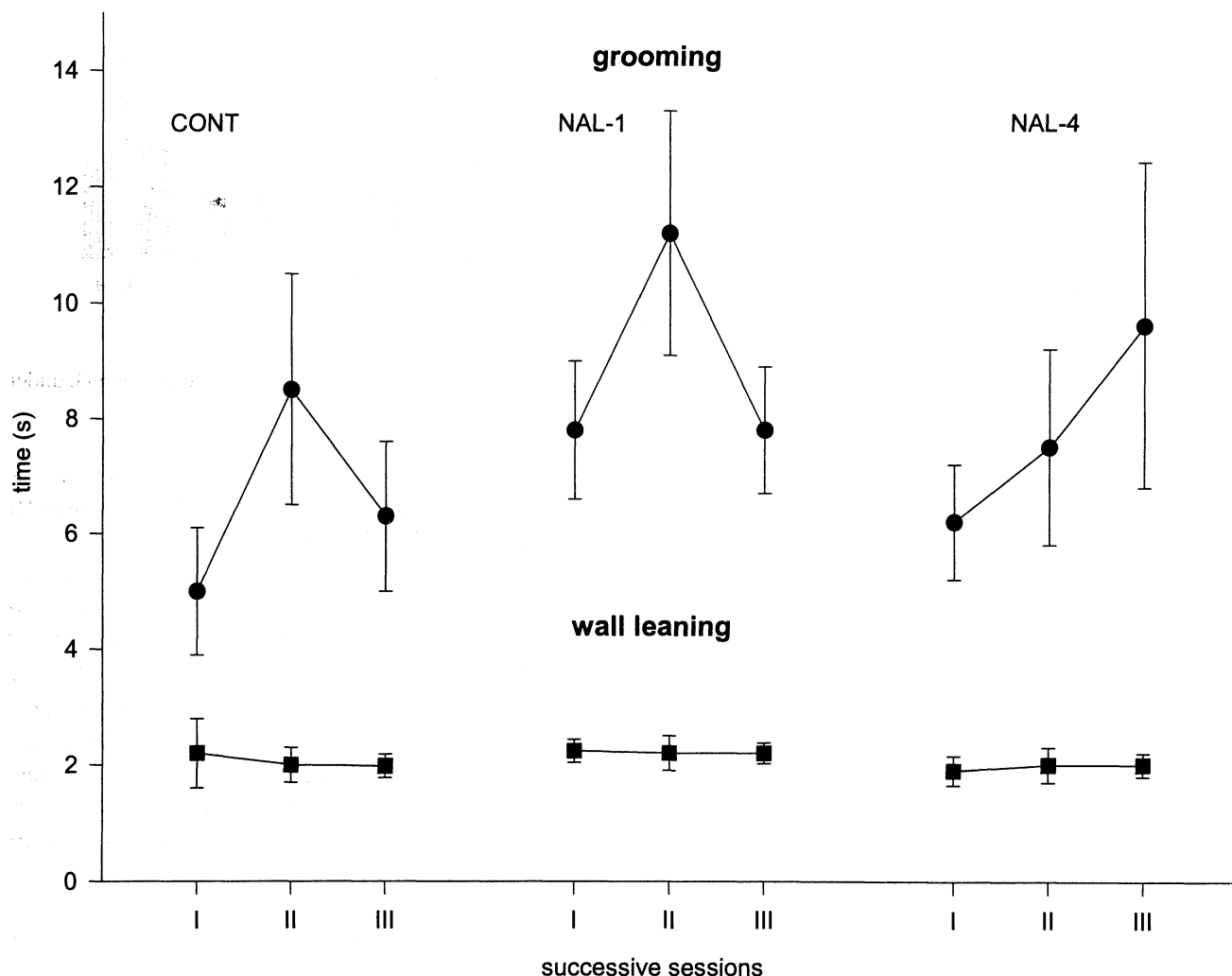


Fig. 4. Mean time of a single episode of grooming and wall leaning by the control and naloxone-treated groups in the three sessions.

tween object approaches reflect the decrease of exploratory motivation we assume that the two-minute period in which there was no contact with an object could be a putative motivational measure for comparison of exploration in the naloxone and saline injected rats' groups. We have found that naloxone injected rats explored the object for a shorter time than did the saline control rats. In the NAL-4 group the period of exploration till criterion was shorter than in the NAL-1 group. These data indicates dose dependent decrease of exploratory motivation by naloxone. Such interpretation is possible since opiates are involved in reward (Stein and Belluzzi 1978). Naloxone decreased the reward value of feeding drinking and social interactions (File 1980, Carey et al. 1981). It may be considered that naloxone interferes with the rewarding properties of novel stimuli, which lose their motivating value faster and cease to reinforce the exploratory behavior.

Different exploration time precluded full comparison of performance of the groups, therefore the analyses were limited to 3 min initial period of the trials in the Session I. Naloxone lowered the number of contacts with an object. No significant dose dependence was found, similarly as in some other studies (e.g. Rodgers and Deacon 1979). In our experiment the decrease of the number of contacts with an object due to naloxone treatment was short-lasting. The significant difference was limited to the very first minute of the trial. When the 3 min period was assessed, the difference was non-significant although the number of contacts in the control group was higher than that in naloxone groups (see Table I). Mean duration of contacts with an object was higher in naloxone treated rats than in saline control assessed either in 1 min or 3 min initial period of the trial. In the first minute of the trial the NAL-1 group differed significantly from the NAL-4 group, whereas in the 3 min period no dose dependence was observed. In all groups mean contact time quickly diminished and in the period of 3 min it was lower than during the first minute of trial I. Lower number of contacts with environmental stimuli and the elongation of contact duration due to naloxone treatment was observed in earlier studies (e.g. Arnsten and Segal 1979). The results of the present experiment differ in that, the naloxone effects on these measures were short-lasting which could be ascribed to procedural differences or to our lower doses of naloxone.

Numerous studies have evaluated the effect of naloxone on memory in various paradigms. Naloxone enhanced retention in a variety of tasks including inhibition

and active avoidance, spatial learning and recognition memory (Izquierdo 1979, Messing et al. 1979, Gallagher 1982, Fulginitti and Cancela 1983, Gallagher et al. 1983). However, some studies showed an impairment (Izquierdo 1980, Turnbull et al. 1983, Łukaszewska and Klepaczewska 1995) or no effect of naloxone administration (Andrews and Holtzman 1988). In our experiment the pre-trial administration of naloxone in dose 1 mg/kg or 4 mg/kg had no effect on recognition memory when it was examined 4 h or 24 h later. Previously drugged rats, similarly to control rats, recognized the familiar object exploring it significantly less than the novel one. This indicated that in Session I under drug condition naloxone-treated rats encoded the sensory information about an object as efficiently as control rats although they spent less time in the arena in the presence of an object. They were able to retain the acquired information for a considerable time. These findings implied that naloxone did not produce any sensory alteration.

The lack of any influence of naloxone on recognition memory might be due to the floor effect. Recognition of large objects is an easy task for rats. In normal rats, object recognition after long delays up to 24 h was reported by Radulska (1993) which is in line with our earlier findings (unpublished). It should be noted that in the present experiment rats were confronted with the same familiar object several times, therefore possibly some long-term memory was involved.

A considerable number of studies examined the effect of naloxone on motor activity in rats. Conflicting findings have been often reported. In some studies a dose dependent reduction of locomotor activity was found (Arnsten and Segal 1979, DeRosset and Holtzman 1982, Rodgers 1982) in others a decrease was not dose dependent (Rodgers and Deacon 1979). The lack of changes in locomotor activity was also reported (File 1980, Galina and Amit 1985, Dokla 1992,). In the present experiment reduction of locomotion was observed in the NAL-4, whereas the NAL-1 group did not differ significantly from the CONT group.

Locomotor activity is often opposed to exploration, however in some conditions animals acquire knowledge about the environment through locomotion. This might be the case in the present experiment. In spite of the fact that rats were well familiarized with the arena before the proper test, they still walked around in experimental sessions. Perhaps they perceived an object and the arena not separately but as an entity - so for them the qualitative aspect of arena was changed in Session I in which an ob-

ject was introduced and this induced exploration of the arena.

In each group a marked decrease of locomotion was observed between the first and the third minute of trial. The steep slope of the curve of intra-trial decrease of locomotion in both, trial I and trial II, and also between-trial decrease point to habituation of exploratory responses to arena.

Wall leaning often considered as attempts to escape from a container is viewed by us as another manifestation of exploration directed to small details of the wall. Animals never tried to go up, but they put their forelegs on the wall and sniffed or looked up. This measure of exploration was diminished by naloxone but only the NAL-4 group differed significantly from the CONT group.

The CONT group and NAL-1 group showed similar pattern of changes in locomotor activity and in wall leaning across the three sessions: Session I under the naloxone influence and further two sessions under no drug conditions - Session II in the afternoon and Session III in the next morning. Figure 3 shows that the CONT and NAL-1 group decreased the locomotion or wall leaning in the afternoon session. This might reflect habituation because the rats were re-introduced to the same arena after considerably short time (4 h). Next morning, after longer interval between the visits to arena the values of locomotion and wall climbing - as the activities expressing exploration returned to initial values due to dishabituation. This pattern of changes could also reflect the diurnal arousal fluctuations. The group treated with 1 mg/kg naloxone (NAL-1 group) was most probably promptly weaned from the drug effect, so that its behavior did not differ from that of the CONT group.

Different pattern of changes was observed in the NAL-4 group. In the afternoon (Session II) rats increased locomotion and wall leaning contrary to the CONT group and NAL-1 group. This might be interpreted as a rebound effect, which followed a release from stronger than in NAL-1 group influence of naloxone. Alternative explanation, however less probable, is that lower activity in the NAL-4 group in Session I provided less information about arena features, and that content is supplemented in Session II. It should be noticed (Figs. 3 and 4) that pattern of changes in locomotion and wall leaning across 3 sessions was exactly the same in the CONT group and in the NAL-1 group. In contrast in the NAL-4 group pattern of changes in wall leaning differed from that in locomotion and was similar to pattern observed in the other two groups. Decreasing effect of naloxone on locomotion

and wall climbing suggests a reflection of a general depression of active motor responding. Similar view was expressed earlier by some authors (e.g. Arnsten and Segal 1979) but denied by others (e.g. Dokla 1992). In our experiment the earlier cessation of the object exploration in naloxone groups than in the control group seems to be in line with the motor depression interpretation. The changes observed in exploration also could be interpreted as a sedative effect of naloxone which caused the lowering of the number of contacts with an object i.e. active approach to an object. The prolonged time of contact with an object could result from lowered flexibility of movement. However the short-lasting effects of naloxone on exploratory measures in the present experiment seems not compatible with this supposition. Frequency of contacts and their duration could be viewed as a reflection of the two different kinds of attention. We reported earlier (Łukaszewska and Klepaczevska 1995) that naloxone affected attention to spatial arrangement of several objects. Attention to single object may consist of two elements: a) drawing attention i.e. approaching an object and b) holding attention i.e. duration of contact with an object. Perhaps naloxone distorts normal relation between the particular elements of attention similarly to found in this paper distortion of diurnal patterns of locomotion and grooming by 4 mg/kg dose of naloxone (NAL-4 group).

It seems probable that naloxone exerts several different effects which interact in some types of behavior.

REFERENCES

- Andrews J.S., Holtzman S.G. (1988) Effects of *d*-amphetamine, morphine, naloxone, and drug combinations on visual discrimination in rats. *Psychopharmacology* 94: 172-177.
- Arnsten A.T., Segal D.S. (1979) Naloxone alters locomotion and interaction with environmental stimuli. *Life Sci.* 25: 1035-1042.
- Carey M.P., Ross J.A., Enns M.P. (1981) Naloxone suppresses feeding and drinking but not wheel running in rats. *Pharmacol. Biochem. Behav.* 14: 569-571.
- DeRossett S.E., Holtzman S.G. (1982) Effects of naloxone and diprenorphine on spontaneous activity in rats and mice. *Pharmacol. Biochem. Behav.* 17: 347-351.
- Dokla C.P.J. (1992) Naloxone reduces social locomotor activity in rats. *Pharmacol. Biochem. Behav.* 43: 1183-1193.
- Galina Z.H., Amit Z. (1985) Interactions between ACTH, morphine, and naloxone and their effects on locomotor be-

- havior. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 9: 691-695.
- File S.E. (1980) Naloxone reduces social and exploratory activity in the rat. *Psychopharmacol.* 71: 41-44.
- Flood J.F., Cherkin A., Morley J.E. (1987) Antagonism of endogenous opioids modulates memory processing. *Brain Res.* 422: 218-234.
- Fulginiti S., Cancela L.M. (1983) Effect of naloxone and amphetamine on acquisition and memory consolidation of active avoidance responses in rats. *Psychopharmacology* 79: 45-48.
- Gallagher M. (1982) Naloxone enhancement of memory processes: effects of other opiate antagonists. *Behav. Neural Biol.* 35: 375-382.
- Gallagher M., Kapp B.S. (1978) Manipulation of opiate activity in the amygdala alters memory processes. *Life Sci.* 23: 1973-1978.
- Gallagher M., King R.A., Young N.B. (1983) Opiate antagonists improve spatial memory. *Science* 221: 975-976.
- Izquierdo I. (1979) Effects of naloxone and morphine on various forms of memory in the rat: possible role of endogenous opiate mechanisms in memory consolidation. *Psychopharmacology* 66: 199-203.
- Izquierdo I. (1980) Effect of β -endorphin and naloxone on acquisition, memory, and retrieval of shuttle avoidance and habituation learning in rats. *Psychopharmacology* 69: 111-115.
- Katz R.J., Gelbart J. (1978) Endogenous opiates and behavioral responses to environmental novelty. *Behav. Biol.* 24: 338-348.
- Koek W., Slangen J.L. (1984) Acute effects of naloxone and naltrexone, but lack of delayed effects, on exploratory behavior in the rat. *Psychopharmacol.* 84: 383-387.
- Łukaszewska I., Klepaczevska A. (1995) Naloxone impaired spatial attention in rats. *Ena Satellite Symposium, Amsterdam.*
- Messing R.B., Jensen R.A., Martinez J.L., Spiehler Jr. V.R., Vasquez B.J., Soumireu-Mourat B., Liang K.C., McGaugh J.L. (1979) Naloxone enhancement of memory. *Behav. Neural Biol.* 27: 266-275.
- Radulska A. (1993) Habituation of object exploration as a model of memory in the rat (in Polish). Master Thesis, Warsaw University, Warsaw.
- Rodgers R.J. (1982) Delayed effects of naloxone on responsiveness to environmental novelty in rats. *Psychopharmacology* 78: 230-233.
- Rodgers R.J., Deacon R.M.J. (1979) Effect of naloxone on the behavior of rats exposed to a novel environment. *Psychopharmacol.* 65: 103-105.
- Turnbull B.A., Hill D.L., Miller L.H., McElroy J., Feldman R.S. (1983) Effect of high doses of naloxone on shuttle avoidance acquisition in rats. *Pharmacol. Biochem. Behav.* 19: 423-426.

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