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Naloxone impairs spatial performance in rats

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Short communication

Abstract. Naloxone-injected (1.0 mg/kg) and saline-injected control rats were subjected to a two-trial test of object localization memory. In trial I rats were allowed to explore for 5 min an enclosed T-maze with an object (plastic bottle) placed in one maze arm. Then, the object was removed and after a 20-min retention interval rats were faced with two empty arms of the same maze (trial II). Control rats showed good retention of the place occupied by the object, displaying a significant preference (74%, $P<0.032$) for the arm which previously contained the object. Naloxone treated rats responded at chance levels (53%). An accurate performance in this task is normally based on information provided by spatial cues outside the maze (cognitive map), so that a random performance of Naloxone treated rats could supposedly be related to some disorders in the internal representation of the environment.

Key words: spatial memory, cognitive map, exploration, naloxone, rat

There is ample evidence that endogenous opioids are capable of modulating memory processes. Effects exerted by naloxone, an opioid antagonist, have been investigated in a variety of testing procedures with sometimes conflicting results. Naloxone facilitated retention of both inhibitory and active avoidance (Kapp and Gallagher 1978, Izquierdo 1979, Messing et al. 1979, Flood et al. 1987) and also improved non-aversive tasks like spatial learning and the habituation of rearing response (Izquierdo 1980, Gallagher et al. 1983, Fanelli et al. 1985). Naloxone had no effect on a visual discrimination test (Andrews and Holzman 1988) or within-session habituation (Koek 1984). In some studies (Izquierdo 1980, Turnbull et al. 1983) naloxone impaired shuttle avoidance acquisition. In our previous study (in preparation) no naloxone effect on object recognition was found, whereas in another experiment (Łukaszewska and Klepaczewska 1995) naloxone-injected rats failed to react to object displacement, which we interpreted as a failure in spatial attention.

The purpose of this experiment was to examine the effect of naloxone on the rats' reaction to removal of an object from the explored environment. The task described here is really a one-session task. It does not involve at all the learning of a rule since it is entirely based on a spontaneous behavior.

The animals used in the experiment were 40 naive male Wistar rats about 180 days old. They were group--housed in plastic cages and had free access to food and water. They were kept under 12:12 light/dark cycle (7 a.m. light on). The animals were habituated to handling by the experimenter.

Experiments were carried out in a T-maze with white painted arms. T-maze stem was 30 cm long and 11.3 cm wide; the arms were 30 cm long and 11.3 cm wide, and the walls were 29 cm high. A video camera above the apparatus was connected to a video recorder and a monitor. The testing environment was homogenous in terms of proximal stimuli but structured in terms of distal stimuli.

The test was preceded by examination of directional preferences under no drug conditions in the same T-maze and thus the animals were familiarized with the apparatus before the test. To determine directional preference three trials were performed, separated by 2 h intervals. The choice of the same arm 2 or 3 times was considered as a preference for the respective direction.

The test consisted of two trials - exposure (trial I) and choice (trial II), separated by a 20-min interval.

Trial I: in the exposure trial lasting 5 min, a new object was placed in one arm; it was a plastic dark-blue bottle (ϕ 6.3 cm, 21.5 cm high). Rats could walk the maze and freely explore its stem and both arms. For each rat, an object was placed in the arm opposite to the directional preferences as determined on the previous day.

During the interval between trial I and trial II the rat was temporarily removed from the maze. The object was removed from the maze arm.

Trial II: after the 20-min break the rat was reintroduced into the maze and faced with a choice between two visually alike empty arms. The arm that the animal entered with all four feet was considered its choice. After the choice the rat could walk the maze arms during 1 min.

The apparatus was cleaned before testing the next rat. One test only was performed on a given rat.

Rats were randomly divided into Naloxone and Control groups. Rats in the Naloxone group received injections of 1 mg/kg naloxone in saline solution. The dose of 1 mg/kg of naloxone was selected because this dose was used in a majority of studies on spatial performance in rats. The Control group received matched volumes of saline. Rats were injected intraperitoneally, 20-min prior to test.

TABLE I

Behavioral measures during 5-min exploration of a T-maze. One arm contained an object

Group	Time spent in arm (in s) with object	Time spent in arm (in s) empty	Number of shifts between the arms	Object exploration (in s)
Control	52.1	39.2	11.9	19.3
Naloxone	54.7	59.2	12.5	19.6

Data are means. No significant difference was observed between the data of the Control and the Naloxone groups.

In the exposure trial (trial I) the groups did not differ significantly in any measure during the 5-min trial (Table I). Both groups spent the same amount of time in the maze arms showing no significant preference for the arm containing an object. Time spent on object exploration was the same in either group. The number of shifts between the maze arms reflecting motor activity did not differ between the groups either. It could be concluded, therefore, that 1 mg/kg naloxone did not influence the exploratory or motor activity in the experimental situation of trial I.

In trial II rats faced the two identical maze arms, both empty now because the object had been removed. Control rats detected the change and showed significant preference (74%, $P<0.032$, binomial test) of choices of the arm which previously contained the object. Such a detection implies that internal representation of the T-maze with an object in one arm was formed in trial I and compared with the actual perception. In other words, Control rats noticed the absence of the object and could recall its previous position. The discrepancy between the memorized and present situation inclined them to investigate the place of change (Berlyne 1950).

The Naloxone rats responded in trial II at chance levels showing 53% of choices of the maze arm which contained an object in trial I. Table II presents the behavior of the Naloxone and Control groups, during 1 min after the choice. Subgroups of rats selecting the arm from which an object was removed (emptied arm) we denoted by "+", the subgroups selecting the arm which was empty in both trials we denoted as "-". The choice latency of the Naloxone group was significantly lower (5.8 s) than that of the Control group - 15.0 s ($P<0.002$, Mann-Whitney, two-tailed). The choice latency did not depend on the kind of arm choice, since latencies did not significantly differ between subgroup "+" and subgroup "-" either in the Control or in the Naloxone group. Rats from each group and subgroup were persistent in their choice, since they spent more time in the initially chosen arm. Wilcoxon test indicated significant differences (see Table II), except control "-" subgroup which could not be analysed by Wilcoxon test because of a too small number of subjects. The longer time spent in the initially chosen arm could not be attributed to passive stay in that arm. Rats shifted between the maze arms and the stem, entering either arm several times. Again, the number of visits was significantly higher (see Table II) in the arm of the first choice in each subgroup of rats. This clear preference to one arm may indicate that rats were aware of the lack of the object and displayed searching activity. Control rats reacted according to their correct internal representation. The retrieval of the relevant information after long retention interval is not an easy task, so that the Control rats hesitate while choosing the arm, which resulted in a considerable choice latency. Naloxone rats presumably search also for an object but they made false guesses as to the place from where an object was removed. It is supposed that the failure could be attributed to some defects of their cognitive maps. This might cause a random retrieval of information about the position of the object (left arm, right arm) and a random performance: short latencies of the arm choices could be

TABLE II

The behavior of rats which selected the emptied (+) or empty (-) maze arm during 1 min after the choice

Subgroups	Choice latency	Time spent in "+"arm		"-"arm	Number of visits in "+"arm		"-"arm
Control +	15.1	19.4	$P<0.001$	3.2	1.7	$P<0.005$	0.6
	NS						
Control -	14.8	4.8	x	14.8	0.6	x	1.6
Naloxone +	8.7	18.7	$P<0.005$	4.4	1.8	$P<0.05$	0.7
	NS						
Naloxone -	2.25	10.4	$P<0.01$	25.5	1.0	$P<0.01$	2.4

Time is given in seconds; "+" and "-" denotes of maze arms which in trial I contained and did not contain an object, respectively (see text); x, small number of subjects in this subgroup prevents Wilcoxon statistic.

a consequence of a random selection. An alternative interpretation, e.g. a more rapid decay of memory trace, is also possible. However, our previous finding that naloxone-treated rats did not react to object displacement (Łukaszewska and Klepaczewska 1995) supports the notion of the impairment of their cognitive maps. Therefore, we are in favour of this hypothesis.

The result of this experiment showing an inferior performance of naloxone-treated rats in a spatial task which requires an intact cognitive map, is at variance with the findings of Galagher et al. (1983) and Fanelli et al. (1985) showing naloxone-facilitated learning in an eight-arm radial maze. It is possible that such a discrepancy is related to different problems being investigated. In the experiments of Gallagher et al. (1983) and Fanelli et al. (1985) a transfer of radial maze from one room to another after the four choices caused an interference between the retention of the original environmental map and the gradual construction of the new one. Presumably, this was the main factor responsible for slowing down the learning of normal rats. If one assumes that naloxone impairs the cognitive map of the environment in the first room the drug administered after four choices would remove the impediment in performance in further choices in the another room. The effectiveness of naloxone is short-lasting (Messing et al. 1979), then the drug would have no effect on the rats' performance after 6 h delay.

The present experiment does not offer the advantage for Naloxone rats from the impairment of spatial representation, because the task consists of a single test which requires the retrieval of information from an intact cognitive map. Therefore any imperfection of the cognitive map would impair performance.

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