*Pharmacology Biochemistry & Behavior*, Vol. 23, pp. 199–202, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

# Differential Effect of Naloxone on Food and Self-Stimulation Rewarded Acquisition of a Behavioral Response Pattern

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VAN WOLFSWINKEL, L. AND J. M. VAN REE. *Differential effect of naloxone on food and self-stimulation rewarded acquisition of a behavioral response pattern.* PHARMACOL BIOCHEM BEHAV 23(2) 199–202, 1985.—The involvement of endogenous opioids in self-stimulation reward was investigated by repeated administration of the opioid antagonist naloxone to rats during acquisition of a behavioral response pattern that was rewarded with electrical (self-)stimulation of the ventral tegmental area. A control experiment was performed using food deprived rats in which a comparable response pattern was rewarded with food pellets. The response patterns consisted of gradually decreasing amounts of reward per response, which could be reset to maximal reward by another response. It was found that naloxone disrupted the acquisition of the stimulation rewarded response pattern, while it did not influence the food rewarded behavior. It is suggested that endorphin systems are actively involved in the acquisition of self-stimulation reward procedures, and that this involvement may be specific for self-stimulation reward.

Acquisition Food reward Naloxone Opioids Self-stimulation

OPIOIDS have since long been known to possess rewarding properties which appear to be comparable, at least in part, to the reward of electrical stimulation [12]. The discovery of endogenous peptides with opiate-like rewarding activities (endorphins) [1,9] has stimulated the investigation of the function of endorphins in brain stimulation reward. However, experiments using the opioid receptor antagonist naloxone have revealed that self-stimulation is still feasible even in the presence of high doses of this drug [2,7]. Previous experiments in rats with electrodes in the ventral tegmental area have indicated that the effect of naloxone on self-stimulation thresholds is diminished when these rats have been used for other self-stimulation experiments before, while the administration of morphine induces a greater threshold change as compared to naive animals [11]. This suggests that the function of endorphin systems may change, depending on the experience with or the time spent on self-stimulation.

The present experiment was designed to investigate the effects of naloxone in rats that had very little experience with self-stimulation. We used a relatively high dose of naloxone to block most opioid receptor subtypes [13]. The drug was given to animals while in the acquisition phase of a behavioral paradigm which had been used to investigate response rate independent self-stimulation threshold changes in previous experiments. During acquisition the animal is on "threshold behavior" as far as the new behavioral response is concerned. Since it is known, e.g. from avoidance behavior tests, that endorphins affect acquisition of behavior [4, 5, 8], an experiment was performed to control for effects on the acquisition process *per se*. In this test the acquisition of a behavioral paradigm, that was comparable to the self-stimulation procedure, was reinforced with food pellets. It was found that naloxone blocked the acquisition of self-stimulation behavior but not that of the food rewarded behavior.

## METHOD

For the experiments 34 male Wistar rats from our own breeding stock were used. They weighed 200–250 grams at the beginning of the experiments. They were individually housed in transparent cages under standard conditions. Half of the rats were used for the self-stimulation experiments, the others for the food reward tests. Tap water was continuously available and laboratory food either ad lib (self-stimulation group) or restricted (food reward group).

### *Apparatus*

All experiments were conducted in an experimental cage that was made from aluminium with a Plexiglas front door (Campden Instruments Ltd. U.K., model 410). Two side by side levers protruded into the cage, 6 cm above the stainless steel grid floor. Between the levers a food dispenser was present which could be illuminated. Above the levers a white lamp was mounted. The experiments were controlled by 24 V electromechanical equipment connected to a DEC PDP8 computer.

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*Self-Stimulation*

For self-stimulation a spring shielded lead connected by a mercury swivel was present which could be fixed to the electrode on the skull of the animal. The stimulator (Janssen Scientific Instruments, Belgium, type ST) produced stimulus trains of 500 msec duration, with bipolar pulses at a frequency of 100 Hz. Pulse duration and interval between the positive and the negative pulse were 0.5 msec. Bipolar twisted stainless steel electrodes were made from 200 $\mu$m wire insulated except at the cross section at the tip. The electrodes were aimed at the ventral tegmental area (coordinates according to De Groot (see [6]) were A: 2.4, D: −3.6, L: 1.0). The operation was performed under Hypnorm® anaesthesia using standard stereotaxic procedures. The electrode was fixed to the skull with acrylic dental cement. The rats were allowed to recover from the operation for at least one week.

The rats were trained to work for stimulation in a reset procedure, in which the first response on the right-hand lever was rewarded with a high current (maximal current), and this current was decreased by 5% of the maximal current after every response. The current could be reset to maximal by a response on the left-hand lever. During stimulation the lamp above the right lever was off. The lamp above the reset (left) lever was off only after a reset response until the first right lever response was made. The training procedure was preceded by one to three pretraining sessions. The rat was placed in the box and a response on either lever was rewarded with maximal current. The current was set individually for each rat by the experimentator according to the behavior of the rat. The behavior was shaped until 5 consecutive lever presses were made without interference of the experimentator. When this occurred, the session ended and the rat was not used until the first training session. The rats that did not perform these 5 responses in one of the 3 pretraining sessions were not used for further experimentation. Training started on the day after the 3rd pretraining session. The rats were randomly assigned into two groups. Injections were given subcutaneously in a volume of 1 ml/kg, 10 minutes before each training session. One group was injected with naloxone, 10 mg/kg, the other group with vehicle (saline). All injections were administered blind to the experimentator. The rats were trained for 15 minutes daily. During the training procedure both levers could be pressed. The first 100 stimulus trains could be obtained by responding on any lever. Thereafter, for another 100 stimuli, one lever was rewarded and the rat was taught to switch from one lever to the other after every 10 responses. Then, 10 rewarded responses on the right lever had to be followed by one stimulation-rewarded response on the left lever for a total of 100 responses. For another 100 stimulations, the left lever was not rewarded with stimulation but had to be pressed once to make another 10 maximal current stimulus trains available on the right lever. Eventually, training was continued using the reset procedure as described above. Each new session started on the level that the rats had been able to complete the session before. The experimentator could optimize the individual maximal currents whenever needed in his view. The criterion for acquiring the behavioral pattern was: Within a session the rat had to reset the current 5 times by one to three responses on the reset lever at each occasion of reset, and each reset had to be followed by responding for stimulation.

After completion of the experiments the rats were decapitated, their brains quickly removed from the skulls and stored on 10% formalin. The location of the electrode was verified in thionin stained frozen sections of 100 $\mu$m thickness.

*Food Reward*

The rats were food deprived to 80% of their initial body weight and kept on this weight by giving them food supplements several hours after the experimental session. The reset procedure that the rats were trained for was as follows: the rats had to press a lever for 45 mg food pellets on a ratio that started with FR:5 and that was multiplied by a factor of 1.2 after every reward, and thus increased gradually to a maximum ratio of FR:20. The ratio could be reset to FR:5 by a response on the reset lever (left lever). The light above the right lever was on until food was available, then the light in the food dispenser was on. When the food dispenser was emptied, the dispenser lamp went off and the lamp above the lever was turned on. The lamp above the left lever was on when the ratio was higher than FR:5 and thus could be reset.

The sessions lasted 15 minutes. First, the non-treated food-deprived rats were placed in the box and food was available on a FR:1 schedule. As soon as 5 responses were made without intervention by the experimentator in one of the three pretraining sessions, that session ended and the rats were not tested further until the first training session. The rats were randomly assigned into two groups after pretraining and they were injected with either naloxone or saline 10 minutes before each daily session. Treatment was performed blindly. The ratio was gradually increased to FR:20. Only one lever was rewarded, the levers being alternated every day. When the rat had reached the FR:20 schedule, the ratio was set to FR:5 and pressing the right lever was rewarded with food while the left could be used to reset the ratio to FR:5. The criterion for acquisition of the reset procedure was: The rat had to reset the ratio 5 times within one session by pressing the reset lever one to three times, every reset being followed by responding on the right lever and consumption of the food pellet.

*Statistics*

The differences in numbers of the placebo and naloxone treated rats that passed the criterion on the training days were analysed using Fishers exact test.

## RESULTS

*Histology*

The location of the electrode tips of the self-stimulation acquisition group is shown in Fig. 1. The electrodes were in or close to the ventral tegmental area. The electrode locations of the naloxone and the control groups were not different.

*Self-Stimulation*

Figure 2A shows the percentage of animals from each treatment group that reached the criterion of the self-stimulation procedure, for every treatment day, starting with the first day of the treatment. On day 2 about 35% of the rats appeared to reach the criterion. On subsequent days the naloxone treated animals performed worse than the saline treated rats. Their performance seems to decrease after the 2nd day. This deficit was still present on the 5th day (Fig. 2A) and remained till the end of the observation period (the 8th day). The rats that did not learn the procedure did re-

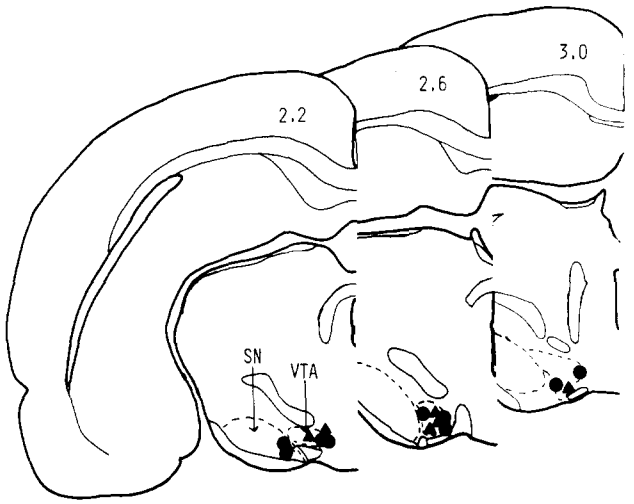


FIG. 1. Histologic examination of the site of the tips of the electrodes in the self-stimulation acquisition experiment. Sections have been redrawn from Pellegrino *et al.* [6]. ●=Naloxone treated rats; ▲=saline treated rats.

spond for a high current, but refused to press the reset lever. Apart from the difference in acquisition, no other differences in behavior were obvious. Two experiments were done with an interval of several weeks. No differences in outcome with respect to effects of naloxone were observed between the two experiments and the data were therefore combined.

*Food Reward*

The effects of naloxone on food rewarded behavior are shown in Fig. 2B. Two experiments were done, several weeks apart. Because of a slight difference in the start but not in the pattern of acquisition among the two experiments, which was consistently present in both the naloxone and placebo treated groups, and in order to make comparisons with the self-stimulation acquisition the data were combined by taking as day two for each experiment the first day on which the first saline treated rat passed the criterion (about on the 5th day of treatment). No difference in acquisition rate between saline and naloxone treated rats was observed. Like in the self-stimulation experiment, treatments in the food reward experiments did not induce behavioral changes to an extent that the experimentator could distinguish between naloxone and placebo treated rats.

## DISCUSSION

Acquisition of a complex behavior that is rewarded with electrical stimulation of the ventral tegmental area appears to be disrupted when training is performed in the presence of naloxone. This suggests an involvement of endorphins in either the acquisition of the new behavioral pattern or in the reward of self-stimulation. Since naloxone had no effect at all on the acquisition of a comparable behavioral procedure with food as reinforcer, opioid antagonism may attenuate the reward of self-stimulation rather than the acquisition of the behavioral response *per se*. The two procedures are comparable, in that during responding on the rewarded lever the amount of work for a given amount of reinforcement increases and that this work can be attenuated by a reset response. Differences between the two procedures are, however, present as well. Self-stimulation reward has unique properties in that the stimulation follows the response immediately and is rewarding in non-deprived animals. Moreover, the reward of self-stimulation probably lasts not much longer than the stimulation and, as it is related to current intensity it can easily be modified. In contrast, between lever pressing and actual food reward some time elapses, and to obtain a reasonable high response rate on a FR:1 schedule even a small food pellet is too large. As can be inferred from the number of sessions needed to reach the criterion, the rats acquired the food rewarded schedule somewhat slower than the self-stimulation schedule. But these differences can hardly explain the differential effect of naloxone on self-stimulation and food rewarded acquisition of lever pressing behavior. The influence of naloxone on self-stimulation as found in the present experiment is more pronounced than that observed in previous experiments by ourselves and others [2,10]. In some reports, however, a greater suppression of response rate by naloxone has been described [1,3]. This differential effect of naloxone may be due to the degree of experience of the rats with self-stimulation.

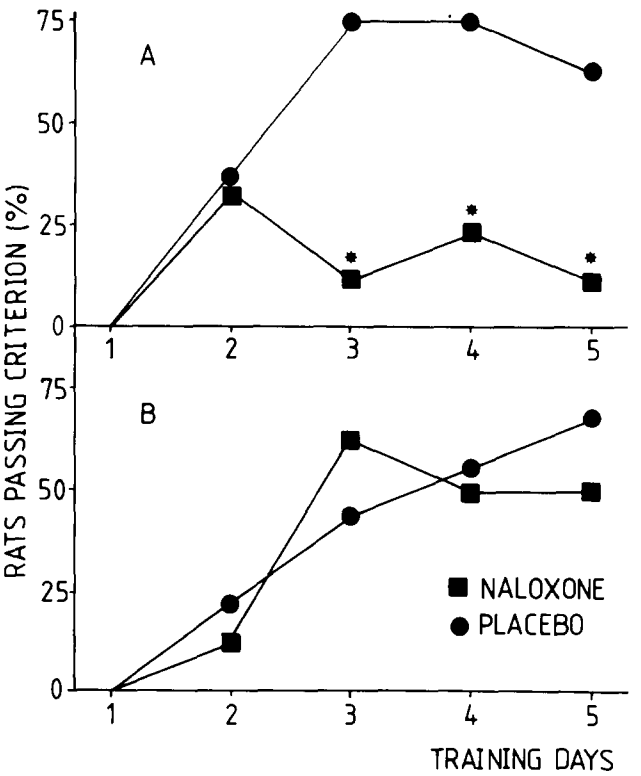


FIG. 2. Acquisition of a response pattern rewarded with either self-stimulation (A) or food (B). Shown is the percentage of rats from each group that passed the preset criterion on the training days. The rats were treated with naloxone (10 mg/kg) or placebo before every training session. In A (self-stimulation) treatment started on day one, in B (food reward) day 2 is the first day on which the first placebo treated rat passed the criterion. Number of animals in both experiments: naloxone: 9; placebo: 8. *$p<0.05$, Fishers exact test.

The present findings support the idea that the endorphin systems are involved in self-stimulation particularly in the phase in which self-stimulation behavior is acquired. In this learning period endorphin systems seem to be more concerned in self-stimulation reward than in food rewarded be-

havior. In view of the similarities between brain stimulation reward and drug self-administration [12] it is tempting to speculate that endorphins are predominantly active during the initial stage of addiction.

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