

*Short communication*

INTRAHYPOTHALAMIC MICROINJECTIONS OF NORADRENALINE  
WITH AND WITHOUT INDUCTION OF THE ALIMENTARY DRIVE  
AS A REWARD IN A T MAZE LEARNING IN RATS

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*Abstract.* Noradrenaline injected into the perifornical region of the anterior part of the lateral hypothalamus in rats can serve as a reinforcement in a T maze learning, whether it induces the activation of the alimentary drive or not. It follows that the main role of noradrenaline in this brain area consists not so much in induction of the alimentary drive as in activation of the very process which is essential for the reinforcement. It is claimed that intrahypothalamic microinjections of noradrenaline evoke pleasurable states which constitute a virtual reinforcement of motivated behavior.

In our recent paper (8) we have found that noradrenaline (NA) injected into the perifornical region of the anterior part of the lateral hypothalamus (LH) evokes an eating response and can serve as a reinforcement in a T maze learning. However, it was found that in about 30% of cases NA injected into this region does not produce an eating response (17). Therefore, it was interesting to investigate whether these intrahypothalamic microinjections of NA, which do not induce activation of the alimentary drive, have reinforcing properties for motivated behavior or not. If they do, it would follow that NA when injected into this region not so much induces the alimentary drive as rather exerts reinforcing action. The aim of this paper was to solve this problem. For this purpose the reinforcing properties of two categories

of intrahypothalamic NA microinjections, inducing and noninducing the activation of the alimentary drive, were tested in a T maze situation.

A large group of male hooded rats weighing 250–300 g was stereotaxically implanted with chronic bilateral cannulas aimed at the perifornical region of the anterior part of the lateral hypothalamus (LH). Implantation was performed under Nembutal anesthesia (20 mg/kg) according to the procedure described in detail by Slangen and Miller (29). The rats were held in the stereotaxic apparatus with the tooth bar 3.1 mm above the intra-aural line, and the following coordinates were used: 0.0 mm (bregma) anterior, 1.3 mm lateral to the center of the saggital sinus, and 8.2 mm down from the surface of the skull. This part of the brain is known to elicit a reliable eating response to NA microinjections (3, 4, 16, 29) in sated rats.

After one week recovery period the effect of NA microinjections was tested with respect to eating response. NA was given in 0.5  $\mu$ l injections containing 60 nmoles (21  $\mu$ g) of l-noradrenaline bitartrate (Chemie Linz Ag, Austria) dissolved in normal saline. For proper experiment ten rats were chosen: five which reacted to NA microinjections with a marked eating response (eaters), and five which did not eat after NA microinjections (non-eaters). Experimental sessions were conducted daily on food and water sated animals, always at the same time (10 a.m.).

Before the experiment the eating responses to unilateral NA or saline microinjections were tested twice for each cannula in a counter-balanced sequence once a day. During this period as well as later during the chemostimulation in a T maze situation, free access to water and chow pellets was given for 45 min after injections, and the chow intake was then measured.

After completion of cannula testing all rats were given ten choices in a T maze, one a day, in order to find their spontaneous preference for one of the two arms of the maze. Then, the chemostimulation experiment began which lasted 15 or 16 days. Each choice of the previously nonpreferred side of the T maze was rewarded with an NA microinjection to the perifornical LH, while choosing of the other arm was followed with a saline microinjection through the same cannula, both being delivered as soon as the animal reached the end of the maze.

For a statistical analysis of the results Student's *t*-test for independent samples was applied.

Figure 1 shows an eating response to intrahypothalamic NA microinjections. Only the group of eaters reacted to this injection with a marked eating response of  $2.00 \pm 0.22$  g. Microinjections of saline in

this group gave no eating response or a negligible one, amounting to  $0.03 \pm 0.02$  g ( $P < 0.001$ ). In the group of non-eaters NA microinjections were ineffective in eliciting an eating response ( $0.05 \pm 0.03$  g), similarly as saline microinjections ( $0.18 \pm 0.11$  g). In this group the effects of these two kinds of microinjections did not differ statistically, while the difference between eaters and non-eaters in their eating response to NA microinjections was statistically highly significant ( $P < 0.001$ ). With the passage of time eating responses to NA diminished slightly, and at the end of the experiment they even differed statistically when compared to the effects of first microinjections ( $P < 0.02$ ). However, the difference between effects of NA and saline microinjections remained statistically highly significant ( $P < 0.001$ ) till the last day of the experiment.

In the T maze experiment, during the ten days when rats had free but unrewarded choice of the arm of the maze, the spontaneous preference of one of two arms established itself gradually, and finally on the last day none of the rats of both groups chose the nonpreferred side. Starting from the day, in the second phase of the experiment, when each of rats incidentally turned towards the nonpreferred side and received an intrahypothalamic NA microinjection, they chose this particular side more and more frequently with each succeeding day, and

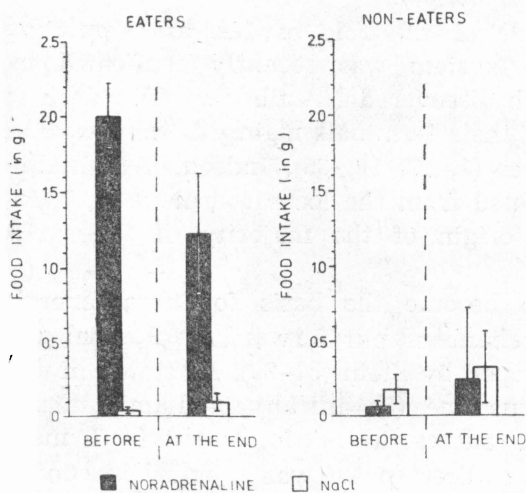


Fig. 1. Effect of noradrenergic stimulation of the hypothalamus on food intake before and at the end of T maze experiment (mean  $\pm$  SE,  $n = 5$ ).

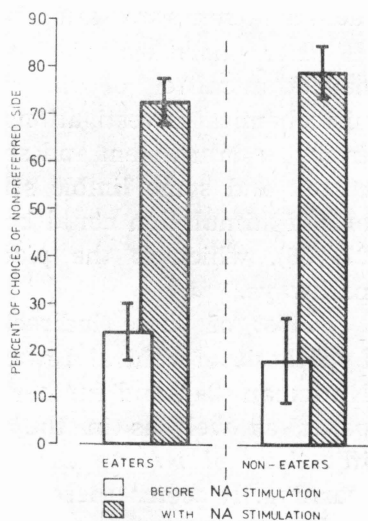


Fig. 2. Reversal of preference in T maze induced by noradrenergic stimulation of the hypothalamus as a reward for choosing the nonpreferred side (mean  $\pm$  SE,  $n = 5$ ).

finally on the last day of the experiment all of them chose the previously nonpreferred side. It occurred that this change of preference was irrespective of eating response to NA and the same in both groups.

Figure 2 summarizes the results of the experiment. When uninfluenced by intrahypothalamic NA stimulation, the eaters chose the nonpreferred side of the T maze in  $24 \pm 6\%$  of trials and the non-eaters in  $18 \pm 9\%$ . With NA chemostimulation as a reward this side was chosen as many as  $73 \pm 5\%$  by eaters, and  $79 \pm 6\%$  by non-eaters. Differences in the T maze performance of both groups due to application of intrahypothalamic NA microinjection as a reward were highly significant ( $P < 0.001$ ). Both groups, eaters and non-eaters, behaved identically in this respect and did not differ statistically between themselves.

A number of indirect data showing that catecholamines can function as neurotransmitters of the reward system have been gathered for the last two decades. Drugs such as amphetamine (30, 34), cocaine (6) or inhibitors of monoamine oxidase (25) that potentiate transmission of catecholamines, facilitate self-stimulation, whereas drugs that inhibit catecholamine transmission such as chlorpromazine (20, 21, 34) or  $\alpha$ -methyl-p-tyrosine (26), suppress self-stimulation. These data were supported by findings which pointed to NA as a neurotransmitter of the reward system: it was found that intraventricular injections of NA facilitated self-stimulation behavior (22, 36).

The pharmacological evidence of the role of NA as a putative neurotransmitter of the reward system was recently confirmed by histochemical investigations which discovered NA fiber systems derived from the brain stem and sending their terminals mainly to the cerebral cortex and some limbic structures (1, 12, 18, 35). Indeed, a high rate of self-stimulation could be obtained from the locus coeruleus (2, 7, 11, 27, 28), which is the place of origin of the majority of these NA pathways.

Most of these indirect data become the basis for noradrenergic hypothesis of central reward mechanisms put forward by Poschel and Ninteman (24), and further developed by Stein (31-33). As stated in this paper as well as in the previous one (8), intrahypothalamic microinjections of NA appear to be a successful reinforcement in T maze learning. It seems therefore that a direct proof was supplied in confirmation that NA acts as a neurotransmitter of the reward system, independently of its alimentary drive activating action which was found earlier (3, 4, 13-16, 19, 29). In our opinion NA may act as a neurochemical substrate of the process of hedonesthesia, i.e., the central nervous process which determines hedonic value of stimuli depending on a given state of need (10). Perhaps NA acts as a neurotransmitter

which shifts the hedonic value of stimuli towards pleasure and evokes pleasurable states, which according to the hedonic theory (5, 9, 23, 37) constitute a virtual reinforcement of motivated behavior. Due to this action NA may function as a neurotransmitter of the reward system, which according to Cytawa and Trojnar (10) should rather be called the pleasure system of the brain.

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