24. G. L. Fel'dman, G. M. Fedorenko, and V. N. Gusatinskii, "Study of the topography and level of protein synthesis in large pyramidal neurons during sleep and arousal," Tsitologiya, 21, No. 4, 429-433 (1979).
25. V. N. Chernigovskii, Habituation in Visceral Systems," Izd. Nauka, Leningrad (1980).
26. J. Eccles, The Physiology of Synapses, Academic Press (1964).
27. R. Davies and R. Keynes, "A coupled sodium-potassium pump," in: Membrane Transport and Metabolism, Academic Press, New York (1961), pp. 336-340.
28. E. Evarts, "Activity of individual cerebral neurons during sleep and arousal," in: Sleep and Altered State of Consciousness, Proc. Sleep Assoc., Vol. 45, Baltimore (1967), pp. 319-338.

AVOIDANCE CONDITIONING IN RATS FOLLOWING CHANGES IN ACTIVITY OF THE NORADRENERGIC AND CHOLINERGIC SYSTEMS OF THE BRAIN

N. N. Martynov and O. A. Bessonova

UDC 612.821+612.814

Bilateral coagulation of the ventral noradrenergic pathways of the brain in male rats of the Wistar strain disrupted the conditioned passive avoidance response after unconditioned stimulation with a current of 0.75 mA. Injecting 10 mg/kg galantamine into these rats 20 min before training and increasing the unconditioned stimulus to 3 mA improved subsequent avoidance responses. Injecting galantamine under analogous conditions into normal rats impaired this reaction. Disruption of the conditioned avoidance response following the operation may be due to a change in the intracentral interrelation of the noradrenergic and cholinergic systems of the brain.

KEY WORDS: ventral noradrenergic pathways; noradrenergic and cholinergic systems of the brain; conditioned reaction of passive avoidance.

Data are available at present on the involvement of the central cholinergic structures in emotional "fright" responses during a conditioned avoidance stimulus [6, 8]. Thus, when small doses of anticholinesterase preparations are administered, the development and production of conditioned avoidance responses improves [6, 12], and the emotional "fright" response intensifies [6, 10]. A number of papers demonstrated that anticholinergic preparations suppress the conditioned avoidance response more effectively than substances that block the action of the noradrenergic system of the brain [6, 8, 9]. In this connection a hypothesis was formulated about the primary significance of the cholinergic systems of the brain in conditioned avoidance responses [6, 7].

We also have data on the essential role of central noradrenergic systems in the formation and manifestation of the conditioned emotional response of "fright" [4, 14]. Some authors believe that it is specifically the activation of the noradrenergic systems of the brain that is most significant for the formation of negative emotional reactions [4]. Meanwhile, papers have appeared recently which note that the central noradrenergic system may participate in regulating the sensitivity of the cholinergic neurons of the brain to various influences [2, 7, 19]. In this light, analysis of the interaction of central noraderenergic and cholinergic systems in the process of learning avoidance reactions is of great significance.

The purpose of the present work was to elucidate the participation of central cholinergic systems in the conditioned avoidance responses of rats with selective noradrenergic denervation of diencephalon structures.

Laboratory of Functional Neuromorphology, Institute of Physiology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Fiziologicheskii Zhurnal SSSR imeni I. M. Sechenova, Vol. 68, No. 3, pp. 297-302, March, 1982. Original article submitted April 8, 1981.

 0097-0549/84/1405-0368$08.50

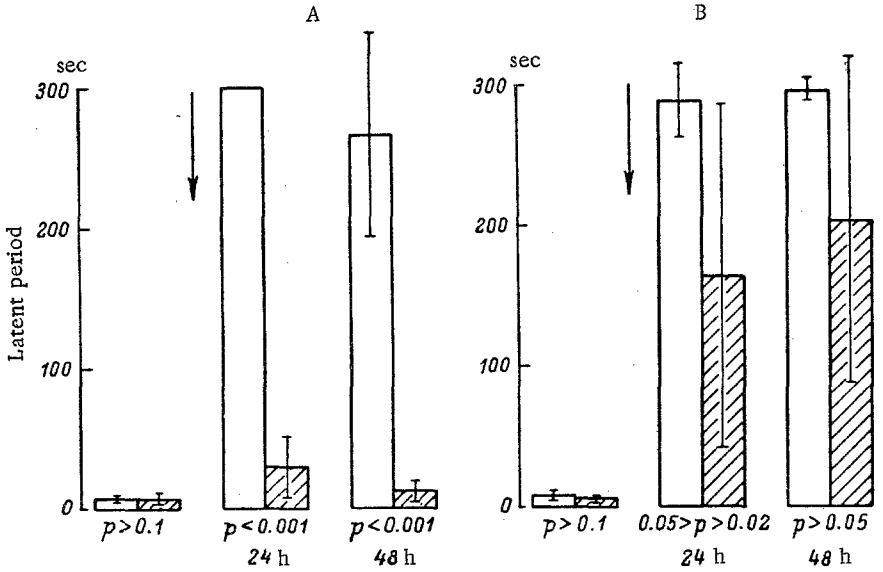

Fig. 1. Conditioned response of passive avoidance after coagulation of ventral noradrenergic pathways following training with different parameters of the current. A) Training with a 0.75 mA current; B) training with a 3 mA current. Open blocks — nonoperated animals; cross-hatched blocks — operated animals

METHODS

The experiments involved 97 male rats of the Wistar strain, weighing 150-200 g. Coagulation of the ventral noradrenergic pathways was done under Nembutal narcosis (40 mg/kg, intraabdominally) in a stereotaxic apparatus along coordinates of the atlas of monoaminergic pathways of the rat brain [20]: A = 1020, L = 1.6, H = 1.4, with a constant current of 1 mA for 10 sec. The group of pseudooperated animals consisted of rats that had electrodes inserted bilaterally along the coordinates of the ventral noradrenergic pathways, but with no current applied. In 12-14 days after the operation, the rats were trained in the conditioned response of passive avoidance. The control was animals that had not been operated on. Development of the conditioned response took place in an experimental chamber divided into light and dark halves with an opening between [17]. For 2 days before training the animals were placed in the experimental chamber daily for 5 min. Training began on the third day: The animals were given a shock in the dark half of the chamber. Immediately after the rat went into the safe, light section, it was taken from the chamber; the training procedure ended here. In some experiments training was done with a current of 0.75 mA, in others, with 3 mA. Testing the retention of the response was done 24 and 48 h after training.

When the experiments were completed, the animals were killed, and the brain was dissected out and fixed in 10% formalin for further morphological examination of the coagulation area. In processing the results, we used only the rats with complete bilateral coagulation of the ventral noradrenergic pathways.

To affect the activity of the central cholinergic systems, we used the following pharmacological preparations: galantamine, a blocker of acetylcholinesterase (10 mg/kg), which increased the level of acetylcholine in the brain, and scopolamine, a blocker of M-cholinoreceptors (30 mg/kg) [5, 6]. The preparations were administered intraperitoneally 20 min before training.

Statistical processing of the result was done according to Student's criterion.

RESULTS AND DISCUSSION

These experiments demonstrated that when the animals were placed into the experimental chamber before training, the control, pseudooperated rats, and operated rats exhibited no substantial difference in the latent period of entering the dark section.

In animals with bilateral coagulation of the ventral noradrenergic pathways, we found a significant disturbance of the conditioned avoidance response in testing 24 to 48 h after training

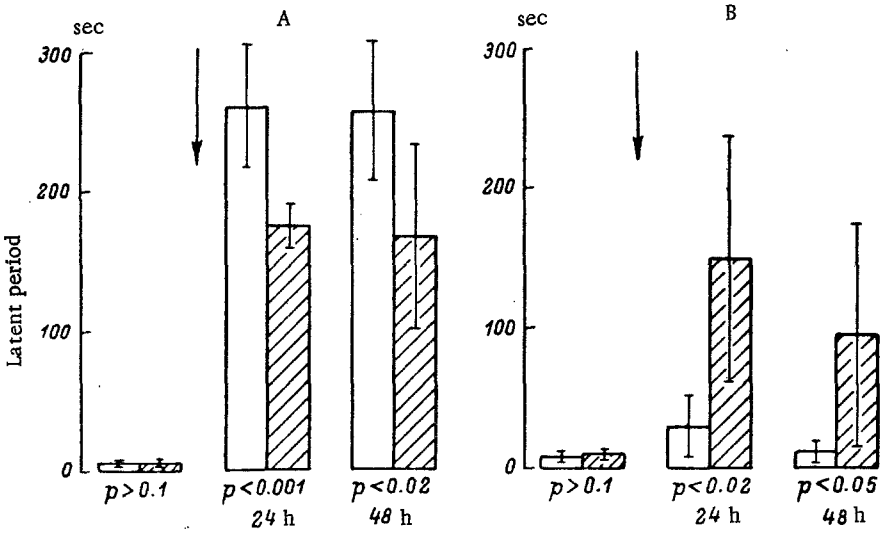

Fig. 2. Effect of galantamine on conditioned avoidance response in nonoperated and operated animals following training with a 0.75 mA current. A) Nonoperated animals; B) animals with coagulation of the ventral noradrenargic pathways. Open blocks — training without galantamine; cross-hatched blocks — training with galantamine (10 mg/kg, intraperitoneally 20 min before training).

with an 0.75 mA current. Thus, if the latent period of entering the dark, "dangerous" section of the chamber rose sharply in the nonoperated rats, in the operated animals, this index remained practically unchanged (Fig. 1A). It must be noted that behavior of the pseudooperated rats in the experimental chamber after training was the same as that of the nonoperated animals.

In subsequent experiments we established that in the operated rats, the capacity for the conditioned avoidance response was not completely lost. We demonstrated that when the stronger unconditioned stimulus (3 mA current) was used during training, there was a substantial improvement of subsequent reproduction of the conditioned passive avoidance response in animals with coagulation of the ventral noradrenergic pathways (Fig. 1B).

Disruption of the conditioned avoidance response, observed in operated rats after training with weak current, was evidently not connected with a change in sensitivity to the current. Some evidence for this is the presence of a distinct motor reaction to the unconditioned stimulus in the animals with lesion of the ventral noradrenergic pathways.

The observed changes in the conditioned avoidance response are probably not connected with a disruption of motor activity either. Actually the operated animals and the control animals certainly did not differ in motor activity when tested in the experimental chamber during the familiarization period. It must also be noted that the operated rats were able to carry out the conditioned passive avoidance response after training with a stronger current (3 mA).

As we demonstrated earlier [1], in 12-14 days after lesion of the ventral noradrenergic pathways, there is a definite decrease in the level of fluorescence of noradrenalin in the denervated structures of the diencephalon. It is known that during this period there is a more complete degeneration of the transected axons of the noradrenergic neurons of the brain [13, 18]. In this connection, it is possible that in rats with a deficit of central noradrenalin, improvement in the conditioned avoidance response after a strong unconditioned stimulus is due to the more effective activation of other chemoreactive systems. From data in the literature on the substantial contribution of the central cholinergic system to learning processes, including the avoidance response [6, 8, 15, 16], we proposed that central cholinergic structures may play a significant role in producing the effect we observed.

To detect participation of the cholinergic system of the brain in conditioned avoidance responses after coagulation of the ventral noradrenergic pathways, we compared the effect of cho-

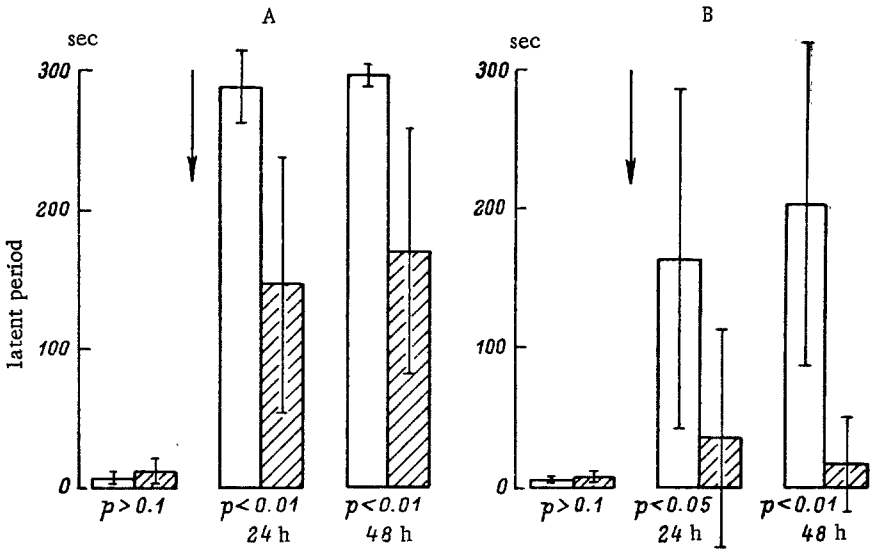

Fig. 3. Effect of scopolamine on the passive avoidance reaction in nonoperated and operated animals with training with a 3 mA current. A) Nonoperated animals; B) animals with coagulated ventral noradrenergic pathways. Open blocks — training without scopolamine; cross-hatched blocks — training with scopolamine (30 mg/kg, intraperitoneally 20 min before training).

linergic preparations on conditioned reflex activity of the nonoperated and operated rats. As the experiments indicated, if the nonoperated rats were preliminarily injected with galantamine, an anticholinesterase substance, in a dose of 10 mg/kg 20 min before training with a 0.75 mA current, there was a certain deterioration in subsequent reproduction of the conditioned passive avoidance response (Fig. 2A). On the other hand, after training with a weak current with the effect of galantamine, the operated rats showed a substantial increase in the latent period of entering the dark section of the experimental chamber as compared with the operated rats that were not injected with the preparation before training (Fig. 2B). Thus in experiments with the galantamine injections there was a difference in the effect of this preparation on the conditioned avoidance response of the nonoperated and operated animals.

The results of deterioration of avoidance response in the nonoperated rats after injection of galantamine in a dose of 10 mg/kg agree with data in the literature conserning the disruption of conditioned reflex activity resulting from large doses of anticholinesterase preparations [6, 12]. Some investigators believe that this effect may be linked to the overexcitation of the central cholinergic structures [3, 15].

It is probable that in the operated rats, the dose of galantamine used did not excessively activate the cholinergic structures of the brain, and as a result, as distinct from the nonoperated animals, there was no disruption of the conditioned avoidance response. The results presented are a basis for proposing that after coagulation of the ventral noradrenergic pathways, there is evidently a change in the functional activity not only of the noradrenergic, but also of the cholinergic system of the brain.

This hypothesis is confirmed by data from experiments with blocking of M-cholinoreceptors with scopolamine. In this case also the preparation injected had a different effect in groups of operated and nonoperated rats on subsequent reproduction of a conditioned passive avoidance response. Thus, injection of 30 mg/kg scopolamine 20 min before training with a 3 mA current adversely affected the production of a conditioned passive avoidance response in control animals and in operated animals, but in operated rats, these disruptions were expressed much more strongly (Fig. 3A, B). These results agree with data of N. V. Orlova [11] on changes in effect of scopolamine on training rats after disruption of the noradrenergic neurons of the macula cerulea.

Thus our results indicate that the noradrenergic component of the structures of the diencephalon participates in the conditioned avoidance response. But disruption of the conditioned passive avoidance response observed after bilateral coagulation of the ventral noradrenergic pathways does not seem to be very deep since its development improves substantially

with a change in the conditions of developing this reaction (a stronger unconditioned stimulus, activation of the cholinergic system). Significant differences in effect of cholinergic preparations on conditioned avoidance response in nonoperated and operated rats indicate that after noradrenergic denervation of structures of the diencephalon there is a change in the functional activity not only of noradrenergic, but also of the cholinergic systems of the brain. These results obviously can serve to confirm the hypothesis concerning the regulatory role of noradrenargic mediation with respect to the cholinergic neuronal system.

LITERATURE CITED

1. O. A. Bessonova and N. N. Martynov, "Change in fluorescence of central noradrenalin in conditioned avoidance response at different times after coagulation of ventral noradrenergic pathways of the brain," Neirofiziologiya, 13, 4, 418-420 (1981).
2. A. Yu. Budantsev, "Functional interaction of monoaminergic and cholinergic innervation of the limbic system. (Morphological model)." in: Neurochemistry and Physiology of Synaptic Processes [in Russian], Pushchino (1976), pp. 86-100.
3. S. N. Gulikov, M. A. Razumova, and A. T. Selivanova, "The mechanism of disruption of higher nervous activity due to anticholinesterase substances," Farmakol. Toksikol., 31, 2, 145-148 (1968).
4. E. A. Gromova, Emotional Memory and Its Mechanisms [in Russian], Nauka, Moscow (1980) p. 181.
5. R. Yu. Il'yuchenok, Neurohumoral Mechanisms of the Reticular Formation of the Brain Stem [in Russian], Nauka, Moscow (1965), p. 258.
6. R. Yu. Il'yuchenok, Pharmacology of Behavior and Memory [in Russian], Nauka, Novosibirsk (1972), p. 222.
7. R. I. Kruglikov, "Certain neurochemical mechanisms of the process of consolidation," in: Gagarsk Discussions, 7th Conference, 1978, [in Russian], Tbilisi (1979) 7, 498-507.
8. R. I. Kruglikov and G. M. Dolganov, "Effect of scopolamine on formation, preservation, and reproduction of temporary connections," Zh. Vyssh. Nervn. Deyat., 22, 4, 837-842. (1972).
9. R. I. Kruglikov and N. V. Orlova, "Effect of decrease in content of noradrenalin in the brain on formation and fixation of temporary connections," Zh. Vyssh. Nerv. Deyat., 26, 1, 120-126 (1976).
10. M. D. Mashkovskii and R. Yu. Il'yuchenok, "The problem of effect of galantamine on the central nervous system," Zh. Nevropatol. Psikhiat., 61, 2, 166-175 (1961).
11. N. V. Orlova, "Interaction of noradrenergic and cholinergic mechanisms of the brain in the processes of formation and fixation of temporary connections," in: Memory and Trace Processes [in Russian], Proceedings of the 4th All-Union Conference, Pushchino (1979), p. 58.
12. A. T. Selivanova, Effect of Cholinergic Substances on Higher Nervous Activity [in Russian], Meditsina, Leningrad (1969), p. 215.
13. N.-E, Andern, K. Fuxe, and K. Larsson, "Effect of large mesencephalic-diencephalic lesions on the noradrenaline, dopamine, and 5-hydroxytryptamine neurons of the central nervous system," Experientia (Basel), 22, 12, 842-843 (1966).
14. T. J. Crow and S. Wendlandt, "Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6-OH-dopamine," Nature, 259, 5338, 42-44 (1976).
15. A. J. Deutsch, "The cholinergic synapse and the site of memory," Science, 174, 4011, 788-791 (1971).
16. S. D. Glick, T. W. Mittag, and J. P. Green, "Central cholinergic correlates of impaired learning," Neuropharmacology, 12, 4, 291-296 (1973).
17. M. E. Jarvik and R. Kopp, "An improved one-trial passive avoidance learning situation," Psychol. Rep., 21, 2, 221-224 (1967).
18. R. J. Moore and A. Heller, "Monoamine levels and neuronal degeneration in rat brain following lateral hypothalamic lesions," J. Pharmacol. Exp. Ther., 156, 1, 12-24 (1967).
19. J. Nagy and L. Decsi, "Transmitter background of an emotional reaction of the cat," Activ. Nerv. Super., 19, 2, 122-123 (1977).
20. U. Ungerstedt, "Stereotaxic mapping of the monoamine pathways in the rat brain," Acta Physiol. Scand., Suppl. 367, 1-48 (1971).