DIFFERENCES IN EFFECTS OF 6-HYDROXYDOPAMINE ON LEARNING IN RATS WHEN INJECTED INTRAVENTRICULARLY AND LOCALLY INTO INDIVIDUAL NUCLEI OF THE BRAIN CATECHOLAMINERGIC SYSTEM

E. A. Gromova, T. P. Semenova, and N. I. Grishchenko

UDC 612.821.1/.3-06:616.822.1.014.46:612.452.019

KEY WORDS: 6-hydroxydopamine; learning; emotions; reinforcement.

In previous investigations the writers discovered some particular features of the role of the brain catecholaminergic systems in learning by animals with emotionally different reinforcement [1, 4, 11]. However, effects of factors acting on noradrenergic and dopaminergic systems were not differentiated in these experiments.

The distinguishing between these effects in the regulation of learning and memory processes, which could be achieved with the aid of local injections of the neurotoxin 6-hydroxydopamine (6-OHDA), was the aim of the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 72 male Wistar rats weighing 200-230 g. In the experiments of series I the characteristics of the animals' learning pattern were studied when pain was used as reinforcement, whereas in series II food was used for this purpose.

The model of learning with pain reinforcement that was chosen was the conditioned active avoidance reflex (CAAR) of nociceptive stimulation by an electric shock applied through a grid in the floor of the experimental chamber. The criterion of learning was a 70% level of performance of the reflex by the rats 2 days running.

The model of learning with food reinforcement chosen was the conditioned reflex of bilateral alternation of visits (CRBAV) to the place where food was obtained [3].

The reflexes mentioned above were formed in the same experimental chamber. In both cases the conditioned response consisted of leaving the starting compartment to visit one of the two platforms of the experimental chamber, fixed to its side walls. The stability of the skill produced was tested in experiments involving its extinction and subsequent restoration. From 10 to 20 days before training, 6-OHDA (from Sigma, USA) was injected into various parts of the animals' brain, where it induced degeneration of catecholaminergic terminals and fibers [6, 7, 9]. The compound was dissolved in physiological saline with the addition of 0.1% ascorbic acid in the cold immediately before injection into each animal. The solution was injected by means of a microsyringe through steel cannulas implanted into the animals' brain 3-4 days before injection of the compound, using stereotaxic coordinates [8]. To differentiate between the role of the individual catecholaminergic systems in training of the animals, 6-OHDA was injected into the lateral ventricles (blocking all the brain catecholaminergic systems), into the region of the locus coeruleus (blocking the coerulo-cortical adrenergic bundle), and into the substantia nigra — the main source of the dopaminergic innervation of the forebrain. The animals were divided correspondingly into three experimental groups. The rats of group 1 received 6-OHDA in a dose of 150 μg/10 μl into each ventricle. 6-OHDA was injected into the animals of group 2 in the region of the locus coeruleus (A_6 according to Dahlstrom and Fuxe [5]) in a dose of 10 μg/2 μl into each locus. 6-OHDA was injected into the substantia nigra (A_9) of the animals of group 3 bilaterally in the same dose. The rate

Laboratory of Neurotransmitter Systems, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 6-8, August, 1983. Original article submitted January 12, 1982.

0007-4888/83/9608-1033$07.50

TABLE 1. Effect of Injection of 6-OHDA into Different Brain Regions on Training of Rats in CRBAV to the Site of Food Reinforcement

Experimental conditions	Number of rats in group	Number of visits achieving criterion of learning	Preservation of skill during extinction	
			latent period, sec	level of presence of response
Control	11	72.7	17.3	71.0%
Injection of 6-OHDA				
Into lateral ventricles	9	300.0**	—	—
Into region A_6	5	33.0*	16.8	75.0%
Into region A_9	6	65.0	22.6*	55.2%

Note. Significance of difference between data by Mann–Whitney U test: *P < 0.01; **P < 0.001.

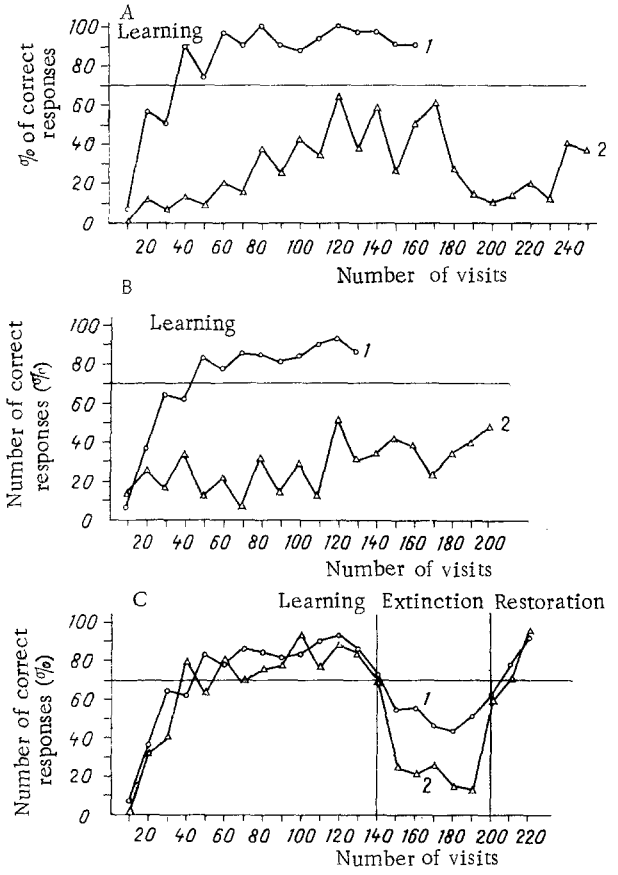

Fig. 1. Time course of formation, extinction, and restoration of CAAR in rats after injection of 6-OHDA into different brain regions. A) 6-OHDA (150 µg/10 µl) injected into lateral ventricles, B) 6-OHDA (10 µg/2 ml) injected into locus coeruleus (A_6), C) 6-OHDA (10 µg/µl) injected into substantia nigra (A_9). Abscissa, number of visits; ordinate, number of correct responses in 10 visits (in %). 1) Control, 2) rats receiving 6-OHDA. A, B) Significance of differences between control and experiment by the signs test at the P < 0.001 level.

of injection of the compound in all cases was 2 μl/min. The operation of implanting the direction cannulas, through which equivalent volumes of physiological saline with the addition of ascorbic acid were injected, was performed on control animals. The results of all the experiments were subjected to statistical analysis by the Mann–Whitney test and the signs test [2].

EXPERIMENTAL RESULTS

The results of the experiments of series I, reflecting the character of CAAR formation in rats receiving injections of 6-OHDA into different parts of the brain are given in Fig. 1.

Analysis of the course of the curves shows that injury to the brain catecholaminergic system as a whole or to its noradrenergic part made the formation of CAAR impossible in the rats (Fig. 1A, B), despite the animals' making 200-250 visits in each case. Control animals achieved the criterion of learning after 40 visits. The observations showed that animals of both experimental groups were exposed to electric shocks for 4.5-6.5 times longer than the control rats. The number of avoidance reflexes in these animals during the first 100 visits was three times less, and the latent period was 2-2.5 times longer than in the control.

By contrast, destruction of the region containing dopaminergic neurons did not affect the process of CAAR formation, but the resistance of the rats' skill to extinction was significantly reduced (Fig. 1C).

The results of the experiments of series I thus showed that selective injury to noradrenergic or catecholaminergic neurons in the brain disturbed the formation of responses to painful reinforcement, whereas destruction of dopaminergic neurons, which had no effect on the formation of this response, significantly worsened its preservation.

Data on the effect of 6-OHDA on learning in rats with food reinforcement (experiments of series II) are given in Table 1. Intraventricular injection of 6-OHDA prevented CRBAV formation. The animals did not reach the criterion of learning even after 300 visits, whereas this reflex was formed in the control animals on average after 72.7 visits. Selective injection of 6-OHDA into nuclei of the noradrenergic system, on the other hand, caused the response to be formed three times faster, without affecting its resistance to extinction. By contrast, in rats receiving injections of 6-OHDA into a region of concentration of dopaminergic neurons (A_9), despite the earlier achievement of the criterion of learning, the response formed in the experiments with extinction was significantly less stable.

Comparison of the data showing the effect of the different methods of injection of 6-OHDA on learning in rats with emotionally different reinforcement thus revealed a dissimilarity in its effects. Combined injury to ascending noradrenergic and dopaminergic projection systems due to injection of 6-OHDA into the lateral ventricles sharply disturbed the animals' learning process irrespective of the emotional sign of the reinforcement used.

Local injection of 6-OHDA into region A_9 had no effect on skill formation but severely impaired their preservation, likewise independently of the emotional sign of the reinforcement. Conversely, injection of 6-OHDA into region A_6 made CAAR formation more difficult, but facilitated CRBAV formation.

These results as a whole are in good agreement with those of the study of effects of blockers of catecholamine synthesis — α-methyltyrosine and disulfiram — on learning in animals [4] and they confirm the view regarding reciprocity of functional relations between the serotoninergic and catecholaminergic brain systems [1]. By using local action of 6-OHDA on noradrenergic and dopaminergic structures it was thus shown that this reciprocity characterizes interaction of the serotoninergic system not with the brain catecholaminergic system as a whole, but with its noradrenergic component.

LITERATURE CITED

1. E. A. Gromova, Emotional Memory and Its Mechanisms [in Russian], Moscow (1980).
2. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria to Evaluate Differences between Two Groups of Observations in Medical and Biological Research [in Russian], Moscow (1969).
3. T. P. Semenova, Zh. Vyssh. Nerv. Deyat., 26, 1322 (1976).
4. T. N. Semenova and N. L. Vekshina, Byull. Éksp. Biol. Med., No. 6, 643 (1978).
5. A. Dahlstrom and K. Fuxe, Acta Physiol. Scand., 232, Suppl. 1 (1964).

6. L. Descarries and G. Saucier, Brain Res., 37, 310 (1972).
7. L. Descarries, A. Beaudet, and J. De Champlain, in: Chemical Tools in Catecholamine Research, 1, 101 (1975).
8. E. Fifkova and J. Maršala, in: Electrophysiological Methods in Biological Research, New York (1967), p. 653.
9. J. C. Hedreen, Brain Res., 157, 129 (1978).
10. J. C. Hedreen and J. P. Chalmers, Brain Res., 47, 1 (1972).
11. E. A. Gromova, in: Akademie der Wissenschaften der DDR. Abhandlungen, Berlin, No. 5, 269 (1979).

STEPPING MOVEMENTS INDUCED IN CATS BY STIMULATION OF THE DORSOLATERAL FUNICULUS OF THE SPINAL CORD

O. V. Kazennikov, M. L. Shik, and G. V. Yakovleva

UDC 612.766-06:612.832-063

KEY WORDS: spinal cord; dorsolateral funiculus; locomotion.

Locomotion can be induced in the mesencephalic cat by stimulating the locomotor region in the brain stem [6, 7, 9, 12]. For spinal stepping generators to be activated under these circumstances, the ventrolateral funiculi must be intact, whereas destruction of the dorsolateral funiculus (DLF) at the C1 level does not prevent the effect [13]. In the turtle, however, swimming movements can be induced by stimulation of DLS [8, 14]. Sherrington observed stepping movements of the ipsilateral hind limb in the decapitated cat in response to stimulation of DLF at the level of spinal cord section [10, 11]. In the curarized, precollicularly decerebrated cat, after division of the spinal cord in the lower thoracic region, stimulation of DLF or of the lateral funiculus at the C3 level induces rhythmic discharges in the nerves to the muscles of the ipsilateral forelimb ("fictitious stepping"). After hemisection of the spinal cord rostrally to the site of stimulation the discharges continued, although they became irregular [15]. DLF and the lateral funiculus in the cat, just as in the turtle, thus contain fibers whose stimulation can activate limb stepping generators. Under these experimental conditions, however, it was impossible to determine whether the generator is activated directly by the stimulated fibers or through propriospinal neurons.

In the present investigation stepping movements of the hind limb were induced by stimulation of DLF in an uncurarized mesencephalic cat. With this technique stimulation of DLF remains effective even after injury to it caudally (at the L1 level) and rostrally (at the C2 level) to the site of stimulation.

EXPERIMENTAL METHOD

After precollicular postmammillary decerebration [6] one or two laminectomies were performed at different levels from C1 to L2. The animal's head was fixed in a stereotaxic apparatus, and the spine was fixed in two places. The cat's limbs were resting on the belt of a treadmill. By means of a manipulator, a tungsten electrode in glass insulation, 50 μ in diameter, was inserted into the dorsolateral funiculus. Monopolar stimulation was carried out with square pulses of negative polarity, 0.4 msec in duration, with a frequency of 60 sec^{-1}, and with a strength of 5-20 μA. Destruction in the region of the effective point was carried out by passing a steady current of 200 μA through the same electrode for 3 min, but if only labeling was required, a current of 10 μA was passed for 2 min. After fixation of the spinal cord in formalin transverse sections were cut to a thickness of 60 μ on a microtome with freezing stage, and after clearing with glycerin, they were photographed.

Laboratory of Physiology of Movement, Institute of Problems in Information Transmission, Academy of Sciences of the USSR, Moscow. Department of Mathematical Methods in Biology, A. N. Belozerskii Interfaculty Research Laboratory, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 8-10, August, 1983. Original article submitted February 1, 1983.

 0007-4888/83/9608-1036$07.50