# Effect of Neuroleptic Haloperidol, L-DOPA Precursor Dopamine, and Neurotoxic Dopamine Analog 6-OHDA on Acquisition of Conditioned Defensive Reflex in Edible Snail

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The effect of neuroleptic haloperidol, a dopamine precursor L-DOPA, and dopamine analog 6-OHDA on the development of conditioned defensive reflex was studied in edible snails. Injection of L-DOPA to intact snails and to snails pretreated with 6-OHDA 2 h before learning session decelerated acquisition of the conditioned reflex and exerted a toxic action. In contrast, injection of 6-OHDA or haloperidol did not affect acquisition of the conditioned reflex.

**Key Words:** *dopamine; haloperidol; L-DOPA, 6-OHDA; conditioned defensive reflex*

Attention to the study of cerebral dopamine and the mechanisms of central dopaminergic transmission is related to their important role in motor, cognitive, and neuroendocrine functions and their possible involvement into pathogenesis of some neuropsychic disorders such as Alzheimer or Parkinson diseases and schizophrenia [7]. The dopaminergic system of the brain modulates various important cerebral functions including emotions and motivation; it is a system of reward and consolidation. In addition, the dopamine system is involved in learning and memory [1,10]. Changes in activity of the dopaminergic systems induced by stimulation and destruction of the cerebral structures or by the pharmacological agents selectively affecting this system pronouncedly modify behavior of the animals [12]. The most popular dopamine hypothesis relates the therapeutic effect of neuroleptics to their blocking action of the dopamine receptors and consequently, moderation of pathologically enhanced tone of the cerebral dopamine system [4,7]. Haloperidol (HAL) was reported to decrease the content of dopamine in the nervous tissue [8,12]. Amino acid tyrosine, a precursor of dopamine and other catecholamines, is produced during enzymatic hydroxylation of phenylalanine or is directly supplied with the food. Hydroxylation of tyrosine by hydroxylase yields L-3,4-dihydroxyphenylalanine (L-DOPA), which is therefore a biogenic substance and a precursor of dopamine. L-DOPA is routinely used in experiments and in clinical practice for increasing the content of dopamine in the organism [6]. Dopamine itself cannot be used to this end, because it poorly crosses the blood-brain barrier. In contrast, L-DOPA enters CNS where it is decarboxylated and transformed into dopamine. The latter is accumulated in the basal ganglia and stimulates dopamine receptors [6]. Pronounced achievements in the study of dopamine functions and its role in activity of the nervous system resulted from the use of 6-hydroxydopamine (6-OHDA), a neurotoxic analog of dopamine. This agent is accumulated in dopaminergic cells and selectively destroys dopamine elements in the nervous system [9].

The aim of this study was to examine the role of the dopaminergic system in the development of conditioned defensive reflex (CDR) in edible snail and to investigate the effects of neuroleptic HAL, dopamine

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precursor L-DOPA, and dopamine-depleting neurotoxin 6-OHDA on the process of learning. We also studied the mechanism of action of these substances, which exert pronounced effects on the organism and is widely used in modern medicine.

## MATERIALS AND METHODS

Mollusks are useful objects for physiological, pharmacological, and biological studies of general properties of neuronal structures. In our study, the experiments were performed on terrestrial snail *Helix lucorum* (*Pulmonata; Gastropoda*). The snails were active at least 2 weeks before the start of the experiments. They were kept in a terrarium under humid air at 18-22°C and *ad libitum* food supply. Apparently healthy and active snails of approximately equal weight (25 g) were randomized into groups. The described experimental conditions were maintained during all stages of the study. The control and experimental groups were kept either in common or in individual terrariums in the same room under identical environmental conditions. There were no differences in the data related to common or individual maintenance of the snails.

Solutions of neuroleptic HAL, dopamine-depleting neurotoxin 6-OHDA, or the dopamine precursor L-DOPA were daily injected into the internal space of the snail near the sinus node (0.1 ml). For all experimental groups, the controls were the intact snails or the snails receiving the same volume of physiological saline for edible snails containing (in mM): 80 NaCl, 4 KCl, 10 $CaCl_2$, 5 $MgCl_2$, 5 $NaHCO_3$ according to the same scheme. HAL solution (1 mg/kg) was injected daily 3 h before CDR training. Neurotoxin 6-OHDA was injected in a single dose (30 mg/kg) 5 days before the onset of conditioning. It was dissolved in 0.1 ml physiological saline for edible snail and supplemented with 0.1% ascorbic acid as the antioxidant. L-DOPA was injected daily 2 h before the onset of conditioning in concentrations of 0.2, 4, and 20 mg/kg.

Typical CDR in response to tapping on the shell was trained as described elsewhere [5]. The conditioned stimulus was tapping on the shell that produced no defensive response under normal conditions. Unconditioned stimulus was insufflations of air into the orifice of the pulmonary cavity, which provoked unconditioned defensive response of pneumostome closure. The reinforcing stimulation was applied at the end of the conditioned stimulus. Combined stimulation was presented with the interval of 2-4 min. This training resulted in complete closing of pneumostome in response to the conditioned stimulus, which was considered as the positive response. The reflex was considered as acquired if any of 30 conditioned stimuli induced complete closing of the breathing opening.

The results were processed statistically and presented as *M*±*SEM*. Significance was assessed by Student *t* and Mann–Whitney *U* tests.

## RESULTS

The role of dopamine during learning was examined by two routines: 1) injection of dopamine precursor L-DOPA and 2) injection of L-DOPA to the snails with depleted total dopamine in the nervous system produced by pretreatment with 6-OHDA. L-DOPA was injected daily 2 h before the start of conditioning in concentrations of 0.2, 4, and 20 mg/kg. After injection of L-DOPA, activity of the snails was reduced throughout the experiment; they consumed less amount of feed, did not open the pneumostome for a long time, did not stick to the pebbles, and did not came out from the shell. The muscle system of the snails was flaccid.

Injection of L-DOPA to experimental snails 2 h before the start of CDR training impaired learning. Control snails injected with 0.1 ml physiological saline demonstrated better results of learning compared to experimental animals. Control snails ($n$=8) completely acquired the conditioned reflex after presentation of 250 combined stimuli, while experimental snails injected with 0.2 mg/kg L-DOPA ($n$=8) demonstrated only 70% performance after presentation of 330 combined stimuli. Increasing the concentration of L-DOPA to 4 ($n$=8) and 20 mg/kg ($n$=8) insignificantly affected the results of conditioning: learning score reached the plateau at 60 and 55%, respectively (Fig. 1). The development of CDR in snails treated with 6-OHDA showed that injection of this neurotoxin 5 days before the onset of conditioning produced no effect on dynamics of CDR acquisition. The snails ($n$=8) attained

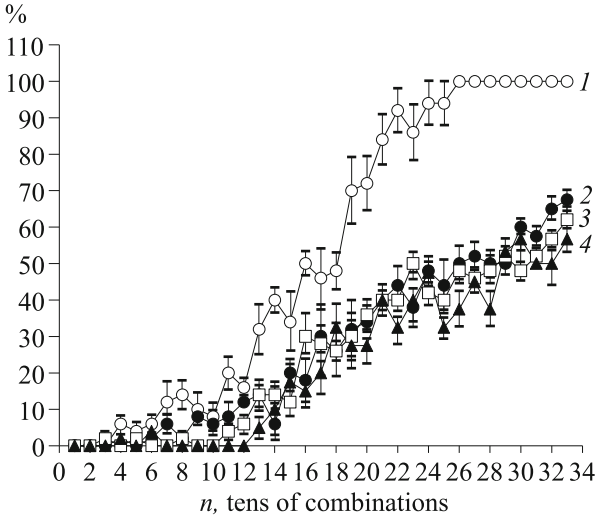


**Fig. 1**. Effect of L-DOPA on CDR conditioning in intact snails. *1*) physiological saline; *2*) 0.2 mg/kg L-DOPA; *3*) 4 mg/kg L-DOPA; *4*) 20 mg/kg L-DOPA.

learning criterion after 250 presentations of combined stimuli (Fig. 2). However, CDR did not develop in 6-OHDA-pretreated snails receiving injection of 0.2, 4, and 20 mg/kg L-DOPA. After injection of 0.2 mg/kg L-DOPA, the snails ($n$=8) demonstrated only 50% performance after presentation of 330 combined stimuli. After increasing the concentration of L-DOPA to 4 mg/kg ($n$=8), the learning curve did not exceed 10% performance. Further increase of L-DOPA concentration to 20 mg/kg ($n$=8) yielded 30% CDR performance (Fig. 2).

Thus, injection of L-DOPA (0.2, 4, and 20 mg/kg) to intact and 6-OHDA-pretreated snails 2 h before the start of conditioning decelerated acquisition of the conditioned reflex and produced a toxic effect. Injection of 6-OHDA alone produced no effect on acquisition of CDR. These data showed that a surplus of dopamine in the organism impedes learning while its deficiency produces no effect on acquisition of the conditioned reflex.

It is known that injection of a low dose of disulfiram or L-DOPA before passive avoidance conditioning in rats impaired learning, but improves retrieval of the memory trace on the next day. The corresponding studies showed that pharmacological up-regulation of the dopaminergic reinforcement system radically changes animal behavior and improves memory consolidation processes [2].

HAL was injected into the region of sinus node in a concentration of 1 mg/kg every day 3 h before the start of conditioning session. Chronic introduction of HAL induced no changes in the dynamics of acquisition of CDR. The experimental snails ($n$=10) were completely conditioned after 260 combined stimuli, while the control snails ($n$=10) became conditioned after 250 combined stimuli (Fig. 3).

The experiments on vertebrates showed that blockade of D2-receptors with HAL (0.5 mg/kg) 1 h before conditioning promoted long-term retrieval of conditioned reflex in submissive mice, while it rapidly decayed in the control mice. By contrast, the ability to maintain stable retrieval of memory trace was impaired if the animal were conditioned after HAL injection [3]. Numerous data indicates that HAL predominantly impaired acquisition and retention of the conditioned response in tests with aversive motivation [13]. However, HAL not always inhibited learning and retention of the memory trace. In some behavioral paradigms, HAL prevented experimentally induced memory deficiency [1,14]. It looks like HAL-induced improvement of memory was predominantly observed in cases, when it was initially disturbed. In these studies, the influence of initial behavioral strategy on the effect of HAL on conditioning was not taken into consideration. It is

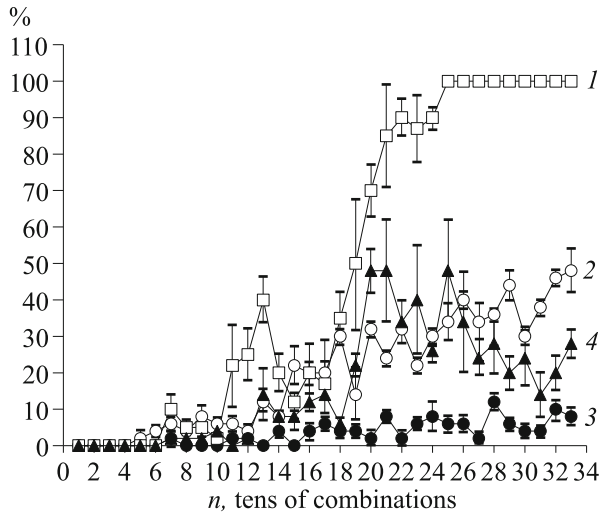


**Fig. 2**. Effect of L-DOPA CDR conditioning in snails pretreated with 6-OHDA. *1*) 6-OHDA+physiological saline; *2*) 6-OHDA+0.2 mg/kg L-DOPA; *3*) 6-OHDA+4 mg/kg L-DOPA; *4*) 6-OHDA+20 mg/kg L-DOPA.

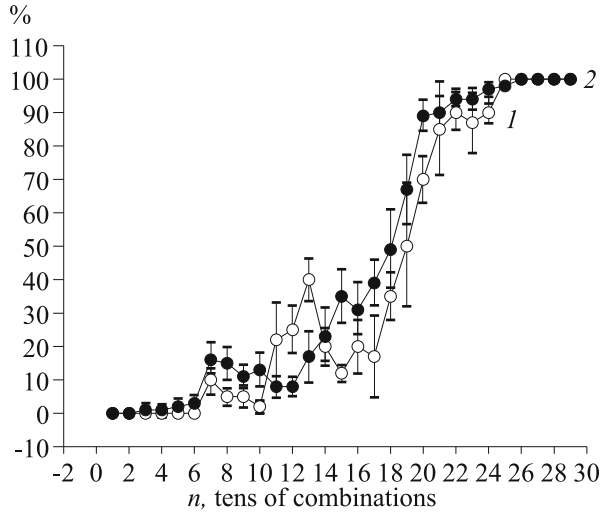


**Fig. 3**. Effect of injections of physiological saline (*1*) or HAL (*2*) CDR conditioning in snails.

known that the moderate level of anxiety promotes rapid memory trace formation and its long-term retention, while excessive anxiety results in memory impairment [13], which can be prevented by preliminary blockade of D2-receptors by HAL [11]. Thus, we showed that chronic administration of HAL does not modify the dynamics of conditioning.

There is no doubt that in order to realize cognitive activity, it is necessary to maintain a certain level of dopamine activity for optimal retrieval of the memory trace. This conclusion results from numerous data on the opposite effects exerted by the same neuropharmacological dopamine influences on learning [13,15]. In many cases, such diversity of the effects does not depend on the choice of preparations, its dosage, or

learning paradigm, but results from initial functional state of the whole organism determined by different levels of anxiety, emotional reactivity, and resistance to aversive stress factors [3].

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