# Effect of Chronic Haloperidol on Electrical Parameters of Command Neurons in *Helix Lucorum*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 248-251, September, 2003
Original article submitted March 13, 2003

We studied the effects of neuroleptic haloperidol on electric characteristics (resting potential, action potential threshold, and critical level of depolarization) of command neurons LPa3, RPa3, LPa2, and RPa2 responsible for defense behavior in edible snail *Helix Lucorum*. Chronic administration of haloperidol led to hyperpolarization shift of the membrane potential and increased the threshold of action potential in these neurons. The data indicate a new possible mechanism of sedative action of neuroleptics based on hyperpolarization of some cerebral neurons, in particular, neurons responsible for some behavioral functions.

**Key Words:** *dopamine; haloperidol; neuroleptics; membrane potential; threshold potential*

The mechanisms of neuropathological abnormalities are now intensively studied in modern biology. Localization of changes at the cellular level is the major question of these studies. This allows modeling of elementary mechanisms providing the basis of the formation and realization of behavioral responses [4,7]. An actual problem of psychopharmacology is elucidation of the mechanism underlying the effects of neuroleptics (NL). NL are grouped according to their chemical structure: phenothiazine derivatives (aminazine, trifluoperazine, thioridazine *etc*.), butyrophenone derivatives (haloperidol (HAL), spiperone, droperidol, *etc*.), and derivatives of thioxanthene, dibenzazepine, benzamide, aminotetralin, *etc*. [6,8]. NL are effectively used in clinical practice, but the mechanisms of their action remain unclear.

The effect of NL on the reticular formation of the medulla oblongata plays a key role in the central action of NL. However, the effects of NL are not related exclusively to this cerebral structure. Versatile effects of NL result from their action on generation and conduction of nerve signals in various elements of the central and peripheral nervous system [10,13]. Among neurochemical mechanisms of NL effects, the most studied is the action of NL on transmitter processes in the brain. The NL groups differ by their effect on the synthesis, storing, release, and utilization of neurotransmitters and their interaction with the receptors in various cerebral structures [6]. The most popular dopamine theory explains the therapeutic efficiency of HAL by blockade of dopamine receptors and decrease in pathologically enhanced tone of the cerebral dopaminergic system [9]. Another mechanism of NL action is based on the ability of NL to decrease dopamine content in the nervous tissue [14]. Most experiments with NL were performed on mollusks and annelids. Long-term application of HAL in experimental mollusks decreased both dopamine (markedly) and serotonin (transiently) levels, while chlorpromazine modulated primarily the content of serotonin. Recent data explain the therapeutic effect of NL by depletion of dopamine or serotonin stores in neurons [10]. This depletion caused by long-term administration of NL probably underlies correction of dopamine-serotonin balance in damaged cerebral regions.

Disturbance in the dopaminergic system in edible snail caused by a neurotoxin (6-hydroxydopamine) resulted in abnormal development of such form of plas-

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0007-4888/03/1363-0216$25.00 

ticity as long-term sensitization [1]. In addition, injection of 6-hydroxydopamine induced depolarization of command neurons. This suggests that this neurotoxin affects the dopaminergic system by modulating electrical parameters of some neurons. HAL also affects the dopamine system [6,9]. It was hypothesized that HAL can also affect parameters of electrical activity of some identified nervous cells. Our aim was to study electrophysiological effects of neuroleptic HAL, in particular, modulation of electrical activity of neurons (resting potential, threshold of action potential generation, and critical level of depolarization).

## MATERIALS AND METHODS

Experiments were carried out on edible snails *Helix Lucorum*. They were kept at room temperature in a glass terrarium with humid air and food *ad libitum*. The animals were active for at least two weeks before the experiment. HAL was daily injected in a dose of 1 mg/kg body weight for 7 days. Injections (0.1 ml) were performed with a syringe into the visceral cavity near the sinus node. The control snails received physiological saline according to the same protocol. Each group comprised 10 or 11 snails. On the next day after the last injection, electrical parameters of command neurons LPa3, RPa3, LPa2, and RPa2 responsible for defense behavior [2] were recorded. A total of 42 neurons were studied. Before isolation of CNS, the snails were cooled in ice-cold water for 15-30 min. The signals were recorded with intracellular glass microelectrodes with a resistance of 5-30 MΩ. The following parameters of electrical activity were measured: resting membrane potential $V_m$, threshold potential $V_t$, and critical level of depolarization ($E_c$). $E_c$ and $V_t$ were measured in evoked spike relatively to $V_m$. The biopotentials were fed into computer via a digitizer. The data were processed statistically using Student's *t* test and Mann—Whitney's *U* test.

## RESULTS

Since command neurons of *Helix Lucorum* are normally silent, action potentials were evoked by 1-sec rectangular current pulses delivered via the recording microelectrode (Fig. 1). The amplitude of the stimulation current was minimum for triggering spike and varied from 1.7 to 3.5 mA. Injection of HAL hyperpolarized LPa3, RPa3, LPa2, and RPa2 neurons. Seven injections of HAL shifted $V_m$ to -67.1±0.8 mV *vs.* -59.7±0.7 mV in control snails receiving physiological saline (Fig. 2); $V_t$ increased by about 2 mV (Fig. 2), while $E_c$ was shifted by about 6 mV (Fig. 3).

According to widely accepted dopamine hypothesis, the principal property of NL is their potency to decrease the efficiency of dopaminergic transmission via blockade of dopamine receptors. This hypothesis was tested in many behavioral, electrophysiological, and biochemical studies [6,9]. Previous studies showed that HAL increases the content of dopamine metabolites in the brain. It was hypothesized that NL blocked dopamine receptors, while enhanced dopamine metabolism reflected activation of dopamine synthesis in neurons as an adaptive response compensating the block of receptors [6]. D. A. Sakharov *et al.* describes a new mechanism of action of these agents: their ability to decrease the content of dopamine and/or serotonin in the nervous tissue [10,14]. It was established

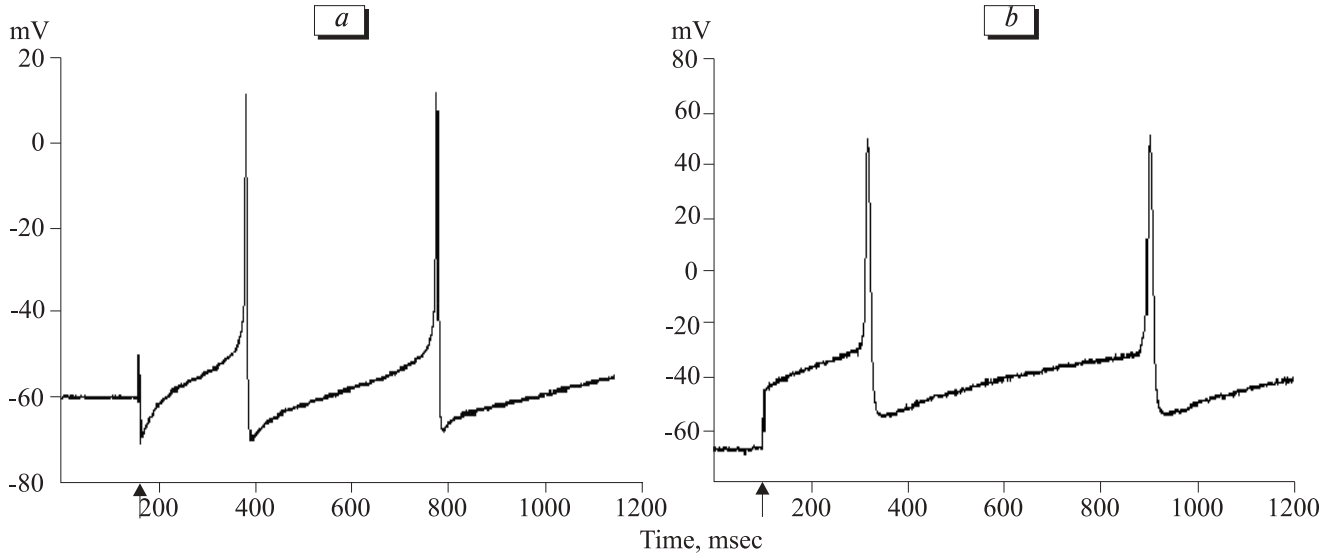


**Fig. 1**. Effect of haloperidol on activity of command neurons: *a*) intact snail (stimulation current 3 mA); *b*) experimental snail after 7 days of chronic haloperidol injections (stimulation current 2.2 mA). Arrow marks the onset of stimulation.

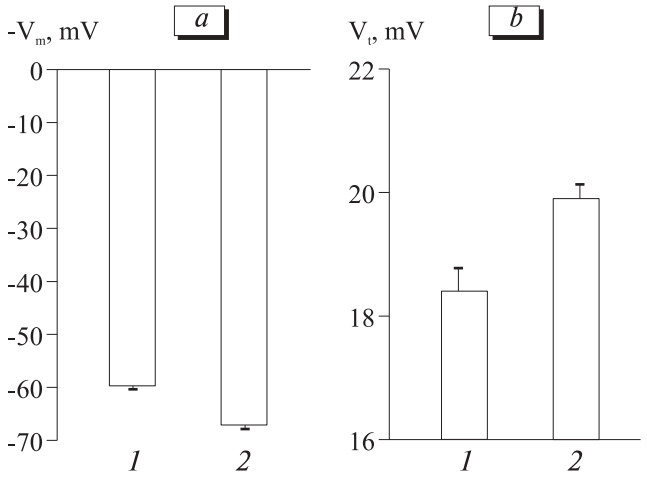


**Fig. 2**. Effect of haloperidol on resting potential ($V_m$, *a*) and threshold of spike generation ($V_t$, *b*) in defense command neurons LPa3, RPa3, LPa2, and RPa2 in *Helix Lucorum*. Here and in Fig. 3: measurements were made after 7-day administration of physiological saline (*1*) or haloperidol (1 mg/kg, *2*).

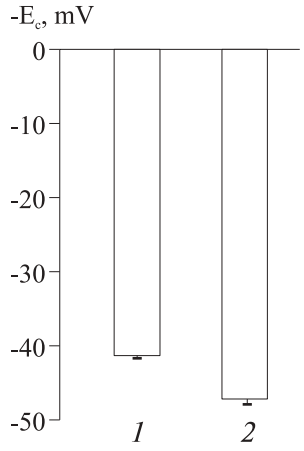


**Fig. 3**. Effect of haloperidol on critical level of depolarization ($E_c$) in defense command neurons of *Helix Lucorum*.

that motor disturbances caused by chronic administration of HAL were similar to those appearing under conditions of dopamine deficiency. It was shown that exposure of *Lymnaea Stagnalis* snail to high micromolar concentration of HAL markedly decreased dopamine level in the nervous system. It was found that the behavioral effect of HAL under certain conditions is explained by depletion of dopamine stores rather than by blockade of dopamine receptors [13]. Affinity binding of receptors with HAL and chlorpromazine can be explained by selective binding with neurons of different populations (chlorpromazine binds to 5-$HT_2$ serotonin receptors and HAL binds to $D_2$ dopamine receptors) [12]. The decrease in catecholamine level (dopamine included) after administration of amphetamine induces depolarization of defensive command neurons in edible snail [3].

Thus, chronic HAL induces hyperpolarizing shift in $V_m$ in defensive neurons of edible snail and increases the spike generation threshold. The absence of additivity in the changes of $V_m$ and $V_t$ attests to changes in $E_c$, the parameter resistant to many pharmacological preparations. The possibility of inhibitory action of NL is confirmed by decreased spike repetition rate under conditions of chronic administration of chlorpromazine [10]. There are data indicating the presence of dopaminergic cells in the nervous system of snails [5]. The observed neurotropic effects of HAL are probably mediated through inhibition of calcium, sodium, and potassium currents in the plasmalemma, as it was demonstrated in experiments with HAL-treated neuroblastoma cells [11].

These data suggest that the sedative effect of NL apart from other known mechanisms can be based on hyperpolarization of cerebral neurons, in particular, cells responsible for some behavioral functions.

The study was supported by the Russian Foundation for Basic Research (grant No. 00-04-48707) and Commission of Russian Academy of Sciences for Young Researchers (grant No. 289).

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