Neuroscience and Behavioral Physiology, Vol. 28, No. 3, 1998

ACTIVITY OF SUBSTANTIA NIGRA NEURONS IN THE CAT BRAIN DURING A SELF-INITIATED BEHAVIORAL ACT

V. G. Sidyakin, V. B. Pavlenko, A. M. Kulichenko, É. V. Gorelova, and O. M. Pavlenko

Cats were trained to perform a self-initiated behavioral act in the form of an operant food-obtaining reflex with defined time requirements. Activity was recorded from 50 dopaminergic neurons (identified in terms of their low frequency of background activity and long action potentials) and 67 nondopaminergic neurons of the substantia nigra and adjacent region. Dopaminergic neurons were the more responsive. Prior to EMG activation, the activity of 33 (66%) of these cells changed, and 44 (88%) showed changes in activity on movement. Dopaminergic neurons showed increased activity during the period of waiting for the conditioned stimulus, predicting the release of reinforcement or its absence. These cells were more frequently activated in response to a positive signal and reinforcement and were more frequently inhibited in the absence of reinforcement. The high reactivity of dopaminergic neurons during execution of a movement task could be explained by the involvement of a cognitive component, i.e., determining the point at which the movement should start.

Key words: *Substantia nigra, dopaminergic neurons, conditioning, voluntary movement.*

The substantia nigra of the midbrain, as well as its adjacent parts of the reticular formation and the ventral tegmentum is known to contain most of the dopaminergic neurons of the central nervous system (groups A8-A10). The axons of these cells innervate widespread regions of the hindbrain, which allows them to take active part in controlling the body's higher behavioral functions [1, 7]. Dopaminergic neurons of the monkey and cat midbrain respond to conditioned stimuli, to presentation of reinforcement, and to new, unexpected stimuli [8, 9, 15, 16]. They represent one of the most important branches of the control system for self-stimulation behavior [17], which indicates that they are involved in organizing the processes of attention and motivation.

The substantia nigra, forming part of the nigrostriatal system, is traditionally associated with sensorimotor integration and the control of voluntary movements [4, 7]. Studies of the overall electrical activity of the brain in the region of the substantia nigra have demonstrated development of slow negative waves of the readiness potential type over the period of 0.5 sec before a movement starts; these are among the highest-amplitude waves of all those recorded in subcortical structures and in various parts of the cortex [9]. At the same time, studies of the spike activity of neurons in this structure gave the opposite results. Thus, execution of a movement was associated with observation of nondopaminergic neurons of the reticular part of the substantia nigra in the monkey [14] and of unidentified cells in this structure in humans [2]. The activity of dopaminergic neurons in monkeys increased before the start of voluntary movements, but the process involved only a small proportion of these neurons. The response was tonic in nature and increased significantly only at the moment of achieving the desired aim [10, 13]. These studies, along with investigations of motor responses launched by conditioned stimuli, led to the hypothesis that dopaminergic neurons are associated not with voluntary movements but with stimuli signaling reinforcement expected on completion of a task [8, 15]. However, the activity of dopaminergic neurons has not been studied in conditions in which the

Department of Human and Animal Physiology and Biophysics, Simferopol' State University, 4 Yaltinskaya, 333036 Simferopol', Ukraine. Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 83, No. 1-2, pp. 28-34, January-February, 1997. Original article submitted September 19, 1996.

 0097-0549/98/2803-0238$20.00

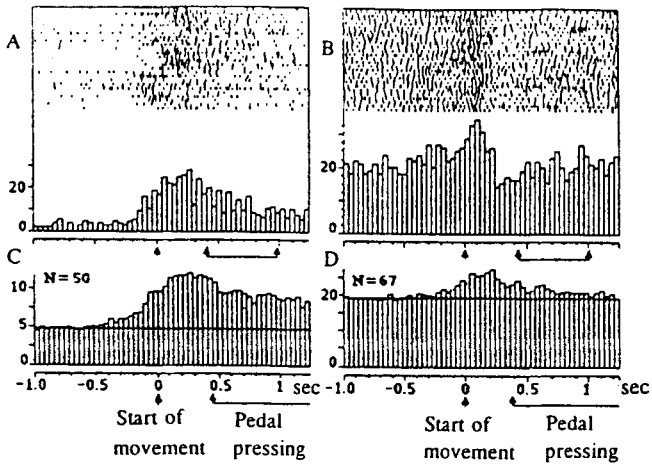

Fig. 1. Responses of dopaminergic (*A*, *C*) and nondopaminergic (*B*, *D*) neurons in the cat substantia nigra, centered on the moment at which movement started. *A*, *B*) Activity of individual neurons (above: sweep diagram; below: corresponding histogram); *C*, *D*) averaged normalized histograms for all cells in the populations. The abscissa shows time, sec; the ordinate shows the number of spikes per sec. Horizontal lines indicate initial activity levels. *N* is the number of neurons.

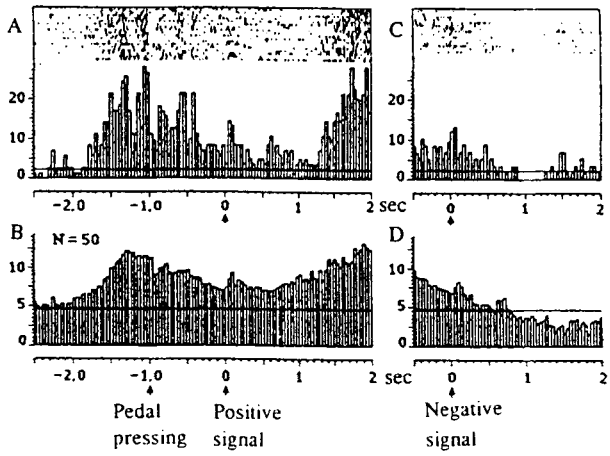

Fig. 2. Responses of cat substantia nigra dopaminergic neurons to positive (*A*, *B*) and negative (*C*, *D*) conditioned signals. *A*, *C*) Activity of a single (the same) neuron; *B*, *D*) activity of all cells in the population. For further explanation see caption to Fig. 1.

substantia nigra produces these high-amplitude potentials — in self-initiated purposive movements in which reinforcement depends on the success of executing the movements in a defined period of time [9].

Thus, the aim of the present work was to attempt to determine which dopaminergic neurons of the substantia nigra region of the cat are involved in the realization of self-initiated behavioral acts in the form of an operant conditioned reflex following defined time characteristics; additionally, we aimed to study the nature of the responses of dopaminergic and other neurons of this region during execution of a behavioral task with cognitive components.

METHODS

Experiments were performed using two adult cats trained to obtain reinforcement ('Viskas' granules) by lifting the right forepaw from a support platform and using it to press a pedal. Attempts were analyzed and reinforced only when the

TABLE 1. Activity of 50 Dopaminergic (Group 1) and 67 Nondopaminergic (Group 2) Cat Substantia Nigra Neurons at Different Stages of the Behavioral Act

Stage of the behavioral act	Activation		Inhibition	
	Group 1	Group 2	Group 1	Group 2
Preparation for movement	26(52.0)	26(38.8)	7(14.0)	9(13.4)
Execution of movement	32(64.0)	34(50.8)	12(24.0)	22(32.8)
Pedal pressing	24(48.0)	21(31.3)	8(16.0)	19(28.4)
Waiting for the signal	29(58.0)	18(26.8)	8(16.0)	23(34.3)
Positive signal	30(60.0)	12(31.3)	3(6.0)	11(16.4)
Reinforcement	35(70.0)	36(53.7)	—	8(11.9)
Negative signal	10(20.0)	13(19.4)	7(14.0)	10(14.9)
Absence of reinforcement	4(8.0)	10(14.9)	25(50.0)	22(32.8)

Notes. Numbers in parenthesis are percentages relative to the total number of neurons in the group.

TABLE 2. Comparison of the Magnitudes ($\bar{x} \pm S_{\bar{x}}$) of Excitatory and Inhibitory Responses of Dopaminergic (Group 1) and Nondopaminergic (Group 2) Cat Substantia Nigra Neurons at Different Stages of the Behavioral Act

Stage of the behavioral act	Activation		Inhibition	
	Group 1	Group 2	Group 1	Group 2
Preparation for movement	218.6±49.4	51.1±7.5**	−35.5±7.0	−38.9±4.6
Execution of movement	489.0±176.5	77.3±12.6**	−51.0±9.4	−41.5±5.8
Pedal pressing	787.7±357.2	79.0±13.4**	−64.1±9.7	−37.4±4.6
Waiting for the signal	533.8±199.3	61.0±11.5**	−35.7±11.8	−24.9±4.1*
Positive signal	350.7±177.2	48.0±11.1**	−56.9±56.9	−22.1±6.7**
Reinforcement	317.0±130.9	50.8±8.0**	—	−35.3±10.9
Negative signal	199.3±69.7	66.2±18.7**	−68.1±14.0	−27.8±7.3*
Absence of reinforcement	189.1±25.5	28.6±9.9**	−49.4±6.4	−21.0±7.5*

Notes. Significance of differences: $^{*}p < 0.05$; $^{**}p < 0.01$.

animal had previously kept the paw on the support for at least 4 sec and when the time taken to move the paw from the support to completing the pedal press was less than 1 sec. Movements could not be too frequent: a pedal press occurring less than 12 sec after the previous pressing was regarded as erroneous and was not reinforced, though it was included in the analysis. When attempts were completed correctly, a positive conditioned sound signal, a tone of 1600 Hz lasting 0.2 sec, was presented 1 sec later, followed after a further 1-1.5 sec by food reinforcement provided with forceps. When attempts were incorrect, a negative conditioned sound signal, a tone of 400 Hz, was presented.

After training, guide cannulae were inserted into the brain under general anesthesia, the tip being placed 5 mm above the upper limit of the substantia nigra. A manipulator stage was attached to the skull, and electromyogram (EMG) electrodes were attached s.c. for recording right limb muscle activity. Neuron activity was recorded on the side contralateral to the working limb at coordinates A = 6-7, L = 3.5-5, H = 5-7 mm, which is the position of the compact zone of the substantia nigra [10]. The recording zone contained group A9 and parts of groups A8 and A10 [7]. Extracellular recording was carried out using a mobile silver microconductor (diameter 12 μm) in a glass insulator (70 μm), sharpened like an injection needle tip. Spike activity and EMG traces were amplified, digitized, and, along with event markers for the platform, pedal presses, and sound signals, were fed into the computer controlling the experiment. Peristimulus histograms were constructed by centering relative to the start of movement (EMG activation) and the moments at which signals arrived from the probes. The numbers of performances ranged from 15 to 30 and bin widths were 8-40 msec. Histograms were used to assess the numbers of responding neurons and magnitudes of their responses. Response sizes were determined in terms of the difference between the number of spikes in the time window during development of the response and the number of spikes over the same period of time during the initial period. Differences, along with signs relative to the initial activity level (increases or decreases), were expressed as a percentages. The initial period was selected between 1 and 2 sec before the onset of movement. The magnitudes of responses from different groups of neurons were compared using the Mann-Whitney criterion.

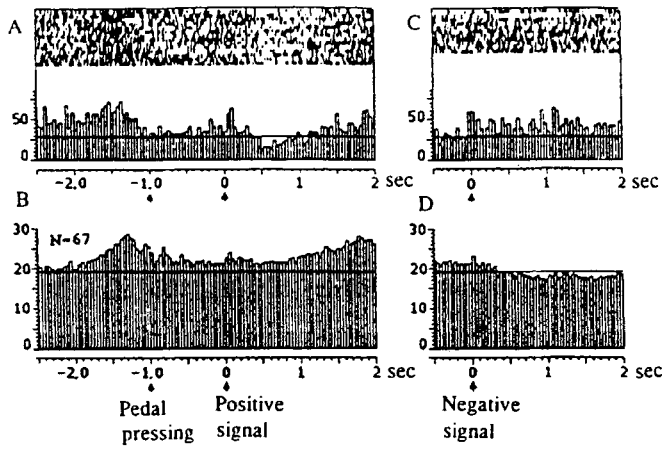

Fig. 3. Responses of cat substantia nigra nondopaminergic neurons to positive (*A*, *B*) and negative (*C*, *D*) conditioned signals. For further explanation see caption to Fig. 1.

Recording sites were checked after experiments by inducing an electrocoagulation marker by replacing the microconductor with a lacquer-insulated tungsten electrode (the tip was not insulated); animals were euthanased and brain sections were cut on a cryomicrotome. Other details of the methods used here have been published previously [3].

RESULTS

Self-initiated behavioral responses performed by the animals had the following characteristics: the interval between attempts averaged 17.6 ± 0.4 sec (results are presented as mean ± error of the mean), with a range of 7.6-129.2 sec. The earliest EMG changes in right limb muscles were seen 240 msec before the paw left the support. The time between the paw leaving the support and pressing the pedal was 296.0 ± 4.0 msec (with extremes of 152.4 ± 994.1 msec). The proportion of correctly executed, reinforced attempts varied from 45% to 80%.

A total of 117 neurons were recorded in the areas of interest. The background spike frequency in 50 of these (recorded at rest over 3 min) was no more than 8.0 Hz, and averaged 4.6 ± 0.4 Hz. Spikes were polyphasic and relatively long-lasting, the duration being 2.5-5.0 msec. On the basis of these parameters, the cells were identified as dopaminergic neurons [8, 16]. The spontaneous spike frequency of the remaining 67 neurons was 21.7 ± 1.2 Hz (with extremes of 10.5 and 62.5 Hz). Action potential duration was 1.5-3.0 msec. These cells were regarded as nondopaminergic neurons.

The neurons recorded could change their activity during the behavioral act. The most marked were responses associated with launching and performing the voluntary movement, receipt of reinforcement, and (to a lesser extent), response to the conditioned sound signals presaging the provision or nonprovision of reinforcement (Figs. 1-3). The following time characteristics were noted on comparison with the key points of the behavioral act. Dopaminergic neurons were activated 275.6 ± 37.3 msec before the start of the movement (with extremes of 40 and 760 msec); inhibitory responses started at 447.1 ± 101.5 msec (range 60-660 msec). Nondopaminergic neurons were activated 317.4 ± 48.8 msec (range 20-910 msec) and inhibited 441.6 ± 80.7 msec (range 50-680 msec) before movement. Changes in activity could last throughout the entire movement (Fig. 1, *A*, *C*, *D*). The latent periods of inhibitory and activatory responses to the conditioned stimuli in both groups of neurons different insignificantly, and averaged 101.9 ± 12.5 msec (range 20-240 msec) for the positive signal and 76.2 ± 23.0 msec (range 10-230 msec) for the negative signal. Responses lasted no more than 150-250 msec. Since reinforcement was provided manually by the experimenter, the latent periods of responses to its presentation could not be measured.

The behavioral act studied here was divided into several stages. Consideration of plots of neuron responses led to selection of the following time windows for assessment of the sizes of activity changes for most neurons: preparation for movement (−300 to 0 msec before EMG activation); execution of movement (0 to 390 msec after onset of EMG activation); pedal pressing (0 to 255 msec after sensor activation); waiting for the conditioned signal (−745 to 0 msec after signal presen-

tation); response to conditioned signal (20 to 160 msec after the start of the signal); response to reinforcement or its absence (1 to 2 sec after presentation of the signal). Data on the numbers of dopaminergic and nondopaminergic neurons showing activation and inhibition at these stages are presented in Tables 1 and 2 respectively, along with comparisons of the extents of the changes.

Attention is drawn to the observation that a significant proportion of substantia nigra cells, including more than half of the dopaminergic cells (66.0%) showed activity changes before movement started. During execution of movements, the number of responding neurons within this group increased to 88.0% (Table 1). The extent of the movement-associated responses of these neurons was significantly greater than that of nondopaminergic neurons (Table 2), which was evident both in terms of individual nerve cells and in terms of normalized peristimulus histograms which summed the activities of all members of cell populations (Fig. 1, *A-D*). It is also of interest to note that after the pedal was pressed and the animals were waiting for the conditioned signal, all dopaminergic neurons retained activity levels 1.5-2 times higher than the initial level, while activity in the population of nondopaminergic neurons returned almost to the initial level (Fig. 2, *B*; Fig. 3, *B*). This difference in the patterns of population histograms can be explained in terms of the fact that at this stage of the behavioral act, more than half of all the dopaminergic neurons were activated, while nondopaminergic neurons were inhibited.

Neurons in the substantia nigra responded to both the positive and the negative conditioned signals. Responses of dopaminergic neurons to the positive conditioned signal were characterized by a larger proportion of activated nerve cells and larger excitatory responses than was the case for responses to the negative signal. Such differences were not seen for nondopaminergic neurons. Activation in response to presentation of reinforcement and inhibition in response to nonpresentation was most typical of dopaminergic neurons (Tables 1 and 2; Fig. 2).

DISCUSSION

The results of the present study showed that cat substantia nigra dopaminergic neurons are characterized by relatively powerful responses associated with the launching and execution of voluntary movements in conditions of a time-dependent, self-initiated behavioral act. The magnitude of these responses may be associated with the fact that execution of the movement task involved a cognitive component: determination of the moment at which movement should start. Thus, dopaminergic neurons, along with other cells (dopaminergic cells possibly playing the key role) can take part in generating the slow potential recorded in this region, similar to the cortical readiness potential [5]. The functional role of this type of activation of the substantia nigra is in the neocortex-striatum-thalamic nuclei-neocortex control loop. A number of authors have suggested that modulation of spike activity flowing along this chain by the substantia nigra, at the level of the striatal part, is the cause of variations in neocortical excitability, which are expressed as slow cortical potentials [6, 11].

The changes in activity of dopaminergic neurons seen here, which develop during the wait for the conditioned signals, signal presentation, and receipt (or not) of reinforcement demonstrate the involvement of this population of neurons in the processes by which reinforcement is perceived. Midbrain dopaminergic neurons have been found to have similar properties in primates [8, 15]. However, in primates, responses to conditioned stimuli presaging reinforcement can completely replace the response to presentation [9]. Dopaminergic neurons in the substantia nigra of the cat responded less actively to the conditioned stimuli, which may indicate a lesser involvement in the conditioning process as compared with neurons in the monkey. Nonetheless, the differences in the response patterns for positive and negative conditioned stimuli were clearly demonstrated.

Another noteworthy observation was the significantly larger range of changes in the activity of dopaminergic neurons, as compared with other substantia nigra neurons, at different stages of the behavioral act. The greater magnitude of both inhibitory and excitatory responses provides an optimal signal:noise ratio, which apparently allows dopaminergic neurons to have more efficient influences on neuron in other structures receiving dopaminergic inputs.

REFERENCES

1. V. A. Otellin and É. B. Arushanyan, The Nigrostriatal System [in Russian], Meditsina, Moscow (1989).
2. S. N. Raeva, "Neuronal mechanisms of word-controlled movements," in: Mechanisms of the Activity of the Human Brain. Part 1. Human Neurophysiology [in Russian], N. P. Bekhterev (ed.), Nauka, Leningrad (1988).

3. T. V. Khitrova-Orlova, V. G. Sidyakin, A. M. Kulichenko, and V. B. Pavlenko, "The anticipatory activity of cat parietal associative cortex activity in different types of voluntary movements," Fiziol. Zh. im. I. M. Sechenova, **81**, No. 4, 70-75 (1995).
4. K. B. Shapovalova, "Possible neurophysiological and neurochemical mechanisms for the involvement of the striatum in the initiation and control of voluntary movements," Fiziol. Zh. SSSR, **71**, No. 5, 537-553 (1985).
5. H. Bauer and C. Rebert, "Preliminary study on subcortical slow potentials related to the readiness potential in monkey," Int. J. Psychophysiol., **9**, No. 3, 269-278 (1990).
6. N. Birbaumer, T. Elbert, A. Canavan, and B. Rockstron, "Slow potentials of the cerebral cortex and behaviour," Physiological Rev., **70**, No. 1, 1-41 (1990).
7. S. Dunnet and T. Robbins, "The functional role of mesotelencephalic dopamine systems," Biol. Rev., **67**, No. 1, 51-59 (1992).
8. T. Ljunberg, P. Apicella, and W. Schultz, "Responses of monkey midbrain dopamine neurons during delayed alternation performance," Brain Res., **567**, No. 2, 337-341 (1991).
9. J. Mirenowicz and W. Schultz, "Importance of unpredictability for forward responses in primate dopamine neurons," J. Neurophysiol., **72**, No. 2, 1024-1027 (1994).
10. H. Nishino, T. Ono, K. Muramoto, M. Fucuda, and K. Sasaki, "Neuronal activity in the ventral tegmental area during motivated for press feeding in the monkey," Brain Res., **413**, No. 2, 302-313 (1987).
11. C. Rebert, J. Diechl, and M. Matteucci, "The distribution and detection of brain slow potentials and their relationship to neuroglial activities and higher mental functions," in: Slow Potential Changes in the Human Brain, W. McCallum, and S. Curry (eds.), New York (1993).
12. F. Reinoso-Suarez, Topographischer Hirnatlas der Katze [in German], Darmstadt (1961).
13. R. Romo and W. Schultz, "Dopamine neurons of the monkey midbrain: contingencies of the responses to active touch during self-initiated arm movements," J. Neurophysiol., **63**, No. 3, 592-606 (1990).
14. W. Schultz, "Activity of pars reticulata neurons of monkey substantia nigra in relation to motor, sensory and complex events," J. Neurophysiol., **55**, No. 4, 660-667 (1986).
15. W. Schultz, P. Apicella, and T. Ljunberg, "Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task," J. Neurosci., **13**, No. 3, 900-913 (1993).
16. R. Strecker, G. Steinfels, and B. Jacobs, "Dopaminergic unit activity in freely moving cats: lack of relationship to feeding, satiety and glucose injections," Brain Res., **260**, No. 2, 317-321 (1983).
17. R. Wise, "Intracranial self-stimulation: mapping against the lateral boundaries of the dopaminergic cells of substantia nigra," Brain Res., **213**, 190-194 (1981).