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Microelectrophysiological Studies of the Ratio of Excitatory to Inhibitory Synaptic Processes in the Corticonigral Projection in a Model of Parkinson's

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Experiments on 23 white male rats (250 g) analyzed the spike activity of individual neurons in the substantia nigra pars compacta (SNc, 242 neurons, n = 11) and substantia nigra pars reticulata (SNr, 289 neurons, n = 12) during high-frequency stimulation of the primary motor cortex (M1)in normal animals and in animals with a rotenone model of Parkinson's disease (BP). SNc neurons in the model of PD showed a complete absence of depressor effects induced by stimulation, though tetanic potentiation was accompanied by posttetanic potentiation and depression at levels 1.65 and 2.02 times greater than in normal animals. In SNr neurons in normal animals, tetanic potentiation, accompanied by post-tetanic potentiation and depression, was 2.37 times greater than tetanic depression, while in the model of PD the levels of both depressor and excitatory activity induced by stimulation were below normal. Spike activity frequency in SNc and SNr neurons preceding and accompanying stimulation was significantly greater than normal in the model of PD. This is evidence for excitotoxicity accompanying neurodegenerative damage, which is completed by neuron apoptosis and death. In SNr neurons, both depressor and excitatory reactions accompanying stimulation were markedly dominant over those in SNc neurons, which is evidence for more extensive cortical projections to the SNr. Furthermore, SNc neurons demonstrated greater susceptibility to pathological changes due to poststimulus depressor effects than SNr neurons, with formation of more marked excitatory effects, which is evidence of a greater involvement of the SNc in PD. In the model of PD, lacking stimulation-induced depressor effects and more marked excitatory effects in SNc neurons, SNr neurons retained their depressor reactions and relatively decreased excitatory reactions, which is evidence of a lower level of susceptibility of SNr neurons to excitotoxicity, extreme increases in the excitability of surviving neurons compensating for the lack of excitation of dead cells.

Keywords: substantia nigra compacta (SNc), substantia nigra reticulata (SNr), rotenone model of Parkinson's disease (PD), primary motor cortex (M1), single-unit spike activity, programmed mathematical analysis.

The substantia nigra (SN) is an important neuronal structure responsible for mediating regulation of the activity of the basal ganglia (BG). The posteromedial part of the SN – the pars compacta (SNc) – among the dopaminergic (DA) nuclei of the brain [1], is mainly connected with the dorsal striatum [2]. The anterolateral zone of the SN – the pars reticulata (SNr) – consists of GABAergic neurons receiving afferents from the striatum and subthalamic nucleus and in turn projecting to the ventral anterior thalamic nucleus [3]. The SN, regulating the BG [4], in pathological conditions promotes the development of a variety of neurological diseases, particularly Parkinson's disease (PD) [5], schizophrenia [6], and pathological tendencies and harmful addictions [7]. The SNc and SNr have been shown to have extensive subcortical networks [8–10]. Data from rodents indicate that the SNr is a large output element of the BG, receiving information from the motor cortex via direct inhibitory connections, indirect excitatory connections via the pallidum

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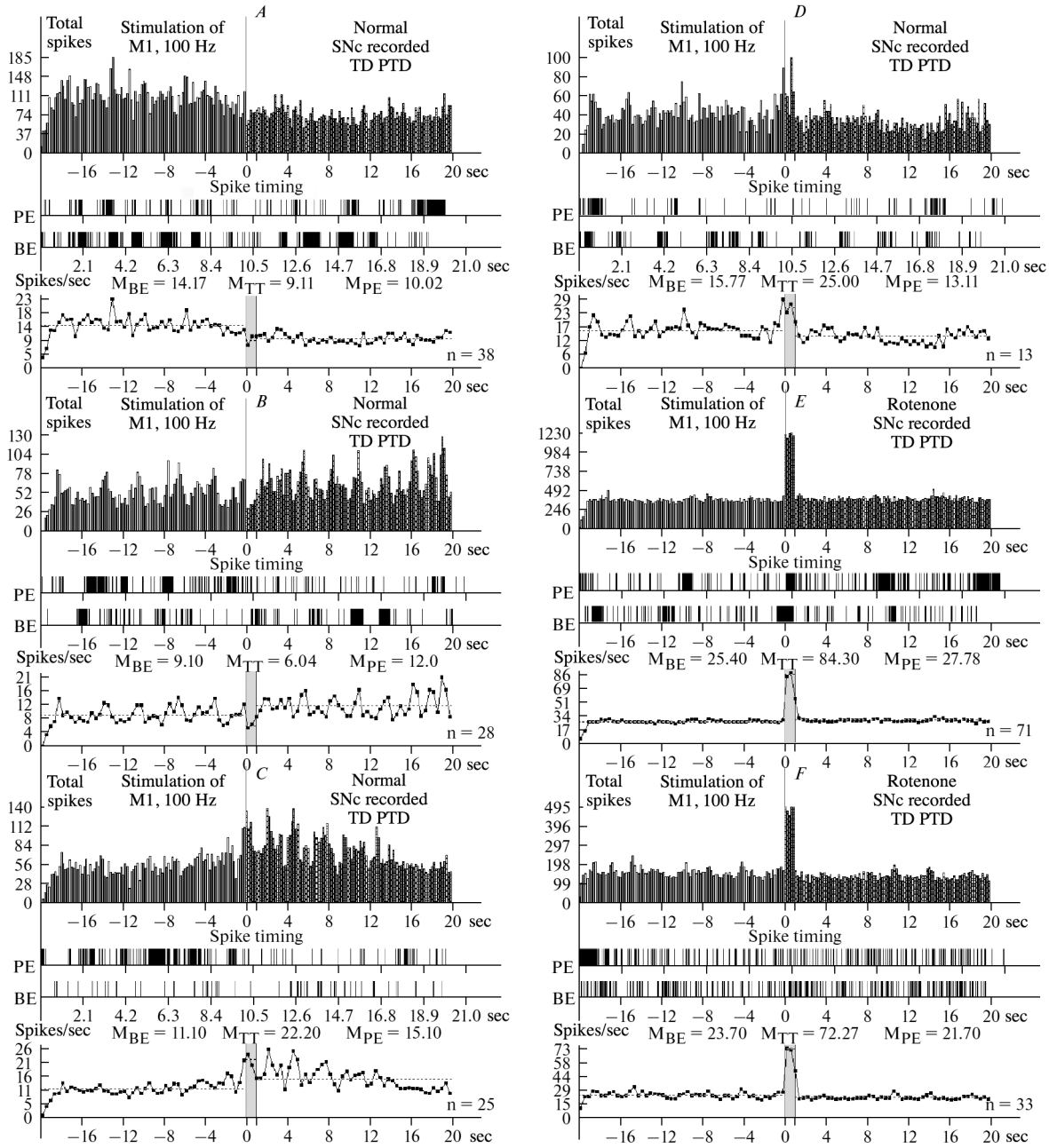

Fig. 1. *A–F*) Histograms showing total spikes preceding and accompanying poststimulus activity: depressor – TD PTD (*A*), depressor-excitatory – TD PTP (*B*), excitatory – TP PTP (*C*, *E*), in combination with depressor – TP PTD (*D*, *F*), in real time for 20 sec (before and after stimulation) of SNc neurons evoked by HFS of M1 in normal animals (*A–D*) and in a model of PD (*E*, *F*). Here and henceforth: plots of spike frequency shown in histograms with mean values (M) for time segments before stimulation (BE, before event), during tetanization (TT, time of tetanization), and after stimulation (PE, post event). Numbers of tests (*n*) are shows at right of plots.

and subthalamus, and direct excitatory connections via the subthalamus [11]. Recent studies have demonstrated the existence of a corticonigral projection in humans. MRI studies seeking to differentiate the interactions of the SNc and SNr with the cerebral cortex via the thalamus demonstrated a connection of the SNc with the prefrontal cortex (PFC) and of the SNr mainly with the motor and premotor cortex [9]. Connections between the SN and various brain structures (the corpus callosum, primary sensory cortex, premotor cortex, caudate nucleus, putamen, nucleus accumbens of the septum the temporal-occipital lobes, the ventral part of the pons, the anterior lobe of the cerebellum, and the external capsule) have been demonstrated [12]. Various reports have indicated that unilateral extirpation of the frontal cortex is accompanied by depletion of glutamic acid in the ipsilateral SN, without any change in the GABA content, which is

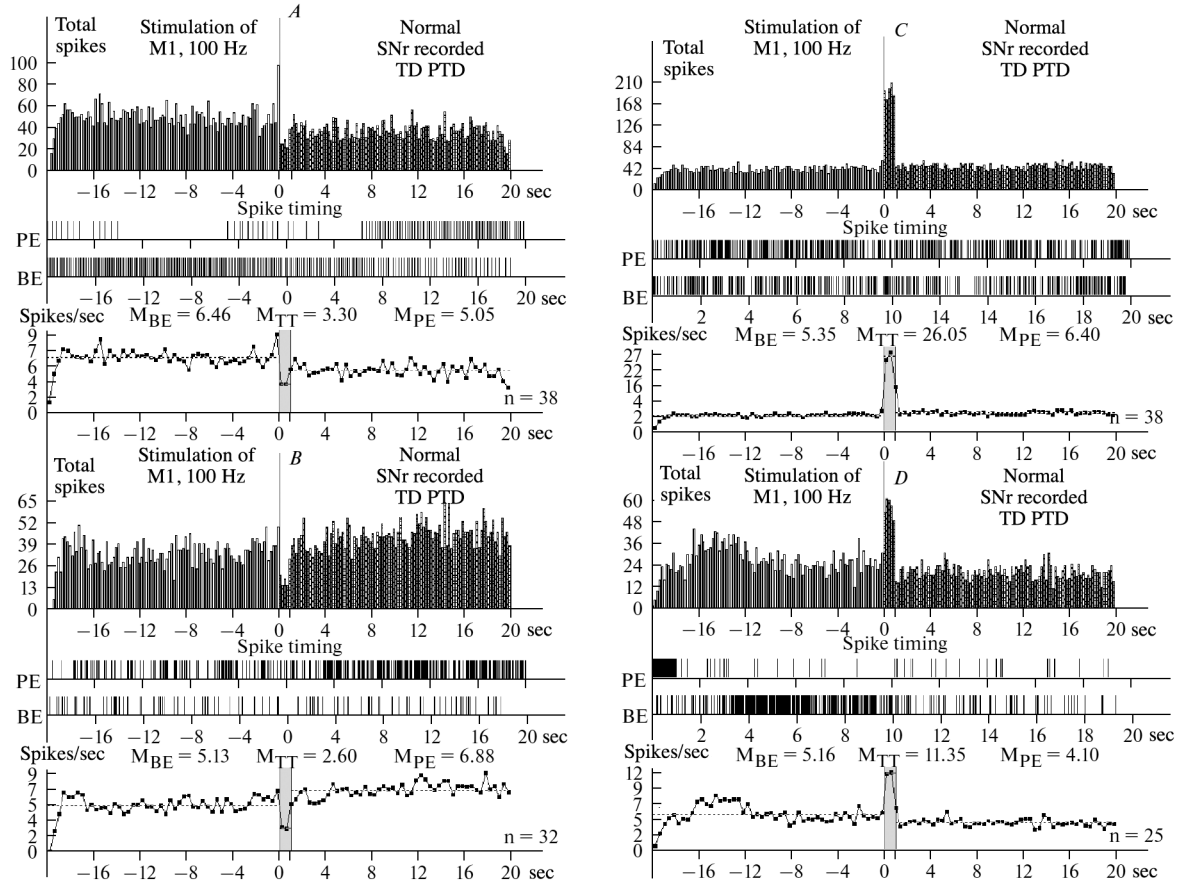

Fig. 2. *A–D*) Histograms showing total spikes preceding and accompanying poststimulus activity: depressor (*A*), depressor-excitatory (*B*), excitatory (*C*), combined with depressor – TP PTD (*D*), in real time for 20 sec (before and after stimulation) of SNr neurons evoked by HFS of M1 in normal animals. Numbers of tests (*n*) are shows at right of plots.

evidence that glutamate operates as a neurotransmitter in the corticonigral tract [13]. Despite a relatively small cortical input to the midbrain in primates, it is rich in glutamate receptors [14]. Cortical control of the midbrain is an important mechanism by which the glutamatergic input is controlled by DA cells [15]. The connections of the SN with the cerebral cortex in humans have been shown to involve the prefrontal cortex (PFC), the pre- and postcentral gyrus, and the superior parietal lobe, which allows us to address the mechanism of development of the pathophysiology of such neurological diseases as PD, schizophrenia, and pathological dependence. Thus, the hypothesis that the SN is not only part of the network of the subcortical BG but is also connected with the cortex via an additional corticonigral pathway in humans is reinforced, strengthening existing data obtained in animals [16]. Thus, the existence of a direct corticonigral connection is demonstrated, and while this has been described in detail in cats, rodents, and primates, it has only been proposed in humans. This connection may be the basis for further biochemical and physiological studies of the regulation of the basal ganglia. There is still inadequate evidence for more marked connections between the motor and premotor cortex with the SNr, as compared with the SNc, on the basis of the state-of-the-art tractographic studies in humans noted above. The objective of the present study was to carry out further investigations, using normal animals and those with a model of PD, of the relative extents of corticonigral connections in microelectrophysiological studies using the ratio of excitatory and inhibitory processes in SNc and SNr neurons as an example, in conditions of activation of the primary motor cortex (M1), which may provide a new understanding of the mechanisms controlling the motor and cognitive functions of the brain at the level of the brainstem in conditions of altered plasticity in neurodegenerative diseases.

Methods. Electrophysiological studies were carried out using 23 white mongrel male rats (230 ± 30 g) in two experimental series: intact (n = 11) and those with a model of PD induced by unilateral administration of rotenone for four weeks (n = 12). Rotenone was given under Nembutal anesthesia (40 mg/kg, i.p.) at a dose of 12 μg in 0.5 μl of Dimexid (dimethylsulfoxide) (at a rate of 0.1 μl/min) into the medial forebrain bundle at stereotaxic atlas [17] coordinates AP +0.2, L ±1.8, DV +8 mm). Experiments were conducted

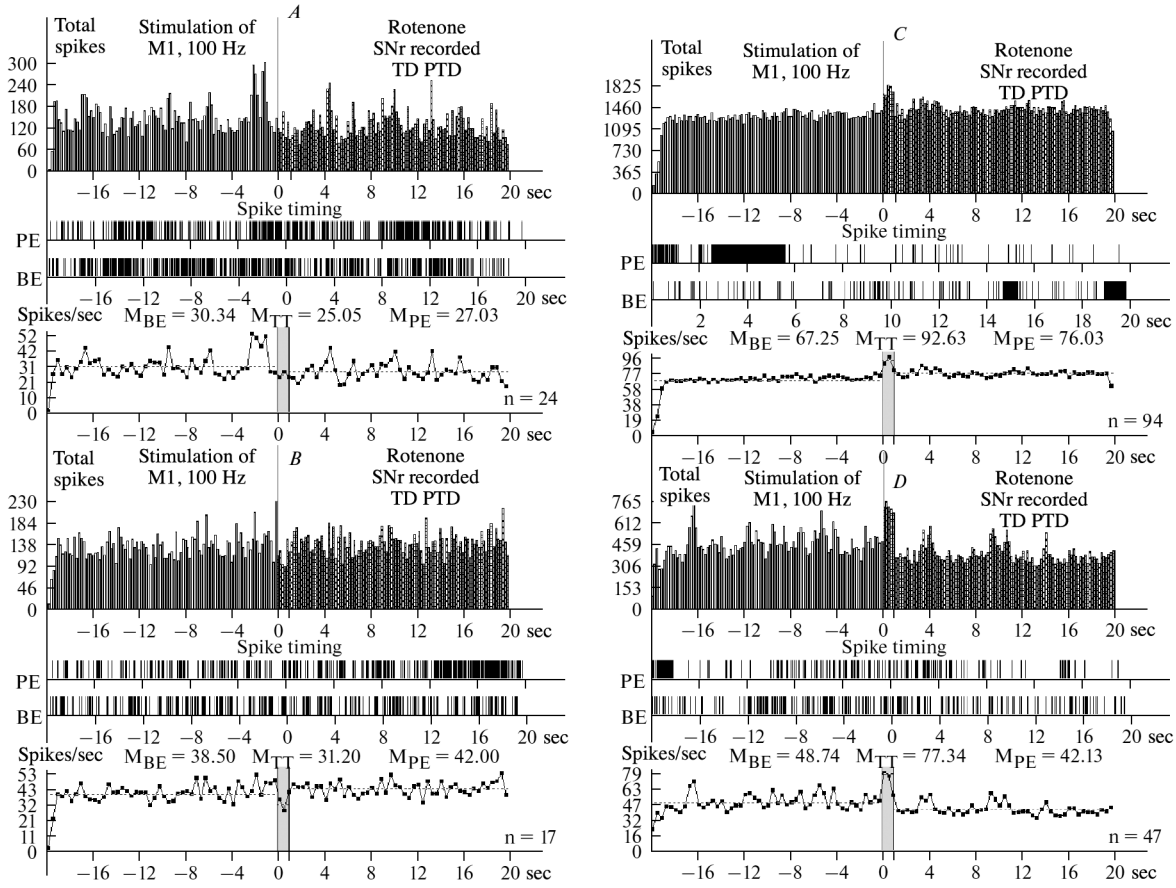

Fig. 3. *A–D*) Histograms showing total spikes preceding and accompanying poststimulus activity: depressor (*A*), depressor-excitatory (*B*), excitatory (*C*), combined with depressor (*D*), in real time for 20 sec (before and after stimulation) of SNr neurons evoked by HFS of M1 in the model of PD. Numbers of tests (*n*) are shows at right of plots.

in compliance with the principles of the Basel Declaration and the recommendations of the ARRIVE guidelines [18]. The skull was trepanned from the bregma to the lambda and the dura mater was opened in the stereotaxic apparatus. After craniotomy, the stimulating electrode was implanted in the ipsilateral M1 at stereotaxic atlas coordinates AP +2.1, L ±2.6, DV +1.6 mm and glass microelectrodes with tip diameter 1–2 μm filled with 2 M NaCl were implanted in the SNc (AP –5.0, L ±2.0, DV +8.1 mm) and SNr (AP –5.1, L ±2.0, SDV +8.6 mm) for extracellular recording of single-neuron spike activity. High-frequency stimulation (HFS) was applied to the MI using square-wave current pulses (duration 0.05 msec, amplitude 0.12–0.18 mV, current strength 0.32 mA, frequency 100 Hz, duration 1 sec). Surgery was carried out under urethane (1.5 g/kg, i.p.) anesthesia in the following sequence: the skull was fixed in the stereotaxic apparatus, craniotomy was carried out with removal of bone from the bregma to the lambda and separation of the dura mater. Animals were initially immobilized with 1% Dithylin (suxamethonium iodide, 25 mg/kg, i.p.) and transferred to mechanical ventilation. Overall, activity was recorded from a total of 531 neurons.

Neuron activity was apparent as tetanic depression and potentiation (TD and TP), which were accompanied by post-tetanic depression and potentiation (PTD and PTP). Single-unit spike activity was recorded from 531 SNc and SNr neurons. A special algorithm was used to calculate mean spike frequencies. Multilevel statistical analysis was then run for the pre- and poststimulus time periods. Spike activity in the selected groups was compared by plotting overall and averaged peristimulus (PETH Average) histograms and Frequency Average histograms. Data were analyzed using a specially developed algorithm. The uniformity of pairs of independent datasets was monitored using Student's *t* test. As the number of spikes was greater than 30, the distribution could be regarded as asymptotically normal, such that use of Student's test was appropriate, and this showed that changes in mean spike parameters in the plots were significant. Critical values in comparison with values in the normal distribution at significance levels of 0.05, 0.01, and 0.001 showed that in most cases changes were statistically significant at $p < 0.05$ or below.

Results. Comparative analysis was performed on the spike activity of single neurons in the SNc and SNr in re-

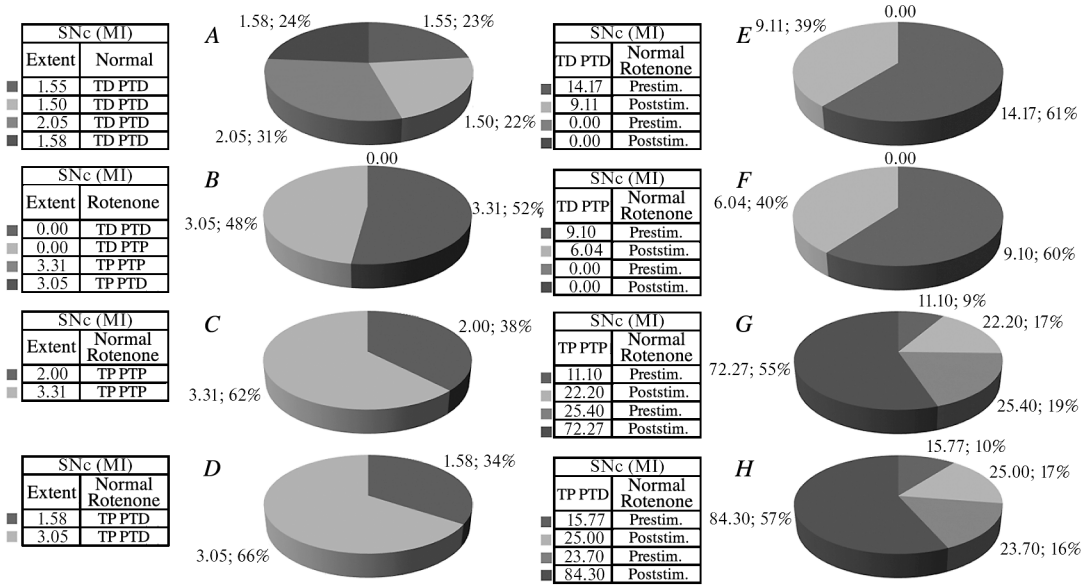

Fig. 4. *A–H*) Percentage ratio of extents of depressor (*A*), depressor-excitatory (*B*), excitatory (*C*), and excitatory-depressor (*D*) changes in mean frequency of spike activity in single neurons in the SNc preceding and accompanying HFS of M1 in a model of PD as compared with normal animals.

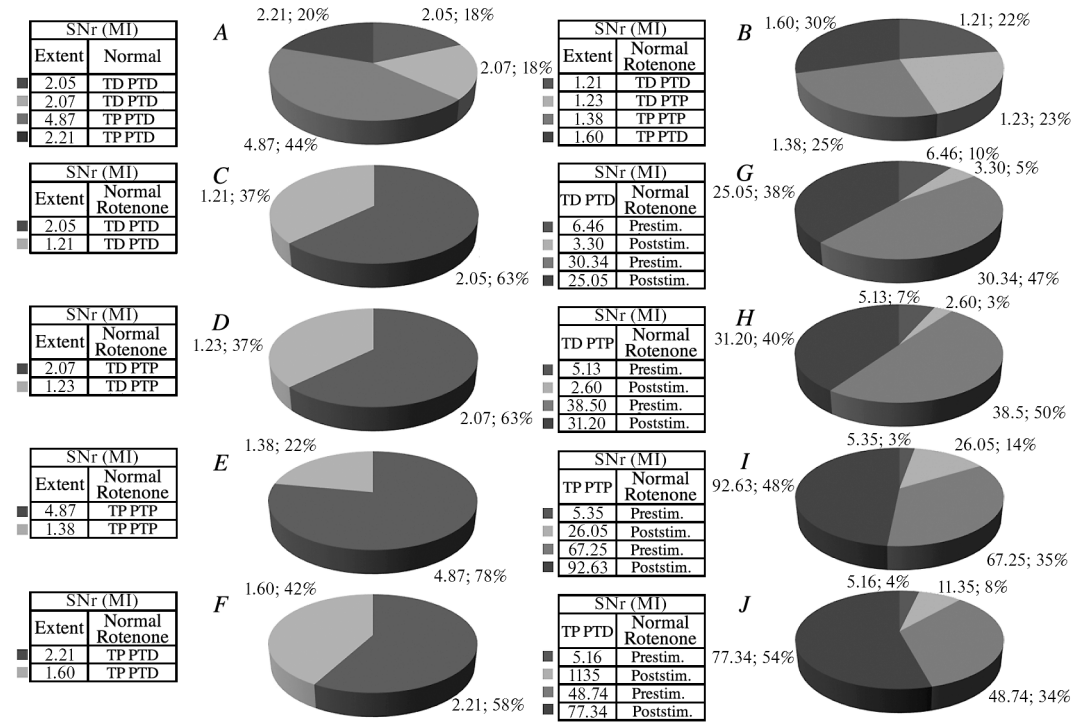

Fig. 5. *A–J*) Percentage ratio of extents of depressor (*A*), depressor-excitatory (*B*), excitatory (*C*), and excitatory-depressor (*D*) changes in mean frequency of spike activity in single neurons in the SNr preceding and accompanying HFS of M1 in a model of PD as compared with normal animals.

sponse to HFS of M1 in normal animals (107 neurons, $n = 4$ and 135 neurons, $n = 7$, respectively) and in a model of PD (105 neurons, $n = 5$ and 184 neurons, $n = 7$). These series showed the following changes in inhibitory and excitatory tetanic post-tetanic reactions in both poststimulus sequences (TD PTD, TD PTP and TP PTP, TP PTD).

Assessment of the relative extents of these effects, exemplified by plots of the mean frequencies of spikes extract-

ed on the basis of rasters of pre- and poststimulus changes in spike activity in different directions of SNc neurons during HFS in M1 in normal animals, showing mean numerical values in real time for the periods of 20 sec before and after stimulation, including the duration of HFS, yielded values (Figs. 1–3) presented as disk plots for assessment of extents as percentages (Figs. 4 and 5).

SNc neurons responded to HFS of M1 in normal animals with decreases (TD) and increases (TP) compared with prestimulus activity in one- and two-directional poststimulus sequences within the following limits. In the depressor sequence, TD reached a 1.55-fold reduction in prestimulus activity, in the depressor-excitatory sequence there was a 1.5-fold decrease (Fig. 1, *A*, *B*; Fig. 4, *A*); in the excitatory sequence TP exceeded prestimulus activity by a factor of 2.05 and in the excitatory-depressive sequence by a factor of 1.58 (Fig. 1, *C*, *D*; Fig. 4, *A*).

In the model of PD, poststimulus signs of depressor activity in SNc neurons were completely absent. Excitatory poststimulus activity TP in two sequences reached 3.31 and 3.05 times prestimulus activity. Excitotoxicity in the SNc neurons was apparent in the model of PD, evidencing neurodegenerative damage to these cells. SNr neurons responded to HFS of M1 with decreases (TD) and increases (TP) in prestimulus activity in one- and two-directional poststimulus sequences within the following limits. In depressor sequences, TD reached 2.05-fold decreases, while in depressor-excitation sequences it decreased by a factor of 2.07 (Fig. 2, *A*, *B*; Fig. 5, *A*). In excitatory sequences, TP changed by up to a 5.07-fold increase, while in excitatory-depressor sequences increases were by a factor of up to 2.20 (Fig. 2, *C*, *D*; Fig. 5, *A*). In other words, TP in SNr neurons was greater than TD, predominantly in one-directional sequences. In the model of PD, TD in SNr neurons in both sequences reached only 1.21- and 1.23-fold decreases in prestimulus activity (Fig. 3, *A*, *B*; Fig. 5, *B–D*). However, TP in both sequences also changed within a small range, with 1.40- and 1.60-fold increases (Fig. 3, *C*, *D*; Fig. 5, *B*, *E*, *F*). In other words, in SNr neurons, the levels of both depressor and excitatory poststimulus activity in the model of PD were below normal. In SNc neurons, on HFS of M1, the frequency of prestimulus activity preceding the two excitatory poststimulus signs of activity in the model of PD, as compared with normal, reached values exceeding those in normal animals by 25.4 and 23.7 vs. 11.10 and 15.77, i.e., 2.30- and 1.50-fold (Fig. 1; Fig. 4, *G*, *H*). As regards poststimulus SNc neuron activity frequencies accompanying poststimulus excitatory activity, these were of the order of 84.3 and 72.27 vs 22.2 and 25.0, i.e., in pathology they reached significant levels exceeding the normal by factors of 3.25 and 3.37 (Fig. 1, *G*, *H*). Thus, comparative analysis of pre- and poststimulus Synaptic neuron activity frequency in the model of PD, as compared with normal animals, led to the conclusion that excitation occurred, accompanied by unavoidable and significant neurodegenerative damage.

In SNr neurons exposed to HFS of M1, the frequency of prestimulus activity preceding the two poststimulus depressor sequences were, in the model of PD as compared with normal, 30.34 and 38.5 vs 6.46 and 5.13, thus exceeding normal by factors of 4.7 and 7.5 (Fig. 2; Fig. 3; Fig. 5, *G*, *H*). SNr neuron activity frequencies preceding the two excitatory sequences in the model of PD, as compared with normal animals, were 67.25 and 48.74 vs. 5.35 and 5.16, i.e., 12.57 and even 9.44 times greater than normal (Fig. 2; Fig. 3; Fig. 5, *I*, *J*). Poststimulus frequencies of SNr neuron activity accompanying the two corresponding depressor sequences in the model of PD, as compared with normal animals, were 25.05 and 31.2 vs. 3.3 and 2.6 (7.6 and 12 times greater than normal), while the frequencies accompanying the excitatory sequences were 92.63 and 77.34 vs. 26.05 and 11.35 (3.55 and 6.81 times greater than normal) (Fig. 5, *G–J*). In other words, both pre- and poststimulus frequencies of SNr neuron activation in pathology were markedly greater than normal, more so in relation to prestimulus baseline activity, which is evidence of compensatory excitotoxicity promoting pronounced neurodegeneration leading to neuron apoptosis and death.

Discussion. According to current concepts, rotenone induces selective degeneration of the nigrostriatal dopaminergic pathway, selective oxidative damage to the corpus callosum, and formation of ubiquitin- and α-synuclein-positive inclusions in nigral cells, which are similar to Lewy bodies in PD [19]. In turn, "excitotoxicity" in neurodegenerative diseases, including PD, results from overactivation of glutamate NMDA and AMPA receptors, leading to serious damage to neurons [20], with neuron death [21, 22]. This occurs as a result of the unavoidable development of a whole series of adverse phenomena, including impairment to calcium "buffering," free radical generation, activation of mitochondrial permeability, and secondary excitotoxicity [23]. As compensation for loss of excitation due to neuron death, it ultimately leads to the apoptosis and death of surviving neurons. With the aim of preventing unavoidable excitotoxicity, there is a need to restore and enhance poststimulus depressor effects, which have a protective function, and decrease extreme excitation [24].

The present experiments conducted a comparative analysis of the spike activity of single neurons in the SNc and SNr in response to HFS of M1 in normal animals and in animals with a model of PD. Analysis of the relative frequencies of the above-noted depressor and excitatory effects, with assessment of plots of mean spike frequencies presented as disk diagrams (%) led to the following conclusions. In SNr neurons exposed to activation of the M1 cortex, overall both depressor and excitatory poststimulus reactions were sharply predominant over those in SNc neurons (2.05, 2.07, 4.87, and 2.21 vs. 1.55, 1.5, 2.0, and 1.58), which is evidence of more marked cortical projections to the SNr than the SNc. Furthermore, in pathology there was greater deficit of depressor poststimulus changes in synaptic neuron activity as compared with the SNr, with formation

of extreme excitatory activity (excitotoxicity). In the model of PD, as compared with normal, there was a complete absence of depressor poststimulus effects, along with more marked excitatory effects, in SNc neurons (3.05 and 3.32 vs. 2.0 and 1.58), while SNr neurons retained depressor poststimulus reactions, though they were smaller, and excitatory reactions were also decreased (1.21, 1.23, 1.38, and 1.6 in pathology vs. 2.05, 2.07, 4.87, and 2.21 in normal animals, which is also evidence o lower susceptibility of SNr neurons to excitotoxicity than SNc neurons.

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