DOI 10.1007/s11055-016-0228-7
Neuroscience and Behavioral Physiology, Vol. 46, No. 3, March, 2016

Changes in the Number of Neurons in the Rat Motor Cortex and Movement Activity with Age

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UDC 611.813.1.018:599.323.4

Translated from Morfologiya, Vol. 147, No. 3, pp. 7–10, May–June, 2015. Original article submitted September 20, 2014.

A programmable Laboras apparatus, consisting of an automated behavioral reaction recording system, was used to record movement activity in male rats aged one, eight, and 16 months studied in groups of 12, with subsequent determination of the numbers of neuron bodies in layer V of the motor cortex in preparations stained by the Nissl method. Different age groups were found to have different numbers of neurons in the motor cortex. The greatest number was seen in animals aged eight months. Movement activity correlated with the number of neurons.

Keywords: motor cortex, number of neurons, locomotion, Laboras, histophysiology.

There are few studies of the motor cortex in which physiological methods of analyzing the activity of the motor cortex are combined with studies of its cytoarchitectonics. Donoghue and Wise [10] discriminated zones of the motor cortex which could be identified both by intracortical microstimulation and cytoarchitectonic properties, though no detailed description of the structure of these zones was provided. Ivliev [4] identified a relationship between the actions of atropine on the motor cortex in rats and their ability to acquire a motor skill. Ipekchyan and Badalyan [6] reported morphological studies demonstrating that the primary motor and primary sensory cortex are two independent cortical centers with different morphofunctional organization. Reports in recent years have been dominated by physiologically orientated investigations [12, 15, 17], while histophysiological studies of the CNS are few in number [1].

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The aim of the present work was to identify the relationship between the number of neurons in the motor cortex in laboratory rats and their movement activity.

Materials and Methods

Experiments were performed on outbred male rats aged one, eight, and 16 months (12 animals in each group) in compliance with the "Regulations for Studies Using Experimental Animals" (USSR Ministry of Health Decree No. 755 of February 12, 1977). Animals were obtained from the Andreevka animal house of the Scientific Center of Biomedical Technologies, Russian Academy of Medical Sciences and were kept in standard animal house conditions [7]. Movement activity was recorded using a programmable Laboras system (Metris, Netherlands) [8]. The locomotion of each animal was recorded separately from 20:00 to 24:00 with subsequent assessment of the mean duration of movements in the group. Animals were sacrifi ced with carbon dioxide the day after the experiment. Brains were fi xed in Carnaud's fl uid and paraffi n sections were stained with 1% aqueous cresyl violet in acetate buffer by the Nissl method. The motor cortex was identifi ed using the Paxinos and Watson atlas [14], after which the numbers of neurons in layer V of the cortex were counted using a Carl Zeiss Axioscop 2 microscope (Germany). Counts were made on images of six microscope fi elds (objective ×40, ocular ×20)

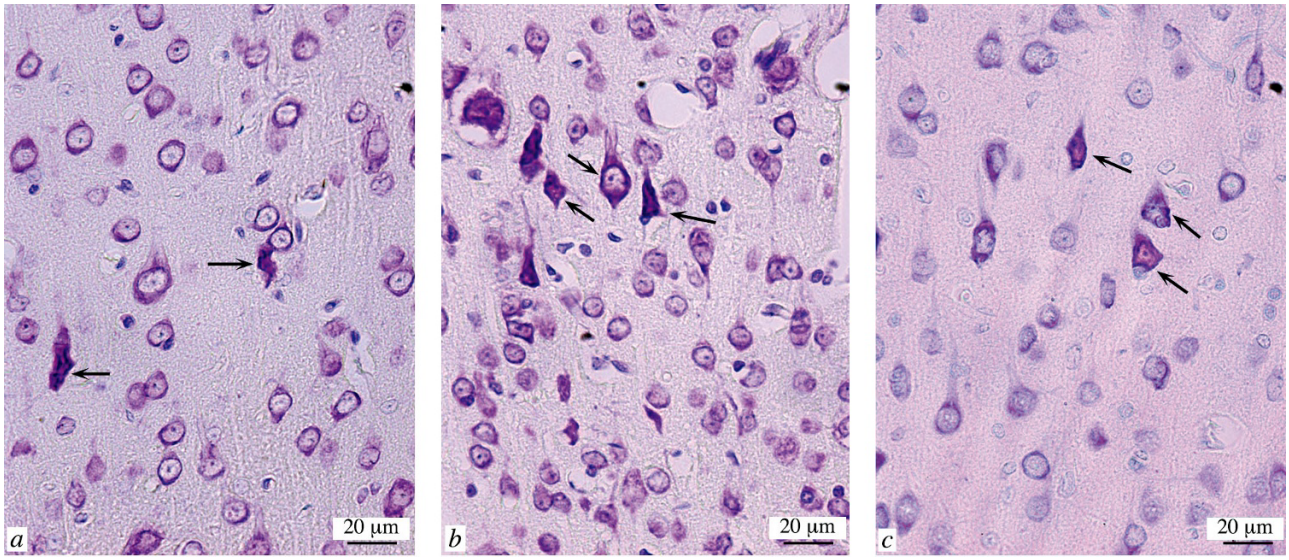

Fig. 1. Layer V of the motor cortex of male rats aged one month (*a*), eight months (*b*), and 16 months (*c*). Arrows show structural differentiated neurons of type. Stained with cresyl violet.

prepared using the AxioVision image processing system (Carl Zeiss, Germany). The mean number of neurons per microscope field was counted and correlational relationships between locomotor activity and the number of neurons in the motor cortex were studied. Motor cortex neuron counts and movement activity were analyzed in each animal separately. Trends in linear and second-order polynomial models were identified and correlation coefficients (r) and determinations (R^2) were analyzed in terms of the following levels: I – linear, II and higher-order – polynomial. Models were used to characterize the general direction of changes in study values. Mathematical analysis of the experimental data was run in Excel [2].

Results

Layer V of the motor cortex consists of neurons (mostly pyramidal cells) of different sizes, along with glial cells. Two types of neurons could be discriminated on sections, in terms of their staining properties: type 1 consisted of cells with clearly stained cytoplasm, while type 2 consisted of cells with weakly stained cytoplasm. Sections of layer V of the motor cortex in rats at one month contained few neurons, and the bodies of most of them were round, with weakly staining cytoplasm forming a narrow rim around the nucleus; processes were not seen (Fig. 1, *a*). Neurons in eight-month-old individuals were much larger and were commonly pyramidal in shape, and some cells had intensely staining cytoplasm containing granules of chromatophilic material (see Fig. 1, *b*). By 16 months, the motor cortex showed decreases in the numbers of neurons of both types 1 and 2, though the numbers remained greater than in animals aged one month (see Fig. 1, *c*).

Studies showed that maximal mobility during a fixed (4 h) period of time was seen in males aged eight months, while the lowest level of activity was in aged, 16-month-old, animals. The mean number of neuron bodies changed in the same direction in different age groups. The smallest value for this measure was seen in rats aged one month and the largest in animal aged eight months; the value decreased by age 16 months but not to the level seen in individuals aged one month (Table 1). Thus, comparison of the age dynamics of mean (for age group) functional and morphometric properties identified a linear relationship between motor cortex neuron density and the animals' mobility: larger numbers of neurons correlated with higher levels of movement activity.

Graphical analysis of neuron content in the motor cortex and movement activity in each animal individually showed the group of one-month-old rats differed from those of other ages in having a significant degree of heterogeneity. This group included individuals which were different from each other. Thus, animals of subgroup 1 displayed weak movement activity on the background of a low neuron count, while subgroup 2 had a higher neuron density and moved more, and subgroup 3 had the greatest neuron density but a shorter duration of movement (Fig. 2). With aging (to eight and 16 months), movement activity correlated linearly with the number of neurons in the motor cortex.

Discussion

The present studies assessed the content of neurons in the rat motor cortex and compared the results with their movement activity as evaluated using a programmable Laboras apparatus – which avoids the subjectivity inherent in the open field test [3]. The results obtained were consistent with data published by various authors. Thus, Klein et al. [11] showed that training of animals to a movement skill was associated with functional reorganization of the corre-

TABLE 1. Durations of Movements and Numbers of Neuron Bodies in Layer V of the Motor Cortex of Male Rats of Different Age Groups ($\bar{x} \pm s_{\bar{x}}$, per microscope field, objective ×40, ocular ×20)

Parameter	Age, months		
	1	8	16
Duration of movements over 4 h, sec	185 ± 16	291 ± 5*	161.4 ± 2.2*,**
Number of neuron bodies in layer V of the motor cortex	46.1 ± 0.6	80.9 ± 1.1*	53.2 ± 0.6*,**
Correlation coefficient	0.25	0.89	0.87
Coefficient of determination	0.91	0.79	0.75

*Significant differences compared with values in one-month-old rats, $p \le 0.05$. **Significant differences compared with values in eight-month-old rats, $p \le 0.05$.

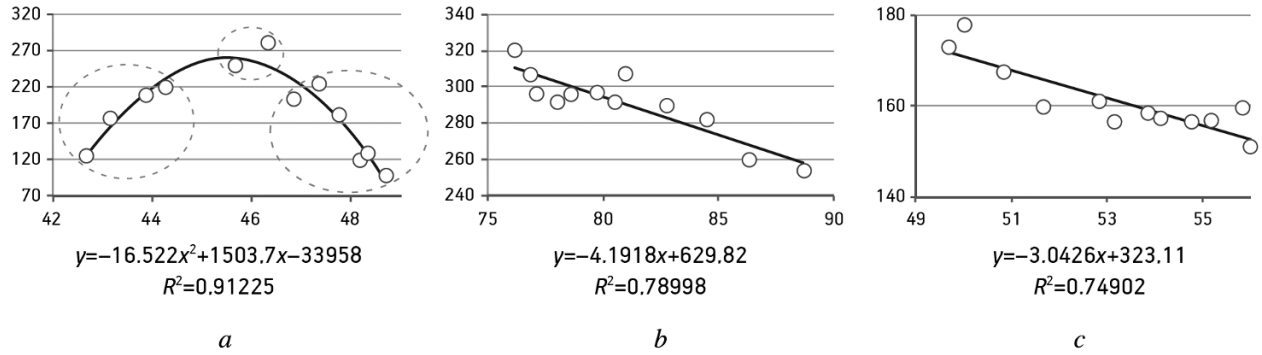

Fig. 2. Duration of locomotor reactions and numbers of neurons in the motor cortex (layer V) in male rats aged one month (ovals show the three subgroups of animals) (*a*), eight months (*b*), and 16 months (*c*). The abscissas show numbers of neurons (per microscope field); the ordinates show reaction durations (sec); $y = ax^2 + bx + c$ is the equation for the model describing the relationship between locomotor reaction duration and the number of neurons in the motor cortex, and R^2 is the coefficient of determination, providing evidence that the model used here is appropriate.

sponding representation areas in the motor cortex. In a detailed review, Ioffe [5] came to the conclusion that the motor cortex, responsible for executing motor acts, is plastic and can undergo functional rearrangement during the training process. Tierney and Nelson [16] reported studies addressing brain development in the first years of life in humans and presented data showing that the age-related numbers of interneuronal contacts in different brain structures are linked with the formation of the skills corresponding to these structures. Yarygin brothers [9] reviewed the literature on CNS morphogenesis and summarized the evidence showing that neurons have their own dividing precursors, which have the properties of stem cells. On these grounds, motor cortex plasticity can be regarded as due particularly to not only the number of synaptic contacts, but also the number of neurons.

The results of our studies support this suggestion. With age, the number of neurons in the motor cortex and the duration of movement activity in male rats increase and decrease in parallel, and are interdependent. This is due to the plasticity of brain structures, which provides the basis for changes in both the number of neurons and the number of their connections with each other [11–13].

Graphical plots showing the individual values for animals identified a number of properties of the interaction between these parameters. The heterogeneity and different directions seen in the youngest animals are probably due to different rates of differentiation of the motor cortex in different individuals. The number of neurons in subgroup 1 of one-month-old rats is evidently sufficient for the necessary range of movements to be performed. Animals of subgroup 3 probably start to assimilate motor skills somewhat earlier and acquire experience of reaching targets using a smaller number of movements, with a greater degree of purposefulness. This explanation may be supported by the fact that males of this subgroup start to show, like rats aged eight and 16 months, a tendency to a direct relationship between the duration of movements and the number of neurons.

We are inclined to explain the variability in individual values in young male animals seen here in the basis of genetic heterogeneity in the rate of development of the motor cortex and the establishment of the motor functions. The nonlinear nature of the interaction between the number of neurons in the motor cortex and movement activity in rats aged one month was very significantly described by a second-order polynomial model. This distinctive characteristic of male rats in the first month of life, as compared with animals of other groups, is evidence for the development of intense and important reorganization processes in the motor cortex by this age.

Thus, the plasticity in neuron localization in the motor cortex in male rats was different in different age groups: minimal in one-month-old individuals, increased by eight months of life, and decreased in older animals. The mobility of animals of different age groups correlated linearly with the number of neurons in the motor cortex. Within the corresponding groups, movement activity in rats aged eight and 16 months correlated linearly with the number of neurons; the analogous relationship in males aged one month was nonlinear and was described by a second-order polynomial model.

The authors would like to thank senior scientist Master of Medical Sciences Assistant Professor T. A. Belousova for assistance with scientific editing of this article.

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