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Age- and sex-related dynamics of structural and functional motor behaviour interactions in striatum neurons in rats

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Abstract

Aim. To study the age-related dynamics of structural and functional interactions of striatal neurons in the implementation of acts of motor behaviour in rats of both sexes.

Materials and methods. The study was carried out on 36 Wistar rats of both sexes aged 2, 7 and 16 months (n = 6 per group). In animals of all groups, locomotor activity was determined using a Laboras device (Metris, the Netherlands) for 15 minutes, after which the brain was sampled to determine the number and size of neurons in the striatum. The median and interquartile range of the index of motor activity and the number of neurons were determined, and to study the relationship between these indicators, a correlation and regression analysis was performed with the construction of linear and polynomial trends, and the coefficient of determination R^2 was calculated.

Results. The size of neurons did not change significantly with age in the rats of both sexes. The number of neurons differed statistically in the rats of different sexes in all age groups. In male rats, the maximum number of neurons was noted at the age of 7 months with a decrease to 16 months. In female rats, the maximum number of neurons was recorded at the age of 2 months with a further decrease to 7 and 16 months. According to the regression analysis, a linear strong relationship (R^2 = 0.80 for males, R^2 = 0.79 for females) was established between the number of neurons in the striatum and motor activity in 2-month-old animals. At the age of 7 and 16 months the relationship is non-linear.

Conclusion. The number of neurons in the striatum is subject to sex and age dynamics, while their size remains unchanged from 2 to 16 months. For animals of both sexes, a decrease in the role of the striatum in providing motor activity in the process of growing up was noted. This relationship reaches its maximum in 2-month-old rats and then decreases.

Keywords: motor acts; striatum; morpho-functional interactions; behavioral reactions; age neuromorphology
MeSH terms:
CORPUS STRIATUM – ANATOMY & HISTOLOGY
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Возрастная динамика структурно-функциональных взаимодействий нейронов стриатума в реализации актов двигательного поведения у крыс обоего пола

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Аннотация

Цель. Изучить возрастную динамику структурно-функциональных взаимодействий нейронов стриатума в реализации актов двигательного поведения у крыс обоего пола.

Материалы и методы. Исследование проведено на 36 крысах линии Wistar обоего пола возрастом 2, 7 и 16 месяцев (*n* = 6 в группе). У животных всех групп определяли двигательную активность с помощью прибора Laboras (Metris, Нидерланды) в течение 15 мин., после чего осуществляли забор мозга с целью определения количества и размеров нейронов в стриатуме. Определяли медиану и интерквартильный размах показателя двигательной активности и количества нейронов; для изучения связи этих показателей проводили корреляционный и регрессионный анализ с построением линейных и полиномиальных трендов, вычислялся коэффициент детерминации R^2.

Результаты. Размеры нейронов с возрастом значимо не изменялись у крыс обоего пола. Число нейронов статистически отличалось у крыс разного пола во всех возрастных группах. У крыс-самцов максимальное число нейронов отмечено в возрасте 7 мес. со снижением к 16 мес. У крыс-самок максимальное число нейронов зарегистрировано в возрасте 2 мес. с дальнейшим снижением к 7 и 16 мес. По данным регрессионного анализа установлена линейная сильная связь (R^2 = 0,80 для самцов, R^2 = 0,79 для самок) между количеством нейронов в стриатуме и двигательной активностью у 2-месячных животных. В возрасте 7 и 16 мес. связь имеет нелинейный характер.

Заключение. Количество нейронов в стриатуме подвержено половой и возрастной динамике, в то время как их размер остается неизменным с 2 до 16 мес. Для животных обоего пола отмечено снижение роли стриатума в обеспечении двигательной активности в процессе взросления. Эта связь максимальна у 2-месячных крыс и в дальнейшем снижается.

Ключевые слова: двигательные акты; стриатум; морфофункциональные взаимодействия; поведенческие реакции; возрастная нейроморфология

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During ontogenesis in rats, the development of behavioural mechanisms changes significantly because of age and brain structure. Rats of different age and sex groups exhibit a different histological structure of the brain, thus largely determining the manifestations of the behavioural activity [1].

The striatum is a structural union of subcortical brain formations comprising the caudate nucleus and putamen of lentiform nucleus, which are responsible for the oldest psychomotor functions. It is interesting that the cortical structures of the brain coordinate complex motor acts though their evolutionary formation is more recent [2].

Ratios between morphological, functional, and quantitative parameters for basal ganglia (striatum) determine the mechanisms of cortical and subcortical involvement into locomotor activity during ontogenesis in rats (comparing with previous studies of motor cortex). The analysis of these mechanisms would allow for the evaluation of the dynamics of behavioural reactions in rats of different age and sex. The approach seems to be effective if we use rat models to look through evolutionary issues of the development of brain structures.

The study aims to evaluate the dynamics of structural and functional interactions for striatum neurons in motor behaviour in rats of both sexes of different age groups.

MATERIALS AND METHODS

Animals

Our experimental study is conducted with 36 Wistar rats: 18 males and 18 females, divided into 6 groups (6 rats in any group) by ages of 2, 7, and 16 months [3]. We have selected the animals of the corresponding age that represent three age categories: young, grown and old rats. The animals were received from the Andreevka site of Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency, Russia. The study was approved by our local ethical committee at Sechenov University (Protocol No. 03–19, February 13, 2019).

We followed the rules of EU Directive for the Protection of the Vertebrate Animals used for Experimental and other Scientific Purposes 86/609 / EES and ethical principles[1].

Each group of the six rats was placed in a separate standard polycarbonate cage of 16 300 cm^3, and all included steel lattice covers with a section for feeding, steel label holders, and plastic drinkers with tips. The animals received feeding by a certified balanced granular feed for rodents (Ltd Laboratorkorm, Russia) *ad libitum*. They also received filtered tap water *ad libitum* in standard autoclaved drinking bottles with steel tips. Special rooms for laboratory animal keeping allowed us to control environmental conditions (20–26 °C, relative humidity of 30–70 %). The rooms had a 12-hour lighting cycle while filtration rate of the room air volume was equal to 10 times per hour.

Behavioural activity of rats

Assessment of motor activity acts was carried out with a Laboras (Metris, Netherlands) device, which is a non-invasive system to automatically recognize and analyse the behavioural reactions of laboratory animals such as movement, immobility, vertical standing, grooming, drinking, eating, and locomotion [4]. As a result, the quantity and duration of these actions are recorded. Our study measured the motor activity for 15 minutes.

Morphological study of the cerebral cortex

After recording the behavioural reactions, the animals were euthanized in a gas chamber to remove the brain. Fixation of brain in Carnoy's liquid and section staining was carried out according to the approach we have recently described [5].

The calculation of the nerve cell quantity and size was performed by an Axiolab microscope with an installed Axiocam camera and AxiOVision image formation system (Carl Zeiss, Germany). The evaluation of nerve cell content in the striatum of rats included a registration of nerve cell quantity and vertical size by methods of morphometric analysis for different ages and sexes [6, 7, 8]. Two brain sections of every animal were made, while 12 fields of vision for each were processed. We identified the structures with the help of the G. Paxinos & C. Watson atlas [9].

Statistical analysis

Assessment of motor activity and calculation of nerve cell quantity and size were carried out for each of the 6 groups. The normal nature of distribution was

[1] https://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm (date of access: 17.03.2020).

evaluated by the Kolmogorov–Smirnov test. The data are represented as medians and interquartile range (25th, 75th percentile). Comparison of the groups involved Mann–Whitney *U*-test.

The relations of the parameters were studied by correlation dependence. We calculated correlation and determination factors, while relation force was evaluated by Cheddok scale. The coefficient of determination was calculated to confirm the correspondence of the model to the values of obtained data. General tendencies in the dynamics of parameters were calculated by regression analysis with a trend drawing (linear and polynomial) [4]. We evaluated data about nerve cell quantity in the striatum (12 averaged values) and motor activity analysis (2 records from any animal).

For statistical data processing, we used Microsoft Excel software (Microsoft, USA) and Origin Pro (Origin-Lab Corporation, USA).

RESULTS

Morphological features of the striatum

Brain sections (cresyl violet Nissl staining) of ventral portions in basal ganglia include many transverse nerve fibres (turned caudally) with some clusters of neurons among them. The cells are usually round with a light colour of the cytoplasm; their processes are not contrasted well. The maximal nerve cell content is found at 7 months in males and gradually decreases in older individuals. The number of neurons in females increases earlier but slightly decreases after that. Brain sections of 7-month-old rats show an increase of pyramidal nerve cell quantity where the cytoplasm contains basophilic granules. The striatum experiences an age-dependent decrease of total nerve cell number (Fig. 1).

Values of quantitative and dimensional parameters for striatal neurons in rats of different age and sex groups show the dynamics of basal ganglia development during the ontogenesis. According to our data, nerve cell size remains the same in any sex and age group.

The data (in the table) indicate the heterogeneity of striatal neuron number for rats of different age and sex groups. The nerve cell content of basal ganglia in 2-month-old rats is various with a slight decrease at the age of 7 months in females. while in males of the same age, there was an increase of nerve cell number. By 16 months, a general decrease is typical for nerve cell number in both sexes.

Motor activity

The recording of motor activity allowed to describe the locomotor behaviour of rats, as well as to compare the data with nerve cell number in brain areas that are responsible for the regulation of the behaviour (especially striatum).

The study revealed 7-month-old males to exhibit a maximal mobility while the minimal one was typical for

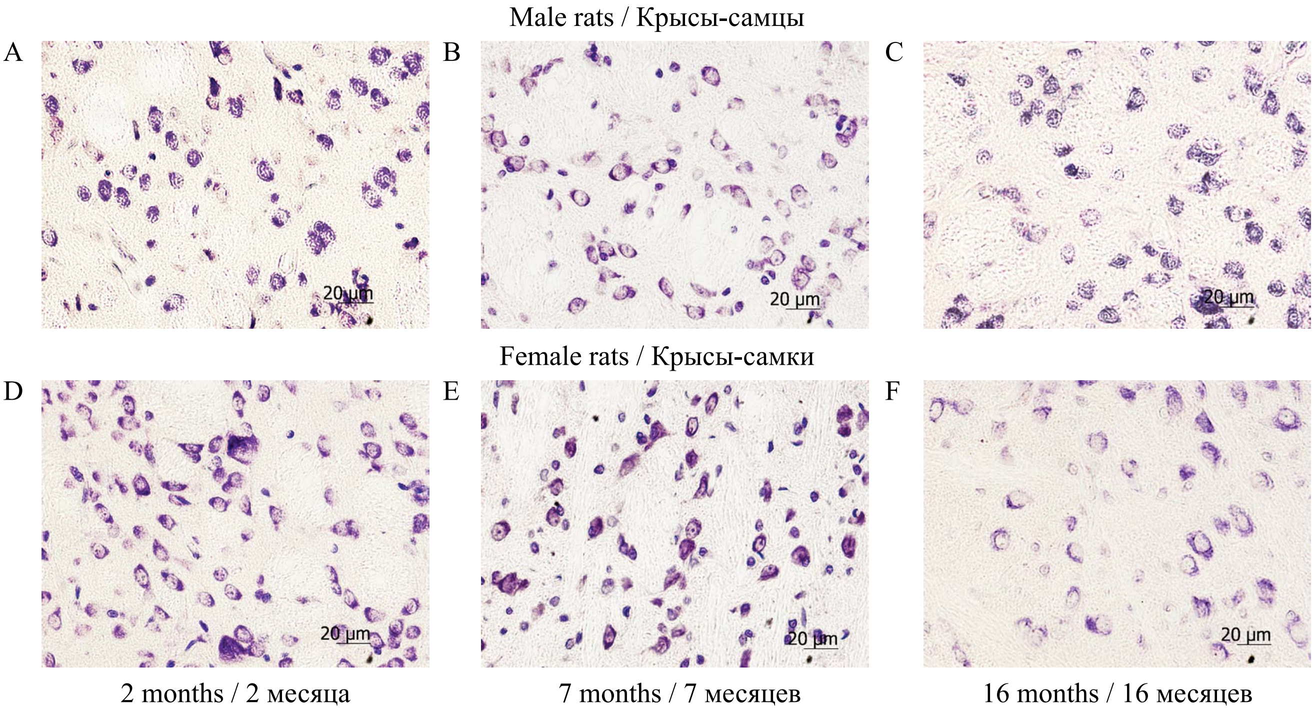

FIG. 1. The striatum fragments in rats for both sexes and different age groups. Cresyl violet stain, oc. 20, obj. 40.
РИС. 1. Фрагменты стриатума крыс обоего пола в разных возрастных группах. Окраска крезиловым фиолетовым, ок. 20, об. 40.

Table. **Nerve cell quantity and size in rat striatum for both sexes of different age groups**

Таблица. **Количество и размеры нейронов стриатума крыс обоего пола в разных возрастных группах**

Age, months / Возраст, месяцы	Nerve cell quantity / Количество нейронов		*p* value / Значение *p*	Nerve cell size, μm / Размер нейронов, мкм		*p* value / Значение *p*
	♂ (*n* = 18)	♀ (*n* = 18)		♂ (*n* = 18)	♀ (*n* = 18)	
2	43 (41; 45)	62 (59; 64)	<0,05	9 (9; 10)	9 (8; 11)	n.s.
7	61 (58; 65)	53 (50; 56)	<0,05	9 (8; 12)	9 (8; 11)	n.s.
16	52 (51; 53)	42 (41; 44)	<0,05	10 (9; 11)	11 (10; 11)	n.s.
p value / Значение *p*	<0.05	<0.05		n.s.	n.s.	

Note: n.s. – not significant.
Примечание: n.s. – not significant (не значимо)

16-month-old animals of both sexes. A similar pattern was found for average nerve cell number: the lowest value was registered in 2-month-old rats whilst highest was special for 7-month-old animals. In 16-month-old rats it decreased to become comparable with values in young rats.

Correlation between motor activity and nerve cell quantity in the striatum

To evaluate the locomotor activity of rats, we used the results of mobility recording. The correlation of this parameter with nerve cell number in the striatum is shown further (Fig. 2). The rats of both sexes expose a strong positive correlation at the age of 2 months, at 7 months it decreases, and reaches minimal values at 16 months.

The regression analysis demonstrated the strongest correlation between the content of neurons in the basal ganglia and motor activity in 2-month-old rats of each sex. The parameters also have a remarkable correlation in 7-month-old animals, while in 16-month-old rats such a phenomenon is not so popular with a *U*-shaped graph (Fig. 2).

DISCUSSION

Our data illustrate the changing influence of morphological and quantitative striatal parameters on animal behaviour between ages and sexes [10–13].

The basal ganglia of the brain are heterogeneous by their molecular content and functional role. These subcortical structures are involved in motor functions, decision-making, training, motivation, behaviour, and memory [10, 14].

The striatum functions as a primary impulse-generating centre of basal ganglia. It consists mainly of projective gamma-aminobutyric acid (GABA) nerve cells or medial spinous neurons. They are divided into two populations with separate final projective tracts, which are opposite to modulate the output structures of basal ganglia. The striatum also includes some number of interneurons, including cholinergic interneurons [15, 16].

The striatum receives the input data from the cerebral cortex and thalamus, sending it via the thalamus by associative and projective tracts back to the cortex and subcortical structures. Striatal region re-joins glutamatergic input with impulse of dopamine neurons from the midbrain to determine the vital role of the striatum in education and decision-making [17].

The motor cortex is a key structure of the frontal brain, and is responsible for motor skill education, voluntary motor activity, motor acts planning, and muscle memory [18–20]. The implementation of these specific functions is due to a remarkable plasticity, as well as to the tight links with other areas of the brain [21, 22]. In particular, morpho-anatomical structures of the motor cortex involve a neural network between motor cortex, premotor cortex, sensor regions of neocortex, and basal ganglia in rats [23, 24]. These relations between the cortex and basal ganglia in motor actions have been examined in a number of studies.

The basal ganglia are closely related to neocortex (especially motor cortex) via oligosynaptic loops. The signal ways of these loops mainly converge in motor areas of the frontal cortex and are mainly divided at the subcortical level. It means that there may be a functional relationship between striatum and motor cortex in motor functions [24].

The motor cortex and striatum in motor function and behaviour of rats are described by numerous studies. Mechanisms of links between these parts of the brain are also important besides the role of structures in the regulation of activity [25, 26]. It is considered that basal ganglia (BG) together with other subcortical structures (BG-subcortical pathway) are responsible for stereotypical movements and innate forms of behaviour in rats [19]. There are more and more facts about the role of striatum in the regulation of behaviour, for example, the influence of basal ganglia in motor acts of rats by trial-and-error training [11, 19] has been proved. Acquired motor skills (their formation and implementation) is under the control of the motor cortex, which carries out its control system through the thalamus (due to thalamocortical ways) [27–29].

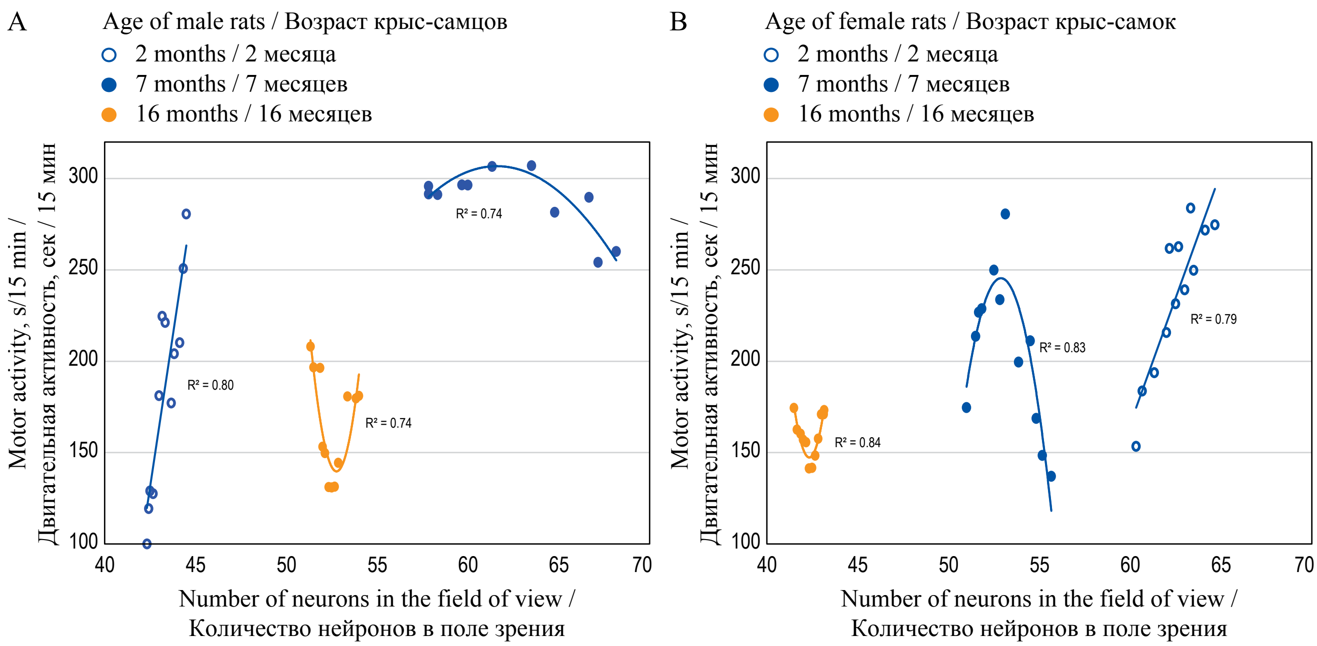

FIG. 2. Length of locomotor reactions and nerve cell quantity in the striatum of rats of both sexes in different age groups.
РИС. 2. Продолжительность локомоторных реакций и количество нейронов стриатума крыс обоего пола в разных возрастных группах.

Note. R^2 – coefficient of determination.
Примечание. R^2 – коэффициент детерминации.

There is also data about the role of the striatum in the development of various motor disorders associated with Parkinson disease [30, 31].

Many studies tried to identify the correlation between the number of neurons in the motor cortex and striatum and motor activity in rats of different age groups. Our previous study showed the presence of structural-functional connections between the motor cortex and motor activity, which was, however, more expressed in adult animals. At the same time, we may assume the principal role of the striatum in motor behaviour in young animals [32]. Most articles are devoted to the study of rats from birth to adolescence, since the greatest changes in their behaviour are observed in this period. Mengler et al. indicates that the brain of rats experiences a rapid growth during the first months after birth [33]. This is confirmed by a statistical assessment with the construction of a growth curve. Moreover, after 2 months of postnatal development, the volume of the brain does not change significantly. Analysing the volumetric graphs of the cortex and basal ganglia in the study, we can conclude that they show a significant increase from 3 weeks to 1-month, continuous growth from 1 month to 2 months, and the absence of significant changes from 2 months to 3 months. In the striatum, the time-dependent development scale in DWI (diffuse-weighted image) apparently corresponds to myelinization, which is visualized by histological study when identifying fibre bundles in nervous tissue sections stained with cresyl violet and BGII [33]. Despite the active use of immunohistochemical methods in the nervous system, classical neuromorphology remains a relevant approach to study the structures of the central nervous system [34].

Most researchers agree that the cortex and basal ganglia during the postnatal development of rats together determine behaviour in different age periods [23, 33]. The motor cortex of the brain in rats is influenced not only by peripheral afferents and prefrontal associative regions, but also by basal ganglia. The assessment of the level of these ascending influences (basal ganglia and cortex) requires tests in different age groups such as motor tests of balance, navigation in labyrinths, punching, as well as the study of the sexual behaviour of rats (during puberty at the age of 6–7 months) [35].

According to the study results, the regulation of motor activity in many brain regions is principally due to the nerve cell number in subcortical structures (striatum) of young animals (2 months). At the same time, there is an age-dependent increase of positive correlation between locomotor activity and the number of neurons in the structures of the cerebral cortex [18, 20, 21, 24]. This confirms our previous data about the role of the motor cortex in motor behaviour in rats during ontogenesis for different sexes [32].

CONCLUSION

The number of neurons in the striatum is sex- and age-related, while their size remains unchanged for the studied life points. For animals of both sexes, an age-dependent decrease of striatal role in motor activity is noted. This parameter is maximal in 2-month-old rats, slightly decreasing by 7 months and further by 16 months of life.

The pattern can be explained by various morphofunctional processes of the cortex in young individuals at antenatal ontogenesis. As a result of brain structure development in the first month of postnatal life, the dominant role in the regulation of behaviour (motor activity in particular) passes from the subcortical structures to the cortical ones.

AUTHOR CONTRIBUTIONS

Gennadii A. Piavchenko participated in the planning of the experiment and its implementation. Varvara A. Kudryavtseva and Aleksandra V. Moiseeva studied the literature, analyzed its data and wrote an introduction and discussion of the article. Svetlana G. Mukhamedova and Sergey L. Kuznetsov exercised overall control over the implementation of the study, participated in the discussion of the data obtained, general editing of the text of the article. All authors approved the final version of the publication.

ВКЛАД АВТОРОВ

Г.А. Пьявченко участвовал в планировании эксперимента, его проведении. В.А. Кудрявцева и А.В. Моисеева изучили литературные источники, провели анализ данных литературы и написали введение и обсуждение статьи. С.Г. Мухамедова и С.Л. Кузнецов осуществляли общий контроль над выполнением исследования, участвовали в обсуждении полученных данных, общем редактировании текста статьи. Все авторы утвердили окончательную версию статьи.

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