Research Article

The neuronal density in the rostral pole of substantia nigra pars compacta in Wistar Albino rats from Rijswijk rats: A link to spike-wave seizures

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Abstract

This study aimed to investigate the role of the nigrostriatal dopaminergic system in the modulation of absence epilepsy. Immunochemical analysis of the rostral pole of the substantia nigra pars compacta (SNpc) was conducted on 13 adult male Wistar Albino rats from Rijswijk rats. The rostral pole of the SNpc included the dorsal and lateral parts. The neuronal density in the dorsal part was higher than in the lateral part. The ratio of dopaminergic to non-dopaminergic neurons in the lateral part of the SNpc was 1:1, while in the dorsal part, it was around 1.9:1. All rats exhibited spontaneous spike-wave discharges (SWDs) on their electrocorticograms. SWDs are known to be a hallmark of absence seizures in both human patients and rat models. In this study, we found that the number and duration of SWDs were negatively correlated with dopaminergic and non-dopaminergic neurons only in the lateral part of the SNpc. However, in the dorsal part of the SNpc, no correlations were found between neuronal density and the severity of absence epilepsy. Our findings suggest that the lateral SNpc may be involved in modulating the severity of absence epilepsy in genetically prone subjects. This contributes to a better understanding of the role of the nigrostriatal dopaminergic system in the absence of epilepsy.

Keywords: Absence epilepsy, Electrocorticography, Substantia nigra pars compacta, WAG/Rij, Tyrosine hydroxylase, Spike-wave discharges

1. INTRODUCTION

Absence epilepsy is a genetically pre-determined, non-convulsive type of epilepsy that falls under the category of idiopathic generalized epilepsies (IGEs), according to the recent classification established by the International League against epilepsy [1]. There are four types of IGE: childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and epilepsy with generalized tonic–clonic seizures. These syndromes have some similarities in their clinical presentation, but they share common electroencephalographic (EEG) patterns, such as a normal background activity with 2.5–6 Hz generalized spike-wave discharges (SWDs) [2-4]. The genetic Wistar Albino rats from Rijswijk (WAG/Rij) rat model are well-established and widely recognized model for studying absence epilepsy [5-10]. This rat model displays face, predictive, and construct validities, which are essential for models of human diseases [5,7,8]. WAG/Rij rats are genetically predisposed to developing SWDs (face validity) [6,11,12]. Antiabsence pharmacotherapy has shown similar effectiveness in WAG/Rij rats as in human patients, demonstrating the predictive validity of this model [7,13-16]. WAG/Rij rats are known to have a broader spectrum of the disease beyond just the epileptic phenotype (the construct validity) [9,17,18]. The usage of WAG/Rij rats in biomedical research offers a unique opportunity to explore the neurobiology underlying the absence of epilepsy.

Electrocorticograms (ECoGs) in WAG/Rij rats exhibit spontaneous high-voltage 7–10 Hz SWDs, which are considered hallmarks of absence epilepsy [6,8,19]. The morphology of SWDs in WAG/Rij rats is similar to that in human patients [12,20]. Abnormal high-voltage spike-wave oscillations are known to be modulated by dopamine [21-23]. The role of dopamine in the modulation of SWDs remains uncertain as demonstrated in the WAG/Rij

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rat model [21-27]. There are two major dopaminergic systems in the brain: mesolimbic and nigrostriatal [28-30]. The mesolimbic dopaminergic pathway involves dopamine neurons in the ventral tegmental area that projects to the nucleus accumbens and other brain regions. The mesolimbic system is mainly involved in the regulation of emotions, motivation, rewards, and addictions [31,32]. The nigrostriatal dopaminergic pathway originates from the substantia nigra pars compacta (SNpc) and projects to the dorsal striatum. The nigrostriatal system plays a crucial role in controlling voluntary movement as part of the basal ganglia circuitry [33].

We studied the association between the severity of the absence of epilepsy and the cellular content of SNpc in WAG/Rij rats and obtained contradictory results [34,35]. In both studies, the SNpc was examined in brain slices stained with Nissl. A positive correlation was found in 6 WAG/Rij rats aged 10 months between the number of neurons in the SNpc (window 200 × 200 μm) and the number of SWDs [34]. An opposite, negative correlation was observed in 9 epileptic WAG/Rij rats between the total number of neurons in SNpc and the number of SWDs [35]. These studies have several shortcomings: (1) Nissl stained all cells, including dopaminergic and non-dopaminergic neurons; (2) they had a low number of subjects and a high variation in seizure severity; and (3) the morphological heterogeneity of the SNpc was ignored. The current study overcame previous limitations by implementing an immunohistochemical (IHC) methodology and using a rigorous experimental design. In particular, first, for dopaminergic neuron identification, we employed tyrosine hydroxylase (TH) immunostaining. TH is a key enzyme involved in dopamine synthesis and serves as a reliable marker for dopaminergic neurons [36,37]. While TH is also present in noradrenaline neurons, it has been established that TH-positive neurons in the SNpc are exclusively dopaminergic [38]. Second, the current study involved 13 adult WAG/Rij rats that displayed spontaneous absence epilepsy. These rats underwent epidural recordings over 24-hperiod, allowing for long-term analysis of epileptic activity. Third, the current investigation conducted a more detailed morphological analysis of IHC-stained slices, focusing on the dorsal and lateral parts of the SNpc. These two parts were selected for analysis, with spatial heterogeneity of SNpc in humans and rodents taken into account [38-45]. The dorsal part of SNpc in rodents contains a unique DA1B subtype of dopaminergic neurons [40,41]. In addition, specific neuronal populations in the dorsal and lateral parts of SNpc differ in the expression of various TH gene expressions [44]. Conventionally, the SNpc has been considered a homogeneous structure, but recent evidence suggests that it is composed of multiple sub-regions with distinct morphological and functional properties [38,40,42-45]. In 2011, Khudoerkov *et al*. conducted a volumetric reconstruction of the SNpc in adult rat brains [46]. Their findings clearly indicated that dividing the SNpc into dorsal, ventral, lateral, and medial regions could provide a more in-depth understanding of its structure and function. Considering that the SNpc contains both dopaminergic and non-dopaminergic neurons, we used IHC+Nissl staining to analyze the detailed topography of the rostral pole of the SNpc and ascertain the dopaminergic profile of the neurons.

The present study aimed to investigate the complex role of the nigrostriatal dopaminergic system in the development of absence epilepsy. We examined the link between two neuronal populations in the rostral pole of the SNpc and the severity of absence epilepsy in symptomatic WAG/Rij rats. We analyzed the density of TH-positive and TH-negative neurons in the dorsal and lateral parts of the SNpc and their correlations with the severity of absence epilepsy. In this study, we selected specific parts of the SNpc based on morphometric parameters obtained from healthy Wistar rats [46], i.e., the dorsal and lateral parts, since the dorsal part is known to occupy around 75% of the SNC volume and comprised large, densely packed neurons arranged in two layers [46] and the lateral part of the SNpc comprised approximately 15% of the SNpc and contained large, slightly elongated neurons [46]. Our results shed light on the role of the rostral pole of SNpc in the intricate mechanisms underlying the absence of epilepsy.

2. MATERIALS AND METHODS

2.1. Animals

The research was carried out on 13 male WAG/Rij rats (body weight 400 – 500 g) that were bred and housed at the Institute of Higher Nervous Activity and Neurophysiology RAS in Moscow, Russia. The rats were kept in a vivarium and maintained under standard conditions with a 12/12 h light/dark cycle and with free access to food and water. All experiments were conducted in accordance with directive 2010/63/EU on the protection of animals used for scientific purposes. All parts of this study were approved by the Institutional Ethics Committee (protocol #4 from October 26, 2021, and protocol #1 from February 11, 2022).

2.2. EEG examination

At the age of 9–10 months, all animals were implanted with electrodes to record ECoGs. The surgery was performed under chloral hydrate anesthesia (DiaM, Russia, 4% solution in 0.9% NaCl) administered through intraperitoneal injection at a dose of 325 mg/kg. Lidocaine (20 mg/mL, Russia) was used for local analgesia to relieve pre-surgical pain because it has a rapid onset of action. Stainless steel screw electrodes were secured to the skull and placed epidurally over the right and left frontal cortices (AP 2; L ± 2.5) and occipital cortices (AP −5; L 4). All coordinates were given in millimeters relative

to the bregma. The reference electrode was implanted over the cerebellum. After the surgery, the rats were allowed to recover for a minimum of 10 days. Following the surgical procedure, the rodents were given intramuscular injections of metamizole (FSSCI Microgen, Russia) at a dosage of 25 mg/kg to manage pain. Afterward, they were housed individually to avoid any damage to the electrode connectors. Effort was made to reduce the distress and suffering of the animals to minimal levels.

Continuous ECoG recordings were performed in freely moving rats during a period of approximately 24 h. Each rat was placed in a plexiglass recording cage (25 × 60 × 60 cm). The rats' head electrode connector was linked to a multi-channel amplifier (PowerLab 4/35, ADInstruments, Australia) through a swivel contact. The recordings were digitized at a rate of 400 samples per second per channel, filtered between 0.5 and 200 Hz, and saved to a hard drive. The data were then processed in the time and frequency domain using LabChart v8 software.

SWDs were detected in ECoG recordings according to standard criteria established by van Luijtelaar and Coenen in 1986 [11]. SWDs were characterized by a sequence of bilaterally synchronous high-voltage repetitive spikes and waves with a frequency of 7–10 Hz and a minimum duration of 2 s. In the frontal channels, SWDs exhibited the greatest amplitude, and information derived from occipital signals was supplementary (Figure 1). To ensure accurate detection, we employed power spectrum analysis. The power spectrum of frontal SWDs exhibited a prominent 7 – 10 Hz component and its frequency harmonic at 14 – 20 Hz (Figure 1).

SWDs were visually identified based on time and frequency domain information during an 8-h period from 0 to 8 am (corresponding to the dark phase of the circadian cycle). The number and total duration of spike-wave activity were calculated, as well as the average duration of a single discharge.

2.3. IHC examination

Upon completion of the ECoG recordings, the rats were anesthetized with a lethal dose of chloral hydrate. Subsequently, the rats were perfused with heparinized saline, followed by buffered histological formalin. Afterward, the rats were decapitated. Their brains were quickly extracted and frozen in isopentane on dry ice. Coronal brain slices (20 µm thick) were cut using a cryostat (CTB 6, Medite GmbH, Burgdorf Germany) at −18°C. Brain slices were mounted on glass microscope slides (Superfrost®Plus Gold, Menzel GmbH, Braunschweig, Germany). The IHC analysis was performed on brain slices obtained at levels −4.8 and −5.2 mm relative to the bregma. At this level, the dorsal and lateral parts of the SNpc could be visualized (Figure 2A and B), as confirmed by the rat brain atlas [47] and the results of SNpc volume reconstruction obtained in Wistar rats [46].

IHC staining was performed using the anti-TH rabbit polyclonal antibody (Abcam, ab112) to identify dopaminergic neurons, following the standard protocol for frozen sections [48]. All reagents were applied manually with a pipette. All incubations were carried out in a humidified chamber to prevent tissues from drying out. To determine the working dilution of the primary antibody, a range of dilutions was tested, and 1:750 was found to be optimal. To block endogenous peroxidase, sections were pre-treated with 3% hydrogen peroxide in tris-buffered saline (TBS). Then, to block endogenous immunoglobulins, sections were pre-incubated in 10% normal serum with 1% bovine serum albumin in TBS. Finally, the primary antibody diluted in TBS was applied and incubated overnight at 4°C. To enhance visualization, the detection was performed using the rabbit-specific HRP/DAB (ABC) Detection IHC Kit (Abcam, ab64261), followed by a light Nissl staining with cresyl violet as the final step.

For the negative control, phosphate-buffered saline was used instead of the primary antibody.

We digitized the brain slices using a Keyence BZ9000E microscope and analyzed images with ImageJ software. For each rat, three consecutive slices were analyzed. The dorsal and lateral parts of the SNpc were identified on the basis of the rat brain atlas [47] (Figure 2A). We measured the area of each part and counted the number of TH-positive (brown) and TH-negative (blue) neurons (Figure 2A and B). Then, we calculated the neuronal densities for each area.

2.4. Statistical analysis

All data were presented as mean ± SD. The Kolmogorov–Smirnov test was used to test the normality of the distribution, with a $P > 0.05$ indicating normally distributed variables. The repeated measures analysis of variance (ANOVA) and Duncan *post hoc* test were used for the analysis of neuronal density. Pearson's test was utilized for correlation analysis.

3. RESULTS

3.1. Density of dopaminergic and non-dopaminergic neurons

TH was used here as a reliable marker for dopaminergic neurons [36,37]. Neurons that stained positive for TH were considered dopaminergic, while neurons that stained negative for TH and were visualized with Nissl staining were taken as non-dopaminergic neurons. The density of dopaminergic and non-dopaminergic neurons was analyzed separately on each of three slices per rat on the left and right sides using repeated measures ANOVA (Figure 3A and B). The factor "slice" was used as a within-effect factor in RM ANOVA, and it did not show significant interactions with TH reactivity ($F_{1;200} = 0.91$, $P > 0.05$). Therefore, IHC staining was homogenous across slices.

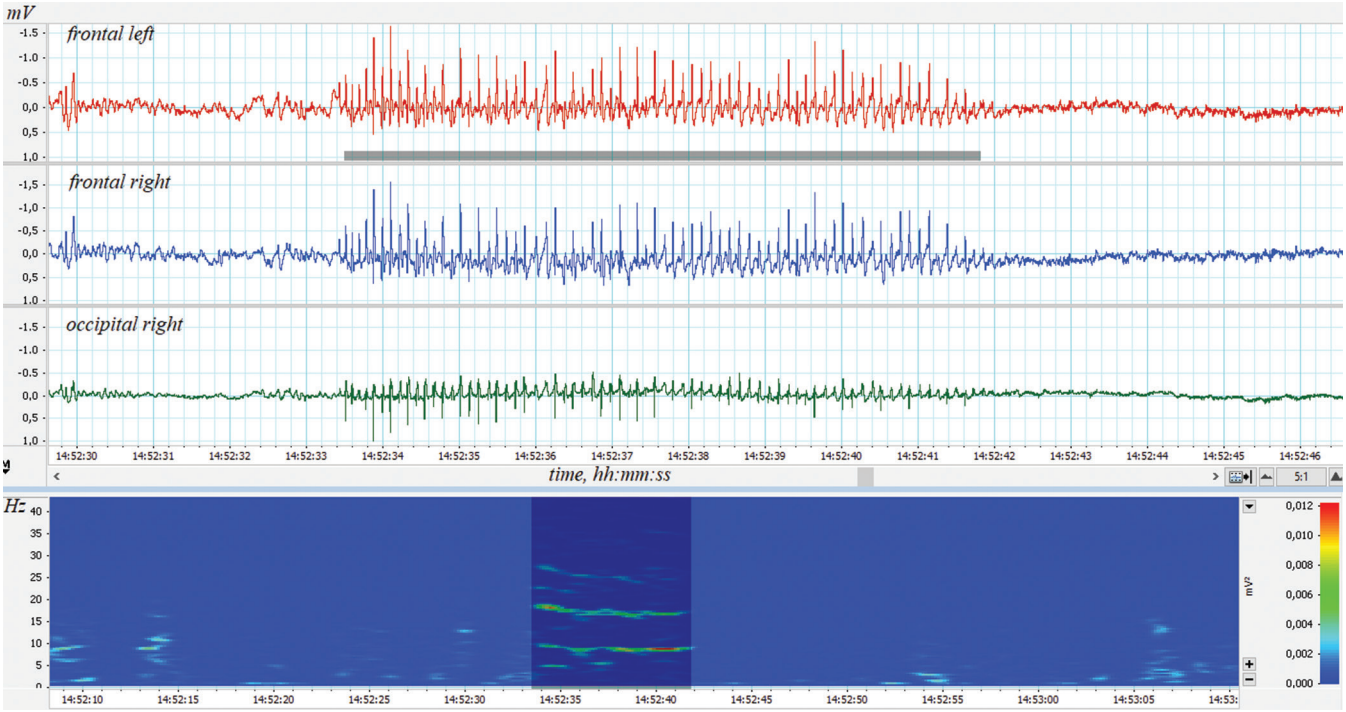

Figure 1. Spontaneous SWDs recorded in WAG/Rij rats were visualized in the time and frequency domains using LabChart v8 software. Top: A three-channel epidural ECoG recording obtained from the left frontal, right frontal, and occipital cortical locations. High-voltage SWD in the left frontal ECoG channel is highlighted. Please note bilaterally symmetrical waveforms of SWDs. Bottom: The power spectrum of the frontal left ECoG, in which the SWDs is highlighted.

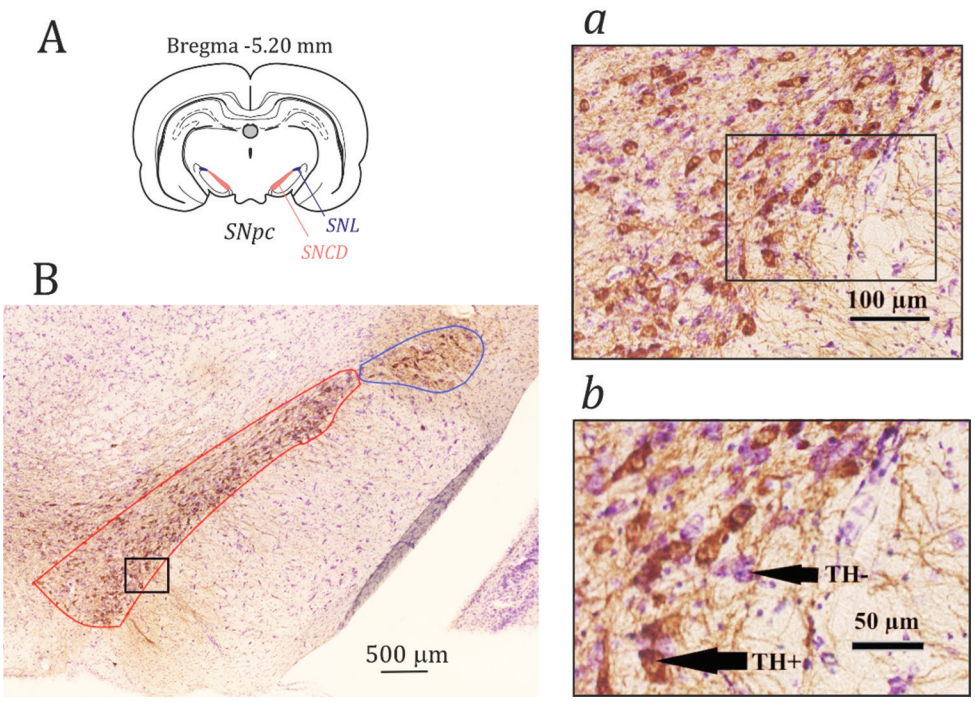

Figure 2. The cytomorphological analysis of the SNpc in rat brain slices. Brain slices were immunohistochemically stained with antibodies to TH and counterstained with cresyl violet (Nissl staining). (A) The schematic representation of brain slice obtained at the level of −5.20 mm from bregma. At this level, the SNpc included the dorsal part (SNCD) and the lateral part (SNL). The same abbreviations were used in Paxinos's Rat Brain Atlas [47]. (B) Section of native brain slice in which SNCD was outlined in red and SNL outlined in blue. The insertion *a* shows the enlarged fragment of B, in which TH-positive neurons are stained in brown and TH-negative are counterstained with cresyl violet in blue. The insertion *b* shows the enlarged fragment of *a*, in which TH-positive and TH-negative neurons are indicated by arrows.
SNpc: Substantia nigra pars compacta; SNCD: Substantia nigra compact part, dorsal tier; TH: Tyrosine hydroxylase; SNL: Substantia nigra lateral part.

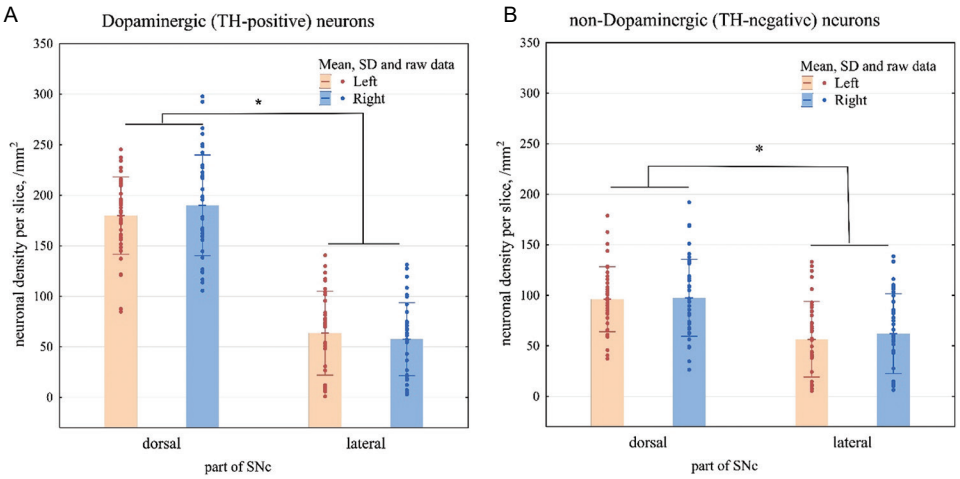

Figure 3. Density of neurons as measured in dorsal and lateral parts of the rostral pole of SNpc. Raw data points display all data obtained (three slices per rat/side, n = 13 rats). The density of both TH-positive neurons (A) and TH-negative neurons (B) was significantly higher in the dorsal part of the SNpc than in the lateral part. *significant differences with $P < 1\ e^{-6}$.
SNpc: Substantia nigra pars compacta; TH: Tyrosine hydroxylase.

No significant differences in neuronal density between the left and right hemispheres were found for either dopaminergic neurons ($F_{1;48} = 0.54$, $P > 0.05$) or non-dopaminergic neurons ($F_{1;48} = 0.08$, $P > 0.05$). Across both hemispheres, the dorsal part of the SNpc displayed a higher neuronal density compared to its lateral counterpart (Figure 3 and Table 1). This observation held true for both dopaminergic ($F_{1;48} = 193.3$, $P < 1e^{-6}$, Figure 3A) and non-dopaminergic neurons ($F_{1;48} = 34.7$, $P = 3.6e^{-6}$, Figure 3B).

Interactions between the factor of "part of SNpc" and "TH-reactivity" were significant ($F_{1;100} = 64.6$, $P < 1e^{-6}$), suggesting differences across TH-positive and TH-negative neuronal populations within each specific area. Duncan *post hoc* analysis was used for further statistical assessment, and Table 1 summarizes the results.

Our findings suggested that the rostral pole of the SNpc could be divided into two specific areas, each being characterized by the following features:

1. The dorsal part of the SNpc showed a higher neuronal density compared to the lateral part
2. In the lateral part of the SNpc, the ratio of dopaminergic to non-dopaminergic neurons was 1:1. In contrast, in the dorsal part, this ratio was approximately 1.9:1.

3.2. Neurons in the SNpc and spike-wave seizure activity

As no differences were found in neuronal density between the left and right hemispheres, the data were pooled together to calculate the average neuronal density per area and per rat. We conducted a correlation study using Pearson's method to analyze the relationship between neuronal density data from two separate areas in the SNpc and epileptic activity. To characterize epileptic activity, we computed the number of SWDs in 8 h' intervals (0–8 a.m., dark phase), the total duration of SWDs in 8 h, and the mean duration of a single SWD.

Table 1. The density of neurons (per square millimeter, mean±SD) as measured in the dorsal and lateral parts of the SNpc in symptomatic WAG/Rij rats at the levels of −4.8 and −5.2 mm relative to bregma [47]

Type of neurons	Part of the SNpc	
	Dorsal	Lateral
TH-positive	185±44	61±39*
TH-negative	97±35#	59±38**

Notes:#Significant difference found in the neuronal density between TH-positive and TH-negative neurons in the dorsal part of the SNpc (*post hoc* Duncan test, P=0.00012). *Neuronal density of TH-positive neurons in the lateral part was lower than in the dorsal part (*post hoc* Duncan test, P=0.00005). **Neuronal density of TH-negative neurons in the lateral part was lower than in the dorsal part (*post hoc* Duncan test, P=0.00006).
TH: Tyrosine hydroxylase; SNpc: Substantia nigra pars compacta.

Pearson's analysis (Figure 4) indicated the neuronal density in the lateral part of the SNpc bore significant correlations with the number of SWDs, with the total seizure activity, and with the average duration of a single discharge. In the lateral part of the SNpc, negative correlations were found for both dopaminergic and non-dopaminergic neurons (Figure 4, left plots). In the dorsal part of the SNpc, neuronal density showed no significant correlation with the measured parameters of SWDs (Figure 4, right plots).

4. DISCUSSION

In this study, we conducted an IHC analysis of the rostral pole of the SNpc and observed a heterogeneous distribution of TH-positive (dopaminergic) and TH-negative (non-dopaminergic) neurons. We examined the neuronal density in the dorsal and lateral parts of the SNpc in relation to the

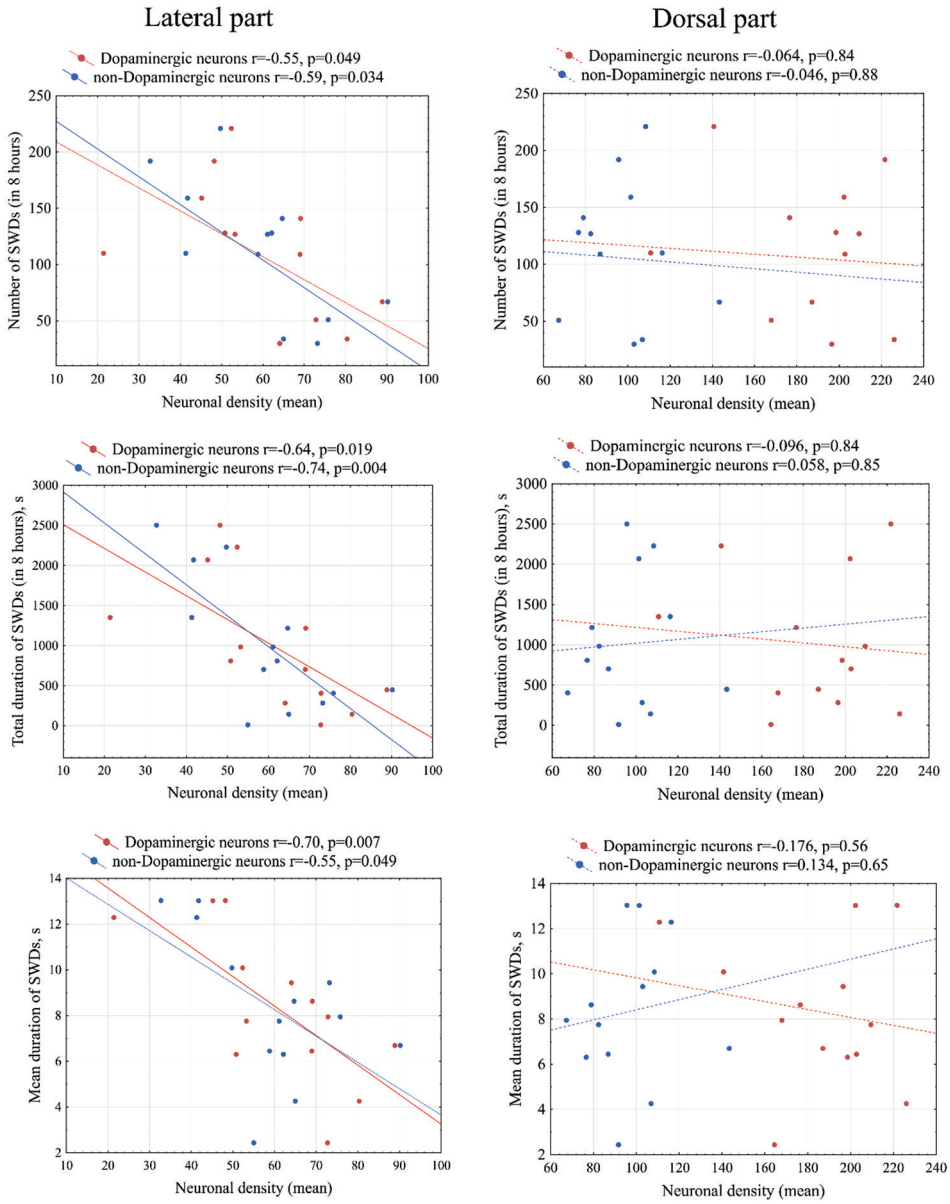

Figure 4. Pearson analysis revealed correlations between neuronal density in the rostral pole of SNpc and the parameters of spike-wave discharges (n=13 rats). In this pole, the lateral and dorsal parts of the SNpc were immunohistochemical-visualized for tyrosine hydroxylase and analyzed. Neuronal density (x-axis) refers to the number of neurons packed into a specific area of the SNpc. The correlation coefficients and *P*-values are shown above each corresponding plot.
SNpc: Substantia nigra pars compacta.

severity of absence epilepsy in symptomatic WAG/Rij rats. Our primary finding was that the density of neurons (both dopaminergic and non-dopaminergic) in the lateral SNpc, but not the dorsal SNpc, was negatively correlated with the number of epileptic discharges and the total duration of epileptic activity. This implies that subjects with more severe epilepsy had less neurons in the lateral SNpc and the lateral SNpc might be involved in the modulation of the severity of absence epilepsy in genetically-prone subjects.

In this study, IHC analysis of the rostral pole of the SNpc in WAG/Rij rats revealed two distinct parts within this structure: the dorsal part and the lateral part. Volume reconstruction of SNpc in male Wistar rats weighing 200–220 g showed that dopaminergic (TH-positive) neurons were concentrated in three major parts: dorsal, lateral, and ventral [46]. In the above-mentioned study, the greatest ratio of dopaminergic to non-dopaminergic neurons was found in the dorsal SNpc, being 1.45:1, which is consistent with our data. Within the lateral SNpc, the ratio of dopaminergic to non-dopaminergic neurons was nearly 1:1. In contrast, the dorsal SNpc exhibited a ratio of 1.9:1 between dopaminergic and non-dopaminergic neurons. In the lateral SNpc, this ratio in Wistar rats was 0.54:1 [46], but in our WAG/Rij rats, it was 1:1. The difference

between our results and those reported in Wistar rats [46] could be ascribed to the influence of age and epilepsy factors. Another key distinction was that the aforementioned study in Wistar rats presented the data of the entire SNpc, while our analysis was limited to the rostral pole of the SNpc.

Our data suggest that the nigrostriatal dopaminergic system might modulate the absence of seizures in genetically prone subjects. We found a remarkably prominent negative correlation ($r = -0.7$) between the neuronal density in the lateral SNpc and the duration of SWDs. This implies the potential involvement of the lateral SNpc in the sustenance of epileptic spike-wave activity. The role of the dopaminergic pathway in absence epilepsy was studied in rats with spontaneous absence epilepsy, including WAG/Rij rats, apomorphine-susceptible (APO-SUS), and apomorphine-unsusceptible (APO-UNSUS) rat lines of outbred Wistar [21,23]. In 2000, de Bruin *et al*. demonstrated that the absence of epilepsy was more severe in APO-SUS rats than in WAG/Rij rats and APO-UNSUS rats. In other words, the distribution of seizure severity was in the following order APO-SUS rats > WAG/Rij rats > APO-UNSUS rats [21]. In 1992, Cools and Peeters investigated SWDs over 48-h intervals in APO-SUS [23] and concluded that both a relatively low dopaminergic activity of the nigrostriatal system and a relatively high dopaminergic activity of the mesolimbic system underlay the higher incidence and duration of SWDs in APO-SUS rats [23]. In studying the degeneration of the nigrostriatal dopaminergic pathway in Genetic Absence Epilepsy rats from Strasbourg, an injection of 6-hydroxydopamine hydrobromide into the medial forebrain bundle led to a significant increase in both the total duration and the average duration of SWDs compared to control values [49]. Our results showed that in drug-naïve WAG/Rij rats, the number and duration of SWDs were negatively correlated with neuronal density only in the lateral part of the SNpc. Interestingly, both dopaminergic and non-dopaminergic neurons in the lateral SNpc appeared to be involved in the modulation of the severity of absence epilepsy in WAG/Rij rats. Future studies are required to disclose intricate mechanisms underlying SNpc-related modulation of absence epilepsy. This will help us better understand the complex interactions between dopaminergic and other neurotransmitter systems within the basal ganglia-thalamocortical neuronal circuitry.

In this study, we found that the neuronal density (both dopaminergic and non-dopaminergic neurons) in the lateral SNpc was lower than that of the neurons in the dorsal SNpc. It is known that a single dopaminergic neuron in the SNpc may give rise to more than 300,000 axon terminals [50]. Therefore, even a slight decrease in the number of neurons can lead to serious consequences.

The correlation between neuronal density and disease severity is not a new concept. For instance, in the substantia nigra, this correlation has been studied extensively for the research of Parkinson's disease [39,51,52]. The novelty of our work lies in the fact that we were the first to link the severity of absence epilepsy with the morphological characteristics of different parts of the SNpc.

Here, we detailed the statistics on seizure activity in freely moving rats. Information about epileptic SWDs was collected over an 8-h period. Since the recordings were made using implanted electrodes, the signal-to-noise ratio of the recorded electrocorticographic signals was very high, and epileptic discharges were easily discernible in terms of both the time and frequency domains.

In this study, we conducted an in-depth analysis of brain slices stained with TH and counterstained them with Nissl, thereby providing crucial insights into the intricate neuroanatomical organization of the SNpc. Here, we analyzed only the rostral pole of the SNpc. Notably, both the dorsal and lateral parts of the rostral pole of the SNpc exhibited the presence of TH-negative neurons, which were effectively visualized through Nissl staining. Our previous studies, done only in the dorsal SNpc, showed somehow conflicting results, suggesting that it was highly probable that there was no significant correlation between the neuronal density and the severity of absence epilepsy for this structure. However, this did not correspond with the data about the disturbances of the dopaminergic nigrostriatal brain system. The present research not only stated the absence of correlations for the dorsal SNpc but also showed that deviations might simply relate to another part of SNpc.

5. CONCLUSION

This study demonstrated that the rostral part of the SNpc is heterogeneous in terms of TH-positive (dopaminergic) and TH-negative (non-dopaminergic) neurons. Within this region, the density of both dopaminergic and non-dopaminergic neurons was relatively lower in the lateral part than in the dorsal part. In the lateral part of the SNpc, the ratio of dopaminergic to non-dopaminergic neurons was 1:1, while in the dorsal part, this ratio was approximately 1.9:1.

Intriguingly, the density of neurons (both dopaminergic and non-dopaminergic) in the lateral SNpc, but not in the dorsal SNpc, exhibited negative correlations with the number of epileptic discharges and the total duration of epileptic activity. This suggests that the lateral SNpc, in contrast to the dorsal SNpc, may play a role in determining the degree of severity of absence epilepsy. Furthermore, a reduced density of both dopaminergic and non-dopaminergic neurons within the lateral SNpc could lead to impaired inhibitory control of the thalamocortical neuronal network.

The intricate relationship between the spatial distribution of midbrain dopaminergic neurons and their roles in the development of epilepsy is an important area of study. Further research is warranted to investigate and understand the distinct phenotypic characteristics of different subpopulations of nigral dopaminergic neurons.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

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Formal analysis: Evgenia Sitnikova
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Investigation: Lidia M. Birioukova, Darya A. Tsvetaeva
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Resources: Lidia M. Birioukova
Validation: Lidia M. Birioukova, Evgenia Sitnikova
Visualization: Evgenia Sitnikova
Writing – original draft: Evgenia Sitnikova
Writing – review & editing: Lidia M. Birioukova, Evgenia Sitnikova

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences (protocol № 4 from October 26, 2021, and protocol № 1 from 11 February 2022).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

Data used in this work are available from the corresponding author upon reasonable request.

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