

# Morphometric and densitometric characteristics of the brainstem locus coeruleus neurons nuclei in rats with experimental arterial hypertension

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

## Key words:

densitometry, carionometry, brainstem, locus coeruleus, arterial hypertension, rats.

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**The aim** of our study was to establish the morphodensitometric features of locus coeruleus (LC) neurons nuclei in the brain stem of rats with arterial hypertension of various origin (essential – SHR and endocrine-salt hypertension (ESH)).

**Materials and methods.** The study was carried out on 30 mature male rats: 10 control Wistar rats, 10 Wistar rats with modeled endocrine-salt AH (ESH) and 10 spontaneously hypertensive rats (SHR) with genetically determined hypertension. The histochemical method of staining with galocyanine-chrome alum by Einerson was used for the evaluation of morphodensitometric characteristics of the neurons nuclei: the nucleus area, the content and concentration of nucleic acids (NA) in the nucleus.

**Results.** It was found that the highest indices were observed in rats of the SHR line while in rats with ESH the content, RNA concentration and nucleus area decreased. In SHR rats with essential hypertension high synthetic activity was observed in the LC neurons with an increase of the content and concentration of NA.

**Conclusions.** Morphometric and densitometric characteristics of the of the brain stem LC in AH depend on the etiological factor and pathogenesis of hypertension. In essential AH of SHR there is a high synthetic activity in the brainstem LC neurons nuclei along with an increase in the content and concentration of NA. In symptomatic AH in rats with ESH, on the contrary, the structure activity is decreased and characterized by the reduction in nucleus size and NA content in it.

## Ключові слова:

денситометрія, каріометрія, мозковий стовбур, locus coeruleus, артеріальна гіпертензія, щури.

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## Морфоденситометричні характеристики ядер нейронів блакитної плями стовбура мозку щурів при експериментальній артеріальній гіпертензії

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**Мета роботи** – встановити особливості морфоденситометричних параметрів ядер нейронів блакитної плями стовбура мозку щурів при артеріальній гіпертензії різного генезу (есенціальній – щури лінії SHR, ендокринно-сольовій).

**Матеріали та методи.** Дослідження виконали на статевозрілих 30 щурах-самцях, серед них 20 тварин лінії Wistar, яких поділили на дві групи: 10 – контроль, 10 – змодельована ендокринно-сольова АГ (ЕСГ); 10 щурів лінії SHR із генетично детермінованою АГ. Для дослідження морфоденситометричних характеристик ядер нейронів блакитної плями (БП) використовували гістохімічний метод забарвлення галоціанін-хромовими галунами за Ейнарсоном. Оцінювали такі показники ядер нейронів: площа ядра, вміст і концентрація нуклеїнових кислот (НК) в ядрі.

**Результати.** Встановили, що найвищі показники спостерігали в щурів лінії SHR, а в щурів з ЕСГ показники вмісту, концентрації НК і площі зменшилися.

**Висновки.** Морфоденситометричні характеристики нейронів БП стовбура мозку при артеріальній гіпертензії залежать від її етіологічного чинника та патогенезу. При есенціальній АГ у щурів лінії SHR спостерігали високу синтетичну активність в ядрах нейронів БП стовбура мозку зі збільшенням вмісту та концентрації НК. При симптоматичній АГ у щурів з ЕСГ активність структури знижується та характеризується зменшенням вмісту НК і площі ядра нейрона.

## Ключевые слова:

денситометрия, каріометрия, ствол мозга, locus coeruleus, артериальная гипертензия, крысы.

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## Морфоденситометрические характеристики ядер нейронов голубого пятна ствола мозга крыс при экспериментальной артериальной гипертензии

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**Цель работы** – установить особенности морфоденситометрических параметров ядер нейронов голубого пятна ствола мозга крыс при артериальной гипертензии различного генеза (эссенциальной – крысы линии SHR, эндокринно-солевой).

**Материалы и методы.** Исследование проведено на половозрелых 30 крысах-самцах, среди которых 20 животных линии Wistar, разделенных на две группы: 10 – контроль, 10 – со смоделированной эндокринно-солевой АГ (ЭСГ); и 10 крыс линии SHR с генетически детерминированной АГ. Для исследования морфоденситометрических характеристик ядер нейронов голубого пятна (ГП) использовали гистохимические методы окраски галлоцианин-хромовыми квасцами по Эйнарсону. Оценивали следующие показатели ядер нейронов: площадь ядра, содержание и концентрация нуклеиновых кислот (НК) в ядре.

**Результаты.** В ходе исследования установили, что наиболее высокие показатели наблюдали у крыс линии SHR; у крыс с ЭСГ показатели содержания, концентрации РНК и площади уменьшились.

**Выводы.** Морфоденситометрические характеристики нейронов ГП ствола мозга при артериальной гипертензии зависят от ее этиологического фактора и патогенеза. При эссенциальной АГ у крыс линии SHR отмечена высокая синтетическая активность в ядрах нейронов ГП ствола мозга с увеличением содержания и концентрации НК. При симптоматической АГ у крыс с ЭСГ активность структуры снижается и характеризуется уменьшением содержания НК и площади ядра нейрона.

Arterial hypertension (AH) is one of the most common human chronic diseases. In Ukraine, according to epidemiological studies, age-standardized prevalence of hypertension in the urban population is 29.6 % both for men and women. Among the rural population the incidence of hypertension is higher – 36.3 %, in particular 37.9 % for men and 35.1 % for women. Also, the interest in this disease can be explained by its widespread, serious and frequent complications which lead to high lethality [1]. Considering the abovementioned, the relevance of this nosology comprehensive study of is beyond any doubt. Today, for further understanding of hypertension pathogenesis, the research of the functional activity of neurons which are the key centers of blood pressure (BP) regulation in AH of various origin is the object of many scientific studies [2–4]. One of such important central regulators of BP is locus coeruleus (LC) – the noradrenergic brainstem structure, the functional variety of which is well described in the scientific literature [5,6]. Indeed, it has been reported that its neuronal system plays an important role in the regulation of the sleep-wake cycle and BP, facilitates learning and memorizing processes as well as regulates pain sensitivity [7,8]. Also mechanisms of LC implication in BP control are described in various scientific works [9,10].

However, the issue of LC structure functional capacity in arterial hypertension of different etiopathogenesis, in our opinion, is not sufficiently covered in the scientific literature. The morphofunctional state of the neuron could be measured indirectly, without applying direct electrophysiological methods, by analyzing the nucleus size and nucleic acids (NA) concentration in it, which

demonstrates the overall synthetic activity and functional state of the cell [11,12]. Therefore, this study is based on the corresponding histochemical method application.

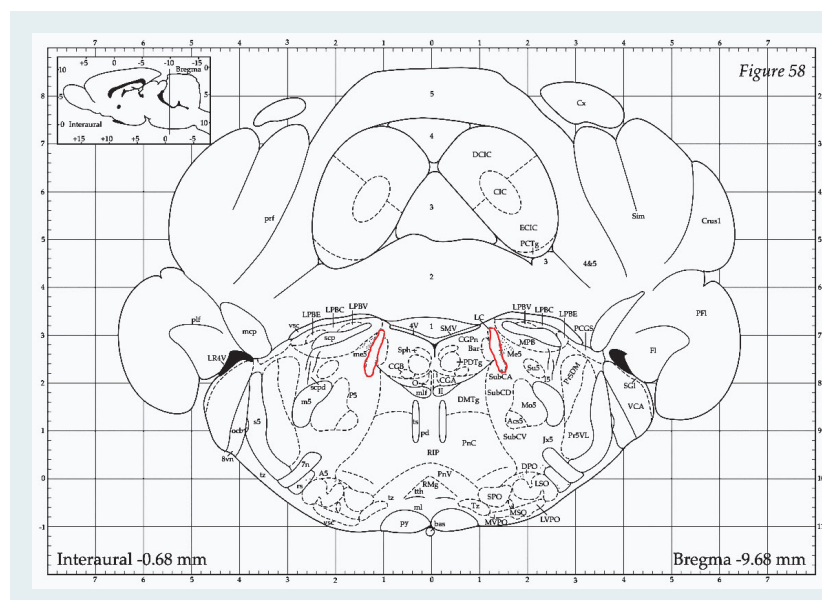
## Objective

To determine the peculiarities of morphometric and densitometric parameters of brainstem LC neurons nuclei in rats with AH of various genesis (essential – SHR and endocrine-salt hypertension).

## Materials and methods

The study was carried out on 30 mature male rats: 10 control Wistar rats, 10 Wistar rats with modeled endocrine-salt AH (ESH) and 10 spontaneously hypertensive rats (SHR) with genetically determined hypertension. To date, the SHR have been widely viewed as one that most closely corresponds to human essential hypertension [13].

ESH was induced by intraperitoneal injection of prednisolone (twice a day for 30 days: at 7 am – 2 mg/kg, at 20 pm – 4 mg/kg) with 5 ml of 2.3 % NaCl solution forced intake [14]. This model is similar to the secondary human AH in terms of endocrine abnormalities. The mean BP on the 21<sup>st</sup> day of modeled ESH was  $137.8 \pm 5.0$  mm Hg, while it was  $83.8 \pm 5.0$  mm Hg in the control. In SHR BP was  $125.8 \pm 5.0$  mm Hg throughout the monitoring period. At the end of experiment the animals were immediately sacrificed via decapitation after being anesthetized with aethaminalum-natrium at a dose of 40 mg/kg body weight intraperitoneally. The study object in experimental animals was the medulla oblongata. The experimental



**Fig. 1.** Topography of the brainstem LC structure in rats (by Paxinos G.). The rat brain in stereotaxic coordinates / G. Paxinos, Ch. Watson – Academic Press, 1998. – P. 474.).

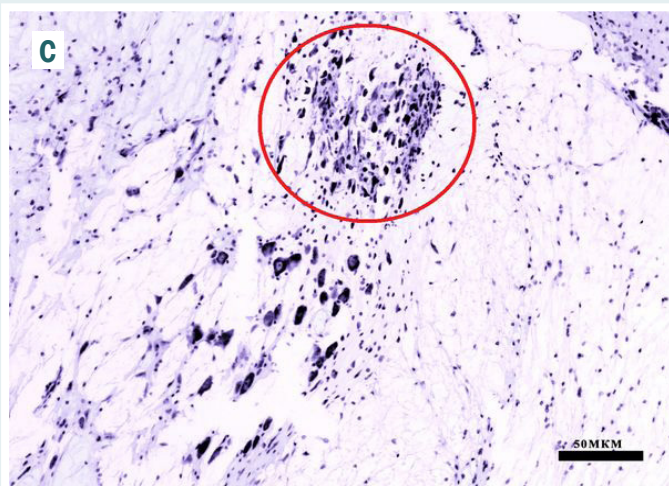
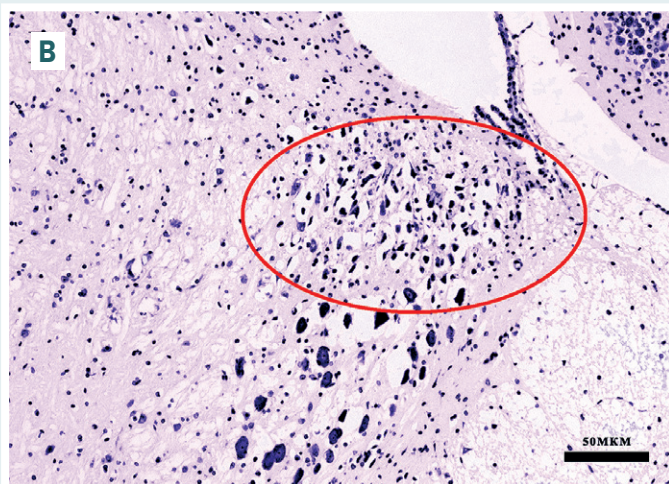
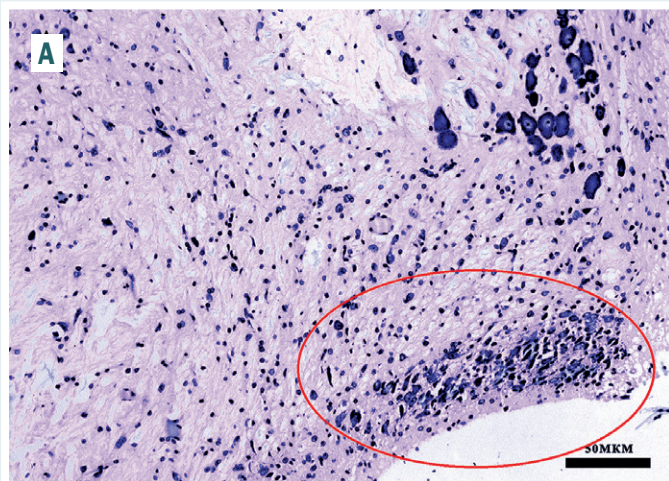


Fig. 2. Neurons of LC in rats of the experimental groups (stained with galloyanine-chrome alum by Einarson):

A: in the control group rats; B: in the SHR; C: in the rats with induced ESH.

part of the study was carried out exactly in accordance with the National "General Ethical Principles of Animal Experimentation" (Ukraine, 2001), in agreement with the Directive 2010/63EU of the European Parliament and

of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The topographical identification of the brainstem LC neurons was performed using the stereotactic atlas of the rat brain (Fig. 1) [15]. To determine the NA content in the nuclei of neurons (mainly the heterogenous RNA), the 5 μm sections were stained in galloyanine-chrome alum by Einarson [16] and mounted with Eukitt (O. Kindler GmbH & Co, Freiburg, Germany) (Fig. 2).

The study of sections stained for NA was performed in visible spectrum on the Axiolmager-M2 microscope (Carl Zeiss, Germany). The images taken with the COHU 4922 (COHU Inc., USA) sensitive camera were recorded as a computer file. The interactive mode was used to identify nucleus-containing neurons and the zone of "interest" whereas automatic calculations helped to find the morphometric and densitometric parameters of the nucleus – its area (μm<sup>2</sup>) and optical density of NA (Uif), which characterizes the NA content in the cell nucleus section, concentration of NA in the nucleus (Uif/μm<sup>2</sup>), which indirectly reflects the neuron functional activity, were calculated automatically. All these characteristics were determined for each neuron. At least 100 cells from each series were subjected to analysis. Microphotographs of the neurons were processed using the Image J software.

**Statistical analysis.** All experimental data obtained were calculated using EXCEL 7.0 (Microsoft Corp., USA) and AtteStat free software package [17]. The arithmetic mean value (M), its variance and the standard error of the mean (m) were calculated for all indicators. Parametric statistical methods (Student t-test for the sample with normal distribution) and non-parametric (Mann–Whitney test for the sample with non-normal distribution) were used to determine the reliability of differences in study results between the experimental and control groups of rats. The differences were considered to be statistically reliable at a value of P < 0.05 [18].

## Results

The study of morphometric and densitometric characteristics of the LC neurons nuclei has found some peculiarities in the indices of the experimental groups in relation to the control group as well as the differences between the experimental groups of rats with AH (Table 1, Fig. 2).

Thus, the content of NA in the LC neuron nuclei of SHR was reliably higher by 21.68 % in comparison with the control index. At the same time, the opposite dynamics was revealed in rats with ESH in relation to control animals, namely a reliable 14.23 % decrease in the nuclear NA content. The difference in NA content between the experimental groups was 29.51 % less in the rats with ESH than in the SHR group (Table 1).

As for the area of LC neurons nuclei, there were no reliable changes in SHR compared to the control group, while in the rats with ESH a reliable 12.65 % decrease in the nucleus area was observed. There was no reliable difference between the experimental groups (Table 1).

The NA concentration in the nuclei of LC neurons was also reliably 33.38 % higher only in the SHR compared to the control index while in the rats with ESH there was no significant difference with the control animals. There

**Table 1.** Morphometric and densitometric indices of the brainstem LC neurons nuclei in rats of the experimental groups (M ± m)

Group of rats / Characteristic under study	Control (n = 10)	SHR (n = 10)	ESH (n = 10)
NA content (Uif)	21.36 ± 0.59	25.99 ± 1.16*	18.32 ± 0.79#
NA concentration (Uif /μm <sup>2</sup> )	0.33 ± 0.03	0.44 ± 0.04*	0.35 ± 0.11#
Nuclear area (μm <sup>2</sup> )	70.14 ± 1.87	65.92 ± 2.62	61.27 ± 2.46*

\*: indicates a significant difference in the parameters (P < 0.05) of the experimental group rats relative to the control; #: indicates a reliable difference in the parameters (P < 0.05) between rats of the experimental groups with AH.

was a reliable difference between indices of the AH groups. In the SHR the NA concentration in the nuclei of LC neurons was 19.3 % higher compared to the rats with ESH (Table 1).

## Discussion

The results of these studies suggest that such differences in the morphometric and densitometric indices of the LC neurons nuclei of the experimental rats have resulted from different pathogenetic peculiarities of the AH development and course in SHR (known as a model of essential hypertension) and in the endocrine-salt model of symptomatic AH. The results obtained are supported by other studies. I. Kourtesis et al. have showed the significantly increased number of noradrenergic granules and their size in the LC neurons of SHR as compared to rats with normal BP, which proves this structure neurons activation in essential AH [19]. Concerning the indices of rats with induced symptomatic ESH, which significantly differed from the previous model, it should be noted that the induction was performed by repeated injections of prednisolone, which has a cortisol-like effect and causes a clinical manifestation of hypercorticism. It has already been demonstrated that chronic hypercorticism leads to a decrease in the content of biogenic amines, including norepinephrine (NE), in the brain [20]. The researchers explained that mainly by the increased monoamine oxidase-A (MAO-A) activity which accelerates the NE degradation. The fact of feedback between NE and cortisol has turned out to be interesting: a decrease in the content of NE increases the level of cortisol [21]. In other words, in this situation we observe the classic pathophysiological *circulus vitiosus*.

Taking into account other links of the ESH pathogenesis – hypernatremia and hypervolemia, it is worth pointing out that Svensson et al. have found by electrophysiological methods that the activity of LC neurons decreases with an increase in circulating blood volume. The researchers have explained that by the increasing activity of vagal afferents and atrial receptors, they also have shown that rat LC single neurons can correspondingly respond to even minor blood volume fluctuations, also suggesting that this structure is involved in circulating blood volume regulation [22].

To sum up, the possible decrease in the LC activity, as the primary source of NE in the brain, could be due to a high level of cortisol and a simultaneous increase in the inhibitory structures activity. Turning to our study, we see that the decrease in the NA content and the nuclear area of LC neurons in rats with ESH are in logical agreement with the results obtained by other researchers.

## Conclusions

Based on the results of our study, the following conclusions can be made:

1. Morphometric and densitometric characteristics of the of the brain stem LC in AH depend on the etiological factor and pathogenesis of hypertension.
2. In essential AH of SHR there is a high synthetic activity in the brainstem LC neurons nuclei along with an increase in the content and concentration of NA.
3. In symptomatic AH in rats with ESH, on the contrary, the structure activity is decreased and characterized by the reduction in nucleus size and NA content in it.

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