

RESEARCH ARTICLE

The differences in the structure of the motor nucleus of the medial gastrocnemius muscle in male and female rats

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ABSTRACT

There are many reports describing sexual dissimilarities in the CNS, particularly in the brain and cortical regions. However, knowledge regarding sexual dissimilarities in the spinal cord and in particular in the hindlimb muscle-motoneuron connectivity controlling locomotion is limited. In order to recognise sex differences in the architecture of the medial gastrocnemius (MG) motor nucleus in rats of the same age, retrograde-labelled motoneurons were identified following a bath of the proximal stump of the transected MG nerve in horseradish peroxidase. The rostrocaudal distribution of motoneurons along the spinal cord and on transverse sections as well as the size and density of motoneurons in the motor nucleus were determined from serial microscopic images. It was shown that the MG pool extended throughout the L4–L6 segments, with a length that was 32% greater in males. The position of the moto pool within the ventral horn of the spinal cord transversal sections was also different in both sexes: the pool was located more dorsally and laterally in males. Three size categories of motoneurons with different soma diameters were distinguished: α_1 (27.5–40.0 μm), α_2 (>40.0 μm) and γ (<27.5 μm). The density of α (α_1 and α_2) motoneurons as well as γ motoneurons was higher in females, by about 13% and 23%, respectively. The number of α_1 motoneurons was 8% higher in females, whereas there were 46% more α_2 motoneurons in males. The most significant differences in the distribution concerned α_2 motoneurons, which revealed a lower density in the rostral parts of the MG motor nucleus in females. It was therefore concluded that the length of the MG motor nucleus was shorter, whereas the mean density of α and γ motoneurons was higher in females.

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1. Introduction

Sexual dimorphism in the organisation of structures of the central nervous system is apparent within some nuclei of the brain in females and males and concerns neuronal differences, including a volume and cell number, morphology, neurotransmitter systems, molecular signalling, and gene expression (Ahmed et al., 2008; Lenz et al., 2012). Sex dissimilarities also occur in the thickness of the cerebral cortex (Luders et al., 2005) as well as in the tissue volume and proportions of grey to white matter in some regions of the human brain (Nopoulos et al., 2000). In comparison to the higher levels of the nervous system, little is known about sexually dimorphic differences at the spinal cord level. In particular, there are still

gaps in knowledge of distinctions in the motor innervation of the limb muscles, including locomotion muscles of the hindlimb. To our knowledge, only the paper by Leslie et al. (1991) described sexual dimorphism concerning spinal motoneurons innervating the rat foot muscle, the flexor digitorum brevis. These authors concentrated on alterations of the number and soma size of motoneurons and cross-sectional areas of the motor nucleus, i.e. the retrodorsolateral nucleus of the lumbar spinal cord, in an effect of hormone manipulation.

Motoneurons of the spinal cord innervating a given muscle are segregated into discrete columns of cell bodies in the ventral horn. The spinal localisation of the forelimb motor nuclei was studied in a number of species, e.g. iguana and mouse (Ryan et al., 1998; Tosolini et al., 2013), rat (Tosolini and Morris, 2012), cat (Fritz et al., 1986; Hörner and Kümmel, 1993), and humans (Routal and Pal, 1999). In contrast, the rostrocaudal distribution of motoneurons within the spinal cord, which innervate the hindlimb muscles, is known to a smaller extent. The available data concern the motor nuclei of the peroneus longus (Donselaar et al., 1985; Kernell et al., 1985), soleus

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(Burke et al., 1977), pelvic floor and proximal hindlimb muscles (Vanderhorst and Holstege, 1997), and lateral gastrocnemius muscles (Weeks and English, 1985) in cats, the gracilis, biceps femoris, gluteus maximus, vastus medialis, semitendinosus, vastus lateralis, gastrocnemius, and tibialis anterior muscles in the mouse (Mohan et al., 2014) and rat (Mohan et al., 2015), short plantar (Crockett et al., 1987) and tibialis anterior muscles in the rat (Peyronnard and Charron, 1983), as well as several frog (Hulshof et al., 1987) and turtle (Ruigrok and Crowe, 1984) muscles.

With regard to the medial gastrocnemius (MG) muscle, the spatial distribution of MG motoneurons in the motor nucleus was investigated in cats (Burke et al., 1977). In rats, Hashizume et al. (1988), when studying age-related changes in the number and size of motoneurons, revealed the extent of the MG motor nucleus along the spinal cord, whereas Peyronnard et al. (1986) showed the topographical arrangement of the spinal motor nuclei for six muscles including MG. Thus, it should be stressed that no comparison of the morphological organisation of the MG motor nucleus between males and females has been reported.

The MG muscle in the rat model has been a subject of both neuroanatomical and functional studies in our laboratory over a long period. In our recent report (Mierzejewska-Krzyżowska et al., 2014), we determined the differences in the number and size of α and γ motoneurons of the MG muscle in female and male rats. The study revealed that the MG motor nucleus in males contains more α motoneurons but a similar number of γ motoneurons, and that the size of α motoneurons is larger in males than in females.

The present study has focused on understanding the organisation of the motor nucleus innervating the MG muscle, which is crucial for knee joint flexion, in male and female rats, to recognise sex differences. In detail, the study concerns: (1) determination of the rostrocaudal extent of the MG motor nucleus, (2) the diversity of motoneuron size, (3) their distribution and participation in the structure of the MG motor nucleus, and (4) its topography. Taking into account the larger mass of the brain and spinal cord as well as the higher number of MG motoneurons in males compared to female rats (Mierzejewska-Krzyżowska et al., 2014), it is reasonable to expect that the MG motor nucleus also has a greater length in this sex. Until now, no attempt has been made to map the topographic positions of the MG motor nucleus in female and male rats. To accomplish this, the retrograde neuronal tracer horseradish peroxidase (HRP) was applied to the MG nerve using the physiological ability of transected axons to take up exogenous substances and carry them by axonal transport to the parent somata.

2. Materials and methods

All experimental procedures and animal care methods were performed in accordance with the European Union guidelines and the Polish Law on the Protection of Animals. All experimental procedures were approved by the Local Ethics Committee for Experiments on Animals in Poznań (Permission Number: 42/2011). Maximal efforts were made to minimise suffering of animals.

2.1. Animals

Six-month-old (young adult) Wistar rats, including eight males (mass 420–530 g) and eight females (mass 210–320 g), served as subjects. During the experiments, all animals were treated equally and housed under the same conditions. The animals were maintained in standard laboratory cages (two per cage) in an animal air-conditioned house with a constant temperature of $22 \pm 2^\circ\text{C}$, a humidity of $55 \pm 10\%$ and a 12:12 h light/dark cycle. The rats were fed ad libitum with a complete laboratory diet (certificated fodder, Labofeed B, Poland) and had free access to tap water throughout the study period, ensuring a balanced nutrient diet.

2.2. Surgery

The surgical procedures were carried out under general anaesthesia via an intraperitoneal injection of sodium pentobarbital (initial dose of 60 mg/kg, supplemented with additional doses of 10 mg/kg when required). The depth of anaesthesia was controlled by assessing withdrawal and pinna reflexes. Under aseptic conditions, the following steps of preparation were made to reach the nerve which supplies the MG muscle: (1) cutting the skin together with underlying muscles at the level of popliteal fossa, (2) isolation of the primary nerve branch to the MG muscle by microdissection, and (3) cutting the nerve branch close to the entry to the muscle. Immediately after this, the end of the proximal stump of the nerve branch was dipped for 90 min in a little hollow in a polystyrene plate which contained approximately 2 μl of a fresh solution of 30% HRP (Sigma type VI, catalogue number P8375) dissolved in sterile saline delivered using a Hamilton microsyringe. The wound was covered with a gauze pad infiltrated with a sterile Ringer's solution. After exposure to HRP, the MG nerve was washed with saline and the incision was closed with sutures. After that, the animals were returned to their home cages.

2.3. Perfusion

After a 72 h survival period, the animals were deeply re-anaesthetised and sacrificed by transcardiac perfusion with 500 ml of warm phosphate-buffered saline mixed with heparin (37°C , max. 15 min), followed immediately by 500 ml of cold fixative mixture (1.25% glutaraldehyde and 1.0% paraformaldehyde in 0.2 M phosphate buffer, pH 7.4, 4°C , max. 25 min) and finally with 500 ml phosphate buffer containing 10% sucrose at 4°C . After perfusion, the vertebral column was exposed from the dorsal side and the L1–L6 spinous processes were identified. Following a laminectomy performed sequentially from S1 to Th10 vertebra, the dorsal roots of the corresponding spinal segments became visible and were carefully isolated. Next, each spinal root from the right side (contralateral to the staining) was marked with ink at point of its entry to the dorsal horn of the spinal cord. The borders between L3/L4, L4/L5, L5/L6 and L6/S1 segments were defined as half-way between the adjacent roots and demarcated by a thin pin (Minutien Pins, FST) inserted into the dorsal white matter for easy identification during the cutting of sections. Then, the brain and the spinal cord were removed from the skull and the vertebral canal and weighed. The lumbo-sacral fragment (L3–S1 segments) was dissected out from the spinal cord and placed in cold 30% sucrose buffer for about 20 h for cryoprotection.

2.4. Histochemistry

The single block of the spinal cord, including the L4–L6 segments was cut in the transversal plane, into 90 μm thick serial sections, using a freezing microtome (L3 and S1 segments were not cut). All sections from individual segment were taken in serial order and each was collected separately in dishes containing 0.1 M phosphate buffer. Sections were processed histochemically to visualise HRP activity with the chromogen tetramethyl benzidine using the procedure reported by Mesulam (1978). After processing, sections were mounted on chrom-alum-gelatinised slides in serial order and then lightly counterstained with neutral red solution, cleared in xylene and cover-slipped with DePeX.

2.5. Identification of labelled motoneurons

Labelled motoneurons were counted in all serial sections (Jenaval, Carl Zeiss, Germany, bright-field illumination) under $10\times$ and $25\times$ magnification using a Sony CCD-Iris camera. The digi-

tised images were then transferred to the MultiScanBase system for Windows (Computer Scanning System, version 14.02, Warsaw, Poland). In addition, for determination of the position of the MG motor nucleus on transverse sections through the lumbar spinal cord, the Optiphot-2 (Japan) microscope with a Nikon DS-Fi1c camera and NIS-Elements Basic Research software was used. The number of labelled motoneurons was determined in each section and the sum of these enabled the total number of motoneurons in the MG motor nucleus to be established. The applied retrograde tracing technique method has certain imperfections. That is, there is the possibility of double-counting the labelled motoneurons in two adjacent sections, which can lead to the overestimation of motoneuron numbers. In this study, to minimise this error, HRP-labelled motoneurons were counted only when the nucleus, i.e. the central non-labelled region of the cell body, was noticeable (Hashizume et al., 1988). On the other hand, an underestimation of cell count can occur. This is difficult to resolve and emerges from the fact that not all axons exposed to the same degree of tracer take it up and transport it to cell bodies, because many factors can negatively influence the effectiveness of this mechanism (Keizer and Kuypers, 1984).

The calculation of an average soma diameter of motoneurons (one-half of the sum of the measured maximum and minimum orthogonal diameters) was performed according to the protocol of Burke et al. (1982).

2.6. Statistical methods

In order to evaluate sex differences in the structure of the MG motor nucleus in the rat, statistical analysis was performed using the Microsoft Excel 2010 and Statistica 12.0 software (StatSoft, Tulsa, OK, USA). The results are presented as means, standard deviations (SD) and variability ranges. P-values describing significance of differences are also reported.

The length of MG motor nucleus, number and density of motoneurons in MG motor nucleus in male and female rats were tested using the Shapiro–Wilk test (normality of distribution) and Levene's test (equality of variances), and the T-test was used to determine between-group differences.

The rostral, middle and caudal position of the MG motor nucleus in transverse sections through the spinal cord in both sexes was tested by Mann–Whitney U-test.

Statistical analysis of a rostrocaudal distribution of the three categories of motoneurons within the MG motor nucleus was performed using the T-test and ANOVA rank Kruskal–Wallis test, indicating significant differences between females and males.

3. Results

All of the retrograde-labelled motoneurons innervating the MG muscle in female and male rats were present in the dorsolateral region of the ventral horn of the ipsilateral spinal cord. They were visible through the L4–L6 lumbar segments. Motoneurons had intensively stained cell bodies and dendrites; for this reason, they were easy to identify. They were differentiated taking into account the soma size and number as well as rostrocaudal extension in the motor nucleus.

3.1. Distribution and density of motoneurons, and length of the motor nucleus

In order to analyse the distribution of motoneurons within the MG motor nucleus, the studied motoneurons were divided into three size categories: γ and α size, with α motoneurons being additionally divided into smaller (α_1) and larger (α_2).

Fig. 1A and B shows the distribution of the diameter of motoneuron bodies in one female and one male rat. The borderline value of diameter for γ/α categorisation was 27.5 μm , whereas for α_1/α_2 division it was 40.0 μm (Mierzejewska-Krzyżowska et al., 2014).

In the categorisation of α motoneurons, differentiation into α_1 and α_2 groups was established approximately halfway between the largest and smallest diameters. There was no difference in this value for the classification of motoneuron type between males and females.

In order to analyse the distribution of the size of three categories of motoneurons along the spinal cord, the motor nucleus for each animal was divided into five parts (I–V from rostral to caudal); each contained the same number of microscopic sections (from 3 to 10) depending on the total number of sections with labelled motoneurons. The total number of sections which were included in analyses concerning five successive sections was: 23, 24, 27, 29, 31, 35, 36, and 43 in female rats and 34, 38, 40, 42, 44, 48, and 49 in male rats for five parts from I to V, respectively. In each section, the number of labelled motoneurons was counted. Examples of the results for one female and one male rat are presented in Fig. 1C and D, respectively. In females, the MG motor nucleus contained labelled cells distributed within 27 sections, whereas in males this was within 40 sections. Thus, the rostrocaudal extension of the male MG motor nucleus was 32% larger than that in females. Microscopic images of HRP-labelled motoneurons from the middle part of the motor nucleus innervating the MG muscle in both sexes are shown in Fig. 1E for females and Fig. 1F for males.

Table 1 summarises data concerning the length of the MG motor nucleus and the density of motoneurons in this nucleus in both sexes. The MG motor nucleus in males was larger (on average 25%) than in females (difference was statistically significant).

The density of motoneurons in the MG motor nucleus was determined as the mean number of motoneurons per microscopic section. The results showed that for all three categories of motoneurons, the density was greater in females, i.e. 23% for γ motoneurons and 13% for α (α_1 and α_2) and also 16% for the sum of α and γ motoneurons (all differences were statistically non-significant).

3.2. Number and participation of three sizes of motoneuron categories in the structure of the motor nucleus

Table 2 shows the average number of α_1 and α_2 motoneurons in the MG motor nucleus of females and males. The number of α_1 motoneurons was slightly higher (about 8%) in the female motor nucleus, but the difference was not significant. The number of α_2 motoneurons, in turn, was significantly larger (difference of 46%) in males. The number of γ motoneurons (no sex differences) was determined in our previous paper (Mierzejewska-Krzyżowska et al., 2014).

Fig. 2A presents data on the percentage participation of the three size categories of motoneurons along the spinal cord that was divided into five (I–V) parts for the female and male MG motor nucleus. Statistical analysis (ANOVA rank Kruskal–Wallis) showed that there are differences between females and males. The differences were between the extreme parts and the middle part (I and III, and V and III) in the case of females and males. For γ motoneurons, they were the largest between parts I and III, and III and V in males, and for α_2 motoneurons between parts I and III, and I and IV in females (differences were statistically significant). The smallest differences (statistically non-significant) were observed in the distribution of α_1 motoneurons in both sexes. In both sexes, the higher number of motoneurons for each category was observed in the middle part of the motor nucleus, whereas the lower number of neurons was noted for parts I and V (Fig. 2B). The lowest number of motoneurons observed in the extreme parts of the MG motor nucleus, i.e. part I (the rostral pole) and part V (the caudal pole)

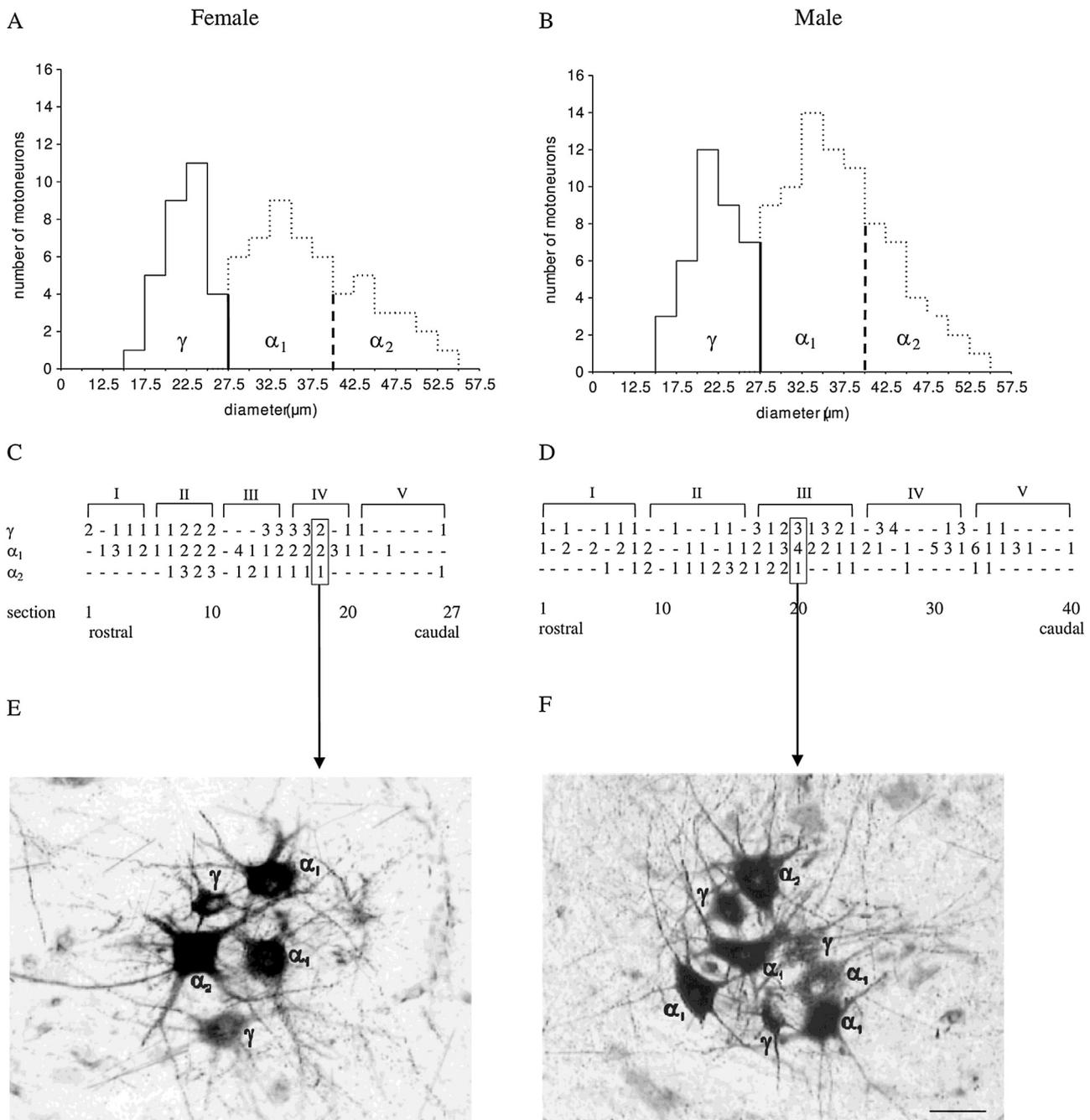


Fig. 1. Distribution of motoneurons of female (left) and male (right) rats. A and B, Sample histograms for diameters for one female (A) and one male (B) rat. Note the bimodal distribution of diameters that enabled them to be divided into γ (left peak in histogram, solid line) and α (right peak in histogram, dotted lines) motoneurons. The interrupted critical line at $40\ \mu\text{m}$ denotes α_1 and α_2 subpopulations. C and D, distribution of the three size categories of motoneurons in a scheme which illustrates the rostrocaudal extent of the MG motor column in female (C) and male (D) spinal cords. Numbers indicate the presence of motoneurons belonging to each of the three size categories and dashes denote a lack of them. The numbers of successive sections (1–27) and (1–40) are shown below. Brackets above data indicate sections included in parts I–V. E and F, Microphotographs of transverse sections through the MG motor nucleus of female (E) and male (F) rats taken from the middle part. Scale bar represents $40\ \mu\text{m}$ for E and F.

Table 1
Mean values, standard deviations and variability ranges for the length of the MG motor nucleus and the density of motoneurons in this nucleus in male and female rats. The significance of differences between male and female properties is indicated by asterisks: **—significant difference, $p < 0.01$; n.s.—non-significant difference, $p > 0.05$; T-test.

Sex	The length of the MG motor nucleus [mm]	Density of motoneurons in the MG motor nucleus [number of motoneurons per section]		
		$\gamma + \alpha$	γ	$\alpha (\alpha_1 + \alpha_2)$
Females (n = 8)	2.78 ± 0.62	2.82 ± 0.70	0.92 ± 0.21	1.88 ± 0.54
	1.98–3.87	1.85–3.83	0.58–1.25	1.1–2.72
Males (n = 8)	3.69 ± 0.56	2.36 ± 0.43	0.71 ± 0.26	1.63 ± 0.25
	3.06–4.77	1.88–2.95	0.33–1.14	1.33–2.02
	**	n.s	n.s	n.s

Table 2

Mean values, standard deviations and variability ranges for a number of α_1 and α_2 motoneurons in the MG motor nucleus and the α_1 / α_2 ratio in male and female rats. The significance of differences between male and female properties is indicated by asterisks: **—significant difference, $p < 0.01$; *—significant difference, $p < 0.05$; n.s.—non-significant difference, $p > 0.05$; T-test.

Sex	Number of $\gamma + \alpha$ motoneurons in MG motor nucleus	Number of α_1 motoneurons in the MG motor nucleus	Number of α_2 motoneurons in the MG motor nucleus	α_1 / α_2
Females (n=8)	83.7 ± 7.8 75–93	39.5 ± 6.4 33–52	15.8 ± 4.9 7–23	2.50
Males (n=8)	94.6 ± 8.5 85–109 *	36.5 ± 10.0 20–55 n.s.	29.4 ± 8.7 18–46 **	1.24

The data in the first column were published earlier (Mierzejewska-Krzyżowska et al., 2014).

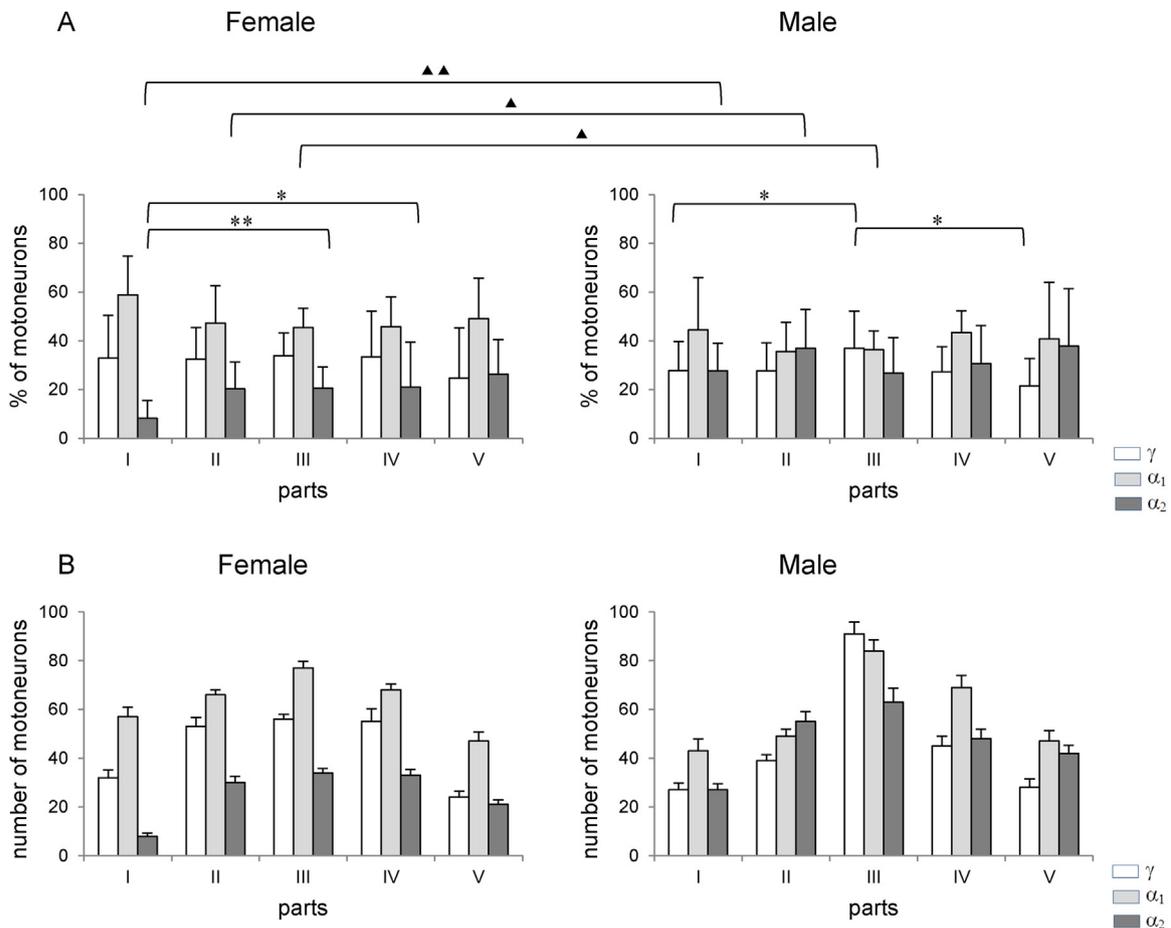


Fig. 2. Rostrocaudal distribution of the three categories of motoneurons within the motor nucleus. A, The distribution of the three types of motoneurons: γ and α_1 and α_2 in the MG nucleus in females (left) and males (right), expressed as a percentage in 5 parts of the motor nucleus. Part I is the most rostral, whereas part V is the most caudal. Data are presented as mean values (bars) and standard deviations (frame). The significance of differences between females and males is indicated by triangles: \blacktriangle —significant difference, $p < 0.01$; \blacktriangle —significant difference, $p < 0.05$; ANOVA rank Kruskal–Wallis as well as separately for females and males is indicated by asterisks: **—significant difference, $p < 0.01$; *—significant difference, $p < 0.05$, T-test. B, The total number of γ and α_1 and α_2 motoneurons studied in five parts of the MG motor nucleus in female (left) and male (right) rats.

in both sexes confirms the known observation that the number of cells in the motor nucleus decreases in the direction of both poles.

The present study shows that the number of motoneurons in the rostrocaudal extent of the motor nucleus was not the same for all three types in females and males (Fig. 2B). In order to compare the distribution of motoneurons within individual categories between females and males, the results were translated into percentages. Thus, concerning α_1 motoneurons in females, the largest percentage participation (25%) in the structure of the motor nucleus was in its central (3) part, while it was 18% and 15% in the rostral and caudal poles, respectively. The data for males were quite similar:

29%, 15% and 16% in the central part, and rostral and caudal poles, respectively. The largest α motoneurons, i.e. α_2 , had the same and highest share in both sexes (27%) in the central part of the nucleus and a very similar share in the caudal pole (18% females, 17% males). However, participation of this category of motoneurons in the rostral pole considerably varied and was 1% in females and 12% in males. With regard to γ motoneurons, they share a similar degree of rostral (16% and 12%) and caudal (11% and 12%) poles of the motor nucleus in females and males, respectively, but they were apparently more numerous in the central part in males (40% versus 26%).

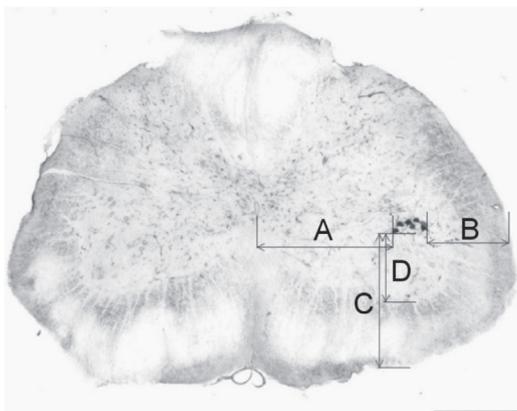


Fig. 3. Parameters describing the location of the motor nucleus. Photomicrograph of a transverse section through the rat spinal cord with HRP-labelled motoneurons of the MG motor nucleus in its middle level (part III). A = Horizontal distance from the extreme medial motoneuron of the MG motor nucleus to the ventral median fissure, B = horizontal distance to the outer margin of the cord from the extreme lateral motoneuron, C = vertical distance from the extreme ventral motoneuron to the ventral margin of the cord, D = vertical distance from the extreme ventral motoneuron to the dorsal margin of the white matter. Scale bar = 500 μm .

3.3. Topography of the motor nucleus

Fig. 3 demonstrates the position of the MG motor nucleus in a sample transverse section of the lumbar spinal cord and measured parameters on the basis of method by Ryan et al. (1997). Table 3 presents the results of four measurements made to determine the location of the MG motor nucleus in female and male rats. The mean value of the horizontal distance from the extreme medial motoneuron of the MG motor nucleus to the ventral median fissure (A) was 15% higher in males in the rostral portion of the nucleus (difference statistically significant), whereas in its middle and caudal portions, this value was statistically not significant in both sexes. The mean value of the horizontal distance to the outer margin of the cord from the extreme lateral motoneurons (B) was distinctly different in both

sexes in the caudal portion (difference was statistically significant) and 13% higher in females, whereas in its rostral and middle portions it was statistically not significant in both females and males. The vertical distance from the extreme ventral motoneurons of MG motor nucleus to the ventral margin of the cord (C) was 8% higher in males in the middle portion of the motor nucleus (difference statistically significant). The vertical distance from the extreme ventral motoneurons of this nucleus to the dorsal margin of the white matter (D) was greater in males than in females, and the differences were 17% in the middle portion, 15% in the caudal portion (both differences were statistically significant), and 11% (difference was statistically non-significant) in the rostral portion.

4. Discussion

The main and novel finding of the present study was the determination of differences in the structure of the motor nucleus innervating the MG muscle in female and male rats, which is longer for males, although the density of motoneurons is higher for females.

With regard to morphological and functional properties, two basic types of motoneurons, α and γ , can be distinguished (Bryan et al., 1972; Burke et al., 1982). In the present investigation, apart from γ motoneurons, two additional subpopulations, α_1 and α_2 motoneurons, were recognised within the α type, taking into consideration the soma size. Thus, the border value between γ and α motoneurons was 27.5 μm and between α_1 and α_2 motoneurons was 40 μm .

Concerning α motoneurons, to our knowledge, only the paper by Burke et al. (1977) referred to the division of α motoneurons into two subpopulations on the basis of their diameter in the MG nucleus, but only in female cats. The population with the smaller average soma diameter between 38.5 μm and 55 μm was called “small α ” and the other population with diameters larger than 55 μm was termed “large α ”. The percentage share of small α cells amounted to 36% and large α cells to 38%. The γ motoneurons constituted the remaining percentage (26%). The two populations of

Table 3
Mean values and standard deviations for the rostral, middle and caudal position of the MG motor nucleus in transverse sections through the spinal cord in male and female rats. A–D, See explanation in Fig. 3. The significance of differences between females and males properties are indicated by asterisks, **—significant difference, $p < 0.01$, *—significant difference, $p < 0.05$, n.s.—non-significant difference, $p > 0.05$, Mann–Whitney U-test.

Sex		Rostral [μm]	Middle [μm]	Caudal [μm]
Female (n=8)	A	649.1 \pm 79.7	697.9 \pm 46.1	642.2 \pm 101.6
	B	474.8 \pm 43.1	434.9 \pm 74.6	457.8 \pm 90.3
	C	679.2 \pm 100.3	578.9 \pm 115.2	606.3 \pm 75.7
	D	302.5 \pm 52.3	268.9 \pm 63.2	236.2 \pm 42.7
Male (n=8)	A	764.1 \pm 82.6	696.5 \pm 79.8	645.9 \pm 48.1
	B	467.5 \pm 69.4	436.8 \pm 53.4	397.7 \pm 33.3
	C	632.1 \pm 45.65	632.3 \pm 101.1	638.3 \pm 58.6
	D	338.9 \pm 34.5	322.9 \pm 59.7	278.9 \pm 47.2

small α and large α cells correspond to α_1 and α_2 motoneurons in the present study, respectively. In comparison to our study in female rats, the participation of α_1 motoneurons in the structure of the MG nucleus was higher and amounted to 48%, whereas it was lower for α_2 motoneurons, amounting to 19%.

In the paper by Muramatsu et al. (2012), the number and size of MG motoneurons in 22 week-old male rats in the diabetes and control groups were compared. The variation in the total number of MG motoneurons in control animals was between 95 and 136. The score was inconspicuously higher than in our previous study in male Wistar rats aged 6 months, where the variation in the total number of α and γ motoneurons innervating the studied muscle was 85–109 (Mierzejewska-Krzyżowska et al., 2014). In addition, interesting observations concerning the similar distribution of the average soma diameter of motoneurons in the MG nucleus were made. Both in control rats (Muramatsu et al., 2012), and the rats used herein, the border between the groups of smaller γ and larger α motoneurons was 30 μm and 27.5 μm , respectively. The range in soma size of the γ and α motoneurons was similar as well, ranging from 14 to 30 μm and 30 to 56 μm (Muramatsu et al., 2012), and 12.5 to 27.5 μm and 27.5 to 55 μm in our present and previous studies (Mierzejewska-Krzyżowska et al., 2014).

In a previous study on the anatomy of the motor nuclei for hindlimb muscles, i.e. MG and soleus (SOL) in female cats, the number and size of motoneurons were identified (Burke et al., 1977). For both muscles, the similar bimodal distribution of γ and α motoneurons (with a transition at 35–40 μm between small and large cell subpopulations) was evident. The cut-off point for the MG and SOL was arbitrarily assumed at 38.5 μm to distinguish between γ and α motoneurons. The smallest MG motoneurons had a minimal diameter of 18 μm and the largest ones a maximal diameter of 78 μm . The average number of motoneurons in the MG motor nucleus was 174, including 44 γ and 130 α , which was confirmed by the latter observations concerning the proportion of these types of motoneurons. Namely, a similar proportion of γ and α motoneurons, i.e. about 30% versus 70%, was established in male rats (Hashizume et al., 1988; Muramatsu et al., 2012). In our recent report (Mierzejewska-Krzyżowska et al., 2014), this finding was also coincident in males, whereas the participation of γ motoneurons in comparison to α ones in females was slightly higher (33% versus 67%, respectively).

Moreover, the present study provides evidence for the existence of sex differences in the proportion of α_1 and α_2 motoneurons (Table 2). The following relationships were found when the number of α_1 motoneurons was compared to α_2 motoneurons in both sexes. The calculation showed that the ratio of α_1 to α_2 motoneurons was twice as high in females (females: 315/126 = 2.50; males: 292/235 = 1.24).

No other report has described two sets of α motoneurons neither in the rat nor considering sex differences in this species herein. However, according to Burke et al. (1982), it can be expected that the α_1 category contains a higher proportion of type S motoneurons and FR motor units, whereas the α_2 category contains a higher proportion FF motor units. A physiological study revealed that the rat MG muscle in males contains 12% S, 37% FR and 51% FF motor units, whereas in females there was a higher proportion of S and lower proportion of FF motor units (26, 36 and 33% of S, FR and FF units, respectively) (Celichowski and Drzymała, 2006). The present morphological results are in accordance with the above data and indicate that α_1 motoneurons (category containing slow motor units) are more numerous in females. However, with regards to α_2 motoneurons in the rats of both sexes, the apparent domination of α_2 motoneurons in males indicates the greater contribution of fast motor units. Moreover, the difference in the size of motoneurons seems to correlate with the differences in a force of motor units which are higher in male muscles (Celichowski and Drzymała-Celichowska, 2007) and, additionally, male motoneu-

rons innervate higher number of muscle fibres (the mean value of innervation ratio are 153 and 207 for females and males, respectively) (Mierzejewska-Krzyżowska et al., 2011).

Only one report, in adult female cats, provides evidence for the longitudinal distribution of large α (α_2), small α (α_1) and γ motoneuron types in the MG motor nucleus (Burke et al., 1977). Three parts, rostral, middle and caudal, were distinguished in this nucleus, and the percentage of cells in each size category was estimated with respect to these three parts. The data indicate that the percentages varied somewhat along the length of the nucleus. The highest percentage participation of large α motoneurons (about 50%) and the minor participation of small α motoneurons (28%) was observed in the rostral third of the nucleus. In addition, there was a relative increase of γ motoneurons in the caudal third of nucleus, which constituted the highest proportion of the motoneuron population (up to 40%). Our results in female rats indicate different relationships. Namely, in the rostral part of the MG motor nucleus, α_2 motoneurons created the least numerous population (only 8%) in contrast to α_1 motoneurons (58%), and γ motoneurons constituted 26% of the motoneuron pool in the caudal part. The comparison of the percentage participation of motoneuron types between homologous parts indicated that the most important sex differences concerned the proportion of α_2 and α_1 motoneurons. In the case of α_2 motoneurons in females and males, the differences in part 1 were 8% and 28%, and were 20% and 37% in part 2, respectively. With regard to α_1 motoneurons, differences were only significant in part 3, amounting to 50% (females) and 35% (males).

In the present study, motoneurons innervating the MG of rats were located ipsilaterally between the L4 and L6 levels in the spinal ventral horn. The length of the MG motor nucleus (distance between the most caudal and rostral motoneurons) was greater in males than in females: 3.69 versus 2.78 mm. This localisation of the MG motor nucleus is similar to that observed by Mohan et al. (2015) in adult female Long Evans rats; however, it was studied for the entire gastrocnemius muscle, but not for its medial and lateral heads separately. Our findings correspond to those of Hashizume et al. (1988) in male Fisher rats (age of 10–13 months), where the MG motor nucleus was included within the L4–L6 segments, but mainly the L5. However, in this breed, in spite of the older age, the length of the nucleus was smaller (about 2.8 mm). In male Wistar rats (22 weeks), the longitudinal cell column of MG motoneurons was largely found between L4 and L5, and occasionally, a small number of motoneurons extended into the L6 spinal segment. However, in comparison to our results, the rostrocaudal extension of MG nucleus was also smaller (about 2.8 mm) (Muramatsu et al., 2012). These discrepancies in the length of the nucleus may arise from the older and greater mass of the rats used in our examinations. In young adult female cats, the MG nucleus had a length of about 8 mm and was present almost equally in the L7 and S1 segments, or almost entirely within L7, in two individual cases (Burke et al., 1977). Reconstruction of the MG motor nucleus in female cats (Weeks and English, 1987) enables both the rostrocaudal and mediolateral extents of the MG nucleus to be recognised. Retrograde-labelled cells as parents for the MG motor nucleus were distributed through the caudal half of L7 to the rostral half of S1, within the lateral group of motoneurons in Rexed's lamina IX. Its rostrocaudal length averaged 7 mm and the mediolateral width (at the widest point) averaged 1 mm.

The next aim of the present study was a comparison of the topography of the MG motor nucleus on the transverse plane in male and female rats. The location of the motor column innervating the hindlimb muscles has been described in numerous species of vertebrates. Our observations are in general accordance with data on the organisation of mammalian motor nuclei provided by Romanes (1951). Motoneurons innervating a particular hindlimb muscle are clustered in longitudinally arranged motor pools. The

distribution of the various motor pools demonstrates a somatotopical organisation of motoneurons within the lumbar spinal cord. According to this, the MG nucleus occupies the lateral column of the ventral horn region. The reconstruction of the motoneuron columns in the lumbar spinal cord of rats (Nicolopoulos-Stourmaras and Iles, 1984) was made for 23 muscles or muscle groups of the hindlimb, including the triceps group, i.e. soleus column and gastrocnemius columns. The deep posterior muscles of the calf (crus) have motor columns which are located more rostral than those of the triceps surae. Within the triceps group, the soleus column was often distributed more rostral than the gastrocnemius columns. Motoneurons extended over the lumbar segment three (L3) to the lumbar segment four (L4).

The present study compared the position of MG motoneuron pools in a rostrocaudal direction as well as in the dorsoventral and mediolateral extents in both female and male rats and revealed differences in the dorsoventral distribution. In males, motoneuron clusters occupied a more dorsal position predominantly through the middle and caudal levels of the cord. The rostral and caudal portions of the male motor nucleus were positioned more laterally, but the position of motoneuron pools in the middle portion was similar in both sexes. It is likely that the above sex differences appeared from different positions of sexual centres within the spinal segments (Tobin and Joubert, 1988).

In conclusion, the present study provided evidence for sex differences in the organisation of the MG motor nucleus in rats, considering its length and localisation within the spinal segments, density of motoneurons and proportion of α_1/α_2 motoneurons. The main findings showed that the number of α_2 motoneurons was 46% higher in males and the length of the nucleus in males was also larger by about one quarter. In addition, the density of α and γ motoneurons was higher in females, by about 16%.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.aanat.2018.09.002>.

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