The cytoarchitectonic and neuronal structure of the red nucleus in guinea pig: Nissl and Golgi studies

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The present studies were carried out on the brains of adult guinea pigs, Dunkin-Hartley strain. On the basis of preparations, they were stained according to the Nissl and the Klüver-Barrera methods; a short description of the cytoarchitecture and the characteristics of the rubral cells were written. The red nucleus (RN) of the guinea pig is 1.2 mm in length. Three cellular parts in RN, and three classes (A, B, C) of the rubral cells were distinguished. Taking into consideration the predominant cell size, RN was divided into magnocellular part (RNm), parvocellular part (RNp) and intermediate part (RNi). On the basis of Golgi impregnated preparations four neuronal types (I, II, III, IV) were distinguished. To sum up, in the guinea pig were observed: the large, mainly multipolar (type I) and bipolar (type II) spiny being coarse (class A) in Nissl material; the medium-sized, triangular, aspiny (type III) corresponding to the fine cells (class B); and the small, both spiny and aspiny neurons (type IV), which are the fine or achromatic cells (classes B or C) in Nissl stained slices. The highest degree of dendritic branching was observed in type I, whereas the lowest in cells of types III and IV.

key words: red nucleus, cytoarchitectonic, types of neurons, Nissl and Golgi studies, guinea pig

INTRODUCTION

The red nucleus appears in terrestrial tetrapods [25,31]. This centre is a link in the neuronal loops [10,16], involved in processing motor [26,36] and somatosensory information [23]. RN has two (magnocellular and parvocellular) components, which are activated by different dominant nervous centres [19,20]. The parvocellular (RNp) part, having an integrative nature [25,37], is better developed in mammals, in which motor activity has become more intentional [16,25]. The magnocellular (RNm) part, conforming information for muscle tone and co-ordination [16,26], is older philogenetically, and is affected by age, in contrast to the parvocellular part [9,30,33]. The latter undergoes changes caused by maternal alcohol [37]. In general, the red nucleus receives central [8,25] and exteroceptive connections [23,25,36], and has reciprocal connections with the cerebellum [5]. The magnocellular units project directly to motoneurons and are the origin of the rubrospinal tract [9,12,24,26,32]. Some evidence is available about the topographical organisation [8,12,22–24,31,32,35] and action [10,26] of these projections, their neurotransmitters [2,3,7] and ultrastructure [26,29,30], as well as the function of the red nucleus [14,21,23,25,36,37]. On the basis of those experimental
works, some morphological and cytological data of the red nucleus are known, but cytoarchitectonics were more elaborated in rat [28,29,32], in mouse [33], in rabbit [22], bison [34], camel [1], in domestic animals [cit. 34], in monkey [6,14,15], in man [10,25] and in non-mammalian species [17], but the differentiation of RN cells in Golgi pictures was not carried out in all animals studied. Undoubtedly the Golgi methods, which make perikarya and their processes visible simultaneously [4], are helpful in an examination of the above-mentioned projections and are the most useful technique in a study of the neuronal types of structural entities. The characteristic features of such entities include specific populations of neuron types, dendritic configurations, neurotransmitters, specific connections between neuronal populations, etc. [11]. Because of the paucity of detailed examinations about the neuronal structure of the red nucleus in guinea pig, the present studies were undertaken in order to complete those data.

MATERIAL AND METHODS
The present study was carried out on 6 adult female guinea pigs, Dunkin-Hartley strain, from The Research Institute of the Polish Mother’s Health Centre in Łódź. The animals were overdosed with sodium pentobarbital (80 mg/kg) and perfused transcardially with phosphate and then formaldehyde buffered solutions at pH 7.4. The paraffin transversal and sagittal blocks with mesencephalons were cut into 10 µm thick sections and stained by the Nissl as well as Klüver-Barrera methods. The brains prepared for Golgi impregnation were sectioned into 5–10 mm thick blocks and processed according to the Bagiński and Golgi-Kopsch techniques, and then cut into 60 µm thick sections both in sagittal and transversal planes. The sections were analysed in the light microscope to select well-impregnated cells for Golgi mapping according to localisation within discernible parts of the examined nucleus. The microscopic images of selected neurons were digitally recorded by means of camera that was coupled with microscope and image processing system (VIST-Wikom, Warsaw). From 30 to 100 such digital microphotographs were taken at different focus layers of the section for each neuron. On the basis of these digitzed series the computerised reconstructions of microscopic images were made (Figs. 3a–6a). Afterwards the neuropil was removed to clarify the picture and to show the characteristic features of each type of neuron (Figs. 3b–6b).

RESULTS

Cytoarchitectonics in Nissl picture (Fig. 1, 2)
The red nucleus in guinea pig appears at the back of the posterior edge of the fibres of the oculomotor nerve and extends forward for about 1.2 mm. On the basis of predominant cell size, RN was divided into magnocellular part (RNm), parvocellular part (RNP) and intermediate part (RNi). RNm is the main, non-divided, ventrally located part, whereas the remaining part of RN shows subdivisions that are variable in their shapes, number and location, but in

Figure 1. The microphotograph of the cross-section at the mid-length of the red nucleus. Klüver-Barrera method. n 3 — oculomotor nucleus, f 3 — oculomotor fibres, m, i, p-part (magnocellular, intermediate and parvocellular) of the red nucleus (RN), Ip — interpeduncular nucleus, Sn — substantia nigra.

Figure 2. The three (A, B, C) categories of rubral cells; Klüver-Barrera method. Magn. 800×.
general, they lie above the magnocellular part. Its med-
dial side adjoins the oculomotor nerve III, and the ret-
roflexus fascicle in front, whereas its ventral side ad-
joins strictly the medial lemniscus. The posterior por-
tion of RN is constituted of RNm, which is in the form
of a circular, well distinguishable group of large inten-
sively staining cells. At a short distance from the pos-
terior end of the red nucleus there appears a morpho-
logically outlined group of small cells (RNp), dorsolat-
erally located. Cross-section of RN enlarges, and its cells
segregate in such a way, that RNi appears dorsally. This
part mainly consists of medium-sized cells, but small
and a few large cells are observed too. These three
groups on the cross-section are arranged in a
ring-like fashion. In the middle portion of the red
nucleus its cells disperse (especially within RNi) and
whole RN diminishes. The fibres of the oculomotor
nerve travel along the medial side of RN. The anterior
portion of RN is composed of mixed cells, but the large
cells rarely reach their maximum sizes. Forwards its cells
disperse and are replaced by the cells of the prerubral
field (Forel field). Generally, the RNm is composed of
large cells, RNi of medium size neurons and single large
and small, whereas the small cells preponderate in RNp.
However, throughout the entire extent of the red nu-
cleus, different cells were occasionally present in vari-
ous parts. On the basis of Nissl preparations, three cat-
ergories of neurons are observed in the red nucleus:

A) Coarse, large (40–65 \( \mu m \)) and medium-sized
(25–40 \( \mu m \) in long axis) intensively staining multipo-
lar, fusiform and oval-shaped. The cells contain
a large, bright, sometimes slightly stained nucleus
with deeply staining nucleolus. The cytoplasm con-
tains plenty of large-grained tigroid substance, which
forms bands, nets or clots, distributed throughout
the cytoplasm. The tigroid substance usually pene-
trates deeply into the dendritic trunks. The cells of
the maximum sizes are characteristic for RNm, but
similar, less numerous and smaller rounded cells
(about 25 \( \mu m \) in size) are present within both the
magnocellular and intermediate parts, in the anteri-
or sector of the red nucleus.

B) Fine cells, measuring from 15 to 50 \( \mu m \). Most of
them are fusiform or multipolar, but not stellate in shape.
Their cytoplasm contains moderate amount of fine Nissl
granules, which are usually irregularly distributed and
most often concentrate along the cell circumference.
The round or oval-shaped cell nucleus, pale or slightly
deeper stained, contains dark stained nucleolus.

C) Achromatic cells, small and medium-sized,
from 10 to 25 \( \mu m \). The cells are round or oval, some-
times irregular in shape and contain bright nucleus
and small amount of cytoplasm, and scanty small-
grained or homogenous tigroid substance, which
forms a thin ring around the cell nucleus, or, usually
in the smallest cells (10–12 \( \mu m \)), it is located at one
side of the cell nucleus.

The neuron types in Golgi picture
On the basis of the shape and size of perikaryon, the
pattern of dendritic tree and axon, and the presence
of dendritic appendages, four types of neurons were dis-
tinguished in the red nucleus of the guinea pig:

Type I (Fig. 3) — multipolar cells; spiny; large and
medium-sized. They have stellate and quadrangular
perikarya measuring from 35 to 65 \( \mu m \) in the long-
est axis. The large stellate cells have 4 or 5 thick,
rarely conical, dendritic trunks, which at the distance
of 10–40 \( \mu m \) from the soma (even within one neu-
ron) divide into secondary and subsequently up to
quaternary dendrites. Dendritic branches are usually
longer than their parent dendrite. In that case,
when the length of the secondary dendrites is equal
with their dendritic trunks, tertiary and quaternary
dendrites are prominently longer. The dendritic
branches, especially their distal portions, are vari-
cose and possess knob-like or claw-like spines (Fig.
3a). The dendritic trunks are rather devoid of spines,
but undivided primary dendrites may be covered with
single spinous processes. An axon conically arises
from the dendritic trunk or from the soma, and on
the sagittal sections directs downstairs, whereas a
few visible thinner collaterals perpendicularly go
away from the axon and run forwards in sagittal
plane. These collaterals possess delicate synaptic
buttons. The axons have cones of various sizes and
length. The medium-sized multipolar cells have pri-
mary dendrites, which most often divide twice. The
first point of branching is usually placed at the shorter
distance (6–20 \( \mu m \)) from the soma than the point of
branching in dendritic trunks of those large cells.
Some dendrites do not divide and extend up to 120
\( \mu m \). Sometimes the primary dendrite of the quadran-
ular cell at first gives off thinner collaterals, and
then sends an axon towards the parvocellular part
of RN. In the posterior sections of RN within the par-
vocellular part (RNp) the axon directs upwards.
The cells located in the ventro-medial side of RN (on
the cross-sections), give off axons medially, towards
the oculomotor nerve fibres. The dendritic pattern is
more clearly visible in the sagittal sections than in
the cross-sections (mainly dendrites up to second-
ary order are visible). The dendritic tree of the type I
neurons is oval or ball-shaped.
Type II (Fig. 4) — bipolar cells; fusiform and pear-shaped; spiny; from large to medium size. These neurons are similar to the type I neurons, but differ in the number of primary dendrites and the pattern of the dendritic tree. They usually possess 2 thick primary dendrites, or rarely 1, in these cases, the axon arises from one of the two poles of the perikaryon. One of the two dendrites is often long and undivided (up to 140 µm), whereas the other may branch close to the soma, or after 6–30 µm from the cell body. The dendrites of secondary order issue collaterals, or at a different distance (12–85 µm) from the point of the first division, may divide again into relatively long (up to 180 µm) tertiary branches. Dendrites follow a straight route but they are varicose and possess different spine-like protrusions, especially on their distal portions. The dendritic tree has a stream-like form. The axon usually arises from the

Figure 3. Multipolar neurons of type I; scale bars — 30 µm.
a) Non-clarified microscopic images; Golgi impregnation; curved arrows show an enlargements of the dendritic spines (the leftward — a knob-like spine; the rightward — a claw-like spine).
b) Clarified computerised reconstructions; arrow — axon.
c) Microphotograph of Nissl-stained perikaryon; x — additionally the fine cell (b) at the bottom.
dendritic trunk, but it was also observed to arise from the secondary dendrite or directly from the soma. On the sagittal sections, in the posterior sector of the red nucleus, the axon of RNm cells directs downwards and backwards, whereas in its anterior sector usually runs upwards.

Type III (Fig. 5) — triangular; aspiny; small to medium size (17–37 \( \mu \text{m} \)). Within this type are also observed quadrangular perikarya, but all neurons usually have 3 thin dendritic trunks. These trunks ramify either close to the soma or at some distance (25–35 \( \mu \text{m} \)) from the cell body, into secondary dendrites, which may divide once again and may extend for about 165 \( \mu \text{m} \). Rarely, dendrites divide into as far as the quaternary branches. Generally, the dendrites of the triangular cells possess different varicosities, but they are devoid of spines. The dendritic tree is oval-shaped. Their axons arise from the cell

\begin{figure}
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\includegraphics[width=\textwidth]{bipolar_neurons.png}
\caption{Bipolar neurons of type II; scale bars — 30 \( \mu \text{m} \).
\textit{a)} Non-clarified microscopic images; Golgi impregnation.
\textit{b)} Clarified computerised reconstructions; arrow — axon.
\textit{c)} Microphotograph of Nissl-stained perikaryon; x — additionally the achromatic cell (c) on the right.}
\end{figure}
body or from the base of the dendritic trunk and direct backwards and downwards on the sagittal sections. On the cross-sections, in RNp, axons are mainly directed ventromedially towards the magnocellular part, whereas the axons of neurons located within the RNm run medially. In the anterior sector of the red nucleus, the small cells frequently give off their wavy axons upwards.

Type IV (Fig. 6) — oval, fusiform and pear-shaped cells; medium-sized and small (10–25 µm), although the majority of them measure about 18–20 µm in long axis. These cells may possess spinous or smooth dendrites. Many oval cells (medium-sized) possess 3 primary dendrites of various thicknesses. The primary dendrites are similar to dendrites of the type III neurons, but dendrites of the type IV cells have knob-
like spines, although sparsely distributed. The thin dendritic trunks are smooth or varicose, and may issue collaterals or dichotomously ramify into wavy long secondary dendrites. Sometimes the dendritic trunks ramify into three dendritic branches of secondary order, which further dichotomously divide once or remain undivided. The axon arises from the soma or from the dendritic trunk usually at a distance of 10–13 µm from the perikaryon. The fusiform small cells have 2 rather thin, long primary dendrites, which mostly divide only once. Dendrites divide near to the soma (up to 5 µm) and run without ramifying for the distance of 80–90 µm, or at first give off collaterals with spinous processes, and next divide into secondary branches after 140 µm of their routes. One of the two secondary dendrites of
the same parent dendrite may possess spiny, whereas the other one — none. The stream-like dendritic tree of these neurons located in RNm is usually, approximately, vertically oriented. The pear-shaped perikarya possess only one thick, usually short primary dendrite, which may send the axon after 7–30 μm of its route, and next gives off collaterals, or runs without ramifying even for 200 μm. The distal dendrites are covered with spine-like protrusions, but single protrusions are present on dendritic trunk. The majority of the smallest cells (14 μm in diameter) are found in the RNp, but they are also found throughout the red nucleus. On the sagittal plane of the red nucleus, axons are directed either forwards or backwards, whereas on the cross-section mainly on the lateral side, but in both planes usually direct upwards.

**DISCUSSION**

The red nucleus is composed of two philogenetically different neuronal populations, the older — the magnocellular part and the newer — the parvocellular one, but the possibility that some small neurons are also philogenetically old is not ruled out [16]. Generally in mammals, the large cells constitute the caudal levels of RN, whereas in lizard [17] they are located reversely in an antero-caudal direction. In the guinea pig the large cells (class A in Nissl material, and types I and II in Golgi preparations) form a conspicuous cell group in caudal portion of RN, but in contrast to rat [28] and monkey [14], the magnocellular elements are still present in the anterior portion of RN in the guinea pig and albino rat [32]. The remaining neurons of the red nucleus in guinea pig are segregated into two relatively clear components: the dorsolateral — real parvocellular group (RNp) and the dorsomedial group (RNi), which mainly consists of medium-sized cells. In monkey [14], the medium-sized cells constitute the dorsolateral group, having characteristics of the magnocellular units. In contrast to the dorsomedial (RNi) cell group of the guinea pig, the analogue part of RN in rat contains the giant and large neurons [28]. From the cytological point of view, probably, the dorsomedial part and the lateral horn of the camel’s red nucleus [1] are comparable with the RNi and RNp parts respectively in the guinea pig. Some difficulties occur, when it comes to comparing various subdivisions of RN [1,14,22,28], due to the different criteria used by researchers, namely their connections [21], physiological or cytoarchitectural features in the whole nucleus [14], or only in one of the two parts: the magnocellular [28] or in the parvocellular part [1,21]. For example, in rat [28] the whole RN, but in camel [1] only the parvocellular part of RN, has three subdivisions. An additional problem arises in the precise delineation of the distinguished parts in the red nucleus, especially in such mammals as rat [32] and rabbit [22], in contrast to the primates [14,15]. In some animals (for example, in cat) a topographic correlation exists for different projections of RN sub-nuclei [21] and the somaesthetic map exists for various RN parts: dorsal — for face, ventral — for limbs [23]. Musculatory control in big animals [1,34] reflects the degree of development of RN, which is expected to be well developed, except in cetacean species, due to the reduction of their limbs [18]. The locomotor movements are correlated with rhythmic discharges of the RN cells [14,36], and spike amplitude is correlated with the cell size [14]. Cells of RNm in man are smaller than those in the red nucleus of Tursiop truncatus [18], but the largest cells are found in bison [34]. In the red nucleus of the guinea pig, the range of cell sizes is slightly greater than in monkey [14], but coincides with rat cell sizes [28,29]. The limits of cell sizes between the distinguished neuronal types are sharp in bison [34], but they overlap in the guinea pig and rat [32]. The ratio of small to large cells is species dependent [10], however, the exact diameters of the neurons are not easy to establish in Golgi material since it is difficult to differentiate the cell body from the dendrite. The different numbers of neuronal categories were distinguished in Golgi preparations of examined animals, viz. 3 types in lizard [17] and bison [34], 4 types in rat [28,29] and the guinea pig, whereas in cat [30] even 9 neuronal subpopulations were described. Generally, in the guinea pig there have been distinguished: the large, spiny neurons (type I and II) being coarse (class A) in Nissl material; the medium-sized, aspiny (type III) — fine cells (class B); and the small, either spiny or aspiny neurons (type IV), which are the fine or achromatic cells (class B or C) in Nissl stained slices. In RNm there are commonly observed multipolar cells of the I type, which have up to 5 dendritic trunks in the guinea pig, but in rat those neurons possess 7 dendritic trunks [28], in bison 8 [34] and only 4 in lizard [17]. The radiate dendritic pattern of these multipolar neurons may appear to be profitable for an afferent of heterogenous origin [27]. GABA-ergic fibres constitute a major afferent input to RN-large-sized neurons, which receive also serotoninergic innervation [2]. On the other hand, the cells of I and II neuronal types in the guinea pig, are the efferent cells of RNm, whereas the efferent...
cells of RNp are some neurons of types III and IV according to King’s criterion [15]. The magnocellular units probably are related with the cerebellum [5] and extrapyramidal tract through the rubrospinal tract [1, 9, 32]. The direct monosynaptic connections to motoneurons [26] i.e. the rubrospinal tract, take origin from the large, coarse cells of entire rostrocaudal axis of the red nucleus [32]. Similar distribution is shown by the large cells associating with hialuronic acid, which may have a special role in plasticity responses [7]. On the other hand the human rubrospinal tract arises both from small and large neurons [10]. The morphology of the small neurons in guinea pig corresponds to that of the lizard [17] as regards the degree of dendritic branching in analogue cell types. The dendrites within neurons of the III and IV types branch infrequently, similarly to small cells in rat [28], but a few neurons in IV type have the dendritic trunks ramifying into three secondary branches. In the guinea pig, within the population of small neurons (type IV) in Golgi material, there are more morphological varieties than in the population of small cells visible in Nissl stained preparations. Ramon-Moliner [27] noticed heterogeneity for short-axon cells (Golgi type II) and stated that there is no correlation between the dendritic pattern and the axonal pattern of the same neuron. To the common features of the small neurons (interneurons) belong: the round or ovoid shapes, poor ramification of dendrites, the paucity of organelles, pale staining cytoplasm (achromatic cells), almost total lack of visible processes and also dispersion of these cells throughout a nucleus, also RN [27, 28, 32]. As regards the presence of dendritic spines, only the cells of the type III are devoid of them, however there exists a discrepancy between light and electron microscopy studies in this respect [17]. EM-studies [29] confirm the presence of different terminals either on the same dendritic spine and on the cells varying in their sizes. Some cells of the red nucleus contain iron [10], the large cells — hialuronic acid [7], round and oval (prominently multipolar) — monoamines [6] and a specific receptor subtype, which can act as a modulator [3]. On the periphery of RN, the catecholamine fibres with varicosities are found along its lateral edge, and serotonin ones at the caudal pole of this nucleus [6], but GABA-ergic terminals (probably mainly originating from local interneurons and contacting with large neurons) were detected throughout the red nucleus [2]. In summary, all researchers distinguish the magnocellular and parvocellular components in the red nucleus, but subdivisions of this nucleus (especially RNp) and their topographical arrangement differ from species to species. The most similar features were noted in the red nucleus of two laboratory rodents: albino rat and the guinea pig. Undoubtedly the cytoarchitectonic subdivisions and their neuronal structure of RN reflect structural and functional differences in the organisation of the motor system according to “the principle of proper mass” and are related to the relative importance of the behaviours in the species [11]. They are related to the great variety and number of modifications of behavioural activities that occur in different species, for example, man, camel, cat, rat, cetacean species and the guinea pig. It may be suggested that the adaptational transformation of rubral neurons in mammals shows the division of RN into at least two size-related cell groups, which may contain dispersed small cells, so-called interneurons. This may suggest the evolutionary trends in dendroarchitectonic pattern, manifested by the increasing number of dendritic trunks in the large cells (such as those in type I in the guinea pig) as well as by the increasing number of dendritic spines and probably their specialisation (for example visible in rat [28]).

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