

ABSTRACT

Keywords: *rachidian bulb, sheep, cranial nerves, cranial nerve nuclei, bulbar nuclei, neurons, seriated sections, optical microscopy, electronic microscopy.*

The following doctoral thesis, titled “*Contributions to the morphology of the rachidian bulb nuclei in ovines*”, has been worked upon within the Doctoral School of the University of Agricultural Sciences and Veterinary Medicine “Ion Ionescu de la Brad” Iași between 2004-2012 and is structured conforming to current legal regulations in two main parts: part I titled “*Literature review*” containing 35 pages and representing 29% and part II titled “*Personal research*”, spanning 122 pages and representing 71%.

In **Part I**, which contains five chapters, information is presented from consulted literature in the field on the subject of the thesis, information also used for interpreting the data obtained in the second part. This part contains eight figures and two tables selected as suggestive for detailing the synthesized information.

Chapter I – “*The development of the central nervous system*” presents “The morphogenesis of the nervous system” with the following subchapters: “*Differentiation of the neural tube*” and “*Formation of derived elements from the ectoblast, neural crests and neural tube*”. In the “*Histogenesis of the nervous system*” emphasis has been placed on the formation of nervous tissue and its components.

Chapter II – “*The morphology of the myelencephalon*” – contains anatomic relations between the bulb and other components of the nervous system, morphologic aspects of the bulb and its internal structure represented by white matter and grey matter cords.

Chapter III – “*Cranial nerves*” presents the twelve pairs of cranial nerves, classified from a functional standpoint in sensory, motor and mixed nerves, from a phylogenetic one, related to function and distribution region, in archencephalic, epibranchial, branchial and hypobranchial, and grouped in two great categories in relation to their apparent origin: “cranial nerves with bulbar origin” and “cranial nerves with extrabulbar origin”.

Chapter IV – *“The histology and cytology of the encephalon”* focuses on the structure of grey matter and white matter, with emphasis on the specialized morphofunctional unit of the nervous tissue, represented by the neuron. The chapter also contains morphological aspects of cytoplasmic extensions, tigroid bodies and glial cells.

Chapter V – *“Lesions in the nuclei of the rachidian bulb in ovines affected by the prion protein”* – describes succinctly (within the frame of prionic diseases) scrapie, a disease that affects ovines in which histologic lesions are encountered in the bulb, and methods used to diagnose this disease.

Part II is structured in 5 chapters (chapters VI – X) and consists of Chapter VI – *“Aim and objectives”*, Chapter VII – *“Material and methods”*, Chapter VIII – *“Research on the anatomy, conformation and anatomic relations of the rachidian bulb with other segments of the brain stem in ovine”*, Chapter IX – *“Research on the identification, distribution, cytology and ultrastructure of neurons from the nuclei from which arise the cranial nerves IX – XII”*, Chapter X – *“Research on the identification, distribution, cytological and ultrastructural aspects of the neurons from the rachidian bulb nuclei other than those belonging to cranial nerves V-XII” the final conclusions* ending this part.

Research has been performed between 2006 and 2011 on 137 cases of ovines of various ages, ranging from 2 to 7 years, samples being taken in various slaughterhouses as well as the necropsy hall of the Pathology Laboratory of the Sanitary Veterinary and Food Safety Laboratory within D.S.V.S.A. Bacău.

In chapter VI – *“Aim and objectives”* the reason for the choice of subject is presented. The main goal of the thesis is that of providing a practical guide for all interested veterinary physicians and especially for physicians from the national network of laboratories specialized in diagnosing prion diseases.

Research was performed in order to point out the distribution, cytology and ultrastructure of neurons of nuclei from the rachidian bulb in sheep with the idea of providing a systematization of all the studied bulbar nuclei, both cranial nerves nuclei and bulbar nerves nuclei.

Thus, main objectives and activities corresponding to the thesis were set from the beginning by mutual agreement with the coordinator of the doctoral thesis and were represented by:

- ✚ description of the anatomic conformation of the bulb and its relations to the other segments of the brain stem.
- ✚ highlighting the apparent origin of cranial nerves from the rachidian bulb.
- ✚ making of seriated sections of 5 μm on 1 mm each on a caudo-cranial direction at the apparent origin of each cranial nerve of bulbar origin and in specific nuclei.

✚ research on the distribution, cytology and ultrastructure of bulbar nerve neurons, both cranial nerves and bulbar nerves.

This doctoral thesis provides novel elements, the subject having not been previously studied nationally especially in sheep, the only known sources of information consisting of a sketch of bulbar, pontine and mesencephalic nuclei in cattle provided by Fontaine and Parrodi in 1991 and a study of nuclei in horse (Barrone, 2004).

We mention that the National Laboratory of Reference, specialized in prion diseases diagnosis, recommends the obex, at which some of the bulbar nuclei are evident, for pathological exams in Sanitary-Veterinary Laboratories for all necropsied ovines. Through the present work we provide a study of all nuclei, belonging to cranial nerves and bulbar. Thus, a cytological map of the bulbar nuclei structure totaling 15 nuclei has been realized.

For the numerical evaluation of neurons in each nucleus or region taken into study seriated sections have been made, containing 1 mm (1000 μm = 200 sections of 5 μm) in depth from the structure of each nucleus, cranio-caudally.

All this microscopic research required a very high amount of laboratory work that led to the readings that have been performed very thoroughly and with great effort, and then registered in the tables, in order to be systematized and then represented by histograms with the aim of being used in medical-veterinary diagnosis practice.

Perikaryons were measured by microscope, and with this, all dimensions were noted and histograms were executed. Nuclei of the neurons were measured with the intent of establishing the average dimension, then the nucleolus and Nissl bodies in the neuroplasma. Disposition of tigroid bodies has been described in each of the studied neurons within the respective nucleus. Each structure has its own histograms by which the dynamics of these structures have been emphasized.

Chapter VII – “**Material and methods**” presents the casuistic based on which the research was performed, and methods that were used.

The 137 samples of ovine rachidian bulb were taken from authorized slaughterhouses and originated from clinically healthy animals that had been slaughtered for human consumption. Another part of the study material consisted of brain samples from sheep, where the rachidian bulb was sampled - sheep from farms and populace households, brought to the Pathology Laboratory for necropsy and diagnosis.

Sections were executed from: 9 cases aged between 2 and 7 years for the trigeminus nerve nucleus, 9 cases aged between 2 and 7 years for the abducens nerve nuclei, 9 cases aged between 3 and 7 years for the facial nerve nucleus, 9 sheep aged between 2 and 7 years for the vestibulocochlear nerve nuclei, 9 cases aged between 2 and 6 years for the glossopharyngeal

nerve nucleus, 10 cases aged between 3 and 7 years for the vagus nerve nucleus, 9 cases aged between 2 and 7 years for the spinal nerve nucleus, 10 cases aged between 3 and 6 years for the hypoglossal nerve nucleus, 9 cases aged between 2 and 7 years for the solitary tract nucleus, 9 cases aged between 2 and 6 years for the nucleus ambiguus, 9 cases aged between 2 and 6 years for the nucleus gracilis, 9 cases aged between 2 and 7 years for the nuclei cuneatus, 9 cases aged between 2 and 7 years for the reticular formation, 9 sheep aged between 2 and 6 years for the lateral fascicle nucleus and 9 cases aged between 2 and 7 years for the olivary nuclei.

Two sampling techniques were used for the rachidian bulb samples: whole brain sample technique and foramen magnum technique. The choice of one or another was decided depending on the period that had occurred from the time of slaughter or death until the beginning of laboratory work. In the case of samples originating in animals slaughtered approx. 12 hours before, the “whole brain sample” technique was used, and in the case of heads obtained immediately after slaughter the “foramen magnum” technique was used.

In order to perform histological research on rachidian bulb tissue sampling and sub-sampling was executed, the obtained fragments passing through a succession of steps that are part of the histological processing technique of tissue samples.

Fragments were processed in order to obtain the microsections that were then subjected to histological staining methods. There were two staining techniques used, specific to the central nervous system: the Nissl stain for highlighting the neurons, their distribution and the dimensions of the tigroid granulations. The other complementary method is represented by silver staining that highlights neurofibrillary structures.

Histological sections stained as such were embedded in Canada balm, labeled and then examined at the x10 ocular and 10x, 40x and 100x objective at the transmitted light Motic B1 Series microscope, then microphotographed with camera Moticom 1000.

Electronic microscopy research was performed by using the transmission electronic microscope TEM Phillips CM 100. Samples were prepared as according to working steps and were examined at the electronic microscope. We focused on electronmicroscopically highlighting the nervous tissue structures at the level of the rachidian bulb.

Chapter VIII – “**Research on the anatomy, conformation and anatomic relations of the rachidian bulb with other segments of the brain stem in ovines**” is structured in two subchapters, the first one describing the rachidian bulb anatomically and the second one systematizing identified bulbar nuclei in seriated sections executed on the bulb.

As in other species, the rachidian bulb of sheep is located in the cranium in its posterior part in the caudal plane of the occipital bone, in continuation of the spinal cord, as there is no clear demarcation between this and the bulb, hence the other denomination that it bears, “medulla

oblongata”. Especially in its caudal part the bulb surface has a similar configuration to the external configuration of the spinal cord.

In its cranial part, the bulb is continued with the pons towards which it progressively develops and widens, from which it is separated by the bulbo-pontine groove.

The rachidian bulb is cone-shaped with the great base directed cranially, being flattened dorso-ventrally, with a length of 2,5 – 3 cm and weighing approximately 30 g.

At a first examination we remark that it presents two faces, a dorsal one and a ventral one, and two edges, rounded and thick. Transversally the ventral face is passed by a groove known in literature as the median ventral groove. On the edges of the ventral median groove there are two prominences named bulbar pyramids that are found in the continuation of the ventral cords of the medulla. The bulbar pyramids cross to the apex of the bulb forming the bulbar decussation, where they end. Before crossing, the pyramids become thinner, by the protrusion at the surface of eminences named bulbar olives.

Laterally to the bulbar pyramids, before the pons, there is a prominent formation with trapezoidal aspect that anatomists call *trapezoid body*. Behind the trapezoid body, which is prominent in sheep, there are two rounded eminences, known as the facial tubercle and the acoustic tubercle. The facial tubercle is less apparent while the acoustic tubercle, relatively prominent, tends to embrace the rostral extremity of the caudal cerebellar peduncle.

On the dorsal face there are evident the gracilis and cuneatus fascicles diverging into a right angle and end at the level of the homonymous tubercles where the corresponding nuclei are found. Cuneiform tubercles are prominent, well delimited laterally by an apparent groove. The obex is apparent.

The rachidian bulb is the zone where a great part of the cranial nerves have their apparent origin.

On the ventral face, on the lateral edge of the ventral lateral groove, is the point where the roots of several cranial nerves are apparent. Thus, at the cranial extremity in contact with the pons, behind the prepontine groove there is the root of the abducens nerve, the sixth cranial nerve. Its root emerges several millimeters more to the outside than the lateral edge of the pyramids.

At the caudal extremity laterally from the groove, close to the bulbar decussation, the root of the hypoglossal nerve is apparent, the twelfth and final cranial nerve which is aligned longitudinally.

The trapezoid body is crossed by the fibers of the roots of two cranial nerves. Laterally from this is where the apparent origins of the facial nerve and the seventh cranial nerve are

found, a bit more caudo-laterally from the root of nerve VII, the root of the vestibulocochlear nerve (the eighth cranial nerve) can be noticed.

On the lateral sides of the bulb, in line with the dorso-lateral grooves, directed antero-posterior towards the caudal extremity of the rachidian bulb, through many continuously linearly disposed roots the cranial nerves emerge - glossopharyngeal, vagus and vagus accessory, respectively the IX-th, X-th and XI-th pairs of cranial nerves.

In sections executed in the apparent origin of bulbar nerves, the presence of well-contoured, darker colored nuclei of gray-brownish color is highlighted macroscopically.

In the superior part of the rachidian bulb, occupying its entire lateral edge, the neurons of the trigeminus nucleus and its tract are distributed, the superior limit provided by the dorsal cochlear nucleus and inferior limit provided by the motor nucleus of the facial nerve.

The nuclei of the abducens nerve, in sections executed in the apparent origin of the abducens nerve, are located in the superior region of the bulb close to the median raphe.

The vestibulocochlear nerve nuclei, both vestibular and cochlear ones are situated in the superior part of the bulb in the plane of the vestibular area placed at the lateral apex of the bulbar trigone.

In the superior part of the bulb there are also apparent the vagus, hypoglossal, solitary tract, gracilis and cuneatus nuclei.

In the middle part are distributed, on facing sides of the median raphe, the neurons of the reticular formation.

In sections executed in the anterior portion of the rachidian bulb in its middle region the motor nucleus of the facial nerve is apparent, continuing in sections from the middle portion of the bulb with the nucleus ambiguus, and towards the caudal part, in sections executed near the cranial root of the spinal nerve, with the spinal nucleus, its cranial portion.

Near the nucleus ambiguus, the lateral fascicle nucleus is also apparent in the middle part of the bulb. In the ventral portion of the bulb we notice the olivary complex formed by three layers of nuclei disposed one upon another.

In Chapter IX – ***“Research on the identification, distribution, cytology and ultrastructure of neurons from the nuclei from which arise the cranial nerves IX – XII”*** – the nuclei of cranial nerves of bulbar origin, respectively VI – XII, have been identified and assessed individually, under the aspects of neuron distribution, cytology, and ultrastructure. Although the apparent origin of the greatest cranial nerve, the trigeminus, was not included in the bulb, references have been made since the axons of the Gasser sensitive ganglion located on its sensitive root form synapses with sensitive neurons and also form a long tract that passes through the bulb.

In this chapter the following have been studied: the nucleus and the spinal tract of the trigeminus nerve, the abducens nerve nuclei, the motor nucleus of the facial nerve, the vestibulocochlear nerve nuclei, both vestibular and cochlear, the aboral salivatory nucleus, the vagus nerve nucleus, spinal nerve nucleus and hypoglossal nerve nucleus.

The nucleus of the trigeminus nerve is a large nucleus, well-contoured, rich in neurons of various forms ranging from pyramidal to fusiform and oval, of relatively large dimensions (38,5 μm), and a centrally located large nucleus (17,5 μm) with an intensely basophile nucleolus of approximately 2,5 μm .

Neuron counting has been done depending on the average size of perikaryons, in this case being 38,5 μm in seriated section. Eight sections with the average dimension of 5 μm have been executed (38,5 : 5 \approx 8). Neuron counting has been done at each eighth section of the 25 sections (1 mm = 200 sections of 5 μm : 8 = 25 sections). From each eighth section resulted a number of 80-86 neurons, which summed in the 25 sections give a total count of approximately 2145 neurons within a 1 mm fragment of the respective nucleus, caudo-cranial. The same procedure has been applied in each assessed nucleus in the carried research.

Abducens nerve nuclei are small but evident nuclei, with medium and large size neurons, with polymorphous aspect where the triangle shape is the most prominent, while also encountering oval-shaped, extension rich neurons. The perikaryon in the abducens nerve main nucleus measures approximately 40 μm , and in the secondary nucleus of the abducens nerve it measures 39 μm . The approximate total count of neurons within a 1 mm fragment from the assessed main nucleus of the abducens nerve on 25 of 200 seriated sections reached 883. In the secondary nucleus of the abducens nerve the approximate total count of neurons in a 1 mm fragment, also assessed on 25 of 200 seriated sections, reached 825.

The motor nucleus of the facial nerve is a relatively large nucleus with well represented neurons, compactly disposed in the microscopic field, with the average perikaryon size of 23 μm , 10 μm for the nucleus and 2,5 μm in the nucleolus. Nissl bodies are visible all over the perikaryon cytoplasm and at the base of the dendrites, structured in blocks with the average dimension of 1,7 μm . The approximate total count of neurons within a 1 mm fragment of the motor nucleus of the facial nerve, assessed on 40 of 200 seriated sections reached 1810.

The vestibulocochlear nerve nuclei, studied in the carried research, are represented by the dorsal cochlear bulbar nucleus, the ventral cochlear bulbar nucleus and the vestibular nuclei. The average sizes in the dorsal cochlear bulbar nucleus are 36 μm for perikaryons, 16,2 μm for the nucleus, 2,8 μm for nucleoli and 1,5 μm for Nissl bodies. The approximate total count of neurons within a 1 mm fragment in the dorsal cochlear bulbar nucleus, assessed on 28 of the 200 seriated sections, reached 1206. The average sizes in the ventral cochlear bulbar nucleus are 35 μm for

perikaryons, 15,2 μm for nuclei, 2,4 μm for nucleoli and 1,6 μm for Nissl bodies. The approximate total neuron count within a 1 mm fragment in the ventral cochlear bulbar nucleus assessed on 28 of the 200 seriated sections reached 1232. The average sizes in the vestibular nuclei are: 21,1 μm for perikaryons, 13,4 μm for nuclei, 3 μm for nucleoli and 1,4 μm for Nissl bodies. The approximate total count of neurons within a 1 mm fragment of the vestibular nuclei, assessed on 50 of 200 seriated sections reached 2300.

The parasympathetic nucleus of the glossopharyngeal nerve has relatively large sized neurons, around 58 μm in perikaryons, with a centrally located nucleus of approximately 25 μm and a basophilic nucleolus by the average size of 2,6 μm . Tigroid bodies are structured in blocks in the neuronal soma and at the bases of the dendrites, with sizes between 1,1 – 1,9 μm . The approximate total count of neurons within a 1 mm fragment of the parasympathetic nucleus of the glossopharyngeal nerve, assessed on 16 of 200 seriated sections, reached 584.

The dorsal vagus nucleus is a small nucleus, composed of neurons with an average size of 31 μm , of triangular and multiangular shape, with nervous extensions forming a well-defined neuropil and subtly reveals a rich gliocyte population. The perikaryon nucleus is large, vesiculous and nucleolated with an average dimension of 9,1 μm with an intensely basophilic nucleolus with an average size of 3 μm . The approximate total neuron count within a 1 mm fragment of the vagus nucleus, assessed on 33 of 200 seriated sections reached 990.

Spinal nucleus neurons have an average size of 28,2 μm and their shapes vary from round to oval and even triangular and multiangular with long and evident dendritic extensions. Tigroid bodies, found in the perikaryon and in the first segment of the extensions, have an average dimension of 1,6 μm , but there is also evidence of Nissl bodies of large sizes up to even 2,3 μm at some neurons disposed in blocks. The approximate total neuron count within a 1 mm fragment of the *spinal nucleus*, assessed on 33 of 200 seriated sections, reached 1386.

The hypoglossal nerve nucleus is formed by a restrained group of neurons, of small and medium size, with an average size of 37,4 μm , generally oval and triangular, some neurons from its compositions presenting evident nervous extensions. The size of the Nissl bodies disposed in the perikaryon and in the dendrites of the hypoglossal nerve nucleus neurons is 1,9 μm . The approximate total neuron count within a 1 mm fragment of the hypoglossal nerve nucleus, assessed on 28 of 200 seriated sections, reached 476.

In Chapter X – “**Research on the identification, distribution, cytological and ultrastructural aspects of the neurons from the rachidian bulb nuclei other than those belonging to cranial nerves V-XII**” there were other nuclei than those belonging to the V-VII cranial nerves, nuclei noted as belonging to the rachidian bulb, that were individually identified and assessed.

Aside from bulbar nuclei belonging to the V-XII cranial nerves (described in the ninth chapter), there are also several nuclei from which some that occur only in the rachidian bulb and others that have equivalents in other segments of the brain stem and even at medullar level.

In this chapter the following nuclei have been studied: *the solitary tract nucleus, nucleus ambiguous, nucleus gracilis, nuclei cuneatus, the lateral fascicle nucleus, the reticular formation and the olivary nuclei*. For the most part of these nuclei, research implied emphasizing them in seriated sections executed at the obex level. Only for the *nucleus gracilis* and the *nuclei cuneatus* have there been executed seriated sections also in the caudal portion of the rachidian bulb. It is necessary for us to mention that the solitary tract nucleus receiving sensitive fibers from the nerves VII, IX and X and the nucleus ambiguous sending motor fibers to nerves IX, X and XI (component neurons forming a synapse with neurons from the motor or sensitive components of several cranial nerves) could not be described as nuclei of a single cranial nerve and for that reason they are found in this chapter. Thus they can be considered as nuclei belonging to the bulb.

By making a systematization of these nuclei it is noted that they can be grouped into three distinct regions, the first group being the upper bulbar nuclei comprising *the solitary tract nucleus, the nucleus gracilis* and *nuclei cuneatus*. The following group of nuclei is the one of median bulbar nuclei comprising *the reticular formation, the nucleus ambiguous* and *the lateral fascicle nucleus*, and the group of the lower bulbar nuclei formed exclusively by the *olivary nuclei* which in turn can be grouped into upper, middle and lower. Certainly in the histological slides examined on sections executed at this bulbar level there have also been visualized some nuclei of the cranial nerves that references were made to in the ninth chapter, and which were reminded in the tenth chapter only from a topographical standpoint, some of them topohistologically neighboring the nuclei described in this chapter.

The solitary tract nucleus is a well-contoured nucleus of oval shape, formed by polymorphous neurons, the oval ones most prominent, with relatively large sizes (31,5 μm), and a large nucleus (13,8 μm), disposed centrally, round or oval and euchromatic with an intensely basophilic nucleolus of approximately 2,4 μm . Nissl bodies visible in the perikaryon neuroplasma and at the base of dendritic extensions have an average size of 1,7 μm . The approximate total neuron count within a 1 mm fragment of the solitary tract nucleus, assessed on 33 of 200 seriated sections, reached 2275.

The *nucleus ambiguous* is a small but compact-looking nucleus, well-defined, where medium and large size neurons are found in greatest amounts, with many expansions and abundant chromatophilic substance. Within the *nucleus ambiguous* the average sizes are 34,1 μm for perikaryons, 16, 5 μm for nuclei and 4,3 μm for nucleoli. Nissl bodies are distributed non-

uniformly in the neuron cytoplasm under the form of blocks, granules, with an average size of approximately 2,3 μm . The approximate total neuron count within a 1 mm fragment of the *nucleus ambiguus*, assessed on 29 of 200 seriated sections, reached 726.

The *nucleus gracilis* is formed by small and middle-sized neurons with thick expansions, with tigroid bodies disposed all over the cytoplasmic mass and extending in dendritic extensions. The size of neuron perikaryons within the *nucleus gracilis* is 23,5 μm , the nucleus size is 15,6 μm and the nucleolus size is 4,1 μm . The approximate total neuron count within a 1 mm fragment of the *nucleus gracilis*, assessed on 40 of 200 seriated sections, reached 560.

Nucleus cuneatus is formed by diffuse neuronal cells, imprecisely delimited, of medium and small sizes, generally with polymorphous aspect and a centrally disposed large nucleus. The perikaryon size of neurons within the *nucleus cuneatus* is 13,8 μm , the size of the nucleus is 8,9 μm , the size of the nucleolus is 2,3 μm and the size of the Nissl bodies is 1,2 μm . The approximate total neuron count within a 1 mm fragment of the *nuclei cuneatus*, assessed on 67 of 200 seriated sections, reached 2010.

The neurons of the *reticular formation* are multipolar, large sized and with long and thick expansions. The size of neuron perikaryons within the *reticular formation* is 38,3 μm , the nucleus size is 16 μm , the nucleolus size is 3 μm and the Nissl body size is 1,4 μm . The approximate total neuron count within a 1 mm fragment of the *reticular formation* assessed on 25 of 200 seriated sections reached 1275.

The *lateral fascicle nucleus* is a nucleus formed by neurons of small and medium sizes, the average perikaryon size of approximately 22,5 μm , generally oval-shaped, but with multiangular shapes also noteworthy. Within the *lateral fascicle nucleus*, the neurons have the nucleus size of 9,2 μm , the nucleolus size of 3 μm and the Nissl body size of 1,2 μm . The approximate total neuron count within a 1 mm fragment of the *lateral fascicle nucleus* assessed on 40 of 200 seriated sections reached 1560.

The *olivary complex* contains the upper olivary nuclei, the middle olivary nuclei and the lower olivary nuclei and it is a large nucleus under the aspect of the neuronal population, but with small and middle sized neurons of pyramidal and multipolar shape and short extensions. The perikaryon size in the *olivary nuclei* is 18,2 μm , the nucleus size is 13,4 μm , the nucleolus size is 2,5 μm and the Nissl body size is 1,8 μm . The approximate total neuron count within a 1 mm fragment of the olivary complex assessed on 50 of 200 seriated sections reached 4250.