CYTOARCHITECTURE AND ACETYLCHOLINESTERASE ACTIVITY OF THE AMYGDALOID NUCLEI IN THE DOG

Anna KOSMAL and Liliana NITECKA

Department of Neurophysiology, Nencki Institute of Experimental Biology Warsaw, and
Department of Anatomy, Institute of Medical Biology, School of Medicine Gdańsk, Poland

Abstract. The cellular structure and distribution of histochemically demonstrated acetylcholinesterase (AChE) activity were studied in the amygdaloid body of 9 dogs. Cytoarchitectonic observations were made in series of paraffin and celloidin sections stained with cresyl violet. For the demonstration of the acetylcholinesterase activity, modifications of Koelle method were used. The general pattern of morphological structure of the dog's amygdaloid body is similar to that in other mammalian species. The corticomedial group of the nuclei was characterized generally by cytoarchitectonic uniformity of small, lightly stained cells and low intensity of the AChE reaction, except for the nucleus of the lateral olfactory tract and the lateral part of the central nucleus. The latter showed further differentiation in both cellular arrangement and distribution of AChE activity and may be divided into three subdivisions. The basolateral group of nuclei was characterized by higher differentiation of the cellular arrangement and distribution of the AChE activity. The highest enzyme activity was observed in the basal magnocellular nucleus. These findings support the homology of particular amygdaloid nuclei in various mammalian species.

INTRODUCTION

The amygdaloid body is one of the structures of the limbic system, involved in many vital functions of animals. Behavioral and electrophysiological investigations carried out on rats (10), cats (6, 16, 21, 22,
Morphological examinations of the amygdaloid body in various mammalian species show that the functional differentiation is reflected in the morphology of this structure. Moreover, considerable differences in the cellular composition of the amygdaloid nuclei have been found in various species of mammals (2, 10, 11, 17). Histochemical studies, particularly of the distribution of the acetylcholinesterase (AChE) activity, allow determination of homologous amygdaloid subdivisions as well as definitions of accurate boundaries of its nuclei in various species (11, 12, 26, 28, 30).

In contrast to physiological studies (3–6) there are few data concerning the morphological structure of the amygdaloid body in the dog. Maksymowicz (23) and Miodoński (24), on the basis of myeloarchitectonic investigations, have made a general division of the dog’s amygdala into particular nuclei according to the nomenclature of Johnston (15). The present paper deals with the cellular structure of particular nuclei and distribution of histochemically demonstrable acetylcholinesterase activity in the dog’s amygdaloid body.

**MATERIAL AND METHODS**

The cellular structure of the amygdaloid body was studied in five series of 10 μm thick paraffin sections and 50 μm thick celloidin sections stained with cresyl violet. Acetylcholinesterase activity was determined in brain sections by the method of Koelle as modified by Gerebtzoff (7). Four brains were perfused intravitaly with Baker fluid and blocks of tissue about 10 mm thick were fixed for 8–10 h in the same fluid at a temperature of 4°. Frozen 30 μm thick sections were incubated 3–4 h at 37° and a pH of 6.8. The incubation mixture and other reagents were prepared according to Gerebtzoff’s (7) description. Moreover, unfixed cryostat sections of the dogs’ amygdalae were incubated according to the Koelle copper thiocholine method, modified by Mellgren and Srebro (25).

**RESULTS**

On the basis of cytoarchitectonic observations two main regions in the dog’s amygdala can be distinguished: corticomedial and basolateral. The corticomedial part consist of the following nuclei: nucleus of the lateral olfactory tract, medial, cortical and central nuclei and the anterior amygdaloid area. The basolateral part on the other hand, consists of: basal magnocellular, basal parvocellular and lateral nuclei.
Neurons in the corticomedial part are typically smaller than those in the basolateral part. The corticomedial part occupies the anterior and dorsomedial region of the amygdala. The basolateral part is characterized by greater variability in the types of neurons and occupies the lateral and posterior regions of the amygdala. In addition to the above-listed nuclei, concentrations of small-sized neurons called intercalatae massae or intercalatae nuclei were noticed in various regions of the amygdala.

On the basis of AChE activity, three groups of nuclei are recognized: (i) nuclei with high AChE activity: basal magnocellular, the lateral part of the central nucleus, nucleus of the lateral olfactory tract; (ii) nuclei with moderate AChE activity: basal parvocellular, cortical nucleus, the medial part of the central nucleus, and the anterior amygdaloid area; (iii) nuclei with low AChE activity: medial and the intermediate part of the central nucleus.

The nucleus of the lateral olfactory tract (N, Fig. 1IC) is placed medially in the most anterior region of the amygdala. The cells of this nucleus differ from the neighboring cells of the nucleus diagonal band in the intensity of staining with cresyl violet and in their more dense arrangement (Fig. 1IA). The cell bodies were 12 to 16 μm in diameter when measured in material fixed with formalin (Fig. 2A). The intensity of its enzyme reaction to AChE is more than moderate. The cells of the nucleus are invisible, which suggests that the AChE activity is either equally localized in the cells and neuropil or exclusively in the latter (Fig. 1IB).

The anterior amygdaloid area (Aa, Fig. 1IC) is located laterally to the nucleus of the lateral olfactory tract and extends posteriorly, with no clearcut limit, into the nuclei of the corticomedial part of the amygdala (Fig. 1IA). It is composed of small (8–10 μm in diameter) and slightly larger (12–14 μm and 18–20 μm in diameter) neurons with the Nissl substance more clearly visible than in the neurons of the medial nucleus (Fig. 2B). The AChE activity in this area is of moderate intensity as in the medial part of the central nucleus which seems to be the direct posterior extension of the anterior amygdaloid area (Fig. 1IB). There are no well-marked local differences in the intensity of the AChE activity in the anterior part of this area. In the posterior part of the anterior amygdaloid area, two regions of different AChE activity can be distinguished: a dorsal and a ventral region. The histochemical reaction for AChE is stronger in the dorsal, and weaker in the ventral region with the perikarya of cells being invisible in both regions.

The medial nucleus (M, Fig. 1IC) is placed laterally and posteriorly to the nucleus of the lateral olfactory tract and anterior amygdaloid
area and directly adjacent to fibers of the optic tract. The medial nucleus is composed of very small cells lightly stained with cresyl violet. Their bodies are of 9 to 12 μm in diameter (Fig. 2C). On the surface of the nucleus a rather large layer of densely-packed cells can be observed (see double arrow in Fig. 1IIA). The medial nucleus in the dogs is most developed in the anterior portion of the amygdala and disappears in its central part in which the well-shaped stria terminalis can be easily distinguished. In the medial nucleus and also in the intermediate part of the central nucleus the enzyme reaction to AChE is weaker than in any other part of the amygdala. Only on the surface of the nucleus a very narrow band of higher AChE activity was found. This band does not seem, however, to represent the superficially, more densely packed cells of this nucleus. Rather, it merely compares the plexal layer of the periamygdalar cortex, and is, therefore, mainly represented by the nerve fibers rich in AChE (Fig. 1IIB).

The cortical nucleus (Co, Fig. 1II, IIIC) occupies the superficial, ventromedial part of the amygdala. In some respect it is the medial continuation of the pyriform cortex. The border between the pyriform cortex and cortical nucleus is delimited by the amygdaloid fissure which is, however, poorly seen on the surface of the brain (Fa, Fig. 1III, IIIC). The cellular architecture of the amygdaloid fissure is characterized by scattered cells which are different from the compactness of cells in layers II and III, typical for the pyriform cortex. The cortical nucleus is not homogeneous over its entire area. In the lateral part of the nucleus two layers of cells can be discerned. A thin external layer, showed a compact arrangement of cells and thereby may be considered equivalent to layers II and III of the pyriform cortex. The larger internal layer consisted of scattered cells and may be equivalent to layer IV of the pyriform cortex (Fig. 2D). The medial part of the cortical nucleus was occupied by a large cluster of cells in a compact group (Fig. 2E). Over the entire area of the cortical nucleus, both smaller and larger cells were seen, with the diameter of 13–14 μm and 17–18 μm respectively.

Dorsally, the cortical nucleus merges with the ventral part of the basal parvocellular nucleus. It was impossible to delimit the border between these two nuclei on the basis of the cellular structure, because the cells of the internal layer of the cortical nucleus intermingled with the cells of the ventral part of the basal parvocellular nucleus with no clearcut boundary. However, the intensity of the AChE activity lower in the cortical nucleus than in basal parvocellular, reveals the existence of a relatively clear boundary between those nuclei (Fig. 1III, IIIB). This boundary is, however, less evident more anteriorly; i.e., in the region in which the borders of all nuclei are indistinct. Posteriorly, the AChE
activity in the cortical nucleus increases slightly. Only along the periphery of the nucleus was there a indistinct zone of low activity which represents its superficial layer. Uniformly low AChE activity in the lateral and medial parts of the cortical nucleus did not give support to its cytoarchitectonic subdivision into two parts.

The central nucleus (Cm; Ci, Cl, Fig. III–IVC) is situated in the dorsal part of the amygdala just below the putamen. The border between these two structures can hardly be defined on the basis of cytoarchitectural differences. The only admissible distinguishing criterion was that the central nucleus lacked the large, darkly stained neurons, which, although in small numbers, were found in the putamen. The small-sized neurons which quantitatively dominated are very similar in both structures with respect to their size and staining properties in Nissl method.

However, from the detailed observations of the cellular structure of the central nucleus it may be concluded that this nucleus is not homogeneous and can be divided into three parts: medial, intermediate and lateral what is supported by localization of AChE activity (see below). In its medial part there are distinctly delimited groups of fairly large cells with the somata of about 14–16 μm in diameter. Scattered between them there are not numerous very small cells of 8–10 μm in diameter (Fig. 2F).

Instead, the entire remaining region of the central nucleus is composed of small, lightly stained neurons, 12–14 μm in diameter, and some single neurons, 9 and 17μm in diameter. However, larger neurons are more numerous in the lateral part of this nucleus than in its intermediate part (Fig. 2GH). The AChE activity localization in the central nucleus of the amygdala in the dog is different in these three parts similar to previously investigated species of animals (26) (Fig. 1II, IIIIB).

1. The medial part is characterized by the AChE enzyme reaction of moderate intensity, which is slightly higher in its dorsal zone and lower in the ventral zone adjacent to the medial nucleus. Single cells of higher activity are visible here.

2. Almost no AChE activity can be observed in the intermediate part of the central nucleus.

3. Conversely, in the lateral part, providing the transitional area between the amygdala and the putamen, the AChE activity is the highest and nearly comparable to that in the basal magnocellular nucleus. However, the AChE activity is less intense here than in the putamen, so that the border between the two structures can be recognized.

The basal magnocellular nucleus (Bm, Fig. 1II–IVC) is situated centrally in the amygdala. It is easily distinguished from the surrounding
nuclei due to its typical, triangular shape and large, dark stained neurons (Fig. 1III-IVA). It boarders the lateral nucleus on the lateral side, the lateral part of the central nucleus on the dorsomedial side, while ventrally it is adjacent to the basal parvocellular nucleus. More detailed observations of cytoarchitecture of the basal magnocellular nucleus reveals that it is composed not only of characteristically large and well stained neurons (their somata are ca. 28 \( \mu \text{m} \) in diameter), but also of medium-sized (18–22 \( \mu \text{m} \) in diameter) and small neurons (8–11 \( \mu \text{m} \) in diameter) (Fig. 2I). The cellular arrangement of the nucleus is generally homogeneous. However, some separate group of different neurons was, oddly enough, located inside this nucleus (Fig. 1III-A).

The AChE activity in the basal magnocellular nucleus is far higher than in the adjacent amygdaloid nuclei (Fig. 1III-IVB). The activity was merely moderate only in the anterior extension of this nucleus, where the boundaries of the nucleus are not clearly visible. In the posterior portion of the basal magnocellular nucleus there is a small area of slightly lower AChE activity; this area is a wedge-shaped ventrolateral area of the nucleus, placed between the lateral and basal parvocellular nucleus.

The basal parvocellular nucleus (Bp, Fig. 1III-IVC) is located in the dog’s ventral amygdala in relation to the basal magnocellular nucleus, not medially as in primates. The great variety of sizes of neurons is a characteristic feature of this nucleus. The somata of large neurons are 22–24 \( \mu \text{m} \) in diameter, 15–16 \( \mu \text{m} \) in the medium size cells and about 8–11 \( \mu \text{m} \) in the smallest neurons (Fig. 2J). The dorsal boundary of the nucleus is clearly delimited in the posterior part, being slightly less distinct only in the anterior amygdala. There is the area of gradual diminution of the size of neurons from the basal magnocellular to the basal parvocellular nucleus, which is similar to that described in other mammals by Koikegami (17). In the inferior part of the basal parvocellular nucleus, the cells became slightly scattered and linked with the internal, dorsal layer of the cortical nucleus. Similarly, its lateral portion extended into the lateral nucleus without any distinct borderline. The AChE enzyme reaction is of moderate intensity in the basal parvocellular nucleus and is not equal over the entire area of this structure, with slightly lower reaction in the centrolateral part and higher in the medial and posterior part of the nucleus (Fig. 1III-IVB). The boundaries of this nucleus could be delimited in the central and posterior part of the amygdala.

The lateral nucleus (L, Fig. 1III-IVC) occupies the entire lateral region of the amygdala. On the medial side it borders the lateral part of the
central nucleus and the basal magno- and parvocellular nuclei. Laterally and posteriorly it is limited by the fibers of the external capsule. The lateral nucleus is not homogeneous with respect to its cellular structure. In the anterolateral part of the amygdala, between the bundles of fibers, the medium-size dark stained neurons appeared. They should be considered as belonging to the lateral nucleus. In the central and posterior part of the nucleus these relatively large, dark stained neurons form a thin layer adjacent to the external capsule (Fig. 1II–IVA shown by arrow). The bodies of these neurons were 17–19 μm in diameter. In between the dark stained rather large neurons, less numerous and scattered neurons of 14–16 μm in diameter and very small ones, 8–10 μm in diameter were observed (Fig. 2K). Towards the center of the amygdala, in the region adjacent to the basal magnocellular nucleus there is a zone of rather scattered cells of various shapes, sizes and intensity of staining. In this medial part of the nucleus the most numerous were the medium-size, rather lightly stained with cresyl violet neurons of 13–16 μm in diameter and very small neurons of 8–10 μm in diameter (Fig. 2L). The AChE activity is moderate in most of the lateral nucleus. A somewhat stronger AChE enzyme reaction is observed in the anterior part of the nucleus as well as in its ventral and posterolateral portion (Fig. 1II–IVB).

The massae intercalatae (I, Fig. 1IIC) was found in great numbers in various parts of the amygdala. Moreover, especially large and numerous clusters of these neurons were observed as isolated densely packed groups of small neurons in the anterior part of the amygdala, between the medial and central nuclei, as well as between the central and basal magnocellular nuclei. In addition, small groups of extremely small neurons were seen on the surface of the lateral nucleus.

Regarding their cytoarchitectonic arrangement these groups are not identical. The most numerous are the groups of very small, lightly stained neurons, the somata of which measured approximately 8 μm in diameter (Fig. 2M). In addition, between the medial, central and basal magnocellular nuclei a small group of slightly larger, less compact and darker stained neurons were found (Fig. 2N). Usually, the clusters of intercalated neurons were seen accompanying a bundle of fibers on the borders of various nuclei. The only exception is a group of neurons in the region of the basal magnocellular nucleus. The existence of the isolated group of neurons within the basal nucleus had not been previously reported in other species of animals. In the dog, the groups of neurons of massae intercalatae are very numerous and variable in their cellular arrangement, which makes the amygdala even more differentiated from a cytoarchitectonic view. In the preparations stained by the Koelle's method, the intercalated masses were clearly distinct from the other
nuclei of the amygdala due to their relatively high AChE activity. The lowest AChE reaction was seen in the massae intercalatae composed of definitely larger cells.

DISCUSSION

Parallel investigation of the cellular structure and the level of AChE activity allowed the establishment of the general pattern of the cytoarchitectonic division of the dog's amygdala as well as the boundaries between the respective amygdaloid nuclei. On the basis of our results, the general division of the dog amygdala into corticomedial and basolateral parts may be accepted, according to Johnston's (15) nomenclature for other mammalian species. Moreover, in both regions of the amygdaloid body, the distribution of AChE activity and the cellular arrangement in particular nuclei suggest further internal differentiation of this structure.

Our observations of the cellular structure and AChE activity of the nucleus of the lateral olfactory tract, indicating uniformity of this nucleus, did not support the subdivision as mentioned by Koikegami (17). A similar observation has also been reported by Hall and Geneser-Jensen (11) in their histochemical study of the amygdala in the guinea pig. High AChE activity of this nucleus in the dog may, to some extent, be a result of the activity of cells of the nucleus itself or of the afferent system of fibers (20, 31). The anterior amygdaloid area was distinguished for the first time by Gurdijan (9) and then by other researchers in various species of animals on the basis of embryological (13, 14), cytoarchitectonic and histochemical (12, 17, 27, 29) investigations. However, it is still a very unclearly delimited area.

The medial nucleus of the amygdala is composed of small lightly tectonic and histochemical (12, 17, 27, 29) investigations. However, it is structure is concerned. Compact arrangement of the cells in the medial part of the nucleus has also been reported by Hall and Geneser-Jensen (11). The superficially thin layer of the enhanced AChE activity is probably related to the afferent fibers reaching this nucleus, similar to what has been noted in the cortical nucleus as a continuation of the plexiform layer of the pyriform cortex. Conversely, no differences in the AChE activity is observed in the medial and lateral parts of the cortical nucleus. For this reason, notwithstanding some variations of the neuronal arrangement, it seems appropriate to consider this nucleus as a homogeneous entity, delimited by the region of a uniformly low level of AChE activity. Koikegami (17) applies the term “cortical nucleus” for two different structures depending on the species; either for the group
of cells situated most medially (and according to Hall, representing medial part of the cortical nucleus) or for the group of cells lying deeply in the nucleus above the pyriform cortex which then extends far in the medial direction.

In the corticomedial part of the amygdala, the most controversial data concern the central nucleus, probably due to differences of its structure in various species. In our material we were able to distinguish two cytoarchitectonically different parts in this nucleus, as has been usually described in other species (17, 30). However, in the lateral part of the nucleus, some cytological differences between its dorsal and ventral region also exist. On the other hand, striking differences in the level of AChE activity suggest the existence of three subdivisions in this nucleus, which support the results obtained by Nitecka (26–29) in other species. Therefore it seems justified to divide the central nucleus of dogs into three parts: medial, intermediate, and lateral. The lateral part shows the highest enzyme activity, so that the border with the putamen is still difficult to define, although comparing the level of AChE activity can be delimited with greater precision. This portion of the nucleus in some species was denoted as C-p (caudate-putamen nucleus) (17); in the guinea pig it was labelled as X-zone (11), and in the cat as the lateral part (12). Ben-Ari et al. (1) suggested that this region should be regarded as an extension of the putamen. Nevertheless, this problem needs further investigation. The basal magnocellular nucleus is characterized in dogs by a very high AChE activity level being equal over the entire area of this nucleus. The distinct character of both cellular composition and AChE activity level permits the precise delimiting of the borders of the nucleus. The separate group of cells situated in the central region of the basal magnocellular nucleus were not accompanied by a change in the intensity of the AChE reaction. It is only the small extension of the posteroverentral part of the basal magnocellular nucleus, situated between the lateral nucleus and the basal parvocellular nucleus, where AChE activity is weaker. This zone may be compared with part X of the basal magnocellular nucleus described in rats (28) and other species (27, 29) as a small area of the weaker activity, being a transition from basal magnocellular nucleus to the deep layer of the entorhinal cortex. It seems, however, that the intensity of the activity in this zone is higher than in adjacent lateral and basal parvocellular nuclei. Possible, it is due to the passage of the more active fibers which cross this area on their way from or to the entorhinal cortex. In the basal parvocellular nucleus the AChE activity level is considerably lower than that in the dorsally located basal magnocellular nucleus, although a few zones of slightly different intensities of the enzyme reaction might be discerned
in it. However, on the basis of cytoarchitectonics, no separate groups of cells could be discerned. Probably, this nucleus receives fibers of different AChE activity from various structures, and neuropil in its particular areas indicate the different AChE reaction. In the lateral nucleus, a slightly higher reaction is observed in the pericapsular part composed of large neurons, while markedly higher activity was observed in the anterodorsal part of the nucleus. The dorsal cluster of neurons, situated laterally to the putamen, corresponds to the "nucleus putaminalis", as named by Maksymowicz (23) and Miodoński (24), and it forms the lateroanterior continuation of this "magnocellular" pericapsular part of the lateral nucleus. Our findings support in some respect the differentiation of the lateral nucleus into the dorsal and ventral parts. Such a division had been suggested on the basis of both the electrophysiological investigations on cats (6, 33), behavioral studies in dogs (4, 5) and anatomical studies carried out by Golgi's method in cats (32). In the dog, studies of the efferent connections of the amygdala (18, 19), have supported this subdivision of the lateral nucleus. We have found that the intercalated masses in the dog's amygdala are especially numerous and characterized by the cytoarchitectonic differentiation. The intercalated groups lying in the anterior part of the amygdala are very large. On the other hand, along the borders of almost all nuclei, including those in the posterior part of the amygdaloid body, there are small groups of densely-packed, very tiny neurons. A very large intercalated group, composed of slightly larger and less densely-packed cells situated in the anterior portion of the amygdala between the medial, central and basal magnocellular nuclei, might be equivalent to the group X described by Koikegami (17) in the tiger. It must be emphasized that in these groups, contrary to what was observed in some other species of animals and in humans (27), the AChE enzyme reaction is markedly higher.

Taking into consideration both the cellular structure and the distribution of the AChE activity, we may conclude that the dog's amygdaloid complex is constituted according to a general pattern that is common to mammalian species. However, it must be pointed out that some nuclei, e.g., central, basal parvocellular and lateral, are characterized by further internal differentiation.

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Anna Kosmal, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw, Poland.
Liliana Nitecka, Institute of Medical Biology, School of Medicine, Dębinki 1, 80-211 Gdańsk, Poland.
LIST OF ABBREVIATIONS

Aa  anterior amygdaloid area
Bm  basal magnocellular nucleus
Bp  basal parvocellular nucleus
Ci  central nucleus, intermediate part
Cl  central nucleus, lateral part
Cm  central nucleus, medial part
Co  cortical nucleus
Fa  amygdaloid fissura
I   massae intercalatae
L   lateral nucleus
M   medial nucleus
N   nucleus of lateral olfactory tract
Fig. 1. Cytoarchitecture (A), distribution of AChE activity (B), and scheme of the amygdaloid nuclei (C) within the dog's amygdaloid complex. Four frontal sections from different anteroposterior positions at approximately the same levels are shown. Arrow in II, III, IVA — point to pericapsular part of the lateral nucleus. Double arrow in IIA — point to superficial, densely-packed cells in medial nucleus.
Fig. 2 (continued)
Fig. 2. Cellular structure of the particular amygdaloid nuclei in the dog. A, nucleus of lateral olfactory tract; B, anterior amygdaloid area; C, medial nucleus; D, cortical nucleus, lateral part; E, cortical nucleus, medial part; F, central nucleus, medial part; G, central nucleus, lateral part; H, central nucleus, intermediate part; I, basal magnocellular nucleus; J, basal parvocellular nucleus; K, lateral nucleus, pericapsular part; L, lateral nucleus, internal part, M, N, massae intercalatae.