Acta Veterinaria (Bepgrad), Vpl. 47. Np. 5-6, 361-370, 1997.

UDK 619:611.018.8

THE CENTRAL NUCLEUS OF THE AMYGDALOID COMPLEX IN THE RAT BRAIN

OLIVERA LOZANČE, D. DREKIĆ and DIJANA DJELIĆ

Department of Anatomy, Faculty of Veterinary Medicine, Beograd

(Received, 13. August 1997.)

The present investigation attempts to define the main adbivisions of the central nucleus (CN) of the amygdaloid complex (AC) by some histological methods and sterological parameters. Since recent studies indicate that distinct neuropeptides and projections are associated with discrete portions of the central amygdaloid nucleus, a detailed investigation of the cytoarchitecture of the CN should contribute to a better understanding of its combined structure and organization. Qualitative and quantitative analyses of the rat CN suggest that it consists of two main lateral and medial subdivisions. In addition, this is the first morphometric study that recognizes the differences in the investigated stereological parameters of the CN lateral and medial subregions and sex dimorphism in the quantitative parameters of the lateral subregion of the rat CN.

Key words: central nucleus, amygdaloid complex, subregions, differences, stereology, rats

INTRODUCTION

The central nucleus (CN) is located in the dorsal portion of the rat amygdaloid complex (AC). Recent anatomical and histochemical studies have provided evidence that the CN represents the major part of a single anatomical entity commonly referred to as the "extended amygdala" including the bed nucleus of the stria terminalis and substantia innominata (Alheid and Heimer, 1988; Sun and Cassel, 1993; Hyytia and Koob, 1995). Stimulation and ablation studies suggest that the CN is an important forebrain autonomic structure which participates in numerous adaptive behaviours including arousal, feeding, avoidance and the defence reaction, and produces visceral responses that accompany such behaviours including changes in heart rate, blood pressure, respiration and gastrointestinal function (Le Doux et al., 1988; Bernard et al., 1997; Petrov et al., 1994; Turton et al., 1996; Bohus et al., 1996a).

The cytoarchitecture of the AC, including the CN has been studied in numerous species mostly using Nissl and Golgi-stained brains. In that way, the earliest descriptions of the rat central nucleus (Johnston, 1923; Gurdian, 1928), describe the CN of the rat as a homogenous, undifferentiated mass in the medial

part of the amygdala. Later investigators, on the cat (Fox, 1940), rat (Brodal, 1947; Uchida, 1950; Koikagami, 1963) and several other species, have recognized medial and lateral subdivisions, but these reports differ in the exact delineation of each subdivision. In the guinea pig, Hall and Genser-Jensen (1971) noticed a region, area X, at the junction of the CN and putamen. McDonald (1982) and Cassell et al., (1986) identified four main subdivisions on the basis of quantitative analysis of Nissl-stained material. McDonald defined:medial, lateral, lateralcapsular and ventral subdivisions of the CN of the AC. DeOlmos (1990) subdivided the CN into lateral (main-central core, main-anterodorsal apical, capsular-dorsal, capsular intermedial and Cassell et al., (1986): Medial, lateral, lateralcapsular and ventral and paracapsular parts) and medial subdivisions (dorsal and ventral parts).

It is clear that there is confusion regarding the manner in which the CN should be subdivided. The present investigation attempts to define subregions of the central nucleus of the rat AC by correlating some of the currently employed morphometric parameters.

MATERIAL AND METHODS

The cytoarchitecture and morphometric analysis of the central nucleus of the amygdaloid complex were studied in the brains of twenty adult Wistar rats (10 males and 10 females). The animals were sacrificed on the 62nd day of life under ether narcosis. After that, the brains were removed and amygdaloid complexes with surrounding parts of brain were isolated and fixed in Bouin solution. Standard paraffin embedding was done and serial microtome transversal sections (5 μm thick) were stained with hematoxylin-eosin, and by the Klüver-Barrera and Herlant methods. On Klüver-Barerra stained sections, using light microscopy, we determined the precise localization and defined subregions of the central amygdaloid nucleus. The definition and description of the main subdivisions of the central nucleus of the amygdaloid complex folow the scheme (lateral/medial and anterior/posterior neurogenetic gradients) suggested by Krettek and Price (1978) and Bayer (1980).

For stereological analyses, two morphological parameters, the volume density /Vv/ (mm^0) of lateral and medial parts of CN cell nuclei, cytoplasm, neuropil and the numerical density /Nv/ (mm^{-3}) of cell were determined, using the Weibel multipurpose test system (P:42). Sampling was performed so that the rostral (the first), central (the middle) and posterior (the last) hematoxylin-eosin sections of the amygdaloid complex were used. For each lateral and medial part of the CN AC, 25 test fields were chosen (from anterior, central and posterior levels of the CN AC) by intermittent sampling (Kališnik, 1985). Statistical significance was tested with Student's t-test.

RESULTS

The central nucleus consisted of a neuronal structure surrounded by fibers of the longitudinal association bundle laterally and ventrally which sharply demar-

cated it from the lateral (LN), basolateral (BLN) and basomedial nuclei (BMN). Within the amygdaloid complex the central nucleus of the AC was medially separated from the medial nucleus (MN) by fibers of the stria terminalis (Figure 1.). In our Klüver Barrera-preparations, the central nucleus in adult mature rats was clearly distinguished from neurons of the other nearby nuclei within the AC (bacomedial, basolateral, lateral and medial) and other similar neuronal structures (putamen, claustrum, substantia innominata and area amygdaloidea anterior). Throughout its rostro-caudal extent, the central nucleus always lay dorsal in the anterior, the middle and the posterior part of the AC in adult rat brain. At its rostral (anterior) and caudal (pasterior) poles, it blended with the anterior amygdaloid areas and the putamen, respectively.

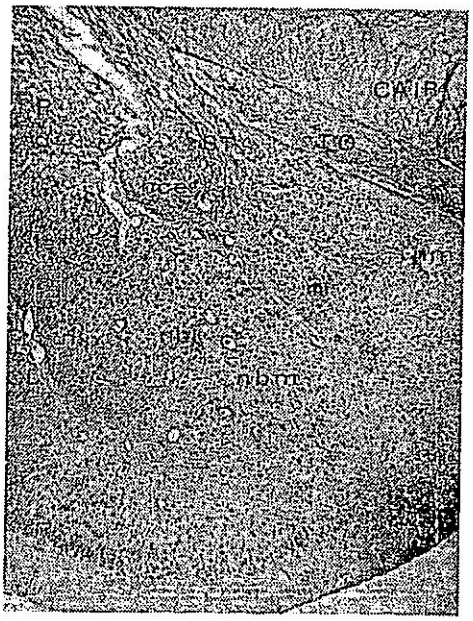

Figure 1. Nucleus centralis (nce) with surrounding neuronal structure in the adult rat brain: nm-nucleus medialis; nbl-nucleus basolateralis; nbm-nucleus basomedialis; nla-nucleus lateralis anterior; nlp-nucleus lateralis posterior; nco-nucleus corticalis; mi- massa intercalata; ST-stria terminalis; TO- tractus opticus; ce-capsula extema, CL - claustrum, P-putamen; CAIR-capsula intema; 44x, H&E.

On the basis of differences in topographic location and differences in cell density, neuron body size and density, we distinguished two main subdivisions: the medial and lateral parts of the CN. Most of the medial subdivision lay dorsomedially to the lateral part of the CN. It was interposed between the latter, medial, lateral and basolateral nuclei. The medial part of the CN was composed primarily of neurons that lay with their maximal diameter oriented in parallel with fibers of stria terminalis. The lateral subregion of the CN consisted of the neuronal

mass which was located in the middle and posterior part of the AC forming a round-oval shaped structure between the medial partof the CN and the basolateral nucleus of the AC (Figure 2.). The population of neurons in the lateral

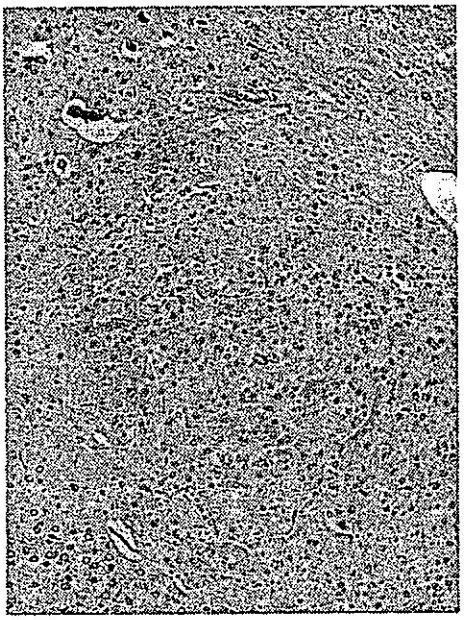

Figure 2. Nucleus centralis (lateral subregion), 108x, H&E.

region was more numerous than the neuronal population of the medial CN subregion. In our Klüver Barrera preparations, this substructure of the CN was easily recognized surrounded by a capsule of tightly organized myelinated fibers projecting towards ansa lenticularis and stria terminalis at the medial site. On Herlant cell preparations, the myelin capsule of the lateral subregion was more difficult to observe. However, rounded and slightly oval neurons in this subregion were clearly recognized. These neurons had an intermedialry size (slightly larger than neurons of the MN and significantly smeller than neurons of the BLN that lean an the lateral and ventrolateral site of the CN subregion).

Stereological results. The results of the stereological investigations of the lateral and medial subregions of the CN AC in male and female rats are shown in Tables 1. and 2. In the male rats the data demonstrated that the volume density of neuronal nuclei and cytoplasm in the medial part of the CN was significantly ($p< 0.05$) greater than that in the lateral part of this nucleus. In contrast, the volume density of neuropil and its numerical density were significantly ($p<0.001$) greater in the lateral than in the medial part of the CN. In the female rats, there were no differences ($p>0.05$) in the volume density of neuronal nuclei, cytoplasm

and neuropil between the medial and lateral and lateral parts of the CN AC. As in the male, the numerical density was significantly ($p<0.001$) greater in the lateral subregion of the CN AC.

Table 1. Summarized results of stereological measurements of the CN in adult male rats: A/ - lateral subregion; B/-medial subregion.

Table 1. Males

A/lateral subregion	Σx	$\bar{x}$	±SD	V_v (mm^0)	V_v (%)	N_v (mm^{-3})
nucleus	41	1.64	0.97	0.039	3.90	
cytoplasm	33	1.32	1.00	0.031	3.14	
neuropil	980	39.20	1.20	0.930	93.00	
N^0 of neurons	893	35.72	1.88			6.372×10^4
B/medial subregion						
nucleus	56	2.24	0.94	0.053	5.30	
cytoplasm	49	1.96	1.11	0.046	4.60	
neuropil	945	37.80	1.41	0.900	90.00	
N^0 of neurons	842	33.68	2.07			5.901×10^4

Table 2. Summarized results of stereological measuerments of the CN in adult female rats: A/- lateral subregion; B/ - medial subregion.

Table 2. Females

A/lateral subregion	Σx	$\bar{x}$	±SD	V_v (mm^0)	V_v (%)	N_v (mm^{-3})
nucleus	55	2.20	0.89	0.0523	5.23	
cytoplasm	47	1.88	1.10	0.0447	4.47	
neuropil	948	37.92	1.54	0.9028	90.28	
N^0 of neurons	943	37.72	1.94			6.881×10^4
B/medial subregion						
nucleus	57	2.28	1.00	0.0542	5.42	
cytoplasm	45	1.80	0.98	0.0428	4.28	
neuropil	946	37.84	1.43	0.9000	90.00	
N^0 of neurons	819	32.76	2.50			5.685×10^4

Sx – Total Number of Sections
$\bar{x}$ – Mean Value
SD – Standard Deviation
V_v – Volume Density
N_v – Numerical Density

Since data in the literature showed that AC was a sexually dimorphic region, we analysed the numerical values of the stereological parameters (volume and numerical densities) within the lateral and medial subregions of the CN AC in mature male and female rats. The data demonstrated that there were no significant ($p>0.05$) differences in the investigated volume densities of cytoplasm, of neuropil and of cell nuclei between adult male and female rats in the medial

subregion of the CN AC. The numerical density of cells in the same medial part also, did not show significant differences ($p>0.05$) between values for males compared to female rats: Nv -in the female was 5.685×10^4 mm^{-3} and in the male 5.901×10^4 /mm^{-3}/. The results of stereological measurement of the medial subregion of the CN are summarized in Table 1. B/ and 2. B/. The volume densities of the cytoplasm and the neuronal nuclei in the lateral part of the CN were not significantly ($p>0.05$) different between adult male and female rats. The volume density of the neuropil in the males was significantly ($p<0.05$) greater than that in the females. Thus, the volume density of the neuropil in males was about 3% greater than that in females. The numerical density of cells in the lateral subregion of the CN also was significantly ($p<0.001$) greater in males compared to female rats; the increase was about 8%. The results of stereological measurements of the lateral subregion of the CN are summarized in Table 1. A/and 2. A/

DISCUSSION

One of the basic problems in neurobiology today is the exact conformation of the morphologic and functional characteristics of different parts of the central nervous system, especially the amygdaloid complex with its structural and functional complexity. The central nucleus of the amygdaloid complex, its morphology and subdivisions, has recently atracted considerable attention because it has been found to contain a vast array of different neuropeptides and is unique among the amygdaloid nuclei in its extensive connections with visceral and monoaminergic nuclei of the brainsteam (Kainu et al., 1993; Bohus et al., 1996b). Cytoarchitectural studies of the CN have been conducted in a variety of species using Nissl and Golgi techniques; these investigations have revealed that the CN in all species consists of subdivisions. Detailed hodological and immunohistochemical studies, conducted primarily in the rat, have demonstrated that each subdivision exhibits a characteristic set of connections and neuropeptides (Veening et al., 1984; Gray and Magnuson, 1992; McDonald et al., 1995; McDonald et al., 1996).

In virtually all mammals it is possible to recognize a medial subdivision of the CN located just lateral to the medial amygdaloid nucleus, and a lateral portion located just medial to the caudoventral caudoputamen. In the rat, the lateral portion can be subdivided into two subdivisions: a lateral subdivision proper located just lateral to medial subdivision and a lateral subcapsular subdivision that encapsulates the lateral half of the CL (McDonald, 1982). Although, McDonald (1982) and Cassell et al. (1991) suggested that the central amygdaloid nucleus consist of four subdivisions, we entertain the opinion that only the subdivision of neurons into new groups inside the lateral and medial subregions has occurred as deOlmos (1990) has established. Our morphological results and cytoarchitectonic description of the CN AC in adult rat brain correspond to those described in many previous morphological, histochemical and immunocytochemical studies (Krettek and Price, 1978; Cassell et al., 1986; deOlmos, 1990; Roder and Ciriello, 1993). These studies have identified a broad division of the CN into lateral and medial zones. This division forms the basis for

the general terminlogy describing the anatomy of the CN in several species, which we accepted too. Medial/lateral differences in the morphology of the neurons (Hall, 1972; McDonald, 1982) and in the distribution of intraamygdaloid afferents (Krettek and Price, 1978; de Olmos, 1990; Pitkänen et al., 1995), opoid peptides (Gray et al., 1984), catecholamines (Fallon,1981), acetylcholinesterase (BenAri et al., 1977; Amaral and Bassett, 1989) and neurons exibiting GABA-like immunoreactivity (McDonald and Augustine, 1993; Sun and Cassell, 1993), suggested that the cytoarchitectonic division of the CN into medial and lateral zones may reflect some basic organizational feature of the CN. The study by Veening et al. (1984) indicates that medial/lateral trends may also be fundamental to the organization of certain populations of projection and peptide-containing neurons of the CN. Our results showed the existence of medial and lateral subregions of the CN AC in adult, sexual mature rats. These subregions were determined on the basis of their rostrocaudal extension, localization and differences in cell density, neuron body size and orientation. We also identified a medial/lateral difference in the investigated stereological parameters: the volume density of neuronal nuclei and cytoplasm in the medial part of the CN were significantly greater than that in lateral part of this nuclei in males. On the contrary, the volume density of the neuropil and numerical density were significantly greater in the lateral than in the medial part of the CN in both male and female rats.

Having in mind experimental data that the AC is a sexually dimorphic region, we also analysed the numerical values of the stereological parameters for lateral and medial subregions of the CN statistically. The results showed that the volume density of the neuropil in the adult males was significantly ($p<0.01$) greater than that in the sexually mature females; the numerical density of cells in the lateral subregion of the CN also was significantly ($p<0.001$) greater in males compared to female rats. These results demonstrate for the first time that, like the posterodorsal part of the NM, the lateral subregion of the CN AC represents a sexually dimorphic area of the amygdaloid complex in the adult rat brain.

REFERENCES

1. *Alheid, G. F. and Heimer, L.* 1988. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience, 27 (1)*, 1-29.

2. Amaral, D. G. and Bassett, J. L. 1989. Cholinergic innervation of the monkey amygdala: an immunohistochemical analysis with antisera to choline acetyltransferase. *J. Comp. Neurol, 281 (3)*, 337-61.

3. *Bayer, A. S.* 1980. Quantitative H-thymidine radiographic analyses of neurogenesis in the rat amygdala. *J. Comp. Neurol., 194*, 845-75.

4. *Ben-Anri, Y., LeGal La Salle,G. and Kanazava, I* 1977. Regional distribution of substance P within the amygdaloid complex and bed nucleus of the stria terminalis. *Neurosci. Lett., 4*, 299-302.

5. *Bernard, J. F., Alden, M. and Besson, J. M.* 1993. The organization of the efferent projection from the pontine parabrachial area to the amygdaloid compleex: a Phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. J. Comp. Neurol., 8 (2), 201-29.

6. *Bohus, B., Koolhaas, J. M., Luisten, P. G., Korte, S. M., Roozendaal, B. and Wiesma, A.* 1996a. The neurobiology of the central nucleus of the amygdala in relation to neuroendocrine and autonomic outflow. *Prog. Brain Res., 107,* 447-60.

7. *Bohus, B., Koolhaas, J. M., Korte, S. M., Roozendaal, B. and Wiersma, A.* 1996b. Forebrain pathways and their behavioral interactions with neuroendocrine and cardiovascular function in the rat. *Clin. Exp. Pharmacol. Physiol., 23 (2)* 177-82.

8. *Brodal, A.* 1974. The amygdaloid nucleus in the rat. *J. Comp. Neurol., 87:* 1-16.

9. *Cassell, M. D., Gray, T. S. and Kiss, J. Z.* 1986. Neuronal architecture in the rat central nucleus of the amygdala: cytological, hodological, and immunocytochemical study. *J. Comp. Neurol., 246,* 478-99.

10. *Cassell, M. D. and Roberts, G. W.* 1991. Ultrastructural evidence for an olfactory - autonomic pathway through the rat central amygdaloid nuclei. *Neurosci. Lett.,1,* 100-4.

11. *De Olmos, J.* 1990. Amygdala. The human nervous system. Instituto de investigacion medica. Mercedes Y Martin Ferreyra Cordoba, *Argentina, Academic Press. Inc.,* 583-710.

12. *Fallon, J. H.* 1981. Histochemical characterization of dopaminergic, noradrenergic and serotonergic projections to the amygdala. In Y. Ben-Ari(ed): *The amygdaloid Complex INSERM Symposium No. 20, Amsterdam: Elsevier/North Holland, pp.* 175-183.

13. *Fox, C. A.* 1940. Certain basal telencephalic centers in the cat. *Journal Comp. Neurol, 72,* 1-16.

14. *Gray, T. S. and Magnuson, D. J.* 1992. Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. *Peptides, 13, 3,* 451-460.

15. *Gray, T. S., Cassel, M. D., Nilaver, G., Zimmerman, E. A. and Wialiams, T. H.* 1984. The distribution and ultrastructure of VIP-immunoreacitivty in the central nucleus of the rat amygdala. *Neuroscience, 11, 2,* 399-408.

16. *Gurdijan, E. S.* 1928. The corpus striatum in the rat. *Journal Comp. Neurol., 45,* 249-281.

17. *Hall, E. and Genser-Jensen, F. A.* 1971. Distribution of acetylcholinesterase and monoaminooxidase in the amygdala of the guinea pig. - *Z. Zellforsch, 120,* 204-221.

18. *Hall, E.* 1972. The amygdala of the cat. A Golgi study. *Z. Zellforsch, Microscop. Anat., 102,* 40-48.

19. *Hyytia and Koob, G. F.* 1995., GABA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *J.Pharmacol., 283, 1-3,* 151-159.

20. *Johnston, J. B.* 1923. Further contributions to the study of the evolution of the forebrain. *Journal Comp. Neurol. 35,* 337-481.

21. *Kainu, T., Honkaniemi, J., Gustafsson, J. A., Rechardt, L. and Pelto-Huikko, M.* 1993. Co-localization of peptide-like immunoreactivities with glucocorticoid receptor-and Fos-like immunoreactivities in the rat parabrachial nucleus. *Brain Res, 615,* 245-51.

22. *Kališnik, M.* 1985. Temelji stereologije, predelana in razširjena izdaja. Ljubljana.

23. *Koikagami, H.* 1963. Amygdala and other related limbic structures: experimental studies on the anatomy and function. I. Anatomical researches with some neurophysiological observations. *Acta Med. Biol. (Niigata), 10,* 161-169.

24. *Krettek, J. E. and Price, J. L.* 1978. A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. *J. Comp. Neurol. 178.* 255-280.

25. *LeDoux, J. E., Iwata, J., Cicchetti, P. and Reis, D. J.* 1988. Different projections of the central amygdaloid nuclei mediate autonomic and behavioral correlates of conditioned fear. *J.Neurosci, 8,* 2517-29.

26. *McDonald, A. J.* 1982. Cytoarchitecture of the central amygdaloid nucleus. *J. Comp. Neurol., 208,* 401-18.

27. *McDonald, A. J., Mascagni, F. and Augustine, J. R.* 1995. Neuropeptide Y and somatostatin-like immunoreactivity in neurons of monkey amygdala. *Neuronscience, 44,* 959-82.

28. *McDonald, A. J. and Augustine, J. R.* 1993. Localization of GABA-like immunoreactivity in the monkey amygdala. *Neuroscience, 52,* 2, 281-94.

29. *McDonald, A. J., Mascagni, F. and Guo, L.* 1996. Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience, 71,* 55-75.

30. *Petrov, T., Jhamandas, J. H. and Krukoff, T. L.* 1994. Electrical stimulation of the central nucleus of the amygdala induces fos-like immunoreactivity in the hypothalamus of the rat: a quantitative study. *Brain Res. Mol. Brain, 22, 1-4,* 333-40.

31. *Pitkänen, A., Stefanacci, L., Farb, C. R., Go, G. G., LeDoux, J. E. and Amaral, D. G.* 1995. Intrinsic connections of the rat amygdaloid complex: projections originating in the lateral nucleus. *J. Comp. Neurol., 356,* 2, 288-310.

32. *Roders, S. and Ciriello, J.* 1993. Contribution of the bed nucleus of the stria terminalis to the cardiovascular responses elicited by stimulation of the amygdala. *J. Auton. Nerv. Syst., 45,* 61-75.

33. *Sun, N. and Cassel, M. D.* 1993. Instrict GABA-ergic neurons in the rat central extended amygdala. *J. Comp. Neurol, 3,* 381-404.

34. *Turton, M. D., O'Shea, D., Gunn, I.,* Beak, S. A., S. A., Edwards, C. M., Meeran, K., Choi, S. J., Taylor, G. M., Heath, M. M., Lambert, P. D., Wilding, J. P., Smith, D. M., Ghatei, M. A., Herbert, J. and Bloom, S. R. 1996. A role for glucagon-like-peptide-1 in the central regulation of feeding. *Nature, 379, 6560,* 69-72.

35. *Uchida, Y.* 1950. A contribution to the comparative anatomy of the amygdaloid nuclei in mammals, especially in rodents. Part I. Rat and mouse. Folia Psychiat. *Neurol. Japon (Niigata), 4,* 25-42.

36. *Veening, J. G., Swanson, W. L. and Sawchenko, P. E.* 1984. The organization of projections from central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport immunohistochemical study. *Brain Res., 303,* 337-357.

CENTRALNI NUKLEUS AMIGDALOIDNOG KOMPLEKSA U MOZGU PACOVA

OLIVERA LOZANČE, D. DREKIĆ i DIJANA DJELIĆ

SADRŽAJ

Sadašnje istraživanje pokušava da definiše glavne subregione centralnog nukleusa (CN) amigdaloidnog kompleksa (AK) pomoću nekih histoloških metoda i stereoloških parametara. S obzirom da novija istraživanja ukazuju da su različiti neuropeptidi i projekcije u vezi sa određenim delovima centralnog amigdaloidnog nukleusa, detaljnija istraživanja citoarhitekture CN trebalo bi da doprinesu boljem razumevanju njegove složene strukture i organizacije. Kvalitativne i kvantitativne analize CN pacova ukazuju da se on sastoji iz dva glavna subregiona: lateralnog i medijalnog. Ovo je ujedno i prva morfometrijska studija koja na ovaj način, kvantitativno, prepoznaje razlike u vrednostima ispitivanih stereoloških parametara lateralnog i medijalnog subregiona i ukazuje na polni dimorfizam lateralnog subregiona centralnog nukleusa u pacova.