ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research Paper Acta Neurobiol Exp 2013, 73: 260–279

Expression of the calcium-binding proteins in the central, medial and cortical nuclei of the rabbit amygdaloid complex during postnatal development

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Calbindin-D28k (CB), parvalbumin (PV) and calretinin (CR) are calcium-binding proteins (CaBPs) considered to be markers for certain subpopulations of neurons in the central nervous system. The aim of this study was to describe the pattern of distribution of CB-, PV- and CR-immunoreactive elements in the rabbit corticomedial amygdaloid complex during the postnatal period. The time course of changes in CaBPs expression during maturation of the selected nuclei indicates their diversity. During the first month after birth, CaBPs expression stabilizes earliest in the anterior cortical and then in the medial nuclei. Later, during the second month of postnatal life, the posteromedial and posterolateral cortical nuclei maturate. The central nucleus requires a considerably longer time to reach maturity – about three months are needed to stabilize CaBPs expression in all its subdivisions. This nucleus also shows the most differentiated, time-dependent distribution of CaBPs-immunoreactivity (especially CB), distinct in its divisions. The differences in the CaBPs immunoreactivity confirm previous reports concerning dissimilar origin and development, and also reflect the diversity of connectivity of the amygdaloid body – the collection of nuclei, considered as one functional integrity.

Key words: calbindin-D28k, calretinin, parvalbumin, amygdala, rabbit, development

INTRODUCTION

Calbindin-D28k (CB), parvalbumin (PV) and calretinin (CR) are members of the EF-hand family of calcium-binding proteins (CaBPs). Although their exact function in neuronal metabolism has yet to be elucidated, it has been proved that they: (1) contribute to calcium homeostasis through their capacity to buffer intracellular calcium ions, (2) control, at least to a certain extent, the excitability of neurons (Celio 1990, Camp and Wijesinghe 2009) and (3) modulate synaptic plasticity (Caillard et al. 2000). CB, PV and CR abundantly occur (and co-occur) in various neuronal subpopulations in the central nervous system (Andressen et al. 1993, Hof et al. 1999), which may reflect their various functions. Numerous studies have revealed that patterns of distribution of CB-ir and PV-ir neurons in the primate basal forebrain and midbrain are complementary and suggest that these two CaBPs may work synergistically (Parent et al. 1996, Jones et al. 2001).

The amygdala is a multinuclear complex that lies at the junction of the cerebral cortex and basal forebrain. This complex involves more than ten nuclei and cortical areas, each of which has its unique, peculiar morphological features and physiological role. Despite many years of study, there is no division of the amygdala, which is unambiguously accepted by all researchers. Among many proposals present in the literature there are two that are most commonly used. According to the first one, the amygdaloid body is divided into two main parts: the corticomedial part, considered to be the phylogenetically older part (consists of central, medial and cortical nuclei) and the basolateral part, which is the phylogenetically younger part (consists of basolateral complex nuclei) (Humphrey

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Received 31 August 2012, accepted 12 February 2013

1936, Fox 1940, Crosby and Humphrey 1941). According to the second proposal, the amygdala is formed by three groups of nuclei: deep (basolateral complex), superficial (medial and cortical nuclei) and other (central nucleus) (Price et al. 1987, Amaral et al. 1992, Pitkanen et al. 1997). On the basis of developmental and histological data McDonald (1998) distinguished the centromedial part, which is comprised of central and medial nuclei. Taking into account the similarities of reciprocal connections Alhaied and colleagues (Alhaied and Heimer 1988, Alhaied et al. 1995, Alhaied 2003) added other structures to the centromedial part of the amygdala: bed nucleus of stria terminalis, caudodorsal regions of the substantia innominata, and for the description of all of those structures they introduced the term "extended amygdala". In 1998 Swanson and Petrovich questioned the correctness of combining the amygdala nuclei into a single structure. On the basis of anatomical, electrophysiological and histochemical data they proposed that the central and medial nuclei are a postero-ventral continuation of striatum – mainly because their projecting neurons are GABA-ergic, similar to the projecting neurons of striatum, while the rest of the amygdala is a cortical region (cortex/claustrum and olfactory cortex) because, as in the cortex, projecting neurons are glutaminergic and interneurons are GABA-ergic (Swanson and Petrovich 1998, Swanson 2000). Swanson and Petrovich (1998) claimed that the nuclei of the amygdala belong to four different functional systems: (1) The main olfactory system, which comprises nuclei receiving reach projections from the main olfactory bulb – anterior cortical and posterolateral cortical nuclei. (2) The accessory olfactory system, which comprises nuclei that are the target of projections from the accessory olfactory bulb (related to the pheromone system) - medial and posteromedial cortical nuclei. The central nucleus possesses numerous projections into the structures engaged in the control of the autonomic system, which belong to the (3) autonomic system, while lateral and basolateral nuclei, closely connected with frontal and temporal cortical areas, co-create (4) the cortical frontotemporal system.

The division of the rabbit amygdaloid complex into two main nuclear groups: the corticomedial and basolateral, and even into specific nuclei, generally corresponds to partitioning of the amygdala in other species (Humphrey 1968, 1972, Krettek and Price 1978a, de Olmos et al. 1985, Morys et al. 1999a, Sah et al. 2003, Legaz et al. 2005, Brummelte et al. 2007). Moreover, the anatomical divisions of this structure reflect their functional differentiation.

Recently different aspects of the basolateral amygdaloid complex have been extensively studied, due to its involvement in compound functions during emotional learning and conditioned fear. Neurons containing CaBPs, both principal projection neurons as well as the distinct subpopulations of non-pyramidal neurons, have to play an important role to fulfil those functions. The distribution of CaBPs-ir neurons within the amygdala, particularly in its basolateral complex, has been described (McDonald 1994, 1997, Setzer and Ulfig 1999, Morys et al. 1999b, Berdel and Morys 2000, Kemppainen and Pitkänen 2000, McDonald and Betette 2001, McDonald and Mascagni 2001, Pitkänen and Kemppainen 2002, Legaz et al. 2005, Muller et al. 2005, Brummelte et al. 2007), but data from the corticomedial complex are much more scarce (Sidorowicz et al. 1996, Kemppainen and Pitkänen 2000, Pitkänen and Kemppainen 2002, Guirado et al. 2008).

The nuclei of the corticomedial amygdaloid complex (especially the medial nucleus) play an important role in the convergence of sensory (especially olfactory), autonomic and endocrine inputs from the hypothalamus and brain stem, thus they are possibly engaged in various aspects of social signaling, reproductive, maternal and parental behavior and, as a part of a larger integrated system, may control some forms of aggression (Newman 1999). The olfactory inputs *via* the olfactory bulb reach the anterior and posterolateral cortical nuclei (Price et al. 1991, Carmichael et al. 1994, McDonald 1998) whereas inputs from the vomeronasal organ *via* the accessory olfactory bulb terminate in the medial nucleus and posteromedial cortical nucleus.

The medial nucleus is best characterized by its connections with the accessory olfactory system and medial hypothalamus (Kevetter and Winans 1981a). Moreover, almost all the nuclei of the corticomedial amygdaloid complex project to the medial nucleus (Pitkänen 2000). Among all the nuclei of the amygdaloid complex, the medial nucleus is also a target of the most extensive projections from the contralateral amygdala (Pitkänen 2000) being for them the gateway to brainstem centers (de Olmos et al. 1985).

The central nucleus receives efferents from almost all ipsilateral amygdaloid nuclei (Pitkänen 2000), and

it plays a pivotal role as the main output station of the amygdala (Martina et al. 1999). Its activation induces autonomic responses such as freezing, fear-potentiated startle and release of stress hormones (Davis 1992) by its influence on diverse brain structures through divergent projections.

During the last two decades, the central and medial nuclei have been widely examined in the context of their participation in responses to stressing stimuli (Petrovich and Swanson 1997, Dayas et al. 1999, Davis 2000, LeDoux 2000, Petrovich et al. 2001, Davern and Head 2011).

The functions of the cortical and centromedial nuclei of the amygdala are not completely established yet, probably because the grounds for the functioning – the neuronal organization in their various aspects – is also not clear. There are many reports concerning the anatomy and delineation of cortical and centromedial amygdaloid nuclei in the rabbit (Uchida 1950, Urban and Richard 1972, Girgis and Shih-Chang 1981, Kapp et al. 1985, Shek et al. 1986), but the most detailed description was encompassed by Jagalska-Majewska and colleagues (2001). However, there are no immunohistochemical studies of the development of the rabbit corticomedial complex.

Comparative knowledge about the development of diverse structures in different species, based on the determination of both similarities and differences, allows selection of the most similar animal models for biological studies. For example, rabbit along with the human are the few species which show teratogenic effects of thalidomide, which were not detected with previously tested animals (Wells et al. 2005, Ito et al. 2011). In neurobiological studies, especially prenatal developmental toxicity studies, rabbit is frequently used as the second species of choice, after rodents: rats and mice. As a consequence, there is data available about the prenatal development of rabbits, but hardly any information is available about postnatal development (Wolterbeek and Waalkens-Berendsen 2011). Recently, postnatal developmental toxicity studies of amygdala seem to be of importance (Hewitson et al. 2010, Novella and Hines 2011), thus, the aim of our study was to describe the onset and pattern of CB, PV and CR expression during postnatal development of the cortical and centromedial nuclei in the rabbit amygdala by means of immunohistochemical methods.

METHODS

Subjects

The studies were carried out on 36 male New Zealand White rabbits, divided according to the days of postnatal life into nine experimental groups: P0, P4, P7, P14, P21, P30, P60, P90 and P360. In each group the brains of four animals were studied. The care and treatment of animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health, European Communities Council Directive 86/609/EEC as well as by the Local Ethics Committee. Animals were deeply anesthetized with Thiopental (80 mg/kg body weight i.p.) and chloral hydrate (40 mg/kg i.p.); adequate measures were taken to minimize pain and discomfort. Then they were perfused transcardially with 150–250 ml (depending on the age of the animal) of cold 0.9% NaCl followed by 500 ml (groups P0, P4, P7, P14) or 1000 ml (remaining age groups) 4% solution of paraformaldehyde in 0.1 M phosphate buffer. Immediately after perfusion the brains were removed from the skulls and refrigerated in 30% solution of sucrose in 0.1 M phosphate buffer until sunk. The brains were then frozen and cut into 40µm-thick coronal serial sections with a cryostat Jung 1800 (Leica, Germany). The sets of sections were taken for anti-PV, anti-CB and anti-CR immunohistochemistry.

Immunohistological procedures

Protocols for staining were in accordance with those previously published (Wójcik et al. 2004a,b, 2007). Free floating sections were incubated in a solution composed of 0.3% Triton X-100 and 3% normal goat serum (NGS) for PV and CB or 3% normal donkey serum (NDS) for CR in 0.01M PBS (pH = 7.2) for 1 hour. The sections were then incubated at 4°C with primary antibodies: mouse anti-PV (diluted 1:1 000, lot No. 033K4846, Sigma, USA) or mouse anti-CB (diluted 1:1 000, lot No. 082K4879, Sigma, USA) or goat anti-CR (diluted 1:1 000, lot No. 1§.1, Swant, Switzerland) in 0.01M PBS (pH = 7.2) containing 3% solution of NGS or NDS and 0.1% Triton X-100. After 48 hours the sections were washed with PBS and incubated with the secondary antibodies: goat anti-mouse (lot No. 32319) or donkey anti-goat (lot No. 40988) coupled to Cy3 (diluted 1:800, Jackson, USA) for 2

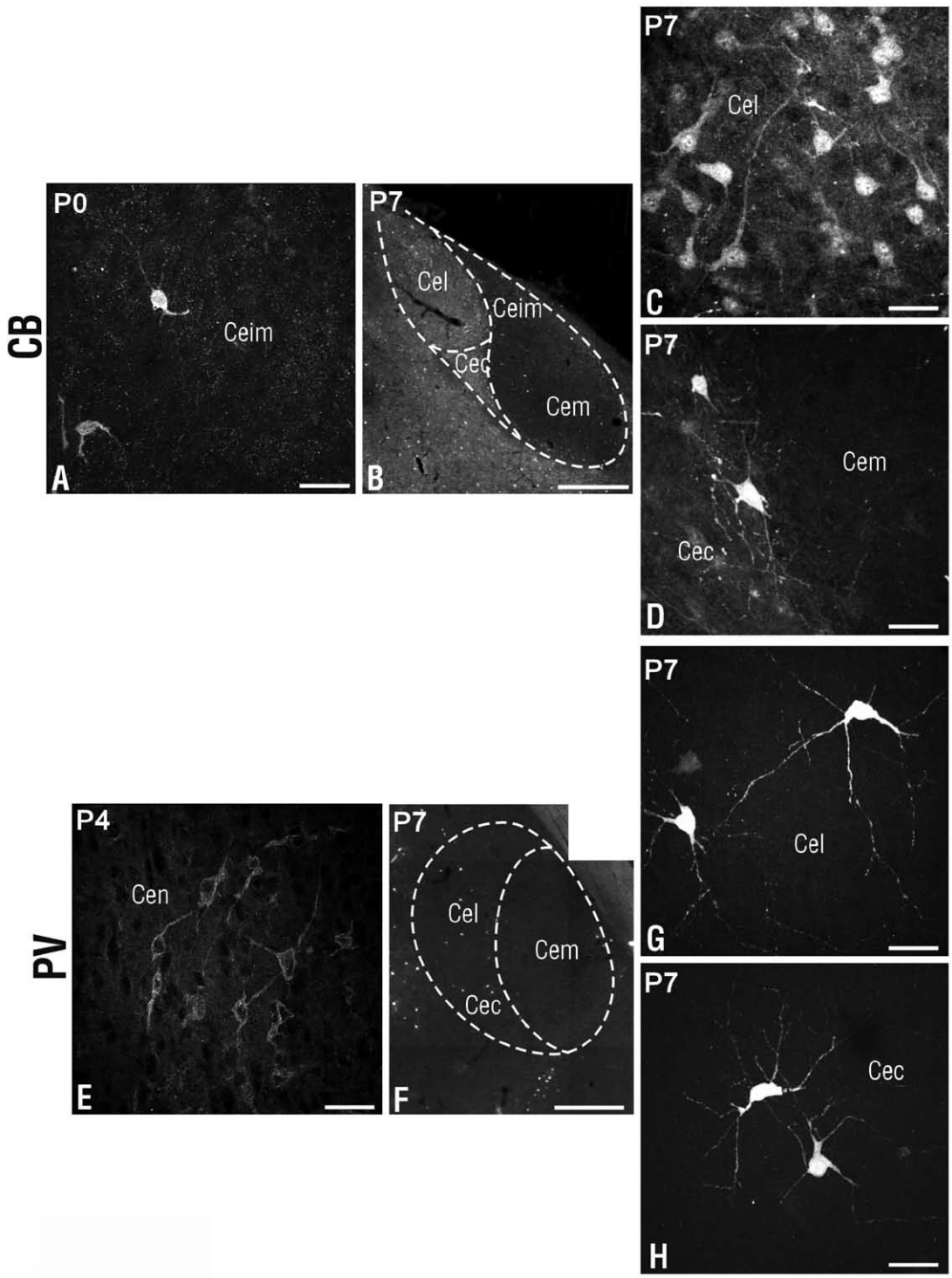

Fig. 1. (A–D) Calbindin-D28k (CB) and (E–H) Parvalbumin (PV) imunoreactivity in the central nucleus (Cen) of the rabbit amygdala. (A) Oval CB-ir cells observed in intermediate subdivision of the central nucleus (Ceim) from P0; (B) Characteristic shape and CB-ir of each division of Cen on coronal sections on P7; (C) CB-ir cells with well visible proximal parts of processes on P7 in the lateral (Cel) and (D) capsular (Cec) subdivisions of Cen; (E) Numerous PV-ir cells of immature morphology resembling migrating neurons within the central nucleus on P4; (F) Distribution of PV-ir elements within central nucleus on coronal section on P7; (G) PV-ir cells of almost mature morphology in the lateral; (H) and capsular subdivisions of Cen. Scale bars are 0.05 mm (A, C–E, G, H) and 0.5 mm (B, F).

hours. Finally, they were washed with 0.01M PBS, mounted onto gelatine-coated slides, air-dried, and cover slipped with Keiser Gelatine (Merck, Germany).

Controls for staining specificity were: omission of the primary antibody, its replacement with non-immune sera or irrelevant antibody. They always resulted in a complete lack of the immunostained elements.

Qualitative and quantitative analyses

Immunohistochemically stained sections were studied using a fluorescent microscope BX-51 (Olympus, Japan) and a confocal laser scanning microscope (CLSM) – system Radiance 2100 (Bio-Rad, UK) equipped with an Argon/Krypton ion laser (American Laser Corporation, USA) and mounted on a light microscope Eclipse 600 (Nikon, Japan). An excitation filter 568 and emission long-pass filter E570LP were used to detect Cy3 fluorescence. CLSM images were obtained using 40× and 60× oil immersion objective lenses of N.A.=1.3 and 1.4, respectively. The optimal iris was used for each magnification. In each case only sections completely stained with fluorescence were taken into account. The images were recorded on a hard drive and analysed using LaserSharp 2000 software (Bio-Rad, UK). In order to evaluate the level of the expression of studied CaBPs, in particular nuclei of the corticomedial complex, a semi-quantitative method was applied. Either neuronal density or neuropil intensity of PV-, CB- and CR-ir was determined in a "blind" study by two independent observers (SW and AL). The density of the perikarya immunostaining were scored as: − absent, −/+ very low, + low, ++ moderate, +++ high. The evaluation of the images performed with the CellSens Dimension 1.5 imaging software (Olympus) revealed that level "−/+ very low" corresponded to a density from 1 to 25, "+ low" – from 26 to 50,"++ moderate" – from 51 to 200, and "+++ high" with more than 200 positively stained perikarya per mm^2. The intensity of neuropil staining were scored as: − absent, −/+ very low, + low, ++ moderate, and +++ high, similar to the previously published criteria (Kemppainen and Pitkänen 2000).

RESULTS

All studied CaBPs were present within neuronal cell bodies and elements of neuropil – fibers and terminals. Neurons containing CaBPs in the corticomedial amygdala belonged to morphologically differentiated types. During the early postnatal period (P4–P7), cells containing CaBPs with characteristic morphology of immature migratory cells with fusiform shape and leading process were observed. They corresponded to those described earlier by Frassoni and colleagues (2000). The majority of stained CaBP-containing cells showed morphology of mature neurons corresponding to those described by Kemppainen and Pitkänen (2000). Among them, multipolar neurons with oval somata prevailed. No neuronal type was characteristic for any studied nucleus of corticomedial amygdala.

Central nucleus (CeN)

The pattern of the CaBPs distribution allowed the differentiation of CeN into parts, which was in accordance with a previous description based on analysis of cresyl violet stained sections (Jagalska-Majewska et al. 2001). The delineation into anti-CB stained sections allowed the differentiation of all four subdivisions of CeN: medial (Cem), capsular (Cec), intermediate (Ceim) and lateral (Cel) (Fig. 1B). Anti-PV staining permitted non-complete partitioning – only into two parts (Fig. 1F). Distribution of the CR-ir elements, due to its homogeneity, did not allow any further subdivision of CeN (data not shown).

Calbindin-D28k

CB-ir allows partitioning of CeN from the beginning of the postnatal period (Fig. 1B, Table I). In all CeN subdivisions CB-ir neurons of similar morphology, mainly multipolar with small and medium round somata, were observed. However, these cells appeared in particular subdivisions of CeN in different time periods. Within Ceim, CB-ir neurons were observed from P0 (Table I, Fig. 1A). Their density increased during the first week of the postnatal life (P7). In the end of the second week the density of CB-ir cells gradually decreased. At P0, the CB-ir of neuropil was low, and then at P7 it was moderate, whereas at P14 it was diminished. In Ceim the level of CB-ir, in both neurons and neuropil, was stabilized since P14 (Table I), and the pattern characteristic for an adult animal was achieved (Table II).

CB-ir cells within the Cel have also appeared from P0 (Table I). During the first postnatal week their density greatly increased, reaching the highest level at P7 (Fig. 1C),

Table I

The dates (P – postnatal day) of first appearance (columns A) and reaching the mature pattern (columns B) of calbindin-D28k, parvalbumin and calretinin-ir neurons and neuropil in central (Cen), medial (Med) and cortical (anterior CoA, posterolateral CoPl, posteromedial CoPm) nuclei of rabbit amygdaloid complex

Nucleus	Subdivision/ Layer	Calbindin-D28k				Parvalbumin				Carletinin			
		neurons		neuropil		neurons		neuropil		neurons		neuropil	
		A	B	A	B	A	B	A	B	A	B	A	B
Cen	Cem	P7	P7	P7	P30	P14	P30	P14	P14	–	–	P0	P30
	Cec	P7	P14	P7	P60	P7	P7	P7	P7	P7	P7	P7	P21
	Ceim	P0	P14	P0	P14	P14	P60	P14	P14	–	–	P7	P60
	Cel	P0	P14	P0	P90	P7	P7	P7	P14	–	–	P0	P60
Med	Md	P0	P14	P0	P21	P7	P14	P21	P30	P0	P0	P0	P0
	Mv	P0	P14	P0	P21	P7	P14	P14	P14	P7	P7	P0	P0
CoA		P0	P7	P0	P0	–	–	–	–	P0	P0	P0	P0
CoPl	I	P7	P60	P0	P30	–	–	P21	P21	P0	P30	P0	P0
	II	P0	P14	P0	P0	P4	P7	P4	P4	P0	P7	P0	P0
	III	P0	P7	P0	P14	P4	P7	P4	P4	P7	P60	P0	P0
CoPm	I	P7	P14	P0	P0	–	–	P14	P14	P0	P21	P0	P7
	II	P0	P14	P0	P60	P4	P30	P14	P14	P0	P0	P0	P0
	III	P0	P0	P0	P0	P4	P30	P14	P14	P0	P0	P0	P0

Table II

Adult pattern (P360) of distribution and density of calbindin-D28k-, parvalbumin- and calretinin-ir neurons and neuropil in central (Cen), medial (Med) and cortical (anterior CoA, posterolateral CoPl, posteromedial CoPm) nuclei of rabbit amygdaloid complex

Nucleus	Subdivision/ Layer	Calbindin-D28k		Parvalbumin		Carletinin	
		neurons	neuropil	neurons	neuropil	neurons	neuropil
Cen	Cem	−/+	−/+	+	−/+	−	+
	Cec	+	+++	+	+	−/+	−/+
	Ceim	++	+	−	−/+	−	+
	Cel	++	+++	+	+	−	+
Med	Md	++	+	−/+	+	+	+
	Mv	+++	+	−	−/+	+	+
CoA		++	+	−	−	+	+
CoPl	I	−/+	−/+	−	−/+	−/+	+++
	II	++	++	+	++	+	+
	III	+++	+	++	−/+	+	+
CoPm	I	++	+	−	−/+	−	−/+
	II	++	++	−/+	−/+	+	+
	III	+	+	−/+	−/+	+	+

The density of cells and neuropil is expressed as: +++ high, ++ moderate, + low, −/+ very low, − absent

then it decreased to a moderate level. Immunoreactivity of neuropil was low until P30; however, it gradually increased reaching a high level in the adult animal (Table II). In the Cem the first scattered CB-ir cells with poorly labeled perikarya appeared at P7 (Table I, Fig. 1B). Their amount did not change until the end of the observation period. Also, the immunoreactivity of neuropil was very low at P7 when single CB-ir puncta were observed (Fig. 1D). Then the transition increase of CB-ir of Cem neuropil was observed during the second and third postnatal weeks. From P30 it was again very low. In the Cec for the first time CB-ir neurons appeared at P7 (Table I, Fig. 1B, 1D). Their density was high, and then from P14 their amount decreased, and until the end of observation period it maintained a low level (Tables I and II). This part of the central nucleus characterized differentiated immunoreactivity of neuropil. From P7 immunoreactivity of neuropil was moderate, then it decreased and maintained a similar level until P30. Finally it reached a high level at P90 (Tables I and II).

Parvalbumin

From P7, PV-ir allowed further subdivision of CeN, but only into two areas: medial and lateral. The former comprised Cem and Ceim, whereas the latter – Cel and Cec (Fig. 1F, Table I). During the first week of postnatal life (P0, P4) PV-ir cells were observed within the whole CeN (Fig. 1E). Their morphology resembled immature migrating cells (relatively poorly stained somata of fusiform shape with short, thick leading process). Within the lateral area of CeN PV-ir cells of adult morphology occurred at P7 (Fig. 1G, 1H). This adult morphology of neurons maintained until the end of the observation period (Table I) and their density was constantly low (Table II). PV-ir of neuropil at P7 was very low (single fibers) in Cel and moderate in Cec. During the second postnatal week it increased in Cel to the level observed in Cec, and then kept at a constant low level until the end of the observation period (Table I, Table II). For the first time, single PV-ir neurons of adult morphology were observed in the medial area of CeN at the end of the second postnatal week (Table I). This pattern of distribution did not change until adulthood, but minor changes were observed at P60 as an increase in the number of PV-ir perikarya to moderate level and prevalence of PV-ir neurons localization to territory corresponding to Ceim. The immunoreactivity of neuropil was noticed from P14, and this level was maintained throughout the remaining age groups (Table I).

Calretinin

CR-ir did not allow any distinct parts of CeN to be distinguished. From P7 single CR-ir neurons were found only in the vicinity of the lateral border of CeN – within the region corresponding to Cec. Thr remaining territory of CeN was characterized by a lack of CR-ir neurons (Table I).

Immunoreactivity of neuropil was initially (P0) the strongest in Cel and very low or absent in the remaining areas of CeN. CR-ir within the neuropil reached the morphology characteristic for adult animal at P21 in Cec, then at P30 in Cem and at about P60 in Cel and Ceim. Adult pattern of CR-ir was at a moderate level in Cel, Cem and Ceim and low in Cec (Table II).

The maturation data (dates of reaching the mature pattern of immunolabeling, separately for perikarya and neuropil) for the studied CaBPs in each subdivision of CeN is presented in Figure 6.

Medial nucleus (Med)

The pattern of CaBPs immunoreactivity did not allow clear differentiation of the medial nucleus into parts, although some discrete differences in the immunoreactivity existed between the ventral (Mv) and dorsal (Md) parts of the medial nucleus (Table I). The distribution of both immunoreactive neurons and neuropil was relatively homogeneous throughout the whole structure.

Calbindin-D28k

On P0 CB-ir neurons were observed (Table I, Fig. 2A) in this nucleus. Their density increased considerable during the first week, reaching the highest level at P7 (Fig. 2B,C) and then decreased during the second week to the moderate level that was observed in the adult animal (Table II). Immunoreactivity of the neuropil in early postnatal age groups P0 and P4 was low, in P7 and P14 groups it was moderate, then it stabilized at P21 again at a low level (Table II).

Parvalbumin

During the first week of postnatal life (P0, P4) PV-ir cells with morphology resembling immature migrating neurons (Fig. 2D) were observed within the whole Med. Although single PV-ir neurons appeared at P7

within the whole nucleus (Fig. 2E,F), their presence in older age groups was confined to the dorsal part of this nucleus (Tables I and II). Immunoreactivity of neuropil was different between the studied parts of Med. Within the Md, PV-ir appeared from P21 and was very low, then at P30 it reached a low level characteristic for adults, whereas within the Mv, neuropil of very low PV-ir were constantly observed already from P14 (Tables I and II).

Calretinin

At P0 and P4 single CR-ir neurons were observed only in Md (Fig. 2G), while from P7 – in the whole nucleus (Table I, Fig. 2H, 2I). In the remaining age groups a few scattered CR-ir neurons were present within the whole Med. The level of immunoreactivity of neuropil in all studied age groups was similar – low or very low (Table II).

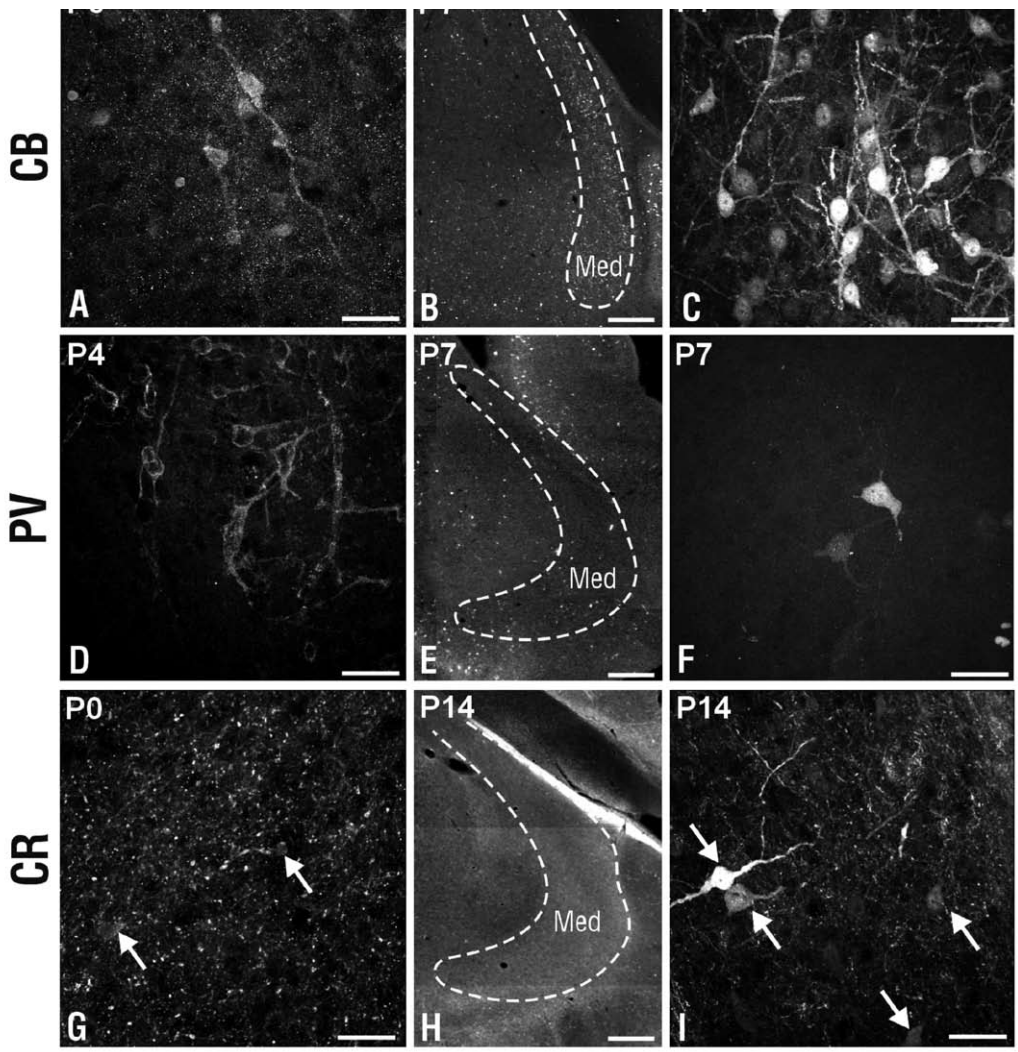

Fig. 2. (A–C) Calbindin-D28k (CB), (D–F) parvalbumin (PV), and (G–I) calretinin (CR) immunoreactivity in the medial nucleus (Med) of the rabbit amygdala. (A) CB-ir neurons observed in Med during the first day of the postnatal life; (B) CB-ir in the Med and neighboring areas at the end of the first postnatal week; (C) Morphology of CB-ir neurons present in Med on P7. The increase of the density during first week of postnatal life was significant. PV-ir cells (D) of immature morphology, probably migrating (F) and mature neurons within Med on P4 and P7, respectively. (E) Characteristic appearance of the medial nucleus on P7; (G) Single CR-ir neurons (indicated by arrows) in the dorsal part of Med on P0; (H) CR-ir in the Med and neighboring areas at the end of the second postnatal week; (I) Single CR-ir neurons (indicated by arrows) and network of CR-ir fibers and immunoreactive puncta in neuropil present in Med on P14. Scale bars are 0.05 mm (A, C, D, F, G, I) and 0.5 mm (B, E, H).

The maturation data (dates of reaching the mature pattern of immunolabeling, separately for perikarya and neuropil) for studied CaBPs in both subdivisions of Med is presented in Figure 6.

Anterior cortical nucleus (CoA)

The pattern of the CB-ir and CR-ir was homogeneous throughout the whole structure, whereas PV staining revealed neither cell bodies nor neuropil (Tables I and II).

During development the anterior cortical nucleus characterized almost no diversity in the expression of CB and CR (Table I). The level of the immunoreactivity of neuropil was low and constant during the whole studied period. However, some age differences concerned the density of stained neurons. CB-ir neurons were present in CoA from P0 (Fig. 3A); nevertheless, their amount significantly increased reaching moderate values at P7 (Fig. 3B,C). CR-ir neurons were also present from P0 (Fig. 3D), but their amount did not change until adulthood (Fig. 3E, Table I).

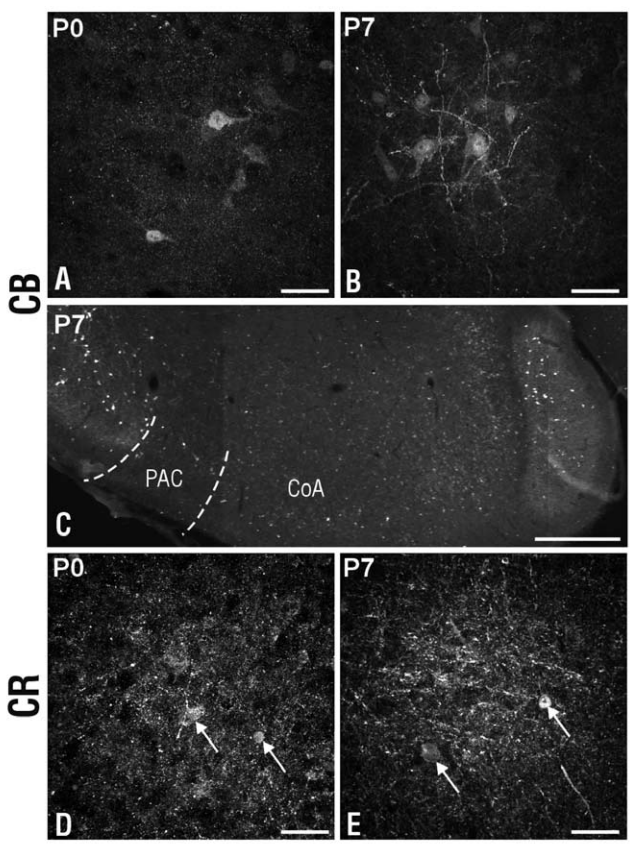

Fig. 3. (A–C) Calbindin-D28k (CB) and (D–E) calretinin (CR) immunoreactivity in the anterior cortical nucleus (CoA) of the rabbit amygdala. (A, B) Increase of the density CB-ir neurons during the first week of the postnatal life; (C) CB-ir in the CoA and neighboring areas at the end of the first postnatal week. (PAC) periamygdaloid cortex. (D, E) Sparse CR-ir neurons, indicated by arrows, observed in CoA. Scale bars are 0.05 mm (A, B, D, E) and 0.5 mm (C).

The maturation data (dates of reaching the mature pattern of immunolabeling, separately for perikarya and neuropil) for studied CaBPs in CoA is presented in Figure 6.

Posterolateral cortical nucleus (CoPl)

The pattern of CaBPs immunoreactivity allowed differentiation of the posterolateral cortical nucleus into three layers, although their best delineation was observed in CB- and PV-stained sections (Fig. 4). CR staining showed differentiation of layer I, whereas the remaining layers characterized somewhat equal immunoreactivity (Fig. 5).

Calbindin-D28k

At P0, CB-ir neurons were observed in layers II and III (Fig. 4A,B). Their density increased during the first two weeks of postnatal life, earlier in the III layer than in the II layer, and stabilized at the levels equal to those observed in the remaining groups (Table I). In layer I some scattered CB-ir neurons were noticed from P7 (Fig. 4D,E), and during two months of postnatal life their amount slightly decreased. During the first week of postnatal life the immunoreactivity of neuropil in CoPl was very low in layer I, moderate in layer II and high in layer III (Table II, Fig. 4A,D). Throughout the whole observation period immunoreactivity of neuropil in layer II did not change significantly, whereas in layer III the level of immunoreactivity decreased considerably at P14. Within neuropil of layer I minor changes occurred, namely a temporary increase of CB-ir, until P30 (Table I). This age pattern did not change until P360 (adult animal).

Parvalbumin

PV-ir neurons in layers II and III were noticed for the first time at P4 – they were more numerous in layer III (Table II, Fig. 4H). Layer I was characterized by a lack of immunoreactive cells until the end of the stud-

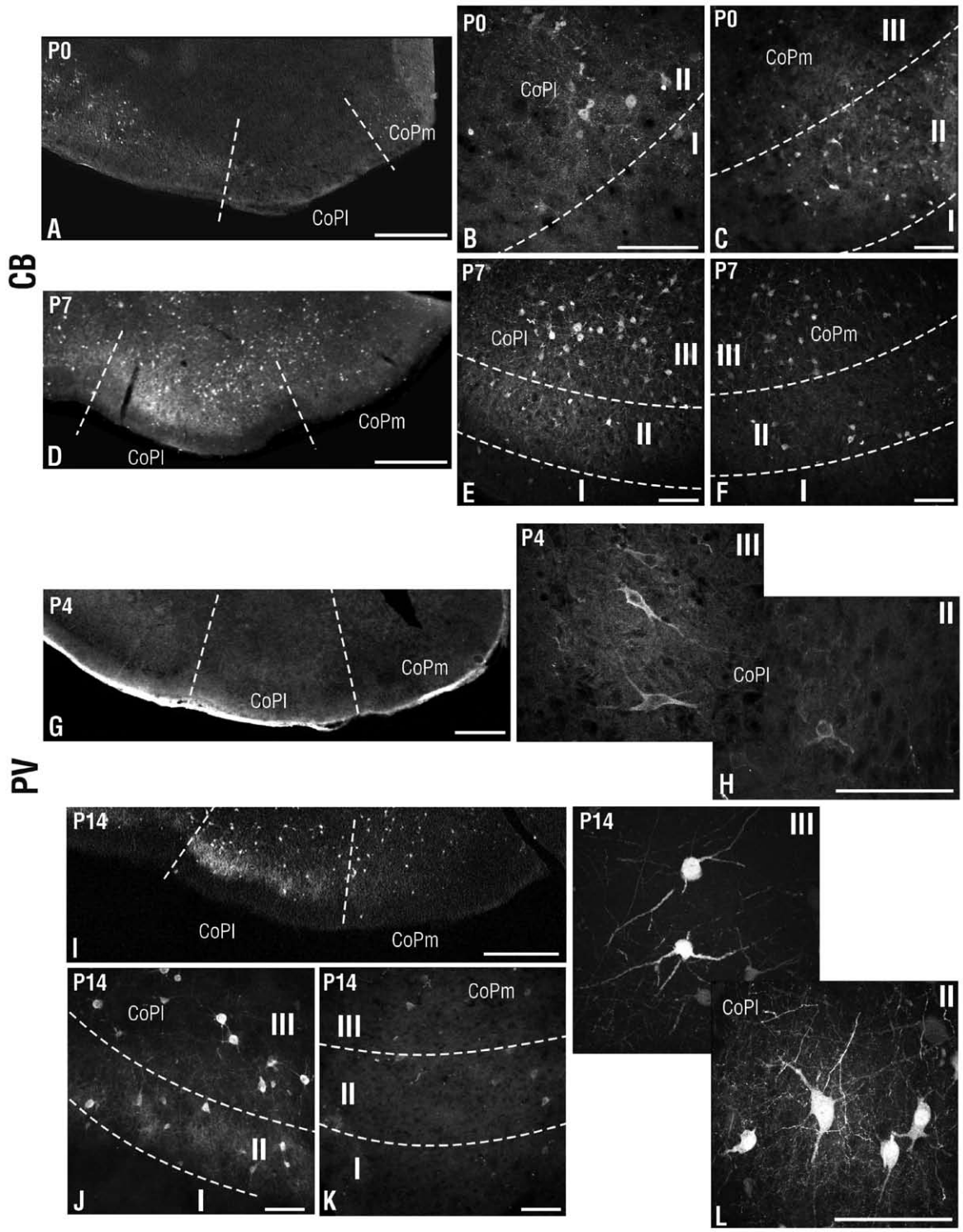

Fig. 4. (A–F) Calbindin-D28k (CB) and (G–L) parvalbumin (PV) immunoreactivity in the posterolateral (CoPl) and posteromedial (CoPm) cortical nuclei of the rabbit amygdala. I, II, III refer to layers I, II and III of CoPl and CoPm. (A–C) on P0 and (D–F) P7 – Distinct three-layered differentiation of both nuclei was present. (G) PV-ir in the CoPl, CoPm and neighboring areas at the fourth day of the postnatal life. (H) Scattered neurons of the immature morphology in the layers III and II in CoPl on P4 and (J–L) of mature morphology on P14. Relatively dense neuronal meshwork in CoPl. Scale bars are 0.5 mm (A, D, G, I) and 0.05 mm (B, C, E, F, H, J–L).

ied period. From P7 in layers II and III the density of the PV-ir cells was slightly higher than that observed at P4 (Fig. 4L) – low and moderate, respectively. This pattern did not change until P360 (Table I).

From the first week of postnatal life the pattern of immunoreactivity of neuropil in layers II and III was the same in all studied age groups (bright stripe visible on Figure 4G is an unspecific staining of pia mater, Table I). In the neuropil of layer I some PV-ir elements were observed from P21, and from this period the pattern of PV-ir characteristic for CoPl showed the differentiation between particular layers, from almost no expression in the layer I, through low expression in layer III, to moderate in layer II (Fig. 4I).

Calretinin

At P0 numerous CR-ir neurons were present in layer I of CoPl (Fig. 5A). Their morphology was very similar to Cajal-Retzius cells. Their density gradually decreased until P30, and in the older age groups only single neurons were observed in this layer (Table II). Scattered CR-ir neurons were observed in layer II from P0, whereas in layer III – from P7 (Fig. 4A,D). From P7 in layer II their density did not change considerably in subsequent age groups (Table I). Minor changes (transient increase followed by decrease) were observed within layer III until P30. From P60 to P360 the pattern of CR-ir neuron distribution was constant in all layers of CoPl.

Immunoreactivity of neuropil within all the layers remained at a relatively constant level from P0 throughout all the observation period. The strongest CR-ir was noticed in layer I, while in layers II and III it was low and similar, respectively (Table II).

The maturation data (dates of reaching the mature pattern of immunolabeling, separately for perikarya and neuropil) for studied CaBPs in each layer of CoPl is presented in Figure 6.

Posteromedial cortical nucleus (CoPm)

Calbindin-D28k

From P0 CB-ir neurons in layers II and III were observed (Fig. 4A,C). In layer II the density of neurons increased during the second week of postnatal life and then remained unchanged, whereas the number of cells in layer III maintained a similar level during the whole period (Tables I and II). In layer I only single CB-ir, weakly stained neurons were observed. They appeared for the first time at P7 (Fig. 4D,F).

During the first four weeks of postnatal life neuropil immunoreactivity was low and similar in all three layers. From P60 distinct differentiation was observed: a moderate level of immunoreactivity characterized layer II, separating layers I and III with low level of immunoreactivity maintained (Table II).

Parvalbumin

PV-ir relatively weakly differentiated layers of CoPm (Fig. 4G,I,K). In layer I throughout the whole observation period no immunoreactive cells were detected. In layers II and III single PV-ir neurons were observed from P4. A transient increase of density of PV-ir cells was observed at P14 and P21; from P30 the density was persistently very low until the end of the observation period (Table I).

PV-ir of neuropil appeared for the first time at P14, but its level was very low and homogenous in the whole nucleus (Fig. 4I,K). This pattern of PV-ir did not undergo any age-dependent changes (Tables I and II).

Calretinin

Although CR-ir neurons were noticed in all three layers from the beginning, throughout the whole observational period, both density of CR-ir cells as well as neuropil immunoreactivity remained low and unchanged in layers II and III (Table II, Fig. 5). The smallest number of CR-ir cells, detectable only to P14, was observed in layer I. Those cells resembled Cajal-Retzius cells. The pattern of neuropil immunoreactivity allowed further subdivision of layer I into two sublayers: superficial and deep, the former was characterized by very high CR expression during almost the whole postnatal period (from P7) (Fig. 5C).

The maturation data (dates of reaching the mature pattern of immunolabeling, separately for perikarya and neuropil) for studied CaBPs in each layer of CoPm is presented in Figure 6.

DISCUSSION

The current study shows that CaBPs expression revealed distinctly heterogeneous time- and intensity-dependent patterns during development in the exam-

ined nuclei. The findings of the present study can be briefly summarized as follows. During the first month after birth, CaBPs expression stabilizes earliest in the anterior cortical and then in the medial nuclei. Later, during the second month of postnatal life, posteromedial and posterolateral cortical nuclei maturate. The central nucleus requires a considerably longer time to reach maturity – about three months are needed to stabilize CaBPs expression in all its subdivisions.

Central nucleus

Various parts of the central nucleus reach the pattern of immunoreactivity present in adult animals in different time periods after birth. Stabilization of studied parameters characteristic for adult animal we observed the earliest, about the end of the first month of postnatal life, within Cem. This part revealed the lowest CaBPs immunoreactivity among all the divisions of CeN. Then, at about the end of the second month of the postnatal life, changes in Cec and Ceim were stabilized. Changes of CaBPs immunoreactivity were observed for the longest period of time (till the third month of the postnatal life) within Cel. In the adult rabbit CeN subdivisions were clearly distinguished, especially in CB staining; their topography corresponded to that observed by Jagalska-Majewska and coworkers (2001) in cresyl violet staining.

During development, expression of CaBPs in particular parts of CeN showed a distinct spatiotemporal pattern. This may be related to the physiological variety and diversification of the connectivity profile of particular parts of CeN (Veening et al. 1984, Sah et al. 2003). Cem, which reached maturity first, is usually thought of as the main amygdaloid output. Due to the presence of descending projection to areas generating autonomic and behavioral responses (Schwaber et al.

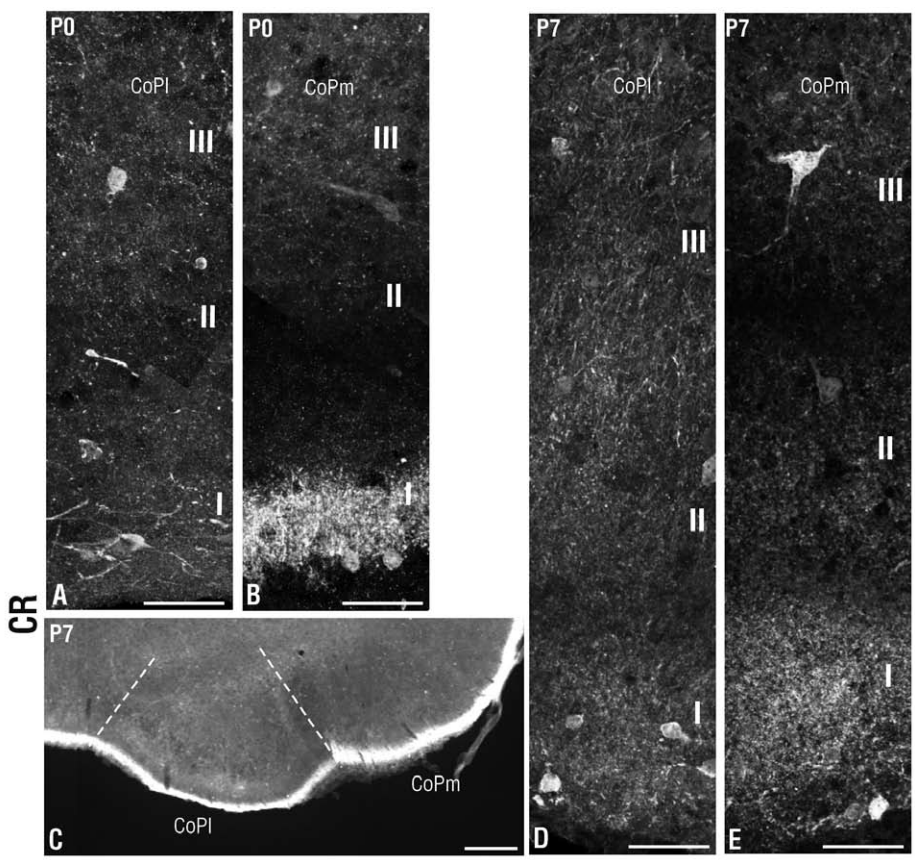

Fig. 5. (A–E) Calretinin (CR) immunoreactivity in the posterolateral (CoPl) and posteromedial (CoPm) cortical nuclei of the rabbit amygdala. I, II, III refer to layers I, II and III of CoPl and CoPm. (A) Numerous neurons resembling Cajal-Retzius cells observed in the layer I CoPl on P0; (B) High CR expression within the superficial part of the layer I of CoPm visible already on P0, and also later (C) with example of a distinct partitioning on P7 (D, E). Scale bars are 0.05 mm (A, B, D, E) and 0.5 mm (C).

1982, Veening et al. 1984, Paredes et al. 2000, Dong et al. 2001, Hall et al. 2001, Salomé et al. 2001), Cem may have an influence on the perineuronal network of those target areas relatively earlier than the remaining parts of amygdala.

Cec is generally believed to be a receiver of information related to most sensory modalities, either directly from the thalamus (LeDoux and Farb 1991) and temporal cortex (Mascagni et al. 1993, McDonald and Mascagni 1996) or indirectly from lateral cortical

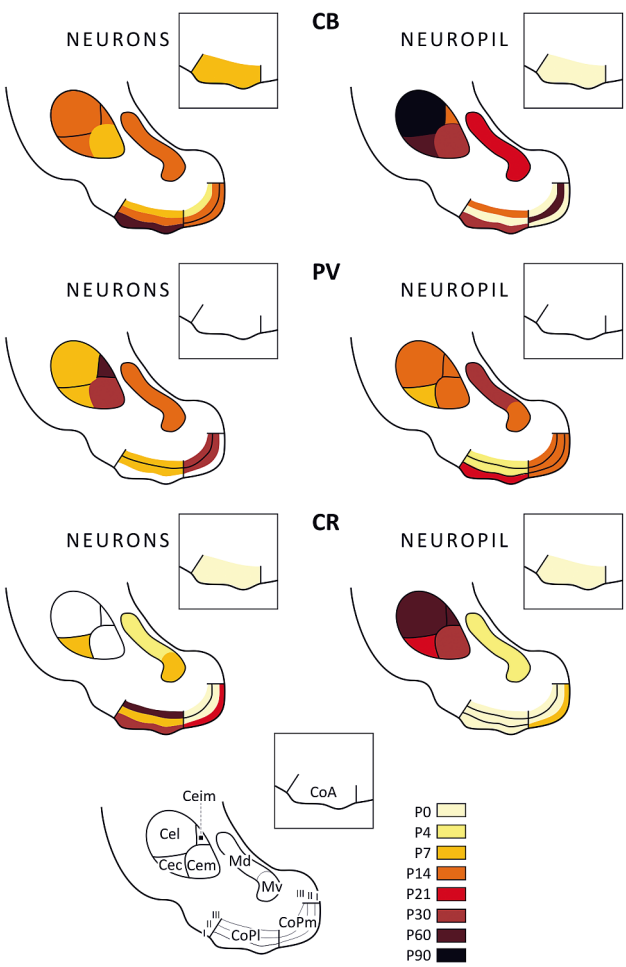

Fig. 6. The summary of maturation data (dates of reaching the mature pattern of immunolabeling; P – postnatal day) of calbindin-D28k (CB), parvalbumin (PV) and calretinin (CR) immunoreactivity within neurons and neuropil in central, medial and cortical nuclei of rabbit amygdaloid complex. (CoA) anterior cortical nucleus; (CoPl) posterolateral cortical nucleus; (CoPm) posteromedial cortical nucleus; (Cem) central nucleus: medial subdivision, (Cec) capsular subdivision, (Ceim) intermediate subdivision, (Cel) lateral subdivision; (Md) medial nucleus: dorsal subdivision, (Mv) ventral subdivision.

(Pitkänen et al. 1995) and medial amygdaloid nuclei (Canteras et al. 1995). Thus, later achievement of maturity by Cec seems to correlate with adjustment of neuronal meshwork in the thalamus, taking place in the first few weeks of postnatal life or, on the other hand, may depend on the shaping of the neuronal structure of the basolateral amygdaloid complex.

The latest stabilization of expression of CaBPs was observed in Cel. This nucleus receives a direct input from the insular cortex (McDonald and Jackson 1987, Yasui et al. 1991, Shi and Cassell 1998) and parabrachial nucleus (Bernard et al. 1993) and sends projections restricted to just three regions: Cem, bed nuclei of the stria terminalis, and the parabrachial nucleus (Petrovich and Swanson 1997). As a result, Cel can mediate aversive tactile and visceral responses processed by these regions. Such a limited area of flow of information may suggest a completely separate course of development of Cel. Moreover, cells of Cel, unlike other subnuclei, are rich in various neuropeptides such as corticotropin-releasing hormone, enkephalin, neurotensin and vasoactive intestinal polypeptide (Veening et al. 1984, Cassell and Gray 1989). Furthermore, GABAergic transmission may play a predominant role in this nucleus (Veinante and Freund-Mercier 1998). This neurotransmitter usually colocalizes with PV and CB. High expression of the former protein confirms an abundance of perineuronal network in Cel, which needs relatively long (at least three months) maturation.

Each CeN subdivision possesses heavy intradivisional projection; the whole CeN reveals unique extensive interdivisional connectivity: Cec projects to Cem, Cel projects to Cem, and Cem projects back to Cec (Jolkkonen and Pitkänen 1998). The intermediate division is the only one that does not interact with the others (Pitkänen et al. 1997). Also, four physiological types of neurons are not distributed uniformly throughout its subdivisions (Martina et al. 1999). The abovementioned data indicate that CeN subdivisions are parts of different neuronal circuits that allow the preservation of their own developmental pattern.

Expression of CaBPs within the corticomedial amygdala was described in the short-beaked echidna (Ashwell et al. 2005), rat (McDonald 1997, Kemppainen and Pitkänen 2000), monkey (Pitkänen and Amaral 1993a) and human (Sorvari et al. 1996a,b, Setzer and Ulfig 1999). Similarly to the rabbit, CeN in the short-beaked echidna, rat and monkey exhibit heterogeneous

expression of CB (Pitkänen and Amaral 1993a, McDonald 1997, Ashwell et al. 2005). However, some differences with regard to expression of CB in Cem and Cel exist. According to our data, only single CB-ir neurons were noticed within rabbit Cem, whereas in the rat moderate to intense neuronal staining was observed in this subdivision. Inversely, the rat Cel possesses only a few positive neurons while in the rabbit their amount was higher. Also, Cel in the short-beaked echidna and monkey was characterized by a high level of CB expression. Contrary to heterogeneous distribution of CB-ir in the central nucleus of the above listed species, CB-ir does not allow its further differentiation in the human, where CeN as a whole is characterized by intense CB-ir neuropil labeling (Sorvari et al. 1996a).

Although PV-ir and CR-ir do not differentiate CeN subdivisions in the rabbit, distinct interspecies discrepancies exist among short-beaked echidna, rat and rabbit in those stainings. Neither PV-ir nor CR-ir somata were found in the amygdala of echidna. In the rat only occasional small PV-ir neurons within CeN were observed, while in the rabbit PV-ir cells were present in all four divisions. No CR-ir neurons except Cec were detected in rabbit CeN, whereas in the rat CR-ir cells were observed in all divisions of CeN, especially within Cem and Ceim.

The discrepancies and similarities in CaBPs expression occurring in the rabbit corticomedial complex in comparison to that in the echidna, rat and primates confirm the developmental progression of complexity of intrinsic neuronal circuits during phylogenesis.

Medial nucleus

The pattern of immunoreactivity of the studied CaBPs indicates that maturation of Med takes place during the first four weeks of postnatal life.

Med is a primary target of the vomeronasal system (Halpern 1987, Martinez-Marcos and Halpern 1999). In turn its efferents terminate in the bed nucleus of the stria terminalis, medial preoptic area of the hypothalamus and the central grey in the midbrain (Krettek and Price 1978b, Kevetter and Winans 1981a). Due to reception of both olfactory and vomeronasal system inputs and relaying them to the lower levels of the central nervous system, the medial nucleus plays a key role in social signaling, reproductive, maternal and parental behavior (Aggleton and Saunders 2000) and fear-related behaviour modulation (Herdade et al. 2006, Davern and Head 2011). As postulated by Newman (2002), the medial amygdala, as the one of the six parts of an integrated subcortical limbic network, subserves the entire spectrum of sex-steroid-modulated social behaviors. It also shows high activity. The majority of authors applying morphological features divide Med into two parts: the antero-ventral and postero-dorsal (de Olmos et al. 1985), while others prefer usage of functional subdivisions: the anterior (chemosensory) and posterior (hormonal) (Gomez and Newman 1991). Due to the fact that in three CaBPs stainings Med showed nearly homogeneous distribution of immunoreactive elements, its further divisions were rather obscured. Moreover, changes of immunoreactivity involved the whole Med.

Except evident lower CR-immunoreactivity, the general pattern of expression of CaBPs in rabbit Med does not differ from that observed in the rat (Kemppainen and Pitkänen 2000) and human (Sorvari et al. 1995, 1996a,b). It seems that due to the close relation to the olfactory system Med acquires the adult pattern of CaBPs expression earlier than CeN.

Anterior cortical nucleus

Our data showed that the period of maturation of the anterior cortical nucleus was the shortest among the studied nuclei of the corticomedial complex – only one week of postnatal life.

In the rabbit, CoA is characterized by a total lack of laminar structure (Jagalska-Majewska et al. 2001), while in the rat (Kemppainen and Pitkanen 2000) and the monkey (Stefanacci and Amaral 2002, Bauman and Amaral 2005) three distinct layers are visible. This interspecies difference was also expressed by the presence of PV-ir neurons in rat and human CoA, contrary to their absence in rabbit.

CoA receives afferents from the main olfactory bulb (Kevetter and Winans 1981b), endopiriform nucleus (Behan and Haberly 1999), midline thalamic nuclei and some of the sensory-related lateral cortical areas, and provides substantial input to the lateral hypothalamic area and bed nucleus of the stria terminalis (Pitkänen 2000). As the structure most closely related to the olfactory system, CoA does not seem to undergo substantial changes during postnatal development. The lack of laminar structure and PV-ir elements indicates simplicity of the intrinsic cytoarchitecture of this nucleus and seems to explain its persistent pattern of CaBPs-ir in almost the whole time of postnatal life.

Posterior cortical nuclei

The maturation period in both cortical posterior nuclei lasts for two months of postnatal life. According to Kevetter and Winans (1981a), CoPm, like Med, is designated to be a part of the vomeronasal amygdala, while CoPl is rather related to the olfactory amygdala. However, their more complicated cytoarchitectonics, layered structure, and presence of PV – the marker of intrinsic network – implicate prolongation (in comparison to CoA) of the time course of their maturation.

All the above-mentioned changes of CaBPs expression in the studied nuclei of amygdala reflect the stages of postnatal physical development in juvenile rabbits. During the first two weeks of rabbit postnatal life major changes related to the maturation of sensory systems (e.g. eye opening (P4-P10) and retina maturation (P13)) and basic motor functions [e.g., body elevation (P4–P10) and head elevation for more than one minute (P7–P14)] occur (Famiglietti and Sundquist 2010, Wolterbeek and Waalkens-Berendsen 2011). Around the third week of postnatal life (Wolterbeek and Waalkens-Berendsen 2011) juvenile rabbits developed more complicated skills necessary for proper behavior execution e.g. hopping (P14–P21). After two months they are ready to start an independent life.

CaBPs as a marker of neuron maturation

Despite the variety of information regarding localization of CaBPs in the brain, knowledge about their exact functions at the neuronal level needs to be extended. It is acknowledged that CaBPs, as important modulators of intracellular calcium dynamics in neurons, may (especially PV) potently modulate short-term synaptic plasticity (Caillard et al. 2000), which establishes a basic issue in the development of the neuronal network. During the development the expression of CB precedes that of PV, while CR appears most precociously (Andressen et al. 1993, reviewed in Legaz et al. 2005). According to many authors PV is related rather with the later stages of development: the appearance of PV-ir indicates at least partial functional maturation of the structure (Nitsch et al. 1990, Solbach and Celio 1991, Seo-Hiraiwa et al. 1995), which can be confirmed by, for example, establishment of the final pattern of the afferent connections (Barker and Dreher 1998).

Among the three studied CaBPs, CR seems to reveal the most conservative and stabile pattern of distribution. Numerous data obtained from various structures of the central nervous system indicate that this protein appears very early in the prenatal life and assists many important developmental events, such as generation of cells, their movements or process outgrowth (Spitzer 1994, Frassoni et al. 1998, Real et al. 2008). These activities are closely correlated with fluctuations of intracellular Ca^{2+} level (Abbott and Jacobowitz 1999); its increase in migrating neurons is essential for their motility (Komuro and Rakic 1996). CR-ir fibres and cells were observed in murine Me already on day 15.5 of the embryological period (Guirado et al. 2008). Setzer and Ulfig (1999) described in man, during development, the earlier appearance of CR-ir in the basolateral complex than in the corticomedial one, which may be related to the process of migration of neurons of the amygdala.

Berdel and Morys (2000) described the occurrence of CB-ir during development of the rat basolateral amygdaloid complex in a two-step way. First, CB-ir was present only in cell bodies, then, two weeks after birth, it appeared in neuropil. Finally, CB-ir was homogeneously distributed in the neuropil. We did not observe such a time course in any nuclei of the corticomedial complex. Moreover, we found that the CB-ir in the neuropil of the posterior cortical nuclei appeared before immunoreactivity in cell bodies.

The diversity of CeN is probably related to its function as the main output from the amygdaloid complex. Sidorowicz and others (1996) proved that prenatal development of the central nucleus in the rat was characterized by a total lack of CB-ir. Some CB-ir neurons appeared at P4 and their density increased until P21. Even taking into account the interspecies differences in morphology and organization of CeN (including intrinsic neuronal network) observed by us, long maturation of this structure in the rabbit might be the result of the longer prenatal period in this species.

The maturation of the central nucleus seems to be also related to maturation of basolateral nuclei – the main source of its afferents. Any changes in the basolateral nuclei anticipate and may possibly force remodelling of the neuronal meshwork in CeN.

In the rabbit CeN a population of PV-ir neurons of adult-like morphology was present from P14. Also in the monkey and man a low density of PV-ir neurons within CeN was observed (Pitkänen and Amaral 1993b, Sorvari et al. 1995). On the contrary, there is a

lack of PV-ir neurons in CeN both during development (Sidorowicz et al. 1996) and in the adult rat (Kemppainen and Pitkänen 2000).

In light of developmental studies the phenomenon of transient expression of CaBPs is of particular important (reviewed in Legaz et al. 2005). The function of the transient expression has been postulated to control cell division, processes outgrowth and cell movement (Andressen et al. 1993). It concerned the cortex and was not confined to any specific calcium binding protein (Hogan and Berman 1993, Schierle et al. 1997, Moon et al. 2002). In rabbit CeN and Med PV-ir neurons of immature morphology resembling migrating cells were noticed only at P0 and P4. These transitory cells may establish a part of the migratory stream described earlier in the rat and human by Bayer and colleagues (1993).

CONCLUSIONS

The pattern of distribution and the level of expression of CaBPs immunoreactivity in the corticomedial complex in the adult rabbit observed by us was rather consistent with those described in other species, but several differences were present.

Various lengths of maturation period in particular nuclei of corticomedial amygdala may result from undoubtedly close relations of its nuclei with the limbic system, possessing a large degree of plasticity (Martin et al. 2000). However, these relations remain on different organization levels.

These differences may also be confirmed by theory of dissimilar origin and development of amygdaloid nuclei (Swanson and Petrovich 1998). According to this theory, CeN and Med can be treated as specialized regions of the striatum, incorporated in autonomic and pheromonal systems, respectively. The remaining nuclei of the corticomedial complex form association parts of the olfactory cortex.

Taken together, these data showed that the developmental patterns of CB, PV and CR immunoreactivity in the corticomedial amygdala are clearly different. CB and PV show complementary patterns of expression, but some temporary differences in their time course appear. CR-ir remains the most conservative – it almost does not undergo major developmental changes. Despite the fact that CaBPs probably play the same role in all the cells, the above-mentioned differences may result from anatomical complexity and functions of particular structures. This diversification, in respect to both time and expression may reflect the different roles of these proteins during development and maturation of the rabbit corticomedial complex.

Therefore, the detailed characteristics of the CaBPs-ir in the corticomedial amygdaloid complex demonstrated in our study may be important in gaining a better appreciation of the role of particular nuclei during early postnatal development.

ACKNOWLEDGMENTS

The authors wish to thank Ms Sylwia Scisłowska, MA, for her help with the preparation of figures. The study was supported by funds from MSHE Grant ST-11.

REFERENCES

Abbott LC, Jacobowitz DM (1999) Developmental expression of calretinin-immunoreactivity in the thalamic eminence of the fetal mouse. Int J Dev Neurosci 17: 331-345.

Aggleton JP and Saunders RC (2000) The amygdala - what's happened in the last decade? In: The Amygdala. A functional analysis (Aggleton JP, Ed.). Oxford University Press, Oxford, UK. p 1–30.

Alheid GF, 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: 1–39.

Alheid GF, DeOlmos SJ, Beltramino CA (1995) Amygdala and extended amygdala. In: The Rat Nervous System (Paxinos G, Ed.). Academic Press, San Diego, CA. p. 495–578.

Alheid GF (2003) Extended Amygdala and Basal Forebrain. Ann NY Acad Sci 985: 185–205.

Amaral DG, Price JL, Pitkanen A, Carmichael ST (1992) Anatomical organisation of the primate amygdaloid complex. In: The Amygdala (Aggleton JP, Ed.). Wiley-Liss, New York, NY. p. 1–66.

Andressen C, Blümcke I, Celio MR (1993) Calcium-binding protein: selective markers of nerve cells. Cell Tiss Res 271: 181–208.

Ashwell KW, Hardman CD, Paxinos G (2005) Cyto- and chemoarchitecture of the amygdala of a monotreme, Tachyglossus aculeatus (the short-beaked echidna). J Chem Neuroanat 30: 82–104.

Barker DA, Dreher B (1998) Spatiotemporal patterns of ontogenetic expression of parvalbumin in the superior colliculi of rats and rabbits. J Comp Neurol 393: 210–230.

Bauman MD, Amaral DG (2005) The distribution of serotonergic fibers in the macaque monkey amygdala: An immunohistochemical study using antisera to 5-hydroksytryptamine. Neuroscience 136: 193–203.

Bayer SA, Altman J, Russo RJ, Zhang X (1993) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14: 83–144.

Behan M, Haberly LB (1999) Intrinsic and efferent connections of the endopiriform nucleus in rat. J Comp Neurol 408: 532–548.

Berdel B, Morys J (2000) Expression of calbindin-D28k and parvalbumin during development of rat's basolateral amygdaloid complex. Int J Dev Neurosci 18: 501–513.

Bernard JF, Alden M, Besson JM (1993) The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex - A phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. J Comp Neurol 329: 201–229.

Brummelte S, Witte V, Teuchert-Noodt G (2007) Postnatal development of GABA and calbindin cells and fibers in the prefrontal cortex and basolateral amygdala of gerbils (Meriones unguiculatus). Int J Dev Neurosci 25: 191–200.

Caillard O, Moreno H, Schwaller B, Llano I, Celio MR, Marty A (2000) Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc Natl Acad Sci U S A 97: 13372–13377.

Camp AJ, Wijesinghe R (2009) Calretinin: modulator of neuronal excitability. Int J Biochem Cell Biol 41: 2118–2121.

Canteras NS, Simerly RB, Swanson LW (1995) Organization of projections from the medial nucleus of the amygdala: A PHAL study in the rat. J Comp Neurol 360: 213–245.

Carmichael ST, Clugnet MC, Price JL (1994) Central olfactory connections in the macaque monkey. J Comp Neurol 346: 403–434.

Cassell MD, Gray TS (1989) Morphology of peptide-immunoreactive neurons in the rat central nucleus of the amygdala. J Comp Neurol 281: 320–333.

Celio MR (1990) Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35: 375–475.

Crosby EC, Humphrey T (1941) Studies of the vertebrate telencephalon II. The nuclear pattern of the anterior olfactory nucleus, tuberculum olfactorium and the amygdaloid complex in adult man. J Comp Neurol 74: 309–352.

Davern PJ, Head GA (2011) Role of the medial amygdala in mediating responses to aversive stimuli leading to hypertension. Clin Exp Pharmacol Physiol 38: 136–143.

Davis M (1992) The role of the amygdala in fear and anxiety. Annu Rev Neurosci 15: 353–375.

Davis M (2000) The role of the amygdala in conditioned and unconditioned fear and anxiety. In: The Amygdala, Vol. 2. (Aggleton JP, Ed.). Oxford University Press, Oxford,UK. p 213–287.

Dayas CV, Buller KM, Day TA (1999) Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. Eur J Neurosci 11: 2312–2322.

de Olmos J, Alheid GF, Beltramino CA (1985) Amygdala. In: The Rat Nervous System (Paxinos G, Ed.). Academic Press, Sydney, AU. p. 223–334.

Dong HW, Petrovich GD, Swanson LW (2001) Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Res Rev 38: 192–246.

Famiglietti EV, Sundquist SJ (2010) Development of excitatory and inhibitory neurotransmitters in transitory cholinergic neurons, starburst amacrine cells, and GABAergic amacrine cells of rabbit retina, with implications for previsual and visual development of retinal ganglion cells. Vis Neurosci 27: 19–42.

Fox CA (1940) Certain basal telencephalic centers in the cat. J Comp Neurol 72: 1–62.

Frassoni C, Arcelli P, Selvaggio M, Spreafico R (1998) Calretinin immunoreactivity in the developing thalamus of the rat: A marker of early generated thalamic cells. Neuroscience 83: 1203–1214.

Frassoni C, Amadeo A, Ortino B, Jaranowska A, Spreafico R (2000) Organization of radial and non-radial glia in the developing rat thalamus. J Comp Neurol 428: 527–542.

Girgis M, Shih-Chang W (1981) Stereotaxic Atlas of the Rabbit Brain. Warren H. Green, Inc. ,St. Louis, MO.

Gomez DM, Newman SW (1991) Medial nucleus of the amygdala in the adult syrian hamster - A quantitative Golgi analysis of gonadal hormonal regulation of neuronal morphology. Anat Rec 231: 498–509.

Guirado S, Real MA, Dávila JC (2008) Distinct immunohistochemically defined areas in the medial amygdala in the developing and adult mouse. Brain Res Bull 75: 214–217.

Hall J, Parkinson JA, Connor TM, Dickinson A, Everitt BJ (2001) Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. Eur J Neurosci 13: 1984–1992.

Halpern M (1987) The organization and function of the vomeronasal system. Annu Rev Neurosci 10: 325–362.

Herdade KC, Strauss CV, Zangrossi Júnior H, Viana MB (2006) Effects of medial amygdala inactivation on a panic-related behavior. Behav Brain Res 172: 316–323.

Hewitson L, Lopresti BJ, Stott C, Mason NS, Tomko J (2010) Influence of pediatric vaccines on amygdala growth and opioid ligand binding in rhesus macaque infants: A pilot study. Acta Neurobiol Exp (Wars) 70: 147–164.

Hof PR, Glezer II, Condé F, Flagg RA, Rubin MB, Nimchinsky EA, Vogt Weisenhorn DM (1999) Cellular distribution of the calcium binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. J Chem Neuroanat 16: 77–116.

Hogan D, Berman NEJ (1993) Transient expression of calbindin-D28k immunoreactivity in layer V pyramidal neurons during postnatal development of kitten cortical areas. Dev Brain Res 74: 177–192.

Humphrey T (1936) The telencephalon of the bat. I. The non-cortical nuclear masses and certain pertinent fiber connections. J Comp Neurol 65: 603–711.

Humphrey T (1968) The development of the human amygdala during early embryonic life. J Comp Neurol 132: 135–166.

Humphrey T (1972) The development of the human amygdaloid complex. In: The Neurobiology of the Amygdala (Eleftherion BE, Ed.). Plenum Press, New York, NY. p 21–77.

Ito T, Ando H, Handa H (2011) Teratogenic effects of thalidomide: molecular mechanisms. Cell Mol Life Sci 68: 1569–1579.

Jagalska-Majewska H, Dziewiatkowski J, Wojcik S, Luczynska A, Kurlapska R, Morys J (2001) The amygdaloid complex of the rabbit - morphological and histochemical study. Folia Morphol 60: 259–280.

Jolkkonen E, Pitkänen A (1998) Intrinsic connections of the rat amygdaloid complex: Projections originating in the central nucleus. J Comp Neurol 395: 53–72.

Jones EG, Lensky KM, Chan VH (2001) Delineation of thalamic nuclei immunoreactive for calcium-binding proteins and around the posterior pole of the ventral posterior complex. Thalamus Rel Syst 1: 213–224.

Kapp BS, Schwaber JS, Driscoll PA (1985) Frontal cortex projections to the amygdaloid central nucleus in the rabbit. Neuroscience 15: 327–346.

Kemppainen S, Pitkänen A (2000) Distribution of parvalbumin, calretinin, and calbindin-D_{28k} immunoreactivity in the rat amygdaloid complex and colocalization with gamma-aminobutyric acid. J Comp Neurol 426: 441–467.

Kevetter GA, Winans SS (1981a) Connections of the corticomedial amygdala in the golden hamster. I. Efferents of the "ventromedial amygdala". J Comp Neurol 197: 81–98.

Kevetter GA, Winans SS (1981b) Connections of the corticomedial amygdala in the Golden hamster: II. Efferents to the "Olfactory Amygdala". J Comp Neurol 197: 99–111.

Komuro H, Rakic P (1996) Intracellular Ca2+ fluctuations modulate the rate of neuronal migration. Neuron 17: 275–285.

Krettek JE, Price JL (1978a) A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. J Comp Neurol 178: 255–280.

Krettek JE, Price JL (1978b) Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J Comp Neurol 178: 225–254.

LeDoux JE (2000) Emotion circuits in the brain. Ann Rev Neurosci 23: 155–184.

LeDoux JE, Farb CR (1991) Neurons of the acoustic thalamus that project to the amygdala contain glutamate. Neurosci Lett 134: 145–149.

Legaz I, Olmos L, Real MA, Guirado S, Davila JC, Medina L (2005) Development of neurons and fibers containing calcium binding proteins in the pallial amygdala of mouse, with special emphasis on those of the basolateral amygdalar complex. J Comp Neurol 488: 492–513.

Martin SJ, Grimwood PD, Morris RGM (2000) Synaptic plasticity and memory: An evaluation of the hypothesis. Annu Rev Neurosci 23: 649–711.

Martina M, Royer S, Paré D (1999) Physiological properties of central medial and central lateral amygdala neurons. J Neurophysiol 82: 1843–1854.

Martinez-Marcos A, Halpern M (1999) Differential projections from the anterior and posterior divisions of the accessory olfactory bulb to the medial amygdala in the opossum, Monodelphis domestica. Eur J Neurosci 11: 3789–3799.

Mascagni F, McDonald AJ, Coleman JR (1993) Corticoamygdaloid and corticocortical projections of the rat temporal cortex: A *phaseolus vulgaris* leucoagglutinin study. Neuroscience 57: 697–715.

McDonald AJ (1994) Calretinin immunoreactive neurons in the basolateral amygdala of the rat and monkey. Brain Res 667: 238–242.

McDonald AJ (1997) Calbindin-D28k immunoreactivity in the rat amygdala. J Comp Neurol 383: 231–244.

McDonald AJ (1998) Cortical pathways to the mammalian amygdala. Prog Neurobiol 55: 257–332.

McDonald AJ, Jackson TR (1987) Amygdaloid connections with posterior insular and temporal cortical areas in the rat. J Comp Neurol 262: 59–77.

McDonald AJ, Mascagni F (1996) Cortico-cortical and cortico-amygdaloid projections of the rat occipital cortex: A *Phaseolus vulgaris* leucoagglutinin study. Neuroscience 71: 37–54.

McDonald AJ, Betette RL **(2001)** Parvalbumin-containing neurons in the rat basolateral amygdala: Morphology and co-localization of calbindin-D_{28k}. Neuroscience 102: 413–425.

McDonald AJ, Mascagni F (2001) Colocalization of calcium-binding proteins and GABA in neurons of the rat basolateral amygdala. Neuroscience 105: 681–693.

Moon JS, Kim JJ, Chang IY, Chung YY, Jun JY, You HJ, Yoon SP (2002) Postnatal development parvalbumin and calbindin D-28k immunoreactivities in the canine anterior cingulate cortex: transient expression in layer V pyramidal cells. Int J Dev Neurosci 20: 511–519.

Morys J, Berdel B, Jagalska-Majewska H, Luczynska A (1999a) The basolateral amygdaloid complex – its development, morphology and functions. Folia Morphol (Wars) 58: 29–46.

Morys J, Berdel B, Kowianski P, Majak K, Tarnawski M, Wisniewski HM (1999b) Relationship of calcium-binding proteins containing neurons and projective neurons in the rat basolateral amygdala. Neurosci Lett 259: 91–94.

Muller JF, Mascagni F, McDonald AJ (2005) Coupled networks of parvalbumin-immunoreactive interneurons in the rat basolateral amygdala. J Neurosci 25: 7366–7376.

Newman SW (1999) The medial extended amygdala in male reproductive behavior - A node in the mammalian social behavior network. Ann N Y Acad Sci 877: 242–257.

Newman SW (2002) Pheromonal signals access the medial extended amygdala: One node in a proposed social behavior network. In: Mammalian Hormone-Behavior System (Pfaff D, Arnold A, Etgen A, Fahrbach SE, Rubin R, Eds). Academic Press, New York, Ny. p 17–32.

Nitsch R, Bergmann I, Kuppers K, Mueller G, Frotscher M (1990) Late appearance of parvalbumin-immunoreactivity in the development of GABAergic neurons in the rat hippocampus. Neurosci Lett 118: 147–150.

Novella S, Hines T (2011) Autism and the amygdala: commentary on Hewitson and coauthors (2010) Acta Neurobiol Exp (Wars) 71: 178–179.

Paredes J, Winters RW, Schneiderman N, McCabe PM (2000) Afferents to the central nucleus of the amygdala and functional subdivisions of the periaqueductal gray: neuroanatomical substrates for affective behavior. Brain Res 887: 157–173.

Parent A, Fortin M, Cote PY, Cicchetti F (1996) Calcium-binding proteins in primate basal ganglia. Neurosci Res 25: 309–334.

Petrovich GD, Swanson LW (1997) Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. Brain Res 763: 247–254.

Petrovich GD, Canteras NS, Swanson LW (2001) Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. Brain Res Rev 38: 247–289.

Pitkänen A (2000) Connectivity of the rat amygdaloid complex. In: The Amygdala: A functional analysis (Aggleton JP, Ed.). Oxford University Press, Oxford, UK. p. 31–115.

Pitkänen A, Amaral DG (1993a) Distribution of calbindin-D(28K) immunoreactivity in the monkey temporal lobe: The amygdaloid complex. J Comp Neurol 331: 199–224.

Pitkänen A, Amaral DG (1993b) Distribution of parvalbumin-immunoreactive cells and fibers in the monkey temporal lobe: The amygdaloid complex. J Comp Neurol 331: 14–36.

Pitkänen A, Kemppainen S (2002) Comparison of the distribution of calcium-binding proteins and intrinsic connectivity in the lateral nucleus of the rat, monkey, and human amygdala. Pharmacol Biochem Behav 71: 369–397.

Pitkänen A, Stefanacci L, Farb CR, Go G-G, LeDoux JE, Amaral DG (1995) Intrinsic connections of the rat amygdaloid complex: Projections originating in the lateral nucleus. J Comp Neurol 356: 288–310.

Pitkänen A, Savander V, LeDoux JE (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci 20: 517–523.

Price JL, Russchen FT, Amaral DG (1987) The limbic region. II: the amygdaloid complex. In: Handbook of Chemical Neuroanatomy, Vol. 5. Integrated Systems of the CNS (Björklund A, Hökfelt T, Swanson LW, Eds). Elsevier, Amsterdam, NL. p. 279–388.

Price JL, Slotnick BM, Revial MF (1991) Olfactory projections to the hypothalamus. J Comp Neurol 306: 447–461.

Real MA, Pineda D, Dávila JC, Guirado S (2008) Development and adult organization of the lateral part of the bed nucleus of the stria terminalis in the chicken. Brain Res Bull 75: 410–413.

Sah P, Faber ES, Lopez DA, Power J (2003) The amygdaloid complex: anatomy and physiology. Physiol Rev 83: 803–834.

Salomé N, Viltart O, Leman S, Sequeira H (2001) Activation of ventrolateral medullary neurons projecting to spinal autonomic areas after chemical stimulation of the central nucleus of amygdala: a neuroanatomical study in the rat. Brain Res 890: 287–295.

Schierle GS, Gander JC, D'Orlando C, Celio MR, Weisenhorn DM (1997) Calretinin-immunoreactivity during postnatal development of the rat isocortex: A qualitative and quantitative study. Cereb Cortex 7: 130–142.

Schwaber JS, Kapp BS, Higgins GA, Rapp PR (1982) Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. J Neurosci 2:1424–1438.

Seo-Hiraiwa ML, Seto-Ohshima A, Kato K (1995) The surface evoked potential and parvalbumin-immunoreactivity in the somatosensory cortex of the developing rat. Dev Psychobiol 28: 337–351.

Setzer M, Ulfig N (1999) Differential expression of calbindin and calretinin in the human fetal amygdala. Microsc Res Tech 46: 1–17.

Shek JW, Wen GY, Wisniewski HM (1986) Atlas of the Rabbit Brain and Spinal Cord. Karger, Basel, CH.

Shi CJ, Cassell MD (1998) Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. J Comp Neurol 399: 440–468.

Sidorowicz M, Dziewiatkowski J, Moryś J, Berdel B, Maciejewska B, Słoniewski P, Zegarska Z. (1996) Calcium binding proteins in the development of central nucleus of amygdaloid body – a morphometric and immunohistochemical study. Folia Morphol (Warsz) 55: 437–439.

Solbach S, Celio MR (1991) Ontogeny of the calcium binding protein parvalbumin in the rat nervous system. Anat Embryol (Berl) 184: 103–124.

Sorvari H, Soininen H, Paljärvi L, Karkola K, Pitkänen A (1995) Distribution of parvalbumin-immunoreactive cells and fibers in the human amygdaloid complex. J Comp Neurol 360: 185–212.

Sorvari H, Soininen H, Pitkänen A (1996a) Calbindin-D28K-immunoreactive cells and fibres in the human amygdaloid complex. Neuroscience 75: 421–443.

Sorvari H, Soininen H, Pitkänen A (1996b) Calretinin-immunoreactive cells and fibers in the human amygdaloid complex. J Comp Neurol 369: 188–208.

Spitzer NC (1994) Spontaneous Ca^{2+} spikes and waves in embryonic neurons: signaling systems for differentiation. Trends Neurosci 17: 115–118.

Stefanacci L, Amaral DG (2002) Some observations on cortical inputs to the Macaque monkey amygdala: An anterograde tracing study. J Comp Neurol 451: 301–323.

Swanson LW (2000) Cerebral hemisphere regulation of motivated behavior. Brain Res 886: 113–164.

Swanson LW, Petrovich GD (1998) What is the amygdala? Trends Neurosci 21: 323–331.

Uchida Y (1950) A contribution to the comparative anatomy of the amygdaloid nuclei in mammals, especially in rodents. Part II: guinea pig, rabbit and squirrel. Folia Psychiat Neurol Jap 4: 91–107.

Urban I, Richard P (1972) A Stereotaxic Atlas of the New Zealand Rabbit's Brain. Charles C. Thomas Publisher LTD, Springfield, IL.

Veening JG, Swanson LW, Sawczenko PE (1984) The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: A combined retrograde transport-immunohistochemical study. Brain Res 303: 337–357.

Veinante P, Freund-Mercier MJ (1998) Intrinsic and extrinsic connections of the rat central extended amygdala: an in vivo electrophysiological study of the central amygdaloid nucleus. Brain Res 794: 188–198.

Wells PG, Bhuller Y, Chen CS, Jeng W, Kasapinovic S, Kennedy JC, Kim PM, Laposa RR, McCallum GP, Nicol CJ, Parman T, Wiley MJ, Wong AW (2005) Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. Toxicol Appl Pharmacol 207 (2 Suppl): 354–366.

Wolterbeek A, Waalkens-Berendsen I (2011) Postnatal physical development in juvenile New-Zealand White rabbits. Reprod Toxicol 32: 178–179.

Wójcik S, Dziewiatkowski J, Spodnik E, Ludkiewicz B, Kowiański P, Spodnik JH, Morys J (2004a) Distribution of immunoreactivity of calcium-binding proteins in the rabbit piriform cortex. Folia Neuropathol 42: 209–220.

Wójcik S, Dziewiatkowski J, Spodnik E, Ludkiewicz B, Domaradzka-Pytel B, Kowiański P, Moryś J (2004b) Analysis of calcium binding protein immunoreactivity in the claustrum and the endopiriform nucleus of the rabbit. Acta Neurobiol Exp (Wars) 64: 449–460.

Wójcik S, Spodnik E, Spodnik JH, Dziewiatkowski J, Moryś J (2007) Distribution of neuronal nitric oxide synthase (nNOS)-immunoreactive elements in the rabbit piriform cortex. Folia Morphol (Warsz) 66: 296–302.

Yasui Y, Breder CD, Saper CB, Cechetto DF (1991) Autonomic responses and efferent pathways from the insular cortex in the rat. J Comp Neurol 303: 355–374.