

# Age-Related Loss of Calcium Binding Proteins in Rabbit Hippocampus

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De JONG, G. I., P. A. NABER, E. A. VAN DER ZEE, L. T. THOMPSON, J. F. DISTERHOFT AND P. G. M. LUITEN. *Age-related loss of calcium binding proteins in rabbit hippocampus*. NEUROBIOL AGING 17(3) 459–465, 1996.—Using immunocytochemistry hippocampal levels of the calcium binding proteins calbindin 28K (CB) and parvalbumin (PV) was studied in young (1 month) to very old (60 month) Albino rabbits. Young (3 month) and senescent (30 month) Wistar rats were also examined to compare the distribution and age dependency of PV and CB in both species. The distribution of PV-ir is similar in the rabbit and rat hippocampus. Aging in both species yielded a small loss of PV-ir in axon terminals. The presence of CB-ir interneurons throughout the hippocampus, and the heavy investment of the dentate gyrus (DG) granular cells with CB-ir was also similar in both species. In rabbits, the number of CB-ir interneurons in the CA1, as well as the density of CB-ir in the DG decreased in the first year of life, and did not change between 12–48 months of age. A secondary reduction in the density of CB-ir in the DG was observed at ages beyond 48 months. A similar loss of CB-ir in the DG occurred in the rat. In the CA1, however, the density of CB-ir was similar in young and aged rats. Another remarkable finding was the total absence of CB-ir in CA1 pyramidal neurons of rabbits at any age. Thus, the distribution and age dependency of PV-ir in the hippocampus is similar in both species. The decline of CB-ir in the DG with advancing age is very prominent and may be related to an altered calcium homeostasis in these cells. However, the absence of CB-ir in the CA1 of rabbits makes a causal role for CB in the functional decline of CA1 pyramidal cells during aging unlikely.

Calbindin 28K    Parvalbumin    Immunocytochemistry    Aging    Hippocampus    Rabbit    Rat

CALCIUM ions are of key importance to neuronal signal transduction as intracellular second messengers, and are involved in a wide variety of different neuronal processes (30). For optimal cell functioning the intracellular  $Ca^{2+}$  concentration is maintained within a narrow range by homeostatic mechanisms. An uncontrolled sustained elevation of free  $Ca^{2+}$  ions is toxic to neurons and may eventually lead to cell death (33). Neurons possess several control mechanisms to prevent such a calcium overload. Besides storage in cellular organelles and extrusion of  $Ca^{2+}$  ions by calcium pumps and ionic exchange (30), neurons contain specific proteins such as parvalbumin (PV) and calbindin-28k (CB), which transiently bind calcium ions with a high affinity (3).

Since the introduction of the calcium hypothesis of aging by Khachaturian in 1984 (16), numerous reports have confirmed dysfunction of neuronal calcium homeostasis during aging. An increased calcium content in whole brain homogenates (24), and a persistent, elevated concentration of free calcium ions in brain synaptosomes (24) and in dorsal root ganglion cells (17) have recently been demonstrated in aged rats. Besides a reduced calcium storage (9) and calcium extrusion capacity (22,23), electrophysiological evidence indicates increased calcium influx through L-type voltage-sensitive calcium channels in hippocampal CA1 neurons of aged rats and rabbits (20,26,27,29,36).

To date, studies concerning aging-related alterations of the content of neuronal calcium binding proteins (CaBP) in the hippocampus have reported variable results, either describing no change (14,25) or a decrease (18,21,38) in CaBP levels. Earlier studies demonstrated that the rabbit hippocampus is an attractive model for the investigation of aging-related neuronal mechanisms (5,6, 26,27), notably of processes underlying learning and memory. For this reason we examined alterations in the immunoreactivity for PV and CB in different hippocampal subregions across a large age range of New Zealand Albino rabbits starting in the early postnatal period up to a presumably senescent age at regular age intervals. The immunoreactivity for both CaBPs was also studied in young and senescent rats in order to compare the distribution and age dependence of PV and CB in both species.

## METHOD

In the present study the immunoreactivity for CaBPs (CaBP-ir) was determined in the hippocampus of female New Zealand Albino rabbits aged 1, 3, 6, 12, 24, or 36 months (each group  $n = 6$ ). Moreover, we examined CaBP-ir in four very old (48 month) and two extremely old (60 month) rabbits. Impaired learning in rabbits has been reported as early as 24–30 months (11,37). Because rabbits may live for up to 8 years (8) we refer to our older rabbits as

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aging rather than senescent. The pattern of CaBP-ir in rabbits was then compared to that in young adult (3 month) or senescent (30 month) male Wistar rats. All animals were regularly checked to ensure good physical health.

#### *Immunohistochemical Procedures*

All animals were deeply anaesthetized with a combination of Ketamine (0.35 ml + 0.44 ml/kg) and Xylazine (0.1 ml + 0.066 ml/kg) (rabbits) or with 6% pentobarbital (rats). Subsequently, the animals were transcardially perfused with a prerinse of heparinized saline, followed by a fixative containing 3% paraformaldehyde, 0.05% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (PB). Brains were cryoprotected overnight at 4°C in 30% buffered sucrose. Coronal sections (20 µm) of the anterior portion and horizontal sections (20 µm) of the posterior part of the hippocampus were cut on a cryostat microtome and collected in 0.01 M phosphate-buffered saline (PBS). The sections were rinsed at least four times between each antibody incubation step with PBS plus 0.5% Triton-X-100 in all immunostainings. Prior to the first antibody incubations, the free floating sections were exposed to 0.01% H<sub>2</sub>O<sub>2</sub> for 15 min and 5% normal sheep serum (NSS, Jackson) for 30 min at room temperature (RT) to suppress nonspecific immunostaining. Then, alternate brain sections were incubated overnight at 4°C with the primary monoclonal antibodies (both from Sigma) mouse IgG antiparvalbumin (immunogen carp muscle parvalbumin; 1:2000 in PBS) and mouse IgG anticalsecalbindin-28K (immunogen chicken gut calbindin-D 28K; 1:400 in PBS). Both antibodies have been extensively characterized, and recognize the calcium bound conformation of PV or CB (1,2), a property that is preserved across species. After rinsing, sections were again incubated for 30 min in 5% NSS and exposed to biotinylated sheep antimouse IgG (Amersham, 1:200 in PBS, 2 h at RT) and streptavidin-HRP (Zymed, 1:200 in PBS, 2 h at RT). Finally, the tissue-bound peroxidase was visualized by reaction with diaminobenzidine (30 mg/100 ml Tris) and 0.01% H<sub>2</sub>O<sub>2</sub>, and the sections mounted for light microscopic inspection. Standard control experiments were performed by omission of the primary antibodies, yielding absence of any detectable immunolabeling.

The immunocytochemistry was performed in parallel to avoid a high level of variation in immunostaining between individual cases. Series of sections of animals of a given species of all different ages tested were processed simultaneously in the same antibody solutions and under the same incubation conditions.

#### *Quantitative Analysis*

Three (in rabbits) or two (in rats) sections from each animal were used for quantitative analysis of the dorsal hippocampus, with emphasis on the CA1 and dentate gyrus (DG). The number of parvalbumin immunoreactive (PV-ir) interneurons was counted in the total area of both CA1 and DG. Also, the number of CB-ir interneurons was determined in the rabbit hippocampal CA1. The boundaries of the inner blade of the DG was defined by an imaginable line that makes a 90° angle with the end of the (good recognizable) granular cells. The boundaries of the CA1 regions were determined by a) a similar line at the site of the thickening of the pyramidal cell layers at borders with CA2 and subiculum (in the case of PV-ir) and b) at the site where the innervation of CA2/3 cells by CB-ir mossy fibers is absent and where the pyramidal cell layer becomes thicker and stained at the site of the subiculum. Within these boundaries the immunopositive cellbodies were counted, which gives an estimate of neuron number. Cell counts are not determined per area because of growth of the brain during development and aging. An estimation of neuron number are, thus, a better measure to overcome the effects of brain volume increase.

The size of transverse sections were standardized by level of cross-section according to atlas coordinates [rats: bregma level -3.3 according to the atlas of Paxinos and Watson (28); rabbits: coronal section 58 according to the atlas of Shek et al. (32)]. The examined sections were chosen according to these atlas coordinates, and due to standardized cutting procedures the analyzed sections were 400 µm apart.

Further, the intensity of immunolabeling for both PV and CB was determined in the hippocampus by measuring the optical density (OD) using an image analyzing system (Zeiss, IBAS). OD was expressed in arbitrary units corresponding to gray levels, and was determined in the pyramidal (CA1) and granular (DG) cell layers. In case of PV-ir within the principal cell layers, only the OD of PV-ir axons and terminals (and not PV-ir cell bodies) were included in the measurement area. In sections stained for CB also the OD of the molecular layer of the DG and of the mossy fiber pathway was measured in the stratum lucidum of the CA3. For PV, the background labeling value was measured in the corpus callosum, which was considered to be devoid of specific immunoprecipitate. For CB, the OD value for the background staining was assessed in the stratum oriens of the CA1. For both PV and CB the OD of the area of interest was related to the background value by the formula  $[\text{OD}(\text{area}) - \text{OD}(\text{background}) / \text{OD}(\text{background})] \times 100$ , thus normalizing the variability in background staining among sections (7).

The mean values from the youngest animals (1 month rabbits or 3 month rats) were used as baseline for an adequate comparison between the effects of aging in rabbit and rat brain. All other values are expressed as a percentage of this young age baseline level. Standard errors of the mean were calculated from the (two or three) section measurements from all animals per group, and thus indicate the level of variation between the different animals within one group. All data were statistically evaluated using one-way analysis of variance (ANOVA), which was, in the case of significant group differences, followed by a Student *t*-test corrected for the use of multiple comparisons.

## RESULTS

### *Parvalbumin*

The distribution of PV-ir in the hippocampus of young adult rabbits appears to be similar to that in the young rat hippocampus. PV was selectively present in nonprincipal neurons in all hippocampal subregions, which is in agreement with earlier descriptions (3). In the CA1, almost all PV-ir perikarya were situated in the strata pyramidale and oriens, with their varicose dendrites extending into the strata radiatum and oriens. The axons of PV-ir interneurons enmeshed the cell bodies of the immunonegative CA1 pyramidal cells (Fig. 1A). In the dentate gyrus (DG), most of the PV-ir cells were localized in the subgranular layer and hilar region. Their dendritic processes can be followed into the hilus and, less prominently, into the molecular layer. As for the principal cells in the cornu ammonis, the immunonegative granular cells situated close to the molecular layer were embedded in a dense network of PV-ir axons and terminals. However, granular cells close to the hilus were not only devoid of immunoreactivity, but were also not innervated by an axosomatic plexus of PV-ir fibers (Fig. 1B).

The estimation of the number of PV-ir neurons in the hippocampal CA1 remained stable across the age range studied in rabbits (Fig. 2A), whereas in the DG a small but significant loss of PV-ir neurons was observed at ages of 12 months and older (Fig. 2A; ANOVA;  $p < 0.030$ ). The relative optical density of PV-ir of the pyramidal and granular cell layers represents the density of PV-ir innervation upon these immunonegative principal cells. In rabbits, this density was not significantly altered with advancing

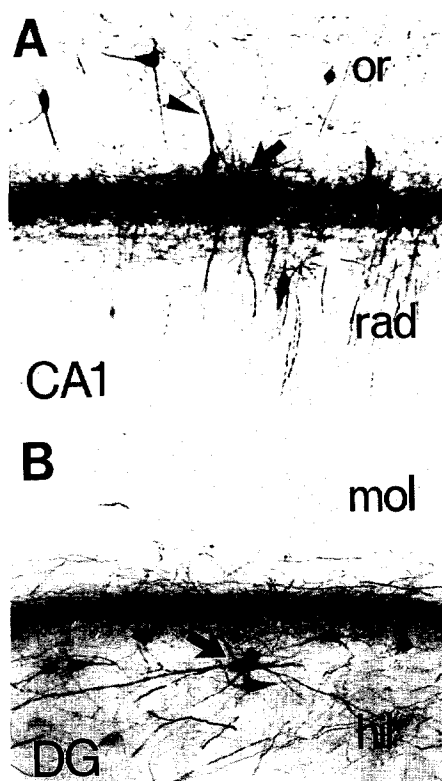


FIG. 1. Photomicrographs of the distribution of parvalbumin immunoreactivity (PV-ir) in young adult rabbits. (A) In the CA1 region PV-ir interneuronal cell bodies (arrows) are mainly localized in stratum oriens (or) and stratum pyramidale (pyr) with dendrites (arrowhead) extending into the stratum oriens and radiatum (rad). Nonlabeled pyramidal cells are enmeshed by PV-ir axons. (B) Granular cells (gran) in the DG are embedded within PV-ir axons. Labeled interneurons (arrow) are extending dendrites into the hilus (hil) and, most notably, into the supragranular zone of the molecular (mol) layer.

age (Fig. 2B). Note, however, the apparent decline in density of PV-ir axons and terminals in both CA1 and DG in the two very old rabbits (Fig. 2B, 60 months).

In our hands, the estimation for number of PV-ir neurons in both CA1 and DG did not differ between young (3 month) and aged (30 month) rats (Fig. 3A). On the other hand, the relative OD of PV-ir in the CA1 pyramidal and in the DG granular cell layer was significantly reduced in the aged rats (Fig. 3B; ANOVA;  $p < 0.006$  and  $p < 0.034$ , respectively).

#### Calbindin-28K

Unlike for PV, the immunocytochemical localization of CB in the hippocampus differs considerably among species (13). Figure 4 gives an overview of the hippocampal and parahippocampal regions in the adult rabbit brain stained for CB. In the cornu ammonis of the rabbit, the CA2–4 areas are characterized by the absence of immunoprecipitate in the pyramidal cells (Fig. 4). In the CA1, almost all pyramidal cells also remain unstained (Figs. 4 and 5B); however, CA1 pyramidal cells close to the subiculum display moderate CB-ir (Fig. 4). Moreover, the stratum pyramidale of the subiculum itself remains immunonegative, whereas a high number of densely CB-ir neurites are present in the pre- and parasubicular regions (Fig. 4). A small number of interneurons was

darkly stained throughout the hippocampus, most prominent in the alveus and stratum oriens of CA1 (Figs. 4 and 5B).

DG granular cells were intensely immunoreactive for CB in young adult rabbits (Figs. 4, 5A, and 6A). In contrast to rats, where all granule cells contain CB with no obvious differentiation in intensity (Fig. 6C), the granular cells lining the hilus in rabbits were more darkly stained for CB than cells closer to the molecular layer (Fig. 6A). The supragranular layer could be identified by the very high density of CB-ir dendrites in close proximity to the granular cells (Fig. 6A). Moreover, based on the density of CB-ir dendrites the dentate stratum moleculare of the rabbit could be subdivided into three layers of which the middle one third displayed the highest intensity of immunolabeling (Figs. 4, 5A, and 6A). The latter subdivision was not present in the dentate molecular layer of the young adult rat (Fig. 6C). The mossy fiber pathway, that consists of the axonal projections from DG to CA3, was also visualized in the rabbit hippocampus (Figs. 4 and 5C).

With aging in rabbits the estimated number of CB-ir interneurons throughout the CA1 gradually and significantly decreased to 47% of young values at the age of 60 months (Fig. 7A; ANOVA;  $p < 0.001$ ). The intensity of CB-ir in the DG granular cells, in their dendrites in the molecular layers, and in their axons in the mossy fiber pathway also markedly declined with advancing age (Fig. 7B; ANOVA;  $p < 0.018$ ,  $p < 0.0001$  and  $p < 0.0001$ , respectively). This reduction was most prominent in the first 12 months, whereafter a plateau was reached. This level was maintained between 12 and 48 months. Thereafter (48–60 months of age) CB-ir further decreased to only 30–40% of the density seen at 1 month (Fig. 7B). The differential distribution of CB within the granular cell layer disappeared, especially in the aging rabbits (Fig. 6B). The three lay-

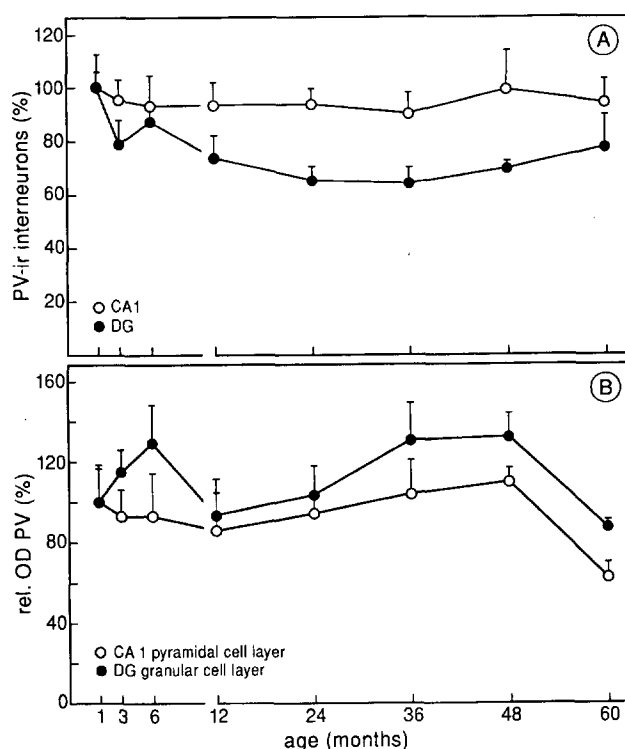


FIG. 2. (A) The relative number of PV-ir interneurons in the CA1 and in the DG, and (B) the relative OD of PV-ir in the CA1 pyramidal cells layer and in the DG granular layer through a large age range of rabbits (in mean  $\pm$  SEM, with the mean value of rats aged 1 month adjusted to 100%).

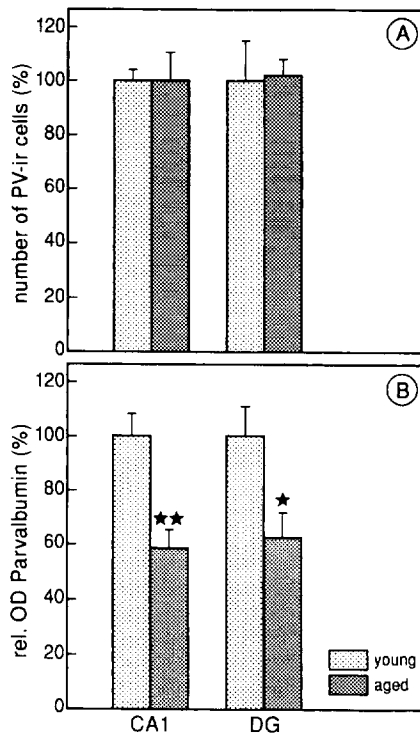


FIG. 3. (A) The relative number of PV-ir interneurons and (B) the relative OD in the CA1 and DG of young (3 month) and aged (30 month) Wistar rats (in mean  $\pm$  SEM with the mean value of young controls adjusted to 100%; \* $p$  < 0.05, \*\* $p$  < 0.01).

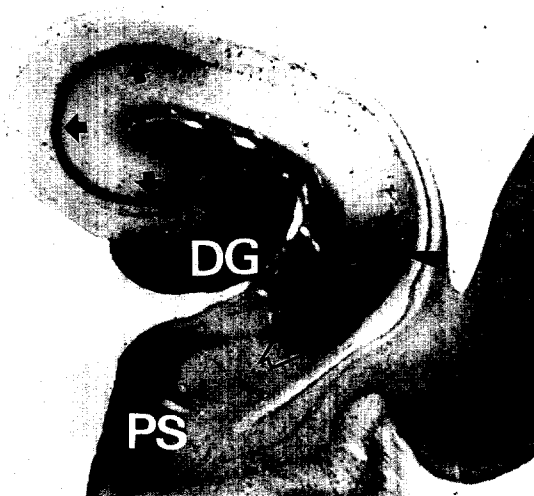


FIG. 4. Horizontal section of the hippocampal and parahippocampal regions within the rabbit brain stained for CB. The dentate gyrus (DG) exhibits a very dense staining pattern. Immunopositive mossy fibers (arrow) arise in the dentate granular cells and innervate the CA3 pyramidal cells, which are devoid of CB-ir. In the CA1 region only the pyramidal cells close to the subiculum (S) display CB-ir (arrowhead), whereas pyramidal cells in the subiculum itself remain unstained (open arrow). In the pre- and parasubiculum (PS) densely CB-ir neurites can be detected.

ered pattern of the dentate stratum moleculare also became less prominent in older rabbits (Fig. 6B). A significantly reduced staining intensity was observed in the DG and in the mossy fibers of aging rats, as well (Fig. 8; ANOVA;  $p$  < 0.001 and  $p$  < 0.0001, respectively). In contrast, the optical density of CB-ir of the rat CA1 pyramidal cell layer did not show any aging-related alterations (Fig. 8).

#### DISCUSSION

The immunoreactivity for the CaBPs parvalbumin (PV) and calbindin (CB) in hippocampal neurons was determined by use of measurements at multiple time points through a significant portion of the lifespan of rabbits. The data obtained from young and aged rats were mainly used to compare the distribution of PV- and CB-ir between rabbit and rat, and to establish similarity in the age-related changes of these CaBPs in the two species.

PV and CB are present in separate subsets of GABA-ergic interneurons throughout the rat hippocampus, with no coexistence of both proteins in the same cell (3,15,19). The localization of both PV- and CB-ir interneurons in the rabbit hippocampus closely resembles that in the rat (3). The present study suggests that in rabbits especially the number of hippocampal CB-ir interneurons decreases with advancing age, whereas the number of PV-ir neurons did not change. Similarly, a stable population of hippocampal PV-ir interneurons was observed when young and aged Wistar rats were compared, which is in agreement with earlier studies (25). Data obtained from human cerebral neocortex also indicates that PV-ir interneurons are resistant against degeneration during aging and in Alzheimer's disease (12).

However, cell number is not a parameter that gives reliable information on subtle intracellular neurochemical or cytoarchitectural alterations. In this respect, the presence of CaBPs in cellular domains such as dendritic processes and axons and terminals may supply meaningful insight regarding the presence of such proteins in cellular regions that dynamically buffer intracellular  $Ca^{2+}$ . The optical density of PV-ir in the pyramidal and granular cell layers in the rabbit and rat hippocampus provides a detailed estimation of the density of PV-ir axons innervating hippocampal principal cells.

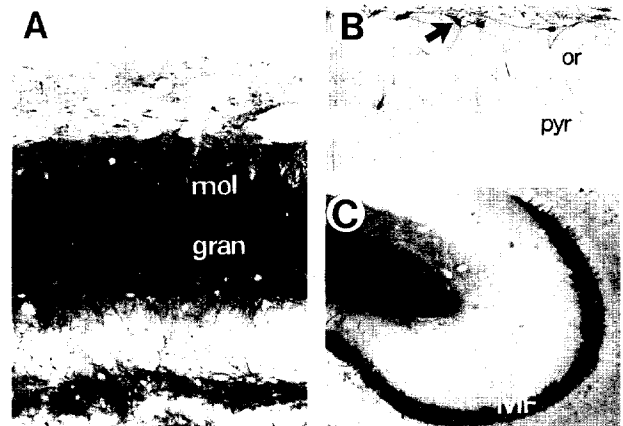


FIG. 5. Photomicrographs of detailed localization of calbindin 28k in the hippocampus of rabbits. (A) In the DG the granular cells (gran) are darkly stained, and also the dendrites in the molecular layer (mol) show an intense immunoreaction. (B) Darkly stained interneurons are found in the stratum oriens (or) of the CA1, whereas the CA1 pyramidal cells (pyr) are devoid of immunoprecipitate. (C) The infrapyramidal mossy fibers (MF) are prominently labeled.

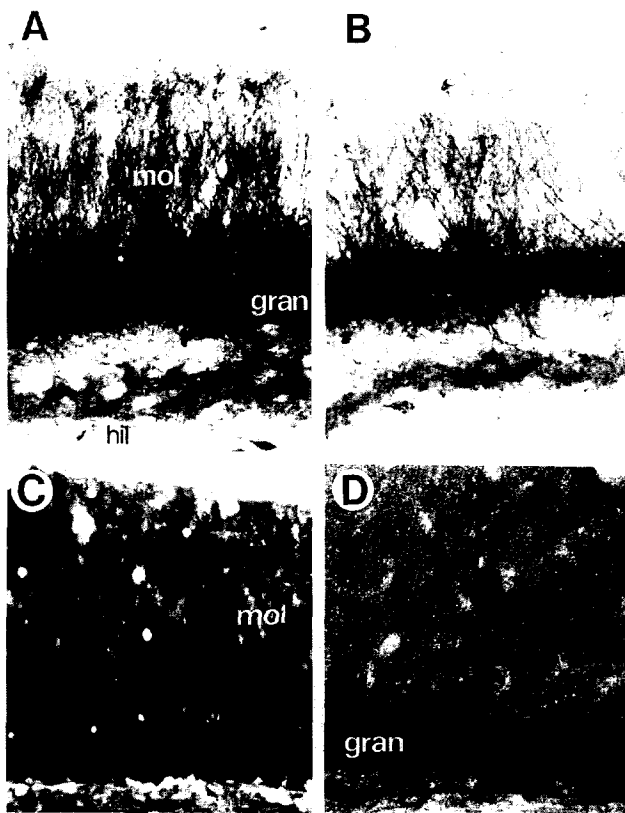


FIG. 6. Photomicrographs of CB-ir in DG of young adult (left panel) and aged (right panel) rabbits (A+B) and rats (C+D). (A) The granular cells (gran) of the DG are darkly stained, and also their dendrites in the molecular layer (mol) are heavily invested with immunoprecipitate in the rabbit. Note that the granular cells lining the hilus are more densely stained, and also the middle third of the molecular layer is characterised by very intense immunolabeling. (B) The CB-ir in DG granular cells (gran) and molecular layer (mol) is prominently reduced in aged rabbits. (C) CB-ir distribution in the rat DG is more equally distributed throughout either the granular or the molecular layer, and (D) the CB-ir in the DG of aged rats is also reduced when compared to young controls.

In both CA1 and DG the density of PV-ir axons and terminals does not alter with advancing age in rabbits, although in the two very old rabbits investigated (60 months) a substantial decline of PV-ir was observed in CA1 and DG. Also, when young and very old rats (30 months) were compared the relative OD was much lower in the CA1 and DG of the senescent animals. Others previously described a reduced amount of PV-ir dendrites throughout the hippocampus in aged Wistar rats (21). Our data suggest to us that the 60-month-old rabbits may most closely compare to the 30-month-old rats, at least regarding the level of PV-ir in the hippocampus.

Thus, aging is accompanied by a loss of PV-ir in neurites of hippocampal interneurons, whereas the number of PV-ir interneuron cell bodies does not alter in the aging rodent hippocampus. In contrast, the number of darkly stained CB-ir interneurons was significantly reduced in the hippocampal CA1 of old rabbits. This decline of CB-ir did, however, not occur in the later stage of the rabbit lifespan, but was manifest in the first 12 months of life, and can, therefore, be categorized as a maturation-related alteration.

In the first 12 months of the rabbit lifespan not only the number of CB-ir interneurons but also the density of CB-ir in the dentate gyrus decreased. In this region the granular cells and their den-

drites (i.e., molecular layer) and axons (mossy fibers) are heavily invested with CB-ir. The high levels of CB-ir in the very young rabbit hippocampus may point to an important role for this protein during development of the hippocampus. Between 12 and 48 months of age the density of CB-ir did not change. At ages beyond 48 months a secondary reduction in the level of CB-ir was observed. In view of the biphasic loss of CB-ir, it is worthwhile to emphasize that the first reduction phase (1–12 months) was more prominent in the neurites of granular cells, for instance, the molecular layer and the mossy fibers, whereas the second reduction phase was equally severe in the granular cell bodies and in their neurites. The latter implies that the content of CB protein throughout the dentate gyrus granular cells is decreased in old rabbits. These findings corroborate qualitative observations from others suggesting that the intensity of CB-ir in the granular cells (7) and mossy fibers in rat is decreased during aging (21). We also observed conspicuously lower optical density values for CB-ir in the dentate gyrus of aged rats. These data indicate that aging-related changes in the neuronal CB content in the dentate is similar in rabbit and rat.

Previous studies demonstrate that morphological alterations during aging in the hippocampus, such as loss of synapses, most consistently occur in the dentate gyrus granular cells (10). This aging-associated loss of synapses in rats can be prevented by chronic administration of L-type calcium channel blockers (4), indicating a role for intracellular calcium ions in the aging process. Using an electrophysiological approach, Reynolds and Carlen also obtained evidence for an increased intracellular calcium concentration in dentate granular cells of aged rats (31). Thus, a relation

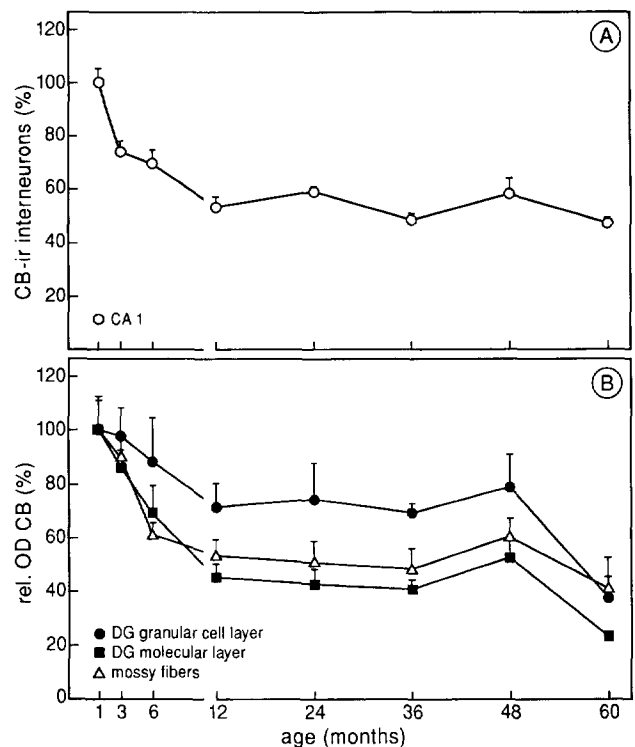


FIG. 7. The relative number of darkly stained CB-ir interneurons in the CA1, and (B) the relative OD of CB-ir in the granular (DG gran) and molecular (DG mol) layers of the DG and in the mossy fiber pathway (MF) in rabbits of ascending ages (in mean  $\pm$  SEM, with the mean value of rats aged 1 month taken as 100%).

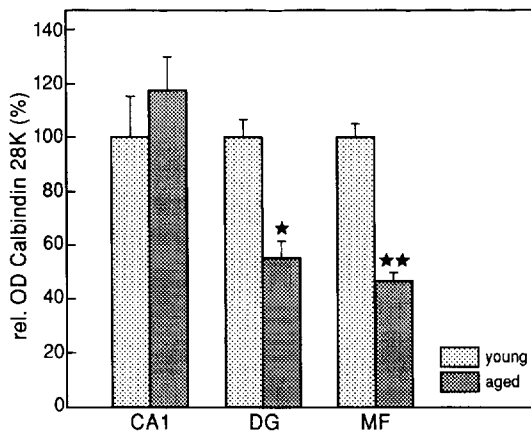


FIG. 8. Relative OD in the CA1, DG, and the mossy fiber pathway (MF) of young (3 month) and aged (30 month) Wistar rats (in mean  $\pm$  SEM, with mean value of young controls adjusted to 100%; \* $p < 0.001$ , \*\* $p < 0.0001$ ).

between the increased intracellular calcium concentration and the decreased content of CB may be present in dentate granular cells of aged rodents. However, a functional relation in this respect seems unlikely because chronic application of the L-type calcium channel blocker nimodipine to aging rats did not influence CB-ir in the dentate gyrus (data not shown).

Pyramidal cells in the hippocampal CA1 are more frequently studied than granular cells to define the neurochemical and neurophysiological characteristics of the aging nerve cell. Detailed electrophysiological analyses of CA1 pyramidal neurons have demonstrated an increased calcium influx in aging rabbits (5,26, 27) and rats (20,29). The properties of CA1 pyramidal cells thus support the calcium hypothesis of aging (5,16), in which an increased intracellular calcium concentration is assumed to be a major trigger of neurodegenerative processes during aging. Previous reports suggest that the intracellular amount of CB plays an important role in the control of neuronal calcium homeostasis (3), and that this content alters with advancing age in CA1 pyramidal cells (7,38). Dutar and co-workers (7) reported less intense CB-ir in CA1 pyramidal cells of aged rats using a polyclonal antibody, which recognized the calcium-free form of CB. They suggested a

shift from the calcium-free to the calcium-bound form of CB during aging. In the present study we applied a monoclonal antibody that recognizes the calcium bound-form (2) of CB, and did not observe an aging-related change in the expression of (calcium-bound) CB in the hippocampal CA1 of aged rats. Our data, in combination with the previous finding of Dutar indicate that the total amount of CB protein in the CA1 is reduced in aged rats, which is in agreement with recent data from others (38).

The literature suggests that the distribution of CB-ir throughout the hippocampus of different species is not uniform (13,34). A most remarkable observation in the present study was the total absence of CB-ir in hippocampal CA1 pyramidal neurons in the rabbit. The lack of CB-ir in these cells was consistent in all rabbits examined, and cannot be attributed to procedural artifacts, as CA1 interneurons and dentate gyrus granular cells were darkly stained in the same sections in which CA1 pyramidal cells were unstained. In addition, we stained sections from the same rabbits with a polyclonal antibody against CB, and did not find any labeling in the CA1 pyramidal cells as well (data not shown). Also in the human hippocampus Sloviter and colleagues, using a polyclonal antibody, could not demonstrate immunostaining in CA1 pyramidal cells (34), however, these findings were followed later by the demonstration of CB mRNA in these pyramidal cells (35).

In conclusion, the distribution and age-dependent levels of PV-ir is similar in the rat and rabbit hippocampus. Aging is accompanied by a loss of PV-ir in axons and terminals, whereas the number of PV-ir interneurons does not alter. The number of CB-ir interneurons declined in the first year of the rabbit lifespan, while the decrease of CB-ir in the dentate gyrus was similar in rabbit and rat brain during aging. However, while the electrophysiological alterations of aged CA1 pyramidal cells from rabbit and rat brain are similar, CB is present in rat but not rabbit hippocampal CA1 pyramidal neurons. Therefore, there is no necessary functional relation between loss of CB and increased calcium action potentials during aging in the CA1 hippocampal pyramidal neurons.

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