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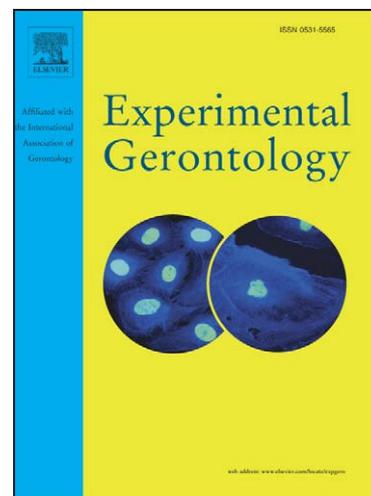
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Changes in parvalbumin immunoreactivity with aging in the central auditory system of the rat

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ABSTRACT

Changes in the levels of calcium binding proteins are known to occur in different parts of the brain during aging. In our study we attempted to define the effect that aging has on the parvalbumin-expressing system of neurons in the higher parts of the central auditory system. Age-related changes in parvalbumin immunoreactivity were investigated in the inferior colliculus (IC), medial geniculate body (MGB) and auditory cortex (AC) in two rat strains, normally aging Long Evans (LE) and fast aging Fischer 344 (F344). The results demonstrate that the changes in PV-immunoreactivity are strain-dependent with an increase in the number of PV-immunoreactive (PV-ir) neurons occurring in the inferior colliculus of old LE rats and a pronounced decline in the number of PV-ir neurons appearing in the auditory cortex of aged F344 animals. In some parts of the AC of old F344 animals no PV-ir neurons were present at all. The number of PV-ir neurons in the MGB in all examined animals was very low independent of the strain and age. The loss of PV-ir neurons in the auditory cortex of Fischer 344 rats with aging may contribute to the substantial deterioration of hearing function in this strain.

INTRODUCTION

The calcium binding proteins (CBP), calbindin (CB), parvalbumin (PV) and calretinin (CR), are among the major fast cytoplasmatic calcium buffers in the central nervous system (for review see Baimbridge et al., 1992). The disruption of neuronal calcium homeostasis and consequent molecular events affect neuronal viability and synaptic plasticity and may represent early steps in the development of neuronal degeneration (Foster, 2007). Changes in calcium homeostasis in the brain during aging are thought to be tightly linked to a decline in neuronal performance (Khachaturian, 1989; Verkhratsky and Toescu, 1998).

The distribution of parvalbumin-immunoreactive (PV-ir) neurons in the central nervous system of the rat was first described in a summary by Celio (1990). PV-ir neurons were found to be present in most parts of the central auditory system with the exception of the medial geniculate body (Celio, 1990; Covenas, et. al., 1991). Their distribution seems to be complementary to calbindin with a predominant distribution of PV in primary and CB in non-primary areas as also described in mice (Cruikshank et al., 2001), monkeys (Jones, 2003) and humans (Tardif et al., 2003). Most PV-ir cells in the central auditory system, as in other parts of the central nervous system, are interneurons (Golgi type I cells) highly colocalized with the neurotransmitter gamma-aminobutyric acid – GABA (Celio, 1986; DeFelipe and Jones, 1992; Kosaka et al., 1987; Kawaguchi and Kubota, 1998).

Several lines of evidence indicate that fast-spiking GABA-ergic cortical basket cells that express parvalbumin and form most of the PV-ir neurons are essential for the generation of gamma oscillations, which are thought to provide a temporal framework for information processing in the brain (Kawaguchi and Kubota, 1998, Bartos et al., 2007). In the hippocampus parvalbumin-expressing basket cells represent approximately 20% of GABA-containing inhibitory interneurons (Freund and Buzsáki, 1996) and form an extensive,

mutually connected interneuronal network interconnected with gap junctions (Fukuda et al., 2006). Decreases in the presence of parvalbumin-ir neurons have been described in several major illnesses of the human brain such as schizophrenia and epilepsy (for review see Woo and Lu, 2006; Lewis et al., 2005). On the other hand, in human neocortex from individuals without any signs of neurological or psychiatric disorders, Bu et al. (2003) found no age-related changes in PV-immunoreactivity with aging in contrast to a decrease in the number of calbindin- and calretinin-ir neurons.

Age-related changes in CBP in different cortical and subcortical structures have also been described in laboratory animals. Hwang et al. (2003) reported a decline in the number of PV-ir neurons together with other morphological changes in the olfactory bulb of aging rats. In the same species a reduction in the cross-sectional area and a decrease in the numerical density of PV-ir neurons were found in the cingulate cortex (Krzywkowski et al., 1995). A significant loss of CB-ir neurons, but only small changes in the occurrence of PV-ir neurons, was observed in the aged rabbit hippocampus (DeJong et al., 1996). In the brain of hamsters, calretinin and parvalbumin mRNA expression remained unchanged during aging, while calbindin – D28k mRNA expression was decreased (Kishimoto et al., 1998). Age-related changes in the levels of the calcium binding proteins are well characterized in the cochlear nuclei and inferior colliculus of mice. The reported changes were strain-dependant for all three proteins in cochlear nuclei (Idrizbegovic 2003,2004) and for calretinin in the IC (Zettel 1997).

The age-related changes in the levels of CBP in the auditory cortex are not well known. In contrast, a substantial decrease with aging in the levels of other markers in the auditory cortex of rats was reported in the case of GABA (Ling et al. 2005), NADPH-diaphorase (Ouda et al. 2003) and acetylcholine (Stemmelin et al., 2000). In our study we evaluate age-related

changes in parvalbumin expression in the higher parts of the central auditory system in two rat strains, normally aging Long Evans (LE) and fast aging Fischer 344 (F344).

MATERIALS AND METHODS

Animals

Nineteen male rats (10 Long Evans, 5 young animals 3-5 months old and 5 aged animals 27-30 months old, and 9 Fischer 344, 5 young animals 3-5 months old and 4 aged animals 22 months old) were used in the experiments. All Fischer 344 rats were purchased at 2 months of age from Charles River Deutschland (Charles River Wiga GmbH, Sulzfeld, Germany) and then reared in-house under standard rearing conditions without any noise exposure. All Long Evans rats were obtained from our local facility at the age of 2 months and reared in-house under the same conditions. No signs of any middle ear infection were present in any animal during their stay in our animal facility. The care and use of animals and all experimental procedures followed the principles of laboratory animal care and were performed in compliance with the guidelines of the Ethical Committee, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, and the Declaration of Helsinki.

Parvalbumin immunohistochemistry

Under deep anesthesia (pentobarbital, 50 mg/kg, i.p.), animals were transcardially perfused with saline followed by 4% paraformaldehyde fixative in 0.1 M phosphate buffer (pH 7.4). The brains were removed within 15 min of perfusion, postfixed 1 hour at 4° C (same fixative) and then cryoprotected with 30% sucrose in phosphate buffer overnight. Coronal sections (40 µm thick) were cut with a freezing microtome.

Free-floating sections were preincubated in a blocking solution and then incubated for 48 h in PBS (4° C) containing anti-parvalbumin (1:1000, Sigma, St. Louis, MO). Sections were incubated first with a biotinylated secondary antibody (Vector), diluted 1:200, for 90 min, and

then with avidin-biotin-peroxidase complex (1:100, Vector) for 1 h at room temperature. The peroxidase reaction was visualized with 0.02% diaminobenzidine and 0.01% hydrogen peroxide for 3 min. Sections were mounted on slides, dehydrated and coverslipped.

Each second section from one animal from each group was stained for Nissl substance to visualize global age-related changes in the total number of neurons and to ensure the positions of the auditory cortical areas.

Stereological quantification

Parvalbumin immunoreactivity was examined in all sections containing the IC, MGB and auditory cortex delineated according to anatomical atlases (Zilles, 1985; Paxinos and Watson, 1998) and with the help of Nissl-stained sections (topographical borders of cortical auditory areas). Sections for the auditory cortex were sampled from bregma -4.2 mm to bregma -5.8 mm (Zilles, 1995), identified by measuring 2.0-2.5 mm from the rhinal fissure for Te3 and 3.5-4.0 mm for Te1. As an auxiliary criterion, the line going through the dorsal margin of the diencephalon demarcated the control dorsal (upper) border of the auditory cortex. For the IC, sections were sampled from bregma -8.0 to -9.2 mm and for the MGB from bregma -5.0 to -6.2 mm (Fig.1A,B,C). To estimate the total number of PV-ir neurons, an unbiased stereological method, the optical fractionator, was used (West et al., 1991; for review see Mayhew and Gundersen, 1996). The optical fractionator method is unaffected by tissue shrinkage, and therefore it was not necessary to measure this variable. Measurements were determined using bright-field microscopy (Leica DMRXA microscope) connected to a video camera, which transmitted the microscopic image to a monitor (Optronics, 1600x1200 px) coupled with Neurolucida software (MicroBrightField). Two sets of motors were connected to the microscope to move the section a known distance in the x- and y-directions. The z-direction was measured using a microcator. A 10x objective lens was used to delineate the regions of interest and the starting position of the counting frame. We adapted the

NeuroLucida-generated grid superimposed on the screen with the use of a random number table to establish the random starting position of the counting frame in the area of interest.

A 100x oil-immersion objective was used and the appropriate counting frame superimposed on the screen. The counting frames used to obtain the sampling had the same size (3880 μm^2). The upper and lower surfaces of the sections were examined to determine if the margins of the tissue sections were depleted of neuronal nuclei, as suggested by Andersen and Gundersen (1999). The height of the optical dissector was constant at 10 μm , and the first and last 5 μm of the section thickness were omitted from the analysis. Approximately 200 neurons per animal in each examined structure were counted (except for the MGB where only 30-50 neurons per animal were counted). Each neuron in the counting frame was counted when its nucleolus came to maximum focus. At maximum focus, the cell body was outlined by a cursor on the computer screen, and the morphological parameters and optical density were measured automatically using NeuroLucida software. Only positive somas that exceeded 5 μm in diameter and with a distinct nucleolus were counted and used for the analysis.

Although the counting of neurons was unbiased, the measurement of their volume might be connected with a small error. Because the sections were cut coronally, the requirement of an isotropic or vertical random orientation of the sections could not be accomplished (for review see Mayhem and Gundersen, 1996). However, in an older review (Gundersen et al., 1988), authors reported that the error should be negligible in such a case. The neuronal volume was estimated using the nucleator technique when the nucleolus was at maximum focus. The volume of the PV-ir soma was then calculated by averaging the third powers of intercept lengths and multiplying this average by $4 \times \pi / 3$ formula (Mayhem and Gundersen, 1996).

Variation in the background immunostaining affects the real values of the optical density of the neuronal soma. In studies of aging, there are not available specific “indifferent

reference structures” in the brain for normalization, hence we calculated the normalized value of the optical density in each section as the ratio between the average optical density of the analyzed PV-ir neurons in the section and the average optical density of a corresponding number of immunonegative cells in the same section (compare Ling et al., 2005). In most cases the immunopositivity of the neurons was explicit; in a few disputable cases, a selected neuron was omitted from the analysis as "non-PV-ir" unless the measured optical density of its soma was higher than double of the average optical density of immunonegative cells in the same section.

Statistical analysis

The number of neurons, normalized values of the optical density and mean neuronal volumes were compared between young and old animals. In addition, comparison was made between Long Evans and Fischer 344 animals.

The results of statistical analysis are expressed as mean \pm S.E.M. The results and the significance of the differences between young and old animals of the same strain and between LE and F344 rats (young vs. young and old vs. old) was assessed by the non-parametric two-tailed Mann-Whitney test with the use of GraphPad Prisma software (version 4.0). P values of 0.05 or less were considered statistically significant.

RESULTS

Inferior colliculus

PV-ir neurons were distributed throughout all three subdivisions of the IC. In the central nucleus of the IC (CIC), oval and disc-shaped neurons predominated with a large variation in the size of the neuronal somas; the intensity of their immunoreactivity varied from low to high. The majority of PV-ir neurons in the external cortex of the IC (EIC) were large spindle

multipolar cells or smaller oval neurons, both in the 2nd and 3rd layers of the EIC. Clusters of immunoreactive somas and neuropil in the 2nd layer of the EIC (Chernock et al., 2004) were clearly visible in the majority of examined animals of both strains. The highest number of PV-ir neurons was found in the CIC (5520/mm³) followed by the EIC (3750/mm³), while only occasional oval PV-ir neurons occurred in the dorsal cortex of the IC – DIC (720/mm³). The mean neuronal volume was largest in the EIC (1260 μm³) followed by the CIC (990 μm³) and DIC (810 μm³). All data in parentheses apply to young LE rats. However, these features were generally present in all examined groups of animals.

Significant age-related changes were found only in the CIC (Fig. 2 A,B). In the old Long Evans rats the number of PV-ir neurons in the CIC was increased by 19% (P=0.03, Fig. 4A). This numerical change was associated with an increase in the optical density of PV-ir somas of 18% (P=0.03) and a slight decrease in the neuronal volume (decrease of 7% from 990 μm³, N.S.; Fig. 4C,E). In the Fischer 344 rats a non-significant tendency towards a decrease in the number of PV-ir neurons and no changes in their optical density with aging were found in the CIC (Fig. 4 B,D). In contrast to the LE rats, the volume of PV-ir neuronal somas was significantly smaller in old F344 animals in comparison with young ones (a decrease of 21% from 1030 to 815 μm³, P=0.03; Fig. 4 E,F). The observed changes could not be attributed to any specific type of neuron.

Medial geniculate body

Both the dorsal and ventral divisions of the MGB contained only sparse small oval parvalbumin-positive neurons with a low intensity of immunostaining. Their number varied over the range of 120-150/mm³ in the ventral and 100-130/mm³ in the dorsal division of the MGB in young animals of both strains. Only a minority of examined sections contained any PV-ir neurons at all. The volume of PV-immunoreactive neurons in both the ventral and

dorsal MGB was approximately $750\text{-}800\ \mu\text{m}^3$. In the medial division of the MGB practically no PV-ir neurons were seen in any of the examined animals regardless of age or strain. No remarkable age-related changes were observed in the parameters of PV-ir neurons in the MGB (Fig. 4).

Auditory cortex

In both strains, PV-ir cells were scattered throughout layers II–VI with only sparse immunoreactive neurons and fibers in layer I. The optical density of neuropil staining was highest in layers II and IV, followed by layer V. Layers III and VI exhibited a markedly lower optical density (Fig. 2 C,E). On average, the neuropil staining was more intense in the Te1 area compared to Te3, but the optical density of PV-ir somas showed no significant difference between these areas. The PV-ir cells were mainly medium-sized and exhibited moderate or strong immunoreactivity with less variability compared to the IC and no significant differences among layers. Most immunoreactive cells in all layers were oval and multipolar elements with diverse orientations of the dendrites. PV-ir cells also included a few triangular and spindle neurons. Primary dendrites could be followed in some neurons up to a distance of $100\ \mu\text{m}$ from the cell body, especially in the case of spindle neurons with processes perpendicular to the cortical surface. The number of PV-neurons was higher in the superficial layers (II-IV) than in the deep layers (V-VI), especially in Te3 area. These features were present in all examined groups of animals

In old Long Evans rats, we found in a few cases sections with no PV-immunoreactive neurons or with a highly reduced number of PV-ir neurons (reduced by more than 50% compared to the average numerical density in young rats) in fields within the Te1 or Te3 area. This resulted in a slight but non-significant average decrease in the numerical density in the Te1 and Te3 areas in old LE rats compared to young ones (Fig. 4A). The optical density of PV-ir somas in old Long Evans animals was non-significantly increased (Fig. 4C).

In contrast to the findings in LE animals, in old F344 rats we observed substantially more sections with no PV-positive neurons or with a substantially reduced number of PV-ir cells (>50%) within the auditory cortical areas Te1 or Te3 involving all layers (Fig. 2D,F). The reduction occurred non-preferentially in all types of PV-ir neurons. In more than one-third of the examined sections containing the Te1 and Te3 areas, we either did not find any PV-ir neurons or else they were very scarce. The parvalbumin-free fields extended through all cortical layers. Examination of serial sections revealed that parvalbumin-free fields occupied in some cases the majority of the Te3, Te1 or both areas in the examined hemisphere (Fig. 3). In all old F344 rats at least one auditory cortical area was affected to a certain degree. In non-affected parts of the auditory cortex only a slight decrease in the number of PV-ir cell was found compared to young animals. However, these large deficits in the local occurrence of PV-ir neurons resulted in a significant reduction of their mean numerical density in the auditory cortex by 31% in comparison with young Fischer 344 rats ($P=0.015$, Fig. 4B). In addition, similar deficits in the number of PV-ir neurons were also found in the visual cortical areas V1 and V2 and the retrosplenial cortex as captured in the examined sections. No fields devoid of PV-ir neurons were observed in young Fischer 344 rats. There were no changes in the optical density of PV-ir neuronal somas in aged F344 animals in comparison with young ones (Fig. 4D). Similarly, the mean volumes of PV-ir neurons in the auditory cortex were not different between young and old rats of either strain (about $950 \mu\text{m}^3$ for LE animals and $900 \mu\text{m}^3$ for F344 rats).

When comparing the Long Evans and Fischer 344 strains (young vs. young and old vs. old animals), no other statistically significant differences were found with the exception of a lower number of PV-ir neurons in the AC of old F344 rats vs. old LE rats ($P=0.03$).

Nissl staining

In sections stained for Nissl substance, no noticeable age-related changes in the total number of neurons were observed in either strain. The number of neurons was found to be about 93 000/mm³ in the IC, 55 000/mm³ in the MGB, and 75 000/mm³ in the AC. However, the number of examined animals (one for each group) was too low for any further statistical analysis. As well, no apparent changes in the thickness of the auditory cortex or in the cross-sectional areas of the IC and MGB were observed in old animals.

DISCUSSION

Our results demonstrate significant age-related changes in parvalbumin immunoreactivity in the upper parts of the auditory pathway in the rat, which comprise changes in the number of PV-ir neurons, their optical density and the volume of PV-ir somas. In summary, the number of PV-ir neurons increased with age in the CIC in the Long Evans strain, whereas in the Fischer 344 strain a significant reduction was observed in the occurrence of PV-ir neurons in the AC with aging. Some parts of the AC in Fischer 344 rats were, in contrast to young animals, completely devoid of parvalbumin immunoreactivity.

Long Evans and Fischer 344 represent two rat strains with a wide variety of morphological, physiological and behavioral differences. For example, similarly to other inbred strains (Wistar, Sprague-Dawley and Dark-Agouti), Fischer 344 rats display large cognitive deficits in different tests of spatial memory in the Morris water maze in contrast to wild rats and Long-Evans rats (Harker and Whishaw, 2002). A more rapid and pronounced deterioration of hearing function with aging is found in the Fischer 344 strain in comparison with Long Evans rats, resulting in larger hearing threshold shifts, a decrease in the amplitude of click-evoked auditory brainstem responses, a diminution of distortion product otoacoustic emissions and a decrease in middle-ear compliance (Popelar et al. 2003, 2006). Age-related

sensory deficits in F344 rats also include visual function, which is damaged due to retinal degeneration (DiLoreto et al, 1994).

In our experiments, significant differences were observed in the age-related changes in parvalbumin immunoreactivity between the F344 and Long Evans strains. The reduction in the numerical density of parvalbumin-ir neurons in the AC in aged F344 rats is in accordance with the prevailing tendency towards a decrease in the number of PV-ir neurons in other parts of the aging brain in rats, for example in the cingulate cortex, hippocampus and olfactory bulb (Krzywkowsky et al., 1995; Shetty and Turner, 1998; Hwang et al., 2003), although focal declines in immunoreactivity were not reported. In old Long Evans rats only a slight reduction in the number of PV-ir neurons was found in the AC, but, in contrast, such neurons were found in elevated numbers in the central nucleus of the IC. What this means for the function of the IC in old Long Evans rats remains to be determined. In any case, a similar increase in the number of PV-ir neurons was found in aged CBA/CaJ mice, a strain with preserved hearing function up to an advanced age, in the cochlear nuclei (Idrizbegovic et al., 2001). The authors argue for recruited new expression during aging in neurons originally immunonegative for calcium binding proteins. The up-regulation of calcium binding proteins should represent a buffering capacity against changes in intracellular calcium levels, while impaired calcium homeostasis is thought to play a critical role in cellular aging (Verkhatsky and Toescu, 1998; Toescu et al., 2004). In addition, the optical density which reflects immunostaining intensity of CIC neurons was found to be increased in Long Evans rats. This may either be connected with an increase in the expression of the protein or the result of an accumulation of parvalbumin due to the shrinkage of the cells (Krzywkowski et al., 1995). Another feature characteristic of aged PV-ir neurons in the rat might be a decline in the size of their somas with aging. In the IC of old Fischer 344 rats, we observed a significant decline in the volume of PV-ir somas, similar to that seen in the medial septum-diagonal band of the

Broca region in the Sprague-Dawley rat strain (Krzywkowski et al., 1995). No apparent age-related changes in the average cross-sectional size of the CIC found in both strains are consistent with previous observations in rat (Caspary et al., 1995) and mice (Willot et al., 1994).

An interesting analogy to the present findings in Long Evans vs. Fischer 344 strains of rat exists in mice when comparing the CBA/CaJ and C57/BL/6J strains. An age-related decline in calbindin-immunoreactivity was found in the medial nucleus of the trapezoid body only in C57 mice, not in CBA mice (O'Neill et al., 1997). With aging both strains show a decline in the number of calbindin-ir neurons in the IC, whereas calretinin-ir neurons increase with age only in the IC of CBA mice, not in C57 mice (Zettel et al., 1997). This up-regulation of calretinin is dependent on preserved sound-evoked activity, since in CBA mice bilaterally deafened in youth, the increase with age does not occur (Zettel et al., 2001). It remains to be investigated whether the age-related increase in the number of parvalbumin-ir neurons in the IC of old Long Evans rats, found in our experiments, is also dependent on sound-evoked activity.

The substantial loss of PV-ir neurons in the auditory cortex in Fischer 344 strain cannot be simply explained by a selective neuronal loss, because in sections stained for Nissl substance no significant numerical changes were present. This finding is in agreement with a paper by Merrill et al. (2001), who reported no significant changes in the total number of neurons in the cortex of old F344 rats in comparison with young animals. In addition, also Poe et al. (2001) in the rat sensorimotor cortex and Helfert et al. (1999) in the rat inferior colliculus did not report any age-related decline in the total quantity of neurons but rather morphological and synaptic changes. Interestingly, staining for calbindin and calretinin did not reveal any numerical changes in the cortical areas of old F344 rats where PV-ir neurons were absent or

nearly absent (our unpublished data), which supports the idea of a predominant selective loss of parvalbumin expression or parvalbumin-expressing neurons.

In the last few years, growing evidence has accumulated about the role of PV-ir neurons in the inhibitory networks generating gamma oscillations, especially in the neocortex and hippocampus, both in man and rat (Bragin et al, 1995; Buzsáki and Draguhn, 2004). Gamma oscillations (30-100 Hz) are thought to represent reference signals for temporal encoding, the sensory binding of features into a coherent percept and also for memory (for review see Bartos et al., 2007). In humans, alteration of GABA inhibitory transmission mediated by PV-ir interneuron networks has been shown to be involved in several disorders of the brain (Woo and Lu, 2006; Lewis et al., 2005). Parallel studies were performed in animal models: In the LPA1-deficient mice model of schizophrenia, a significant decrease in PV and GABA immunoreactivity was present in the entorhinal cortex, associated with a dramatic decrease in the power of gamma oscillations at the same location (Cunningham et al, 2006). In the rat methylazoxymethanol model of schizophrenia, the number of PV-ir neurons was significantly decreased in the prefrontal cortex but not in the hippocampus (Penschuck et al., 2006). In both animal studies, no changes in the number of CB-ir or CR-ir cells were found.

Following this logic, the observed reduction in PV and GABA immunoreactivity in the hippocampus (Shetty and Turner, 1998; Shi et al. 2004) could contribute to the cognitive deficits observed in aged Fischer 344 animals. The cognitive deficits were found mainly in spatial learning but also in fear conditioning and in odor discrimination tasks in this strain (Oler and Markus, 1998; Harker and Whishaw, 2002; Villarreal et al., 2004; LaSarge et al., 2007). In many tasks, a tone stimulus was used for fear conditioning (Oler and Markus, 1998; Villarreal et al., 2004). Since old Fischer 344 rats are hearing-impaired with large hearing losses (Popelar et al., 2003, 2006; Buckiova 2007), the results of those experiments may also have been influenced by the limited hearing abilities of the aged animals. The relationship

between a decline in hippocampal PV-ir neuronal networks and cognitive deficits in the same strain may suggest that the large decline in the number of PV-ir neurons in the auditory cortex in Fischer 344 rats could contribute to the deterioration of hearing function in these animals. In addition, findings of comparable deficits in the visual and retrosplenial cortices might suggest the contribution of PV-ir neuronal loss in the cortex to the general deterioration accompanying aging in F344 rats.

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Legends to figures:**Fig. 1 (A,B,C)**

Topographical overview of the IC, MGB, AC and their subdivisions. Drawings adapted from Paxinos and Watson (1998) and Zilles (1985); (A) – bregma -8.8 mm, (B) – bregma -5.0 mm, (C) – bregma -4.8 mm. Par – Parietal cortex; Hip – Hippocampus; PRh – Perirhinal cortex; V1,2 – Visual cortex 1,2; RSD,RSV – Retrosplenial cortex, dorsal and ventral; MGB-D,V,M – dorsal, ventral and medial division of the MGB.

Fig. 2 (A-F)

Comparison of PV immunoreactivity between young (A,C,E) and old (B,D,F) Fischer 344 rats in the central nucleus of the IC (A,B) and in the primary auditory cortex – Te 1: C,D – all layers, E,F – layers II-IV only. D and F show the reduced occurrence of PV-ir neurons in the auditory cortex of old F344 rats.

Fig. 3

Schematic lateral view of the rat brain (Fischer 344) with the indicated fields devoid of PV-ir cells within the AC in two old animals (the fields passed through all cortical layers). Dotted line - rat FG1; dashed line - rat FR2. Par – parietal cortex.

Fig. 4

Results of the quantitative evaluation of PV immunoreactivity in Long Evans (A,C,E) and Fischer 344 rats (B,D,F). Light grey columns are for young animals, dark grey columns for old animals.

A, B) Number of PV-immunoreactive neurons in the CIC, MGB, and auditory cortex (Te1 + 3) of young and old rats. C, D) Optical density of the somas of PV-immunoreactive neurons in the CIC, MGB, and auditory cortex (Te1 + 3) of young and old rats. E, F) Mean volumes of the somas of PV-immunoreactive neurons in the CIC, MGB, and auditory cortex (Te1 + 3) of young and old rats.

Fig.1A

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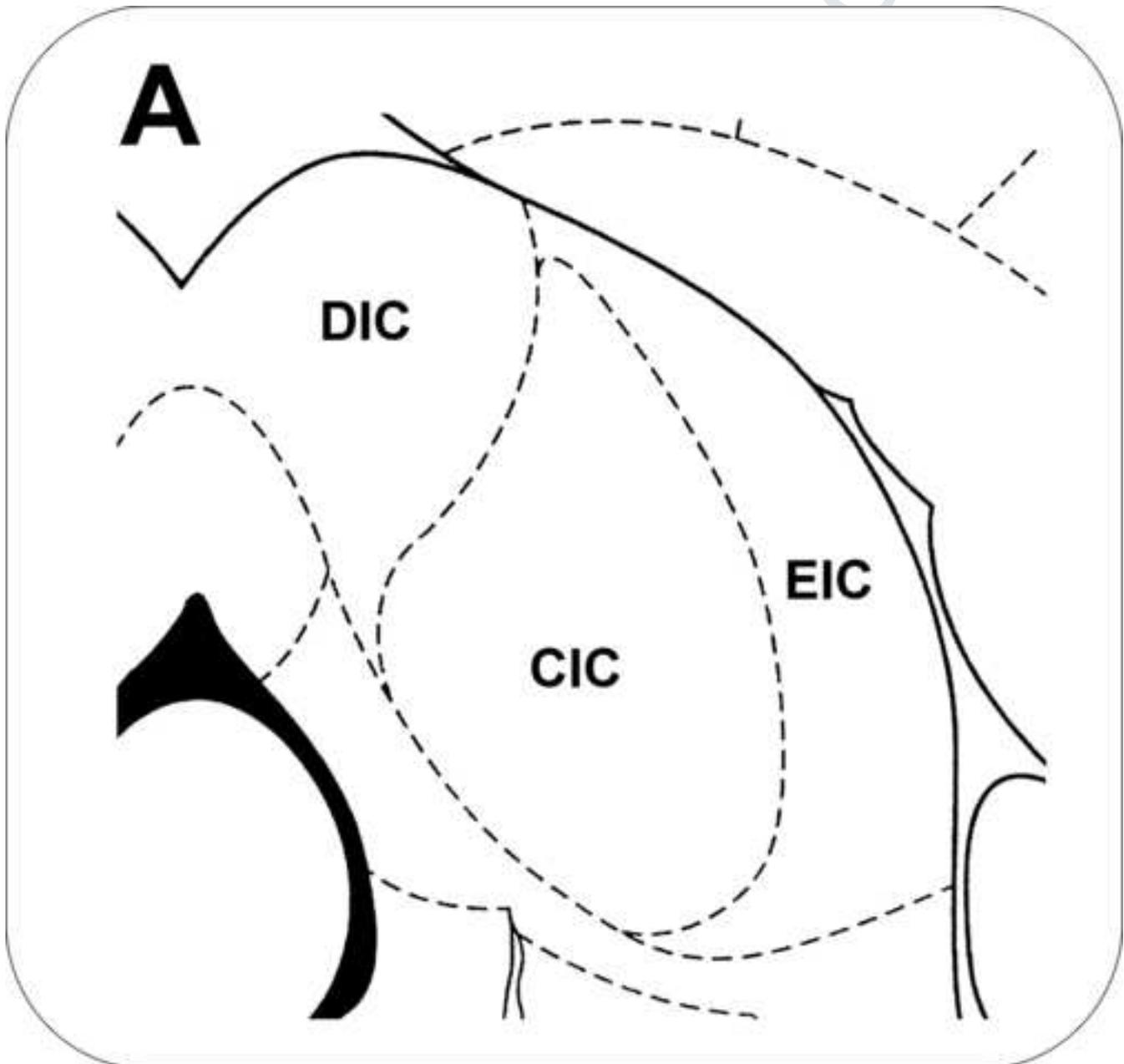


Fig.1B

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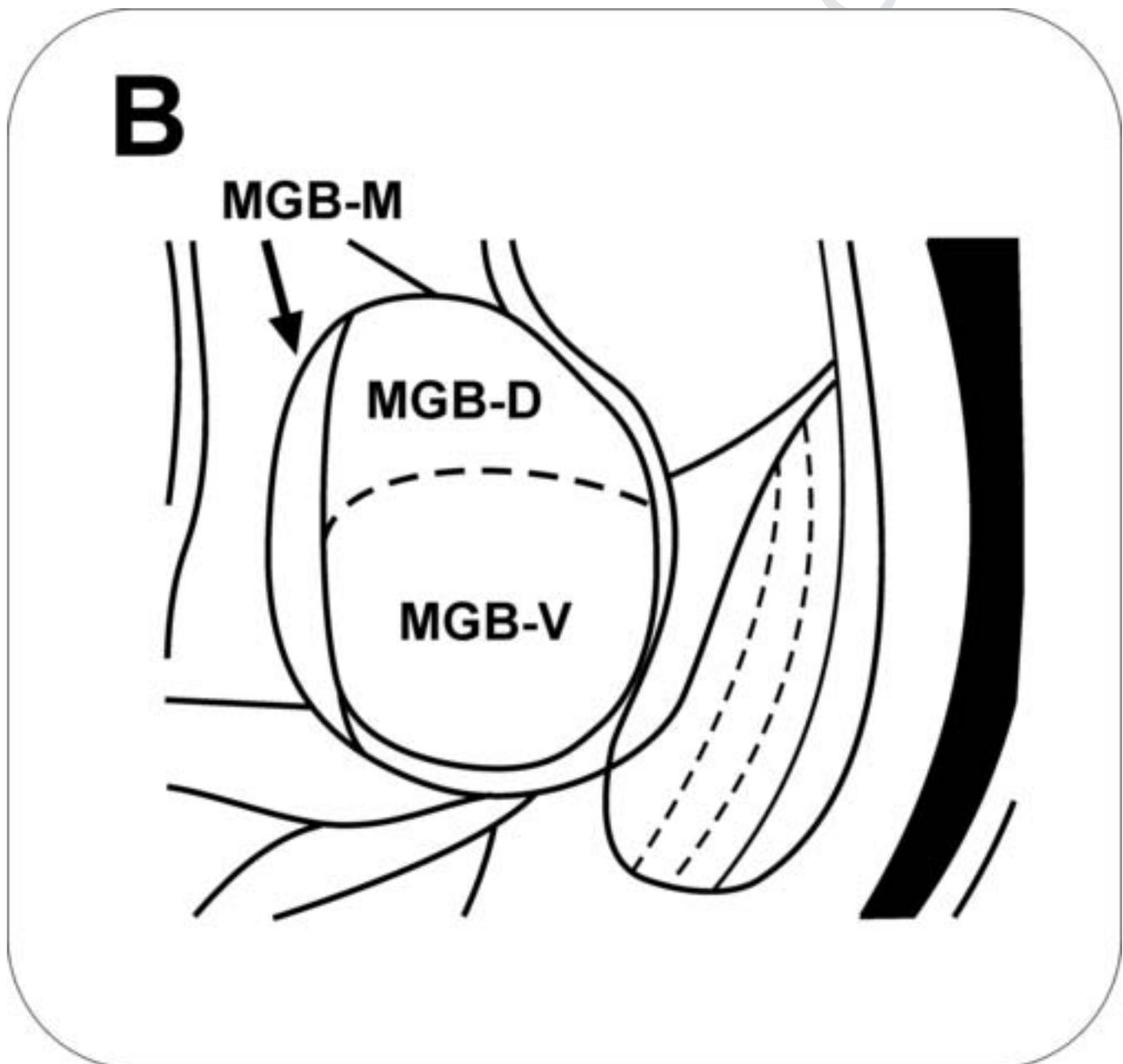


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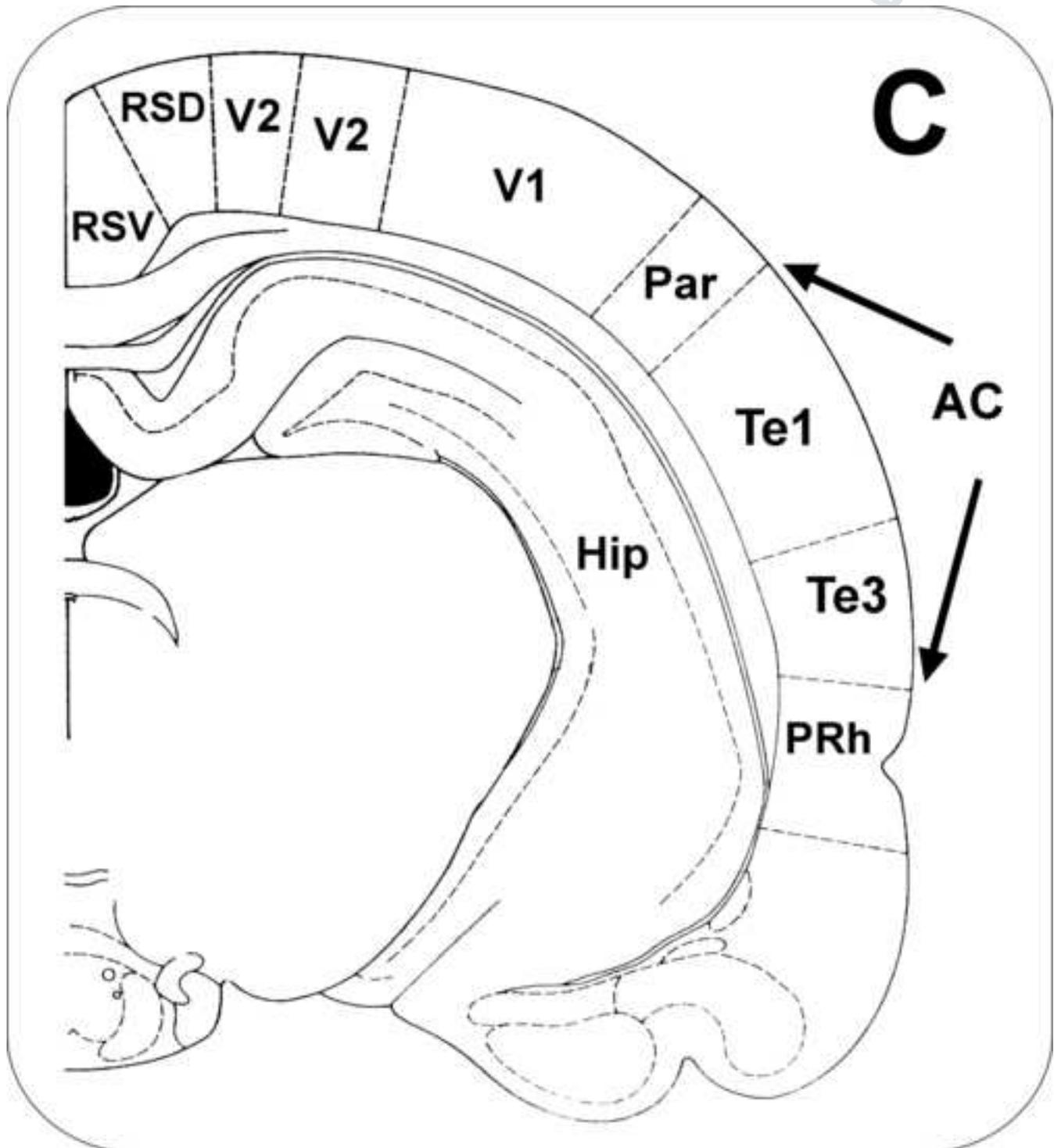


Fig.2A

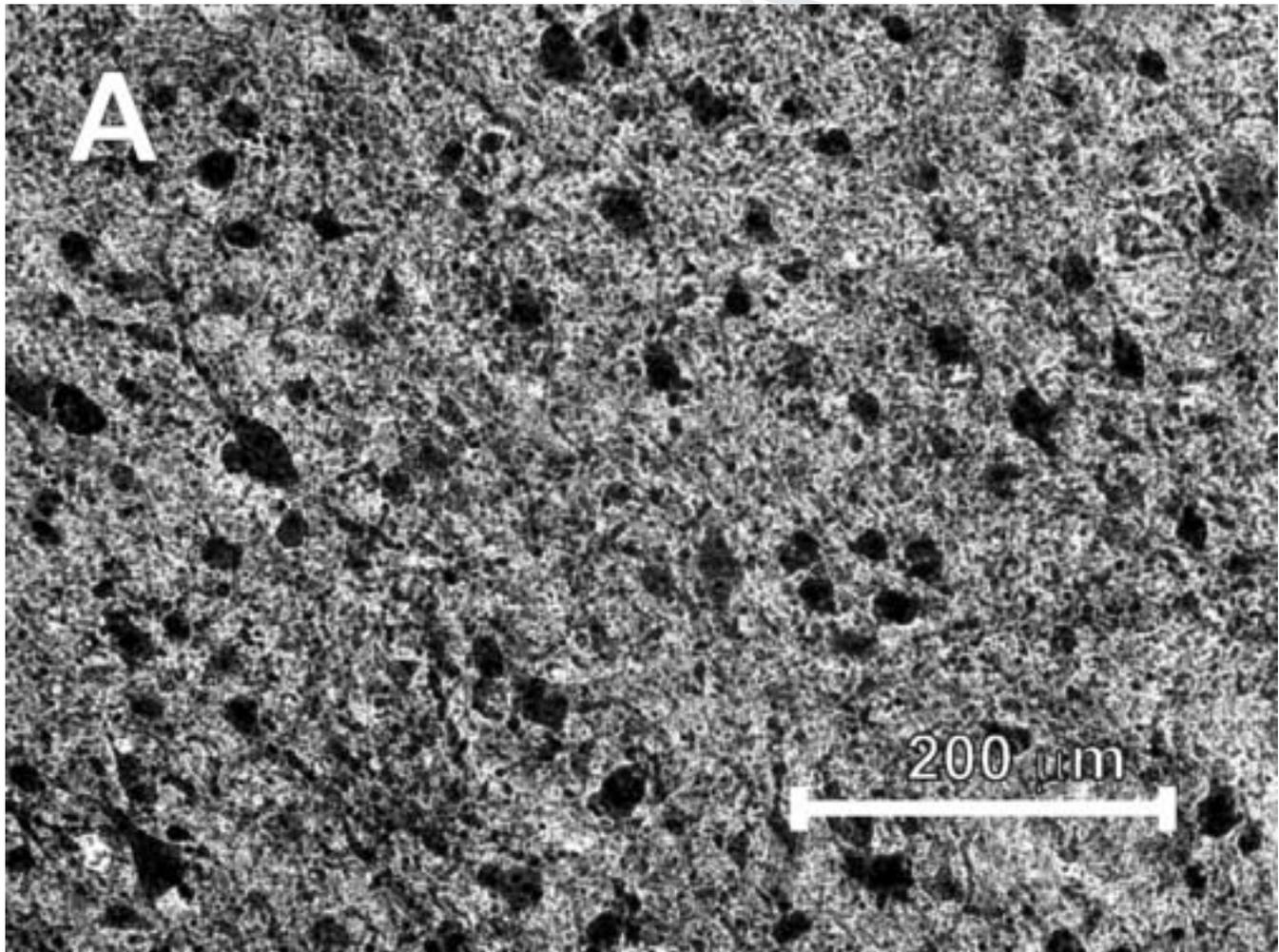


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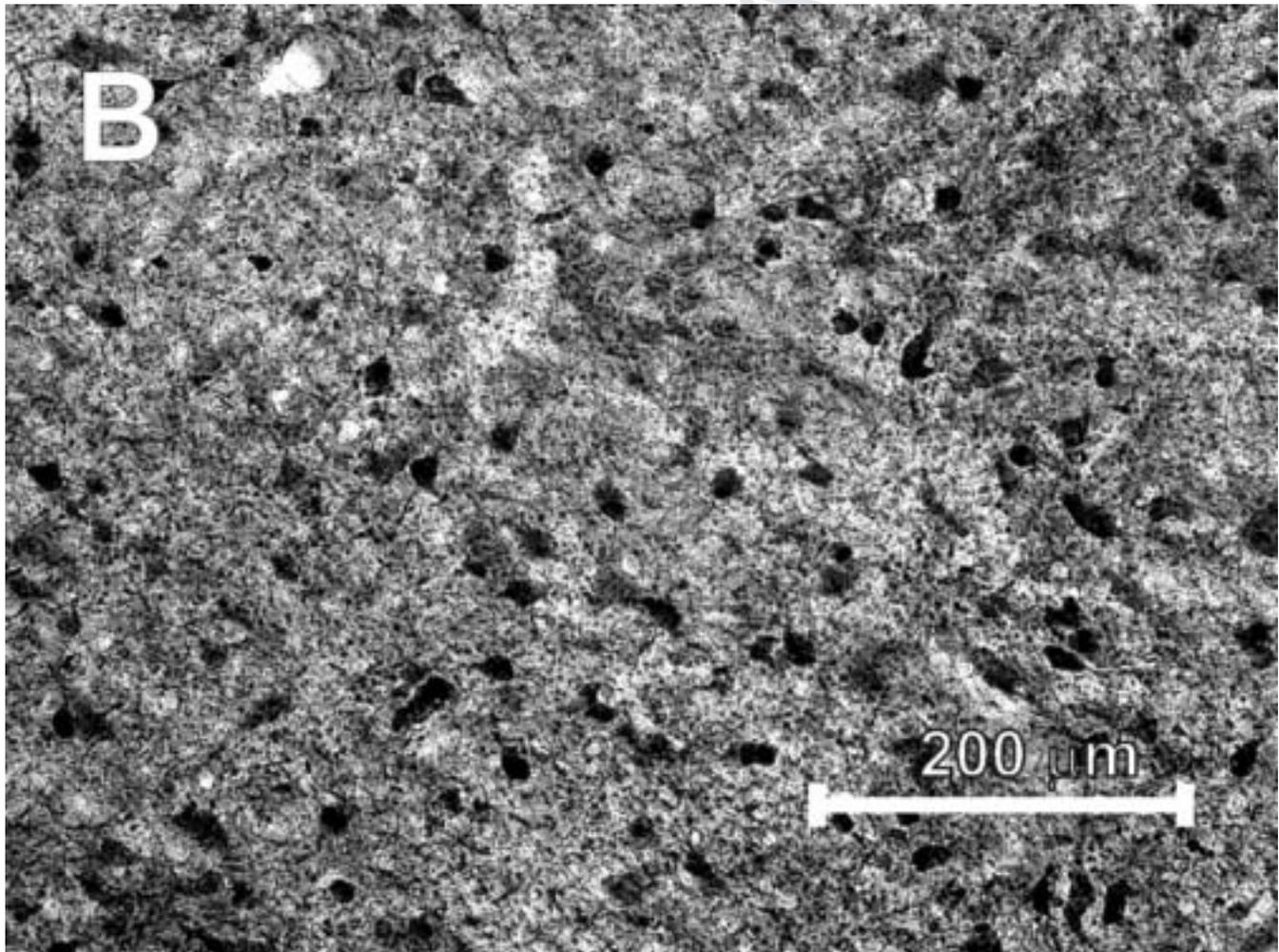


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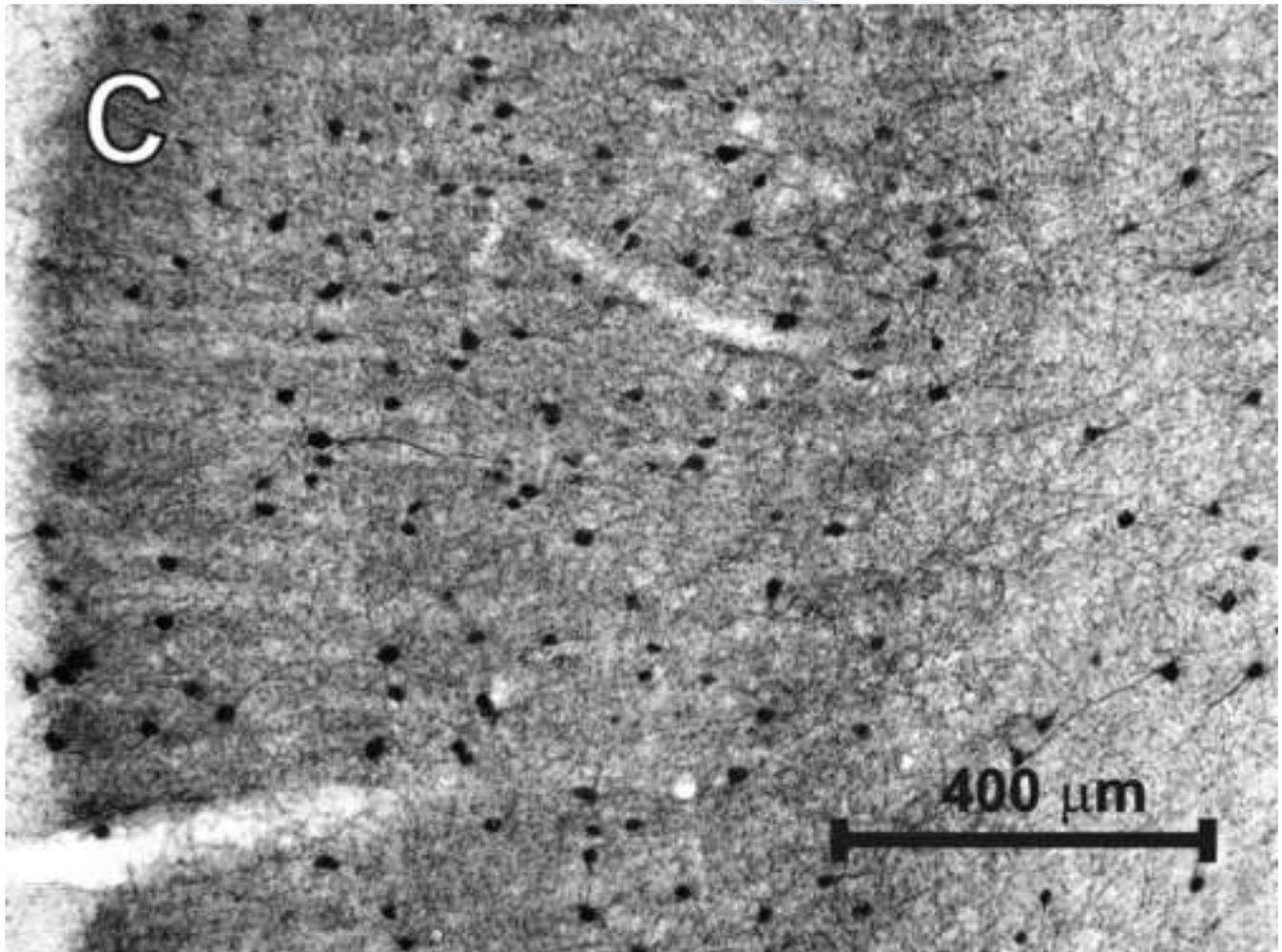


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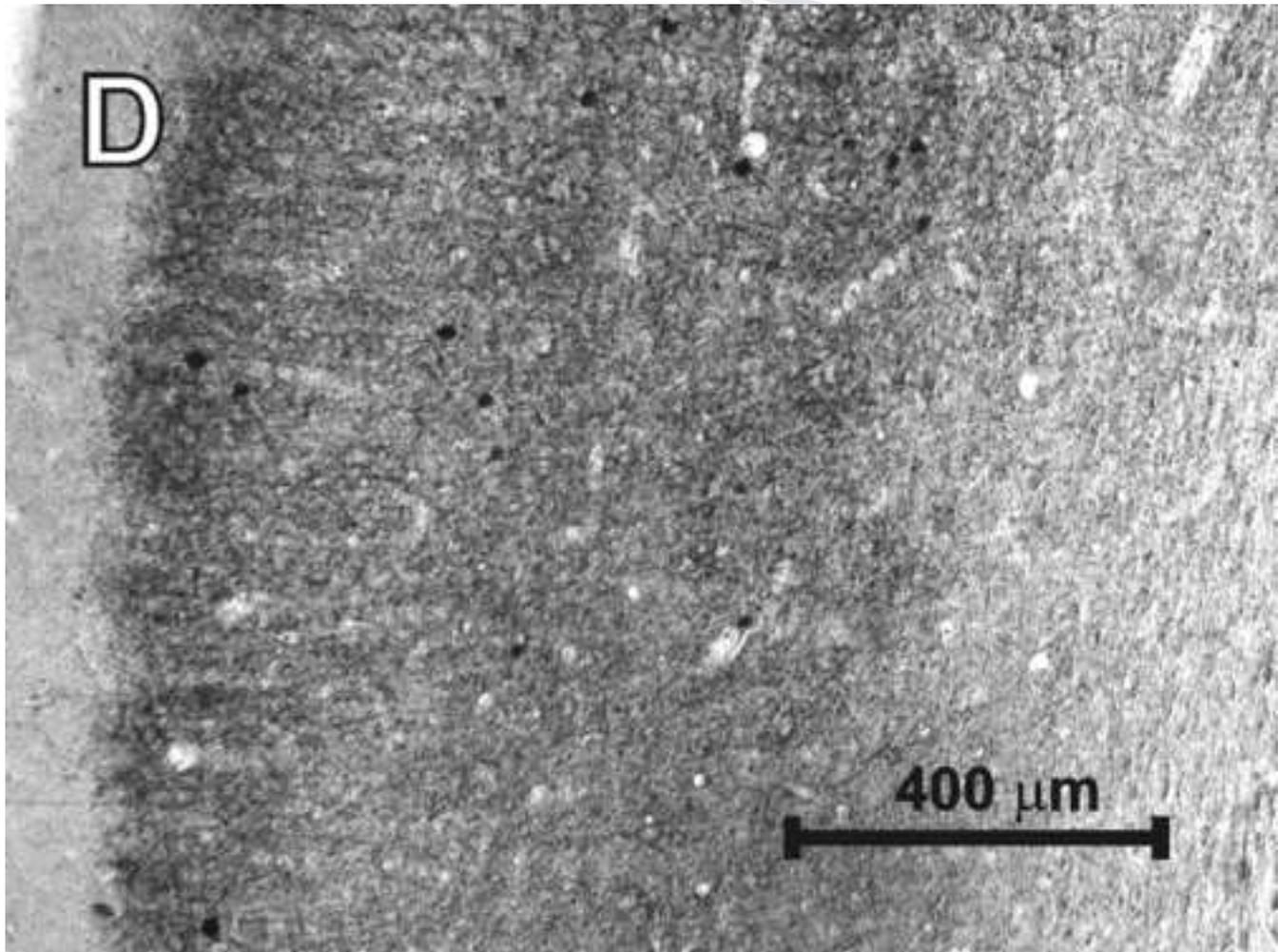


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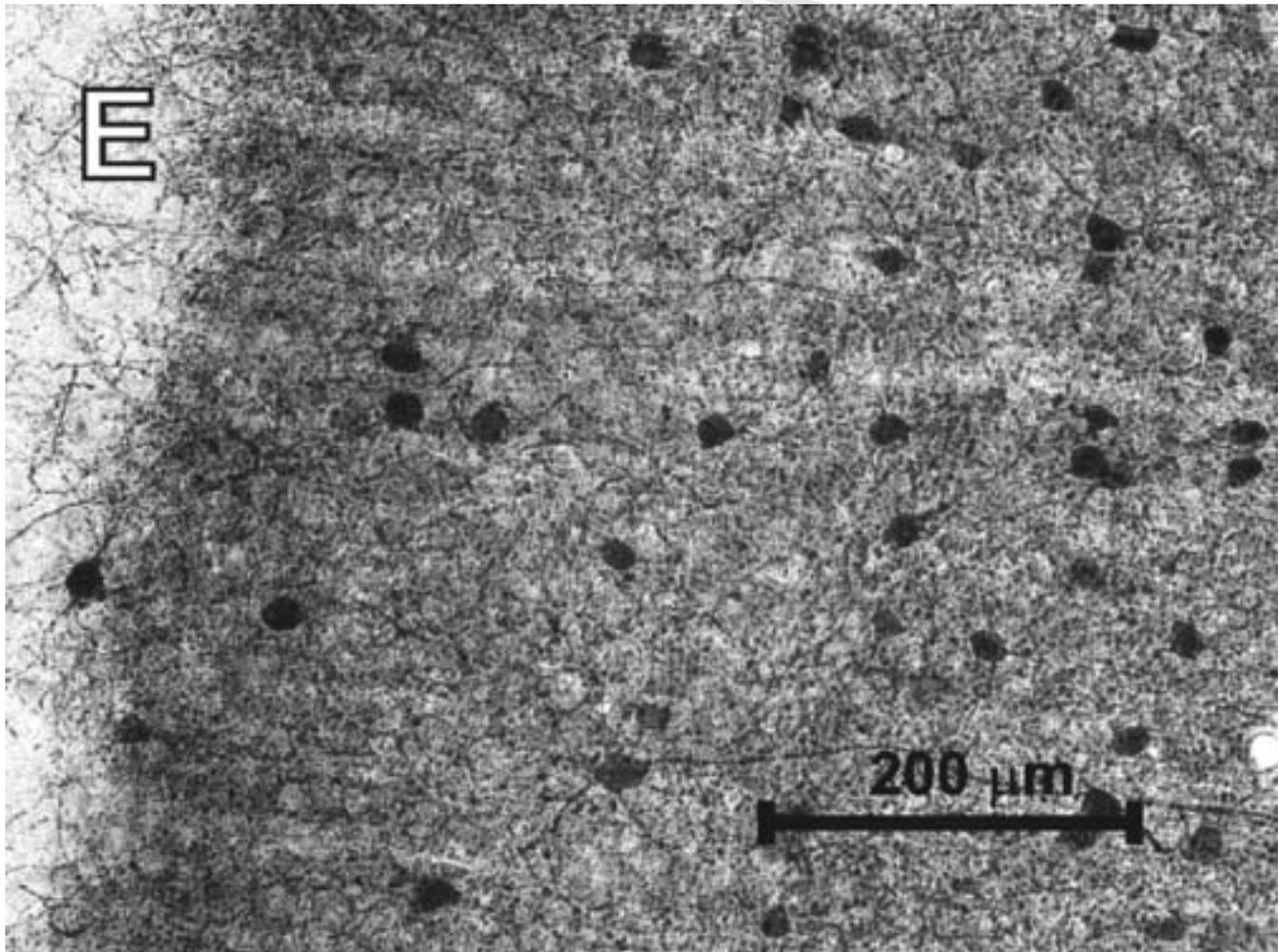


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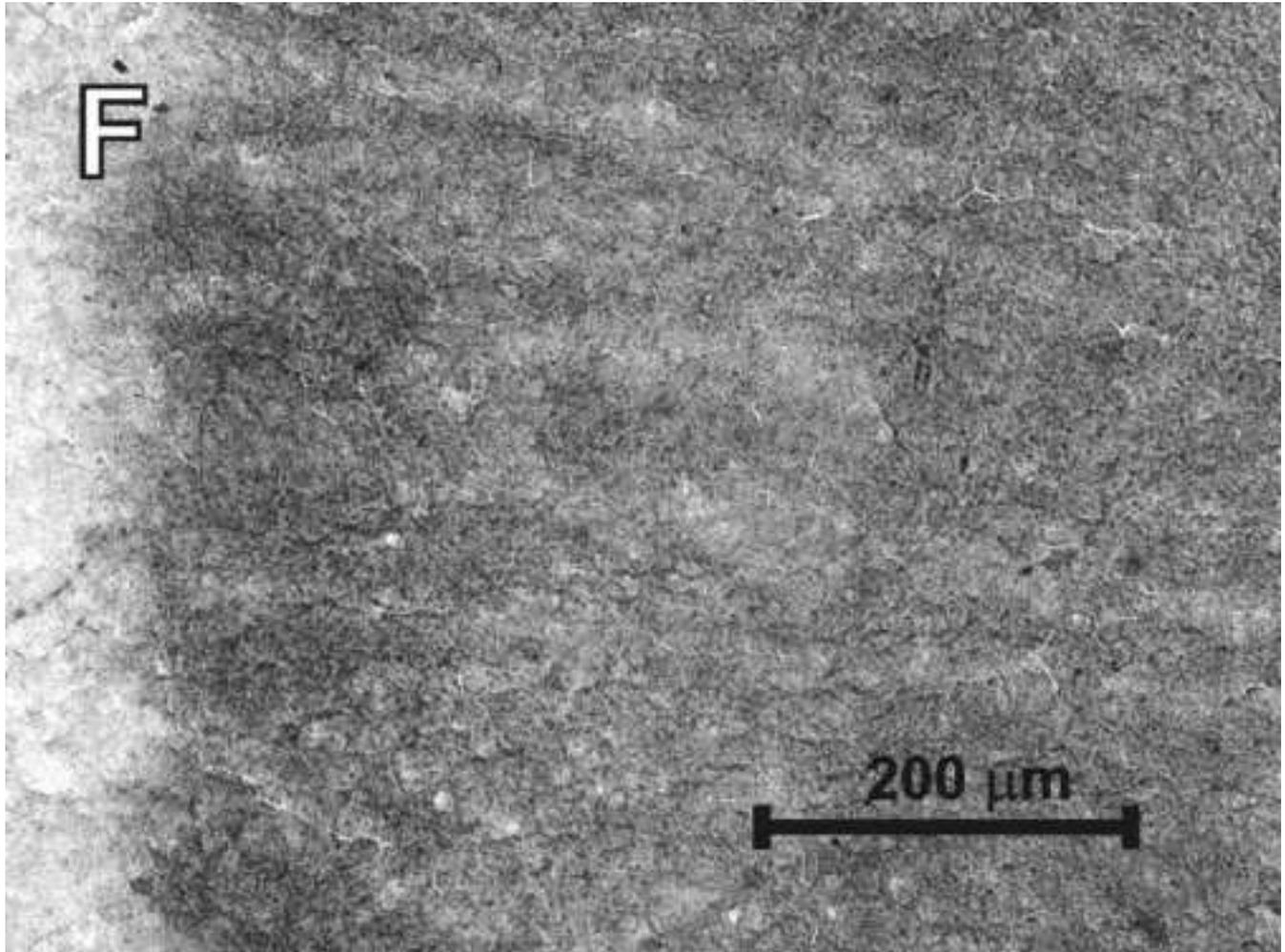


Fig.3

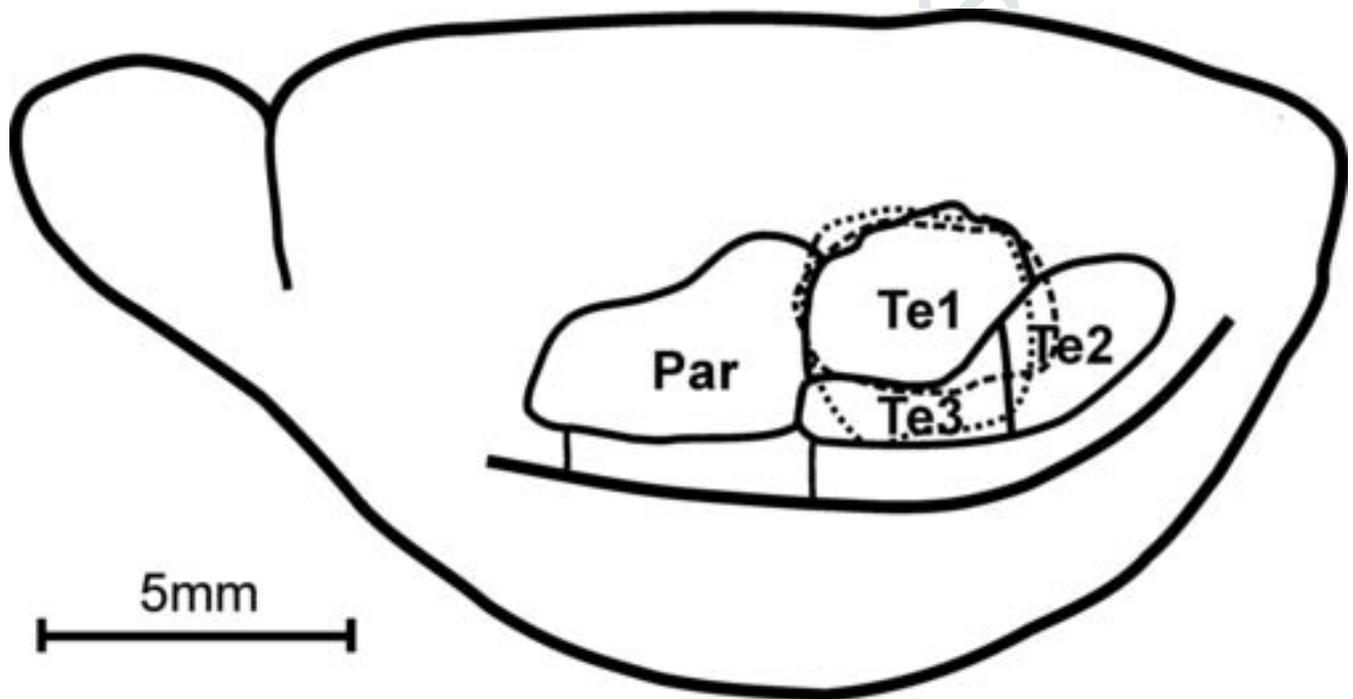


Fig.4A

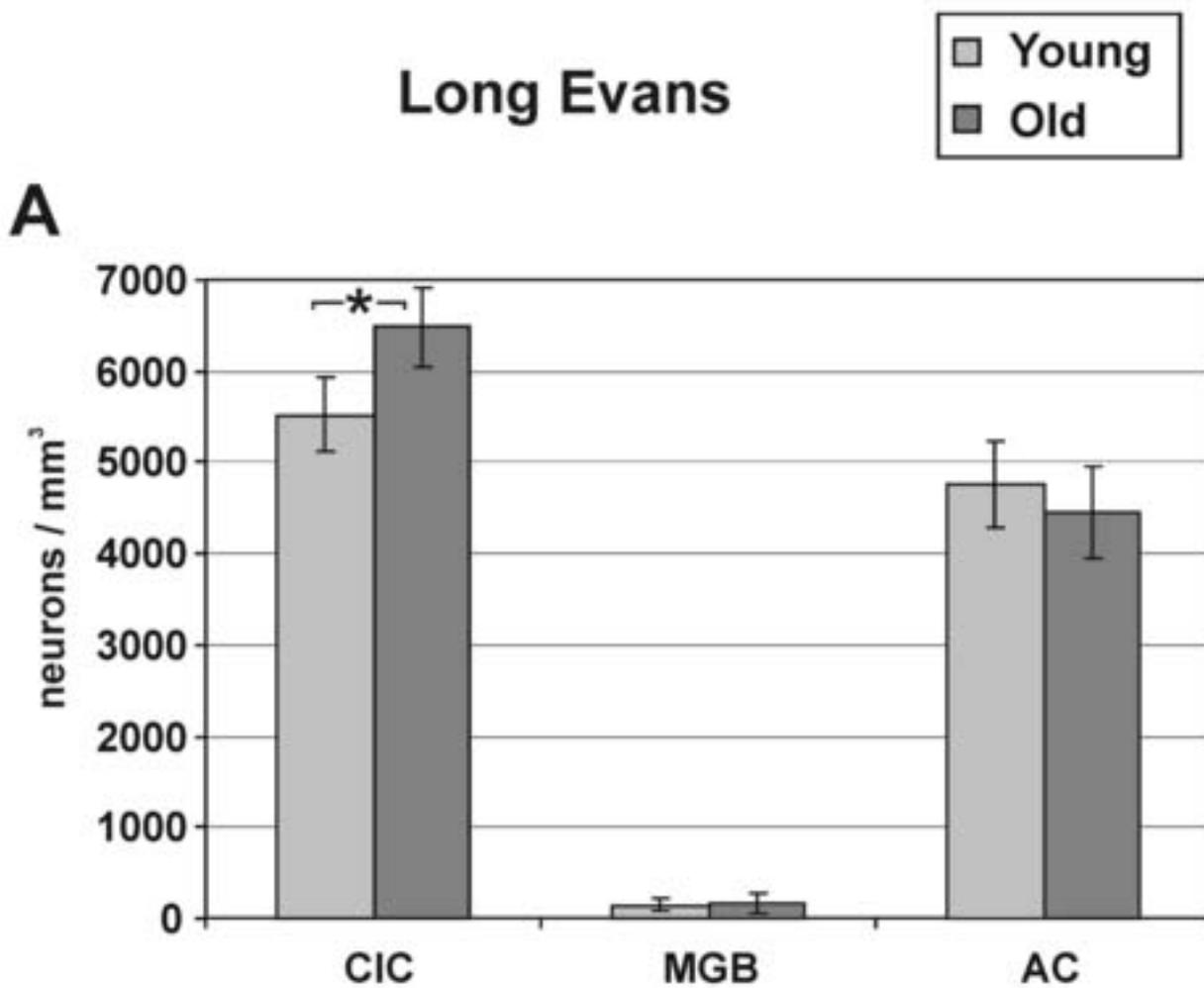


Fig.4B

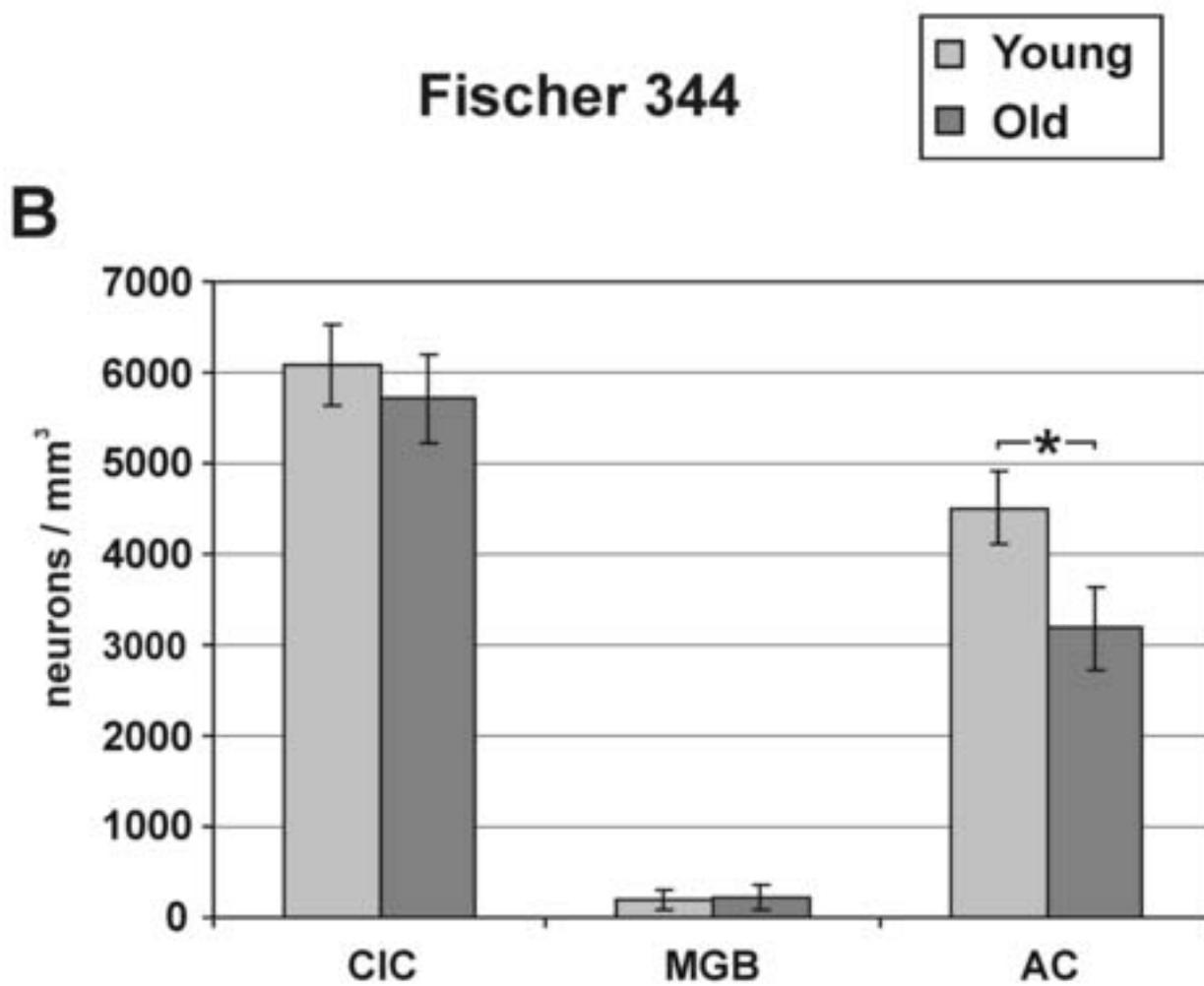


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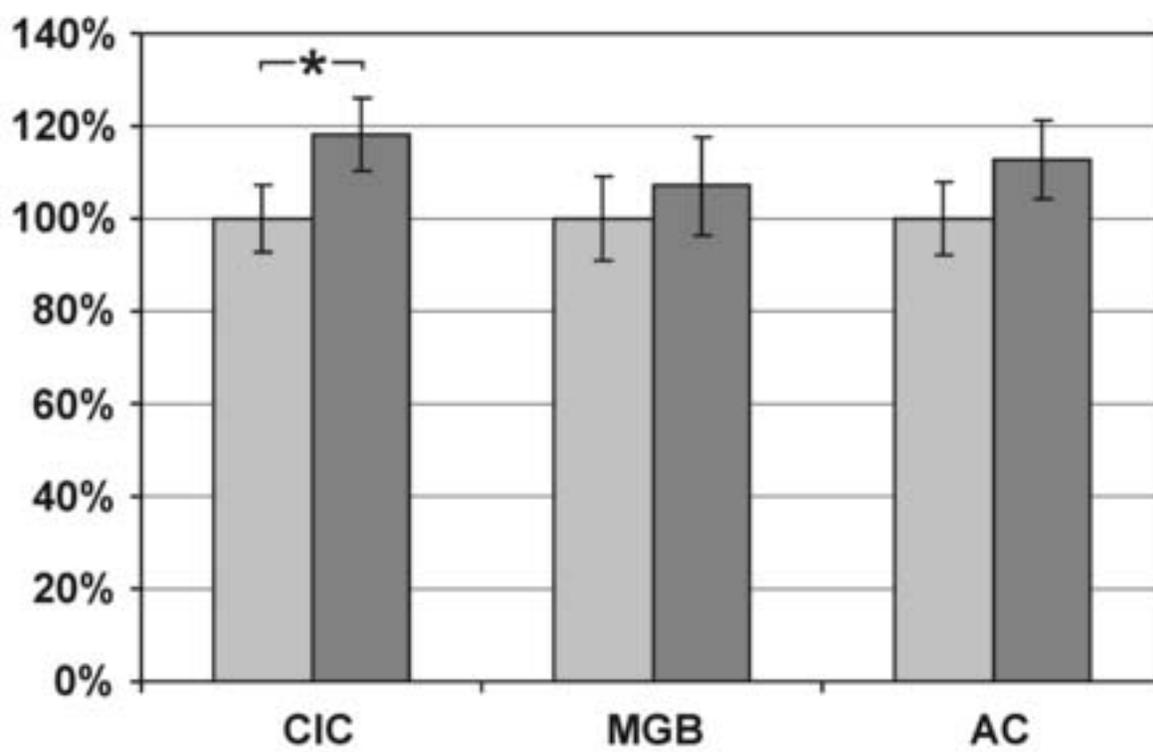
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Fig.4D

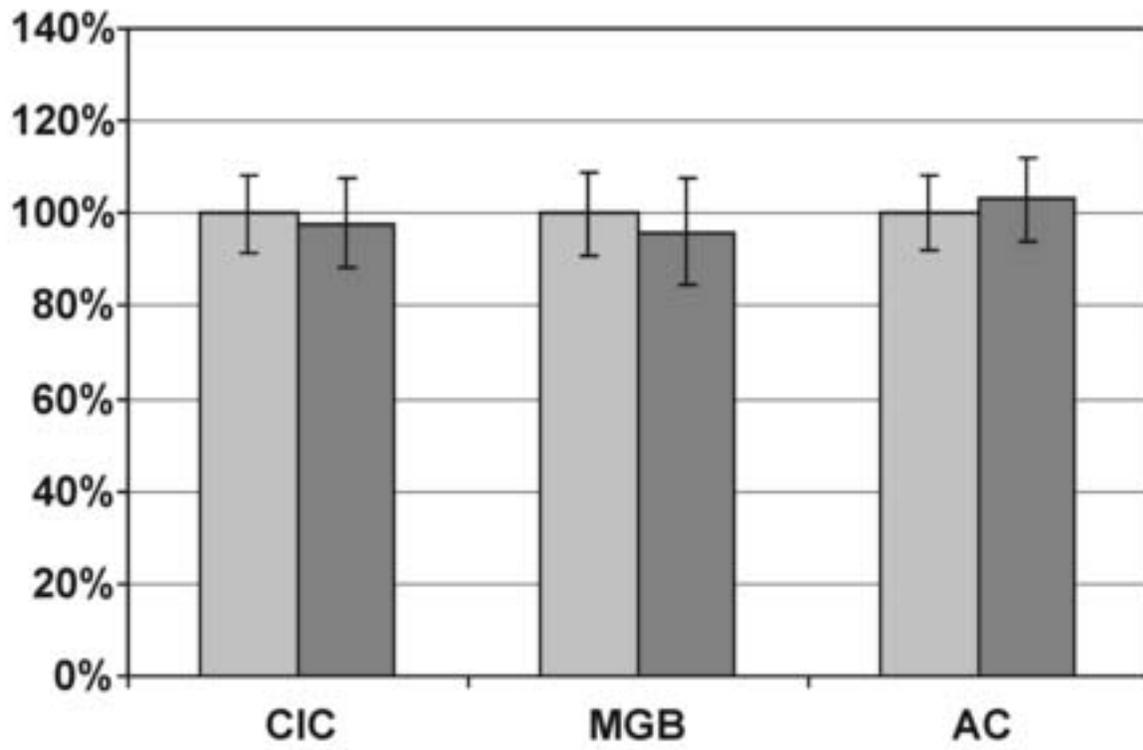
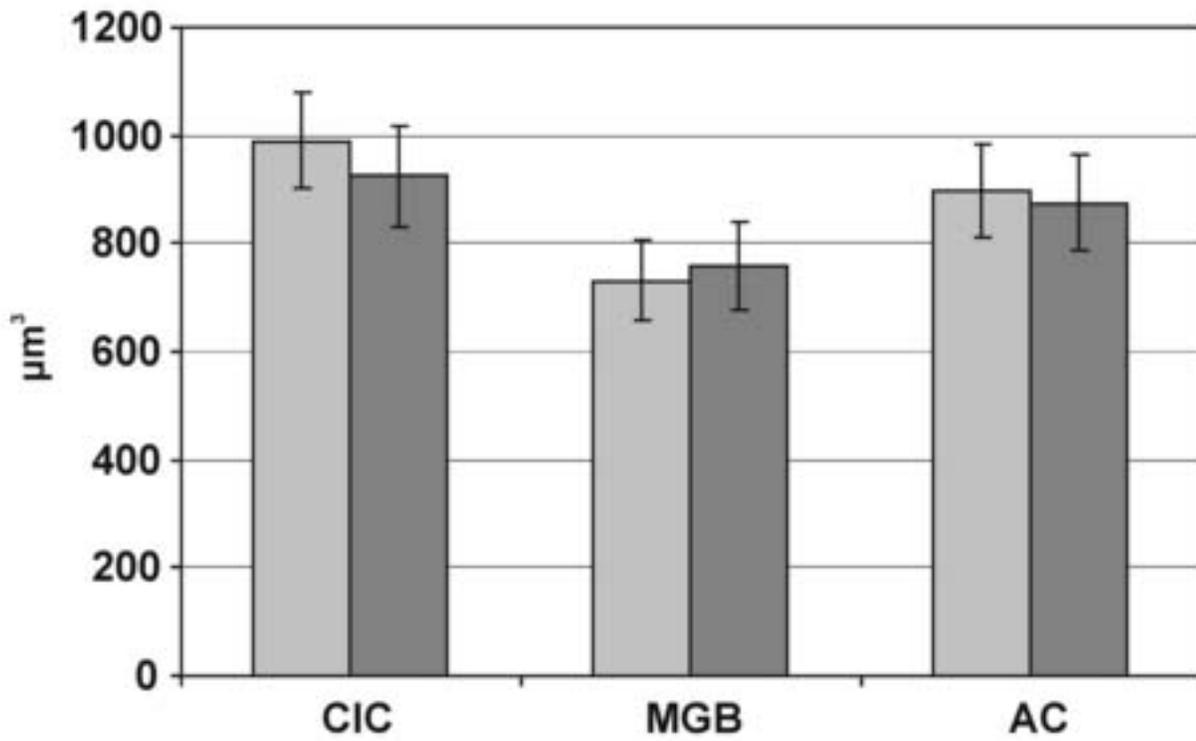
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Fig.4E

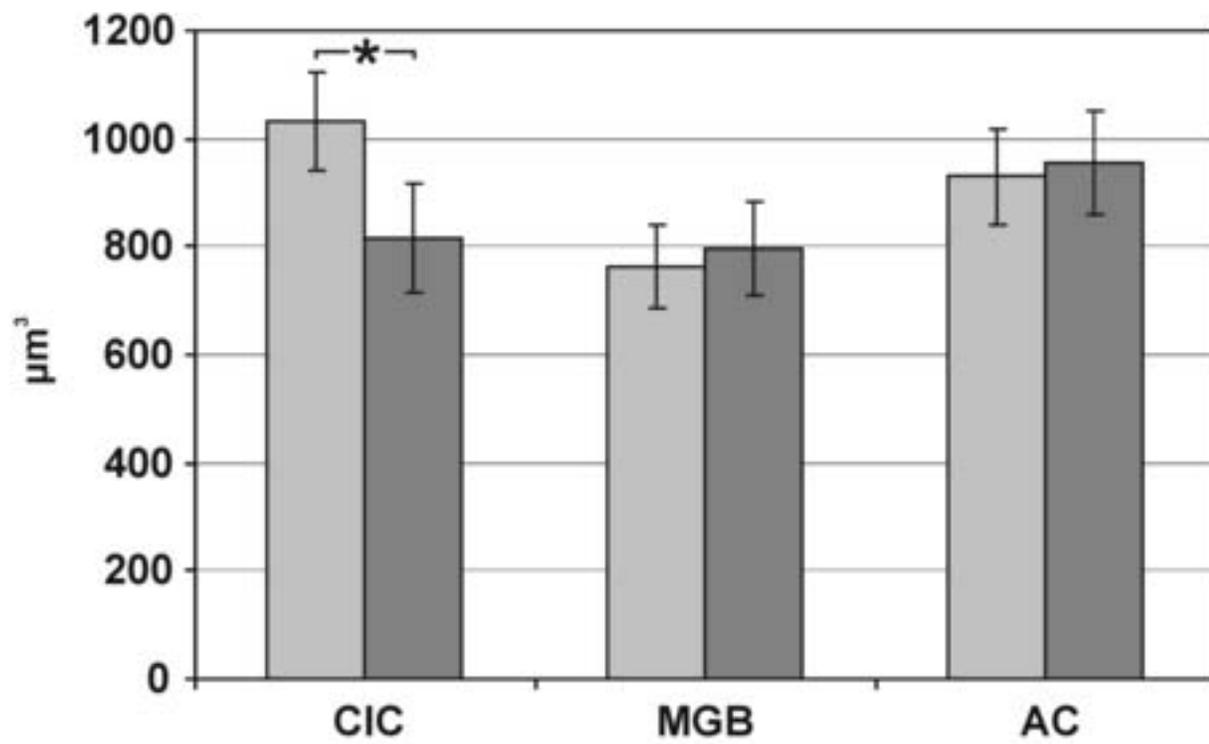
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A

Fig.4F

SCRIPT

F



AC