QUANTITATIVE STUDY OF PERINEURONAL NETS IN THE RETROSPLENIAL CORTEX OF RATS

BY

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SUMMARY

Reticular or net-like perineuronal coatings, enriched with proteoglycans (PGs) and/or glycoproteins (GPs), were demonstrated to ensheath cell surfaces of certain neuronal circuits in the retrosplenic cortex of adult rats. These coated cells, comprising about 26% of total neurons, were expressed either with iron colloid and/or certain plant lectins from Vicia villosa agglutinin 'VVA', Wisteria floribunda agglutinin 'WFA' or Glycine max agglutinin 'SBA'. The mean number of coated retrosplenial neurons per unit area, as labeled by VVA, WFA, SBA, iron colloid or iron colloid/Bodian staining was 6.45 ± 0.87, 5.70 ± 0.73, 3.91 ± 0.53, 4.01 ± 0.42 and 4.43 ± 0.37, respectively. In addition, labeling indices expressed by the aforementioned stains were 16.97%, 15.00%, 10.28%, 10.55% and 11.65%. The labeled neurons underwent a non-significant increase with progression of animal age during the first postnatal year; however, they declined thereafter toward senility. Qualitatively, there were three types of neuronal circuits at the retrosplenial cortex: PGs-, GPs- and PGs/GPs-coated neurons. These coatings comprised 9%, 64% and 27%, respectively. This data indicated that in the retrosplenial cortex of rats, the GPs-coated neurons represented the most prevalent subset among the net-associated neurons that together, might be involved in processing of navigation or episodic memory.

INTRODUCTION

The previous light or electron microscopic studies have demonstrated that many neurons in the central nervous system of human, rats, mice and other animals, including such lower vertebrates as reptiles and fish, are surrounded by a reticular extracellular matrix of PGs and/or GPs.

These pericellular matrices exhibit a characteristic lattice-like structure, called perineuronal nets (PNs) or coats (Murakami et al., 1993, 1994; Celio & Blümcke, 1994). Nerve terminals are thought to reach cell surface of neurons through the holes of PNs (Matsui et al., 1999). Qualitatively, the perineuronal coats

are categorized into three major subdivisions: first, coats exclusively formed of sulfated glycoconjugates (Proteoglycans); second, coats formed of unsulfated glycoconjugates (Glycoproteins) with terminal *N*-acetyl-galactosamine; and third, coat complexes which are formed of sulfated PGs networks intermingled with GPs molecules (Sayed et al., 2002).

The present investigation supplements our previous study and obtains some quantitative data on the pericoated retrosplenial neurons of rats. The granular and non granular subfields of the retrosplenial cortex were used for this study which, provided with various neuronal subsets enriched with perineuronal PGs and/or GPs (Murakami et al., 1996; Sayed, et al., 2002). In human, the retrosplenial cortex is suggested to be involved in navigation and processing of episodic memory (Maguire, 2001); as well as acquisition or extension of the trace conditioned reflex (Weible et al., 2000).

The obtained data would be contributed as baseline for future experimental studies searching for the biological significance of pericoated neurons at this cortical region.

MATERIALS AND METHODS

Animals and tissue processing.

The material for this investigation originated from 58 Wistar rats aging 2 (n= 8), 3 (n= 8), 4 (n= 8), 6 (n= 8), 8 (n= 8), 12 (n= 4) and 18 months (n= 4). The animals were perfused transcardially under ether anesthesia with saline. Thereafter, they were fixed by 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3).

The brains were isolated and 2-3 mm-thick tissue slices which, traversing the granular and agranular subfields of retrosplenial cortex were cut in sagittal or coronal planes and immersed in the aforementioned fixative. After proper fixation, specimens were dehydrated, cleared and embedded in paraffin. Step serial sagittal or coronal sections (5-7 µm in thickness) were prepared and deparaffinized by xylene.

For expression of PNs with sulfated glycoconjugates, the brain sections were treated with a cationic iron colloid (CIC) at pH 1.5 (Murakami et al., 1986) or with CIC/Bodian combination (Murakami et al., 1997). Sections for control reaction were not treated with iron colloid. For PNs with unsulfated glycoconjugates, tissue sections were labeled with lectin *VVA* (Kosaka & Heizmann, 1989), lectin *WFA* (Härtig et al., 1992) or lectin *SBA* (Lüth et al., 1992) and the reaction was demonstrated with diaminobenzidine (Nakagawa et al., 1986). Controls for lectin-stained sections were consisted of adjacent sections treated with phosphate buffer containing no agglutinin.

To allow differentiation of PNs exhibiting unsulfated glycoconjugates (GPs) and/or sulfated glycoconjugates (PGs), the brain sections were labeled with WFA, VVA or SBA and then stained with a cationic iron colloid (Sayed et al., 2002).

Histomorphometry and stereology. The stained sections were quantitatively analyzed under a light microscope using an eye-piece graticule (40X magnification) (Weibel, 1979) and computer adjusted image analysis system.

All stained sections were used for measuring the following parameters.

1-Number of net-associated (coated) neurons, non-coated neurons and the total neuronal density per unit area (UA, 60.15 μm^2). Mean values (absolute densities) were used to calculate the relative (percentage) densities of variables.

2.-Absolute and relative densities of PGs-coated neurons, GPs-coated neurons and neurons exhibiting coat complexes (estimated from VVA/CIC, WFA/CIC and SBA/CIC stained specimens).

3.-Labeling indices of the VVA,WFA, SBA ,CIC or CIC/Bodian staining. The indices were calculated from the number of labeled neurons, divided by the total number of neurons scored, and multiplied by 100. In addition, the intensity and distribution of lectin-bound neurons or CIC and CIC/Bodian-stained nerve cells were documented. The results were obtained for an average of about 55-70 randomly selected fields occupied by the retrosplenial nerve cells. Although tissue shrinkage is known to occur following similar tissue processing techniques, no specific estimation of this shrinkage was made in the present study, and all obtained data were presented in relative units.

Statistical Analysis.

All data obtained from the histomorphometric or stereological measurements were expressed as the means ± SD. Correlation between age groups was assessed by using statistical analysis system (Sas, 1989).

RESULTS

General Findings.

Reticular or net-like coatings of the extracellular matrix were demonstrated to surround somata, proximal dendrites and the axon initial segments of certain neurons at the retrosplenial cortex of adult rats (Fig. 1A,B). The coated neurons at this cortex formed 24-26% of the total neuronal population (Tables 1,3 and Fig. 2).

Qualitatively, there were three types of perineuronal coats differentially labeled with the dual iron colloid/lectin staining: PGs-enriched coats (solely stained with CIC), GPs-enriched coats (solely labeled with lectins) and PGs/GPs-enriched coat complexes (stained with CIC and lectin). These coatings respectively comprised 9%, 64% and 27% (Table 3 and Fig. 3).

Notably, number of PGs/GPs coat complexes was varied with respect to the lectin used. It was the highest in brain sections stained with VVA/CIC or WFA/CIC than SBA/CIC. Number as well as labeling intensity of GPs-coated neurons differed considerably with respect to the lectin used. These parameters were the highest in brain sections treated with lectin VVA or WFA than SBA.

Furthermore, staining intensity of the neuropil also differed and it was much denser by lectin WFA than VVA or SBA. Apart from lectin staining, the dual iron colloid/Bodian staining of PGs coatings presented a relatively higher labeling intensity than the solely iron colloid staining.

Control Experiments.
In all treatments, control incubations resulted in a significant failure of the labeling.

Histomorphometry and stereology.

Histochemical parameters or characteristics of the rat retrosplenial cortex were presented in Tables 1-3 and Figures 2-7. The mean number of labeled neurons (coated neurons) in two months-old rats was 8.45 ± 2.62 per UA. It underwent a non significant increment with age ($r = 0.35$; $P < 0.44$), reaching 10.90 ± 2.62 on 12-months old rats (Tables 1,2 and Fig. 4).

However, they declined thereafter toward senility, reaching 9.21 ± 2.69 in 18-months old rats. Notably, mean number of total neuronal cells per UA was 38.10 ± 2.60 (Table 3). In 2-months old rats the mean value was 42.43 ± 5.45 per UA, underwent a significant decrease with progressing of postnatal age ($r = - 0.97$ and $P < 0.003$), reaching 37.34 ± 5.89 at 18 month of postnatal age (Tables 1,2 and Fig. 3). The absolute density of coated neurons and non-coated neurons per UA was 9.87 ± 0.43 and 28.13 ± 1.80, respectively (Table 3).

Apart from the pericoated neurons, the absolute density of non-coated nerve cells was 33.95 ± 4.16 per UA in 2 months old rats. It showed a significant decrease with postnatal age ($r = - 0.79$; $P < 0.03$), reaching 28.10 ± 3.79 per UA at the age of 18 months (Tables 1,2 and Fig. 4). In consistency, the relative density of coated neurons was 19.92% in two months-old rats, underwent a gradual increase with postnatal age, reaching 28.53% at 12 months (Table 1).

Whereas, the non-coated neurons comprised 80.08% of the total neurons in two months-old rats, showed a gradual decrease with progression of age, reaching 71.47% at the age of 12 months.

The absolute density of GPs-coated neurons, PGs-coated neurons and neuronal cells exhibiting PGs/GPs coat complexes per UA was 6.33 ± 0.34, 0.84 ± 0.07 and 2.70 ± 0.22, respectively (Table 3 and Fig.5). These variables respectively comprising, 16.65%, 2.21% and 7.11% of the total neuronal population (Table 3 and Fig. 6). In relative terms, the GPs-coated neurons comprised 64% of total coated neurons, whereas, the PGs-coated neurons and those exhibiting coat complexes comprised 9% and 27% (Table 3 and Fig. 3).

This data indicated that in the retrosplenial cortex of rats the GPs-coated neurons were the most prevalent circuit among the net-associated neurons.

The mean number of labeled neurons per UA as documented by VVA, WFA, SBA, CIC or CIC/Bodian staining was 6.45 ± 0.87, 5.70 ± 0.73, 3.91 ± 0.53, 4.01 ± 0.37and 4.43 ± 0.42, respectively. In consistency, labeling indices of coated

neurons revealed by the aforementioned stains were 16.97%, 15.00%, 10.28%, 10.55% and 11.65% (Fig. 7). The differences between indices were not statistically significant.

This data indicated that lectin VVA-staining of unsulfated glycoconjugates exhibited the highest labeling index than that presented by lectin WFA or SBA. Apart from the lectin staining, the combined iron colloid/Bodian staining of sulfated glycoconjugates presented a relatively higher labeling index than that expressed by the solely iron colloid staining, and the differences were not statistically significant.

DISCUSSION

In this investigation reticular or net-like coatings were demonstrated to surround 20-28% of retrosplenial neurons in adult rats. These coatings as was described by Sayed et al., (2002) were enriched with PGs and/or GPs and distributed throughout the cortical layers II-V. The lattice-like or fenestrated structure of perineuronal coats has been described at the surfaces of certain neuronal subsets in the literatures (Nakagawa et al., 1986; Kosaka & Heizmann, 1989; Naegele & Katz, 1990; Härtig et al., 1992; Brückner et al., 1993; Brückner et al., 1996; Köppe et al., 1997a,b).

Although the function of perineuronal PGs and/or GPs are not clear, their distribution around a selected number of neurons implies that they may participate in specific activities such as fast spiking signal transduction or generation of polyanionic microenvironment.

The present histochemical or cytochemical investigation confirmed that the epitopes visualized in PNs consisted of sulfated glycoconjugates and/or unsulfated glycoconjugates with terminal *N*-acetylgalactosamine. The coated neurons presented a non significant increase with progression of age, reaching their maximal number in one-year old rats. However, with aging they underwent a gradual decrease in number. This data demonstrates the postnatal changes of PNs in the retrosplenial cortex of rats.

The basic components of perineuronal nets have been demonstrated to be somewhat variable (Köppe et al., 1997b). In addition, clear differences exist between PNs with respect to the quantity of PGs and/or GPs revealed at the microenvironment of the corresponding neurons (Sayed et al., 2002). The differential concentration of these epitopes appears to be a significant factor in regulation of neuronal micromilieu (Köppe et al., 1997) and suggests a definite or certain role for each neuronal circuit (Sayed et al., 2002).

There is a little quantitative data that describes expression of perineuronal GPs in the different brain regions, e.g. 9-15% of nerve cells at the visual cortex of rats exhibits binding sites for lectin SBA on their surface (Peters & Kara, 1985; Lüth et al., 1992). In the present study, the mean number of labeled neurons per UA as estimated from lectin VVA, WFA and SBA-stained preparations, were 6.45 ± 0.87, 5.70 ± 0.73 and 3.91 ± 0.53, respectively. Consistent with this phenomenon,

labeling indices of neuronal cells presented by the aforementioned stains were 16.97%, 15.00% and 10.23%, respectively. This data indicates that the VVA staining presents the highest labeling affinity for the *N*-acetyl-galactosamine containing glycoconjugates; whereas, lectin SBA exhibits the lowest affinity for the same epitope.

In addition, the present morphometric study revealed that the CIC/Bodian staining of sulfated proteoglycans presented a relatively higher labeling intensity than CIC-staining. Their labeling indices were 10.55 and 11.55, respectively. Therefore, the dual CIC/Bodian staining is more strongly recommended for the expression of sulfated proteoglycans than the solely CIC staining. A similar conclusion was drawn by Murakami et al., (1997).

Although the coated neurons comprised a significant population in the central nervous system of mammals, their anatomical and functional significance is still obscure. However, the differential and morphometric characterization of PNs, as presented in this investigation, provides a quantitative information that has special significance when establishing correlation with experimental data.

Nevertheless, there are some putative roles for PGs, which include: stabilization of synapses, signal transduction, generation of polyanionic ion-buffering microenvironment, maintenance of extracellular spaces and concentration of growth or inhibitory factors (Matsui et al., 1999). Interestingly, the coated cells are neither susceptible to the age related changes (e.g. abnormal or hyperphosphorylation) (Härtige et al., 2001) nor to the neurofibrillary changes-characterizing Alzheimer's disease (Brückner et al., 1999).

Furthermore, in the developing nervous tissue, proteoglycans have been suggested to play important roles in various cellular processes such as cell proliferation and migration, neurite formation or neuronal maturation (Margolis and Margolis, 1993; Margolis and Margolis, 1997).

In conclusion, the cerebral cortex of rats possessed certain subsets of PGs, GPs and PGs/GPs-coated neurons, suggesting a significant or specific role for each circuit. In the retrosplenial cortex, the GPs-coated neuronal circuit is more widely spread compared with the other two circuits that together, might be contributing to specific biological role concerned with navigation and processing of episodic memory

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REFERENCES

Brückner G, Brauer K, Härtig W, Wolff JR, Rickmann MJ, Derouiche A, Delpech B, Girard N, Oertel WH, Reichenbach A (1993): Perineuronal nets provide a polyanionic, glia-associated form of microenvironment around certain neurons in many parts of the rat brain. *Glia 8*: 183-200

Brückner G, Härtig W, Kacza J, Seeger J, Welt K, Brauer K (1996):Extracellular matrix organization in various regions of rat brain grey matter.*J Neurocytol 25*: 333-346

Brückner G, Hausen D, Härtig W, Drlicek M, Arendt T, Brauer K (1999) Cortical areas abundantin extracellular matrix chondroitin sulphate proteoglycans are less affected by cytoskeletal changes in Alzheimer's disease. *Neuroscience 92:* 791-805

Celio MR, Blümcke I (1994): Perineuronal nets- a specialized form of extracellular matrix in the adult nervous system. *Brain Res Rev 19*: 128-145

Härtig W, Brauer K, Brückner G (1992): *Wisteria floribunda* agglutinin-labelled nets surround parvalbumin-containing neurons. *NeuroReport 3*: 869-872

Härtig W, Klein C, Brauer K, Schuppel KF, Arendt T, Bigl V, Brückner G (2001) Hyperphosphorylated protein tau is restricted to neurons devoid of perineuronal nets in the cortex of aged bison. *Neurobiol Aging 22:* 25-33

Köppe G, Brückner G, Härtig W, Bigl V (1997a): Developmental patterns of proteoglycan-containing extracellular matrix in perineuronal nets and neuropil of the postnatal rat brain. *Cell Tissue Res 288*: 33-41

Köppe G, Brückner G, Härtig W, Delpech B, Bigl V (1997b): Characterization of proteoglycan-containing perineuronal nets by enzymatic treatments of rat brain sections. *Histochem J 29*: 11-20

Kosaka T, Heizmann CW (1989): Selective staining of a population of parvalbumin containing GABAergic neurons in the rat cerebral cortex by lectins with specific affinity for terminal N-acetylgalactosamine. *Brain Res 483*: 158-163

Lüth H-J, Fischer J, Celio MR (1992): Soybean lectin binding neuron in the visual cortex of the rat contain parvalbumin and are covered by glial nets. *J Neurocytol 21*: 211-221

Maguire EA (2001): The retrosplenial contribution to human navigation: a review of lesion and neuroimaging findings. *Scand J Psychol 42:* 225-238

Margolis RK, Margolis RU (1993): Nervous tissue proteoglycans. *Experientia 49*: 429-446

Margolis RU, Margolis RK (1997): Chondroitin sulfate proteoglycans as mediators of axon growth and pathfinding. *Cell Tissue Res 290*: 343-348

Matsui F, Nishizuka M, Oohira A (1999): Proteoglycans in perineuronal nets. *Acta Histochem Cytochem 32*: 141-147

Murakami T, Taguchi T, Ohtsuka A, Sano K, Kaneshige T, Owen RL, Jones AL (1986): A modified method of fine-granular cationic iron colloid preparation: its use in the light and electron microscopic detection of anionic sites in the rat kidney glomerulus and certain other tissues. *Arch Histol Jap 49*: 12-23

Murakami T, Taguchi T, Ohtsuka A, Kikuta A (1993): Neurons with strongly negative- charged surface-coats in adult rat brain as detected by staining with cationic iron colloid *Arch Histol Cytol 56*: 13-21

Murakami T, Ohtsuka A, Taguchi T (1994): Neurons with intensely negatively charged extracellular matrix in the human visual cortex. *Arch Histol Cytol 57*: 507-522

Murakami T, Ohtsuka A, Piao DX (1996): Perineuronal sulfated proteoglycans in the human brain are identical to Golgi's reticular coating. *Arch Histol Cytol 59*: 233-237

Murakami T, SU WD, Hong LJ, Piao DX, Ohtsuka A, Seo K (1997): Physical development of tissue-reacted cationic iron colloid with protein silver and gold chloride. *Okayama Igakkai Z 109*: 151-156

Murakami T, Murakami T, SU WD, Ohtsuka A, Abe K, Ninomiya Y (1999): Perineuronal nets of proteoglycans in the adult mouse brain are digested by collagenase *Arch Histol Cytol 62*: 199-204

Naegele JR, Katz LC (1990): Cell surface molecules containing N-acetylgalactosamine are associated with basket cells and neurogliaform cells in cat visual cortex. *J Neurosci 10*: 540-557

Nakagawa F, Schulte BA, Spicer SS (1986): Selective cytochemical demonstration of glycoconjugate-containing terminal N-acetylgalactosamine on some brain neurons .*J Comp Neurol 243*: 280-290

Peters A, Kara DA (1985): The neuronal composition of area 17 of rat visual cortex. II. The nonpyramidal cells. *J Comp Neurol 234*: 242-263

Sas (1989): User's Guide: Statistics. *SAS Inst Inc, Cary, NC*

Sayed R, Mubarak W, Ohtsuka A, Taguchi T, Murakami T (2002): Histochemical study of perineuronal nets in the retrosplenial cortex of adult rats. *25th Congress of the Egyptian Society for Cytology and Histology. Fac of Med, Zagazig Unive. January 3, 2002.*

Weibel ER (1979): Stereological methods. Practical methods for biological morphometery. *Volume I. London, New York, Toronto, Sydney, San Francisco: Academic press.*

Weibel AP, McEchron MD, Disterhoft JF (2000): Cortical involvement in acquisition and extinction of trace eye link conditioning. *Behav Neurosci 114:* 1058-1067

LEGENDS

Table 1:

Morphometric analysis of the histochemical characteristics in the retrosplenial cortex of rats.

Table 2:

Relationship between the postnatal age and the total number of neurons together with the number of coated and non-coated neurons

Table 3:

Morphometric analysis of the retrosplenial cortex of 8 months-old rats.

Figure 1:

A- Two proteoglycans-coated neurons. The CIC binding sites surround the perikarya and the initial segments of cell processes. CIC-staining countered by nuclear fast red. X 1132

B- A glycoprotein-coated neuron. VVA-labeling counter-stained by Mayer's hematoxyline. X 990.

Figure 2: Percentage of coated and non-coated neurons.

Figure 3: Percentage of different perineuronal coatings.

Figure 4 Numerical density of coated and non-coated neurons together with the total neuronal density, in relation to the postnatal age.

Figure 5: Absolute numerical density of coated and non-coated neurons together with the total neuronal density.

Figure 6: Relative densities of pericoated neurons, GPs-coated neurons, PGs-coated neurons and neurons exhibiting coat complexes. The percentages are shown.

Figure 7: Labeling indices of VVA, WFA, SBA, CIC and CIC/Bodian staining. The indices are shown.

Table 1: Morphometric parameters of the histochemical characteristics in the retrosplenial cortex of rats$^{\Phi}$

Serial No.	Age (Month)	No. of animal	Total No. of Neurons / UA	No. of Coated Neurons / UA	% of Coated Neurons / UA	No. of Non Coated Neurons / UA	% of Non Coated Neurons / UA
1	2	8	42.43 ±5.45	8.45 ±2.62	19.92	33.95 ±4.16	80.08
2	3	8	41.32 ±6.11	8.75 ±2.53	21.18	32.55 ±4.11	78.82
3	4	8	41.12 ±5.36	9.10 ±2.71	22.14	32.00 ±5.16	77.86
4	6	8	39.81 ±5.82	10.44 ±2.94	26.23	29.36 ±4.22	73.77
5	8	8	38.84 ±4.99	10.50 ±2.61	26.06	27.90 ±4.66	73.94
6	12	4	38.23 ±5.56	10.90 ±2.62	28.53	27.30 ±4.17	71.47
7	18	4	37.34 ±5.89	9.21 ±2.69	24.66	28.10 ±3.79	75.34
Overall Mean			39.71 ±1.79	9.57 ±1.13	24.25	30.14 ±2.79	75.75

Φ VVA/CIC staining
Mean ±SD. UA = (Unit Area, 60.15 μm^2)

Table 2: Relationship between the postnatal age and the total number of neurons together with the number of coated and non-coated neurons

Parameter	Total Number of Neurons (per UA)	Number of Coated Neurons (per UA)	Number of Non Coated Neurons (per UA)
Correlation coefficient (r)	- 0.97	0.35	- 0.79
Probability (P)	0.0003 **HG**	0.44 **NS**	0.03 **S**

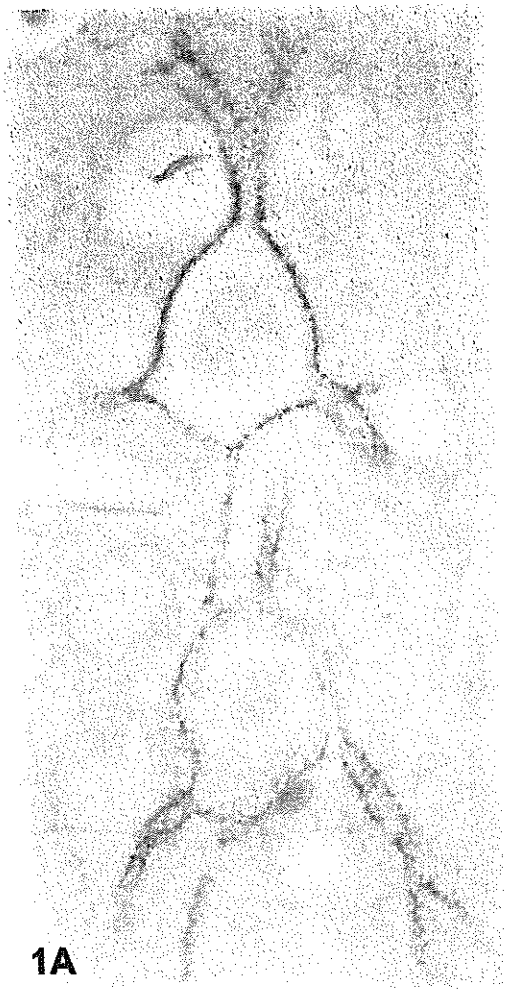
Abbreviations: HG, Highly significant; S, Significant; NS, Non significant

Table 3: Morphometric analysis of the retrosplenial cortex in rats$^{\Phi*}$

Parameter per unit area (60.15 μm^2)	Absolute Density	Relative Density
Total Number of Neurons	38.10 ±2.60	100%
Number of Non Coated Neurons	28.13 ±1.80	74.03%
Number of Coated Neurons	9.87 ±0.43	25.97%
Number of PGs-coated Neurons	0.84 ±0.07	2.21%
Number of GPs-coated Neurons	6.33 ±0.34	16.65%
Number of PGs/GPs-coated Neurons	2.70 ±0.22	7.11%

Φ WFA/CIC staining

* Data estimated from eight months-old rats



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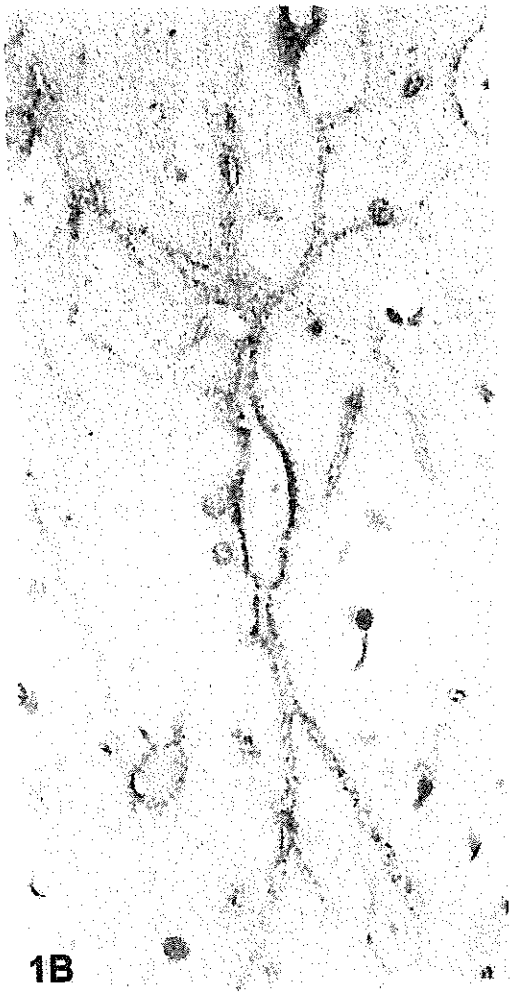

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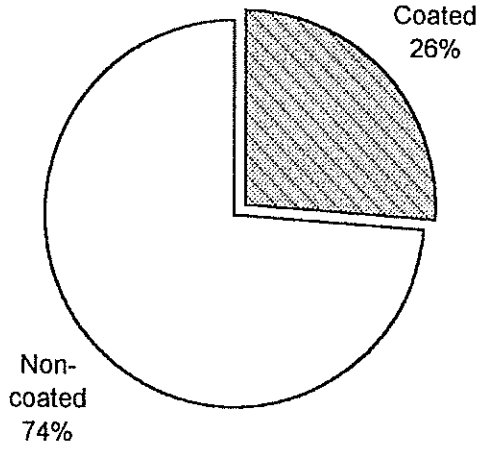
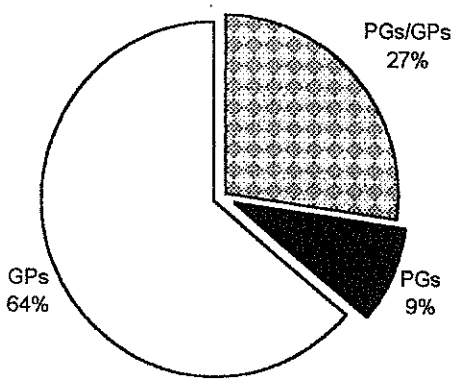
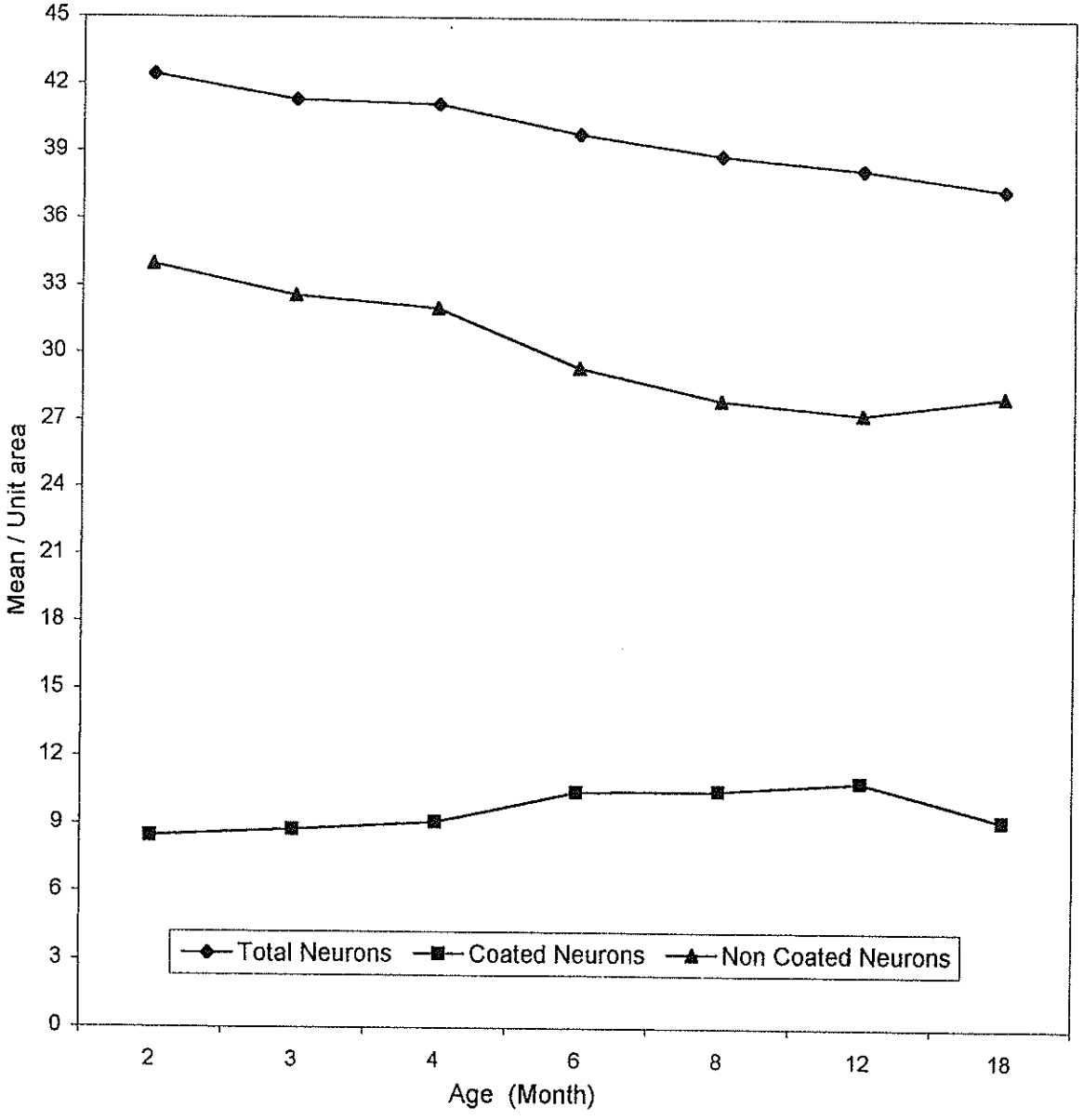

Coated
26%
Non-
coated
74%

2


PGs/GPs
27%
GPs
64%
PGs
9%

3


45
42
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Mean / Unit area
2
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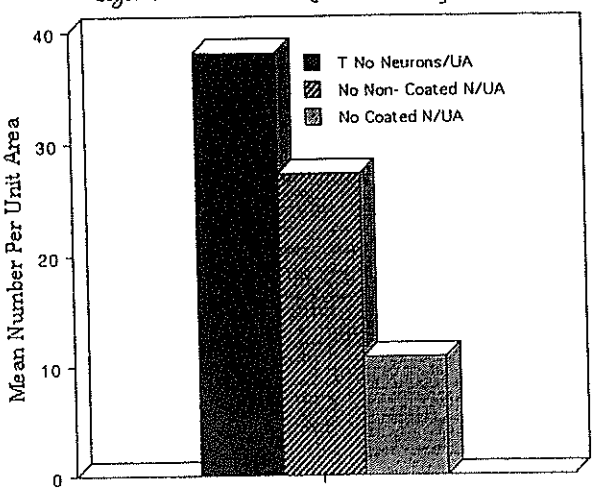
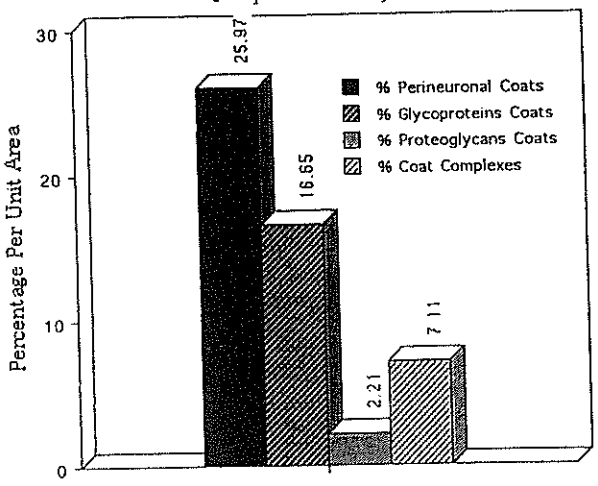
Age (Month)
Total Neurons
Coated Neurons
Non Coated Neurons

Absolute Density of Coated and Non- Coated Neurons Together with the Total Neuronal Density



5

Relative Densities of Neurons Exhibiting Perineuronal Coats in the Retrosplenial Cortex of Rats



6

Labeling Indices of VVA, WFA, SBA, CIC and CIC/Bodian Stainings in the Retrosplenial Cortex of Rats