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Perineuronal sulfated proteoglycans and cell surface glycoproteins in the visual cortex of adult and newborn cats

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Abstract

Sections of the visual cortex of newborn (1-4 weeks after birth) and adult cats were stained with cationic iron colloid, aldehyde fuchsin or lectins (lectin Vicia villosa, soybean and Wisteria floribunda agglutinins). Many neurons in the adult cat visual cortex contained perineuronal sulfated proteoglycans detectable with cationic iron colloid and aldehyde fuchsin, or cell surface glycoproteins reactive to lectins. Double staining indicated that some of the lectin-labeled neurons were not stained with cationic iron colloid, and also that some of the cationic iron colloid-stained neurons were not labeled with lectins. The perineuronal sulfated proteoglycans and cell surface glycoproteins developed 3 weeks after birth. In the newborn cats 1-2 weeks after birth, no neurons were reactive to cationic iron colloid, aldehyde fuchsin or lectins. In the newborn cats 34 weeks after birth, it was clearly observed that the cytoplasm of the glial cells closely associated with the neurons containing the perineuronal sulfated proteoglycans showed an intense reaction to cationic iron colloid and aldehyde fuchsin, and that the Golgi complexes of the neurons with cell surface glycoproteins were intensely labeled with lectins. These findings suggest that the perineuronal sulfated proteoglycans are derived from the associated glial cells, and that the cell surface glycoproteins are produced by the associated nerve cells.

KEYWORDS: cat brain, perineuronal sulfated proteoglycans, cell surface glycoproteins, cationic iron colloid, aldehyde fuchsin, lectin

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Perineuronal Sulfated Proteoglycans and Cell Surface Glycoproteins in the Visual Cortex of Adult and Newborn Cats

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Sections of the visual cortex of newborn (1-4 weeks after birth) and adult cats were stained with cationic iron colloid, aldehyde fuchsin or lectins (lectin *Vicia villosa*, soybean and *Wisteria floribunda* agglutinins). Many neurons in the adult cat visual cortex contained perineuronal sulfated proteoglycans detectable with cationic iron colloid and aldehyde fuchsin, or cell surface glycoproteins reactive to lectins. Double staining indicated that some of the lectin-labeled neurons were not stained with cationic iron colloid, and also that some of the cationic iron colloid-stained neurons were not labeled with lectins. The perineuronal sulfated proteoglycans and cell surface glycoproteins developed 3 weeks after birth. In the newborn cats 1-2 weeks after birth, no neurons were reactive to cationic iron colloid, aldehyde fuchsin or lectins. In the newborn cats 3-4 weeks after birth, it was clearly observed that the cytoplasm of the glial cells closely associated with the neurons containing the perineuronal sulfated proteoglycans showed an intense reaction to cationic iron colloid and aldehyde fuchsin, and that the Golgi complexes of the neurons with cell surface glycoproteins were intensely labeled with lectins. These findings suggest that the perineuronal sulfated proteoglycans are derived from the associated glial cells, and that the cell surface glycoproteins are produced by the associated nerve cells.

Key words: cat brain, perineuronal sulfated proteoglycans, cell surface glycoproteins, cationic iron colloid, aldehyde fuchsin, lectin

Our light and electron microscopic studies of tissue sections stained with cationic iron colloid and aldehyde fuchsin have revealed the occurrence of many neurons with intensely negatively charged surface coatings in the human brain (1-4) and in the brains of cows, cats, rats, mice and other animals, including some lower vertebrates such as frogs and fish (5-13). Similar neurons have also been reported by other authors, most of whom studied the brains of rats and mice with lectin labelings (lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin) or with immunohistochemical stains using antibodies reactive against cartilage (14-19).

Our light and electron microscopic studies, including tissue digestion experiments, of human, rat and mouse brains have further shown that the surface coats develop after birth (7, 12, 13) and consist of sulfated proteoglycans, which are identical to Golgi's reticular coating detectable with silver nitrate (4, 10). These proteoglycans are fairly independent of the cell surface glycoproteins labeled with lectins (3, 9-11).

The present study investigates adult and newborn cat brains to identify the cells producing such perineuronal sulfated proteoglycans and cell surface glycoproteins. The visual cortex or its ganglionic lamina is the object of this study, as it contains many neurons with perineuronal sulfated proteoglycans or cell surface glycoproteins (2, 3).

Materials and Methods

Newborn (1-4 weeks after birth) and adult cats were used. Under ether anesthesia, they were perfused through the ascending aorta with Ringer's solution and with 4.0% paraformaldehyde in 0.1M cacodylate buffer (pH 7.2). From these animals, 2-3mm-thick blocks of tissue traversing the visual cortex were removed. These blocks were fixed again with the 4.0% paraformaldehyde for 4h or longer, embedded in paraffin and cut into 10-

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15 μm-thick sections. These sections were deparaffinized with xylene, and treated as follows:

The deparaffinized sections were stained with cationic iron colloid at a pH value of 1.5 (20), aldehyde fuchsin (21), lectin *Vicia villosa* agglutinin (22, 23) lectin soybean agglutinin (3) or lectin *Wisteria floribunda* agglutinin (18). Some sections from the adult cats were stained doubly with lectins and cationic iron colloid (3, 10). For each lectin labeling, peroxidase activity was demonstrated with diaminobenzidine (22, 23). Controls for lectin-labeled sections consisted of adjacent sections of brain treated with phosphate buffer containing no agglutinin.

Results

Adult cats. The visual cortex of adult cats or its ganglionic lamina contained many neurons whose cell bodies and main processes were coated with perineuronal

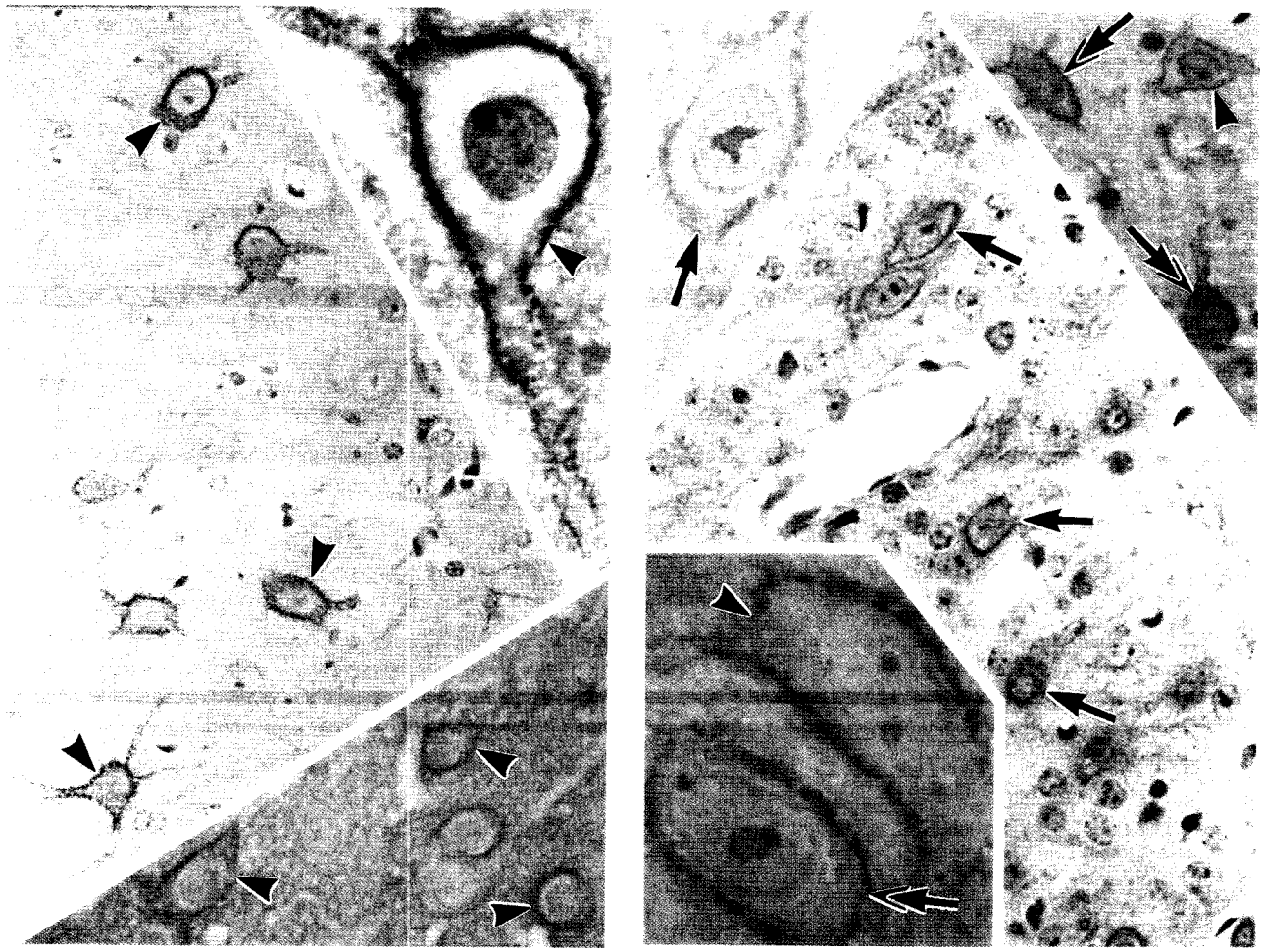

Fig. 1 (Left) A light micrograph survey of the adult cat visual cortex, stained with cationic iron colloid (pH 1.5) and nuclear fast red. Many neurons are coated with perineuronal sulfated proteoglycans reactive to cationic iron colloid (arrowhead). Upper inset shows a closer view of a neuron with perineuronal sulfated proteoglycans stained with cationic iron colloid (arrowhead). Lower inset shows some neurons with perinueronal sulfated proteoglycans stained with aldehyde fuchsin (arrowhead). × 500. Upper inset: × 1,100; Lower inset: × 550.

Fig. 2 (Right) Adult cat visual cortex, stained with lectin *Vicia villosa* agglutinin and Mayer's hematoxylin. Many neurons are coated with cell surface glycoproteins labeled with lectin *Vicia villosa* agglutinin (arrow). Upper-left inset shows a closer view of a neuron with the lectin-labeled surface glycoproteins (arrow). Upper-right and lower insets show the sections of the adult cat visual cortex, which were doubly stained with lectin *Vicia villosa* agglutinin and cationic iron colloid. Many neurons are stained doubly with lectin and colloid (double arrows). Arrowhead indicates neurons stained only with cationic iron colloid. × 500. Upper-left inset: × 1,000; Upper-right inset: × 600; Lower inset: × 1,100.

sulfated proteoglycans reactive to cationic iron colloid (Fig. 1, upper inset) and aldehyde fuchsin (Fig. 1, lower inset). No intracellular structures of these neurons were reactive to cationic iron colloid or aldehyde fuchsin. No glial cells or their intracellular structures were reactive to cationic iron colloid or aldehyde fuchsin.

The ganglionic lamina in the visual cortex of adult cats also contained many neurons, whose cell bodies and main processes were coated with cell surface glycoproteins labeled with lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin (Fig. 2, left-upper inset). No intracelluar structures of these neurons were labeled with lectins. No glial cells and their intracellular structures were also labeled with lectins.

In the double stainings, the majority (75 %) of the neurons stained with cationic iron colloid were also labeled with lectin *Vicia villosa* agglutinin though some (25 %) of them were not labeled with this lectin (Fig. 2, upper and lower insets). In the double staining, it was also observed that some (25 %) of the neurons labeled with lectin *Vicia villosa* agglutinin were not stained with cationic iron colloid (Fig. 2, upper and lower insets). Similar results were also obtained even in the double stainings with lectin soybean agglutinin and cationic iron colloid and with lectin *Wisteria floribunda* agglutinin and cationic iron colloid (data, not shown).

In the control sections, none of the neurons nor any of their intracellular structures were reactive to lectin *Vicia villosa*, soybean and *Wisteria floribunda* agglutinins.

Newborn cats. The visual cortex or its ganglionic lamina of the newborn cats 1–2 weeks after birth contained no neurons reactive to cationic iron colloid, aldehyde fuchsin and lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin.

The ganglionic lamina in the visual cortex of newborn cats 3–4 weeks after birth contained many neurons whose cell bodies and main processes were coated with perineuronal sulfated proteoglycans reactive to cationic iron colloid and aldehyde fuchsin (Fig. 3, upper and lower insets). At this stage, the proteoglycans were lightly stained with cationic iron colloid and aldehyde fuchsin, and were closely associated with the glial cells (satellite astrocytes), the cytoplasm of which was reactive to cationic iron colloid and aldehyde fuchsin (Fig. 3, upper and lower insets). No intracellular structures of the nerve cells were reactive to cationic iron colloid.

The ganglionic lamina in the visual cortex of newborn cats 3–4 weeks after birth also contained many neurons whose cell surfaces were coated with cell surface glycoproteins labeled with lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin (Fig. 4, inset). At this stage, the Golgi complex of the neurons with cell surface

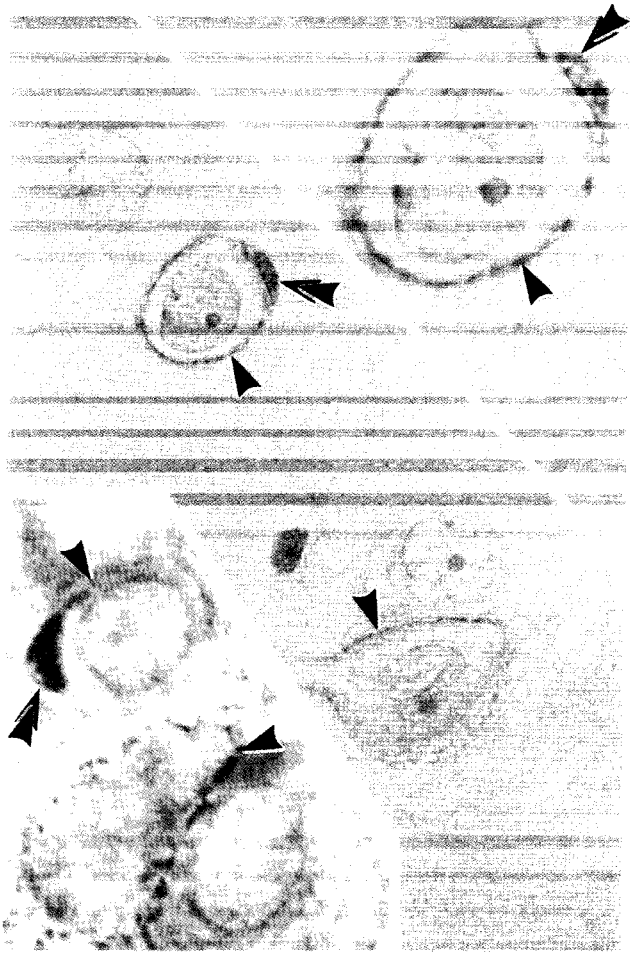

Fig. 3 Newborn cat visual cortex (3 weeks after birth), stained with cationic iron colloid (pH 1.5) and nuclear fast red. Some neurons are coated with perineuronal sulfated proteoglycans lightly reactive to cationic iron colloid (arrowhead). The perineuronal sulfated proteoglycans surrounding the upper neurons are closely associated with the satellite astrocyte (double arrowheads). Upper inset shows a closer view of the upper neuron in the main figure, where the close association of perineuronal sulfated proteoglycans (arrowhead) and satellite astrocytes (double arrowheads) is clearly visible. Lower inset shows a section of the newborn cat visual cortex (3 weeks after birth) which was stained with aldehyde fuchsin. Two neurons with perineuronal sulfated proteoglycans are visible (arrowhead). In the upper neuron, the cytoplasm of the satellite astrocyte is intensely reactive to aldehyde fuchsin (double arrowheads) and closely associated with the perineuronal proteoglycans (arrowhead). × 800. Upper inset: × 1,500; Lower inset: × 1,100.

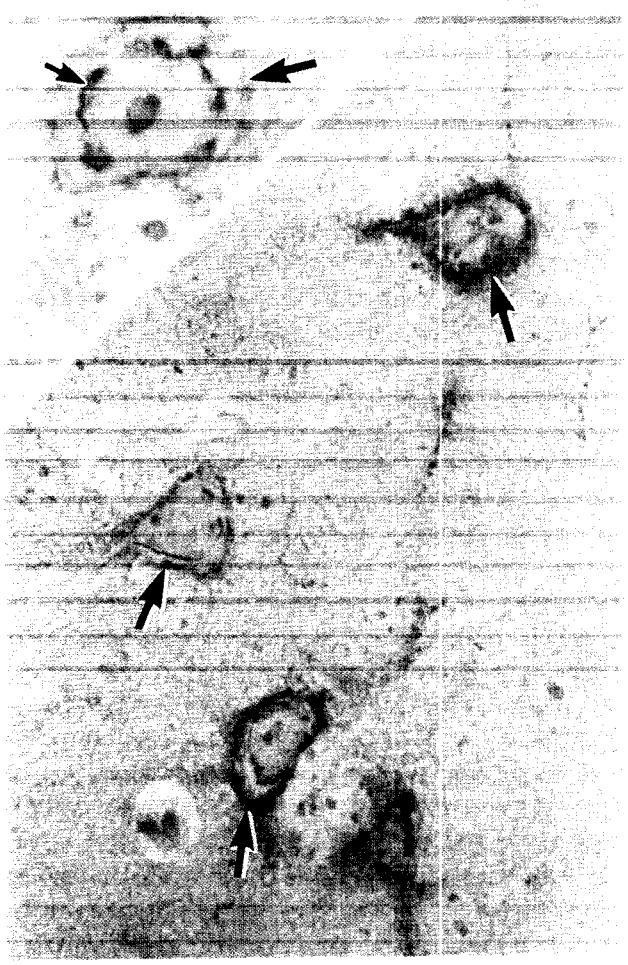

Fig. 4 Newborn cat visual cortex (3 weeks after birth), stained with lectin *Vicia villosa* agglutinin and nuclear fast red. Many neurons are labeled with lectin *Vicia villosa* agglutinin (arrow). Inset shows a closer view of a lectin-labeled neuron (arrow) in the newborn visual cortex (3 weeks after birth). The Golgi's complex of this neuron is intensely labeled with lectin *Vicia villosa* agglutinin (small arrow). × 700. Inset: × 1,200.

glycoproteins (or these cells whose cell surfaces were labeled with lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin) were also labeled with lectin *Vicia villosa* agglutinin (Fig. 4, inset).

In the control sections, neither neurons nor glial cells were reactive to lectin *Vicia villosa* agglutinin.

Discussion

The present study, together with our previous study (8), confirms that the cat brain contains many neurons with intensely negatively charged surface coats or perineuronal sulfated proteoglycans which can be stained with cationic iron colloid and aldehyde fuchsin. Our previous experiments with human, rat and mouse brains have shown that chondroitinase ABC/heparitinase/keratanase digestions thoroughly erase the cationic iron colloid staining potential of the coats though they never interfere with aldehyde fuchsin staining of the coats, indicating that cationic iron colloid and aldehyde fuchsin stain the sulfate groups and core proteins of proteoglycans, respectively (2, 3, 7, 9, 11).

The present double stainings, together with our previous studies of human, rat and mouse brains (2, 3, 9, 11), clearly show that some of the neurons stained with cationic iron colloid are not labeled with lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin. Our previous experiments with human, rat and mouse brains have shown that hyaluronidase digestion never interferes with the lectin labelings of the neurons though it thoroughly erases the cationic iron colloid and aldehyde fuchsin stainings of the neurons, indicating that the cell surface glycoproteins are fairly independent of the perineuronal sulfated proteoglycans (2, 3, 11). Therefore, our present and previous studies supplement the findings by Brückner *et al.* (14, 18), Celio and Blumcke (15), Hartig *et al.* (16) and other authors (17, 19) who have described how neurons with cell surface glycoproteins are identical to neurons with perineuronal sulfated proteoglycans.

The present study demonstrates that in newborn cats 3–4 weeks after birth, the cytoplasm of some glial cells shows an intense reaction to cationic iron colloid and aldehyde fuchsin which preferentially stain perineuronal sulfated proteoglycans. This suggests that these glial cells produce the perineuronal sulfated proteoglycans, supporting the classical view (glial nets) of Golgi in production of the proteoglycans (24, 25). Judging from their position, form and size, the glial cells may be included in the group of satellite astrocytes. Decreased reaction of the glial cells to cationic iron colloid and aldehyde fuchsin in the adult cats suggests a poor supply of proteoglycans in these animals.

The present study further shows that in the newborn cats 3–4 weeks after birth, the Golgi complexes of the neurons whose cell surfaces are labeled with lectin *Vicia villosa*, soybean or *Wisteria floribunda* agglutinin, are also labeled with these lectins. This suggests that the cell surface glycoproteins are produced by the associated nerve cells. The lack of a marked reaction of the Golgi complexes of these neurons to the lectins in the adult

animals suggests a poor supply or production of the surface glycoproteins in these animals.

It is noteworthy that in the newborn cats 1-2 weeks after birth, no neurons are reactive to cationic iron colloid, aldehyde fuchsin and lectin *Vicia villosa* agglutinin. This may indicate that the brain matures at a later stage. Our previous studies indicate that in the rat and mouse, perineuronal sulfated proteoglcyans and cell surface glycoproteins thoroughly develop during the weaning period (12, 13).

It is generally believed that lectin *Vicia villosa*, soybean and *Wisteria floribunda* agglutinins preferentially detect N-acetyl-D-galactosamine. However, our previous observations of serial sections from the human brain indicate that some neurons labeled with lectin *Vicia villosa* agglutinin are not labeled with lectin soybean agglutinin (3). Such observations of lectin-labeled serial sections were omitted from the present study.

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