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The occurrence of neurons with strongly  
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# The occurrence of neurons with strongly negatively charged surface coats in mammalian, avian, reptilian, amphibian and piscine brains.\*

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## Abstract

Neurons with strongly negatively charged surface coats were recognized in mammalian, avian, reptilian, amphibian and piscine brains. Many large-sized neurons had strongly negatively charged surface coats in the visual cortex and brain stem of the cow, cat, guinea pig, mouse, quail and parakeet. Such neurons were also seen in the brain stem of the lower vertebrates such as the house lizard, Japanese terrapin, bullfrog, newt, carp and sweetfish.

**KEYWORDS:** central nervous system, neurons, negatively charged surface coats, proteoglycans

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## The Occurrence of Neurons with Strongly Negatively Charged Surface Coats in Mammalian, Avian, Reptilian, Amphibian and Piscine Brains

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Neurons with strongly negatively charged surface coats were recognized in mammalian, avian, reptilian, amphibian and piscine brains. Many large-sized neurons had strongly negatively charged surface coats in the visual cortex and brain stem of the cow, cat, guinea pig, mouse, quail and parakeet. Such neurons were also seen in the brain stem of the lower vertebrates such as the house lizard, Japanese terrapin, bullfrog, newt, carp and sweetfish.

**Key words:** central nervous system, neurons, negatively charged surface coats, proteoglycans

Our previous papers showed that in humans and in rats, a considerable number of neurons in the central nervous system had strongly negatively charged surface coats (1-4). In this study, we investigated the occurrence of such neurons in the cow, cat, guinea pig, mouse, quail, parakeet, house lizard, terrapin, bullfrog, newt, carp and sweetfish.

### Materials and Methods

Small blocks ( $2 \times 3 \times 4$  mm) were isolated from the visual cortex (optic lobe) and brain stem of adult cows, cats, guinea pigs, mice, quails, parakeets, house lizards, Japanese terrapins, newts, bullfrogs, carps and sweetfish. These blocks were immediately fixed with 2.5 % glutaraldehyde or 10 % formalin in 0.1 M cacodylate buffer (pH 7.2) for 12 h or longer. They were then embedded in paraffin, cut into sections, incubated in our fine cationic iron colloid with pH values of 1.0-1.5 (5), immersed in a mixture of  $K_4Fe(CN)_6$  and HCl for Prussian blue reaction, stained with nuclear fast red or carbol-thionin (1,5), and observed with a transmission light microscope (BH-2, Olympus).

### Results

Incubation in fine cationic iron colloid with pH values of 1.0-1.5 and successive treatment with the mixture of  $K_4Fe(CN)_6$  and HCl to measure the Prussian blue reaction clearly demonstrated the presence of strongly negatively charged surface coats of the nerve cells and their processes under the light microscope (Figs. 1-3). The neurons with strongly negatively charged surface coats were usually large in size and possessed a well-developed nucleus and a well-defined nucleolus (Fig. 1); the cytoplasm of neural somata was well stained with thionin, indicating Nissl bodies (Figs. 1, 3). Distributions of these neurons seemed to vary among species and also among the areas of the brain (Figs. 1-3). Although the present investigation does not explain these differences, some findings hitherto obtained are described below.

**Cow, cat, guinea pig and mouse (mammalia).** In the visual cortex of the cow, cat, guinea pig and mouse, the neurons were preferentially localized in the deep areas, especially in the lamina pyramidalis and ganglionalis (Fig. 1). They were also noted in the brain stem: the nuclei pontis, zona incerta and in certain nuclei in the cerebellum.

**Quail and parakeet (aves).** The neurons were demonstrated in the lamina pyramidalis and ganglionalis of the visual cortex of the quail and parakeet. They were also noted in the brain stem: nucleus principalis precommissuralis, nucleus pretectalis and in certain other nuclei, including pontine nuclei (Fig. 2).

**House lizard and Japanese terrapin (reptilia).** In the house lizard (*Takydromus tatakoides*) and the Japanese terrapin, the neurons were preferentially observed in the ventromedial and lateral areas of the brain stem at a level near the caudal end of the optic lobes. They were not noted in the visual cortex

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of these reptiles.

**Bullfrog and newt (amphibia).** The neurons were preferentially observed in the dorsomedial and dorsolateral areas of the brain stem at a level near the caudal end of the cerebellum. They were not noted in the optic lobe of these animals.

**Sweetfish and carp (pisces).** In the sweetfish (*Plecoglossus altivelis*) and the carp, the neurons were preferentially localized in the area ventral to the aqueduct of the brain stem (Fig. 3). They were not observed in the optic lobe of these fish.

### Discussion

This study demonstrated clearly that the cow, cat, guinea pig, mouse, quail and parakeet had many neurons with strongly negatively charged surface coats in their visual cortex and brain stem. It also demonstrated that the house lizard, Japanese terrapin, bullfrog, newt, carp and sweetfish contained some neurons with strongly negatively charged surface coats in their brain stem, but few in their visual cortex.

The neurons with strongly negatively charged surface coats have been recognized in the hindlimb cortex, visual cortex, hippocampal subiculum, pontine nuclei, zona incerta, intracerebellar nuclei and certain other nuclei of the rat brain (1, 2). These neurons have been identified even in the human brain, especially in the visual cortex (3). Our present and previous studies (1-3), thus, suggest that the neurons can occur widely in the central nervous system through the vertebrates, though their occurrence in the visual and other cerebral cortex might be inconsistent in the reptilian, amphibian and piscine brains. Our recent light microscopic experiments in the rat have shown that the gray matter of the spinal cord, especially

**Fig. 1** Light micrograph of an adult mouse brain section (lamina pyramidalis of visual cortex), which was incubated in cationic iron colloid at pH 1.5, treated for the Prussian blue reaction, and stained with carbol-thionin. Note that two large-sized neurons and their processes are coated with strongly negatively charged substances (arrowheads). Bar = 10  $\mu$ m.

**Fig. 2** An adult quail brain section (pontine area). Cationic iron colloid (pH 1.0)-Prussian blue nuclear fast red staining. Note that some nerve cells and their processes were provided with strongly negatively charged surface-coats (arrowheads). Bar = 10  $\mu$ m.

**Fig. 3** Neurons with strongly negatively charged surface coats as observed in the sweetfish midbrain (arrowheads). Cationic iron colloid (pH 1.5)-Prussian blue carbol-thionin staining. Bar = 10  $\mu$ m.

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the posterior horn, contains some large-sized neurons with strongly negatively charged surface coats (4).

The strongly negatively charged surface coats in the rat brain are sulfated proteoglycans such as chondroitin sulfate proteoglycans (1). This may be supported by our recent findings that the surface coats can be stained with Gomori's aldehyde-fuchsin and digested with hyaluronidase (manuscript in preparation).

Our previous reports also suggest that the neurons are interneurons projecting associational or commissural fibers (1, 2), and that they may be identical with the *Vicia villosa* agglutinin-labelled cells described by Drake *et al.* (6).

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