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Research article

Immunohistochemical localization of serotonin in the superior colliculus of porcupine *(Hystrix cristata)*

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ABSTRACT

The distribution and localization of serotonin immunoreactive cells in the superior colliculus of the porcupine (*Hystrix cristata*) was studied using the peroxidase-antiperoxidase method and an antiserum against serotonin. The study was performed on four superior colliculus' of porcupine. Serotonin immunoreactivity showed a dense body of positively labelled cells throughout the superior colliculus. These serotonin immunoreactive cells had a characteristic arrangement corresponding to the laminar structures of the superior colliculus. In this study, the densest concentration of serotonin immunoreactive cells was found in a single tier located within the stratum zonale (SZ) and upper part of the stratum griseum superficiale (SGS). The second densest is located within stratum griseum intermedium (SGI) and the third appears as an incomplete layer, found only in the medial and central parts of the stratum griseum profundum (SGP). On the basis of these findings, serotonin has been suggested to be an inhibitory neurotransmitter and neuromodulator in the mammalian superior colliculus.

Keywords: superior colliculus, serotonin, porcupine, immunohistochemistry

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INTRODUCTION

The single largest group of mammals is the Rodentia. Most non-flying mammals are rodents. Porcupines are the third largest of the rodents and are members of the family Hystricidae, a small group of Rodentia.[1,2]

The superior colliculus is one of the extrageniculate visual structures and is characterized by a precise lamination and morphologically well defined cell types. The mammalian superior colliculus is the center of visuo-motor integration. It coordinates head and eye movements to visual, auditory, and somatic stimuli. The colliculus consists of seven alternating fibrous and cellular layers[3] that have been grouped into two functionally distinct units: a superficial subdivision and a deep subdivision.[4–6] The superficial layers are stratum zonale (SZ), stratum griseum superficiale (SGS) and stratum opticum (SO). The deep layers are stratum griseum intermedium (SGI), stratum album intermedium (SAI), stratum griseum profundum (SGP) and stratum album profundum (SAP). The superficial layers receive their major input from the retina and the visual cortex and are exclusively related to vision. However, the deeper layers receive auditory, somatic and visual input from numerous cortical and subcortical areas and are involved in head, eye and ear movements.

The distribution of serotonin fibers in the superior colliculus of the rat was first described by[7] who used a fluorescence histochemical method.[8] reported that slices of the superior colliculus picked up serotonin, which was subsequently released by optic tract stimulation.[9] showed that iontophoretically-applied serotonin inhibited neuronal activity in the superior colliculus. On the other hand biochemical investigation has shown that the level of serotonin in the rat superior colliculus is relatively high.[10] On the basis of these findings, serotonin has been suggested to be an inhibitory neurotransmitter or neuromodulator in the mammalian superior colliculus.

Serotonin is a central neurotransmitter involved in a great variety of physiological functions as well as neurological and psychopathological disorders.[11–13] Serotonin has been studied in the superior colliculus of several mammals, including rat, hamster, chipmunk, cat and monkey.[14]

The aim of the present study is to determine the distribution of serotonin immunoreactivity in the superior colliculus of the porcupine (*Hystrix cristata*) and to compare the results to other species. Distribution of serotonin in the porcupine superior colliculus will provide a valuable database for the evaluation of future histological studies.

MATERIALS AND METHODS

Three porcupines (*Hystrix cristata*) were examined in this study. The animals were anesthetized and killed using ether. The superior colliculus of porcupines was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 h before paraffin embedding. Tissues were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. Sections 5 μm thick were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the streptavidin-biotin complex technique.[15] Blocking of endogenous peroxidase activity was carried out with 0.08% hydrogen peroxide (H_2O_2) in methanol for 5 min. In order to block non-specific binding, an incubation with Large Volume Ultra V Blok (Lab Vision co) for 30 min was performed.

Sections were incubated for 16 – 20 h at 4°C in rabbit anti-serotonin (Sigma). Antibody was diluted to 1:500 with PBS containing 0.25% sodium azide and 2.5% bovine serum albumin, respectively. Sections were incubation in biotinlated secondary antiserum (Lab Vision co) for 1 h, followed by rabbit streptavidin-biotin -peroxidase complex (Lab Vision Co) for 1 h, at room temperature. Sections were washed in PBS for 30 min after each incubation and finally immersed in AEC (Dako) chromogen substrate for 10 min. After washing in distilled water, sections were counterstained with Mayer Hematoxylin. Sections were dehydrated and coverslips mounted with squamous mounting medium. Sections were examined with a light microscope and photographs taken.

Negative control sections were treated identically except for the omission of primary antiserum. No reaction product was observed in any of the control sections.

The specificity of each immunohistochemical reaction was determined as recommended by,[16] including the replacement of specific antiserum pre-incubated with its corresponding antigen.

RESULTS

The present study provides the first immunohistochemical determination in the porcupine of serotonin. Our data appears to be consistent with previous results that have been reported for other species of

mammals. Serotonin immunoreactivity was selectively distributed in the superior colliculus of porcupine. Three tiers of serotonin neurons were formed in the porcupine superior colliculus (Figure 1). The densest concentration of serotonin immunoreactive cells was found in a single tier located within the SZ and upper part of the SGS, the second is located within SGI and the third appears as an incomplete layer, found only in the medial and central parts of the SGP (Figures 1–2).

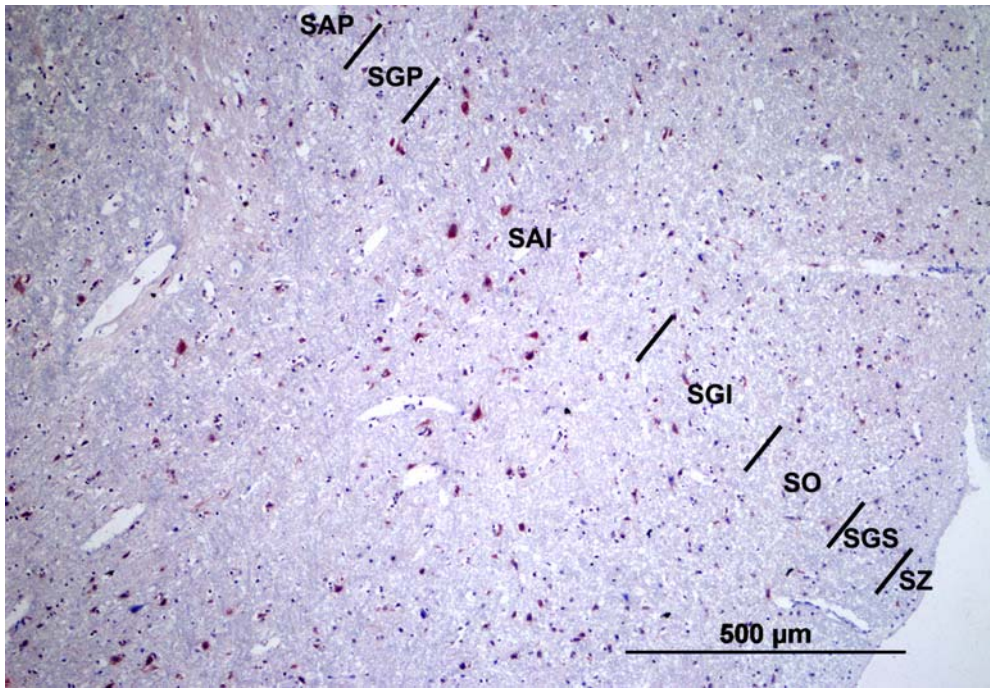

Figure 1. Photomicrographs of serotonin immunoreactive cells in the porcupine Superior colliculus. SZ: stratum zonale, SGS:stratum grizeum superficiale, SO: stratum opticum, SGI: stratum griseum intermedium, SAI: stratum album intermedium, SGP: stratum griseum profundum, SAP: stratum album profundum

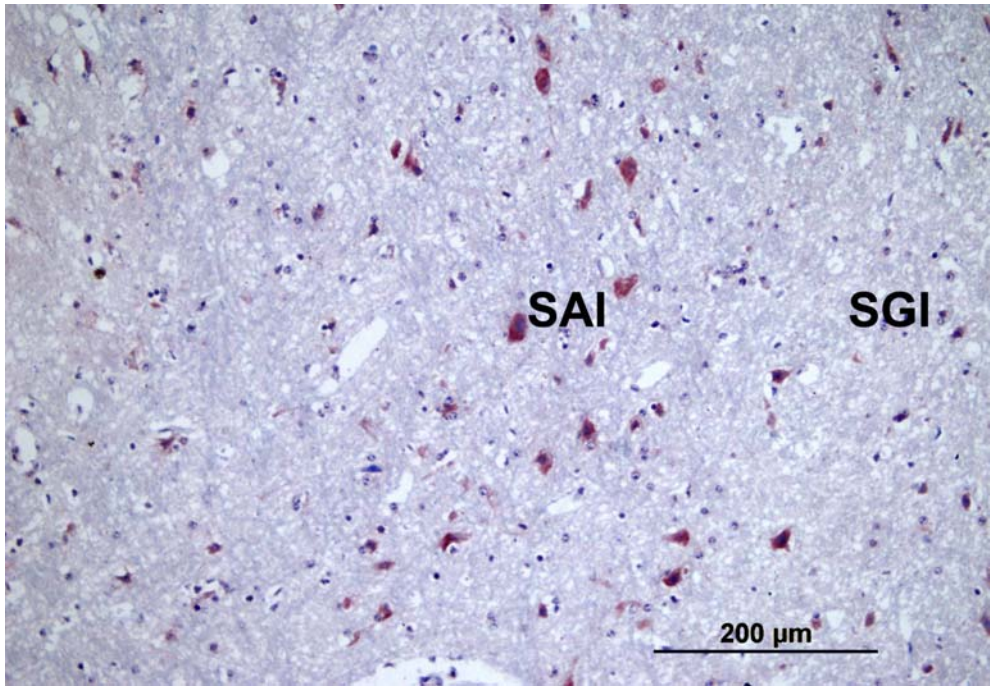

Figure 2. Serotonin immunoreactive cells, stratum album intermedium (SAI) and stratum griseum intermedium (SGI) of the porcupine superior colliculus.

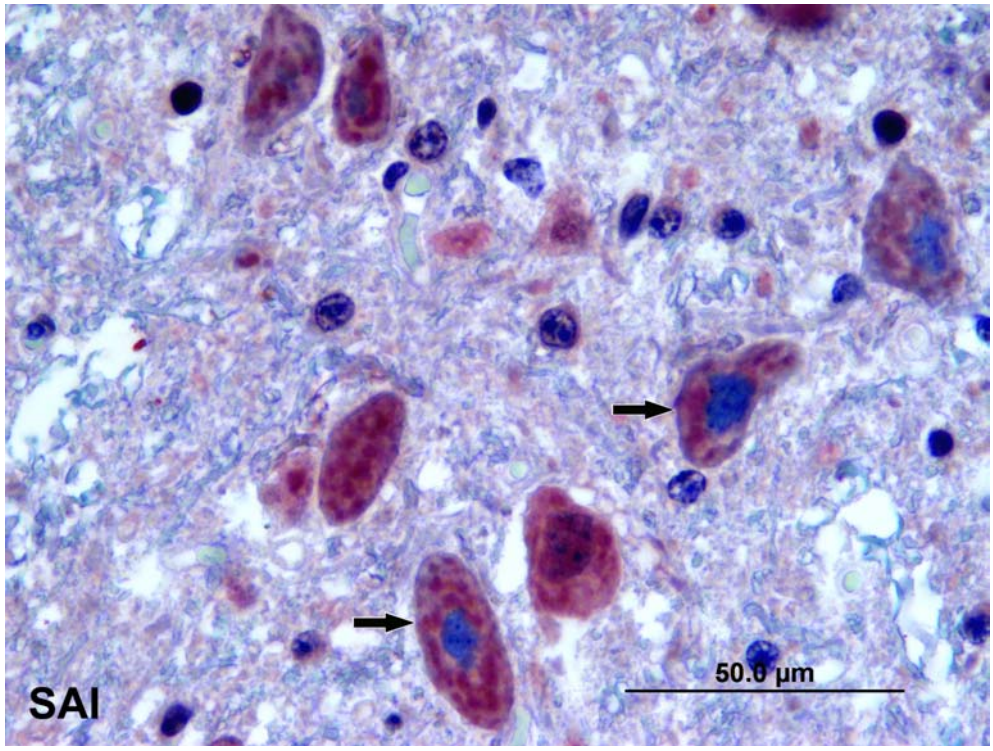

Figure 3. High magnification serotonin immunoreactive neurons (arrows) of the stratum album intermedium (SAI) of the porcupine superior colliculus.

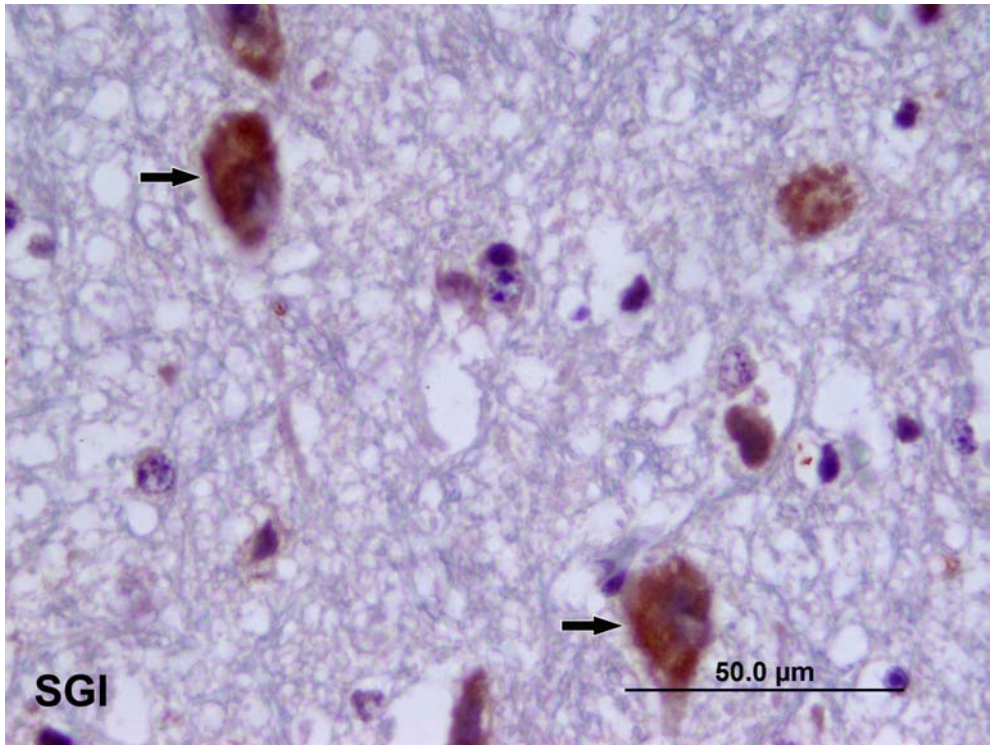

Figure 4. High magnification serotonin immunoreactive neurons (arrows) of the stratum griseum intermedium (SGI) of the porcupine superior colliculus.

This tier of immunoreactive neurons in the lower SGI could be seen throughout the rostrocaudal extent of the superior colliculus (Figure 4). Serotonin immunoreactive cells were more dense in the SAI than in the other SGI (Figures 2, 3). Serotonin immunoreactive neurons were selectively distributed in the porcupine (*Hystrix cristata*) superior colliculus.

DISCUSSION

In the study serotonin-containing fibers were dense in the stratum griseum superficiale of the mammalian superior colliculus.[14,17] In an other study a specific immunolabeling was observed in the superficial layer of the rat superior colliculus.[18] In the chipmunk, the serotonin fibers were denser in the stratum griseum intermedium than in the stratum griseum superficiale.[14]

In phylogenetic studies using immunohistochemistry, the organization of serotonin fibers was laminar in the optic tectum of amhibians and reptiles.[19,20] However, the serotonergic neuron cells in the mammalian superior colliculus were not as markedly laminated as in the submammalian optic tectum.

Recently, using immunohistochemistry,[21] demonstrared that serotonergic fibers and cells were distributed in all layers of the rat superior colliculus. The present study indicate that not only in the rat but also in porcupines examined, serotonergic cells were present throughout the superior colliculus. In our results, the densest concentration of serotonin immunoreactive neuron cells was found in a single tier located within the SZ and upper part of the SGS. The second densest concentration was located within SGI and the third appears as an incomplete layer, found only in the medial and central parts of the SGP, in the porcupines. These are generally in agreement with previous descriptions.[21]

The present study provides the first morphological description in the porcupine (*Hystrix cristata*) of the serotonin. Our results demonstrate that changes in the expression of serotonin occur in the superficial layers of the porcupine. This localization may be a reflection of the functioning of the serotonin as a neuromodulator and neurotransmitter in the superior colliculus of the porcupine.

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