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BRIEF COMMUNICATION

# Activated Astrocytes Display Increased 5-HT2a Receptor Expression in Pathological States

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**In human brain tissues from patients dying with cerebral infarction, hypertensive encephalopathy, Alzheimer's disease, Huntington's disease, frontotemporal dementia, and Creutzfeldt–Jakob disease there is an activation of astrocytes. Such activated astrocytes display GFAP and strong 5-$HT_{2A}$, but not 5-$HT_{2B}$ or 5-$HT_{2C}$, receptor immunoreactivity; this 5-$HT_{2A}$ reaction has not been observed in normal, nonactivated astrocytes. It is suggested that an up-regulation of 5-$HT_{2A}$ receptors may be part of an early response reaction in astrocytes, possibly designed to maintain homeostasis or to induce secondary message pathways involving trophic factors or glycogenolysis.** 

***Key Words:*** **5-$HT_2$ receptors; astrocytes; cerebral infarction; neurodegenerative disease.**

An activation of astrocytes occurs in many situations of nervous system damage which include head injury (1), tissue infarction, and hypertension (2) but this also takes place in the presence of certain neurodegenerative disorders such as Alzheimer's disease (AD) (3), Huntington's disease (HD) (4), frontotemporal dementia (FTD) (5), and Creutzfeldt–Jakob disease (CJD) (6). In morphological terms, this activation usually includes an enlargement of the cell body and an increase in the number, and an extension, of the dendrites; there is also an upregulation of particular proteins and their message, especially glial fibrillary acidic protein GFAP or S-100 protein (1–6).

In the course of a preliminary study on the immunolocalization of 5-$HT_{2A}$ receptor protein (7) we observed a strong immunoreaction in astrocytes in regions of brain tissue injury. This prompted us to perform a wider immunocytochemical survey on the cellular distribution of 5-$HT_2$ receptors in normal human brain and in tissues from patients with cerebral infarction and hypertensive encephalopathy or ones suffering from particular neurodegenerative disorders. In all instances reactive, but not inactivated, astrocytes strongly expressed 5-$HT_{2A}$, but not 5-$HT_{2B}$ or 5-$HT_{2C}$, receptor protein.

Blocks of frontal cortex (BA9) or caudate nucleus (in cases of HD) were cut from the formalin-fixed brains of 45 individuals who, in life, had suffered from a variety of neurological and neuropsychiatric illnesses associated with either cerebrovascular disease (4 cases, mean age 76.0 ± 6.9 years) or neurodegeneration (41 cases comprising, 14 cases AD, mean age 71.0 ± 6.4 years; 11 cases FTD, mean age 60.8 ± 8.9 years; 9 cases HD, mean age 59.8 ± 13.1 years and 7 cases CJD, mean age 59.4 ± 9.1 years) and from 6 other cases free from apparent neurological or psychiatric illness (mean age 76.3 ± 8.4 years). All clinical diagnoses had been confirmed by full pathological investigation (DMAM) at autopsy employing standard criteria (e.g., ADRDA criteria for AD, Lund–Manchester criteria FTD). Consecutive paraffin sections were cut at 6 μm thickness, mounted onto APES-coated slides, and immunostained using a standard avidin–biotin peroxidase methodology (Dako). Primary antibodies used were mouse monoclonals against 5-$HT_{2A}$, 5-$HT_{2B}$, or 5-$HT_{2C}$ receptor proteins (7, 8). To produce these antibodies mice were immunized with the peptides specific to each receptor subtype. The antigens were prepared as glutathionine *S*-transferase fusion proteins to insert into cells using a Baculovirus expression system. The resulting hybridomas were tested for their specificity by ELISA, Western blotting, and immunohistochemistry as detailed elsewhere (7, 8). Incubation in a primary antibody was overnight at 4°C and dilutions were 1/1000. Additionally, a polyclonal antibody to GFAP (Sigma Chemical Company) was used at dilution 1/750, incubation again being overnight at 4°C. GFAP immunoreaction was

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enhanced by microwaving the tissue sections in 0.1 M citrate buffer pH 7.6 (9). 5-$HT_2$ immunoreaction was performed without microwave pretreatment. Negative controls employed omission of primary antibody or substitution with rabbit IgG. The above pretreatment method for tissue sections did not allow satisfactory double staining with antibodies to GFAP and 5-$HT_2$ receptor proteins. In contrast, trypsin digestion (10) of a tissue section followed by incubation with rhodamine and FITC-labeled secondary antibodies permitted successful colocalization of GFAP and 5-$HT_{2A}$ receptor protein.

Control (i.e., neurologically normal) cases displayed no immunoreaction for 5-$HT_{2A}$, 5-$HT_{2B}$, or 5-$HT_{2C}$ receptor, nor for GFAP, except in respect of an occasional astrocyte within the gray or white matter lying close to small arteries displaying a fibrous or hyaline thickening of their walls, in relationship to an occasional deposit of amyloid β protein in the gray matter, or as a thin subpial network of GFAP-positive fibers (data not shown).

In consecutive sections of cerebral infarction there was intensive immunolabeling of GFAP-positive astrocytes for 5-$HT_{2A}$ receptor protein both around the margins of completed (old) infarcts or within areas of cerebral softening relating to more recent or incomplete infarctions (Figs. 1a and 1b). In cases of hypertensive encephalopathy there was intense immunolabeling of astrocytes and their processes around the fibrosed or hyalinized arteries within cerebral white matter with both GFAP (Fig. 1c) and 5-HT-2a (Fig. 1d).

In AD, GFAP-positive astrocytes in and around the Aβ plaques (Fig. 2a) were also strongly positive for 5-$HT_{2A}$ receptor protein (Fig. 2b), as were astrocytes in the caudate nucleus in HD (Figs. 2c and 2d) and in the cerebral cortex in CJD (Figs. 2e and 2f) and in frontotemporal dementia due either to microvacuolar (not shown) or Pick-type (Figs. 2g and 2h) degeneration. In no instance was there astrocytic staining for 5-$HT_{2B}$ or 5-$HT_{2C}$ receptor protein.

Double-staining immunofluorescence of tissue sections further confirmed the colocalization of GFAP and 5-$HT_{2A}$ receptor protein in reactive astrocytes (Fig. 3).

The presence of neurotransmitter receptors on the cell surface of glial cells has been revealed by a variety of methods including calcium imaging, electrophysiol-

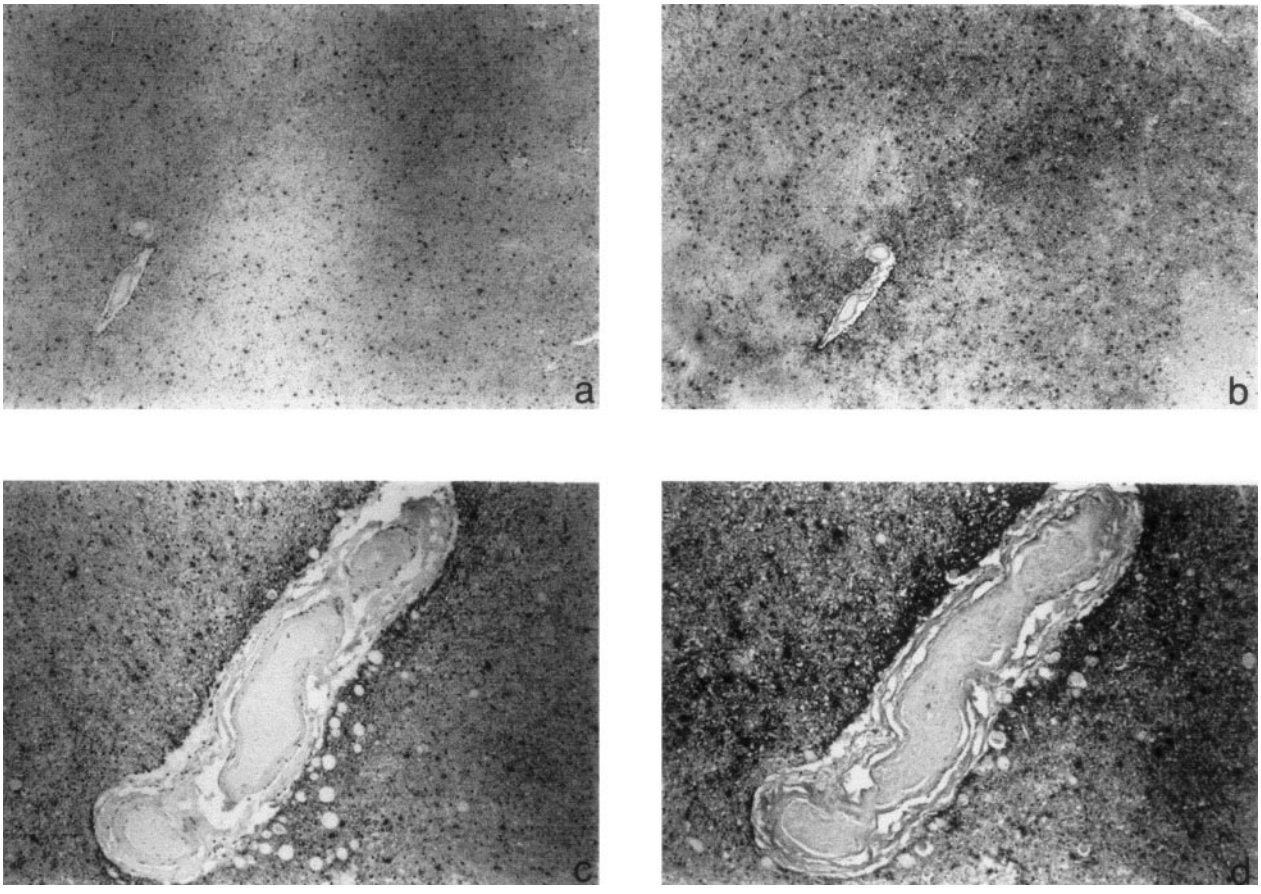


**FIG. 1.** Activated astrocytes in consecutive sections of an area of recent infarction display strong GFAP (a) and 5-HT-2a (b) receptor immunoreaction. There is also pronounced GFAP (c) and 5-HT-2a (d) immunoreaction in astrocytes surrounding a small artery showing hypertensive changes. Immunoperoxidase–hematoxylin; ×30 (a,b); ×150a (c,d).

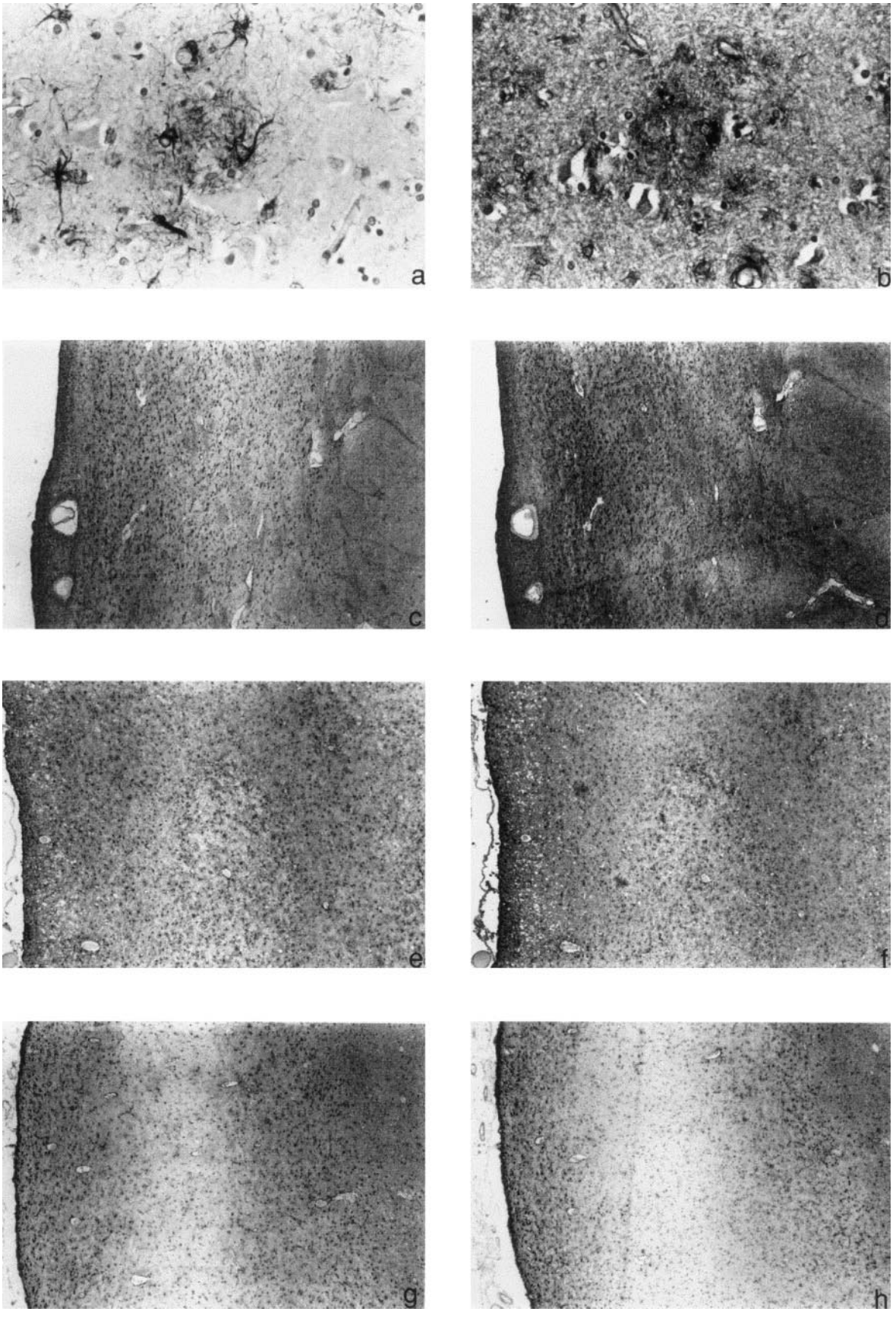


**FIG. 2.** Activated astrocytes in consecutive sections of amyloid plaques in AD (a,b), caudate nucleus in HD (c,d), and frontal cortex in CJD (e,f) and frontotemporal dementia (g,h) show similar patterns of immunoreactivity for both GFAP (a,c,e,g) and 5-HT-2a receptor protein (b,d,f,h). Immunoperoxidase–hematoxylin; ×300 (a,b); ×30 (c–h).

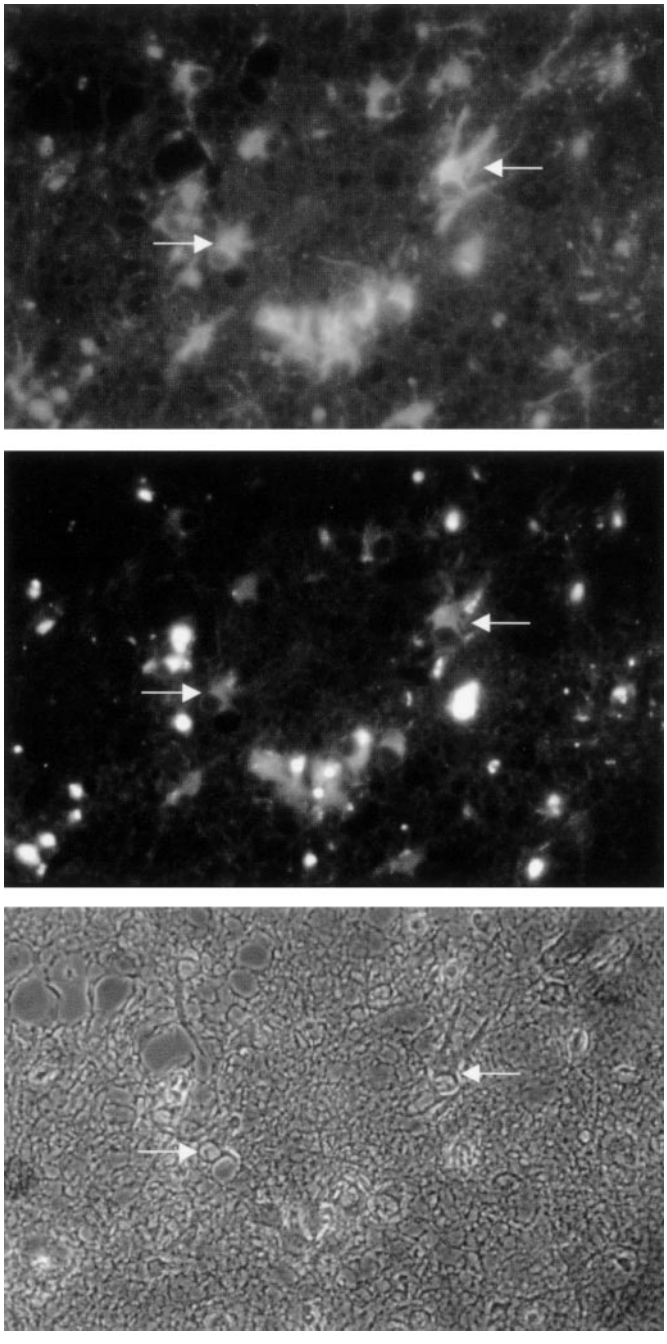

**FIG. 3.** Colocalization of GFAP (top) and 5-$HT_{2A}$ receptor (middle) proteins in reactive astrocytes (arrow). Double-stained immunofluorescent preparation: top (FITC), middle (rhodamine), and bottom phase contrast ×400.

ogy, *in situ* hybridization, and RT-PCR (11–14). Among neurotransmitters, 5-HT was found to interact with specific receptors expressed by astrocytes. Binding to its receptor, 5-HT was shown to cause changes in the cerebral microcirculation (15) and to effect growth and migration of the astrocytes themselves (12, 13, 15). 5-HT can also influence the CNS plasticity by stimulating astrocytic production of S-100β, which is a calcium-binding protein known to induce continuous migration of neurons (16). By RT-PCR, it was found that 5-$HT_{2A}$ receptor was strongly expressed in glioma cells but was expressed very weakly in normal astrocytes, whereas 5-$HT_{2C}$ receptor was expressed in the glioma cell lines but not in normal astrocytes (13). 5-HT was also found to positively modulate glioma cell proliferation, migration, and invasion *in vitro*. Recently, Schwann cells expressing 5-HT receptors were found to be a quiescent and myelinating phenotype and that 5-HT receptors are expressed as a function of differentiation in Schwann cells (17–19).

This study clearly shows that as part of the process of activation following nervous tissue damage astrocytes come to contain increased amounts of 5-$HT_{2A}$ receptor protein. Normal (i.e., nonactivated) astrocytes contain either no 5-$HT_{2A}$ receptor protein or if so express this at levels below the threshold to immunodetection. The change is restricted to astrocytes; microglial cells, even when activated as in AD plaques do not display this property and furthermore the change is limited to 5-$HT_{2A}$ receptor with no (immunohistochemically) detectable changes in 5-$HT_{2B}$ or 5-$HT_{2C}$ receptor occurring within such activated astrocytes. Moreover, nerve cells do not come to contain (express) 5-$HT_{2A}$ receptor protein, even under pathological situations where the astrocyte reaction is florid.

The purpose of, or the triggering mechanism underlying, this aspect of astrocyte activation is not clear. Astrocytes are exquisitely sensitive to neurotransmitters showing physiological responses to noradrenaline, via both α- and β-adrenoceptors, with the number of β-adrenoceptor-binding sites per cell increasing in gliosis (20, 21). They also possess receptors for glutamate and GABA (21). Further, they have uptake systems for all these latter transmitters along with that for 5-HT (23). The CNS function mediated through these properties is one of regulation of brain microenvironment, this being achieved through the balancing of ion flows, particularly $K^+$ and $Cl^-$, and pH between neurons and the extracellular fluid. The catabolism of any excess of released neurotransmitter as a waste product that neurons are unable to deal with may be a further aspect of this homeostatic function.

In pathological states the transmitter uptake systems in neurons may become impaired and the need for homeostatic maintenance of the extracellular fluid by astrocytes may be increased. Reactive changes involving the extension of cell processes and a swelling of the cell body may be the most visible aspect of this activity and the upregulation of the neurotransmitter uptake system, via 5-$HT_2$ and other receptors perhaps being critical to this requirement to deal with excess transmitter in the extracellular fluid. Whether these reactive changes are beneficial or ultimately deleterious to

brain (neuronal) function is not clear but warrant further investigation.

Stimulation of β-adrenoceptors on astrocytes leads to increases in (a) glycogen metabolism, (b) release of taurine, S100 protein and NGF, (c) changes in membrane potential, (d) up-regulation of early response genes, and (e) morphological changes (11). These responses are mediated by an activation of protein kinase A leading to intracellular increases in cAMP through stimulation of adenylate cyclase. The up-regulation of 5-$HT_{2A}$ receptors may form part of this early response procedure in astrocytes following perturbations of the extracellular fluid, in an attempt to restore the external milieu and stabilise neuronal function. Whether activation of 5-HT-2a receptors triggers any secondary message pathways in astrocytes (e.g., release of S-100 or other trophic factor or as part of glycogenolysis in the supply of energy to bolster compromised ionic transport system in neurons) is not known though this may be one (of many) facets to the activation process which crosses the boundaries between the many and varied pathological insults that afflict the brain.

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