5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: Possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites

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ABSTRACT To identify the cortical sites where 5-hydroxytryptamine2A (5-HT2A) serotonin receptors respond to the action of hallucinogens and atypical antipsychotic drugs, we have examined the cellular and subcellular distribution of these receptors in the cerebral cortex of macaque monkeys (with a focus on prefrontal areas) by using light and electron microscopic immunocytochemical techniques. 5-HT2A receptor immunoreactivity was detected in all cortical layers, among which layers II and III and layers V and VI were intensely stained, and layer IV was weakly labeled. The majority of the receptor-labeled cells were pyramidal neurons and the most intense immunolabeling was consistently confined to their parallely aligned proximal apical dendrites that formed two intensely stained bands above and below layer IV. In double-label experiments, 5-HT2A label was found in calbindin D28k-positive, nonphosphorylated-neurofilament-positive, and immuno-negative pyramidal cells, suggesting that probably all pyramidal cells express 5-HT2A receptors. 5-HT2A label was also found in large- and medium-size interneurons, some of which were immuno-positive for calbindin. 5-HT2A receptor label was also associated with axon terminals. These findings reconcile the data on the receptor’s cortical physiology and localization by (i) establishing that 5-HT2A receptors are located postsynaptically and presynaptically, (ii) demonstrating that pyramidal neurons constitute the major 5-HT2A-receptor-expressing cells in the cortex, and (iii) supporting the view that the apical dendritic field proximal to the pyramidal cell soma is the “hot spot” for 5-HT2A receptor-mediated physiological actions relevant to normal and “psychotic” functional states of the cerebral cortex.
staining was identical. For light microscopic experiments, 0.3%
Triton X-100 detergent was added to the antisera solutions and
the staining of some sections was intensified with 0.01% osmium
tetroxide. Cortical areas and layers were delineated according to
the nomenclature of Brodmann (27) and Walker (28) on adjacent
immunoreacted sections counterstained with cresyl violet. The
sections were photographed with a Zeiss Aristoplan microscope.
Prefrontal (area 46) sections from each monkey were postfixed in
1% osmium tetroxide in PB for 45 min, flat-embedded in
Durcupan ACM (Fluka), cut serially into ultrathin sections on an
Ultramicrotome (Reichert), stained with uranyl acetate and lead
citrate, and examined with a JEOL 1010 transmission electron
microscope. Because the nonphosphorylated neurofilament pro-
tein SMI-32 and the calcium-binding protein calbindin D28k
(CB) are excellent markers of pyramidal cell subgroups (29–31),
selected 5-HT2A-immunoreacted prefrontal sections were further
incubated with either SMI-32 (1:5,000 dilution; Sternberger–
Meyer, Jarrettsville, MD) or CB (1:10,000 dilution; Sigma) mouse
antibodies for 2 days at 4°C, treated with biotinylated anti-mouse
antiserum (1:250 dilution; 1 h) and the ABC Elite reagent (1:100
dilution; 1 h), and reacted with a chromogen of different color
than the one used in the first immunostaining. When one of
the primary antisera was omitted, only single staining was observed;
when both were omitted, no staining was detected. The specificity
and use of the CB and SMI-32 antisera have been described (29,
32). The 5-HT2A receptor antibody was produced by PharMingen
by using a recombinant fusion protein between glutathione
S-transferase (GST) and human 5-HT2A receptor (amino acids
1–72) as immunogen. The hybridomas were screened by Phar-
Mingen by using ELISA, immunocytochemistry of frozen rat
brain sections, and Western blot analysis: the 5-HT2A antibody
recognizes the receptor as a 55-kDa band. The specificity of the
band was verified by competition with a 5-HT2A receptor fusion
protein and lack of competition with an irrelevant fusion protein.
The supernatant was also tested against GST protein to rule out
reactivity to this portion of the immunogen. The antibody was
purified from hybridoma culture supernatant by protein G affinity
chromatography. The antibody has specifically shown to react
with human, monkey, and rat 5-HT2A receptor.

RESULTS
5-HT2A receptor immunoreactivity was observed throughout the
anterior (area 24) and posterior (area 23) areas of the cingulate
cortex; prefrontal cortical areas 9, 11, 12, and 46; motor cortex
(areas 4 and 6); temporal cortical areas 21, 22, and 41; insular
cortex; and primary and secondary visual cortex (areas 17 and
18). Throughout the cortical sheet, the receptor labeling featured
a weakly stained band located in layer IV flanked by two intensely
labeled bands in layers II and III and layers V and VI (Fig. 1
B–D). In area 46 of the prefrontal cortex, 5-HT2A labeled pyramidal cells were densely distributed throughout
layers II, III, V, and VI (Fig. 1B). Apparently the majority (if not all) of pyramidal neurons expressed 5-HT2A label. The receptor
label was consistently found to be most intense in the proximal
part of their apical dendrites (Fig. 1B–D). Perikarya and
distal dendritic branches were labeled with moderate intensity
(Fig. 1C and D). Dendritic spines were devoid of the immuno-
label completely or occasionally labeled weakly. 5-HT2A label was
also detected in large- and medium-size (12–30 μm in diameter)
nonpyramidal neurons (Figs. 1C and 2A) through layers II–VI
and very rarely in layer I, but the majority of the labeled
interneurons was located in infragranular layers. The label was
strongest in proximal dendrites.

Electron microscopic analysis confirmed the presence of
5-HT2A receptor labeling in the cytoplasm of pyramidal (Fig. 2C)
and nonpyramidal neurons (Fig. 2B), substantiated that the
receptor label is strongest in their proximal large-diameter
dendrites (Fig. 2C), and revealed 5-HT2A label in axons.
Somata (Fig. 2B and C) and thin (distal) dendrites (Fig. 3
A–D) demonstrates receptor-labeled pyramidal cells (p), unlabeled
(asterisks) and labeled nonpyramidal (np) cells, and receptor-positive fine
processes (their ultrastructure is shown in Fig. 3). Note that the immu-
noreaction is strongest in the apical dendrites (arrows) of pyramidal cells
and relatively weak in perikarya. [Bars = 1 cm (A), 0.5 mm (B), 50 μm
(C), and 20 μm (D).]
A–C) were moderately stained. Dendritic spines were rarely and weakly labeled (Fig. 3A–C). The nucleus, mitochondria, and Golgi apparatus of labeled cells was immunonegative. Labeled nonpyramidal neurons (Fig. 2B) typically exhibited large- or medium-size cell body, infolded nucleus with an intranuclear rod (B3, small arrows), nuclear infolding (B3, open arrows), and axosomatic asymmetric synapses (B1 and B2, arrowheads), three typical features of cortical interneurons. (C) The receptor label concentration is high in the pyramidal cell apical dendrite (arrows) and low in the perikaryal cytoplasm. [Bars = 25 μm (A), 5 μm (B and C), and 0.5 μm (B1–B3).]

In 5-HT2A/CF and 5-HT2A/SMI-32 double-label experiments, both CB-positive supragranular and CB-negative infragranular pyramids (Fig. 4B–E) and both SMI-32-positive (Fig. 4F and G) and SMI-32-negative pyramidal cells contained 5-HT2A label. The double-label patterns accented the intracellular segmenta-

ton of the receptor label. Note in Fig. 4B–G that the color of the 5-HT2A label dominates in the proximal apical dendrites but that CB and SMI-32 labels highlight the cell bodies. The presence of SMI-32 label in distal dendrites (Fig. 4G) indicates that the weak 5HT2A receptor label in the same cell segment is not due to a possibly inadequate antibody penetration, affirming that the intradendritic segmentation of 5-HT2A receptor label is a true feature of pyramidal cells.

**DISCUSSION**

**Postsynaptic Localization of 5-HT2A Receptor.** The findings presented herein clearly demonstrate that the bulk of 5-HT2A receptor-expressing cells in the primate cortex are pyramidal
neurons, and the receptor label is most intense in their apical dendrites, in agreement with recent immunocytochemical findings in the rat cortex (20). Although previous studies using autoradiographic detection of receptor binding (14) or in situ hybridization histochemistry have inferred that pyramidal neurons contribute to the expression of 5-HT2A receptor (8, 11, 17), the fact that, in the present study, neither CB- and SMI-32-positive pyramidal cells nor pyramids lacking these markers are seen without 5-HT2A label indicates the absence of any major 5-HT2A-receptor-lacking pyramidal cell class and suggests the nonselective presence of 5-HT2A receptor in pyramidal cells.

In cortical interneurons, 5-HT2A receptor expression is selective: large- and medium-size interneurons were labeled, whereas many small- and medium-size interneurons were unlabeled. The predominance of 5-HT2A-expressing interneurons in infragranular layers is in agreement with the preservation of 5-HT2A receptor immunoreactivity in the proximal dendrites of pyramidal and nonpyramidal neurons in prefrontal area 46. The neurons were neurochemically identified by using 5-HT2A/CB (B–E) or 5-HT2A/SMI-32 (F–G) double immunocytochemistry. Arrowheads point to dendrites, arrows point to pyramidal cell somata, and open arrows point to nonpyramidal cell bodies. (A) 5-HT2A-positive pyramidal cell in layer III (stained purple with the Vector VIP staining kit). (B and C) Two 5-HT2A/CB double-stained sections from cortical layer III demonstrate that 5-HT2A immunoreactivity (arrowheads) is colocalized with CB immunolabel (arrows) in pyramidal neurons. (B) 5-HT2A label is light brown and CB label is bluish gray. (C) Double labeling is “reverse,” 5-HT2A label is bluish gray, and CB label is light brown. (D and E) Two micrographs from layer V demonstrate that large- (asterisk) and medium-size interneurons (open arrows) colocalize 5-HT2A receptor and CB and show that 5-HT2A receptor is also present in CB-negative pyramidal cells (arrows), typical of the infragranular layers. (D) 5-HT2A, light brown; CB, bluish gray. (E) 5-HT2A, bluish gray; CB, light brown. (F and G) 5-HT2A/SMI-32 double-labeled pyramidal cells from layer III. Note that the gray color of 5-HT2A label dominates only in the proximal apical dendrites (within the frame), whereas thinner dendritic branches and the cell body are overshadowed by the brown-colored SMI-32 staining.

![Fig. 3](image-url)  
**Fig. 3.** Postsynaptic (A–C) and presynaptic (B, D–F) localization of 5-HT2A receptor immunoreactivity in area 46 of the prefrontal cortex. (A and B) 5-HT2A label is localized to dendritic shafts (d), but dendritic spines (s) are generally immunonegative. Unlabeled axons form asymmetric synapses (arrowheads) on these dendrites. (C) A rare example of two weakly labeled dendritic spines (s). (B, D–F) Immunolabeled axon terminals (asterisks) forming asymmetric synapse (E), arrowheads) or containing dense core vesicle (F). 5-HT2A label is restricted to a portion of the axoplasm. (Bars = 0.5 μm.)

![Fig. 4](image-url)  
**Fig. 4.** Color light micrographs demonstrate the accumulation of 5-HT2A receptor immunoreactivity in the proximal dendrites of pyramidal and nonpyramidal neurons in prefrontal area 46. The neurons were neurochemically identified by using 5-HT2A/CB (B–E) or 5-HT2A/SMI-32 (F–G) double immunocytochemistry. Arrowheads point to dendrites, arrows point to pyramidal cell somata, and open arrows point to nonpyramidal cell bodies. (A) 5-HT2A-positive pyramidal cell in layer III (stained purple with the Vector VIP staining kit). (B and C) Two 5-HT2A/CB double-stained sections from cortical layer III demonstrate that 5-HT2A immunoreactivity (arrowheads) is colocalized with CB immunolabel (arrows) in pyramidal neurons. (B) 5-HT2A label is light brown and CB label is bluish gray. (C) Double labeling is “reverse,” 5-HT2A label is bluish gray, and CB label is light brown. (D and E) Two micrographs from layer V demonstrate that large- (asterisk) and medium-size interneurons (open arrows) colocalize 5-HT2A receptor and CB and show that 5-HT2A receptor is also present in CB-negative pyramidal cells (arrows), typical of the infragranular layers. (D) 5-HT2A, light brown; CB, bluish gray. (E) 5-HT2A, bluish gray; CB, light brown. (F and G) 5-HT2A/SMI-32 double-labeled pyramidal cells from layer III. Note that the gray color of 5-HT2A label dominates only in the proximal apical dendrites (within the frame), whereas thinner dendritic branches and the cell body are overshadowed by the brown-colored SMI-32 staining.
studies (19, 34) to detect 5-HT_{2A} receptor in pyramidal cells is harder to reconcile with the rest of the literature and the present data; the antiserum may have recognized an epitope or receptor subunit expressed in interneurons and not pyramidal cells.

**Presynaptic Localization of 5-HT_{2A} Receptor.** The presence of the receptor label in a minor group of asymmetric synapse-forming cortical axons suggests that 5-HT_{2A} receptors may presynaptically modulate excitatory neurotransmission in a discrete cortical axonal system. The presynaptic serotonergic modulation of glutamatergic cortical neurotransmission has been implied by the reduction of serotonin-induced excitatory postsynaptic potentials by an inhibitory metabotropic gluta-

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**5-HT_{2A}-Receptor-Rich Apical Dendrites, a Possible Site for Switching Pyramidal Cell Firing from Normal to “Psychotic.”** It is postulated that 5-HT_{2A} receptors accumulated in the apical dendritic “bottle-neck” segment of pyramidal cells have a strategic role in controlling the rate of dendritic currents that can pass through the apical dendrite and reach the soma. The manipulation of ion channels along the pyramidal cell apical dendrite can amplify or gate this information flow (41–43), and 5-HT_{2A} receptors are able to modulate these ion channels; their activation in the microenvironment of apical dendrites enhances a subthreshold tetrodotoxin-sensitive inward current (22), and this current underlies a postsynaptic amplification of excitatory postsynaptic potentials impinging upon apical dendrites (41, 44). We suggest that this apical dendritic gating mechanism may play an important role in working memory processes and the related behavioral phenomenon called latent inhibition. Working memory reflects a capacity of neurons in association cortices to keep information “on line” (45), whereas latent inhibition is a capacity of all vertebrates to attenuate the rate at which a stimulus can evoke a conditioned response, if the prior exposure of that stimulus has been without consequence for the human or animal subject (46–48). Both phenomena are associated with temporal processing of information that, under normal circumstances, could prevent sensory overload and distractibility; and both phenomena are impaired in schizophrenic patients (for review see refs. 49 and 50). The entire pyramidal dendritic tree is bombarded by sensory and intrinsic inputs but, probably in part due to the gating mechanism of apical dendritic ion channels, only a fraction of these stimuli reaches the soma and results in cell firing during normal behavioral states. A nominal 5-HT_{2A} receptor activation seems beneficial for working memory because 5-HT_{2A} agonists increase activity for preferred directions and reduce activity for nonpreferred directions in pyramidal cells in monkeys performing spatial delayed-response task (G. V. Williams, S. G. Rao, and P.S.G.-R., unpublished data). However, excessive 5-HT_{2A} receptor activation may be disruptive. For example, 5-HT_{2A} agonists impair latent inhibition in rats (51). We suggest that the gating mechanisms of apical dendritic ion channels may be dysfunctional in psychotic behavioral states occurring in the acute “positive” phase of schizophrenia or induced by 5-HT_{2A} agonist drugs (e.g. the powerful “recreational drug 3,4-methylenedioxy-
methamphetamine, also known as “Ecstasy”). Our hypothesis infers that the hallucinogenic-drug-induced state and the acute schizophrenic condition both are concomitant with the inability of pyramidal cells to switch to a resting firing mode after or in the absence of a specific stimulus. An excessive rate of dendritic currents reaching the soma may underlie the pow-

dercial hallucinogenic effect of 5-HT_{2A} agonists. Similarly, hyperactive pyramidal cells may produce the “positive” hallucinosis-like behavioral symptoms observed in acute schizophrenia due to a dysfunctional 5-HT_{2A} receptor system in their apical dendrites. A compromised protein kinase C-mediated 5HT_{2A} signal transduction cascade in the pyramidal cells (17) may send erroneous signals to the apical dendritic ion channels. Willins and Roth recently have shown that the administration of the partial 5-HT_{2A} antagonist drug, clozapine, to the rat cortex down-regulates apical dendritic (but not perikaryal) 5-HT_{2A} receptors (personal communications). Clozapine’s ca-

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**Laminar Localization of 5-HT_{2A} Receptor, a Consequence of Intracellular Segmentation.** It has long been recognized that 5-HT_{2} receptors have a laminar pattern in the cortex (9, 13). The finding that virtually all pyramidal cells exhibit 5-HT_{2A} receptor label makes it unlikely that the laminar pattern would be based on an alternation of 5-HT_{2A}-lacking and 5-HT_{2A}-containing pyramidal cells. Instead, it appears to be a conse-

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**Accumulation of 5-HT_{2A} Receptors in Proximal Apical Dendrites.** The disproportionately high concentration of 5-HT_{2A} receptor labeling in the proximal apical dendritic segment may indicate that the accumulated protein is undergoing transport to other segments of the dendritic tree, but may as well suggest a unique receptivity of this segment to actions mediated by 5-HT_{2A} ligands. Because receptor and ligand concentrations determine physiological effectiveness (40), it is remarkable that serotonin concentrations also appear to be high in the microenvironment of these segments: a dense band of 5-HT_{2} receptors in upper layer V has been found in register with a dense plexus of fine 5-HT axons (14), and dendritic shafts and not spines have been shown to be the most common targets of serotonin-immunolabeled cortical synaptic boutons (37). Consistent with this is the lack of labeling in dendritic spines, suggesting that this segment is not involved in 5-HT_{2A}-mediated neurotransmission. Finally, the “key” physiological finding that microiontophoresis of serotonin only to hot spots near the border of layers IV and V within the apical (but not basi-

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proteins at this site in schizophrenic patients and animal subjects with experimentally induced “psychotic” symptoms.

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