Neurons Expressing 5-HT_2 Receptors in the Rat Brain: Neurochemical Identification of Cell Types by Immunocytochemistry

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The serotonin$_2$ (5-HT_2) receptor has been implicated in a number of behavioral and physiological processes. It may also play a role in cellular development and differentiation, and represents a site of action of hallucinogens and certain psychotherapeutic drugs. To better understand the functions and regulation of the 5-HT_2 receptor, we have undertaken a series of studies in which we attempted to identify the specific cell types that express the receptor. This was accomplished using a variety of double-labeling strategies with an antibody we raised against the rat 5-HT_2 receptor protein. In this review, we recount of some of our previously published findings and present some new data in which we identify subpopulations of cholinergic neurons in the brainstem and gamma-aminobutynic acid (GABA)ergic interneurons in the cortex that express 5-HT_2 receptor immunoreactivity. Developmentally, the appearance of 5-HT_2 receptor immunoreactivity occurs relatively late in the ontogeny of the cells in which it is expressed, mostly in the early postnatal period. This argues against a significant role for this receptor in early development, though it may participate in some aspect of terminal differentiation. We discuss the significance of the cell-type-specific and temporal expression of the 5-HT_2 receptor in the context of current hypotheses of neuropsychiatric disorders such as schizophrenia.
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KEY WORDS: *5-HT_2 receptors; Immunocytochemistry; Gamma-aminobutyric acid; Schizophrenia*

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The post-synaptic effects of serotonin (5-HT) in the brain are transduced by a number of receptor subtypes that have been classified according to various criteria, including G-protein coupling and second messenger effector systems, agonist and antagonist binding profiles, and nucleic acid sequence homology of cloned cDNAs. The 5-HT_2 receptor subtype was one of the first subtypes to be identified based on binding affinity profiles, and yet this receptor has remained one of the more elusive in terms of ascribing to it a behavioral or physiological function. The 5-HT_2 receptor has been implicated in a number of processes in the brain, though none definitively. It is of particular interest because it has also been implicated in the mechanism of action of a variety of therapeutic and recreational pharmacological agents, including hallucinogens (Glennon et al. 1986; Heym and Jacobs 1987) and atypical antipsychotics (Meltzer et al. 1989). Serotonin has also been

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proposed to function not only as a neurotransmitter in the central nervous system, but also as a potential morphogen, mitogen, or other type of regulatory factor in neuronal development (Lauder 1990), and the 5-HT_2 receptor has been demonstrated to promote cellular proliferation, growth and/or differentiation in non-neural cell systems (Nemecek et al. 1986; Julius et al. 1990; Corson et al. 1992). Thus, it is possible that the 5-HT_2 receptor may also transduce some of the developmental effects of 5-HT in the brain.

Without a clear hypothesis of the function of the 5-HT_2 receptor at the cellular and cell-circuit level, however, understanding the regulation of 5-HT_2 receptors and its clinical implications has proven difficult. Thus, in order to better understand the function and regulation of 5-HT_2 receptors in the CNS, we understood a series of studies aimed at identifying the specific cell types within the brain that express 5-HT_2 receptors, and the developmental pattern of receptor expression in these cells. Such an approach requires a high degree of anatomical resolution at the cellular level. Accordingly, we have adopted the strategy of immunocytochemistry using an antibody that we raised against the rat 5-HT_2 receptor protein.

METHODS

Immunocytochemistry

The production and characterization of our polyclonal 5-HT_2 receptor antibody has been described previously (Garlow et al. 1993). Rats brains were perfusion-fixed with 4% paraformaldehyde, and 50 μm cryostat sections were processed free-floating for 5-HT_2 immunocytochemistry. For our standard immunoperoxidase studies (Morilak and Ciaranello 1993b; Morilak et al. 1993), sections were washed in PBS and preincubated in blocking buffer (PBS with 5% BLOTTO, 0.3% Triton X-100). They were then incubated for 2 to 4 hours at 24°C and then 3 days at 4°C in affinity-purified anti-5-HT_2 antibody diluted 1/50 (approximately 2 μg/ml) in blocking buffer containing 0.05% thimerosal. After washing, sections were incubated for 3 hours in biotinylated sheep anti-rabbit IgG (Sigma) diluted 1/250 in blocking buffer, followed by 90 minutes in a streptavidin-horseradish peroxidase conjugate (Jackson Laboratories) diluted 1/3000 in PBS. The immunoperoxidase reaction was then carried out using 3,3'-diaminobenzidine (DAB) as the chromagen.

For our immunofluorescence studies (Morilak and Ciaranello 1993a), primary anti-5-HT_2 antibody was diluted 1/25, and primary anticholine acetyltransferase (ChAT; Chemicon) was diluted 1/1500. Secondary antibodies (Jackson Laboratories), diluted 1/100, were tagged with either fluoroscein or rhodamine.

Postembedding Immunocytochemistry

To reveal gamma-aminobutyric acid (GABA) immunoreactivity together with 5-HT_2 receptor immunoreactivity, we modified the method of Somogyi et al. (Somogyi et al. 1984; Somogyi 1988). Animals were perfused with 4% paraformaldehyde/0.1% glutaraldehyde, and 5-HT_2 immunocytochemistry was performed as above. Floating 50 μm sections were then washed thoroughly and embedded in epoxy resin as previously described (Somogyi and Takagi 1982). The sections were mounted in resin on glass slides and coverslipped. Following evaluation and photography of selected cells in the light microscope, the coverslip was removed and a small area of cortex containing 5-HT_2 receptor-positive cells was cut out and reembedded for sectioning on an ultramicrotome. Adjacent serial 0.5 μm sections were mounted on gelatine-coated slides.

Postembedding immunoprocessing was carried out as described previously (Somogyi et al. 1984; Somogyi 1988) with the addition of proteinase-K treatment (Romjin et al. 1993). Sections were blocked for 1 hour with 20% normal swine serum, and then incubated in anti-GABA antiserum (Hodgson et al. 1985), diluted 1/1000 in 1% normal swine serum for 3 hours. After rinsing, sections were then incubated for 3 hours in horseradish peroxidase-conjugated swine antirabbit secondary antiserum (Vector), diluted 1/50. The second DAB immunoperoxidase reaction was then performed, and the reaction product intensified in dilute OsO_4 for 3 to 5 minutes.

As both the 5-HT_2 and GABA immunoreactivities were revealed using a DAB immunoperoxidase technique, adjacent semithin sections through the same cells were used as controls to ensure that no false positive GABA immunoreactivity resulted from either residual enzyme activity or reaction product generated in the first set of reactions. For adjacent control sections, anti-GABA serum was replaced with nonimmune rabbit serum at the same dilution. No peroxidase reaction product was detected in these sections.

In Situ Hybridization Histochemistry for Glutamic Acid Decarboxylase

In situ hybridization histochemistry was performed to label mRNA for glutamic acid decarboxylase (GAD) as a marker for GABA-synthesizing neurons. A 0.5-kb Bam HI/Eco RV fragment of the rat GAD_{67} cDNA was subcloned into pBluescript (Stratagene) for riboprobe synthesis (parent cDNA clone provided by Dr. Allan Tobin, University of California, Los Angeles). Transcription was carried out using α-^{35}S-UTP to generate radiolabeled riboprobes. Brains were perfused, frozen, and cut as above. Free-floating 30 μm brain sections

were rinsed in PBS, acetylated (0.25% acetic anhydride, 0.1% triethanolamine, pH 8.0), and then rinsed in 1× SSC (150 mmol sodium chloride, 15 mmol sodium citrate, pH 7.2). Hybridization with radiolabeled riboprobe (8×10^7 cpm/ml) was carried out overnight at 55°C in hybridization buffer containing 50% formamide. Sections were then rinsed, treated with RNAse A (20 µg/ml, 37°C for 30 minutes), and carried through a series of washes of increasing stringency (15 minutes each in 1× SSC, 0.5× SSC, 0.2× SSC at 24°C, then 2 × 1 hour in 0.2× SSC, 45% formamide, 55°C). They were then rinsed, mounted on poly-L-lysine coated slides, dipped in photographic emulsion, and exposed at 4°C for 72 hours. The slides were then developed using standard darkroom chemistry and coverslipped for light microscopy. Control sections were hybridized with the sense-strand riboprobe.

RESULTS AND DISCUSSION

Initially, we mapped 5-HT$_2$ receptor immunoreactive neurons throughout the rat brain. We observed populations of immunolabeled neurons in the olfactory bulb, olfactory tubercle, ventral pallidum, Islands of Calleja, nucleus accumbens, neostriatum, hippocampus, piriform and entorhinal cortex, neocortex, and in a limited number of nuclei in the brainstem, including the anterior pretectal nucleus of the thalamus, nucleus tractus solitarius, spinal trigeminal nucleus, and dorsal pontine tegmentum (Morilak et al. 1993). These observations were in good general agreement with previous studies describing 5-HT$_2$ receptor localization using radioligand binding (e.g., Pazos et al. 1985; Schotte and Leysen 1989; Appel et al. 1990), with the added dimension that we were able to describe in detail the morphological characteristics of cells in these regions that expressed immunoreactivity. Moreover, these results allowed us to make inferences regarding the functional identity of subpopulations of 5-HT$_2$-bearing cells in the brain, hypotheses that we then tested using a series of double-labeling strategies. These studies have produced a number of surprising observations, which are described below.

5-HT$_2$-Expressing Cholinergic Neurons in the Dorsal Pons

Our first novel observation was in the dorsal pontine tegmentum, where two dense clusters of immunolabeled neurons were seen in the pedunculopontine and laterodorsal tegmental nuclei (Ppt and Ldt). These cells bore a striking resemblance in both their distribution and their morphology to the cholinergic neurons comprising the Ch5 and Ch6 cell groups, respectively (Armstrong et al. 1983; Mesulam et al. 1983). Thus, we tested the hypothesis that the 5-HT$_2$-expressing cells in the dorsal pons were cholinergic neurons using double immunofluorescence combining our 5-HT$_2$ antibody with an antibody against choline acetyltransferase (ChAT), the synthetic enzyme for acetylcholine, and a marker for cholinergic neurons. With this approach, we found approximately 95 to 100% colocalization of the 5-HT$_2$ immunoreactivity with ChAT immunoreactivity in the Ppt and Ldt (Morilak and Ciaranello 1993a), indicating that these were indeed cholinergic neurons, and that essentially all the cholinergic neurons in these two regions express the 5-HT$_2$ receptor (Figure 1). This was not a nonspecific nor an ubiquitous association, as cholinergic neurons in other regions of the brain, including cranial nerve motor nuclei, basal forebrain, and

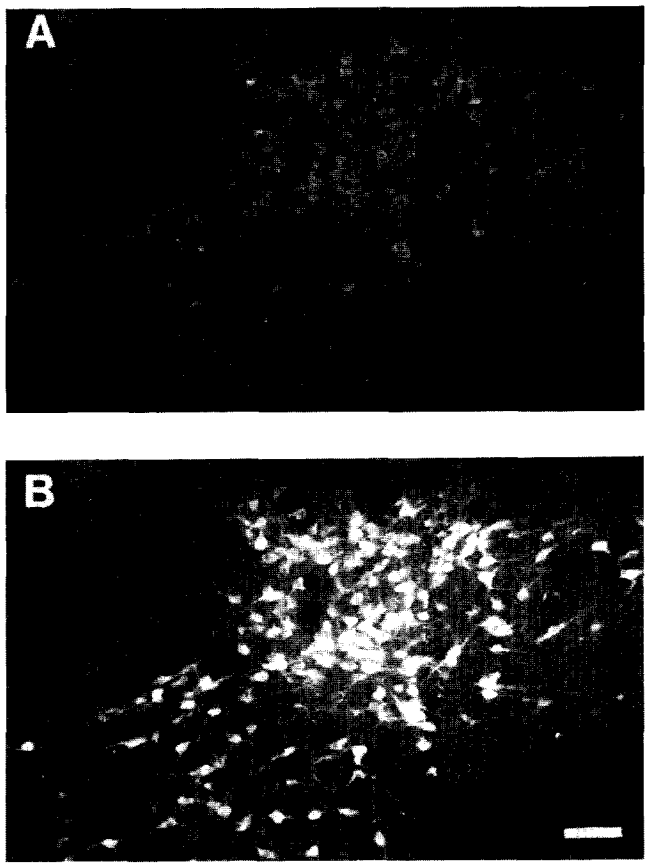

Figure 1. Colocalization of 5-HT$_2$ and ChAT immunoreactivity in the laterodorsal tegmental nucleus. (**A**) Fluorescein-immunofluorescence of 5-HT$_2$-positive neurons. (**B**) Rhodamine-immunofluorescence of ChAT-positive neurons. Even though the ChAT-associated fluorescence is more intense and labels the cells more extensively, analyses of paired color exposures showed that 94% to 99% of the labeled cells in the LDT were double-labeled. Scale bar = 100 µm. Reprinted, with permission, from Morilak and Ciaranello (1993a).

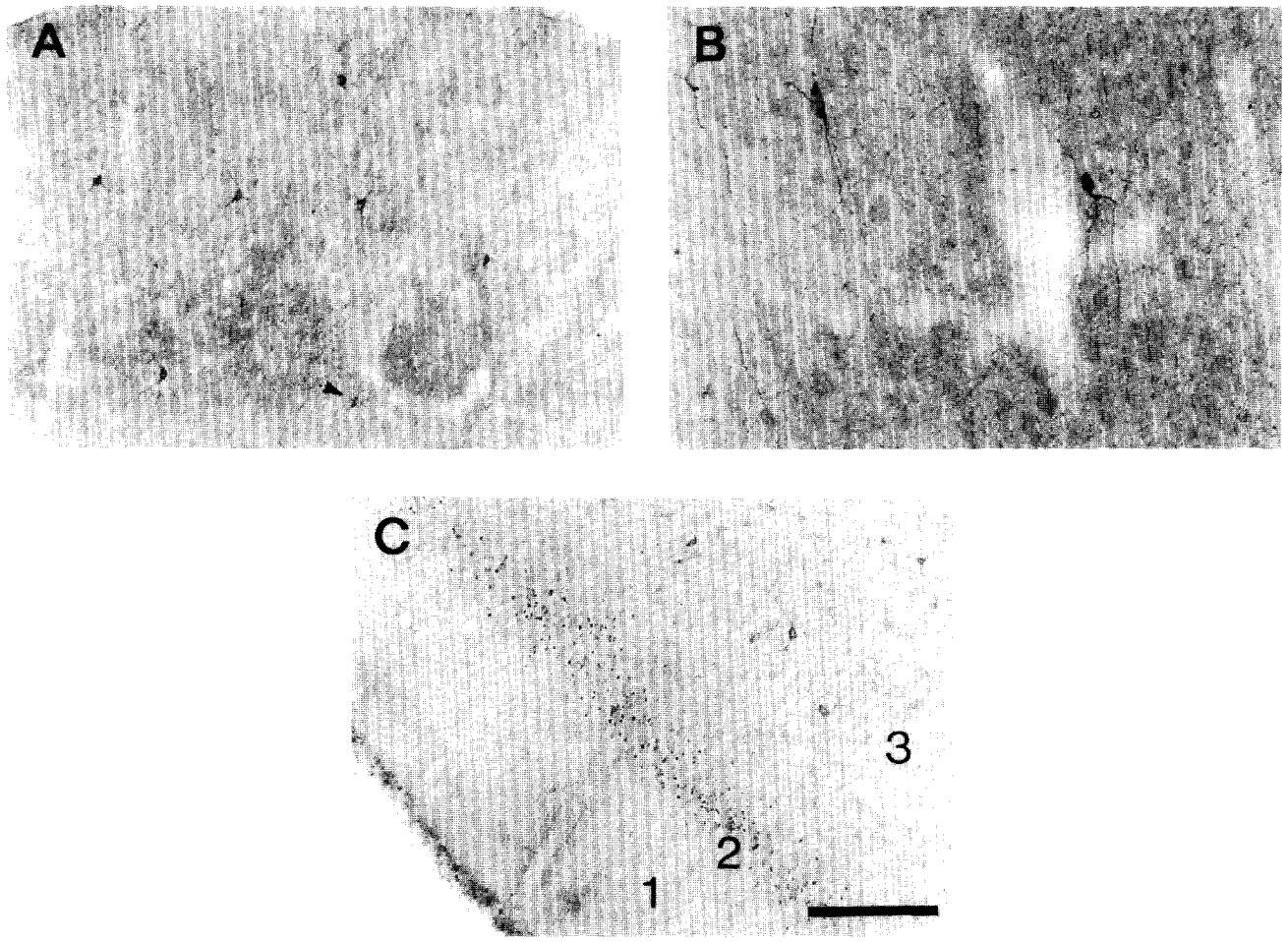

Figure 2. 5-HT_2-immunolabeled cells in cerebral cortex. (**A**) Labeled neurons in layers II to IV of fronto-parietal cortex in a 12-day old rat pup. Note the presence of medium-sized multipolar neurons scattered throughout the area, and a small number of bipolar neurons (arrowhead). (**B**) 5-HT_2-immunopositive cells in the middle parietal cortex of the same animal shown in (**A**). These cells exhibit dendritic fields characteristic of GABAergic interneurons in cortex. The example is taken from an immature brain to illustrate these morphological characteristics because the dendritic processes were more extensively labeled at P12 than in adults. (**C**) 5-HT_2 labeling in pyriform cortex. Medium-sized bipolar and multipolar neurons can be seen in the deep layer of pyriform cortex, similar to those seen in other regions of neocortex. Note also the dense labeling in layer 2. Scale bar: (**A**) 200 μm, (**B**) 100 μm, (**C**) 200 μm.

neostriatum, were not 5-HT_2 positive. Likewise, 5-HT_2-immunoreactive neurons in other regions of the brain were noncholinergic. It is difficult to speculate what the normal function of these 5-HT_2 receptors might be, since to our knowledge a measurable influence of 5-HT_2 receptor activation on the activity of these cells has not been demonstrated. Nonetheless, given the role of the dorsal pontine cholinergic neurons in arousal and sleep regulation, especially as related to thalamocortical sensory processing during rapid-eye-movement (REM) sleep, it is an intriguing possibility that these receptors may represent a substrate for the many autonomic and sleep dysfunctions that often occur in such neuropsychiatric disorders as depression and schizophrenia, in which the 5-HT_2 receptor has been implicated. It is also possible that these receptors may be a heretofore unrecognized site of action of pharmacological hallucinogens, such as LSD, which are known to act at the 5-HT_2 receptor (Glennon et al. 1986; Heym and Jacobs 1987).

5-HT_2-Expressing GABAergic Interneurons in Cerebral Cortex

Our second unexpected observation was in the cerebral cortex, where we found a fairly sparse and uniform distribution of 5-HT_2-immunoreactive neurons throughout layers II to VI (Morilak et al. 1993). Immunolabeled neurons showed very slight dorsal-ventral and rostral-caudal gradients, being slightly more dense in the ventral and rostral regions of cortex.

The relatively even laminar distribution is rather a different pattern of labeling than that seen with radioligand autoradiography. Whereas binding sites do

exist throughout the layers of cortex, they are particularly concentrated in the middle layers (Pazos et al. 1985; Blue et al. 1988; Lidow et al. 1989; Schotte and Leysen 1989; Appel et al. 1990; Francis et al. 1992). We believe this difference arises from the different characteristics of the two labeling techniques. Whereas immunocytochemistry gives an indication of the number of cells expressing the 5-HT$_2$ receptor protein, it is not quantitative with respect to binding site density. In contrast, autoradiography reflects binding site density but provides no indication of cell number, particularly if binding sites are concentrated on limited areas of the cell. Thus, there may be a preferential targeting to 5-HT$_2$ receptors to portions of the dendritic tree that are localized to the middle layers of cortex. Likewise, immunostaining in the cell bodies may be indicative of synthesis and transport of the receptor protein, whereas the active binding sites labeled by radioligands may be stably concentrated in the midlayers. Another factor to consider is that the different ligands used show varying degrees of specificity for the 5-HT$_2$ binding site relative to, for instance, the 5-HT$_{1C}$ binding site. A more thorough discussion of these and related issues is presented in our previous paper (Morilak et al. 1993).

The predominant morphological cell types labeled by the 5-HT$_2$ antibody in the cortex were medium-sized multipolar neurons, with slightly lesser numbers of medium-sized bipolar neurons, both vertically and horizontally oriented (Figures 2A, 2B; see Morilak et al. 1993). In addition to the sparse, even distribution in the cortical grey, a small number of bipolar neurons were also seen in the interstitial white layer underlying the cortex. The one exception to this general pattern of cortical labeling was in piriform and entorhinal cortices. In these regions, in addition to a scattering of medium-sized complex cells in the deep layer (layer III), we also observed a prominent band of small, round, granule-type cells in layer II (Figure 2C).

The nonpyramidal morphology of 5-HT$_2$–immunoreactive neurons throughout the cortex, as well as their somewhat even distribution in layers II to VI, are both suggestive of certain subclasses of GABAergic interneurons (Nagai et al. 1984; Ottersen and Storm-Mathisen 1984; Somogyi et al. 1985; Haberly et al. 1987). Because the pattern of 5-HT$_2$ immunolabeling did not generate the detailed view of the entire dendritic and axonal profile of labeled neurons produced by techniques such as Golgi impregnation, which has been used to define the morphological subclasses of interneurons, we cannot say with certainty which of the major subclasses of GABAergic interneurons are most represented in the population of 5-HT$_2$–expressing cells. Rather, we can only describe certain morphological features that place them broadly into the population of GABAergic interneurons as a whole (Figure 2). The 5-HT$_2$–labeled cells were smaller than pyramidal neurons (approximately 10 to 20 μm diameter). There were a number of bipolar cells. The multipolar neurons that were labeled were stellate in appearance; they did not have a pyramidal shape and did not typically exhibit a recognizable apical dendrite extending toward the pial surface.

The suggestion that cortical 5-HT$_2$–expressing cells are GABAergic interneurons has been corroborated by a number of other investigators using a variety of techniques. Electrophysiological studies have shown that a population of GABAergic interneurons in piriform cortex are excited by 5-HT, acting through a 5-HT$_2$ receptor, resulting in indirect inhibition of pyramidal cells (Sheldon and Aghajanian 1990; Gellman and Aghajanian 1993). Also, in a recent study utilizing selective retrograde lesioning of cortical projection neurons, the lesions were accompanied by a loss of 5-HT$_{1A}$, but not 5-HT$_2$ binding, suggesting that postsynaptic 5-HT$_{1A}$ receptors are localized on pyramidal cells, whereas 5-HT$_2$ binding is restricted to local interneurons (Francis et al. 1992).

In terms of the distribution of 5-HT$_2$ neurons, the correlation with the distribution of GABAergic interneurons is not restricted to cortex. Indeed, populations of GABAergic interneurons have been described in olfactory bulb, basal forebrain, neostriatum, and hippocampus, as well as in piriform, entorhinal, and neocortex with distributions similar to those we have described for 5-HT$_2$–labeled cells (Ribak et al. 1977, 1978; Somogyi et al. 1983, 1985; Ottersen and Storm-Mathisen 1984, 1989), though the number of GABAergic cells in these regions far exceeds the number of 5-HT$_2$–immunoreactive cells.

In Figure 3, we show the distribution of 5-HT$_2$–immunoreactive cells in hippocampus compared to the distribution of GABAergic neurons in the same region labeled by in situ hybridization using the GAD$_{67}$ riboprobe. The two populations of labeled cells overlapped extensively. This correlated distribution was also observed in the basal forebrain and neostriatum, as well as in hippocampus and neocortex. However, the number of GAD-labeled cells was considerably higher than the number of 5-HT$_2$–receptor-bearing neurons in these areas, especially in the cortex.

To directly test the hypothesis suggested by these observations, that cortical and possibly other (e.g., hippocampal) 5-HT$_2$–bearing cells are GABAergic interneurons, required a double-labeling approach. The strategy for double-labeling cells for GABA and 5-HT$_2$ receptor immunoreactivity was not as straightforward as for the cholinergic neurons. The primary difficulty has been incompatibility of fixation techniques: GABA immunocytochemistry requires high concentrations of glutaraldehyde, whereas successful labeling with the 5-HT$_2$ antibody is very sensitive to changes in fixation condition, especially to over-fixation. After attempting many alternative approaches, we adopted a compromise condition that led to suboptimal labeling of

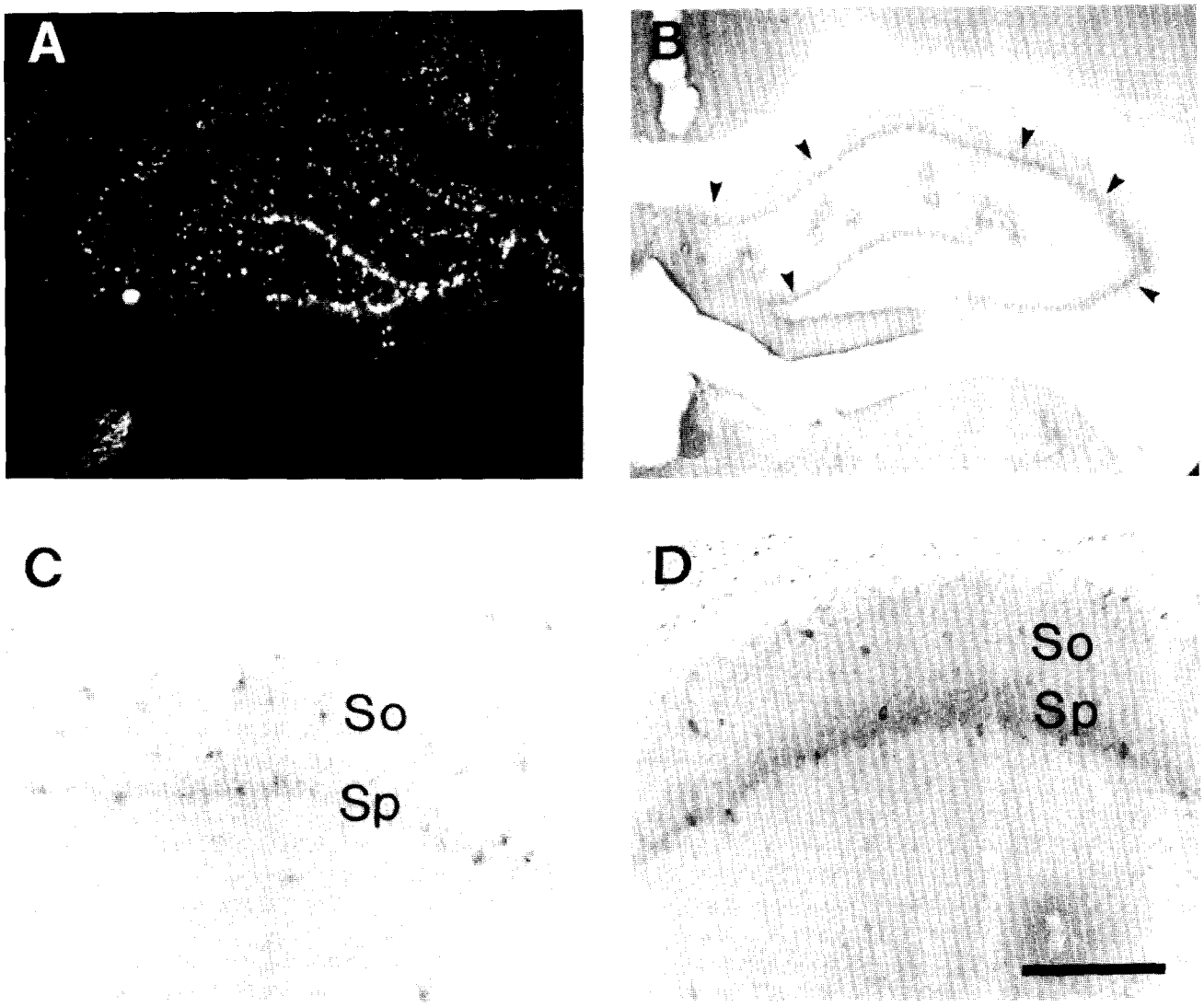

Figure 3. Correlated distribution of cells in hippocampus labeled by 5-HT_2 receptor immunocytochemistry and GAD_{67} in situ hybridization. (**A**) Darkfield low-magnification micrograph of cells showing hybridization signal for GAD_{67} mRNA, indicated by white silver grains. (**B**) Low-magnification micrograph illustrating 5-HT_2–immunoreactive neurons in the same region shown in **A**. Labled cells were seen in the CA1, CA3, and subicular regions, with fewer cells in the dentate gyrus (arrowheads). Although the distribution of 5-HT_2– positive and GAD-labeled cells were similar, the number of GAD-labeled cells far exceeded the number of 5-HT_2– expressing neurons. (**C**) Brightfield micrograph showing GAD_{67} hybridization signal overlying cells in the CA1 region of dorsal hippocampus. Note the concentration of labeled neurons in the stratum pyramidale (Sp) extending up throughout the stratum oriens (So) to the border of the overlying alveus. (**D**) 5-HT_2–immunopositive neurons in the CA1 region of the dorsal hippocampus. Again, note the concentration of cells in the strata pyramidale and oriens, extending to the oreins-alveus border. Scale bar: (**A**) 1 mm, (**B**) 1mm, (**C**) 200 μm, (**D**) 200 μm.

both antigens, by adding only 0.1% glutaraldehyde to the 4% paraformaldehyde fixative. This attenuated the 5-HT_2 labeling somewhat and led to severely attenuated and spotty GABA immunolabeling. Nonetheless, using the postembedding GABA immunoperoxidase technique outlined above, we did observe a small number of double-labeled GAGAergic, 5-HT_2–expressing interneurons in cortex (Figure 4). Although we were unable to reliably quantify the extent of double-labeling due to the fixation conditions, this observation directly verifies that at least a subset of 5-HT_2–positive cells in the cortex are GABAergic. Taken together with the distribution and morphological characteristics exhibited by 5-HT_2–labeled cells, we would conclude that the majority of these cells are GABAergic neurons in the neocortex, and also perhaps in hippocampus, basal forebrain, piriform and entorhinal cortex, and olfactory bulb.

Ontogeny of 5-HT_2 Receptors

Our second major goal was to describe the temporal pattern of 5-HT_2 receptor expression during development. Thus, we conducted an immunocytochemical

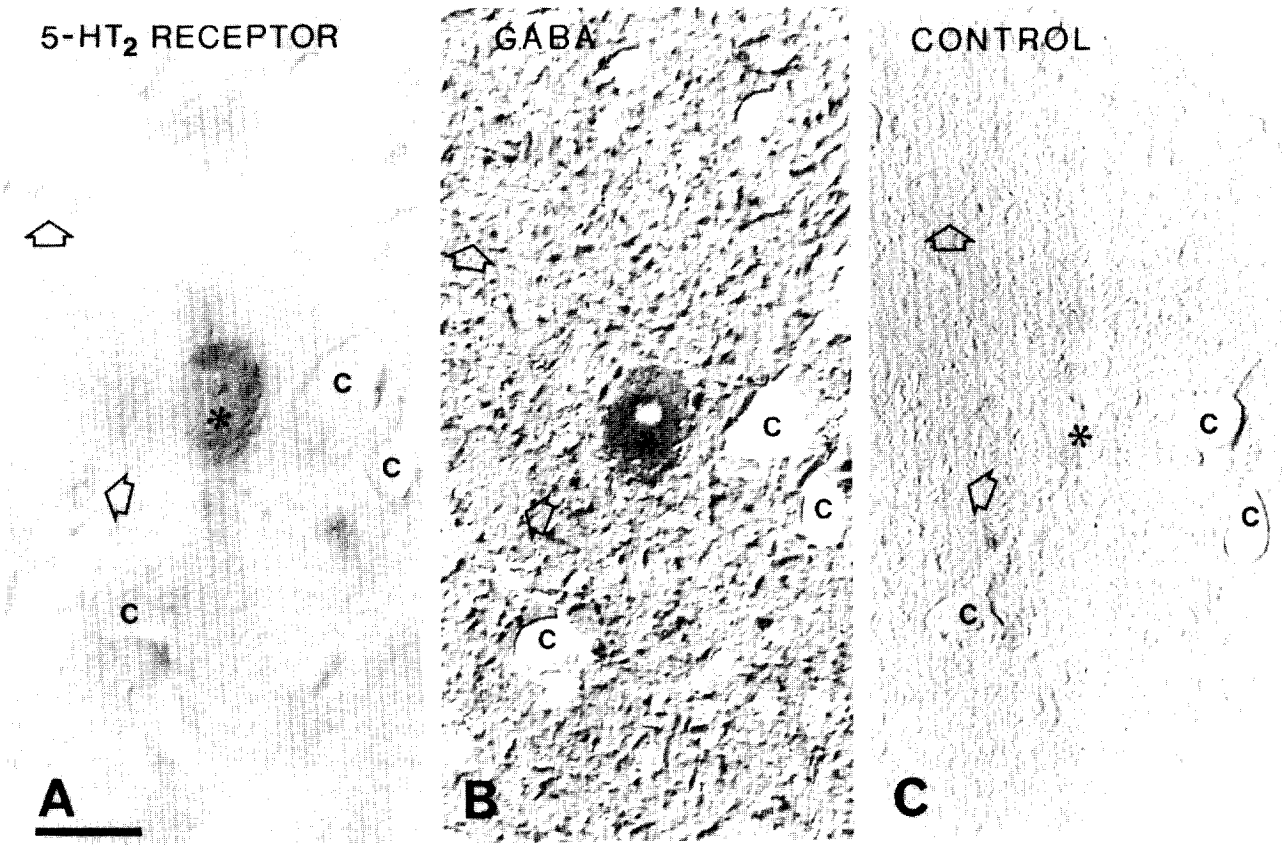

Figure 4. Demonstration of GABA immunoreactivity in a cell showing strong 5-HT$_2$ receptor immunolabeling in cortex. (**A**) A nonpyramidal neuron (*asterisk*) in layer 2 of parietal cortex showing 5-HT$_2$ immunoreactivity in a 50 μm section (preembedding immunoperoxidase reaction). (**B**) Postive GABA immunoreactivity in the same cell revealed in a 0.5 μm section cut from the section shown in **A** (postembedding immunopreoxidase reaction). Note also the numerous GABA-positive varicosities in the surrounding neuropil. (**C**) Adjacent control section through the same cell (*asterisk*), showing that the reaction product seen in **B** is due to anti-GABA serum and not to reagents used in the preembedding reaction. This section was reacted identically to that in B, except that the GABA antiserum was replaced with normal rabbit serum at the same dilution. The preembedding 5-HT$_2$-immunoreaction product seen in A was not detectable in such a thin section through the cell, and no residual peroxidase enzymatic activity remained after embedding and postembedding immunoprocessing. As reference landmarks, some capillaries (c) and cells that are negative for both 5-HT$_2$ and GABA immunoreactivity (*open arrows*) are indicated. Scale bar: 15 μm.

analysis of expression in the brains of rat embryos and pups from the late prenatal period (embryonic day 19, or E19) through the first month postnatally (Morilak and Ciaranello 1993b). The region in which the earliest 5-HT$_2$ receptor immunoreactivity was observed was in the dorsal pons, where very few cells showed light labeling beginning at E19, with substantial labeling evident by the day of birth (gestation is 22 days in the rat). There was a caudal to rostral developmental gradient, with the brainstem showing the earliest immunoreactivity, followed by the caudate on postnatal day 0 (P0, or day of birth), basal forebrain and neocortex between P0 and P4, and hippocampus between P4 and P7. On P4 in the neocortex, a small number of immature looking neurons, with small, primitive neurites, were seen in the middle and deep layers of the cortical plate. The onset of expression was followed by a rapid increase in the number of positive cells throughout all layers of cortex, culminating in a period of "relative hyperexpression." This hyperexpression, lasting approximately 1 to 2 weeks, was manifest as both an elevation in the relative cell density compared to the adult, and also as an elevation in the apparent elaboration of cell processes that were immunolabeled (Figure 5). This was followed by a regression until the adult pattern of expression was evident by about the end of the first month postnatal. Whether the decrease in cell density was a result of dilution by an increasing neuropil density and cortical volume, or represented true cell death, cannot be determined from our data. Likewise, we cannot determine if the decrease in extent of dendritic labeling was due to a decrease in antigen concentration to below detectable limits, or represented dendritic pruning and retraction. Nonetheless, the pattern of developmental expression of the 5-HT$_2$ receptor at the cellular level correlates very closely with more gross but quantitative estimates

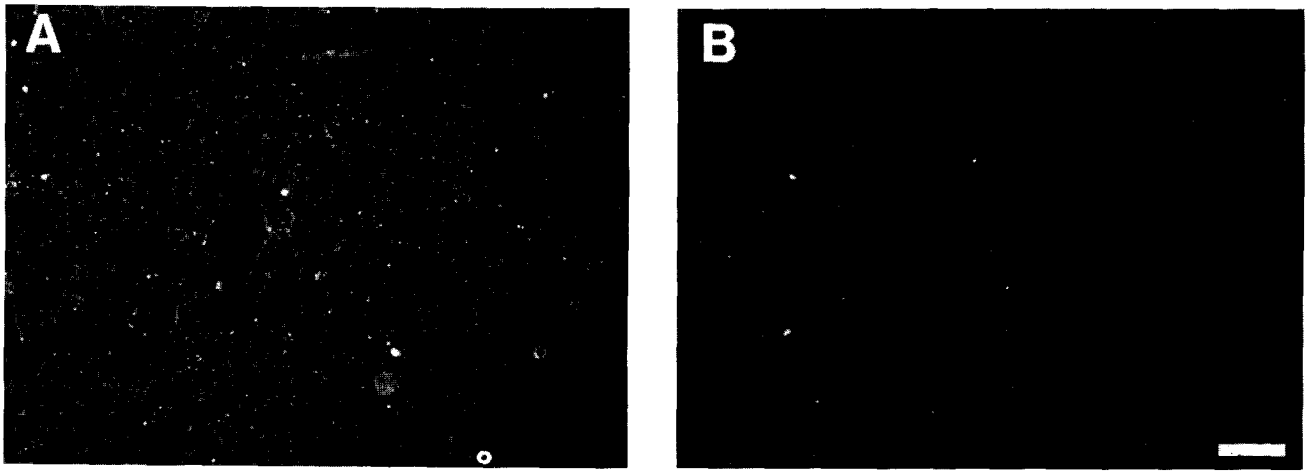

Figure 5. Hyperexpression of 5-HT_2 immunoreactivity in the developing rat neocortex. (**A**) Darkfield illumination of layers IV to V of fronto-parietal cortex in a P12 rat pup. Note the extensive network of labeled processes throughout these layers of cortex, which are not evident in adult cortex (compare to **B**). (**B**) Darkfield illumination of a comparable region of adult fronto-parietal cortex. Even though cell bodies are labeled in the adult cortex, labeling of processes, as seen in this micrograph, are relatively sparse compared to that seen at P12 (**A**). Scale bar: 100 μm.

of receptor expression using whole brain ligand binding and Northern analyses (Roth et al. 1991), suggesting that the changing patterns of immunolabeling we saw during development do indeed reflect functional changes in receptor expression.

Cellular birthdating studies using ^{3}H-thymidine incorporation to monitor cell division have shown that nearly all cortical neurons, both pyramidal projection neurons and local interneurons, will have undergone their final mitosis between E14 and E19 (Miller 1988). Thus, by P0, when we still observed no detectable 5-HT_2 immunoreactivity in the cortex, a number of these neurons will have migrated into appropriate locations in the cortical plate and will have begun to undergo a degree of neuronal differentiation. In our study, when cells were first seen to exhibit 5-HT_2 immunoreactivity, they were located within the regions in which they would ultimately reside, well away from the subventricular germinal zones for these regions. Thus, the main conclusion from these results is that the 5-HT_2 receptor comes on relatively late in the ontogeny of those neurons that express it.

Clinical Significance of the Cell-Type Specific and Temporal Developmental Regulation of 5-HT_2 Receptors

The localization of cortical 5-HT_2 receptors of GABAergic interneurons suggests a very close interaction between the serotonergic and GABAergic transmitter systems in cortical circuits. Their expression of GABAergic interneurons further suggests that the 5-HT_2 receptor would play a modulatory role in the cortex, a suggestion supported by the work of Aghajanian and colleagues (Sheldon and Aghajanian 1990). Thus, disruptions in the normal functioning of 5-HT_2 receptors could produce widespread but potentially subtle alterations in cortical processing.

It has recently been reported that there is a selective deficit of GABAergic interneurons in the cortex of schizophrenics (Benes et al. 1991). One question that arises is what subpopulation of GABAergic interneurons may be affected. The antipsychotic action of many atypical antipsychotics may be mediated, at least in part, by an antagonist action at the 5-HT_2 receptor (Meltzer et al. 1989), suggesting a relative excess of 5-HT_2 activation. Thus, it is possible that the decreased population of GABAergic interneurons in schizophrenia may be non-5-HT_2 expressing, perhaps creating a local imbalance in which the 5-HT_2-activated interneurons are in excess. Though highly speculative, such a scenario would reconcile the apparent loss of GABAergic cells in cortex with the antipsychotic effectiveness of 5-HT_2 receptor blockade.

Also, it is likely that certain forms of schizophrenia may be primarily disorders of neuronal development (Feinberg 1982/83; Weinberger 1987). Related to the selective loss of GABAergic interneurons in cortex, it has also been shown that there is an apparent migrational abnormality in the cortex of schizophrenics; a population of GABAergic interneurons are ectopically localized in the deep layers of cortex and especially in the subcortical white matter, suggesting that they had been arrested in their migration during development (Akhbarian et al. 1993a, 1993b). Whether this is the same population of cells that is missing from the appropriate regions of the cortical grey or not remains to be determined.

There has been some suggestion that the 5-HT_2 receptor may act as a growth factor transducer (Neme-

cek et al. 1986; Julius et al. 1990; Corson et al. 1992), perhaps mediating some of the early developmental effects that have been attributed to serotonin. However, our observations would argue against the 5-HT$_2$ receptor serving such a role in early development. Rather, the late developmental expression of the receptor would imply that if the 5-HT$_2$ receptor has anything to do with development, it may participate in some aspect of terminal differentiation, such as the establishing of appropriate synaptic connectivity of target neurons, perhaps by modulating neuronal activity as it does in the adult. It is possible that such a function, in light of the postnatal pattern of hyperexpression followed by regression, may be relevant to current development hypotheses of schizophrenia, which focus on abnormalities in the postnatal processes of synaptic proliferation, connectivity, and retraction (Feinberg 1982/83). How such a theory might account for the morphological observations discussed above and how it may be understood in terms of the cellular effects of 5-HT$_2$ receptors are not clear at this time. Nonetheless, we feel that such observations can generate testable hypotheses about the structural and functional abnormalities underlying complex neuropsychiatric disorders.

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