The Journal of Neuroscience, May 1, 1997, 17(9):3157–3167

The 5-HT_3 Receptor Is Present in Different Subpopulations of GABAergic Neurons in the Rat Telencephalon

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The type 3 serotonin receptor (5-HT_3R) is a ligand-gated ion channel whose presence in the CNS has been established by radioligand binding, *in situ* hybridization, and immunohistochemical analysis. To analyze further the role of the 5-HT_3R in the CNS, we used *in situ* hybridization and immunocytochemistry to determine that 5-HT_3R-expressing neurons are mainly GABA-containing cells in the rat telencephalon. We determined that 5-HT_3R/GABA-containing neurons do not exhibit somatostatin immunoreactivity but often contain cholecystokinin (CCK) immunoreactivity. 5-HT_3R-expressing cells with CCK immunoreactivity were observed in the neocortex, olfactory cortex, hippocampus, and amygdala. The 5-HT_3R/CCK interneurons represent between 35 and 66% of the total population of CCK-containing cells in the neocortex.

Further characterization of the 5-HT_3R/GABAergic neurons was based on their calcium-binding protein immunoreactivity and showed that these neurons lack parvalbumin (PV) and represent a subpopulation of calbindin (CB)-containing interneurons that were preferentially present in the CA1–CA3 subfield of the hippocampus. Although some 5-HT_3R/GABAergic neurons with calretinin (CR) were found in the neocortex, olfactory cortex, hippocampus, and amygdala, these neurons were more often present in the agranular insular and piriform cortices.

We conclude that the neuronal expression of the 5-HT_3R is selective within the GABA neuron population in the rat telencephalon. These 5-HT_3R-expressing interneurons might contain CCK, CB, and CR. We suggest that serotonin through the 5-HT_3R may regulate GABA and CCK neurotransmission in the telencephalon.

Key words: 5-HT_3R; serotonin; GABA; interneurons; calcium-binding proteins; cholecystokinin

The neurotransmitter serotonin [5-hydroxytryptamine (5-HT)] interacts with several receptors (Hoyer et al., 1994), all of which are G-protein-coupled (Hoyer et al., 1994), with the exception of the 5-HT_3 receptor (5-HT_3R), which is a ligand-gated ion channel (Derkach et al., 1989). Electrophysiological studies on neurons and neuronal cell lines indicate that stimulation of the 5-HT_3R causes a rapid depolarization produced by an increased membrane permeability to monovalent cations (Peters and Lambert, 1989). The cloning of a functional subunit of the 5-HT_3R (subunit A) from the neuroblastoma cell line NCB-20 has confirmed that the 5-HT_3R is a member of the superfamily of ligand-gated ion channels and is structurally related to the nicotinic, $GABA_A$, and NMDA receptors (Manricq et al., 1991).

Radioligand binding studies have detected 5-HT_3R binding sites in the CNS of rodents, primates, and humans (Kilpatrick et al., 1987, 1988, 1989; Waeber et al., 1988, 1989, 1990; Barnes et al., 1989a, 1990; Pratt et al., 1990; Gehlert et al., 1991; Jones et al., 1992; Laporte et al., 1992). High-density 5-HT_3R binding sites have been found in the hindbrain: the nucleus of the tractus solitarius, area postrema, nucleus of the spinal tract of the trigeminal nerve, and dorsal motor nucleus of the vagus (Waeber et al., 1988, 1989, 1990; Barnes et al., 1990; Pratt et al., 1990; Gehlert et al., 1991; Jones et al., 1992; Laporte et al., 1992). Moderately dense 5-HT_3R binding sites have been found consistently in cortex, amygdala, and hippocampus (Kilpatrick et al., 1987, 1988; Waeber et al., 1988; 1989; 1990; Barnes et al., 1989a; Gehlert et al., 1991; Jones et al., 1992; Laporte et al., 1992). In contrast, only a few studies have reported specific binding sites in the nucleus accumbens, striatum, or substantia nigra (Kilpatrick et al., 1987; Waeber et al., 1988; Gehlert et al., 1991; Laporte et al., 1992). The presence of neurons containing 5-HT_3R has been confirmed in some of these brain areas by *in situ* hybridization (Tecott et al., 1993; D. Johnson and S. Heinemann, personal communication) and immunocytochemical analysis (Morales et al., 1996a).

The function of the 5-HT_3R in the CNS as well as in the neuronal circuits in which this receptor might participate remains to be established. The use of 5-HT_3R antagonists in behavioral studies, however, has led to the view that this receptor participates in several pharmacological events, such as anxiolytic, antipsychotic, and cognitive-enhancing actions and facilitation of the withdrawal from drugs of abuse (Carboni et al., 1989; Costall et al., 1990, 1993; Nevins and Anthony, 1994). In addition, activation of 5-HT_3R has been demonstrated to modulate the release of various neurotransmitters in the brain (Barnes et al., 1989b; Blandina et al., 1989; Chen et al., 1991, 1992; Paudice and Raiteri, 1991; Maura et al., 1992).

To obtain information on the functional significance of the 5-HT_3R-containing neurons, we sought to determine additional neurochemical features of the neurons that express this receptor in the telencephalon.

MATERIALS AND METHODS

Tissue preparation. Twenty adult male Sprague Dawley 20 rats (100–120 gm body weight) were anesthetized with choral hydrate (3.5 mg/100 gm body weight) and perfused transcardially with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.3. Brains were post-fixed

Received Oct. 1, 1996; revised Jan. 27, 1997; accepted Feb. 10, 1997.

This work was supported by National Institute on Alcohol Abuse and Alcoholism Grant AA 06420. We thank Drs. David Johnson and Steve Heinemann for the rat 5-HT_3R-A cDNA clone, and Elena Battenberg for her role in early experiments.

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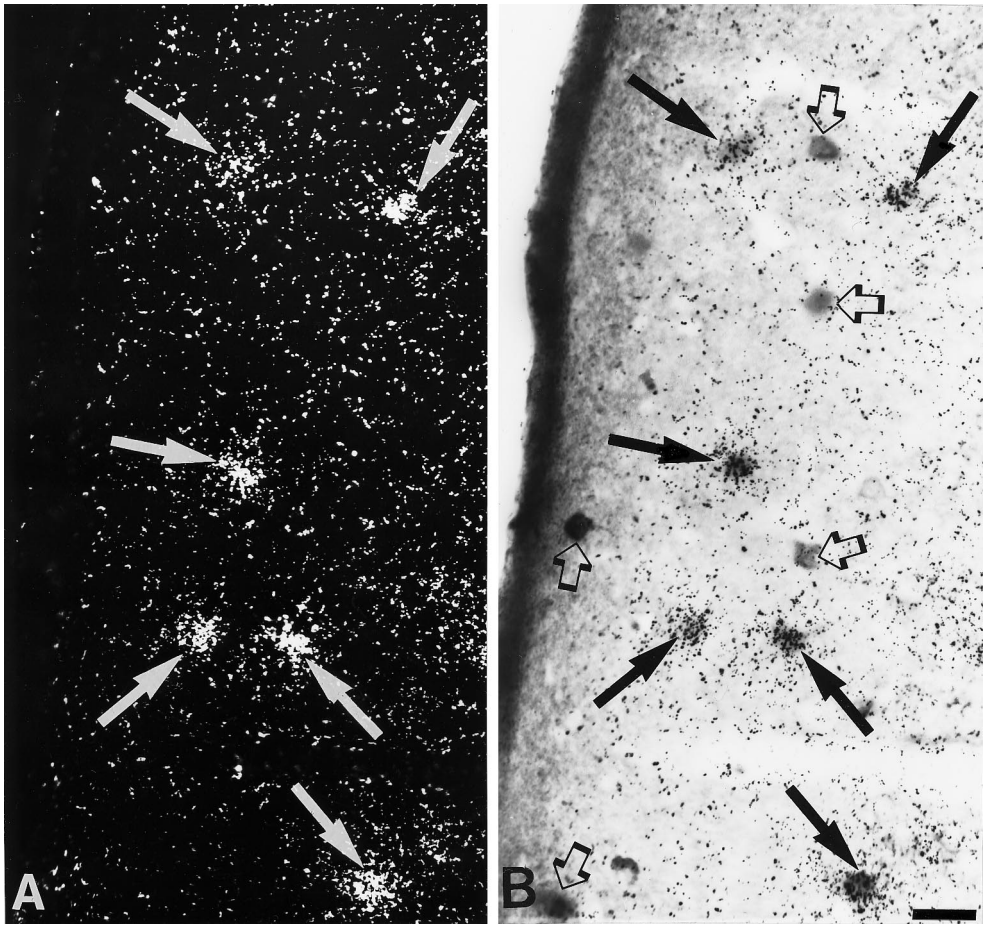

Figure 1. Simultaneous detection of 5-HT$_3$R transcripts and GABA immunoreactivity in layer II of parietal cortex. *A*, Observation of 5-HT$_3$R-expressing cells under epiluminescence microscopy. *B*, Observation of GABA-immunoreactive neurons under bright-field microscopy. In this section, all 5-HT$_3$R-expressing cells showed GABA immunoreactivity (*filled arrows* in *A* and *B*), but not all GABA-immunoreactive neurons contained 5-HT$_3$R transcripts (*open arrows* in *B*). Scale bar, 25 μm.

overnight, rinsed with PB, and sequentially transferred to 12%, 14%, and 16% sucrose solutions. Brains were then frozen on dry ice, and sections of 30–40 μm thickness were obtained on a cryostat.

Probe preparation. [^{35}S]- and [^{33}P]-labeled sense and antisense RNA probes were generated from a 730 basepair (bp) *Pst*I insert (corresponding to nucleotides 1500–2230 of the rat 5HT$_3$R cDNA), using the TransProbe T Kit (Pharmacia, Piscataway, NJ). The [^{35}S]- and [^{33}P]-labeled antisense probes were synthesized separately.

In situ *hybridization–immunocytochemistry labeling. In situ* hybridization combined with immunolabeling was performed as described previously (Morales et al., 1996a). Free-floating cryosections were incubated in PB supplemented with 0.5% Triton X-100 for 10 min, rinsed 2 × 5 min with PB, treated with 0.2N HCl for 10 min, rinsed 2 × 5 min with PB, and then acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min. Sections were rinsed 2 × 5 min with PB and post-fixed with 4% paraformaldehyde for 10 min, and after a final rinse with PB, sections were prehybridized for 3 hr at 55°C in hybridization buffer (50% formamide, 10% dextran sulfate, 5× Denhardt's solution, 0.62 M NaCl, 50 mM dithiothreitol, 10 mM EDTA, 20 mM PIPES, pH 6.8, 0.2% SDS, 250 μg/ml ssDNA, 250 μg/ml tRNA). After prehybridization, sections were hybridized at 55°C for 16 hr in hybridization buffer containing [^{35}S]- and [^{33}P]-labeled single-stranded RNA probes at 10^7 cpm/ml. Sections were treated with RNase A at 4 μg/ml at 37°C for 1 hr, washed in 1 × SSC, 50% formamide at 55°C for 2 hr, and in 0.1 × SSC at 68°C for 1 hr. Sections were rinsed with PB, incubated in 1% bovine serum albumin (BSA) supplemented with 0.3% Triton X-100 in PB for 1 hr, and then incubated with the corresponding primary antibody for 24 hr at 4°C. A previously well characterized rabbit polyclonal antibody against SS, SS-320 (Morrison et al., 1983), was used at dilution 1:3000 (antibody was provided by Dr. R. Benoit, Montreal, Quebec, Canada); anti-GABA

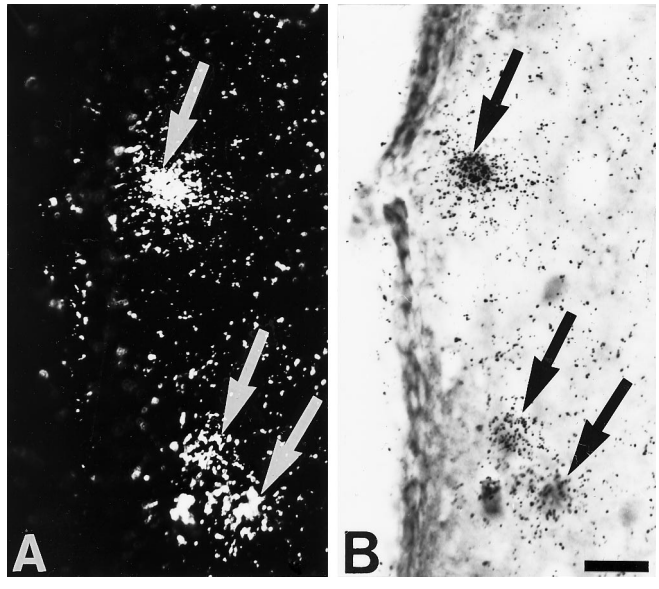

Figure 2. Simultaneous detection of 5-HT$_3$R transcripts and GABA immunoreactivity in the medial septal nucleus. *A*, Observation of 5-HT$_3$R-expressing cells under epiluminescence microscopy. *B*, Observation of GABA-immunoreactive neurons under bright-field microscopy. Double-labeled cells are indicated by *arrows*. Scale bar, 25 μm.

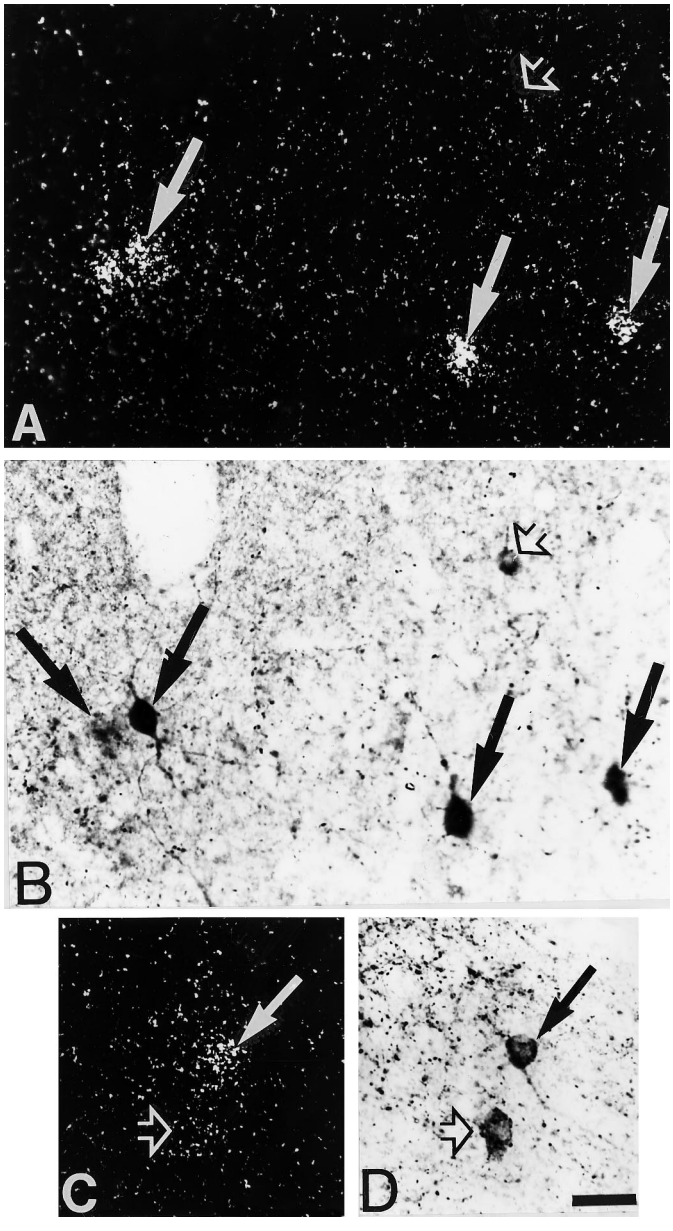

Figure 3. Simultaneous detection of 5-HT$_3$R transcripts and CCK immunoreactivity in the temporal (*A, B*) and piriform (*C, D*) cortices. *A, C*, Observation of 5-HT$_3$R-expressing cells under epiluminescence microscopy. *B, D*, Observation of CCK-immunoreactive neurons under bright-field microscopy. In these sections, several 5-HT$_3$R-expressing cells showed CCK immunoreactivity (*filled arrows*), but not all CCK-immunoreactive neurons contained 5-HT$_3$R transcripts (*open arrows*). Scale bar, 25 μm.

rabbit antibody (Sigma, St. Louis, MO) was used at dilution 1:2000. A specific anti-cholecystokinin (CCK) monoclonal antibody raised in mouse was used at dilution 1:2000 (provided by Dr. D. Goodwillie, Farmitalia Carlo Erba, Nerviano, Italy), and anti-parvalbumin (PV), anti-calbindin (CB) mouse monoclonal, and anti-calretinin (CR) rabbit polyclonal antibodies were used at dilution 1:2000 (Swant).

After sections were rinsed 3 × 10 min in PB, they were processed with an ABC kit (Vector, Burlingame, CA). Material was incubated in a 1:200 dilution of the corresponding biotinylated secondary antibody. After they were rinsed with PB, sections were incubated with avidin–biotinylated horseradish peroxidase for 2 hr. Samples were rinsed, and the peroxidase reaction was developed with 0.05% 3,3-diaminobenzidine-4 HCl (DAB) and 0.003% hydrogen peroxide (H_2O_2). All antibody dilutions were performed in PB supplemented with 1% BSA and 0.3% Triton X-100.

Table 1. Percentage of CCK-immunoreactive neurons expressing the 5-HT$_3$R

Region	Percentage of double-labled neurons[a]
Neocortex	
Motor cortex	49 ± 0.47 (n = 119)
Agranular insular cortex	35 ± 0.47 (n = 25)
Anterior cingulate cortex	60 ± 0.47 (n = 62)
Auditory cortex	36 ± 0.47 (n = 113)
Posterior parietal association cortex	66 ± 0.47 (n = 55)
Prelimbic cortex	55 ± 0.47 (n = 89)
Somatosensory cortex	35 ± 0.47 (n = 70)
Visual cortex	46 ± 0.47 (n = 193)
Olfactory system	
Anterior olfactory nucleus	65 ± 0.47 (n = 45)
Piriform cortex	53 ± 0.47 (n = 102)
Entorhinal cortex	55 ± 0.47 (n = 75)

[a]Total number of CCK-immunopositive cells (n) was counted in three sections each from three different experiments, and the percentage of CCK-immunoreactive cells expressing the 5-HT$_3$R was calculated.

Sections were mounted on coated slides, air-dried, dipped in nuclear track emulsion, and exposed for several weeks before development. Material was photographed under bright-field or epiluminescence microscopy.

When hybridization was performed with sense probes, few silver grains were scattered on the sections. This level of signal was very low and was considered as unspecific background. In contrast, hybridization with antisense probes resulted in a localized signal distribution.

Double immunocytochemistry. Free-floating cryosections were processed as described under immunocytochemistry. Sections were incubated with a specific rabbit polyclonal anti-5-HT$_3$R antibody (0165) at 1:4000 dilution for 24–48 hr at 4°C. We have established previously the specificity of the anti-5-HT$_3$R antibody used in this study (Morales et al., 1996a). After the peroxidase reaction was developed with DAB and H_2O_2, the sections were rinsed with PB, and the remaining hydrogen peroxidase activity was inactivated by incubating sections in methanol containing 0.3% H_2O_2 for 15 min at room temperature. After several rinses with PB, sections were treated with an avidin solution (Vector) to block biotin groups for 1 hr at room temperature. Sections were incubated in the second primary antibody (anti-CCK at 1:2000 dilution) supplemented with biotin-blocking solution (Vector) and incubated for 24 hr at 4°C. After they were rinsed 3 × 10 min in PB, sections were processed with an ABC kit (Vector). Material was incubated in a 1:200 dilution of anti-mouse biotinylated secondary antibody. After sections were rinsed with PB, they were incubated with avidin–biotinylated horseradish peroxidase for 2 hr. Samples were rinsed with 0.1 M sodium phosphate buffer (SPB), pH 6.8, and the peroxidase reaction was developed in 0.01% benzidine dihydrochloride, 0.025% sodium nitroferricyanide, and 0.005% H_2O_2 in SPB (Levey et al., 1986), which results in a granular blue-black reaction product.

Data analysis. Sections processed for *in situ* hybridization and immunocytochemistry were analyzed and photographed with bright-field or epiluminescence microscopy. We performed a semiquantitative analysis of double-labeled cells in the neocortex. A neuron was considered double-labeled when its soma was brown and contained more than 15 silver grains. In pilot experiments we found that 15–20 silver grains represent weak label above background. Double-labeled cells were counted in three random sections of three different experiments, and the percentage of neurons containing both 5-HT$_3$R mRNA and immunoreactivity was calculated from the total population of immunolabeled cells.

RESULTS

We have previously used *in situ* hybridization and immunocytochemistry to show the presence of GABA in neurons that express the 5-HT$_3$R in the neocortex and hippocampus (Morales et al., 1996b). In the present study, we investigated further the neurochemical composition of 5-HT$_3$R-expressing neurons in the telencephalon.

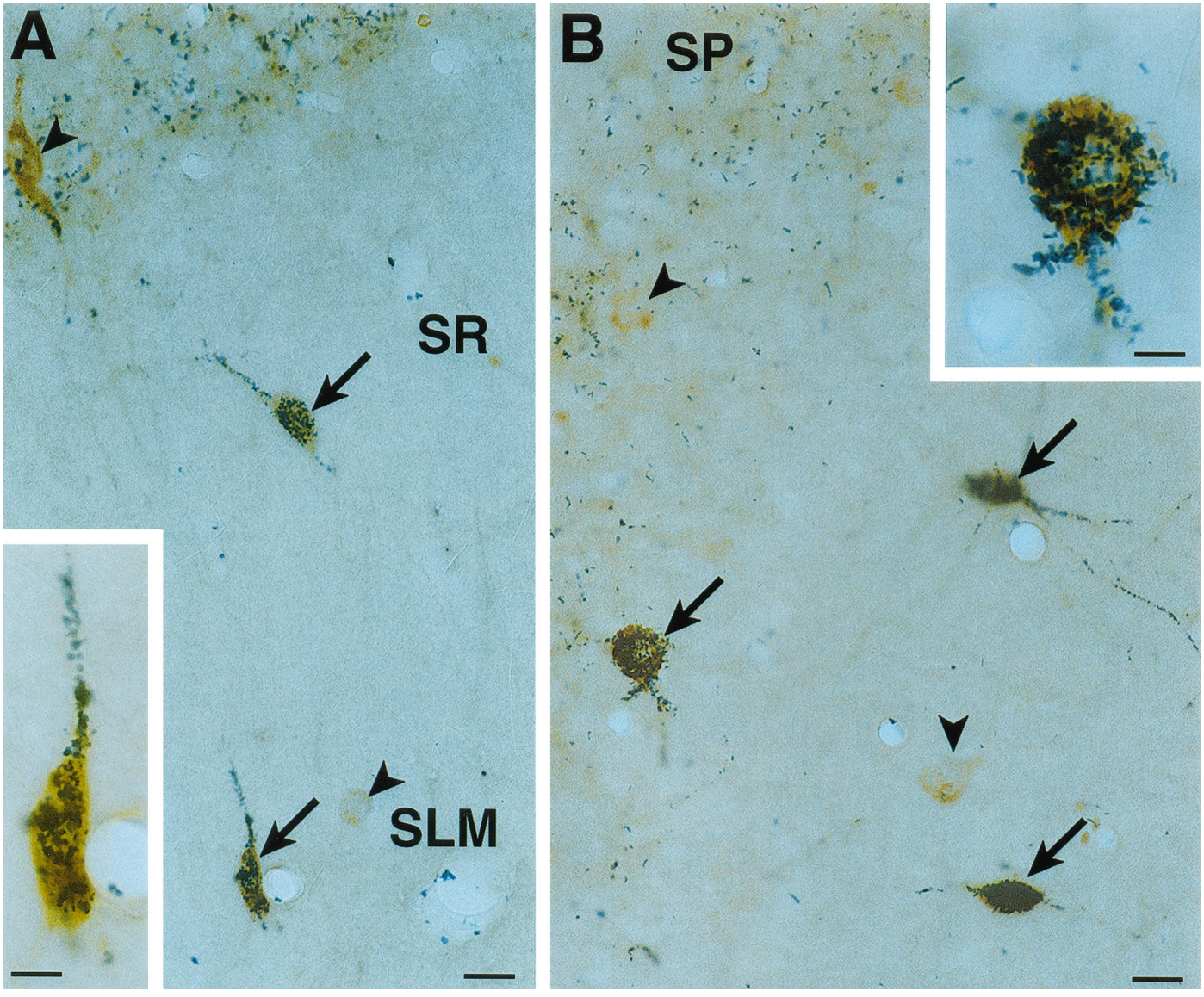

Figure 4. Simultaneous detection of 5-HT$_3$R and CCK immunoreactivity in the CA1 (*A*) and CA3 (*B*) subfields of the hippocampus. Colocalization of 5-HT$_3$R (*brown*) and CCK (*granular blue-black*) immunoproducts was found in several neurons (*arrows*), but not all 5-HT$_3$R-immunoreactive cells contained CCK immunoreactivity (*arrowheads*). *Insets* in *A* and *B* show double-labeled neurons at high magnification. *SR*, St. radiatum; *SLM*, st. lacunosum moleculare. Scale bar, 12.5 μm; for *inset*, 6 μm.

Colocalization of 5-HT$_3$R transcripts and GABA

Coexistence of 5-HT$_3$R transcripts with GABA immunoreactivity was observed in several areas of the telencephalon. These 5-HT$_3$R/GABA neurons were often found in those brain areas that contained cells with high levels of 5-HT$_3$R expression, such as the neocortex (Fig. 1*A,B*), olfactory regions, hippocampal formation, amygdaloid complex, and septal region (Fig. 2*A,B*). Double-labeled cells were also detected occasionally in areas in which neurons contained low levels of 5-HT$_3$R mRNA, such as the corpus callosum, corpus striatum, thalamus, and hypothalamus. A semiquantitative analysis performed from three random sections of three different experiments indicated that between 70 and 95% of the 5-HT$_3$R-expressing cells were GABAergic throughout the telencephalon (n = 550 cells).

The 5-HT$_3$R/GABAergic neurons were found throughout the different layers of the neocortex but were preferentially concentrated in layers II–III and V–VI. This pattern of distribution was observed from the frontal to the occipital regions of the neocortex. Within the olfactory regions, 5-HT$_3$R/GABAergic neurons were detected in the olfactory tubercle, piriform cortex, endopiriform nucleus, anterior olfactory nucleus, and taenia tecta. The 5-HT$_3$R/GABAergic neurons of the hippocampal formation were distributed in all layers of the CA1–CA3 subfields of the hippocampus, dentate gyrus, hilus, and subiculum. The 5-HT$_3$R/GABAergic cells of the amygdaloid complex were found mainly in the lateral, basolateral, basomedial, and cortical amygdaloid nuclei.

Colocalization of 5-HT$_3$R transcripts and neuropeptides

Somatostatin (SS) and CCK are neuropeptides known to coexist with GABA in nonoverlapping neuronal populations in the cortex and hippocampus (Somogyi et al., 1984; Sloviter and Nilaver, 1987). Thus, we investigated whether either of these peptides was

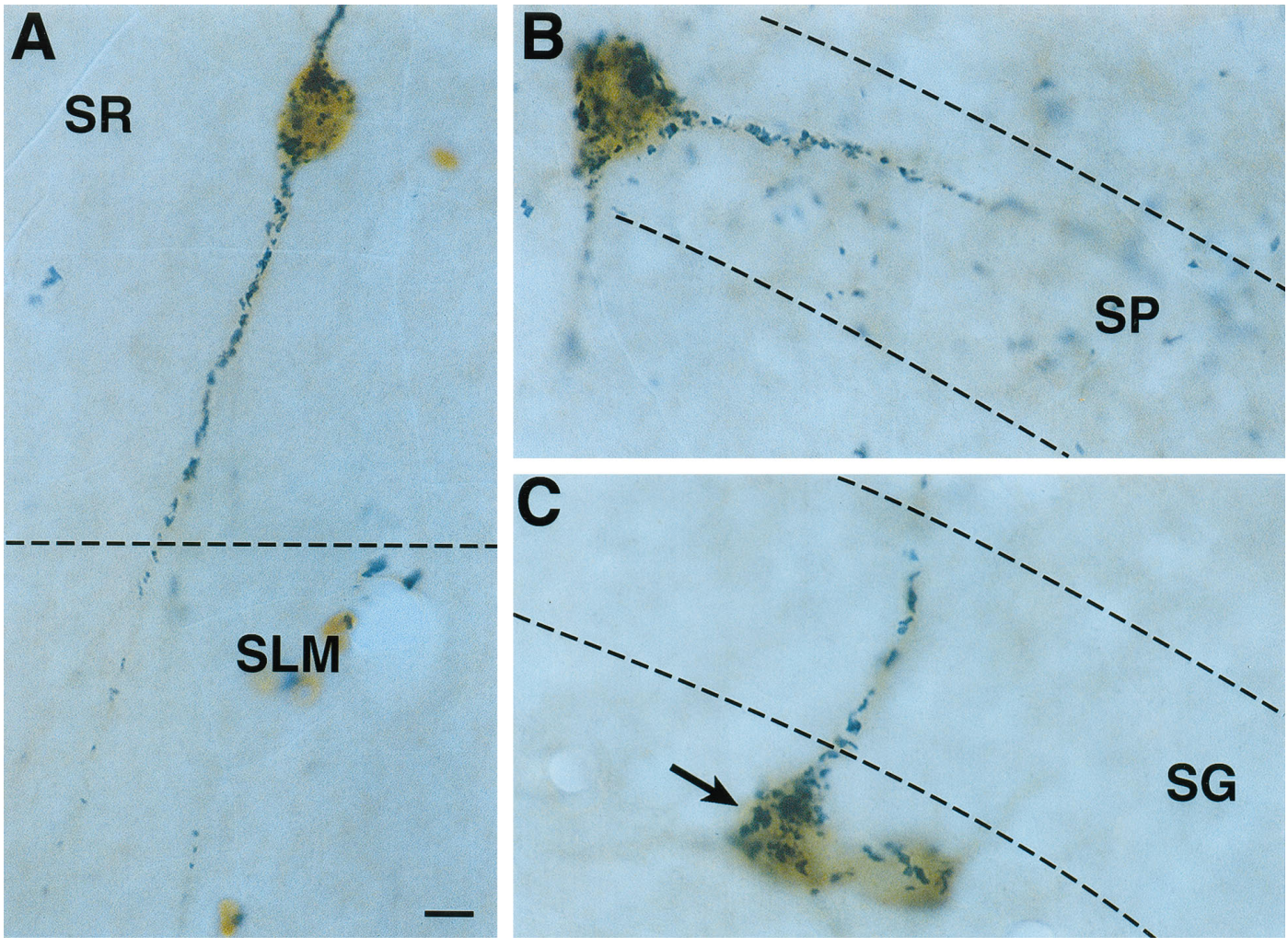

Figure 5. Simultaneous detection of 5-HT$_3$R and CCK immunoreactivity in CA1 (*A, B*) and dentate gyrus (*C*). *A*, Note a 5-HT$_3$R/CCK double-labeled cell in the *SR* extending into the *SLM*. *B*, A 5-HT$_3$R/CCK double-labeled cell in the st. pyramidale (*SP*) extending into the cell layer. *C*, Two 5-HT$_3$R/CCK double-labeled neurons immediately below the st. granulare (*SG*); one of them projects into the granule cell layer (*arrow*). *SR*, St. radiatum; *SLM*, st. lacunosum moleculare. Scale bar, 6 μm.

present in the 5-HT$_3$R-expressing neurons. No SS immunoreactivity was found in any 5-HT$_3$R-expressing neurons, although single SS-immunopositive or 5-HT$_3$R-expressing neurons were identified readily in cortex and hippocampus. In contrast, CCK immunoreactivity was often observed in 5-HT$_3$R-expressing neurons of the neocortex (Fig. 3*A,B*), olfactory regions (Fig. 3*C,D*), hippocampal formation, and amygdaloid complex.

Within the neocortex, 5-HT$_3$R/CCK-labeled cells were distributed mainly in layers II–III, although colocalization was also observed in cells of deeper layers. These double-labeled neurons were found frequently in the motor, prelimbic, and visual cortices (see *n* in Table 1). A semiquantitative analysis indicated that between 35 and 65% of the CCK-containing cells in layers II–III of the neocortex ($n = 726$ cells) express the 5-HT$_3$R (Table 1). We frequently observed 5-HT$_3$R/CCK-labeled cells in the anterior olfactory nucleus and piriform cortex, and a semiquantitative evaluation of these cells showed that 55–65% of the CCK-immunoreactive cells ($n = 147$ cells) contained 5-HT$_3$R transcripts (Table 1). These results indicate that the percentage of 5-HT$_3$R/CCK-labeled neurons is not homogenous within the different cortical areas.

5-HT$_3$R/CCK-labeled cells were found scattered throughout the hippocampus in the stratum (st.) oriens, st. pyramidale, and st. radiatum of the CA1–CA3. Within the st. oriens, double-labeled cells were sometimes located near the alveus or close to the st. pyramidale. The 5-HT$_3$R/CCK-labeled interneurons were located at the border of the granular cell layer in the dentate gyrus. Several nuclei of the amygdaloid complex also have 5-HT$_3$R/CCK neurons, mainly the lateral, basolateral, basomedial, and cortical nuclei.

Colocalization of 5-HT$_3$R and CCK

In another set of experiments, we used an anti-5-HT$_3$R antibody (Morales et al., 1996a) for the simultaneous detection of 5-HT$_3$R and CCK immunoreactivity. We found that some 5-HT$_3$R-immunolabeled neurons were also positive for CCK immunostaining. The pattern of distribution of these double-immunostained cells parallels that observed for CCK-immunoreactive/5-HT$_3$R mRNA-hybridized neurons in the neocortex and the hippocampal formation. Double-immunolabeled cells, however, were difficult to evaluate when the CCK immunoproduct appeared as sparse grains or dense aggregates in the cell bodies, limiting accurate quantitation of double-labeled cells.

Double 5-HT$_3$R/CCK-immunoreactive neurons were scattered in all layers of the neocortex and occasionally were found in the

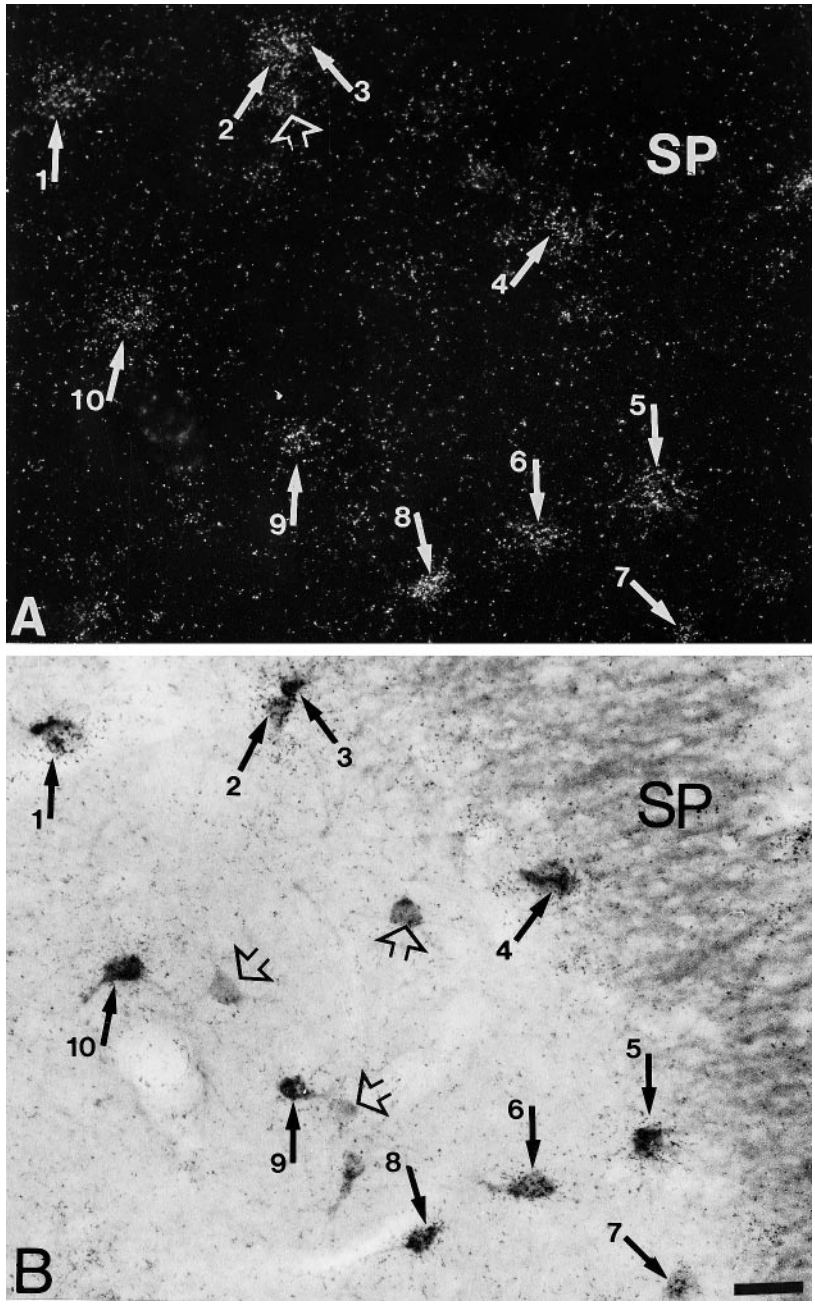

Figure 6. Simultaneous detection of 5-HT_3R transcripts and CB immunoreactivity in CA3. *A*, Observation of 5-HT_3R-expressing cells under epiluminescence microscopy. *B*, Observation of CB-immunoreactive neurons under bright-field microscopy. In this section, the majority of 5-HT_3R-expressing cells showed CB immunoreactivity (*filled arrows* in *A* and *B*), but a few CB-reactive cells were single-labeled for the protein (*open arrows* in *B*). Note a 5-HT_3R-expressing cell without CB immunoreactivity (*open arrow* in *A*). Scale bar, 22 μm.

white matter. Layers II–III contained the highest density of 5-HT_3R/CCK-immunoreactive neurons, and immunoreactive CCK was observed in the perikarya and processes; however, the 5-HT_3R immunoproduct was restricted mainly to the cell bodies.

Double 5-HT_3R/CCK-immunolabeled perikarya were found in the st. oriens, st. pyramidale, st. radiatum, and st. lacunosum moleculare of CA1 (Figs. 4*A*, 5*A,B*) and CA3 (Fig. 4*B*) subfields. The 5-HT_3R/CCK-labeled interneurons contained CCK-immunopositive processes extending from the st. oriens to the st. pyramidale and from the st. radiatum to the st. lacunosum moleculare (Fig. 5*A*). In addition, double 5-HT_3R/CCK cell bodies located in the st. pyramidale of the CA1 subfield showed CCK-labeled processes extending into the st. pyramidale (Fig. 5*B*). Double 5-HT_3R/CCK-immunolabeled perikarya were observed immediately below the granule cell layer of the dentate layer, with CCK-immunopositive processes entering the granule cell layer (Fig. 5*C*).

Colocalization of 5-HT_3R transcripts and Ca^{2+}-binding proteins

GABAergic cells are known to contain different Ca^{2+}-binding proteins, PV, CB, and CR (Baimbridge et al., 1992, for review). Thus, we sought to determine whether any of these proteins were present in 5-HT_3R-expressing neurons. Although no PV immunoreactivity was found in 5-HT_3R-expressing neurons, both CB and CR did colocalize with 5-HT_3R-expressing neurons.

5-HT_3R/CB double-labeled neurons were found occasionally in the entorhinal cortex and basomedial nucleus of the amygdala. In contrast, this type of neuron was often observed in basket cells of the dentate gyrus and the different strata of the hippocampus, preferentially in the st. radiatum of the ventral portion of the CA1 and CA3 subfields (Figs. 6), and a semiquantitative analysis of double-labeled cells indicated that between 33 and 63% of the

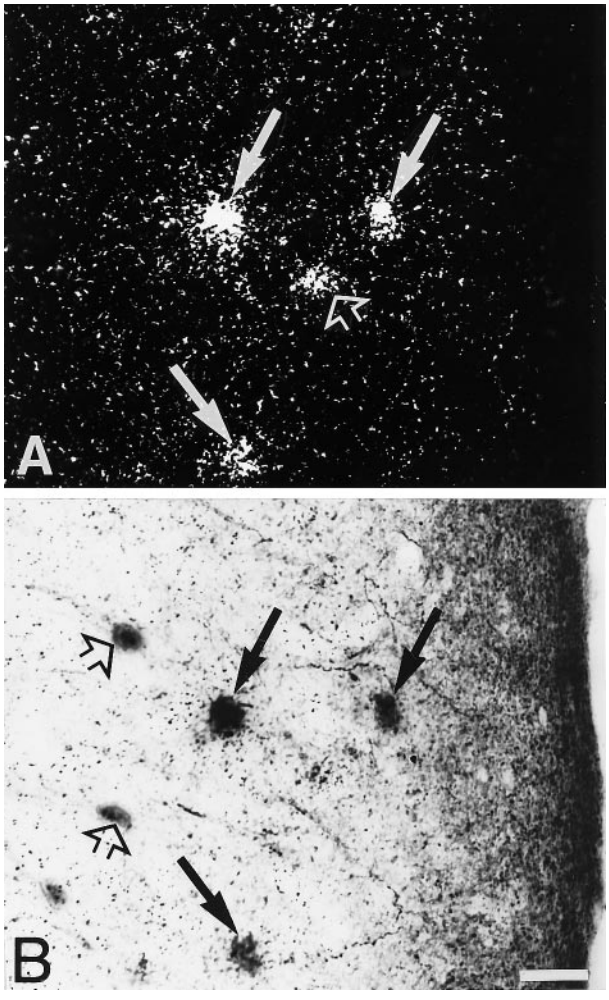

Figure 7. Simultaneous detection of 5-HT$_3$R transcripts and CR immunoreactivity in the occipital cortex. *A*, Observation of 5-HT$_3$R-expressing cells under epiluminescence microscopy. *B*, Observation of CR-immunoreactive neurons under bright field. In this section, several of the 5-HT$_3$R-expressing cells showed CR immunoreactivity (*filled arrows* in *A* and *B*), and few were single-labeled (*open arrows* in *A* and *B*). Scale bar, 25 μm.

CB-immunoreactive cells ($n = 267$ cells) expressed the 5-HT$_3$R in these regions.

5-HT$_3$R/CR double-labeled neurons showed a wider distribution than those for CB. The 5-HT$_3$R/CR-containing neurons were scattered throughout the forebrain: neocortex, olfactory tubercle, endopiriform nucleus, anterior olfactory nucleus, hippocampal formation, posteromedial cortical, and dorsolateral nuclei of the amygdala. 5-HT$_3$R/CR double-labeled neurons, however, were concentrated preferentially in the motor (Figs. 7*A,B*) agranular, perirhinal, insular, and piriform cortices. Within the hippocampal formation, these neurons were distributed mainly in the st. lacunosum moleculare of the subiculum (Fig. 8*A,B*) and CA1–CA3 areas.

DISCUSSION

Characterization OF 5-HT$_3$R/GABA-expressing cells

It has been demonstrated that cortical and hippocampal GABAergic interneurons are a heterogeneous population with regard to their morphology, biochemical composition, and synaptic connections (Wolff and Chronwall, 1982; Freund et al., 1983, 1990; Hendry et al., 1984; Somogyi et al., 1984; Kosaka et al., 1985; Celio, 1986; Sloviter and Nilaver, 1987; Katsumaru et al., 1988). Various biochemical properties of the GABAergic interneurons have been widely used to distinguish subpopulations of GABAergic neurons in cortex and hippocampus (Hendry et al., 1984; Somogyi et al., 1984; Kosaka et al., 1985; Celio, 1986; Sloviter and Nilaver, 1987; Katsumaru et al., 1988; Freund et al., 1990). Subpopulations of GABAergic neurons can be identified according to their calcium-binding protein (i.e., PV, CB, and CR) or neuropeptide content (i.e., SS and CCK). Our data indicate that the 5-HT$_3$R/GABA-expressing neurons belong to a distinct group of GABAergic cells that lack SS and PV but may contain CCK, CB, and CR. These three subgroups of 5-HT$_3$R/GABAergic interneurons have a distinct regional distribution, which is consistent with observations from cortical and hippocampal interneurons showing that PV- and CB-containing neurons form two different populations of GABAergic neurons that do not coexist with CCK (Hendry et al., 1984; Hendry and Jones, 1985; Gulyás et al., 1991). These data support the view that the 5-HT$_3$R-containing neurons are composed of a biochemically heterogeneous subpopulation of neurons that may be involved in different inhibitory circuits.

Coexistence of 5-HT$_3$R and CCK within the same cells

Previous studies have shown that the vast majority of cortical and hippocampal CCK-containing neurons are GABAergic in the rat brain (Hendry et al., 1984; Somogyi et al., 1984; Kosaka et al., 1985). We found that the 5-HT$_3$R, which is expressed in some interneurons, is present in a subpopulation of CCK-immunoreactive neurons in the neocortex, olfactory system, and hippocampal formation.

The colocalization of 5-HT$_3$R and CCK immunoreactivity within some interneurons implies functional interactions between serotonergic terminals and CCK-containing cells. In addition, the findings that 35–66% of the CCK-containing cells in layers II–III of the neocortex and 55–65% of neurons of the anterior olfactory nucleus and piriform cortex express the 5-HT$_3$R suggest that CCK neurotransmission is highly regulated by serotonin in the rat telencephalon. The presence, however, of putative serotonergic contacts on CCK cells has not yet been reported. In contrast, some neurochemical studies indicate that 5-HT$_3$R activation is capable of mediating the release of CCK. Paudice and Raiteri (1991) demonstrated that serotonin or 1-phenylbiguanide, a selective 5-HT$_3$R agonist, enhances the depolarization-evoked release of CCK from synaptosomes prepared from rat cerebral cortex, and this effect was prevented by 5-HT$_3$R antagonists. The 5-HT$_3$R antagonists also prevent the veratrine-evoked release of CCK in frontal cortex of freely moving rats (Raiteri et al., 1993), suggesting that activation of 5-HT$_3$R on CCK-releasing nerve endings might mediate the release of CCK. These pharmacological studies did not show which of the two possible sources of CCK in cortex, an intrinsic source from local CCK-containing cells or an extrinsic one from projections of the mesencephalon, were the source of released CCK. Our data on the presence of 5-HT$_3$R/CCK-containing neurons in cortex and hippocampus suggest that activation of local 5-HT$_3$R/CCK-containing cells might participate in the release of CCK in cortex and hippocampus.

The activity of hippocampal 5-HT$_3$R/CCK-containing neurons is likely to be regulated by the raphe nucleus. In addition, another source of regulation might be provided by the septum, because it has been reported that hippocampal CCK-containing neurons are innervated by GABAergic septal fibers (Gulyás et al., 1990), thus making the 5-HT$_3$R/CCK-containing neurons the target of sub-

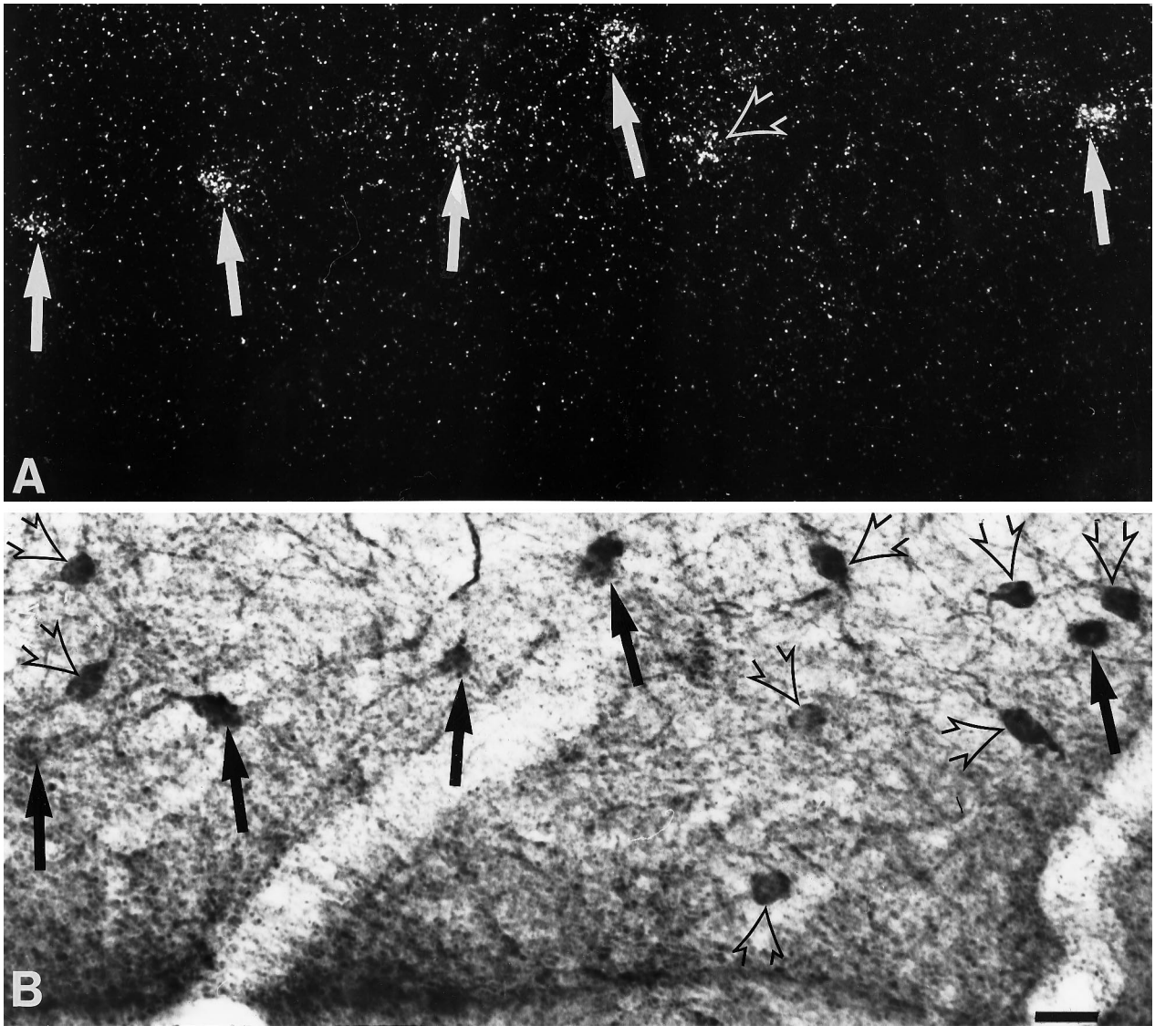

Figure 8. Simultaneous detection of 5-HT$_3$R transcripts and CR immunoreactivity field in the st. lacunosum moleculare of the subiculum. *A*, Observation of 5-HT$_3$R-expressing cells under epiluminescence microscopy. *B*, Observation of CR-immunoreactive neurons under bright field. In this section, the majority of 5-HT$_3$R-expressing cells showed CR immunoreactivity (*filled arrows* in *A* and *B*), and few were single-labeled (*open arrows* in *A* and *B*). Scale bar, 18 μm.

cortical pathways such as those originating in the septum and raphe nucleus.

We also detected 5-HT$_3$R/CCK double-immunolabeled neurons extending into the pyramidal and granule layers of the hippocampus and dentate gyrus. These results suggest that some of the 5-HT$_3$R/GABA/CCK-containing neurons might inhibit principal neurons in these regions. In agreement with this suggestion, it is known that CCK-positive boutons establish symmetrical synaptic contacts with perikarya and dendrites of pyramidal and nonpyramidal neurons in hippocampus (Harris et al., 1985; Hendry and Jones, 1985; Nunzi et al., 1985; Totterdell and Smith, 1986), and that CCK immunoreactivity is found in axonal terminals contacting perikarya of neurons in the cortex (Köhler and Chan-Palay, 1982). The activation of 5-HT$_3$R in CCK-containing interneurons might result in the direct inhibition of principal neurons, thus affecting areas to which these principal neurons project (Fig. 9). This inhibition might occur in pyramidal neurons of the subiculum that project to the nucleus accumbens and are innervated by CCK-immunoreactive terminals (Totterdell and Smith, 1986).

Coexistence of 5-HT$_3$R with Ca^{2+}-binding proteins

The characterization of 5-HT$_3$R/GABAergic neurons on the basis of the content of their calcium-binding proteins suggests that these neurons lack PV but may contain CR and CB.

Some 5-HT$_3$R/CR-containing cells were found in the neocortex, olfactory cortex, hippocampus, and amygdala, but these neurons were more often present in the agranular insular and piriform cortices. In contrast, the 5-HT$_3$R/CB double-labeled neurons were found mainly in the CA1–CA3 subfields of the hippocampus. These results are consistent with previous observations showing that serotonergic median raphe axons selectively innervate the somata and dendritic trees of CB-containing GABAergic interneurons in the CA1 and CA3 subfields of the rat hippocampus, but never those that contain PV (Freund et al., 1990; Halasy et al., 1992; Hornung and Celio, 1992; Miettinen et

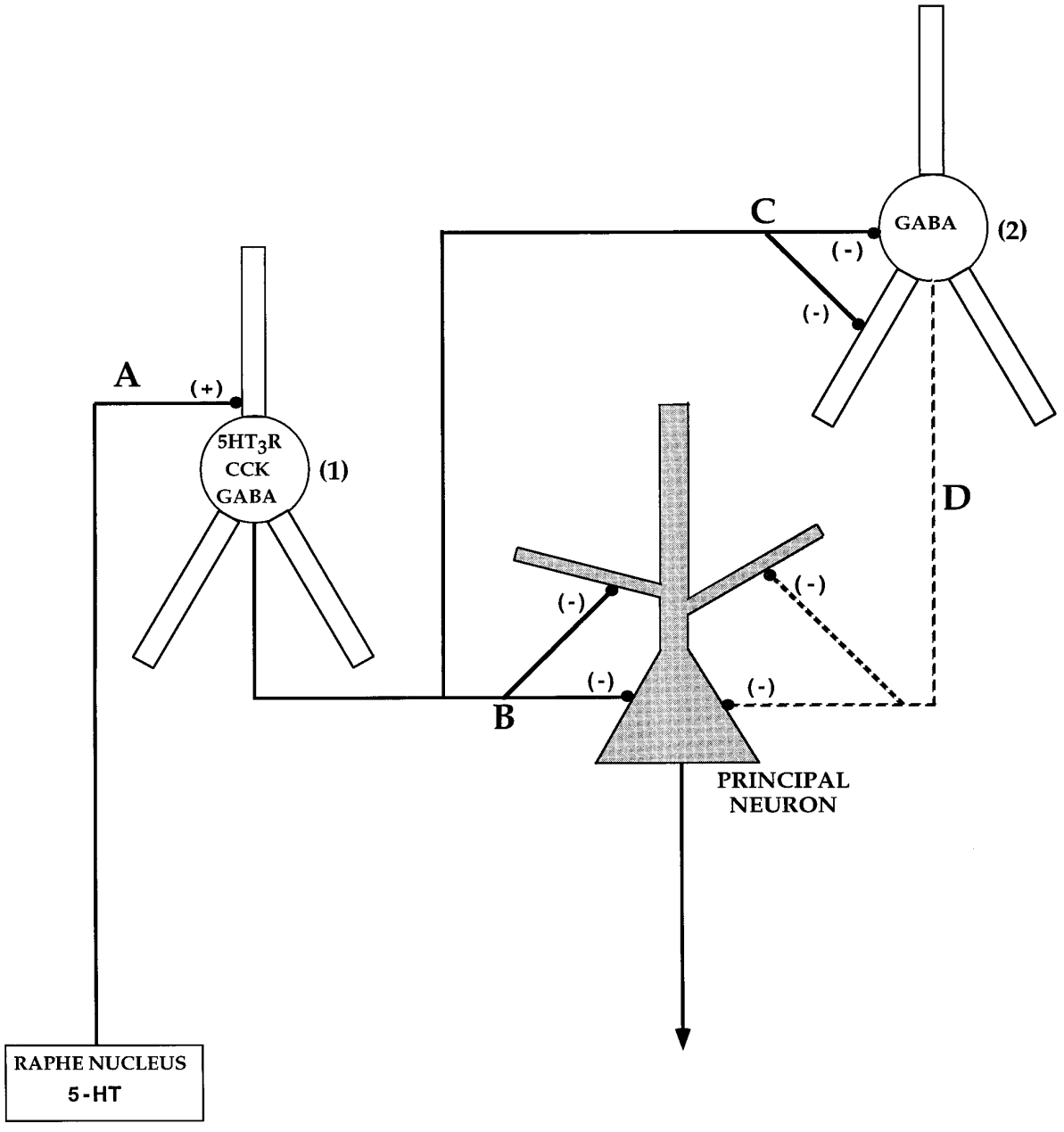

Figure 9. Hypothetical circuitry involving serotonergic activation of 5-HT$_3$R on inhibitory interneurons. Serotonin-immunoreactive terminals make asymmetrical synapses on dendrites of hippocampal and cortical interneurons (Freund et al., 1990; Smiley and Goldman-Rakic, 1996); some of these interneurons containing 5-HT$_3$R and CCK (*1*) might be activated by 5-HT, resulting in inactivation of other interneurons (*2*) and principal neurons. It is known that CCK-positive boutons establish symmetrical synaptic contacts with perikarya and dendrites of pyramidal and nonpyramidal neurons in hippocampus (Harris et al., 1985; Hendry and Jones, 1985; Nunzi et al., 1985; Totterdell and Smith, 1986), and that CCK immunoreactivity is found in axonal terminals innervating the perikarya of neurons in the cortex (Köhler and Chan-Palay, 1982). The activation of 5-HT$_3$R in CCK-containing interneurons (*1*) might result in the direct inhibition of principal neurons, thus affecting areas to which these principal neurons project.

al., 1992). Likewise, CR-immunoreactive neurons of the hippocampus also receive serotonergic synaptic contacts from the median raphe nuclei (Acsády et al., 1993). In addition, the hippocampal CB- and CR-immunoreactive neurons receive innervations from the medial septum (Freund and Antal, 1988; Freund et al., 1990; Gulyás et al., 1990; Acsády et al., 1993). The CB-containing interneurons are mainly basket and axo-axonic cells that make symmetrical synapses on the soma of the principal cells but also contact the axon initial segments, proximal dendrites, and dendritic spines of the pyramidal cells (Katsumaru et al., 1988; DeFelipe et al., 1989). The presence of CB and CR in 5-HT$_3$R-expressing interneurons suggests that these interneurons might belong to those hippocampal CB- and CR-immunoreactive neurons shown previously to be innervated by subcortical inputs from the medial septal and median raphe nuclei. In addition, these 5-HT$_3$R/CB and 5-HT$_3$R/CR interneurons might modulate neuronal transmission of interneurons and principal neurons.

In the present study we demonstrated that the neuronal expression of the 5-HT$_3$R is selective within the GABA neuron population in the rat telencephalon, extending preliminary

observations showing that 5-HT$_3$R transcripts coexist with GABA in the neocortex and hippocampus (Morales et al., 1996b). Despite the extensive colocalization of 5-HT$_3$R transcripts and GABA in the same neurons throughout the telencephalon, the expression of the 5-HT$_3$R is not restricted to GABAergic cells in the CNS; for instance, we have detected 5-HT$_3$R mRNA in some dopaminergic neurons of the mesencephalon (M. Morales and F. E. Bloom, unpublished observations) and in motoneurons of the dorsal horn of the spinal cord (Morales et al., 1996a).

The widespread colocalization of 5-HT$_3$R transcripts and GABA in the same neurons suggests the participation of 5-HT$_3$R in the excitation of inhibitory neurons in several brain regions. Consistent with these observations, previous immunohistochemical studies have shown that serotonin-immunoreactive terminals make asymmetrical synapses on dendrites of hippocampal and cortical interneurons (Freund et al., 1990; Smiley and Goldman-Rakic, 1996). Furthermore, 5-HT directly excites GABAergic interneurons via 5-HT$_3$R and consequently increases the frequency of inhibitory synaptic events recorded in CA1 pyramidal cells of rat hippocampal slices (Ropert and Guy, 1991). Although no similar electrophysiological findings have been reported in cortical areas, it is likely that some of the 5-HT$_3$R/GABAergic cortical neurons will also inhibit cortical pyramidal neurons or interneurons. The enhancement of GABAergic inhibition by activation of 5-HT$_3$R might be relevant for brain regulatory events, because 5-HT$_3$R agonists will inhibit the induction of long-term potentiation (LTP) in CA1 and CA3 (Corradetti et al., 1992; Maeda et al., 1994) through the facilitation of GABAergic neurons (via GABA$_A$ receptors). In addition, intraperitoneal administration of the 5-HT$_3$R antagonist ondansetron increased the frequency of the hippocampal theta rhythm, the induction of LTP in CA1, and the retention of olfactory and spatial memory in freely moving rats, suggesting that 5-HT$_3$R blockage of GABAergic cells results in the disinhibition of pyramidal cells (Stäubli and Xu, 1995). These observations suggest that the 5-HT$_3$R might participate in inhibitory and disinhibitory circuits in the rat telencephalon.

Relations to other 5-HT receptor localizations

The functional role played by the 5-HT$_3$R we have localized to GABA-containing interneurons provides only one facet of what may be viewed as serotonergic synaptic functions in the cerebral cortex and hippocampal formation. Clearly, the 5-HT$_3$R is only one of several 5-HT receptor subclasses whose cellular locations have already been assessed. In several recent studies on the cellular distribution of other 5-HT receptors, only the 5-HT$_2$R (Molineaux et al., 1989; Morilak et al., 1993; Freund and Buzsaki, 1996) has been detected on interneurons in addition to the 5-HT$_3$R; however, the 5-HT$_2$R is also present in principal neurons (Morilak et al., 1993; Hamada et al., 1996). The other major subtypes of 5-HT receptors, such as the 5HT$_{1A}$R (Gerard et al., 1994) and the 5HT$_6$R (Miquel et al., 1996) have been attributed only to the pyramidal neurons of the hippocampus but were not detected in interneurons. Although such studies cannot define precise roles for the different 5-HT-transducing target neurons, the contrasting localization may provide the basis for developing hypotheses for future pharmacological analyses.

In conclusion, we demonstrated that the 5-HT$_3$R-expressing neurons are mainly GABAergic in the rat telencephalon, suggesting that depolarization of these cells by serotonin might regulate inhibitory and disinhibitory circuits in the rat telencephalon. The 5-HT$_3$R-expressing cells might contain CCK, CB, and CR. This biochemical heterogeneity may reflect the participation of these neurons in different inhibitory circuits. In addition, the high degree of coexistence between the 5-HT$_3$R and CCK is indicative of the importance that the 5-HT$_3$R has in regulating CCK neurotransmission in the neocortex.

REFERENCES

Acsády L, Halasy K, Freund TF (1993) Calretinin is present in nonpyramidal cells of the rat hippocampus-III: their inputs from the median raphe and medial septal nuclei. Neuroscience 52:829–841.

Baimbridge KG, Celio MR, Rogers JH (1992) Calcium-binding proteins in the nervous system. Trends Neurosci 15:303–308.

Barnes JM, Barnes NM, Costall B, Ironside JW, Naylor RJ (1989a) Identification and characterization of 5-hydroxytryptamine$_3$ recognition sites in human brain tissue. J Neurochem 53:1787–1793.

Barnes JM, Barnes NM, Costall B, Naylor RJ, Tyers MB (1989b) 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. Nature 338:762–763.

Barnes JM, Barnes NM, Champaneria S, Costall B, Naylor RJ (1990) Characterization and autoradiographic localization of 5-HT$_3$ receptor recognition sites identified with [^{3}H]-(*S*)-zacopride in the forebrain of the rat. Neuropharmacology 29:1037–1045.

Blandina P, Goldfarb J, Craddock-Royal B, Green JP (1989) Release of endogenous dopamine by stimulation of 5-hydroxytryptamine$_3$ receptors in rat striatum. J Pharmacol Exp Ther 251:803–809.

Carboni E, Acquas E, Leone P, Di Chiara G (1989) 5-HT$_3$ receptor antagonists block morphine- and nicotine- but not amphetamine-induced reward. Psychopharmacology 97:175–178.

Celio MR (1986) Parvalbumin in most γ-aminobutyric acid-containing neurons of the rat cerebral cortex. Science 231:995–997.

Chen J, van Praag HM, Gardner EL (1991) Activation of 5-HT$_3$ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res 543:354–357.

Chen J, Paredes W, van Praag HM, Lowinson JH, Gardner EL (1992) Presynaptic dopamine release is enhanced by 5-HT$_3$ receptor activation in medial prefrontal cortex of freely moving rats. Synapse 10:264–266.

Corradetti R, Ballerini L, Pugliese AM, Pepeu G (1992) Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of rat hippocampal slices. Neuroscience 46:511–518.

Costall B, Naylor RJ, Tyers MB (1990) The psychopharmacology of 5-HT$_3$ receptors. J Pharmacol Ther 47:181–202.

Costall B, Domeney AM, Kelly ME, Tomkins DM, Naylor RJ, Wong EH, Smith WL, Whiting RL, Eglen RM (1993) The effect of the 5-HT$_3$ receptor antagonist, RS-42358–197, in animal models of anxiety. Eur J Pharmacol 234:91–99.

DeFelipe J, Hendry SHC, Jones EG (1989) Synapses of double bouquet cells in monkey cerebral cortex visualized by calbindin immunoreactivity. Brain Res 503:49–54.

Derkach V, Suprenant A, Northand RA (1989) 5-HT$_3$ receptors are membrane ion channels. Nature 33:706–709.

Freund TF, Antal M (1988) GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature 336:170–173.

Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6:347–470.

Freund TF, Martin KAC, Smith AD, Somogyi P (1983) Glutamate decarboxylase-immunoreactive terminals of Golgi-impregnated axo-axonic cells and of presumed basket cells in synaptic contact with pyramidal cells of the cat's visual cortex. J Comp Neurol 221:263–278.

Freund TF, Gulyás AI, Acsády L, Görcs T, Tóth K (1990) Serotonergic control of the hippocampus via local inhibitory interneurons. Proc Natl Acad Sci USA 87:8501–8505.

Gehlert DR, Gackenheimer SL, Wong DT, Robertson DW (1991) Localization of 5-HT$_3$ receptors in the rat brain using [^{3}H]LY278584. Brain Res 553:149–154.

Gerard C, Langlois X, Gingrich J, Doucet E, Verge D, Kia HK, Raisman R, Gozlan H, Mestikawy SEL, Hamon M (1994) Production and characterization of polyclonal antibodies recognizing the intracytoplasmic third loop of the 5 hydroxytryptamine 1A receptor. Neuroscience 62:721–739.

Gulyás AI, Görcs TJ, Freund TF (1990) Innervation of different peptide-containing neurons in the hippocampus by GABAergic septal afferents. Neuroscience 37:31–44.

Gulyás AI, Tóth K, Dános P, Freund TF (1991) Subpopulation of GABAergic neurons containing parvalbumin, calbindin D28k, and cholecystokinin in the rat hippocampus. J Comp Neurol 312:371–378.

Halasy K, Miettinen R, Szabat E, Freund TF (1992) GABAergic interneurons are the major postsynaptic targets of median raphe afferents in the rat dentate gyrus. Eur J Neurosci 4:144–153.

Hamada S, Senzaki K, Tabuchi K, Yamamoto H, Yamamoto T, Yoshikawa S, Okano H, Okado N (1996) Localization of 5-HT_{2A} receptor in rat cerebral cortex by immunohistochemistry. Soc Neurosci Abstr 22:1774.

Harris KM, Marshall PE, Landis DMD (1985) Ultrastructural study of cholecystokinin-immunoreactive cells and processes in area CA1 of the rat hippocampus. J Comp Neurol 233:147–158.

Hendry SHC, Jones EG (1985) Morphology of synapses formed by cholecystokinin-immunoreactive axon terminals in regio superior of rat hippocampus. Neuroscience 16:57–68.

Hendry SHC, Jones EG, DeFelipe J, Schmechel D, Brandon C, Emson PC (1984) Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. Proc Natl Acad Sci USA 81:6526–6530.

Hornung JP, Celio MR (1992) The selective innervation by serotonergic axons of calbindin-containing interneurons in the neocortex and hippocampus of the marmoset. J Comp Neurol 320:457–467.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA (1994) International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46:157–203.

Jones DN, Barnes NM, Costall B, Domeney AM, Kilpatrick GJ, Naylor RJ, Tyers MB (1992) The distribution of 5-HT_3 recognition sites in the marmoset brain. Eur J Pharmacol 215:63–67.

Katsumaru H, Kosaka T, Heizmann CW, Hama K (1988) Immunocytochemical study of GABAergic neurons containing the calcium-binding protein parvalbumin in the rat hippocampus. Exp Brain Res 72:347–362.

Kilpatrick GJ, Jones BJ, Tyers MB (1987) Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 330:746–748.

Kilpatrick GJ, Jones BJ, Tyers MB (1988) The distribution of specific binding of the 5-HT_3 receptor ligand [^{3}H]GR65630 in rat brain using quantitative autoradiography. Neurosci Lett 94:156–160.

Kilpatrick GJ, Jones BJ, Tyers MB (1989) Binding of the 5-HT_3 ligand, [^{3}H]GR65630, to rat area postrema, vagus nerve and the brains of several species. Eur J Pharmacol 159:157–164.

Köhler C, Chan-Palay V (1982) The distribution of cholecystokinin-like immunoreactive neurons and nerve terminals in the retrohippocampal region in the rat and guinea-pig. J Comp Neurol 210:136–146.

Kosaka T, Kosaka K, Tateishi K, Hamaoka Y, Yanaihara N, Wu J-Y, Hama K (1985) GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. J Comp Neurol 239:420–430.

Laporte AM, Koscielniak T, Ponchant M, Verge D, Hamon M, Gozlan H (1992) Quantitative autoradiographic mapping of 5-HT_3 receptors in the rat CNS using [^{125}I]iodo-zacopride and [^{3}H]zacopride as radioligands. Synapse 10:271–281.

Levey AI, Bolam JP, Rye DB, Hallenger AE, Demuth RM, Mesulum M-M, Wainer BH (1986) A light and electron microscopic procedure for sequential double antigen localization using diamine benzidine and benzidine dihydrochloride. J Histochem Cytochem 34:1449–1457.

Maeda T, Kaneko S, Satoh M (1994) Inhibitory influence via 5-HT_3 receptors on the induction of LTP in mossy fiber-CA3 system of guinea-pig hippocampal slices. Neurosci Res 18:277–282.

Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D (1991) Primary structure and functional expression of the 5HT_3 receptor, a serotonin-gated ion channel. Science 254:432–437.

Maura G, Andrioli GC, Cavazzani P, Raiteri M (1992) 5-Hydroxytryptamine$_3$ receptors sited on cholinergic axon terminals of human cerebral cortex mediate inhibition of acetylcholine release. J Neurochem 58:2334–2336.

Miettinen R, Gulyás AI, Baimbridge KG, Jacobowitz DM, Freund TF (1992) Calretinin is present in non-pyramidal cells of the rat hippocampus-II: co-existence with other calcium binding proteins and GABA. Neuroscience 48:29–43.

Miquel MC, Lefevre K, Sari Y, Brisorgueil MJ, Calas A, Gerard C, Hamon M, Verge D (1996) Immunocytochemical visualization of 5HT_6 receptors in the rat brain. Soc Neurosci Abstr 22:1782.

Molineaux SM, Jessell TM, Axel R, Julius D (1989) 5HT_{1C} receptor is a prominent serotonin receptor subtype in the central nervous system. Proc Natl Acad Sci USA 86:6793–6797.

Morales M, Battenberg E, de Lecea L, Sanna PP, Bloom FE (1996a) Cellular and subcellular immunolocalization of the type 3 serotonin receptor in the rat central nervous system. Mol Brain Res 36:251–260.

Morales M, Battenberg E, de Lecea L, Bloom FE (1996b) The type 3 serotonin receptor is expressed in a subpopulation of GABAergic neurons in the rat neocortex and hippocampus. Brain Res 731:199–202.

Morilak DA, Garlow SJ, Ciaranello RD (1993) Immunocytochemical localization and description of neurons expressing serotonin 2 receptors in the rat brain. Neuroscience 54:701–717.

Morrison JH, Benoit R, Magistretti PJ, Bloom FE (1983) Immunohistochemical distribution of pro-somatostatin-related peptides in cerebral cortex. Brain Res 262:344–351.

Nevins ME, Anthony EW (1994) Antagonists at the serotonin-$_3$ receptor can reduce the fear-potentiated startle response in the rat: evidence for different types of anxiolytic activity? J Pharmacol Exp Ther 268:248–254.

Nunzi MG, Gorio A, Milan F, Freund TF, Somogyi P, Smith AD (1985) Cholecystokinin-immunoreactive cells form symmetrical synaptic contacts with pyramidal and nonpyramidal neurons in the hippocampus. J Comp Neurol 237:485–505.

Paudice P, Raiteri M (1991) Cholecystokinin release mediated by 5-HT_3 receptors in rat cerebral cortex and nucleus accumbens. Br J Pharmacol 103:1790–1794.

Peters JA, Lambert JJ (1989) Electrophysiology of 5-HT_3 receptors in neuronal cell lines. Trends Pharmacol Sci 10:172–174.

Pratt GD, Bowery NG, Kilpatrick GJ, Leslie RA, Barnes NM, Naylor RJ, Jones BJ, Nelson DR, Palacios JM, Slater P, Reynolds DJM (1990) Consensus meeting agrees distribution of 5-HT_3 receptors in mammalian hindbrain. Trends Pharmacol Sci 11:135–137.

Raiteri M, Paudice P, Vallebuona F (1993) Inhibition by 5-HT_3 receptor antagonists of release of cholecystokinin-like immunoreactivity from the frontal cortex of freely moving rats. Naunyn Schmiedebergs Arch Pharmacol 347:111–114.

Ropert N, Guy N (1991) Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus in vitro. J Physiol (Lond) 441:121–136.

Sloviter R, Nilaver G (1987) Immunocytochemical localization of GABA-, cholecystokinin-, vasoactive intestinal polypeptide-, and somatostatin-like immunoreactivity in the area dentate and hippocampus of the rat. J Comp Neurol 256:42–60.

Smiley JF, Goldman-Rakic PS (1996) Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. J Comp Neurol 367:431–443.

Somogyi P, Hodgson AJ, Smith AD, Nunzi MG, Gorio A, Wu J-Y (1984) Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. J Neurosci 10:2590–2603.

Stäubli U, Xu FB (1995) Effects of 5-HT_3 receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. J Neurosci 15:2445–2452.

Tecott LH, Maricq AV, Julius D (1993) Nervous system distribution of the serotonin 5-HT_3 receptor mRNA. Proc Natl Acad Sci USA 90:1430–1434.

Totterdell S, Smith AD (1986) Cholecystokinin-immunoreactive boutons in synaptic contact with hippocampal pyramidal neurons that project to the nucleus accumbens. Neuroscience 19:181–192.

Waeber C, Dixon K, Hoyer D, Palacios JM (1988) Localization by autoradiography of neuronal 5-HT_3 receptors in the mouse CNS. Eur J Pharmacol 151:351–352.

Waeber C, Hoyer D, Palacios JM (1989) 5-hydroxytryptamine$_3$ receptors in the human brain: autoradiographic visualization using [^{3}H]ICS 205–930. Neuroscience 31:393–400.

Waeber C, Pinkus LM, Palacios JM (1990) The (*S*)-isomer of [^{3}H]zacopride labels 5-HT_3 receptors with high affinity in rat brain. Eur J Pharmacol 181:283–287.

Wolff JR, Chronwall BM (1982) Axosomatic synapses in the visual cortex of adult rat: a comparison between GABA-accumulating and other neurons. J Neurocytol 11:409–425.