41 Holzmann, C. *et al.* (1998) Isolation and characterization of the rat huntingtin promoter. *Biochem. J.* 336, 227–234
42 Alberici, A. *et al.* (1999) Presenilin 1 protein directly interacts with Bcl-2. *J. Biol. Chem.* 274, 30764–30769
43 Passer, B.J. *et al.* (1999) Interaction of Alzheimer's presenilin-1 and presenilin-2 with bcl-X (L). A potential role in modulating the threshold of cell death. *J. Biol. Chem.* 274, 24007–24013
44 Shen, J. *et al.* (1997) Skeletal and CNS defects in presenilin-1-deficient mice. *Cell* 89, 629–639
45 Guo, Q. *et al.* (1999) Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid β-peptide toxicity: central roles of superoxide production and caspase activation. *J. Neurochem.* 72, 1019–1029
46 Naruse, S. *et al.* (1998) Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron* 21, 1213–1221
47 Katayama, T. *et al.* (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. *Nat. Cell Biol.* 1, 479–485
48 Johnston, L.A. and Edgar, B.A. (1998) Wingless and notch regulate cell-cycle arrest in the developing *Drosophila* wing. *Nature* 394, 82–84
49 Nagy, Z.S. *et al.* (1998) The cell division cycle and the pathophysiology of Alzheimer's disease. *Neuroscience* 87, 731–739
50 Stabler, S.M. *et al.* (1999) A myristolated calcium-binding protein that preferentially interacts with Alzheimer's disease presenilin 2 protein. *J. Cell. Biol.* 145, 1277–1292
51 Parshad, R.P. *et al.* (1996) Fluorescent light-induced chromatid breaks distinguish Alzheimer disease cells from normal cells in tissue culture. *Proc. Natl. Acad. Sci. U. S. A.* 93, 5146–5150
52 Rolig, R.L. and McKinnon, P.J. (2000) Linking DNA damage and neurodegeneration. *Trends Neurosci.* 23, 417–424
53 Tabrizi, S.J. and Schapira, A.H. (1999) Secondary abnormalities of mitochondrial DNA associated with neurodegeneration. *Biochem. Soc. Symp.* 66, 99–110
54 Khan, S.M. *et al.* (2000) Alzheimer's disease cybrids replicate beta-amyloid abnormalities through cell death pathways. *Ann. Neurol.* 48, 148–155
55 Boutell, J.M. *et al.* (1999) Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Hum. Mol. Genet.* 8, 1647–1655
56 McCampbell, A. *et al.* (2000) CREB-binding protein sequestration by expanded polyglutamine. *Hum. Mol. Genet.* 9, 2197–2202
57 Ostrerova, N. *et al.* (1999) α-Synuclein shares physical and functional homology with 14-3-3 proteins. *J. Neurosci.* 19, 5782–5791
58 Clayton, D.F. and George, J.M. (1998) The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci.* 6, 249–254
59 Petersen, K. *et al.* (1999) Developmental expression of α-synuclein in rat hippocampus and cerebral cortex. *Neuroscience* 91, 651–659
60 Shimura, H. *et al.* (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat. Genet.* 3, 302–305
61 Arnold, J. *et al.* (1998) Ubiquitin and its role in neurodegeneration. *Prog. Brain Res.* 117, 23–34
62 Leroy, E. *et al.* (1998) The ubiquitin pathway in Parkinson's disease. *Nature* 395, 451–452
63 Taya, S. *et al.* (1999) The deubiquitinating enzyme Fam interacts with and stabilizes β-catenin. *Genes Cells* 4, 757–767
64 Huang, Y. and Fischer-Vize, J.A. (1996) Undifferentiated cells in the developing *Drosophila* eye influence facet assembly and require the Fat facets ubiquitin-specific protease. *Development* 122, 3207–3216
65 Mehler, M.F. and Gokhan, S. (2000) Developmental expression and regulation of neurodegenerative disease proteins in embryonic stem cells, neural progenitor species, and differentiated neurons and glia. *Neurology* 54, A313

Acknowledgements
The authors thank B. Shapiro for artistic expertise, R. Sanchez for performing the protein database searches and K.S. Marder and Z. Kaprielian for critical commentaries. The authors research was supported by grants from the NINDS, NIH. We sincerely regret the fact that space limitations have prevented us from citing the many outstanding experimental studies that have contributed to our evolving view of the relationship between development and neurodegeneration.

5-HT$_3$ receptors and neurotransmitter release in the CNS: a nerve ending story?

Johannes A. van Hooft and Henk P.M. Vijverberg

Serotonin (5-HT) 5-HT$_3$ receptors are ligand-gated ion channels, which are generally thought to be involved in the presynaptic modulation of neurotransmitter release. However, analysis of published data reveals that most of the evidence for the alleged presynaptic role of 5-HT$_3$ receptors in modulating CNS neurotransmitter release is not compelling. Nevertheless, 5-HT$_3$ receptors are present in nerve terminals from some brain regions. The increased basic knowledge of the cellular physiology of central 5-HT$_3$ receptor ligand-gated ion channels provides opportunities for a detailed characterization of the specific presynaptic effects of 5-HT$_3$ receptors. Such reconsideration is required for the full appreciation of the functional role of 5-HT$_3$ receptors in the CNS.

Trends Neurosci. (2000) 23, 605–610

THE PRESYNAPTIC modulation of neurotransmitter release by ionotropic neurotransmitter receptors is a recent focus of interest[1]. The 5-HT$_3$ receptor (Box 1) is a ligand-gated ion channel[2–4]. Postsynaptic 5-HT$_3$ receptors, which have been shown to be present on GABAergic interneurons (Fig. 1), mediate fast synaptic neurotransmission in the CNS (Refs 7,8). In addition, it has been suggested that presynaptic 5-HT$_3$ receptors modulate the release of several neurotransmitters in the CNS (Refs 1,9–11). In order to modulate the release of a specific neurotransmitter by a presynaptic mechanism, the primary requirement is that 5-HT$_3$ receptors are present on the presynaptic terminals. Initial evidence for presynaptic 5-HT$_3$ receptors is derived from autoradiographic studies in the spinal cord[12]. In several brain regions, including the CA1 pyramidal cell layer in the hippocampus, the dorsal motor nucleus of the vagal nerve, the nucleus of the solitary tract and the area postrema, 5-HT$_3$ receptor binding sites were shown to be present using autoradiography[13–16]. *In situ* hybridization studies of the same brain regions[17] have not revealed hybridizing cells, suggesting that 5-HT$_3$ receptors in these regions are also

Johannes A. van Hooft is at the University of Amsterdam, Swammerdam Institute for Life Sciences, Section Neurobiology, PO Box 94084, NL-1090 GB Amsterdam, The Netherlands, and Henk P.M. Vijverberg is at the Research Institute of Toxicology, Utrecht University, PO Box 80.176, NL-3508 TD Utrecht, The Netherlands.

 PII: S0166-2236(00)01662-3

Box 1. Structure and function of 5-HT$_3$ receptors

Molecular properties

Serotonin 5-HT$_3$ receptors belong to the family of ligand-gated ion channels. To date, two subunits of this receptor, 5-HT$_3$R-A and 5-HT$_3$R-B, have been cloned[a,b]. The 5-HT$_3$R-A subunit is a protein with four transmembrane (TM) segments and an extracellular N terminus. By contrast, the structure of the 5-HT$_3$R-B subunit appears to be more complex, because its TM2 segment lacks any structural homology to that of other ligand-gated ion channels[b]. Two splice variants of the 5-HT$_3$R-A subunit have been identified that differ in the presence of six amino acids in the putative large intracellular loop[c], and have different expression profiles during development[d,e]. However, thus far, physiologically relevant functional differences between the splice variants have not been shown.

5-HT$_3$R-A subunits form homopentameric ligand-gated ion channels[f]. Site-directed mutagenesis has demonstrated several functional features of the 5-HT$_3$R-A subunit: the extracellular N terminal domain contains the agonist recognition site[g–i] and the mutation L286T in TM2 alters the kinetics of the current that passes through the ligand-gated ion channel[j].

Native 5-HT$_3$ receptors share many pharmacological and functional properties with homopentameric 5-HT$_3$ receptors in heterologous expression systems. However, specific differences in agonist (and antagonist) profiles and in the permeability to Ca^{2+} ions suggest that native receptors are distinct from homopentameric receptors[k]. Functional heteromeric 5-HT$_3$ receptors with properties distinct from homomeric 5-HT$_3$R-A receptors can be formed in heterologous expression systems by co-expressing 5-HT$_3$R-A subunits either with 5-HT$_3$R-B subunits[b] or with the α4 subunit of the nicotinic ACh receptor[l]. Whether such heteromeric receptors actually occur in the CNS remains to be determined.

Pharmacological properties

The affinity of 5-HT for the agonist recognition site of the 5-HT$_3$ receptor is relatively low (Table I; for an extensive review on 5-HT receptor pharmacology, see Ref. rr). 5-HT might not be the only endogenous ligand, because dopamine (DA) is also a low-affinity agonist of 5-HT$_3$ receptors[m]. Of the various 5-HT$_3$ receptor agonists known, 2-methyl-5-HT, phenylbiguanide and *m*-chlorophenylbiguanide are the more commonly used (Table I). However, highly selective 5-HT$_3$ receptor agonists have not been found. Non-selective effects, observed at agonist concentrations similar to those required for 5-HT$_3$ receptor activation, include reversal of DA transport[n–s] and partial agonism or antagonism of α_2 adrenoceptors[t,u].

Numerous highly potent antagonists of 5-HT$_3$ receptors have been developed. Several common 5-HT$_3$ receptor antagonists are listed in Table I. In general, these compounds are highly selective for 5-HT$_3$ receptors and non-selective effects appear to occur only at concentrations in excess of those required to antagonize 5-HT$_3$ receptors by 100-fold or more. The non-selective effects of 5-HT$_3$ receptor antagonists are of a variable nature and include antagonism of receptors other than 5-HT$_3$ receptors[v–z], agonism of 5-HT$_4$ receptors[aa], and local anaesthetic-like block of ligand- and voltage-gated ion channels[bb–dd].

TABLE I. Functionally effective concentrations of various agonists and antagonists commonly used in 5-HT$_3$ receptor research[a]

Agonists	EC_{50} (μM)	Ref.
5-HT	2.0	k,m,ll
2-methyl-5-HT	5.0–8.0	k,ll
1-phenylbiguanide	22.0	mm
m-chlorophenylbiguanide	0.4	k,ll,mm
Antagonists	**IC_{50} (nM)**	**Ref.**
ondansetron	0.3	nn
tropisetron	0.2	oo
D-tubocurarine	1.0	pp
(S)-zacopride	0.6	qq

[a]EC_{50} values for activation of inward current by agonists and IC_{50} values for inhibition of 5-HT-induced inward current by antagonists are presented. Values are representative for mouse 5-HT$_3$ receptors, and species differences[ff] are not shown.

A notable property of 5-HT$_3$ receptors is the potentiation of agonist effects by alcohols and anaesthetic agents (reviewed in Ref. ee), which might complicate the comparison of *in vitro* data with data obtained *in vivo* from anaesthetized animals.

Functional properties

5-HT$_3$ receptors are non-selective cation channels that mediate transient inward currents and membrane depolarization under physiological conditions (for reviews see Refs ff–hh). In the CNS the functional properties of presynaptic 5-HT$_3$ receptors might differ from those of postsynaptic 5-HT$_3$ receptors. Presynaptic 5-HT$_3$ receptors on striatal synaptosomes are Ca^{2+}-permeable ion channels, and the influx of Ca^{2+} through these channels is responsible for the elevation of intracellular Ca^{2+} induced by stimulation with 5-HT (Ref. ii). Conversely, postsynaptic 5-HT$_3$ receptors on hippocampal interneurons are blocked by Ca^{2+} at negative membrane potentials[jj], similar to the voltage-dependent block by Mg^{2+} of NMDA receptors[kk]. Whether the signal transduction pathway that couples 5-HT$_3$ receptor activation to neurotransmitter release involves Ca^{2+} influx through the ligand-gated ion channels or through voltage-gated Ca^{2+} channels, activated secondarily to 5-HT$_3$ receptor-mediated membrane depolarization, remains to be determined.

References

a Maricq, A.V. *et al.* (1991) Primary structure and functional expression of the 5HT$_3$ receptor, a serotonin-gated ion channel. *Science* 254, 432–437

b Davies, P.A. *et al.* (1999) The 5-HT$_{3B}$ subunit is a major determinant of serotonin-receptor function. *Nature* 397, 359–363

c Hope, A.G. *et al.* (1993) Cloning and functional expression of an apparent splice variant of the murine 5-HT$_3$ receptor A subunit. *Eur. J. Pharmacol.* 245, 187–192

d Emerit, M.B. *et al.* (1995) Differentiation alters the expression of the two splice variants of the serotonin 5-HT$_3$ receptor-A mRNA in NG108-15 cells. *J. Neurochem.* 65, 1917–1925

e Miquel, M.C. *et al.* (1995) Developmental changes in the differential expression of two serotonin 5-HT$_3$ receptor splice variants in the rat. *J. Neurochem.* 65, 475–483

f Boess, F.G. *et al.* (1995) Ultrastructure of the 5-hydroxytryptamine3 receptor. *J. Neurochem.* 64, 1401–1405

g Boess, F.G. *et al.* (1997) Analysis of the ligand binding site of the 5-HT$_3$ receptor using site directed mutagenesis: importance of glutamate 106. *Neuropharmacology* 36, 637–647

h Spier, A.D. and Lummis, S.C.R. (2000) The role of tryptophan residues in the 5-hydroxytryptamine(3) receptor ligand binding domain. *J. Biol. Chem.* 275, 5620–5625

i Mochizuki, S. *et al.* (1999) Identification of a domain affecting agonist potency of *meta*-chlorophenylbiguanide in 5-HT$_3$ receptors. *Eur. J. Pharmacol.* 369, 125–132

j Yakel, J.L. *et al.* (1993) Single amino acid substitution affects desensitization of the 5-hydroxytryptamine type 3 receptor expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U. S. A.* 90, 5030–5033

k van Hooft, J.A. *et al.* (1997) Native serotonin 5-HT$_3$ receptors expressed in *Xenopus* oocytes differ from homopentameric 5-HT$_3$ receptors. *J. Neurochem.* 69, 1318–1321

l van Hooft, J.A. *et al.* (1998) Promiscuous coassembly of serotonin 5-HT$_3$ and nicotinic α4 receptor subunits into Ca^{2+}-permeable ion channels. *Proc. Natl. Acad. Sci. U. S. A.* 95, 11456–11461

m Neijt, H.C. *et al.* (1986) The dopamine response in mouse neuroblastoma cells is mediated by serotonin 5HT$_3$ receptors. *Eur. J. Pharmacol.* 127, 271–274

n Andrews, D.W. *et al.* (1978) The effects of 5-hydroxytryptophan and 5-hydroxytryptamine on dopamine synthesis and release in rat brain striatal synaptosomes. *J. Neurochem.* 30, 465–470

o Schmidt, C.J. and Black, C.K. (1989) The putative 5-HT$_3$ agonist phenylbiguanide induces carrier-mediated release of [^{3}H]dopamine. *Eur. J. Pharmacol.* 167, 309–310

p Yi, S.J. *et al.* (1991) Effect of cocaine and 5-HT$_3$ receptor antagonists on 5-HT-induced [^{3}H]dopamine release from rat striatal synaptosomes. *Eur. J. Pharmacol.* 199, 185–189

q Jacocks, H.M., 3rd and Cox, B.M. (1992) Serotonin-stimulated release of [^{3}H]dopamine via reversal of the dopamine transporter in rat striatum and nucleus accumbens: a comparison with release elicited by potassium, *N*-methyl-D-aspartic acid, glutamic acid and D-amphetamine. *J. Pharmacol. Exp. Ther.* 262, 356–364

r Benuck, M. and Reith, M.E. (1992) Dopamine releasing effect of phenylbiguanide in rat striatal slices. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 345, 666–672

s Campbell, A.D. *et al.* (1995) The 5-HT$_3$ receptor agonist 1-(*m*-chlorophenyl)-biguanide interacts with the dopamine transporter in rat brain synaptosomes. *Eur. J. Pharmacol.* 290, 157–162

t Allgaier, C. *et al.* (1995) Effects of 5-HT receptor agonists on depolarization-induced [^{3}H]-noradrenaline release in rabbit hippocampus and human neocortex. *Br. J. Pharmacol.* 116, 1769–1774

u Schlicker, E. *et al.* (1994) The 5-HT$_3$ receptor agonist 1-(*m*-chlorophenyl)-biguanide facilitates noradrenaline release by blockade of α_2-adrenoceptors in the mouse brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 349, 20–24

v Klein, R.L. *et al.* (1994) Effects of 5-HT$_3$ receptor antagonists on binding and function of mouse and human GABA$_A$ receptors. *Eur. J. Pharmacol.* 268, 237–246

w Squires, R.F. and Saederup, E. (1999) Clozapine's antipsychotic effects do not depend on blockade of 5-HT$_3$ receptors. *Neurochem. Res.* 24, 659–667

x Ye, J.H. *et al.* (1999) Inhibitory effect of ondansetron on glycine response of dissociated rat hippocampal neurons. *J. Pharmacol. Exp. Ther.* 290, 104–111

y Bertrand, D. *et al.* (1990) Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. U. S. A.* 87, 1993–1997

z Zwart, R. and Vijverberg, H.P.M. (1998) Four pharmacologically distinct subtypes of α4β2 nicotinic acetylcholine receptor expressed in *Xenopus laevis* oocytes. *Mol. Pharmacol.* 54, 1124–1131

aa Eglen, R.M. *et al.* (1995) Central 5-HT$_4$ receptors. *Trends Pharmacol. Sci.* 16, 391–398

bb Scholtysik, G. *et al.* (1988) 5-Hydroxytryptamine antagonist ICS 205-930 blocks cardiac potassium, sodium and calcium currents. *J. Pharmacol. Exp. Ther.* 245, 773–778

cc Barann, M. *et al.* (1993) Inhibition by anaesthetics of ^{14}C-guanidinium flux through the voltage-gated sodium channel and the cation channel of the 5-HT$_3$ receptor of N1E-115 neuroblastoma cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 347, 125–132

dd Ye, J.H. *et al.* (1997) Ondansetron exhibits the properties of a local anesthetic. *Anesth. Analg.* 85, 1116–1121

ee Parker, R.M.C. *et al.* (1996) Allosteric modulation of 5-HT$_3$ receptors: focus on alcohols and anaesthetic agents. *Trends Pharmacol. Sci.* 17, 95–99

ff Lambert, J.J. *et al.* (1995) 5-HT$_3$ Receptors. In *Ligand- and Voltage-gated Ion Channels* (North, R.A., ed.), pp. 177–211, CRC Press

gg Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology* 38, 1083–1152

hh Yakel, J.L. The 5-HT$_3$ receptor channel: Function, activation and regulation. In *Pharmacology of Ionic Channel Function: Activators and Inhibitors* (Endo, M., ed.), Springer-Verlag (in press)

ii Rondé, P. and Nichols, R.A. (1998) High calcium permeability of serotonin 5-HT$_3$ receptors on presynaptic nerve terminals from rat striatum. *J. Neurochem.* 70, 1094–1103

jj McMahon, L.L. and Kauer, J.A. (1997) Hippocampal interneurons are excited via serotonin-gated ion channels. *J. Neurophysiol.* 78, 2493–2502

kk Nowak, L. *et al.* (1984) Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307, 462–465

ll van Hooft, J.A. and Vijverberg, H.P.M. (1996) Selection of distinct conformational states of the 5-HT$_3$ receptor by full and partial agonists. *Br. J. Pharmacol.* 117, 839–846

mm Morain, P. *et al.* (1994) Biguanide derivatives: agonist pharmacology at 5-hydroxytryptamine type 3 receptors *in vitro*. *Mol. Pharmacol.* 46, 732–742

nn Lambert, J.J. *et al.* (1989) The properties of 5-HT$_3$ receptors in clonal cell lines studied by patch-clamp techniques. *Br. J. Pharmacol.* 97, 27–40

oo Neijt, H.C. *et al.* (1988) Pharmacological characterization of serotonin 5-HT$_3$ receptor-mediated electrical response in cultured mouse neuroblastoma cells. *Neuropharmacology* 27, 301–307

pp Yakel, J.L. and Jackson, M.B. (1988) 5-HT$_3$ receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron* 1, 615–621

qq Emerit, M.B. *et al.* (1993) Characteristics of [^{14}C]guanidinium accumulation in NG 108-15 cells exposed to serotonin 5-HT$_3$ receptor ligands and substance P. *J. Neurochem.* 60, 2059–2067

rr Hoyer, D. *et al.* (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* 46, 157–203

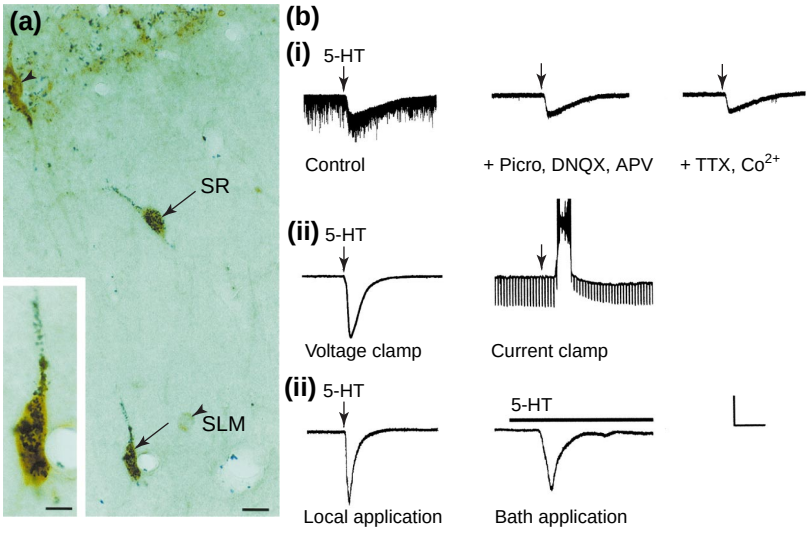

Fig. 1. ***Postsynaptic 5-HT$_3$ receptors.*** **(a)** *Co-localization of 5-HT$_3$ receptors and cholecystokinin (CCK) in neurons in the CA1 area of the rat hippocampus. 5-HT$_3$ receptor (brown) and CCK (granular blue-black) immunoproducts are co-localized in several neurons (arrows), but not all 5-HT$_3$ receptor-immunoreactive cells contain CCK immunoreactivity (arrowheads). The inset shows a double-labeled neuron at high magnification. Scale bar, 12.5 μm; for inset, 6 μm. Abbreviations: SLM, stratum lacunosum moleculare; SR, stratum radiatum.* **(b)** (i) *A 6 s pressure ejection of 100 μM 5-HT near the soma of a voltage-clamped interneuron from stratum radiatum of the rat hippocampal CA1 area elicits an excitatory inward current (left trace) that is unchanged in the presence of ionotropic glutamate receptor antagonists, 6,7-dinitroquinoxaline-2,3dione (DNQX) (10 μM) and ±-2-amino5-phosphonovaleric (±APV) (50 μM), and the GABA$_A$ receptor antagonist picrotoxin (picro) (100 μM) (middle trace).The 5-HT-induced inward current also persists when synaptic transmission is blocked withTTX (1 μM) and Co^{2+} (100 μM) (right trace). These results indicate a direct postsynaptic effect of 5-HT on the recorded cell.* (ii) *Responses to 6-s pressure ejection of 100 μM 5-HT to an interneuron under whole-cell voltage clamp (left trace) and current clamp (right trace) recording conditions. In current clamp, 5-HT elicits a depolarization from the resting membrane potential (−68 mV) to threshold (action potentials are truncated), showing the excitatory action of 5-HT.* (iii) *Comparison of 5-HT responses elicited in the same cell by pressure ejection (left trace) and bath application (right trace) of 100 μM 5-HT. Bar indicates the duration of 5-HT application and clearly demonstrates that the response desensitizes in the continuous presence of 5-HT. Scale bar 12.5 μm; for inset 6 μm.:* (i) *50 pA, 20 s;* (ii) *200 pA, 20 mV, 20 s;* (iii) *100 pA, 20 s.* (a) Reproduced, with permission, from Ref. 5. (b) Reproduced, with permission, from Ref. 6.

located presynaptically. More recently, presynaptic 5-HT$_3$ receptors have been directly demonstrated by immunocytochemistry in subsets of synaptosomes from hippocampus, striatum, amygdala and cerebellum[18,19] (Fig. 2a).

The presynaptic localization of 5-HT$_3$ receptors, at least in some areas of the CNS, suggests that they could be involved in the modulation of neurotransmitter release. Here, we examine the experimental evidence for this alleged role of presynaptic 5-HT$_3$ receptors.

5-HT release

Quantitative autoradiography has shown that the number of 5-HT$_3$ receptors in the amygdala is significantly reduced after chemical lesioning of the serotonergic system with 5,7-dihydroxytryptamine[21]. In other brain regions, for example the hippocampus, cerebral cortex and the striatum, the number of 5-HT$_3$ receptors was unaffected by the destruction of the serotonergic system[21]. Chemical lesioning of dopaminergic and noradrenergic pathways did not affect the number of 5-HT$_3$ receptors in any of the brain regions investigated[21]. Thus, it appears that a significant proportion of the 5-HT$_3$ receptors in the

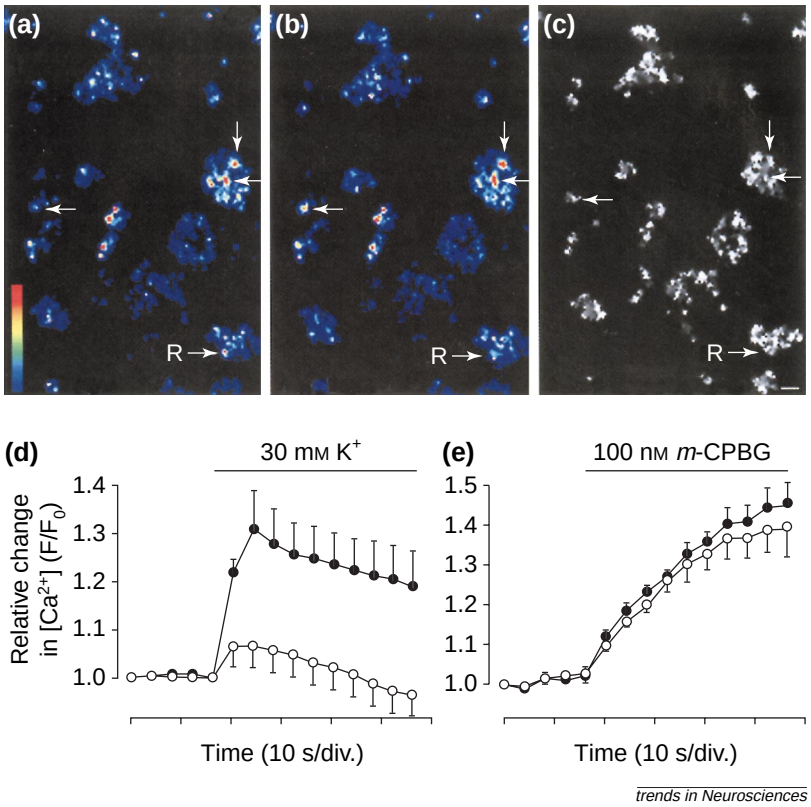

Fig. 2. ***Presynaptic 5-HT$_3$ receptors.*** *5-HT$_3$ receptor-induced changes in striatal synaptosomes monitored by confocal Ca^{2+} imaging using the Ca^{2+} indicator fluo-3.* **(a)**, **(b)** *Confocal images of synaptosomes 16 s before* (a) *and 40 s after* (b) *application of 100 nM of the 5-HT$_3$ agonist* m-*chlorophenylbiguanide* (m-*CPBG). Arrows indicate synaptosomes responding with an increase in intracellular Ca^{2+}. R indicates a synaptosome that does not respond to the application of* m-*CPBG.* **(c)** *Subsequent immunocytochemical identification shows that the structures responding to* m-*CPBG are also immunostained with anti-synaptophysin antibody, confirming that these structures are synaptosomes.* **(d)**,**(e)** *The K^+-evoked* (d), *but not the* m-*CPBG-induced* (e), *increase in Ca^{2+} is inhibited in the presence of the Ca^{2+} channel blockers Cd^{2+} and Co^{2+} (open circles) indicating that presynaptic 5-HT$_3$ receptors are Ca^{2+}-permeable ion channels. Solid circles represent control responses.* Reproduced, with permission, from Ref. 20.

amygdala are located on serotonergic nerve terminals, consistent with the high incidence of 5-HT$_3$ receptors in synaptosomes isolated from the amygdala[19]. In spite of this promising perspective, experiments to demonstrate 5-HT$_3$ receptor-mediated 5-HT release in the amygdala do not appear to have been carried out. By contrast, a 5-HT$_3$ receptor-mediated enhancement of 5-HT release has been reported from other brain regions, for example, hippocampus, frontal cortex, hypothalamus, and raphé nucleus[22–25]. However, the presence of 5-HT$_3$ receptors on serotonergic nerve endings in these brain regions has not been demonstrated thus far.

Dopamine release

A presynaptic mechanism for the modulation of dopamine (DA) release was suggested by the finding that activation of 5-HT$_3$ receptors enhances Ca^{2+}-dependent, basal release of endogenous DA in rat striatal slices, and that this effect is partially inhibited by TTX (Refs 26,27). However, this initial result has not been corroborated in subsequent investigations. By contrast, experimental evidence indicating that striatal DA release is not modulated by presynaptic 5-HT$_3$ receptors has been presented from *in vitro* studies on synaptosomes[28–31] and on slices[32–34] in addition to data from *in vivo* microdialysis studies[35–37]. Several of these studies show that the 5-HT$_3$ receptor agonists used in these experiments enhance DA release by reversing carrier-mediated DA uptake (Box 1). An alternative hypothesis is that the 5-HT$_3$ receptor agonists could enhance DA release only from active DA terminals[38]. This is in contrast with the finding that TTX does not affect 1-phenylbiguanide-induced DA release in the striatum *in vivo*[36]. Although the bulk of evidence indicates that striatal DA release is not controlled by presynaptic 5-HT$_3$ receptors, it has been shown that 5-HT$_3$ receptors are present on a small proportion of synaptosomes isolated from the striatum[19]. However, the nature of the neurotransmitters, contained within these striatal synaptosomes remains to be determined.

Evidence from *in vivo* studies indicates that 5-HT$_3$ receptors are involved in the release of DA in the nucleus accumbens[37,39,40]. Furthermore, it has been suggested that DA release in the nucleus accumbens correlates with the activity of dopaminergic neurons in the ventral tegmental area[41,42]. However, a modulatory effect of 5-HT$_3$ receptors on the firing rate of these neurons has not been demonstrated *in vitro*[43,44]. Thus, the exact location of the 5-HT$_3$ receptors involved in accumbal DA release remains inconclusive, and direct evidence for the involvement of presynaptic 5-HT$_3$ receptors is lacking.

Cholecystokinin and GABA release

Given that the majority of cortical and hippocampal 5-HT$_3$ receptors are located on cholecystokinin (CCK)-containing GABAergic interneurons[5] (Fig. 1), it is probable that activation of these receptors results in the release of GABA in addition to CCK. Release of CCK-like immunoreactivity from synaptosomes in the cortex and nucleus accumbens is enhanced by 5-HT and 1-phenylbiguanide (EC_{50} values <1 nM) and this effect is antagonized by selective 5-HT$_3$ receptor antagonists[45]. However, it has been consistently shown that the 5-HT$_3$ receptor is activated by agonists at concentrations in the micromolar range (Box 1). Moreover, the *in vivo* release of CCK is unaffected by 5-HT$_3$ receptor antagonism[46]. Therefore, the involvement of 5-HT$_3$ receptors in CCK release remains ambiguous.

There is little direct evidence for a presynaptic action of 5-HT$_3$ receptors on GABA release. From electrophysiological experiments, indirect evidence for GABA release, induced by activation of 5-HT$_3$ receptors, has been reported. The frequency of spontaneous IPSPs recorded from hippocampal CA1 pyramidal neurons is enhanced by micromolar concentrations of 5-HT and 2-methyl-5-HT, and these effects are potently antagonized by the 5-HT$_3$ receptor antagonist tropisetron[47]. A similar enhancement of the frequency of both EPSP and IPSPs has been observed in nucleus tractus solitarius neurons, in addition to a direct depolarizing effect of 5-HT and 2-methyl-5-HT on the postsynaptic membrane[48]. By contrast, it has been shown that 5-HT$_2$, and not 5-HT$_3$ receptors mediate the enhancement of IPSC frequency in the hippocampus[49] and cortex[50]. In a study on the modulation of GABA release from hippocampal synaptosomes[51] the identity of the putative 5-HT receptor involved in this modulation was not determined unequivocally. In addition, the reported inhibition of GABA release is the opposite

to what would be expected following the activation of excitatory 5-HT$_3$ receptor ligand-gated cation channels (Box 1). In this respect, it is of interest to note that high concentrations of 5-HT$_3$ receptor antagonists also affect GABA$_A$ receptors (Box 1). Therefore, although the presence of functional postsynaptic 5-HT$_3$ receptors on GABAergic interneurons has been demonstrated[6,52], evidence for presynaptic 5-HT$_3$ receptors in hippocampal nerve endings is restricted to the immunocytochemical studies of 5-HT$_3$ receptors in 3% of hippocampal synaptosomes[19]. Similar to striatal synaptosomes, the nature of the neurotransmitter contained within the hippocampal synaptosomes remains to be determined.

Acetylcholine and noradrenaline release

The involvement of 5-HT$_3$ receptors in the release of ACh and noradrenaline (NA) in rat entorhinal cortex, hippocampus, and several other brain regions has been investigated. Reports on the modulation of ACh release by 5-HT$_3$ receptors vary from inhibition in cortical slices and synaptosomes[53,54], to no effect in cortical slices[55], and to enhancement in the dorsal hippocampus *in vivo*[56]. Similarly, the effects of 5-HT$_3$ receptors in the modulation of NA release also vary[57–59]. Some of these effects on neurotransmitter release might be confounded by additional effects of the 5-HT$_3$ receptor agonists on α_2 adrenoceptors[60,61]. In the absence of structural evidence for the presence of 5-HT$_3$ receptors on terminals or varicosities containing ACh or NA, for example in the hippocampus (reviewed in Ref. 62), it is tempting to speculate that the modulatory effects of 5-HT$_3$ receptors on ACh and NA release are, at least in part, secondary to the release of GABA and CCK from interneurons. It has been shown that inhibition of GABA$_A$ receptors using bicuculline enhances ACh and NA release from cortical slices[63,64]. Such confounding effects, secondary to the excitatory action of postsynaptic 5-HT$_3$ receptor activation in interneurons[6,52], might underlie the variability in the results reported on the modulation of ACh and NA release.

Perspective

Detailed analysis of the available literature indicates that evidence for the alleged role of the 5-HT$_3$ receptor as a presynaptic receptor modulating neurotransmitter release in the CNS is not compelling. Either the exact localization of the 5-HT$_3$ receptors involved in the modulation of the neurotransmitter released is unknown or the nature of the neurotransmitter contained within the presynaptic terminals that express the 5-HT$_3$ receptors remains obscure. In order to progress, information is required on the neurotransmitters contained within the nerve terminals in which 5-HT$_3$ receptors are present. Recently, the presence of 5-HT$_3$ receptors has been shown in 3% of synaptosomes prepared from hippocampus, in 6% of synaptosomes prepared from striatum, and in 30% of synaptosomes prepared from amygdala and cerebellum[19]. Moreover, the 5-HT$_3$ receptors in these preparations were shown to be Ca^{2+}-permeable ion channels[19,20], indicating that they might directly support the exocytosis of neurotransmitter vesicles. Establishing the nature of the neurotransmitters contained within synaptosomes that express 5-HT$_3$ receptors is essential to determine, (1) which neurotransmitters are released from nerve endings *in situ* under the direct control of 5-HT$_3$ receptors and (2) which neurotransmitters are released as a consequence of secondary phenomena. At present, specific tools are available for the identification of 5-HT$_3$ receptors and for demonstrating the co-localization of 5-HT$_3$ receptors with other elements of presynaptic nerve endings. In addition, insight into the structural determinants and heterogeneity of 5-HT$_3$ receptors, and into the specific functional properties of pre- and postsynaptic 5-HT$_3$ receptors is steadily improving (Box 1). Thus, all the ingredients for a detailed experimental reassessment of the contribution of presynaptic 5-HT$_3$ receptors to neurotransmitter release appear to be available.

Selected references

1 MacDermott, A.B. *et al.* (1999) Presynaptic ionotropic receptors and the control of transmitter release. *Annu. Rev. Neurosci.* 22, 443–485
2 Neijt, H.C. *et al.* (1988) Pharmacological characterization of serotonin 5-HT$_3$ receptor-mediated electrical response in cultured mouse neuroblastoma cells. *Neuropharmacology* 27, 301–307
3 Derkach, V. *et al.* (1989) 5-HT$_3$ receptors are membrane ion channels. *Nature* 339, 706–709
4 Maricq, A.V. *et al.* (1991) Primary structure and functional expression of the 5HT$_3$ receptor, a serotonin-gated ion channel. *Science* 254, 432–437
5 Morales, M. and Bloom, F.E. (1997) The 5-HT$_3$ receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci.* 17, 3157–3167
6 McMahon, L.L. and Kauer, J.A. (1997) Hippocampal interneurons are excited via serotonin-gated ion channels. *J. Neurophysiol.* 78, 2493–2502
7 Sugita, S. *et al.* (1992) 5-hydroxytryptamine is a fast excitatory transmitter at 5-HT$_3$ receptors in rat amygdala. *Neuron* 8, 199–203
8 Roerig, B. *et al.* (1997) Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT$_3$ receptors in developing visual cortex. *J. Neurosci.* 17, 8353–8362
9 Lambert, J.J. *et al.* (1995) 5-HT$_3$ Receptors. In *Ligand- and Voltage-gated Ion Channels* (North, R.A., ed.), pp. 177–211, CRC Press
10 Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology* 38, 1083–1152
11 Yakel, J.L. The 5-HT$_3$ Receptor channel: Function, activation and regulation. In *Pharmacology of Ionic Channel Function: Activators and Inhibitors* (Endo, M., ed.), Springer-Verlag (in press)
12 Hamon, M. *et al.* (1989) 5-HT$_3$ receptor binding sites are on capsaicin-sensitive fibres in the rat spinal cord. *Eur. J. Pharmacol.* 164, 315–322
13 Waeber, C. *et al.* (1988) Localisation by autoradiography of neuronal 5-HT$_3$ receptors in the mouse CNS. *Eur. J. Pharmacol.* 151, 351–352
14 Leslie, R.A. *et al.* (1990) Evidence for presynaptic 5-hydroxytryptamine3 recognition sites on vagal afferent terminals in the brainstem of the ferret. *Neuroscience* 38, 667–673
15 Merahi, N. *et al.* (1992) Baroreceptor reflex inhibition induced by the stimulation of serotonin3 receptors in the nucleus tractus solitarius of the rat. *Neuroscience* 46, 91–100
16 Laporte, A.M. *et al.* (1992) Autoradiographic mapping of central 5-HT$_3$ receptors. In *Central and Peripheral 5-HT$_3$ Receptors* (Hamon, M., ed.), pp. 157–187, Academic Press
17 Tecott, L.H. *et al.* (1993) Nervous system distribution of the serotonin 5-HT$_3$ receptor mRNA. *Proc. Natl. Acad. Sci. U. S. A.* 90, 1430–1434
18 Nichols, R.A. and Mollard, P. (1996) Direct observation of serotonin 5-HT$_3$ receptor-induced increases in calcium levels in individual brain nerve terminals. *J. Neurochem.* 67, 581–592
19 Nayak, S.V. *et al.* (1999) Calcium changes induced by presynaptic 5-hydroxytryptamine-3 serotonin receptors on isolated terminals from various regions of the rat brain. *Neuroscience* 91, 107–117
20 Rondé, P. and Nichols, R.A. (1998) High calcium permeability of serotonin 5-HT$_3$ receptors on presynaptic nerve terminals from rat striatum. *J. Neurochem.* 70, 1094–1103
21 Kidd, E.J. *et al.* (1993) 5-HT$_3$ receptors in the rat central nervous system are mainly located on nerve fibres and terminals. *Brain Res.* 612, 289–298

22 Martin, K.F. *et al.* (1992) Opposing roles for 5-HT$_{1B}$ and 5-HT$_3$ receptors in the control of 5-HT release in rat hippocampus *in vivo*. *Br. J. Pharmacol.* 106, 139–142

23 Blier, P. *et al.* (1993) 5-HT$_3$ receptors which modulate [^{3}H]5-HT release in the guinea pig hypothalamus are not autoreceptors. *Synapse* 15, 143–148

24 Haddjeri, N. and Blier, P. (1995) Pre- and post-synaptic effects of the 5-HT$_3$ agonist 2-methyl-5-HT on the 5-HT system in the rat brain. *Synapse* 20, 54–67

25 Bagdy, E. *et al.* (1998) Feedback stimulation of somatodendritic serotonin release: a 5-HT$_3$ receptor-mediated effect in the raphé nuclei of the rat. *Brain Res. Bull.* 45, 203–208

26 Blandina, P. *et al.* (1988) Activation of a 5-HT$_3$ receptor releases dopamine from rat striatal slice. *Eur. J. Pharmacol.* 155, 349–350

27 Blandina, P. *et al.* (1989) Release of endogenous dopamine by stimulation of 5-hydroxytryptamine3 receptors in rat striatum. *J. Pharmacol. Exp. Ther.* 251, 803–809

28 Yi, S.J. *et al.* (1991) Effect of cocaine and 5-HT$_3$ receptor antagonists on 5-HT-induced [^{3}H]dopamine release from rat striatal synaptosomes. *Eur. J. Pharmacol.* 199, 185–189

29 Crespi, D. *et al.* (1997) Carrier-dependent and Ca(2+)-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylendioxymethamphetamine, *p*-chloroamphetamine and (+)-fenfluramine. *Br. J. Pharmacol.* 121, 1735–1743

30 Ng, N.K. *et al.* (1999) Regulation of striatal dopamine release through 5-HT$_1$ and 5-HT$_2$ receptors. *J. Neurosci. Res.* 55, 600–607

31 Sarhan, H. *et al.* (1999) 5-HT$_{1B}$ receptors modulate release of [^{3}H]dopamine from rat striatal synaptosomes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 359, 40–47

32 Schmidt, C.J. and Black, C.K. (1989) The putative 5-HT$_3$ agonist phenylbiguanide induces carrier-mediated release of [^{3}H]dopamine. *Eur. J. Pharmacol.* 167, 309–310

33 Benuck, M. and Reith, M.E. (1992) Dopamine releasing effect of phenylbiguanide in rat striatal slices. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 345, 666–672

34 Jacocks, H.M., 3rd and Cox, B.M. (1992) Serotonin-stimulated release of [^{3}H]dopamine via reversal of the dopamine transporter in rat striatum and nucleus accumbens: a comparison with release elicited by potassium, *N*-methyl-D-aspartic acid, glutamic acid and D-amphetamine. *J. Pharmacol. Exp. Ther.* 262, 356–364

35 Bonhomme, N. *et al.* (1995) Evidence for 5-HT$_4$ receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacology* 34, 267–279

36 Santiago, M. *et al.* (1995) 5-HT$_3$ receptor agonist induced carrier-mediated release of dopamine in rat striatum *in vivo*. *Br. J. Pharmacol.* 116, 1545–1450

37 De Deurwaerdère, P. *et al.* (1998) Opposite change of *in vivo* dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphé nucleus: role of 5-HT$_3$ receptors. *J. Neurosci.* 18, 6528–6538

38 Zazpe, A. *et al.* (1994) Role of 5-HT$_3$ receptors in basal and K(+)-evoked dopamine release from rat olfactory tubercle and striatal slices. *Br. J. Pharmacol.* 113, 968–972

39 Carboni, E. *et al.* (1989) Differential inhibitory effects of a 5-HT$_3$ antagonist on drug-induced stimulation of dopamine release. *Eur. J. Pharmacol.* 164, 515–519

40 Imperato, A. and Angelucci, L. (1989) 5-HT$_3$ receptors control dopamine release in the nucleus accumbens of freely moving rats. *Neurosci. Lett.* 101, 214–217

41 Guan, X.M. and McBride, W.J. (1989) Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. *Brain Res. Bull.* 23, 541–547

42 Mylecharane, E.J. (1996) Ventral tegmental area 5-HT receptors: mesolimbic dopamine release and behavioural studies. *Behav. Brain Res.* 73, 1–5

43 Gifford, A.N. and Wang, R.Y. (1994) The effect of 5-HT$_3$ receptor antagonists on the morphine-induced excitation of A10 dopamine cells: electrophysiological studies. *Brain Res.* 638, 325–328

44 Pessia, M. *et al.* (1994) Actions of 5-hydroxytryptamine on ventral tegmental area neurons of the rat *in vitro*. *Brain Res.* 654, 324–330

45 Paudice, P. and Raiteri, M. (1991) Cholecystokinin release mediated by 5-HT$_3$ receptors in rat cerebral cortex and nucleus accumbens. *Br. J. Pharmacol.* 103, 1790–1794

46 Nevo, I. *et al.* (1996) Stress- and yohimbine-induced release of cholecystokinin in the frontal cortex of the freely moving rat: prevention by diazepam but not ondansetron. *J. Neurochem.* 66, 2041–2049

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

48 Glaum, S.R. *et al.* (1992) 5-Hydroxytryptamine-3 receptors modulate synaptic activity in the rat nucleus tractus solitarius *in vitro*. *Brain Res.* 589, 62–68

49 Shen, R.Y. and Andrade, R. (1998) 5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. *J. Pharmacol. Exp. Ther.* 285, 805–812

50 Zhou, F. and Hablitz, J.J. (1999) Activation of serotonin receptors modulates synaptic transmission in rat cerebral cortex. *J. Neurophysiol.* 82, 2989–2999

51 Cloëz-Tayarani, I. *et al.* (1992) Inhibition of [^{3}H] γ-aminobutyric acid release from guinea-pig hippocampal synaptosomes by serotonergic agents. *Fundam. Clin. Pharmacol.* 6, 333–341

52 Kawa, K. (1994) Distribution and functional properties of 5-HT$_3$ receptors in the rat hippocampal dentate gyrus: a patch-clamp study. *J. Neurophysiol.* 71, 1935–1947

53 Crespi, D. *et al.* (1997) 5-HT$_3$ serotonin hetero-receptors inhibit [^{3}H]acetylcholine release in rat cortical synaptosomes. *Pharmacol. Res.* 35, 351–354

54 Barnes, J.M. *et al.* (1989) 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. *Nature* 338, 762–763

55 Johnson, R.M. *et al.* (1993) 5-HT$_3$ receptor ligands lack modulatory influence on acetylcholine release in rat entorhinal cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 347, 241–247

56 Consolo, S. *et al.* (1994) Serotonergic facilitation of acetylcholine release *in vivo* from rat dorsal hippocampus via serotonin 5-HT$_3$ receptors. *J. Neurochem.* 62, 2254–2261

57 Feuerstein, T.J. and Hertting, G. (1986) Serotonin (5-HT) enhances hippocampal noradrenaline (NA) release: evidence for facilitatory 5-HT receptors within the CNS. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 333, 191–197

58 Blandina, P. *et al.* (1991) Serotonergic modulation of the release of endogenous norepinephrine from rat hypothalamic slices. *J. Pharmacol. Exp. Ther.* 256, 341–347

59 Mongeau, R. *et al.* (1994) Activation of 5-HT$_3$ receptors enhances the electrically evoked release of [^{3}H]noradrenaline in rat brain limbic structures. *Eur. J. Pharmacol.* 256, 269–279

60 Schlicker, E. *et al.* (1994) The 5-HT$_3$ receptor agonist 1-(m-chlorophenyl)-biguanide facilitates noradrenaline release by blockade of alpha 2-adrenoceptors in the mouse brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 349, 20–24

61 Allgaier, C. *et al.* (1995) Effects of 5-HT receptor agonists on depolarization-induced [^{3}H]-noradrenaline release in rabbit hippocampus and human neocortex. *Br. J. Pharmacol.* 116, 1769–1774

62 Vizi, E.S. and Kiss, J.P. (1998) Neurochemistry and pharmacology of the major hippocampal transmitter systems: synaptic and nonsynaptic interactions. *Hippocampus* 8, 566–607

63 Ramírez, M.J. *et al.* (1996) Involvement of GABA systems in acetylcholine release induced by 5-HT$_3$ receptor blockade in slices from rat entorhinal cortex. *Brain Res.* 712, 274–280

64 Fiber, J.M. and Etgen, A.M. (1997) GABA augments basal and electrically stimulated ^{3}H-norepinephrine release in hypothalamic, preoptic area and cortical slices of female rats. *Neurochem. Int.* 31, 769–780

Acknowledgements

The research of J.A. van Hooft has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences. The authors thank M. Joëls for comments on the manuscript.

Letters to the Editor

Trends in Neurosciences: a forum for comment

The Editor welcomes correspondence from readers of *Trends in Neurosciences* on any articles published. Please state clearly whether or not you wish the letter to be considered for publication. If a letter is considered suitable for publication, it will be sent to the author of the original article for their response; both the letter and reply will be published together.

Please note: submission does not guarantee publication.

Please address Letters to:
Dr Siân Lewis, Editor
Trends in Neurosciences, 84 Theobald's Road,
London, UK WC1X 8RR.