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COMMENTARY

SEROTONERGIC MODULATION OF CHOLINERGIC FUNCTION IN THE CENTRAL NERVOUS SYSTEM: COGNITIVE IMPLICATIONS

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Abstract—Accumulating evidence suggests that serotonin may modulate cholinergic function in several regions of the mammalian brain and that these serotonergic/cholinergic interactions influence cognition. The first part of this review is an overview of histological, electrophysiological and pharmacological (*in vitro*, *in vivo*) data indicating that, in several brain regions (e.g., hippocampus, cortex and striatum), there are neuroanatomical substrates for a serotonergic/cholinergic interaction, and that alterations in serotonergic activity may induce functional changes in cholinergic neurons. In the second part, the review focuses on experimental approaches showing or suggesting that central cholinergic and serotonergic mechanisms are cooperating/interacting in the regulation of cognitive functions. These arguments are based on lesion, intracerebral grafting and pharmacological techniques.

It is concluded that not all mnesic perturbations induced by concurrent manipulations of the serotonergic and cholinergic systems can be attributed to a serotonergic modification of the cholinergic system. The cognitive faculties of an organism arise from interactions among several neurotransmitter systems within brain structures such as, for instance, the hippocampus or the cortex, but also from influences on memory of other general functions that may involve cerebral substrates different from those classically related to mnesic functions (e.g., attention, arousal, sensory accuracy, etc.).

Key words: cortex, hippocampus, 5-hydroxytryptamine, memory, pharmacology, striatum.

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Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; AF64A, ethylcholine aziridinium; AMPA, α-amino-3-hydroxy-5-methyl-isoxazole propionate; BIMU 1, endo-N-(8-methyl-8-azabicyclo[3.2.1.]-oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazol-1-carboxamide; BIMU 8, endo-N-(8-methyl-8-azabicyclo[3.2.1.]-oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazol-1-carboxamide)]; CGS 12066B, 7-trifluoromethyl-4-(4-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline; ChAT, choline acetyltransferase; CP 93129, [3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo[3,2-b]pyrid-5-one]; 5-CT, 5-carboxyamidotryptamine; DA, dopamine DAU 6215, [(*endo*-N-8-methyl-8-azabicyclo-[3.2.1.]oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazol-1-carboxamide]; DBB, diagonal band of Broca; (±)-DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 5,7-DHT, 5,7-dihydroxytryptamine; FG-7080, (−)trans 4-(4-fluorophenyl)-3-(4-methoxyphenoxymethyl)piperidine hydrochloride; GR 113808, [1-[2(methylsulphonyl)-amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate; GR 125487, [1-[2(methylsulphonyl)-amino]ethyl]-4-piperidinyl]-methyl-5-fluoro-2-methoxy-1H-indole-3-carboxylate; GR 127935, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1, 2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide; 5-HT, serotonin, 5-hydroxytryptamine; HVSA, high-voltage spindle activity; IP_3, inositol triphosphate; L 694247, 2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1H-indole-3-yl]-ethylamine; LSD, lysergic acid diethylamine; LTP, long term potentiation; LVFA, low voltage fast activity; LY 53857, 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6A,7,8,9,10,10A-octahydroin-dolo-[4,3-FG]-quinolone; mCPBG, 1-(m-chlorophenyl)-biguanide; mCPP, 1-(3-chlorophenyl)piperazine; MDL 72222, 3,5-dichlorobenzoic acid,8-methyl-8-azabicyclo[3,2,1]octan-3-YL ester, methanesulfonate, monohydrate; MDL 73005, 8-(2-[2,3-dihydro-1,4,benzodioxin-2-yl-methylamino]ethyl)-8-azaspiro[4,5]decane-7,9-dione; MDMA, 3,4-methylenedioxymethamphetamine; 2-Me-5-HT, 2-methyl-5-hydroxytryptamine, ecstasy; 5-MeO-DMT, 5-methoxy-dimethyltryptamine; 5-MO-HT, 5-methoxytryptamine; NA, noradrenaline; NAN 190, 1-(2-methoxyphenyl)-4-[4-(2-phtalimido)-butyl]piperazine; NBM, nucleus basalis of Meynert, or magnocellularis; 6-OHDA, 6-hydroxydopamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; pCA, para-chloroamphetamine; pCPA, para-chlorophenylalanine; RSA, rhythmical slow wave activity; RU 24969, 5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]-1H-indole; SB 204070, (1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate; TFMPP, N-(3-trifluoromethylphenyl)piperazine; THA, tetrahydroaminoacridine; TTX, tetrodotoxin; WAY 100135, N-tert-butyl-3-(4-[2-methoxyphenyl]piperazin-1-yl)-2-phenylpropamide.

CONTENTS

1. INTRODUCTION

In the mid-1970s, three independent research groups[34,59,172,173,255] reported that the basal forebrain from patients with Alzheimer's disease (AD) exhibited dramatic degeneration of the cholinergic neurons. This important series of findings has strongly stimulated research on the possible relationship between cholinergic function in the central nervous system and cognitive processes. Interest for an involvement of cholinergic processes in cognitive function had already emerged in the 1960s. However, exponential progress in this field started only about a decade later[13] with data from clinical observations, psychopharmacological studies in animals and humans, and basic neuroscience approaches relying upon lesion techniques.

Animal models of AD have concentrated on the cognitive deficits resulting either from lesions of cholinergic nuclei or cholinergic pathways, or from pharmacological blockade of central cholinergic neurotransmission (e.g., Refs 52, 69, 226). Almost all experimental (animal or clinical) studies undertaken during the 1960s, the 1970s and part of the 1980s might nowadays be considered as pointing to one conclusion, namely that the disruption of central cholinergic functions results in impaired learning and memory. These apparently solid and replicable results led to the "cholinergic hypothesis of learning", a hypothesis that Bartus *et al.*[13] have summarized as: "it (the cholinergic hypothesis) attempts to explain only the most direct, cause–effect relationship associated with the primary symptoms (i.e. memory loss)". Over the past decade, however, conceptual changes and new neurochemical characterization of the neuropathology of AD (e.g., Refs 32, 86, 129) clearly indicated that the "cholinergic hypothesis of learning" is too reductionistic: memory processes are not

solely a matter of cholinergic processes, even if the latter represent a crucial step in learning and memory. Other neurotransmitter systems such as the dopaminergic, GABAergic, noradrenergic and serotonergic ones are now recognized to be involved in cognition and the concept of neurotransmitter interactions (and balance) in determining behavior and cognition has become a major working hypothesis in behavioral neurosciences (e.g., Refs 64, 119, 210, 252). In a recent review, Decker and McGaugh[64] suggest that cognition could be regarded as the output from various components of an integrated system in which several subsystems would share either parallel synergistic roles (the neurobiological processes occurring in parallel in two or more systems which contribute to cognition through additive processes) or serial interactive roles (different neurotransmitters modulate a given neuronal system to determine a particular cognitive faculty). It is also possible that a given cognitive function may result from a combination of both parallel and serial processes in the brain.

As stated by Decker and McGaugh[64] in their conclusion "while our understanding of the cholinergic system and its role in learning and memory is far from complete, we have progressed to the point where it is possible, and necessary, to address the importance of the interplay of neurotransmitter systems in the modulation of memory processes". With regard to such an "interplay", it is clear that research must not only be focused on the modifications of the behavioral (cognitive) outputs generated by conjoined manipulations of different systems of neurotransmitters, but also, on the structural and functional characterization of the neurobiological substrates which allow interactions among the different neurotransmitters to occur. All the types of interactions reviewed by Decker and McGaugh[64] are potential concerns for histological, electrophysiological, neurochemical, pharmacological and behavioral approaches.

Sirviö *et al.*[224] recently reviewed experimental studies demonstrating that serotonin has a modulatory role in cognition. In this review, the authors mainly focused their attention on their own (enormous) experimental contribution to this field. In addition, they summarized the current knowledge on the substrates involved in serotonergic influences on cognition, but, apart from a large series of psychopharmacological arguments, they did not tackle extensively the experimental approaches which allowed this knowledge to emerge. In a review paper, the latter aspect may be very helpful to the reader.

The main goal of the present review is to present a more general (and perhaps more detailed) overview of the neuroanatomical, electrophysiological, pharmacological (both *in vitro* and *in vivo*) and behavioral arguments which demonstrate that serotonergic modulation of cholinergic function can be accepted as a well (though not exhaustively) documented fact with structurally identified and functionally characterized neurobiological substrates. That Steckler and Sahgal[229] have recently published a review on a similar topic (just as the present one had been completely revised) might be regarded as indicative of the importance that this topic has gained over the last few years.

2. NEUROBIOLOGICAL SUBSTRATES

2.1. *Neuroanatomical approaches*

To exhaustively review the distribution of the various serotonergic and cholinergic nuclei, terminals and receptor sites throughout the mammalian brain is beyond the scope of the present synthesis. On these matters, the reader is referred to review articles on serotonergic and cholinergic neuroanatomy, neuropharmacology and neurophysiology (e.g., Refs 1, 7, 36, 38, 39, 96, 111, 140, 166, 209, 230, 233, 238, 246, 253, 259). In this section, attention will be focused on studies reporting the presence of serotonergic terminals or 5-hydroxytryptamine (5-HT) receptors in brain regions involved in cholinergic function, either because these regions are nuclei rich in cholinergic cell bodies (e.g., nucleus basalis magnocellularis, medial septum, diagonal band of Broca, laterodorsal and pedunculopontine tegmental nuclei) or because they are innervated by cholinergic neurons (e.g., cortex, hippocampus, striatum) and, therefore, the locus of a potential serotonergic modulation of cholinergic function.

2.1.1. *Serotonergic terminals in cholinergic nuclei or targets.* Using immunohistochemical methods, Köhler *et al.*[113] have found serotonergic fibers in the septal nuclei. Whereas the ventral region of the lateral septum and a region bordering the medial edge of the islands of Calleja contained serotonergic terminals, the serotonin immunoreactivity in the diagonal band of Broca (DBB) consisted essentially of fibers *en passage*, suggesting that in the DBB, there is no substrate for serotonin to influence cholinergic function. Using antibodies against serotonin, Milner and Veznedaroglu[143] have demonstrated that the septal region of the rat contained numerous 5-HT-immunopositive afferents which formed symmetric (probably inhibitory) synapses with the perikarya and the proximal dendritic segment, as well as asymmetric (probably excitatory) synapses with the distal dendritic segment of neurons that were identified as septohippocampal ones, some of which are likely to be cholinergic. Vertes[240] has injected wheatgerm agglutinin-conjugated horseradish peroxidase into several nuclei of the basal forebrain (including the medial septum and the vertical limb of the DBB) and found retrogradely labeled neurons in various structures of the brainstem including the median and dorsal raphe nuclei. This finding suggests that some of these projections to the forebrain cholinergic

nuclei might be serotonergic. This suggestion was supported by Semba *et al.*[219] who combined immunohistochemistry and retrograde labeling techniques to study the magnocellular basal forebrain afferents originating in the brainstem. They found that after an injection into both the vertical limb of the DBB and the magnocellular preoptic area, a small amount of serotonin- and tyrosine hydroxylase-positive neurons of the dorsal raphe had been labeled. Using similar techniques, Losier and Semba[124] provided additional data demonstrating the presence of serotonergic terminals in the basal forebrain, although the proportion of serotonergic neurons projecting to the basal forebrain was relatively small (< 5%). Jones and Cuello[107] have reported that after an injection of horseradish peroxidase near the basal forebrain cholinergic neurons (globus pallidus, substantia innominata and magnocellular preoptic nucleus) projecting towards cortical structures, some retrograde-labeled neurons were found in the raphe nuclei. In addition, these authors described multiple serotonin-immunoreactive fibers in the vicinity of positive choline acetyltransferase (ChAT)-positive neurons of the basal forebrain. In the hippocampus, one of the major terminal fields of cholinergic neurons in the brain, Oleskevich *et al.*[165] found the immunostained 5-HT innervation to be predominantly non-synaptic (70–80%), suggesting that serotonin may exert an axoaxonic modulation of multiple types of neurochemical terminals, one of which may be GABAergic, another cholinergic. Séguéla *et al.*[218] have provided immunocytochemical evidence that the "entire cortical volume might actually be within reach of the 5-HT transmitter" and that 5-HT might exert diverse and widely distributed actions on cortical functions. Thus, it is tempting to assume that some of the cholinergic neurons innervating the cortex might be under such an influence.

These findings, however, do not demonstrate unequivocally that there are functional links between serotonergic terminals and cholinergic neurons in the septal area and in other cholinergic nuclei or targets of the brain, nor do they provide any information as to which type of 5-HT receptor may be considered as a potential substrate for these putative connections. Additional information may be obtained from studies based on autoradiography and *in situ* hybridization techniques. However, the precise identification of a receptor subtype supposes the existence of specific ligands. As recently reviewed by Hoyer *et al.*[91] and Martin and Humphrey,[131] such ligands do not exist for all receptor subtypes and, among the ligands which are available, some may bind to more than one subtype of 5-HT receptor [e.g., [^{125}I]lysergic acid diethylamine (LSD) recognizes 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6 and 5-HT_7 receptors, all of which are structurally different]. The reader will find an updated review of serotonergic receptor nomenclature and 5-HT receptor ligands in the article by Hoyer *et al.*[91] (see also Section 3.4.1.).

2.1.2. *5-Hydroxytryptamine$_1$ receptors.* Quirion and Richard,[177] using [^{3}H]5-HT, have labeled the 5-HT_1 binding sites in the rat brain. They found 5-HT_1 receptors to be especially located in areas that are densely innervated by cholinergic (but also dopaminergic) terminals, namely the substantia innominata, the ventral pallidum, the striatum, the septal nuclei and the hippocampus. Interestingly, a fimbriaectomy induced a more than 50% decrease of labeled 5-HT_1 receptors in the hippocampus, a finding that Lawrence *et al.*[116] failed to replicate in rats which received ibotenate or α-amino-3-hydroxy-5-methyl-isoxazole propionate (AMPA) injections into the basal forebrain. The latter authors, however, have used [^{3}H]8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) to label receptors of the 5-HT_{1A} subtype and their conclusion was that, in the hippocampus (but also in the cortex), these receptors are located postsynaptically. Zilles *et al.*[261] have found 5-HT_1 receptors to also be present in nuclei such as the septum, the DBB or the nucleus basalis of Meynert (NBM). Chalmers and Watson,[48] combining *in situ* hybridization and receptor autoradiography, found that, compared to other brain structures, the medial septum and the vertical diagonal band exhibited high levels of both 5-HT_{1A} mRNA and 5-HT_{1A} binding sites. Interestingly, these two markers were also found in various regions of the cortex (frontal, frontoparietal, cingulate and entorhinal cortices) and in the hippocampus, but neither was found in the caudate nucleus or in the nucleus accumbens. Overall, in the structures exhibiting high levels of 5-HT_{1A} mRNA and 5-HT_{1A} binding sites, there was a complementary distribution of these two markers, suggesting that this receptor might have both a presynaptic autoregulatory and a postsynaptic regulatory role in serotonergic neurotransmission.

Other studies have also shown both 5-HT_{1A} mRNA and binding sites to be present in the dorsal raphe, the septum, the hippocampus and the entorhinal cortex, as well as in the cerebral cortex, but at a lower density than in the former cerebral regions.[176] Sijbesma *et al.*[220] have compared the distribution of several subtypes of 5-HT receptors in the brain of the rat and the guinea-pig. They found high levels of 5-HT_{1A} receptors in the neocortex and the hippocampus of both species with, however, some species differences in the relative amounts of these receptors. For instance, in regions CA1/CA2 of the hippocampus and in the dorsal subiculum, the level of 5-HT_{1A} receptors was higher in the guinea-pig than in the rat. Conversely, in the dorsolateral septum, the cingulate cortex and the neocortex (laminae IV and V), the level of 5-HT_{1A} receptors was higher in the rat.

In the basal forebrain of the guinea-pig, Khateb *et al.*[109] have provided pharmacological evidence for a serotonergic modulation of the cholinergic neurons in the nucleus basalis which involves 5-HT_{1A} receptors. Additional evidence is obtained from

experiments based on electrophysiological or lesion approaches (see below).

Pazos and Palacios[170] have reported 5-HT_{1B} receptors to be present in the basal ganglia, the substantia nigra and the subiculum of the rat, a finding that Sijbesma *et al.*[220] have confirmed. In the mouse brain, Boschert *et al.*[31] have compared the distribution of 5-HT_{1B} mRNA and 5-HT_{1B} binding sites. They report high levels of 5-HT_{1B} mRNA in the caudate-putamen, but not in the globus pallidus and the substantia nigra where high levels of 5-HT_{1B} binding sites were found. In the hippocampus there was also a mismatch between the localization of the mRNA and that of the corresponding protein, as high levels of 5-HT_{1B} mRNA were found in region CA1 of Ammon's horn, whilst the highest levels of binding sites were found in the subiculum. In the septal area, these authors found moderate levels of 5-HT_{1B} mRNA and weak amounts of 5-HT_{1B} binding sites, suggesting that in this brain region, the mRNA coding for the 5-HT_{1B} receptor is not associated with cholinergic projection sites. In the guinea-pig, a species in which 5-HT_{1B} receptors do not exist (e.g., Refs 31, 244), 5-HT_{1D} receptors were found to be distributed according to a pattern which resembles that of the 5-HT_{1B} ones in the rat. This is an interesting observation as both 5-HT_{1B} and 5-HT_{1D} receptors may share comparable roles in different species.[244] There are, however, a few exceptions in that the substantia nigra, the ventral pallidum and the globus pallidus present relatively more 5-HT_{1B} receptors in rats than 5-HT_{1D} sites in guinea-pigs. Conversely, in the claustrum, the dorsal endopiriform nucleus and the dentate gyrus, the level of 5-HT_{1D} receptors in guinea-pigs is higher than that of 5-HT_{1B} receptors in rats (e.g., Ref. 220).

2.1.3. 5-*Hydroxytryptamine*$_2$ *receptors.* Rat striatum and nucleus accumbens exhibit high densities of 5-HT_2 receptors whereas in the cortex and in the hippocampus, this density is low (see also Ref. 23).[225] Quirion *et al.*[178] have measured [^{3}H]ketanserin binding, a marker for 5-HT_2 receptors, in the anterior cortex, the middle cortex and in the striatum of rats which had sustained lesions of the nucleus basalis. These authors found that the number of 5-HT_2 receptors was reduced in both the anterior (−42%) and middle (−35%) cortex, but not in the striatum, suggesting that in the cortex, there is a certain proportion of these sites located on cholinergic terminals and which could potentially serve as the substrate for serotonergic-cholinergic interactions.

A reduction in the number of 5-HT_2 receptors has also been reported in patients who died from AD,[182] as well as in aged rats.[153] The patients with AD exhibited a reduction in cortical cholinergic markers as well as a diminished binding of [^{3}H]ketanserin.[182] This decrease of ketanserin-binding sites does not necessarily result from the degeneration of cholinergic neurons possessing serotonergic receptors; serotonergic neurons might be concerned as well. However, Reynolds *et al.*[182] could not show a significant reduction in cortical serotonin or 5-hydroxyindolacetic acid concentrations, suggesting that in patients with AD, the serotonergic tone, at least in the cerebral cortex, might be relatively well-preserved (e.g., Ref. 74).

Although in the initial report by Morilak and Ciaranello,[147] few 5-HT_2 receptors were found to be located in the basal forebrain or striatum, the succeeding study found that one of the most abundant population of neurons possessing 5-HT_2 receptors was in the basal forebrain.[148] Thus, part of the pattern of 5-HT_2 receptors in the rat brain overlapped with that of cholinergic neurons or terminals (e.g., in the caudate–putamen or the basal forebrain) and some of the 5-HT_2-positive neurons displayed morphological features which were comparable to those of the cholinergic cell population in these nuclei.[148]

2.1.4. *Other 5-hydroxytryptamine receptors.* 5-HT_3 receptors have been found in brain regions where dendrites, cell bodies or terminals of cholinergic neurons are present. These regions include the entorhinal cortex, the hippocampus and the nucleus accumbens, as well as other regions of the forebrain (e.g., Refs 8, 9, 12, 35, 110, 245, 248). 5-HT_4 receptors have been detected, using autoradiography, in the hippocampus, striatum and substantia nigra of rat and guinea-pig.[25] As mentioned in the review by Martin and Humphrey,[131] messenger RNA coding for 5-HT_{5A} receptors was found in rat cortex and hippocampus. 5-HT_{5B} mRNA was detected in the habenula and in limited regions of the hippocampus (CA1). 5-HT_6 mRNA has been discovered in the striatum, the olfactory tubercule, the cortex and the hippocampus. 5-HT_7 mRNA has been identified in the thalamus, the hippocampus and discrete regions of the limbic system.

Thus, there are structures in the mammalian brain in which both cholinergic and serotonergic neuroanatomical substrates can be identified. These structures include the basal forebrain nuclei (DBB, septal region, nucleus basalis), the laterodorsal and pedunculopontine tegmental nuclei, the hippocampus, the striatum and at least some cortical areas. However, the general picture lacks precision, essentially because the histological or morphological observations often indicate possibilities rather than certitudes. This lack, however, will be partially offset by the results of the more functional approaches described in Sections 2–4.

2.2. *Lesion approaches*

Decker and Thal[65] have analysed the effects of separate or combined lesions of the NBM (ibotenate) or of the dorsal raphe nucleus 5,7-dihydroxytryptamine (5,7-DHT) on cholinergic and serotonergic functional markers in the rat frontal cortex. They report that NBM lesions reduced the release of acetylcholine by about 50% without affecting that of serotonin, raphe lesions reduced the 5-HT release by 49% without affecting that of

acetylcholine, and that both lesions strictly combined these effects. Their conclusion was that the serotonergic projections arising in the dorsal raphe do not play any role in the regulation of neocortical tonic release of acetylcholine. After intraventricular infusion of 5,7-DHT, Alonso and Soubrié[2] determined the density of muscarinic receptors in both the hippocampus and the cerebral cortex and reported these markers to be unchanged. This result would also indicate that there is no direct link between serotonergic and cholinergic terminals in the cortex or the hippocampus. However, Alonso and Soubrié[2] have found that the scopolamine-induced upregulation of hippocampal and cortical muscarinic receptors was totally blocked by 5,7-DHT lesions, an observation that would support an interaction between the serotonergic and cholinergic systems, but essentially in terms of serotonergic modulation of "muscarinic plasticity".

That serotonergic mechanisms might be involved in muscarinic plasticity is also suggested in the report by Earley *et al.*[70] In olfactory bulbectomized rats, these authors have demonstrated that the density of muscarinic receptors was elevated in the amygdaloid cortex, the basal ganglia, the hippocampus, the hypothalamus, the cortex and in olfactory regions. The density of 5-HT_2 receptors was increased in the cortex, the hippocampus and the thalamus. Interestingly, these authors also found that a 35-day-long systemic treatment with mianserin, a 5-HT_2 antagonist, or desipramine, a noradrenaline reuptake inhibitor, attenuated not only the serotonergic upregulation induced by the lesion, but also the muscarinic one.

Quirion and Richard[177] have measured the density of 5-HT_1 receptors in various regions of the rat brain after lesions of either the area including the NBM and the substantia innominata (kainic acid injections) or the fimbria and the fornix (transection). They report that NBM lesions had no effect on the density of the cortical 5-HT_1 receptors, conversely to fimbria–fornix transections which reduced the density of 5-HT_1 receptors by more than 50% in the hippocampus. The authors suggest that 5-HT_1 receptors may be present at the cholinergic terminals of neurons originating in the septal nuclei. However, fimbria–fornix lesions also disrupt serotonergic, GABAergic and other rostral innervation sources of the hippocampus (as well as hippocampal efferents) and the results reported by Quirion and Richard,[177] therefore, should not be regarded only as a consequence of the cholinergic denervation of the hippocampus. This caution is of particular importance since Fischette *et al.*[72] have reported that selective lesions of serotonergic neurons also decreased the 5-HT_1 receptors in the anterior hippocampus (by about 35% in the dentate gyrus and regions CA3–CA4). In addition, the findings by Quirion and Richard[177] do not show which receptor subtype of the 5-HT_1 class is concerned. According to more recent findings, it is probably not the 5-HT_{1A} type. Indeed, Lawrence *et al.*[116] examined 5-HT_{1A} receptors in the rat cortex and hippocampus after cholinergic or serotonergic lesions and concluded that these receptors are not located on either cholinergic or serotonergic nerve terminals. This finding, however, does not preclude that a functional serotonin-acetylcholine link exists, which may be mediated by receptors other than the 5-HT_{1A} ones (in the rat, the 5-HT_1 receptor class also covers 5-HT_{1B}, 5-HT_{1C} (now called 5-HT_{2C}), 5-HT_{1D} and 5-HT_{1F} sites,[91,131] although the distribution of these subtypes does not necessarily correspond to that of cholinergic terminals in the brain).

Lown *et al.*[125] have compared the effects of lesions of either the dorsal and median raphe or the NBM and found that serotonergic lesions (raphe) have no effect on the level of 5-HT_{1A} and 5-HT_2 receptors in the frontal cortex, whereas cholinergic lesions (NBM) increase the level of cortical 5-HT_{1A} and 5-HT_2 receptors, suggesting that a functional interaction may exist between the serotonergic and cholinergic functions in the frontal cortex of rats. The finding that after NBM lesions the number of cortical 5-HT_2 receptors is increased is in line with earlier reports by Quirion *et al.*[178] and by Wenk and Engisch.[251] If one turns to the early study by Robinson,[207] such a link really seems to exist and can be demonstrated by lesion techniques of the mesencephalic serotonergic nuclei. After 5,7-DHT-induced destruction of the dorsal raphe, he found that the acetylcholine turnover was increased both in the hippocampus and the cortex, but not in the striatum, suggesting that the serotonergic neurons from the dorsal raphe produce a tonic inhibition of cholinergic function in the cortex (as do neurons of the median raphe) and the hippocampus (an effect not mediated by neurons of the median raphe).

Another approach consists of determining the serotonergic receptors in cholinergic-denervated regions. Muramatsu *et al.*[151] showed that following a lesion of the medial septum, the binding of [^{3}H] ketanserin was reduced in the hippocampus, whilst that of [^{3}H]5-HT or [^{3}H]8-OH-DPAT was not, a finding suggesting that the cholinergic terminals in the hippocampus possess 5-HT_2 receptors, but not 5-HT_{1A} ones (this conclusion is corroborated by a functional approach presented hereafter).

2.3. *Electrophysiological approaches*

There is a large series of studies showing that, in various regions of the mammalian brain known to contain cell bodies, dendrites or terminals of cholinergic neurons, a local application of serotonergic drugs, or even a serotonergic lesion, may influence the electrophysiological activity of the "resident" cell population. Depending on the brain region considered, this influence may involve various subtypes of serotonergic receptors (e.g., Refs 6, 24, 25, 97, 136, 154, 171, 187, 216, 231, 238, 241). However

only a few articles in the literature provide clear arguments demonstrating that an interaction between serotonergic and cholinergic mechanisms may have functional consequences that can be detected electrophysiologically.

Richter-Levin and Segal[190] have investigated the effects of 5,7-DHT-induced serotonin depletion and partial lesions of the medial septum on feedforward inhibition in the dentate gyrus of rats. Feedforward inhibition is defined as the blockade of the response of the dentate gyrus to perforant path stimulation by prior stimulation of the hippocampal commissural pathway. These authors found that neither type of lesion affected feedforward inhibition. However, a combination of both lesion types, resulted in reduced feedforward inhibition, an effect that could be reversed by intrahippocampal grafts containing serotonergic neurons. Interestingly, aged rats also display reduced feedforward inhibition.[191,192]

Semba *et al.*[219] combined immunohistochemical and retrograde tracing methods with an electrophysiological approach to investigate the activity of neocortical neurons after stimulation of a region including the raphe nucleus. They showed that such a stimulation was able to modulate the activity of some neocortical neurons, probably via cholinergic neurons located in the horizontal limb of the DBB or in the magnocellular preoptic area receiving afferents from the raphe nucleus. However, it must be emphasized that only a minority of the latter afferents were found to be immunoreactive to serotonin.

Vanderwolf and Baker[237] have used another approach. They measured low-voltage fast activity (LVFA: 10–50 Hz, maximal amplitude 0.5 mV) in the cortex and rhythmical slow wave activity (RSA: 7–12 Hz, amplitude depending on the electrode's location) in the hippocampus of rats submitted to various pharmacological treatments (control, atropine and/or parachlorophenylanine (pCPA), or other drug treatments). Cerebral activity in both the cortex and the hippocampus is controlled by cholinergic and serotonergic inputs. These inputs are concurrently active during mobility (spontaneous waking behaviors defined as type 1 behaviors) and generate RSA in the hippocampus as well as LVFA in the cortex. As stated by Vanderwolf and Penava,[239] the activity of these inputs appears to be smaller during awake immobility (type 2 behaviors) and RSA disappears in the hippocampus, whereas LVFA can often be observed in the cortex. When experimental manipulations inactivate either the serotonergic or the cholinergic input, the hippocampal and the cortical activities are modified, although they keep some characteristics of RSA and LVFA during type 1 behaviors. When both inputs are inactivated simultaneously, RSA and LVFA are no longer observed during mobility. These data, nevertheless, do not demonstrate that the modifications observed after concurrent serotonergic and cholinergic inactivations are partly or completely due to the disruption of a serotonergic control of cholinergic function. The data rather suggest that both neurotransmitter systems may co-operate, perhaps in parallel, in generating RSA and LVFA during mobility. In an earlier study, Peck and Vanderwolf had shown that scopolamine-induced muscarinic blockade partly antagonized the effects of raphe stimulations on RSA and LVFA, a finding that also suggests both systems to co-operate in the determination of RSA and LVFA. That also an interaction with electrophysiological relevance might be possible is suggested by some data from the next experiment. In 13 out of 16 animals, Vanderwolf and Baker[237] have found that pCPA-treated rats exhibited normal RSA (i.e. to motor activity and atropine-sensitive). However, in the three remaining pCPA-treated rats, the electrophysiological correlates of activated septohippocampal cholinergic pathways showed an abnormal profile: RSA was of lower frequency (5–7 Hz vs 6–12 Hz) and appeared during immobility. Since immobility RSA is normally resistant to atropine (and therefore is not mediated by muscarinic receptors), the observations reported in these three animals suggest that the serotonergic-depletion might have influenced the functionality of the septohippocampal cholinergic system. This conclusion is in line with an earlier experiment[138] showing that after serotonergic lesions (5,7-DHT), hippocampal theta activity could be elicited by septal stimulations of lower intensity than that necessary in the septum of unlesioned animals. Recently, however, Leung *et al.*[118] have published data showing that pCPA treatment in rats with septal lesions does not change the hippocampal *theta* pattern compared with the pattern found after septal lesions only.

Dringenberg and Vanderwolf[68] have recorded transcallosal evoked potentials (single pulse stimulation of the deep contralateral neocortex) in rats submitted to antiserotonergic (ketanserin, methiothepin) and/or antimuscarinic (scopolamine) drug treatments. A typical evoked potential consisted of an initial negative component and a subsequent positive one. After methiothepin injections (5 mg/kg, i.p.), the authors found the initial component to be increased in duration and amplitude, an effect that they also observed after scopolamine injections (5 mg/kg, i.p.). Whereas the amplitude of this early component was further increased when scopolamine was given in conjunction with methiothepin (same doses), its duration was reduced to about one half the values observed in the no drug condition. Ketanserin also had some effect. However, this effect was less pronounced than after methiothepin injections. Furthermore, it was apparent only on the duration of the late component of the evoked potential during immobility and could be reversed by scopolamine treatment.

Riekkinen *et al.*[198] have recorded the electroencephalographic activity of the neocortex. In rats, they describe this activity as consisting of desynchronized low voltage fast activity during mobility and high-voltage spindle activity (HVSA; 6–10 Hz,

synchronized over both hemispheres) during waking immobility. Riekkinen *et al.*[198] found that whereas 5,7-DHT lesions of the dorsal raphe had no effect on HVSA, lesions of the NBM resulted in increased HVSA, an effect that is also found after scopolamine treatment.[200] When both lesions were combined, the increase of HVSA was found to be larger than after only NBM lesions. The latter finding suggests that in the neocortex, a functional interaction between both the cholinergic and serotonergic systems might, in some respects, be involved in the generation of HVSA. Interestingly, an increase of HVSA has also been found in aged rat,[200] but this latter increase was resistant to ondansetron (GR 38032F), methysergide and alaproclate treatments.

Using another model, Luebke *et al.*[126] have combined a histochemical approach with intracellular and whole-cell patch-clamp recording techniques to assess whether cholinergic neurons in the laterodorsal tegmental nucleus of the rat showed an electrophysiological activity which was subjected to serotonergic modulation. These authors provided evidence that in this nucleus, more than half of the spontaneously bursting cholinergic neurons responded to application of serotonin with membrane hyperpolarization, an effect that could be closely mimicked with a 5-HT_{1A} receptor agonist. These findings demonstrate that in this nucleus, serotonin exerts an inhibitory influence upon cholinergic neurons by acting on 5-HT_{1A} receptors. Levkovitz and Segal[121] have investigated the electrophysiological reactivity of the hippocampal system to stimulation of the perforant pathways (entorhinal afferents) and found that systemic administration of fenfluramine, a serotonin releaser, increased the population spike response resulting from perforant path stimulation. This potentiating effect of fenfluramine was inhibited by atropine pretreatment in a dose-dependent manner. Interestingly, the fenfluramine-induced effect could be mimicked by physostigmine injections and blocked by spiperone. These findings show that the potentiating effect of fenfluramine may be the result of an interaction involving cholinergic and serotonergic mechanisms in the hippocampal formation. Hong and Krnjevic[90] reported that, when iontophoretically applied to the region CA1 of the rat hippocampus, 5-HT was able to inhibit the acetylcholine-induced facilitation of population spikes induced by an electrical fimbrial–commissural stimulation.

Finally, there are also studies showing that serotonergic and cholinergic mechanisms may be conjointly involved in long-term potentiation (LTP) phenomena. Maeda *et al.*[128] have studied LTP in the mossy fiber-CA3 system of the guinea-pig hippocampus. Using hippocampal slices, they demonstrated that an application of 1-(m-chlorophenyl)-biguanide (mCPBG) or 2-methyl-5-hydroxytryptamine (ecstasy, 2-Me-5-HT), two 5-HT_3 receptor agonists, attenuated the magnitude of LTP, whilst an application of granisetron, a 5-HT_3 antagonist, increased this magnitude (see also Ref. 167). Interestingly, the latter effect could be weakened by the muscarinic antagonist atropine applied at a concentration that had no effect by itself.

Altogether, these experiments suggest that a cooperation or even an interaction between the serotonergic and cholinergic systems may have functional consequences that can be detected with electrophysiological recording techniques in at least the cortex and the hippocampus. This is also true in humans (e.g., Ref. 139). The most convincing data, however, come from pharmacological experiments. These experiments will be described in detail in the next section.

2.4. *Pharmacological approaches*

In the middle of the 1970s, a series of experiments demonstrated the existence of a functional link between serotonergic and cholinergic neurons in the rat striatum. The first demonstration that serotonin could influence the activity of cholinergic neurons was reported by Butcher *et al.* in 1976.[40] These authors showed that rats pretreated with para-chlorophenylalanine (pCA) or lesioned in the dorsal raphe nucleus exhibited a decreased rate of striatal acetylcholine synthesis. Supportive data came from the studies by Euvrard *et al.*,[71] Guyenet *et al.*,[80] and Samanin *et al.*,[214] who all showed that systemic administration of serotonin agonists increased the acetylcholine content in the rat striatum, suggesting that serotonin is exerting an inhibitory modulation of cholinergic function in the striatum.

Since that time, essentially three regions of the brain receiving both a serotonergic and a cholinergic innervation have been investigated with *in vitro* and *in vivo* methods: the hippocampus, the cortex and the striatum. These investigations allowed the identification and the characterization of some pharmacological substrates of the link between serotonergic and cholinergic functions in the mammalian brain.

As regards the influence of serotonin on the release of acetylcholine, the type of modulation (activatory, inhibitory) as well as the type of receptor involved in this modulation were found to depend upon several factors including the species (e.g., rat vs guinea-pig), the brain region (e.g., hippocampus vs striatum) and the methods used to perform the experiments (e.g., *in vitro* vs *in vivo*). *In vitro* experiments essentially investigated the effects of drug applications to tissue slices or synaptosomes preloaded with [^{3}H]choline and subsequently exposed to potassium or electrical stimulation. *In vivo* experiments investigated the effects of drugs delivered locally or administered systemically on acetylcholine content (*post mortem* analysis) or release (microdialysis) in various cholinergic targets.

Thus, the *in vitro* experiments mainly characterized the serotonergic mechanisms modulating cholinergic function by an action occurring at the level of the cholinergic terminal. These mechanisms involve

either heteroceptors located on the cholinergic terminals (i.e. a direct modulatory action) or receptors located on terminals other than cholinergic ones which are able to influence the release of a neurotransmitter to which cholinergic function is also sensitive (i.e. an indirect modulatory action). Conversely, the *in vivo* approaches showed effects of serotonin on cholinergic function which may not only be due to direct pre- and/or postjunctional actions, but which may also result from activation or inhibition of complex polysynaptic loops, especially when i.c.v. or systemic administrations of substances affecting the serotonergic systems are used.

2.4.1. *Principal receptors considered and characteristics.* Basically, the *in vitro* and *in vivo* pharmacological studies have investigated the involvement of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_2, 5-HT_3 and 5-HT_4 receptors in the modulation of central cholinergic function. For each family of 5-HT receptors, operational, structural and transductional specificities have been described.[91,131] Briefly, in the brain, the 5-HT_{1A} receptor is predominantly postsynaptic, although it was identified as a somatodendritic receptor in raphe neurones. The 5-HT_{1A} receptor couples to multiple G proteins with preferential mediation by the inhibition of adenylyl cyclase (e.g., Ref. 81). There are numerous agonists available, but the most useful 5-HT_{1A} agonist is 8-OH-DPAT. The number of selective antagonists is more limited and, with exception of WAY 100135 (see Ref. 131), they all seem to have partial agonist activity. Transductional characteristics similar to those of the 5-HT_{1A} receptor are also found for 5-HT_{1B} and 5-HT_{1D} receptors. The 5-HT_{1B} is the autoreceptor involved in presynaptic regulation of serotonin release in the rat brain (e.g., Refs 142, 228), a role that might be assumed by 5-HT_{1D} receptors in the human and guinea-pig brain (e.g., Ref. 93). The 5-HT_{1B} receptor also functions as a terminal heteroreceptor controlling the release of neurotransmitters other than serotonin. The compound CP 93129 is a selective agonist of the 5-HT_{1B} receptor, sumatriptan and L 694,247 of the 5-HT_{1D} receptor. There is no good selective antagonist for the 5-HT_{1B} receptor, GR 127935 being an antagonist of the 5-HT_{1D} receptor. 5-HT_2 receptors are divided into three subclasses (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}) and are coupled to phospholipase C. Their activation leads to stimulation of phosphatidyl inositol and results in an increased IP_3 production (e.g., Ref. 122). There is neither a selective agonist nor a selective antagonist for any of the three subtypes of 5-HT_2 receptors. Among other substances, alpha-Me-5-HT is a non-selective 5-HT_2 agonist, ketanserin, ritanserin, mesulergine and LY 53857 being non-selective 5-HT_2 antagonists. The 5-HT_3 receptor is coupled to an ionic channel regulating the Na^+/K^+ conductance (e.g., Ref. 234). Agonists of this receptor type are 2-Me-5-HT and m-chloro-phenylbiguanide, the most potent one being 3,3,5-trichloro-phenylbiguanide.[146] Antagonists are tropisetron, ondansetron, granisetron and zacopride. Finally, the 5-HT_4 receptor is coupled to adenylyl cyclase, but positively. 5-HT_4 agonists are 5-methoxytryptamine, renzapride, BIMU 1 and BIMU 8. Antagonists are GR 113808 and SB 204070. All these transductional characteristics, agonists and antagonists of the various 5-HT receptor types have been presented in more detail in recent reviews.[91,131]

2.4.2. In vitro *approaches. In vitro* experiments carried out on slices or synaptosomes prepared from specific brain regions of rats, guinea-pigs or humans are summarized in Table 1.

2.4.2.1. Hippocampus. One consensus to emerge is that, in the hippocampus of both the rat and the guinea-pig, an application of serotonin, in the 1–100 μM range, produces a significant inhibition of evoked (induced by exposure to supranormal concentrations of potassium or by electrical field stimulations) release of acetylcholine. Depending upon the studies and the concentration of serotonin applied, the reported inhibition was generally found to vary between 10 and 40%. Since no effects of serotonin have been described on the baseline release of acetylcholine from slices or synaptosomes, it can be considered that the serotonin-induced inhibition is operating when the cholinergic neurons are active.

The most likely type of 5-HT receptor mediating the inhibitory action of serotonin on acetylcholine release is from the 5-HT_1 class (for an updated nomenclature, see Refs 91 and 131). Several arguments support this assertion (see Table 1 for details). First, the inhibitory effects of serotonin can be mimicked by application of agonists showing some affinity for the 5-HT_1 receptor [e.g., 1-(3-chlorophenyl)piperazine (mCPP), RU-24969, N-(3-trifluoromethylphenyl) piperazine (TFMPP)]. Second, the inhibitory effects of serotonin are abolished or dramatically attenuated under the influence of 5-HT_1 antagonists. For instance, the inhibitory effect of TFMPP, a non-selective agonist of 5-HT receptors, is reduced by application of minaprine, a 5-HT_1 antagonist, or by that of other antagonists having an affinity for both the 5-HT_1 and 5-HT_2 sites. Minaprine itself is able to influence the release of acetylcholine by activating protein kinase C.[47]

In the rat, this inhibitory heteroceptor appears to belong to the 5-HT_{1B} type. This is in agreement with the report by Maura and Raiteri[135] who first demonstrated that the inhibitory effect of serotonin is mediated by a 5-HT_1 receptor, who then showed that neither 5-HT_{1A} nor 5-HT_{2C} receptors were involved, and who therefore concluded that the 5-HT_{1B} receptor might be implicated (see also Ref. 180). Their conclusion was supported by observations that CGS 120966B, a rather selective 5-HT_{1B} agonist, concentration-dependently inhibited the evoked release of acetylcholine from rat hippocampal synaptosomes.[27,28] In addition, we recently found that CP 93129, a 5-HT_{1B} agonist,

Table 1. Pharmacological manipulations of serotonergic functions and *in vitro* effects upon acetylcholine release in various regions of the mammalian brain

Experimental preparation	Stimulation	Drugs and properties		[Concentration or dose]	ACh release in % of baseline	References
Hippocampus						
Synaptosomes of rat hippocampus	15 mM KCl	CGS 12066B	(+)5-HT_{1B}	[0.1]	−10%	27
				[1]	−23%	
				[10]	−35%	
		Citalopram	(−)5-HT uptake	[0.1]	0%	
		Tianeptine	(+)5-HT uptake	[10]	0%	
		Tianeptine		[100]	−18%	
		CGS & citalopram		[3]&[10 mg/kg, i.p.]	−25%	
		CGS & tianeptine		[3]&[20 mg/kg, i.p.]	−25%	
		CGS & citalopram		[3]&[1]	0%	
		CGS & tianeptine		[3]&[1]	0%	
Synaptosomes of rat hippocampus	15 mM KCl	CGS 12066B	(+)5-HT_{1B}	[0.01]	−13%	28
				[0.1]	−24%	
				[1]	−33%	
				[10]	−37%	
		CGS 12066B after tianeptine		[0.1]	< −10%	
		injected for two weeks		[1]	−17%	
		(2 × 10 mg/kg, i.p. per day		[10]	−32%	
		CGS 12066B after citalopram		[0.1]	0%	
		injected for two weeks		[1]	−13%	
		(20 mg/kg, i.p. per day)		[10]	−24%	
Synaptosomes of rat hippocampus	15 mM KCl	TFMPP	(+)5-HT n.s.	[100]	−43%	29
		TFMPP & minaprine	(−)5-HT_1 n.s.	[100]&[0.3]	< −10%	
		TFMPP & methiothepin	(−)5-HT_1/5-HT_2 n.s.	[100]&[10]	−18%	
		TFMPP & mesulergine	(−)5-HT_{2C}	[100]&[10]	−38%	
		TFMPP & spiperone	(−)5-HT_{1A}/5-HT_2	[100]&[10]	−47%	
		TFMPP & ketanserin	(−)5-HT_2	[100]&[10]	−46%	
		TFMPP & MDL 72222	(−)5-HT_3	[100]&[10]	−28%	
Synaptosomes of guinea-pig hippocampus	10 mM KCl	TFMPP	(+)5 = HT n.s.	[0.3]	< −10%	84
				[10]	< −10%	
				[100]	> −40%	
				[1000]	−70%	

		CGS 12066B	(+)5-HT_{1b}/5-HT_{1D}	[10]	−23%	
				[30]	−34%	
				[100]	−81%	
		MCPP	(+)5-HT_{1B}/5-HT_{1D}/5-HT_{2C}	[100]	−38%	
		5-CT	(+)5-HT n.s.	[10]	−12%	
		8-OH-DPAT	(+)5-HT_{1A}	[100]	0%	
		Quipazine	(+)5-HT_2	[100]	0%	
		TFMPP & dihydroergotamine	(−)5-HT_1/5-HT_2	[100]&[1]	< −10%	
		TFMPP & metergoline	(−)5-HT_1/5-HT_2	[100]&[0.1]	−23%	
		TFMPP & methysergide	(−)5-HT_1/5-HT_2	[100]&[1]	< −10%	
		TFMPP & yohimbine	(−)5-HT_{1D}	[100]&[10]	< −10%	
		(release of ACh normal under the following treatments: methiothepin, propranolol, ketanserin, spiroperidol, mesulergine, tropisetron)				
Synaptosomes of rat hippocampus	15 mM KCl	5-HT		[0.3]	−30%	135
				[1]	−35%	
				[3]	−40%	
		5-HT & methiothepin	(−)5-HT_1/5-HT_2	[0.3]&[0.3]	−13%	
				[1]&[0.3]	−21%	
		5-HT & propranolol	(−)5-HT_1/β-adren	[0.3]&[1]	−17%	
				[1]&[1]	−21%	
		5-HT & methysergide	(−)5-HT_1/5-HT_2	[0.3]&[1]	−28%	
		5-HT & ketanserin	(−)5-HT_2	[0.3]&[1]	−31%	
		5-HT & spiperone	(−)5-HT_1/5-HT_2	[0.3]&[0.3]	−24%	
		5-HT & RU 24969	(+)5-HT_1	[1]&[n.i.]	−33%	
		5-HT & 8-OH-DPAT	(+)5-HT_{1A}	[1]&[1]	0%	
400-μm-thick slices of rat hippocampus	Electrical	5-HT		[0.1]	−17%	134
				[0.3]	−24%	
				[1]	−30%	
				[10]	−36%	
				[30]	−35%	
		RU 24969	(+)5-HT_1	[0.1]	−10%	
				[1]	−23%	
				[3]	−28%	
				[10]	−33%	
		(±)-DOI	(+)5-HT_2	[1]	0%	
		8-OH-DPAT	(+)5-HT_{1A}	[1]	0%	
		Methiothepin	(−)5-HT_1/5-HT_2	[1]	+32%	
		5-HT & methiothepin		[0.3]&[1]	0%	
		5-HT & ketanserin	(−)5-HT_2	[0.3]&[1]	−25%	
		6-Nitroquipazine	(−)5-HT uptake	[1]	−30%	
		d-Fenfluramine	(+)5-HT release	[10]	−17%	
				[50]	−33%	
				[100]	−38%	
		d-Fenfluramine & methiothepin		[10]&[1]	0%	
				[50]&[1]	−13%	
				[100]&[1]	−17%	

continued overleaf

Table 1. *(Continued)*

Experimental preparation	Stimulation	Drugs and properties		[Concentration or dose]	ACh release in % of baseline	References
50-mg-slices of rat hippocampus	25 mM KCl	5-HT		[1]	−20%	151
				[10]	−30%	
				[100]	−40%	
		(effects abolished by septal lesions but ACh release low in lesioned rats)				
		Minaprine	(+)5-HT_1 n.s.	[10]	0%	
		Mianserin	(−)5-HT_2	[10]	0%	
		Methysergide	(−)5-HT_1/5-HT_2	[10]	0%	
		8-OH-DPAT	(+)5-HT_{1A}	[100]	0%	
		5-HT & minaprine		[100]&[10]	0%	
		5-HT & mianserin		[100]&[1–10]	0%	
		5-HT & methysetgide		[100]&[0.1–10]	0%	
		5-HT & propranolol	(−)5-HT_1/β-adren	[100]&[n.i.]	−40%	
		5-HT & metoclopramine	(−)5-HT_3	[100]&[n.i.]	−40%	
Cortex						
0.35 × 0.35 mm pieces of rat entorhinal cortex	20 mM KCl	5-HT		[2]	0%	10
		Ritanserin	(−)5-HT_2	[1]	0%	
		5-HT & ritanserin		[2]&[1]	−50%	
		2-Me-5-HT	(+)5-HT_3	[2]	0%	
		2-Me-5-HT & ritanserin		[2]&[1]	−46%	
		GR 38032F	(−)5-HT_3	[0.001]	0%	
		2-Me-5-HT & GR 38032F		[2]&[0.001]	+107%	
		2-Me-5-HT & GR 38032F & ritanserin		[2]&[0.001]&[1]	0%	
		Zacopride	(−)5-HT_3	[0.001]	0%	
		2-Me-5-HT & zacopride		[2]&[0.001]	+36%	
		2-Me-5-HT & zacopride & ritanserin		[2]&[0.001]&[1]	0%	
Slices of guinea-pig cortex	Electrical	5-HT		[10–30]	0%	20
				[100]	−17%	
		Methysergide	(−)5-HT_1/5-HT_2	[1]	0%	
		5-HT & methysergide		[100]&[n.i.]	−15%	
300-μm-thick slices of rat entorhinal cortex	20 mM KCl	2-Me-5-HT	(+)5-HT_3	[2]	0%	106
		Ritanserin	(−)5-HT_2	[1]	0%	
		Ondansetron	(−)5-HT_3	[1]	0%	
		2-Me-5-HT & ritanserin		[2]&[1]	0%	
		5-HT & ritanserin		[2]&[1]	−17%	
		2-Me-5-HT & ondansetron		[2]&[1]	0%	

Synaptosomes of human cerebral cortex (front., temp., occip.)	15 mM KCl	5-HT		[0.1]	−34%	133
				[10]	−56%	
		1-Phynilbiguanide	(+)5-HT_3	[1]	−35%	
		8-OH-DPAT	(+)5-HT_{1A}	[1]	0%	
		5-HT & tropisetron	(−)5-HT_3	[0.1]&[0.1]	0%	
		5-HT & ondansetron	(−)5-HT_3	[0.1]&[0.1]	0%	
		5-HT & spiperone	(−)5-HT_2	[1]&[0.1]	−30%	
		5-HT & ketanserin	(−)5-HT_2	[1]&[0.1]	−35%	
Striatum						
350-μm-thick slices of rat nucleus accumbens	30 mM KCl	5-HT		[20]	−28%	60
		5-HT & methysergide	(−)5-HT_1/5-HT_2	[20]&[10]	−28%	
		5-HT & sulpiride	(−)DA	[20]&[10]	0%	
400–μm-thick slices of rat striatum	Drugs	*(application of 5-HT elicits a biphasic response on release of ACh release, the first part being facilitatory and sensitive to TTX, the second being inhibitory and insensitive to TTX)*				21
Facilitatory phase (without TTX)		5-HT		[30]	+50%	
		5-HT & methysergide	(−)5-HT_1/5-HT_2	[30] + [1]	0%	
		5-HT & methiothepin	(−)5-HT_1/5-HT_2	[30] + [1]	0%	
		5-HT & ritanserin	(−)5-HT_2	[30] + [1]	0%	
		5-HT & propranolol	(−)5HT_1/β-adren	[30] + [1]	+50%	
Inhibitory phase (with TTX)		5-HT		[100]	−27%	
		5-HT & methysergide		[100] + [3]	0%	
		5-HT & methiothepin		[100] + [1]	0%	
		5-HT & ritanserin		[100] + [3]	−22%	
		5-HT & propranolol		[100] + [1]	−22%	
		(inhibitory effect abolished by DA depletion)				
	Electrical	5-HT		[100]	−20%	
		5-HT & methysergide		[100] + [3]	0%	
		5-HT & methiothepin		[100] + [1]	0%	
		5-HT & ritanserin		[100] + [3]	−20%	
		5-HT & propranolol		[100] + [1]	−20%	
		(inhibitory effect not abolished by DA depletion)				

continued overleaf

Table 1. *(Continued)*

Experimental preparation	Stimulation	Drugs and properties		[Concentration or dose]	ACh release in % of baseline	References
250-μm-thick slices of rat striatum	25 mM KCl	5-HT		[10]	−26%	77
		LSD	(+)5-HT n.s.	[3]	−26%	
		5-MOHT	(+)5-HT n.s.	[10]	−32%	
		Bufotenin	(+)5-HT n.s.	[10]	−23%	
		N,*N*-dimethyltryptamine	(+)5-HT n.s.	[10]	−25%	
		5-HT & cinanserin	(−)5-HT$_1$/5-HT$_2$	[10]&[10]	0%	
		5-HT & metergolin	(−)5-HT n.s.	[10]&[10]	−23%	
		5-HT & methysergide	(−)5-HT n.s.	[10]&[10]	0%	
		5-HT & methiothepin	(−)5-HT n.s.	[10]&[10]	0%	
		5-HT & spiroperidol	(−)DA n.s.	[10]&[10]	−30%	
		5-HT & haloperidol	(−)DA n.s.	[10]&[10]	−22%	
350-μm-thick slices of the rat striatum: caudal region	Electrical					95
		Quipazine	(−)5-HT uptake	[1]	−19%	
		Quipazine & methysergide	(−)5-HT n.s.	[1]&[10]	0%	
		(effects not sensible to 6-OHDA-induced DA depletion)				
		Fluoxetine	(−)5-HT uptake	[1]	−16%	
		(effect abolished by 5,7-DHT treatment)				
		Fluoxetine & methysergide		[1]&[10]	0%	
		Fluoxetine		[10]	−23%	
		Fluoxetine & methysergide		[10]&[10]	0%	
		(effect not abolished by 5,7-DHT treatment)				
Rostral region		Quipazine	(−)5-HT uptake	[1]	−21%	
		Quipazine & methysergide	(−)5-HT n.s.	[1]&[10]		
80–120 mg slices of rat striatum	20 mM ouabain	Lesion of dorsal and medial raphe			+425%	242
		pCPA-induced 5-HT depletion		[3 × 100mg/kg, i.p.]	+163%	
		5,7-DHT-lesion		[25 μg, i.c.v.]	+247%	
		d-fenfluramine	(+)5-HT release	[0.1]	0%	
				[1]	−38%	
				[10]	−47%	
		(not abolished by 6-OHDA but abolished by 5,7-DHT lesions)				
		mCPP	(+)5-HT n.s.	[0.1]	0%	
				[1]	−48%	
				[10]	−52%	
		(not abolished by 6-OHDA or 5,7-DHT lesions)				
		Cyproheptadine	(−)5-HT n.s.	[5 mg/kg, i.p.]	0%	
				[10 mg/kg, i.p.]	+115%	
				[20 mg/kg, i.p.]	+232%	
		Mianserin	(−)5-HT$_2$	[3.3]	+123%	
		Methysergide	(−)5-HT$_1$/5-HT$_2$	[2.8]	+139%	

(−): antagonist or inhibitor; (+), agonist or activator; [ ]: the concentrations indicated in the square brackets are given in μM, except where indicated; 0% does not necessarily indicate that there is no modification at all as compared to the baseline release, but that the observed modification did not reach statistical significance; &: combination of two or more substances. The pharmacological characteristics of the substances are those mentioned by the authors, not necessarily the ones recognized nowadays. n.i.: information not indicated in the article; n.s.: not selective.

concentration-dependently decreased the electrically evoked release of acetylcholine from hippocampal slices (unpublished observations).

8-OH-DPAT, a specific agonist of 5-HT_{1A} receptors, did not affect the evoked release of acetylcholine (see Refs 84, 134, 151, and personal unpublished observations).

As to other classes of 5-HT receptor (i.e. 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}), all of which are found in the rat brain,[91,131] no data as regards preparations of rat hippocampus exposed to specific agonists or antagonists are, to our knowledge, currently available.

The involvement of 5-HT_2 receptors (i.e. 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}) has also been excluded since: (i) application of 5-HT_2 agonists such as (±)-DOI or quipazine does not affect the evoked release of acetylcholine; and (ii) application of 5-HT_2 antagonists such as ketanserin, methysergide or spiperone (the two latter drugs also act on 5-HT_1 sites) does not abolish the inhibitory effect of 5-HT, 5-HT uptake inhibitors or 5-HT agonists on acetylcholine release from hippocampal preparations.

Finally, there are a few indications suggesting that 5-HT_3 receptors do not seem to be critically involved in a direct presynaptic regulation of acetylcholine release by serotonin. In the presence of MDL 72222, a 5-HT_3 antagonist, the non-selective 5-HT agonist TFMPP was still able to reduce the potassium-evoked release of acetylcholine from hippocampal synaptosomes.[29] However, this reduction was smaller (−28%) than in the absence of MDL 72222 (−43%). Although the participation of 5-HT_3 receptors in regulation of hippocampal acetylcholine release cannot be excluded, it is probably not of critical importance.

In conclusion, the principal receptor involved in serotonin-induced inhibition of acetylcholine release in the rat hippocampus seems to belong to the 5-HT_{1B} type. In the hippocampus of guinea-pigs where the 5-HT_{1B} receptors do not exist (e.g., Ref. 220), the inhibitory serotonergic heteroceptor might be of the 5-HT_{1D} type (see Table 1).

2.4.2.2. Cerebral cortex. Bianchi *et al.*[20] reported that a high concentration of serotonin produced only a small inhibition of electrically evoked release of acetylcholine from slices of guinea-pig cerebral cortex. Barnes *et al.*[10] reported that in the rat entorhinal cortex serotonin alone had no effect on acetylcholine release. However, the co-application of serotonin and ritanserin, a 5-HT_2 antagonist, resulted in a 50% decrease of potassium-induced release of acetylcholine. Whereas the 5-HT_3 agonist, 2-me-5-HT, had no effect when applied alone, addition of ritanserin produced an effect comparable to that found with serotonin and ritanserin. In addition, the evoked release was increased by 107% when 2-me-5-HT was applied in the presence of ondansetron, a 5-HT_3 antagonist, and by 37% in the presence of zacopride, another 5-HT_3 antagonist. Both these facilitatory effects were prevented by addition of ritanserin. These results, however, do not demonstrate that 5-HT_3 receptors are directly involved since a concomitant action on the 5-HT_2 receptor seems to be required to observe a modulation of cholinergic function via the 5-HT_3 receptor. Moreover, Johnson *et al.*[106] failed to replicate some of the effects described by Barnes *et al.*[10] In an *in vitro* study using human frontal, temporal and occipital cortex fragments, Maura *et al.*[133] found serotonin to inhibit potassium evoked release of synaptosomal acetylcholine in a dose dependent manner, with a maximum of −56% at 10 μM. This inhibition was prevented by tropisetron and ondansetron, two specific 5-HT_3 antagonists, but not by spiperone or ketanserin, two drugs blocking the 5-HT_2 receptor. Altogether, the results indicate differences among species as to the modulation of acetylcholine release from cerebral cortex by 5-HT receptors.

2.4.2.3. Striatum. In the striatum, serotonin has also been found to modulate cholinergic function (Table 1). Using striatal slices, Vizi *et al.*[242] reported that the ouabain-induced stimulation of acetylcholine release was reduced by exposure to d-fenfluramine, a 5-HT releaser, as well as to piperazine, a non-selective 5-HT agonist. These effects of d-fenfluramine and piperazine were not observed in rats which sustained 5,7-DHT lesions of the serotonergic neurons but were still present after 6-OHDA-induced depletion of the dopaminergic neurotransmission. When serotonergic antagonists such as mianserin, methysergide or cyproheptadine were applied to the slices during stimulation, the acetylcholine release was enhanced by more than 100%.[242] Serotonergic agonists or 5-HT-uptake inhibitors blocked the electrically-evoked release of acetylcholine.[77,95] The receptor involved in this modulatory effect of serotonin might be of the 5-HT_1 or 5-HT_2 type as methysergide, cyproheptadine, cinanserin and mianserin, which do not distinguish between these subtypes, antagonized this effect.[77]

In the guinea-pig striatum, Bianchi *et al.*[21] reported that application of 5-HT elicited a biphasic response from cholinergic neurons. First, they rapidly responded by increasing (approx. +50%) the release of acetylcholine, an effect which was sensitive to tetrodotoxin (TTX) and, then, the release decreased below baseline (approx. −27%), an effect which was insensitive to TTX. The authors provided some experimental arguments (Table 1) that the facilitatory phase involved a direct activation of 5-HT_2 receptors located on cholinergic terminals, whereas the inhibitory phase was mediated via a 5-HT-induced modulation of dopamine release, a neurotransmitter to which cholinergic terminals in the striatum are also sensitive.[88]

2.4.3. In vivo *approaches*. *In vivo* experiments that demonstrate the influence of serotonin on cholinergic function are summarized in Table 2.

2.4.3.1. Hippocampus. Administration of drugs

Table 2. Pharmacological manipulations of serotonergic functions and *in vivo/ex vivo* effects upon acetylcholine release or content (*post mortem*) in preparations from various regions of the mammalian brain

Experimental preparation	Stimulation	Drugs and properties		[Concentration or dose]	ACh release in % of baseline or ACh content vs control	References
Hippocampus						
Microdialysis in the rat hippocampus	Drugs	Tianeptine	(+)5-HT uptake	[10 mg/kg, i.p.]	0%	18
				[20 mg/kg, i.p.]	0%	
				[30 mg/kg, i.p.]	−40%	
		(the effects observed are abolished by chemical 5HT lesions or metergoline-induced blockade of 5-HT receptors)				
Microdialysis in the rat dorsal hippocampus	Drugs	Fenfluramine	(+)5-HT release	[20 mg/kg, i.p.]: (peak)	+104%	54
		Norfenfluramine	(+)5-HT release	[7.5 mg/kg, i.p.]: (peak)	+125%	
		Citalopram	(−)5-HT uptake	[10; infused]: (peak)	+55%	
		(all aforementioned effects are prevented by a 5,7-DHT-induced lesion)				
		Fenfluramine & tropisetron	(−)5-HT_3	[5 mg/kg, i.p.]&[0.5 mg/kg, i.p.]	0%	
		Fenfluramine & DAU 6215	(−)5-HT_3	[5 mg/kg, i.p.]&[60 μg/kg, i.p.]	0%	
		Fenfluramine & metergoline	(−)5-HT_1/5-HT_2	[5 mg/kg, i.p.]&[2 mg/kg, s.c.] (peak)	+100%	
		2-Me-5-HT	(+)5-HT_3	[250 μg, i.c.v.]: (peak)	+50%	
		2-Me-5-HT & GR 38032F		[250 μg, i.c.v.]&[60 μg/kg, s.c.]	0%	
		Fenfluramine & DAU 6215	(−)5-HT_3	[5 mg/kg, i.p.]&[60 μg/kg, i.p.]	0%	
		2-Me-5-HT & DAU 6215		[250 μg, i.c.v.]&[60 μg/kg, i.p.]	0%	
		BIMU 1	(+)5-HT_4	[0.1, i.c.v.]	−25%	
Microdialysis in the rat hippocampus	Drugs	(−)fenfluramine	(+)5-HT release	[15 mg/kg, i.p.]	+21%	55
		(+)fenfluramine	(+)5-HT release	[7.5 mg/kg, i.p.]	+45%	
Homogenates from the rat hippocampus	Drugs	Quipazine	(−)5-HT uptake	[10 mg/kg, i.p.]	0%	71
Microdialysis in the rat dorsal hippocampus	Drugs (infused)	5-HT		[10]	0%	94
		Clomipramine	(−)5-HT uptake	[2]	0%	
		8-OH-DPAT	(+)5-HT_{1A}	[20]	+50%	
		CGS 12066B	(+)5-HT_{1B}	[100]	−50%	
		NAN 190	(−)5-HT_{1A}	[10]	0%	
		8-OH-DPAT & NAN 190		[20]&[10]	0%	
		CGS 12066B & NAN 190		[100]&[10]	−30%	
		5-HT & NAN 190		[10]&[10]	−30%	
		Clomipramine & NAN 190		[2]&[10]	−25%	

Microdialysis in the rat dorsal hippocampus	Drugs	Apomorphine	(+)DA	[2.0 mg/kg, i.p.]	+170%	156
		Amphetamine	(+)DA release	[2.5 mg/kg, i.p.]	+400%	
		p-chloroamphetamine	(+)5-HT release	[2.5 mg/kg, i.p.]	+160%	
Microdialysis in the rat dorsal hippocampus	Drugs	NA (infused)		[1000]	0%	164
				[2000]	0%	
				[5000]	0%	
		DA		[1000]	+45%	
				[2000]	+96%	
				[1000]	+100%	
		5-HT		[1000]	+70%	
				[2000]	+125%	
				[3000]	+284%	
				[5000]	+260%	
		Apomorphine	(+)DA	[1000]	+72%	
		5-MeO-DMT	(+)5-HT n.s.	[1000]	+310%	
Post mortem determination of acetylcholine levels in the rat hippocampus	Drugs	Quipazine (*effect abolished by lesions of the median raphe nucleus*)	(−)5-HT uptake	[10 mg/kg, i.p.]	+30%	214
Microdialysis in the guinea pig hippocampus	Drugs	8-OH-DPAT	(+)5-HT_{1A}	[1 mg/kg, s.c.]	+137%	256
				[10 mg/kg, s.c.]	+341%	
				[10; infused]	0%	
		Sumatriptan	(+)5-HT_{1D}	[1; infused]	0%	
		Buspirone	(+)5-HT_{1A} p.	[1 mg/kg, s.c.]	+61%	
				[10 mg/kg, s.c.]	+282%	
		Ipsaspirone	(+)5-HT_{1A} p.	[10 mg/kg, s.c.]	+130%	
		MDL 73005	(+)5-HT_{1A} p.	[3 mg/kg, s.c.]	+98%	
				[5 mg/kg, s.c.]	+99%	
				[10 mg/kg, s.c.]	+74%	
		NAN 190	(−)5-HT_{1A}	[3 mg/kg, s.c.)	0%	
		8-OH-DPAT & NAN 190		[1 mg/kg, s.c.]&[3 mg/kg, s.c.]	< +80%	
		Prazosin	(−)alphal-adren	[1 mg/kg, s.c.]	−27%	
Cortex Microdialysis in the rat frontal cortex	Drugs	Tianeptine	(+)5-HT uptake	[10 mg/kg, i.p.]	0%	18
				[10 mg/kg, i.p.]	0%	
				[20 mg/kg, i.p.]	0%	
				[30 mg/kg, i.p.]	−30%	
		(*the effects observed are abolished by chemical 5-HT lesions or metergoline-induced blockade of 5-HT receptors*)				

continued overleaf

Table 2. *(Continued)*

Experimental preparation	Stimulation	Drugs and properties		[Concentration or dose]	ACh release in % of baseline or ACh content vs control	References
Collection of an epidurally applicated Ringer solution on guinea-pig cortex	Drugs	5-HT		[0.2, i.c.v.]	+27%	20
				[0.5, i.c.v.]	+44%	
				[1, i.c.v.]	+56%	
		(these responses are abolished by 5,7-DHT, but not by pCPA serotonergic depletions)				
		Methysergide	(−)5-HT n.s.	[4.25/kg, i.p.]	> +60%	
		5-HT & methysergide		[1, i.c.v.]&[4.25/kg, i.p.]	> +60%	
		Metitepine	(−)5-HT_1 n.s.	[1/kg, i.p.]	> +40%	
				[4.25/kg, i.p.]	+70%	
		5-HT & methiothepin		[1, i.c.v.]&[1/kg, i.p.]	> +40%	
		5-HT & methiothepin		[1, i.c.v.]&[4.25/kg, i.p.]	0%	
		d-Norfenfluramine	(+)5-HT release	[10.4/kg, i.p.]	0%	
		d-Norfenfluramine & methiothepin		[10.4/kg, i.p.] &[4.25/kg, i.p.]	> −30%	
Microdialysis in the guinea-pig cortex	Drugs	8-OH-DPAT	(+)5-HT_{1A}	[0.02 μg, i.c.v.]	> +20%	22
				[0.2 μg, i.c.v.]	> +40%	
				[2 μg, i.c.v.]	> +100%	
				[0.01 mg/kg, s.c.]	0%	
				[0.1 mg/kg, s.c.]	> +60%	
				[1 mg/kg, s.c.]	> +100%	
		RU 24969	(+)5-HT_{1A}/5-HT_{1B}	[0.1 mg/kg, s.c.]	0%	
				[1 mg/kg, s.c.]	+30%	
				[10 mg/kg, s.c.]	> +80%	
		Propranolol	(−)5-HT_1/β-adren.	[5 mg/kg, i.p.]	0%	
		8-OH-DPAT & propranolol		[0.1 mg/kg, s.c.]&[5 mg/kg, i.p.]	0%	
		Ketanserin	(−)5-HT_2	[0.5 mg/kg, i.p.]	0%	
		5-HT & ketanserin		[500 μg, i.c.v.] &[0.5 mg/kg,i.p.]	> +60%	
		2-Me-5-HT	(+)5-HT_3	[250 μg, i.c.v.]	0%	
				[500 μg, i.c.v.]	−25%	
		2-Me-5-HT & tropisetron	(−)5-HT_3	[500 μg, i.c.v.] &[0.5 mg/kg, s.c.]	0%	
		Methiothepin	(−)5-HT_1	[2 mg/kg, i.p.]	> +50%	
		5-HT & methiothepin		[500 μg, i.c.v.]&[2 mg/kg, i.p.]	0%	
		8-OH-DPAT & methiothepin		[0.1 mg/kg, s.c.] &[2 mg/kg, i.p.]	> +50%	

		5-HT & methiothepin & mesulergide	(−)5-HT$_1$	[500 μg, i.c.v.]&[2 mg/kg, i.p.] &[0.5 mg/kg, i.p.]	0%	
		5-HT & methiothepin & ketanserin		[500 μg, i.c.v.]&[2 mg/kg, i.p.] &[0.5 mg/kg, i.p.]	0%	
		5-HT & methiothepin & propranolol		[500 μg, i.c.v.]&[2 mg/kg, i.p.] &[5 mg/kg, i.p.]	0%	
		5-HT & methiothepin & tropisetron		[500 μg, i.c.v.]&[2 mg/kg, i.p.] &[0.5 mg/kg, s.c.]	> +50%	
		2-Me-5-HT & methiothepin		[500 μg, i.c.v.]&[2 mg/kg, i.p.]	0%	
Acetylcholine content in the rat cortex	Drugs	BIMU 1	(+)5-HT$_4$	[0.01, i.c.v.]	0%	53
				[0.03, i.c.v.]	+27%	
				[0.04, i.c.v.]	+90%	
				[0.06, i.c.v.]	+147%	
		BIMU 8	(+)5-HT$_4$	[0.03, i.c.v.]	+35%	
				[0.04, i.c.v.]	+91%	
				[0.06, i.c.v.]	+142%	
		BIMU 1 & GR 125487	(−)5-HT$_4$	[0.04, i.c.v.]&[0.02, i.c.v.]	0%	
		(Neither of these effects is observed in the hippocampus or in the frontal cortex or in the hippocampus)				
Homogenates from the rat cortex	Drugs	Quipazine	(+)5-HT$_2$	[30 mg/kg, i.p.]	0%	71
Collection of an epidurally applicated Ringer solution on rat cortex on guinea-pig cortex		8-OH-DPAT	(+)5-HT$_{1A}$	[1 mg/kg, s.c.]	+33%	223
		8-OH-DPAT & propranolol	(−)5-HT$_1$/β-adren	[1 mg/kg, s.c.]&[5 mg/kg, i.p.]	0%	
		8-OH-DPAT	(+)5-HT$_{1A}$	[0.1 mg/kg, s.c.]	+44%	
	Drugs	8-OH-DPAT & propranolol	(−)5-HT$_1$/β-adren	[0.1 mg/kg, s.c.]&[5 mg/kg, i.p.]	0%	
Striatum Microdialysis in the rat striatum	Drugs	Tianeptine	(+)5-HT uptake	[10 mg/kg, i.p.]	0%	18
				[10 mg/kg, i.p.]	0%	
				[20 mg/kg, i.p.]	0%	
				[30 mg/kg, i.p.]	0%	
Microdialysis in the rat striatum Accumbens	Drugs	(−)Fenfluramine	(+)5-HT release	[15 mg/kg, i.p.]	0%	55
		(+)Fenfluramine	(+)5-HT release	[7.5 mg/kg, i.p.]	+52%	
		(−)Fenfluramine		[15 mg/kg, i.p.]	+23%	
		(+)Fenfluramine		[7.5 mg/kg, i.p.]	+27%	
Brainstem		(−)Fenfluramine		[15 mg/kg, i.p.]	0%	
		(+)Fenfluramine		[7.5 mg/kg, i.p.]	0%	

continued overleaf

Table 2. *(Continued)*

Experimental preparation	Stimulation	Drugs and properties		[Concentration or dose]	ACh release in % of baseline or ACh content vs control	References
Homogenates – from the rat striatum	Drugs	Quipazine	(+) 5-HT_2	[10 mg/kg, i.p.]	+11%	71
				[30 mg/kg, i.p.]	+45%	
		(response unchanged by 6-OHDA-induced depletion of DAergic innervation)				
Tissues punches in frozen slices from rat striatum	Drugs	Quipazine	(+)5-HT_2	[30 mg/kg, i.p.]	up to 150%	80
		(regional specificity of the response: only observed in areas receiving a rich serotonergic innervation)				
Microdialysis in the rat nucleus accumbens	Drugs	5-HT		[100] infused	−25%	179
				[500] infused	−30%	
		Fluoxetine	(−)5-HT uptake	[200] infused	−35%	
				[1000] infused	−40%	
		Propranolol	(−)5-HT_1/β-adren	[50] infused	+26%	
		Fluoxetine & propranolol		[200]&[50]	0%	
		Isoproterenol	(−)β-adren	[not indicated] infused	0%	
		8-OH-DPAT	(+)5-HT_{1A}	[0.1 g/kg, s.c.]	−20%	
				[1 g/kg, s.c.]	−40%	
				[300] infused	−30%	
		(fluoxetine, but not 8-OH-DPAT effects prevented by PCPA treatment)				
Post mortem determination of acetylcholine levels in the rat striatum	Drugs	Quipazine	(−)5-HT uptake	[10 mg/kg, i.p.]	+33%	214
		Quipazine & methergoline	(−)5-HT_1/5-HT_2	[10 mg/kg, i.p.] &[6 mg/kg, i.p.]	0%	
		(effect of quipazine alone attenuated but not abolished by pCPA treatment)				
		Quipazine & cinanserin	(−)5-HT_{1A}/5-HT_2	[10 mg/kg, i.p.] &[10 mg/kg, i.p.]	0%	
		d-Fenfluramine	(+)5-HT release	[7.5 mg/kg, i.p.]	+51%	
		(effect abolished by pCPA treatment)				
Other structures Microdialysis in the rat brainstem	Drugs	(−)Fenfluramine	(+)5-HT release	[15 mg/kg, i.p.]	0%	55
		(+)Fenfluramine	(+)5-HT release	[7.5 mg/kg, i.p.]	0%	
Post mortem determination of acetylcholine levels in the rat: brainstem telencephalon	Drugs	Quipazine	(−)5-HT uptake	[10 mg/kg, i.p.]	0%	214
		Quipazine		[10 mg/kg, i.p.]	0%	

(−): antagonist or inhibitor; (+): agonist or activator; [ ]: the concentrations indicated in the square brackets are given in μM, except where explicitely indicated; 0% does not necessarily indicate that there was no modification at all as compared to the baseline release, but that the observed modification did not reach statistical significance; &: combination of two or more substances; The pharmacological characteristics of the substances are those mentioned by the authors, not necessarily the ones recognized nowadays.

adren, adrenergic; n.i., information not indicated in the article; n.s., not selective; p., partial (agonist or antagonist). All other abbreviations are conventional ones referring to the substances

modifying serotonergic functions in the brain were found to alter the release of acetylcholine from rat hippocampus. For example, Consolo *et al.*[54] and Nilsson *et al.*[156] reported that serotonin release enhancers (e.g., fenfluramine, norfenfluramine or pCA) or uptake inhibitors (e.g., citalopram), injected i.p., more than doubled acetylcholine release (Table 2; see also Ref. 55). These observations are in line with earlier findings of Samanin *et al.*[214] who found quipazine to alter (but not necessarily to increase) the hippocampal content of acetylcholine.

Conversely, the systemic administration of a substance which increases the uptake of serotonin (i.e., tianeptine), thereby reducing the synaptic availability of serotonin, reduced the release of hippocampal acetylcholine.[18] However, when drugs acting directly on serotonergic receptors were infused into the hippocampus,[94] they did not necessarily activate cholinergic function as was found with systemic serotonergic treatments (see above), although Ohue *et al.*[164] reported intrahippocampal infusions of high concentrations (drugs infused through the dialysis probe) of serotonin to enhance the release of acetylcholine, an effect which was mimicked by 5-methoxy-dimethyltryptamine (5-MeO-DMT) (1 mM), a non-selective serotonergic agonist.

According to Izumi *et al.*[94] two types of receptors having opposite effects may be involved in the serotonin-induced modulation of cholinergic function, that is, 5-HT_{1A} receptors enhanced and 5-HT_{1B} receptors attenuated the release of acetylcholine. Both these effects were obtained with intrahippocampal infusions of the drugs, suggesting that these effects originated directly in the hippocampus. The 5-HT_{1B}-mediated effect is in line with *in vitro* findings (see previous section) and might imply a pre-junctional mechanism. An involvement of 5-HT_{1A} receptors in facilitation of hippocampal acetylcholine release has also been found in guinea-pigs[256] with the substances being administered systemically (8-OH-DPAT); this effect, however, was not observed when the drug was administered through the dialysis probe, suggesting that the 8-OH-DPAT-induced effect does not originate in the hippocampus. In this study, sumatriptan, a 5-HT_{1D} agonist, had no effect, an interesting observation since this receptor seems to be the inhibitory heteroreceptor on cholinergic terminals in the guinea-pig hippocampus (see previous section). Facilitation of hippocampal acetylcholine release has also been described as being mediated by 5-HT_3 receptors in, at least, the rat.[54] Indeed, systemic administration of 5-HT_3 agonists was found to enhance cholinergic function in the hippocampus and this effect was abolished by the co-administration of 5-HT_3 antagonists such as ondansetron or DAU 6215. Since all these drugs were administered i.p., s.c. or i.c.v., it cannot be concluded that this 5-HT_3-mediated effect has pharmacological substrates only in the hippocampus, even if 5-HT_3 receptors are present in the hippocampus.[260] Indeed, one possibility would be that activation of 5-HT_3 sites occurs elsewhere in the brain and, via one or more neurons interposed between the serotonergic terminals and the cholinergic targets, induces an increased efflux of acetylcholine. Another type of serotonergic receptors found in the hippocampus[260] is the 5-HT_4 receptor. As recently shown by Consolo *et al.*,[53] this receptor might also be involved in serotonergic modulation of hippocampal cholinergic function. However, this receptor, in contrast to the 5-HT_3 receptor, is inhibitory as regards the efflux of acetylcholine in the hippocampus.[53,54] This study, as with others based on systemic drug administration, does not allow us to conclude that the 5-HT_4-mediated inhibition has its substrate located only within the hippocampus.

2.4.3.2. Cortex. A serotonin-mediated influence on cholinergic function has also been found in both the rat and guinea-pig cortex. In the guinea-pig, using an epidural cup to collect acetylcholine, Bianchi *et al.*[20] have shown that i.c.v. administration of 5-HT induced an increase of acetylcholine release. This effect was abolished after 5,7-DHT-induced serotonergic depletion in the brain, but neither pCPA, nor systemic methiothepin affected this response; d-Norfenfluramine (i.p.) also had no effect.

Bianchi *et al.*,[22] using a microdialysis technique to collect acetylcholine, have shown that i.c.v. or s.c. injection of 8-OH-DPAT, a 5-HT_{1A} agonist, dose-dependently increased the cortical efflux of acetylcholine, whereas i.c.v. injection of 2-Me-5-HT, a 5-HT_3 agonist, decreased this release. Both these effects could be antagonized with drugs that block the 5-HT_3 receptor (see Table 2); ketanserin, a 5-HT_2 antagonist, had no effect. 5-HT_{1A} receptors also facilitated the release of cortical acetylcholine in rats,[223] although with a lower sensitivity than in the guinea-pig and with a method based on collection of epidurally applied Ringer solution. When less specific drugs were used, such as for instance a serotonin uptake activator administered systemically (e.g., tianeptine), Bertorelli *et al.*[18] found the cortical efflux of acetylcholine to be decreased in rats.

In the rat, activation of 5-HT receptors by i.c.v. administration of BIMU 1 or BIMU 8, two selective 5-HT_4 agonists, enhanced cortical release of acetylcholine, an effect which was fully abolished by co-administration of a selective 5-HT_4 antagonist.[53] Although 5-HT_4 receptors have been found in the rat cortex, the route of drug administration (i.c.v.) does not allow to conclude whether the substrates of this enhancing effect are located in the cortex or elsewhere in the brain. Nevertheless, it is noteworthy that activation of 5-HT_4 receptors enhances acetylcholine release in the cortex but decreases it in the hippocampus.

2.4.3.3. Striatum. Finally, a modulatory influence of serotonergic agents upon the cholinergic function has also been found in the striatum as demonstrated in the early reports by Butcher *et al.*,[40] Euvrard *et al.*,[71] Guyenet *et al.*[80] and Samanin *et al.*[214] Overall,

these pioneering findings suggested that serotonin was able to inhibit cholinergic function in the striatum. On rats submitted to *post mortem* determinations of striatal acetylcholine content, Samanin *et al.*[214] found that systemic treatment with quipazine, a 5-HT uptake inhibitor, or d-fenfluramine, a 5-HT release activator, both injected prior to being killed, resulted in increased acetylcholine concentrations in the striatum (+33% and +51%, respectively). This effect was completely blocked by pretreatment with mixed 5-HT_1/5-HT_2 antagonists (metergoline and cinanserin). Consolo *et al.*[55] have observed that systemic injections of fenfluramine induced an increased release of acetylcholine in the nucleus accumbens (but not in the brainstem). In the same report, these authors also showed that systemic fenfluramine increased striatal acetylcholine content, suggesting that the striatal cholinergic neurons are under inhibitory control of serotonergic neurons. Although Bertorelli *et al.*[18] could not influence the efflux of striatal acetylcholine with i.p. injections of tianeptine, a 5-HT uptake activator, Rada *et al.*[179] have shown that infusion of 5-HT as well as of fluoxetine, an inhibitor of 5-HT uptake, resulted in a lower acetylcholine efflux. Since this effect was prevented by propranolol, a mixed 5-HT_1/β-adrenergic antagonist, and mimicked by 8-OH-DPAT, a selective 5-HT_{1A} agonist, it is possible that the inhibitory effect of serotonin on cholinergic function in the striatum is mediated by a 5-HT_{1A} receptor.

2.5. *Summary and conclusions*

This series of results may be summarized as follows. With histological approaches, some regions of the brain were found to receive both a serotonergic and a cholinergic innervation. These regions include the striatum, the cortex and the hippocampus. Many studies have shown that an interaction between serotonergic and cholinergic processes in one or the other of these regions may result in physiological modifications that the manipulation of only one of these systems is unable to mimick qualitatively or quantitatively. The majority of the arguments demonstrating that an interaction between cholinergic and serotonergic processes have a functional relevance and were obtained with pharmacological approaches. These pharmacological studies showed that in rat hippocampus, acetylcholine release is inhibited by activation of intrinsic 5-HT_{1B} and perhaps 5-HT_3 receptors and, under the condition of a systemic activation, can be facilitated via 5-HT_{1A} as well as 5-HT_3 receptors, or inhibited via 5-HT_{1B} (5-HT_{1D} in the guinea-pig) and 5-HT_4 receptors. In the cortex, the release of acetylcholine may be locally controlled by 5-HT_3 inhibitory receptors, whereas systemic activation of 5-HT_{1A} and 5-HT_4 receptors results in facilitation. Finally, in the striatum, local inhibition may be mediated by 5-HT_1 and/or 5-HT_2 receptors and, under the condition of systemic activation, by 5-HT_{1A} receptors. These data are summarized in Table 3, which may be considered as a provisional state of knowledge about the neurobiological substrates that may be involved in the serotonergic control of cholinergic function in the cortex, the hippocampus and the striatum.

All these possible sites through which serotonin is able to influence cholinergic activity in the brain are potential substrates where an interaction between serotonergic and cholinergic neurobiological processes may potentially influence cognitive function, especially in terms of learning and memory. The next section will deal with the experimental arguments supporting the view that cognitive function does, in some respects, depend upon an interaction between serotonergic and cholinergic function. The non-cognitive implication of the central serotonergic systems (e.g., appetite, circadian rhythmicity, nociception, sleep–wakefulness, sexual behavior) are also well documented in the literature, but lay beyond the scope of the present review (information can be found in Refs 49, 58, 78, 105, 141, 149, 185, 232, 249).

3. COGNITIVE IMPLICATIONS

Many experiments have investigated the involvement of central cholinergic function in cognitive processes (e.g., reference list in Refs 52, 69, 221, 226). Similarly, many studies (some being over 30 years old) have also examined the potential involvement of serotonergic systems in learning, memory and, more generally, cognition (e.g., Refs 43, 101, 115, 123, 137, 163, 224, 243, 257, 258). However, apart from the

Table 3. Receptors involved and effect of serotonergic modulation of cholinergic function in the hippocampus, the cortex and the striatum

Methods	Brain region		
	Hippocampus	Cortex	Striatum
In vitro (presynaptic)	Inhibitory 5-HT_{1B}[a]/5-HT_3[b] 5-HT_2	Inhibitory 5-HT_3[c]	Inhibitory 5-HT_1/5-HT_2
In vivo (systemic)	Inhibitory 5-HT_{1B}/5-HT_4		Inhibitory 5-HT_{1A}
	Facilitatory 5-HT_{1A}/5-HT_3	Facilitatory 5-HT_{1A}/5-HT_4	

[a]5-HT_{1D} in the guinea-pig; [b]effect weak but significant in the rat; [c]controversed in rodents, demonstrated in humans.

contribution by Smith[227] (see below), the interaction between serotonergic and cholinergic systems has only been consistently considered for about 10 years as having a potential cognitive relevance and, consequently, investigated more extensively. These experiments have mostly used lesion, grafting and pharmacological approaches. One of the first reviews dealing with that topic was by Segal *et al.* in 1989,[217] but this book chapter was essentially restricted to the cognitive functions of the hippocampus. In addition, since 1989, many other experiments have been performed and these have also been reviewed in another very recent article.[229]

3.1. *Lesion approaches*

For many years, experiments in behavioral neurobiology have used lesion techniques that selectively damage well delineated regions of the brain (nuclei, pathways of target structures) in order to assess the behavioral correlates of this damage. An approach based on neurochemical selectivity of the lesions has become possible only with the emergence of a series of compounds with neurotoxic properties oriented towards neurochemically-defined populations of neurons. For instance, 6-hydroxydopamine (6-OHDA), a substance that selectively destroys catecholaminergic neurons, was discovered in the late 1960s[235] and was used extensively to study the functional involvement of central dopaminergic neurons and to set up a relevant animal model of Parkinson's disease. Subsequently, Baumgartner and collaborators (e.g., Refs 14, 15, 16) introduced 5,6-DHT and 5,7-DHT, two other substances that were able to damage the serotonergic neurons (when noradrenergic neurons are protected by desipramine). The latter compound, 5,7-DHT, which proved to be more useful, has been used extensively to selectively destroy serotonergic neurons in the brain and to investigate the functional correlates of such specific lesions. As regards the cholinergic system, AF64A has opened some promising perspectives, but the specificity of this substance has been firmly questioned (e.g., Refs 73, 82, 83, 127). Nevertheless, it seems that under conditions of appropriate dosage,[72] the toxic effects of AF64A might reach a satisfactory degree of specificity. Another compound has emerged recently which appears to be much more specific for cholinergic neurons in at least the forebrain. It is an immunotoxin oriented towards the nerve growth factor receptor, ^{192}IgG-saporin (e.g., Refs 17, 30, 87, 157). So far, however, and to our knowledge, no experiment has been published about the behavioral correlates of combined serotonergic and cholinergic lesions performed with 5,7-DHT and 192 IgG-saporin, respectively. Therefore, the present section will focus on a series of experiments that have combined classical lesion techniques (e.g., electrolysis, thermocoagulation or intracerebral injections of excitatory amino acids) disrupting the cholinergic fibers of the basal forebrain with serotonergic depletion techniques utilizing 5,7-DHT, pCPA or pCA.[127] The two latter compounds are administered systemically and induce dramatic depletions of central serotonergic function by inhibiting the synthesis of serotonin (pCPA) or by destroying the serotonergic terminals (pCA), while 5,7-DHT, which is administered into the cerebral ventricles or parenchyma, induces neuronal degeneration by acting on the neuronal cell bodies (e.g., Refs 127, 222). The serotonin depletion induced by pCPA and pCA is reversible. This may be a drawback in experiments assessing long term effects. Among other drawbacks of these compounds, it should be noted that pCPA may induce catecholaminergic alterations in most regions of the brain. Also, it does not affect all functional markers of the serotonergic innervation (i.e. 5-HT uptake sites are preserved[66]). Thus, when pCPA is used to damage serotonergic neurons, extreme caution must be taken in interpreting the physiological and/or behavioral effects found (are they really due to serotonergic lesions? What may be the contribution of non-specific toxicity?). Even if pCA is more selective than pCPA, it is noteworthy that its administration initially results in increased release of serotonin and not in serotonergic depletion (e.g., Ref. 108) Thus, appropriate post-administration delays must be used. Another possible drawback is that pCA seems to work only on mature serotonergic neurons.[108] Finally, whether or not pCA induces true nerve terminal degeneration is still subject to debate[127] (but see Ref. 66).

The various approaches combining different types of lesions are based on a principle that consists of comparing the cognitive effects of separate serotonergic or cholinergic depletion with the cognitive effects found when both these depletions are realized together. The underlying idea of these approaches is the following: if a serotonergic/cholinergic interaction has cognitive relevance, the combined depletions should induce behavioral effects that are different (generally more marked) from those induced by each depletion performed separately. The septohippocampal and the basalocortical cholinergic systems have been investigated with this rationale.

3.1.1. *The septohippocampal system.* Nilsson *et al.*[158] have compared some behavioral effects of cholinergic lesions (by radiofrequency) in the medial septum (note that septal lesions also damage fibers *en passage* such as, for instance, those from the cortex to the nucleus accumbens), serotonergic lesions performed with intraventricular injections of 5,7-DHT, and both types of lesions combined. To assess cognitive performance in their rats, Nilsson *et al.*[158] used the Morris water-maze according to a protocol assessing spatial reference memory. The authors found that, in rats with only 5,7-DHT lesions, the spatial learning abilities were not significantly altered, whilst rats with only septal lesions exhibited an impaired ability to find the platform. However, it is noteworthy that, in the rats given both lesions combined, the deficit was significantly larger than that found after

only septal lesions. Richter-Levin and Segal[190] reported data showing that neither a partial electrolytic lesion of the medial septum, nor an intraventricular injection of 5,7-DHT impaired water-maze performance, whether with a reference memory testing procedure or a working memory one. However, when both lesions were combined, both the reference and working memory performances were dramatically impaired. In contrast, in a passive-avoidance task, the rats treated with 5,7-DHT (whether alone or combined with septal lesions) showed better performance than intact control rats or rats given only a septal lesion. More recently, Murtha and Pappas[152] used *N*-methyl-D-aspartate microinjections to disrupt the cholinergic neurons in the medial septum and/or 5,7-DHT (infused in the fimbria–fornix and the cingulate bundle) to destroy the serotonergic innervation of the hippocampus. Their rats were tested in a water-maze and a radial-maze with testing procedures assessing working and reference memories. They found that in the water-maze reference memory test, only the combination of both lesions impaired performance. In the water-maze working memory test, an impairment was observed only in the group with combined lesions when a delay of one minute separated two consecutive trials. With a 10-min delay, a deficit also emerged in the rats with only septal lesions. As regards radial maze performance, all rats learned the reference memory task. However, during the first block of five trials, the rats with only septal lesions showed impaired performance when compared to the unlesioned rats, but those with combined lesions showed an impairment exceeding that of the septal rats. When tested for working memory, the rats with either combined or only septal lesions were impaired over 25 trials, but did not differ from one another. When testing was continued, however, the rats with only septal lesions came closer to the performance of the unlesioned controls, a trend that was not observed in the group with combined lesions.

3.1.2. *The basalocortical system.* Normile *et al.*[162] used rats that sustained ibotenate-induced lesions of the NBM alone or in combination with systemic administration of pCA. Subsequently, these authors assessed the acquisition of a complex spatial discrimination task in a Stone 14-unit T-maze. They found that the rats given pCA alone showed better performance than their unlesioned counterparts. Although NBM lesions had no effect on spatial learning, they prevented the pCA-induced improvement. Riekkinen *et al.*[201] have compared the effects of separate or combined lesions of the dorsal raphe and the NBM on acquisition of a water-maze task in rats. The authors report the dorsal raphe lesions to be ineffective, the NBM lesions to impair performance and both lesions combined to induce an impairment greater than that due to NBM lesions alone. Using passive-avoidance and water-maze tasks, Jäkälä *et al.*[100] have investigated the behavioral effects of either NBM lesions produced by quisqualic acid injections, inhibition of brain serotonin synthesis performed with systemic pCPA treatment, or both types of lesions combined. They found that NBM lesions severely impaired passive-avoidance retention and only slightly disturbed water-maze performance. PCPA alone had no effect in either of these tasks, but potentiated the effects of NBM lesions. Markowska and Wenk[130] also compared the behavioral effects of NBM lesions (injection of ibotenic acid) combined or not with serotonin depletion induced by systemic pCA treatment, but they used a nonspatial memory task (cued delayed non-matching to sample task) and paid attention to both the acute and long-term effects of the experimental treatments (see also Ref. 251). They found that serotonin depletion had no effect on behavioral performance, whether shortly (four weeks) after treatment or later on, when testing was prolonged. In contrast, NBM lesions produced an impairment in the non-spatial memory task. Combined pCA treatment and NBM lesions did not produce a larger deficit than NBM lesions alone. However, when testing was prolonged, rats with NBM lesions showed some improvement over time, whereas those given both NBM lesions and pCA treatment did not. Overall, the effects of NBM lesions combined with serotonin depletion on a delayed non-matching to sample task appear to be rather weak, a conclusion which is in line with another report by Sahgal and Keith[211] who also used a delayed non-matching to sample paradigm in rats given NBM lesions combined to 5,7-DHT-induced serotonin depletion.

3.1.3. *Conclusions.* Thus, this series of experiments suggests that the cognitive effects of cholinergic denervation of the hippocampus (septal lesions) or the neocortex (nucleus basalis lesions) can be exacerbated by concomitant serotonin depletion. Another constant finding in the aforementioned series of experiments is that serotonin depletion alone does not produce detrimental effects on spatial and non-spatial working or reference memory processes. When effects on memory are observed after serotonin depletion, these effects even consist of an improvement of mnesic performance in some tests (e.g., Refs 3, 162).

In the first part of this review, we have emphasized that serotonin could either inhibit (e.g., via presynaptic mechanisms involving heteroceptors) or facilitate (e.g., via polysynaptic loops with a postsynaptic start point) release of acetylcholine. Such mechanisms are present in both the hippocampus and the cortex (see 2.4.2.1. to 2.4.2.2.), although they do not necessarily involve the same types of pre- or postsynaptic receptors (Table 3). Whatever these mechanisms may be, all aforementioned experiments converge towards the conclusion that there may be a serotonergic modulation of cholinergic function implicated in spatial reference and working memory, as well as in non-spatial memory. This conclusion is further

supported by experiments using techniques consisting of grafting neuroanatomically and/or neurochemically defined populations of neurons into denervated structures of the brain. The next section will review some of the most significant contributions about intracerebral transplantation.

3.2. *Intracerebral grafting approaches*

Basically, intracerebral grafts are performed in order to provide an experimentally denervated brain structure with a new innervation originating from the grafted fetal neurons. Although the mechanisms by which such grafts exert functional effects may be multiple (e.g., Ref. 44), an appropriate selection of the anatomical origin of the fetal neurons may help to replace some of the neurochemically-defined afferents disrupted by the lesion. For instance, fimbria–fornix lesions remove a large part of the cholinergic, noradrenergic, GABAergic and serotonergic afferents of the hippocampus (e.g., Refs 45, 46, 75, 76, 102, 104, 112, 114), as well as hippocampal efferent pathways. Such lesions are considered as a possible animal model of AD (e.g., Refs 52, 69, 204). Concerning the "replacement" of the cholinergic afferents, the grafts are prepared from the region of the fetal brain including the medial septum and the DBB, the normal source of the cholinergic innervation of the hippocampus. As concerns the "replacement" of the serotonergic afferents, the grafts are prepared from the mesencephalic raphe, the normal source of the serotonergic innervation of the hippocampus (e.g., Refs 45, 46, 103, 155).

3.2.1. *Grafts rich in serotonergic and cholinergic neurons.* There are several articles based on a grafting approach which clearly suggest that a serotonergic/cholinergic interaction may have cognitive relevance, as was the case for the lesion approaches summarized above. For instance, in rats given lesions of the medial septum and intracerebroventricular injections of 5,7-DHT, Nilsson *et al.*[155] have studied the behavioral effects of septal grafts alone (rich in cholinergic neurons), raphe grafts alone (rich in serotonergic neurons), and of a combination of both types of grafts. All grafts were placed into the hippocampus and cognitive function was assessed in a Morris water-maze according to a reference memory testing protocol, Whereas neither type of single grafts produced beneficial effects on water-maze performance, the combined grafts improved water-maze performance, but only 10 months after grafting, Using a different lesion paradigm (i.e. electrolytic lesions of the fimbria and the dorsal fornix), we also found that combined septal and raphe grafts placed into the denervated hippocampus of rats were able to improve (even to normalize) water-maze probe trial performance (reference memory protocol), an effect that neither of the single grafts was able to produce. Such co-grafts, however, failed to improve spatial working memory assessed in a radial maze task.[45,103]

3.2.2. *Grafts rich in serotonergic neurons.* Richter-Levin *et al.*[186] used a lesion paradigm similar to that used by Nilsson *et al.*[155] but performed grafts of only mesencephalic raphe cells into the hippocampus or the entorhinal cortex. They found the raphe grafts to exert beneficial effects on water-maze performance (reference memory), but only when implanted within the hippocampus; when implanted into the entorhinal cortex, a region linked to the hippocampus via the perforant path, there was no beneficial effect of the grafts. Thus, in the latter experiment, a serotonergic reafferentation of the hippocampus deprived of its cholinergic and serotonergic afferents was sufficient to induce beneficial effects on spatial memory. In a recent experiment (unpublished observations) assessing the effects of fetal raphe neurons implanted into the dorsal hippocampus of rats with fimbria–fornix lesions, we found the grafted rats to show spatial working memory performances which, over 32 trials, had reached a level close to that found in the sham-operated rats (unpublished observations). In another experiment, Richter-Levin and Segal[189] have combined 5,7-DHT lesions with intrahippocampal grafts of mesencephalic raphe. They found that rats treated in this way performed similarly to the intact control rats in a water-maze task. However, systemic treatment with a subamnestic dose of atropine (20 mg/kg, i.p.) induced a clear-cut deficit in lesion-only rats, but no deficit was observed in either the intact control rats or in the rats with 5,7-DHT-induced lesions and intrahippocampal grafts. These observations were confirmed by another study of Richter-Levin and Segal.[190] Among other aspects, this latter study[190] differed from the aforementioned one[189] by the fact that the intrahippocampal raphe grafts performed in 5,7-DHT lesioned rats partially prevented a water-maze performance deficit elicited by systemic (i.p.) atropine treatment, but at a dose of 25 mg/kg.

3.2.3. *Conclusions.* Altogether, these data provide additional support to the view that serotonergic and cholinergic afferents to the hippocampus may interact and that this interaction has cognitive relevance. Actually, following a combined cholinergic and serotonergic denervation of the hippocampus, grafts providing the denervated hippocampus with new cholinergic and serotonergic innervations induce a better recovery than the grafts providing the hippocampus with only one or the other of these innervations. Under some circumstances, it seems that even the sole serotonergic reinnervation of the hippocampus may be sufficient to promote significant recovery of spatial reference as well as working memory performance (but see Ref. 103). Also, serotonin-depleted rats with a new graft-derived serotonergic innervation of the hippocampus are more resistant to the disruptive effects of an antimuscarinic drug than are their lesioned non-grafted counterparts.

However, while all these data demonstrate an implication of serotonergic and cholinergic processes

in cognitive function, and perhaps may fit with the idea of a serotonergic modulation of some cognitive abilities in which cholinergic mechanisms have been recognized to play an important role, they do not contribute to the pharmacological characterization of the substrates involved (e.g., where in the brain, which systems and which receptors?).

In the next part of this review, attention will be focused on experiments that contribute to such a characterization.

3.3. *Pharmacological approaches*

3.3.1. *Non-selective pharmacological approaches.*

3.3.1.1. Cholinergic action in 5-hydroxytryptamine-depleted animals. Vanderwolf, in one of the first such studies, evaluated both the passive-avoidance and water-maze learning performance in intact or pCPA-treated rats exposed to scopolamine (5 mg/kg, s.c.) or atropine (50 mg/kg, i.p.) during acquisition or retention trials.[236] Scopolamine disrupted acquisition more than retention, an effect mimicked by atropine. pCPA was ineffective, but scopolamine produced a larger deficit in pCPA-treated rats than in intact rats. In a recent book chapter, other contributions by Vanderwolf and colleagues have been summarized.[239] They all point to the conclusion that the cognitive effects of muscarinic blockade may be potentiated by serotonergic depletion. Similar findings were reported by Richter-Levin and Segal[188] who combined pCPA-induced 5-HT depletion and systemic atropine (20 mg/kg, i.p.) treatment before testing their rats in the water-maze task. They observed that this subthreshold dose of atropine disrupted spatial learning performance only in pCPA-treated rats. More recently, Matsuno *et al.*[132] have found that a pCA-induced impairment in passive-avoidance learning in mice could be partially reversed by the administration of tetrahydroaminoacridine (efficient dose: 10 mg/kg, p.o.) or physostigmine (efficient doses: 0.25 and 0.5 mg/kg, i.p.), two acetylcholinesterase inhibitors.

Riekkinen *et al.*[203] have tested intact and 5,7-DHT-treated rats for water-maze performance after scopolamine (0.8 mg/kg, i.p.) or pilocarpine (muscarinic agonist; 4 and 10 mg/kg, i.p.) treatment. While 5,7-DHT lesions produced no deficit, scopolamine severely impaired spatial learning in both intact and lesioned rats. In intact rats, the scopolamine-induced deficit was abolished by pilocarpine treatment at a dose of 4 mg/kg (i.p.), whereas in the lesioned rats, a dose of 10 mg/kg was necessary to counterbalance the scopolamine effect. Thus, a 5-HT depletion decreases the efficacy of pilocarpine to reverse a scopolamine-induced spatial reference memory deficit. In another study,[196] Riekkinen *et al.* have reported that scopolamine (0.8 mg/kg, i.p.) and alaproclate (7.5 and 20 mg/kg, i.p.) were able to impair water-maze navigation performance. Furthermore, the combined injections of scopolamine (0.8 mg/kg) and alaproclate (20 mg/kg) produced a greater impairment than after only scopolamine treatment. This effect could be partially reversed with administration of pilocarpine (6 mg/kg). A subamnestic dose of atropine has also been described to produce cognitive perturbations in rats given 5,7-DHT into the ventricles or directly into the fimbria–fornix, while unlesioned rats showed normal performance.[187,191] Riekkinen *et al.*[197] have also demonstrated that nicotinic receptors may be involved in the cholinergic-serotonergic regulation of cognitive function. Indeed, this group[197] has investigated the effects of mecamylamine (10 mg/kg, i.p.) in rats with massive serotonin depletion (pCPA) on passive-avoidance and water-maze performances. Neither performance was affected by pCPA treatment. Given alone in intact rats, mecamylamine delayed spatial learning in the water maze but did not affect passive avoidance. In pCPA-treated rats, however, mecamylamine induced a marked deficit in passive avoidance. Later on, Riekkinen *et al.*[193] reported a study in which they investigated the effects of mecamylamine (7.5 g/kg, i.p.) in pCPA-treated rats on working memory in a water-maze and on a passive-avoidance response. They confirmed that pCPA did not affect water-maze navigation or passive-avoidance performances and showed that mecamylamine impaired (slightly) working memory and (substantially) passive-avoidance performances in intact rats. This effect was counterbalanced or attenuated by nicotine treatment (0.3 mg/kg, i.p.). In pCPA-treated rats, the mecamylamine-induced impairment was more pronounced and the preventive effect of nicotine was less marked than in the intact rats. Riekkinen *et al.*[199] have reported medial septal lesions to impair performance in both a passive-avoidance and a water-maze learning task. These deficits were aggravated by a pCPA-induced serotonin depletion which, alone, did not affect learning. Pre-training trial injections of tetrahydroaminoacridine (THA; 1, 3 and 5 mg/kg, i.p.) or nicotine (0.03, 0.1 and 0.3 mg/kg, i.p.) attenuated the deficit of septal rats at the respective doses of 3 mg/kg (THA), 0.1 and 0.3 mg/kg (nicotine) in both tasks. In rats which had sustained both a septal lesion and a serotonin depletion, only THA was found to exert beneficial effects.

Using rats, Jäkälä *et al.*[99] have examined the effects of pCPA and/or scopolamine treatment on attentional processes measured in the five-choice serial reactional time test. This test measures an animal's ability to detect and respond to brief flashes of light presented in one of five food-rewarded locations. The authors found that scopolamine (0.2 mg/kg, i.p.) did not alter the discriminative accuracy (proportion of appropriate locations detected among all responses) in the rats which did not sustain pCPA-induced lesions. Under standard test conditions (intensity of visual stimulus and speed of presentation fixed), the discriminative accuracy of pCPA-treated rats tended to decrease. Addition of scopolamine treatment did not further impair pCPA-treated rats. Only when

stimulus intensity was reduced or speed of presentation increased did pCPA-treated rats present impaired accuracy. A weak behavioral effect of concurrent serotonergic and cholinergic manipulations has also been reported by Robinson *et al.*[208] in a water-maze learning task, thus on spatial reference memory processes. These authors induced a long-lasting serotonergic depletion by treating rats with high doses of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") and found serotonin-depleted rats to perform similarly to intact control rats. In addition, MDMA-treated rats showed a sensitivity to systemically administered atropine (50 mg/kg, i.p.) that did not differ from that of intact rats. The authors interpret these weak effects as being due to insufficient depletion of cortical serotonin (−73% in the neocortex, −32% in the caudate nucleus).

3.3.1.2. Serotonergic action in acetylcholine-depleted animals. Smith[227] has shown that lesions of both the medial and the lateral septum—the former being rich in cell bodies of cholinergic neurons—facilitated acquisition of a shuttle-box avoidance task, and that this facilitation could be reversed in rats given 5-hydroxytryptophan (105 mg/kg). Smith[227] interpreted this result as due to 5-hydroxytryptophan-induced restoration of the footshock sensitivity, a sensitivity which is decreased by the serotonergic depletion resulting from the lateral and medial septal lesions. However, an alternative interpretation might be that the observed effects in lesioned 5-hydroxytryptophan-treated rats are related to the restoration of some modulatory mechanisms involving both acetylcholine and serotonin.

In a book chapter, Vanderwolf and Penava[239] summarized the main results of the Vanderwolf paper,[236] but also an experiment in which rats were submitted to a swim-to-platform test after concurrent manipulations of the serotonergic and cholinergic systems with various types of serotonergic antagonists and scopolamine. A scopolamine dose of 5 mg/kg (s.c.) impaired performance. Ritanserin (0.1, 1, 10 mg/kg, i.p.), methysergide (5, 10, 20 mg/kg, i.p.) and pizotifen (10, 2 mg/kg, i.p.) failed to affect the severity of the scopolamine-induced deficit at the doses indicated. At 5 mg/kg, however, pizotifen potentiated the deleterious effect of scopolamine, but this potentiation was not verified at higher doses and, therefore, it seems difficult to consider it as a consistent effect.

Using a radial arm maze test (working memory protocol), Miura *et al.*[144] reported that FG-7080, a novel serotonin re-uptake inhibitor (3 mg/kg, p.o.), attenuated the deleterious effects of scopolamine (0.25 mg/kg, s.c.). Also, FG-7080 administered before an acquisition trial was able to attenuate the scopolamine-induced deficit in acquisition of a passive-avoidance response.

3.3.1.3. Other approaches. An interesting study has been reported by Pavone *et al.*[169] Using a one-trial passive-avoidance test, these authors have investigated the mnemonic effects of a post-trial administration of oxotremorine (a muscarinic agonist: 0.005–0.04 mg/kg, i.p.), 5-MeO-DMT (a non-specific serotonergic agonist: 0.5–2 mg/kg, i.p.) or both drugs given together in two strains of mice showing a different sensitivity to cholinergic stimulation (DBA/2 and C57BL/6 mice). Oxotremorine improved retention performance, whereas 5-MeO-DMT impaired it in both strains. In addition, 5-MeO-DMT was able to inhibit the oxotremorine-induced improvement of performance. Altman *et al.*[4] reported that alaproclate, a serotonin uptake inhibitor, was able to potentiate the facilitatory effects of oxotremorine on passive-avoidance performance. In an earlier experiment, Wallis *et al.*[247] have compared the behavioral effects of systemic treatment with the serotonergic antagonist cyproheptadine (1 and 5 mg/kg, s.c.) and the serotonergic agonist (mCPP, 1 and 3 mg, s.c.) in two lines of Sprague–Dawley rats differing in their sensitivity to anticholinesterase treatment. Whilst cyproheptadine had no significant effect on water-rewarded operant responding, mCPP produced a dose-dependent decrease in responding. Interestingly, the latter effect was more pronounced in the line of rats showing the highest sensitivity to anticholinesterase treatment, a finding suggesting that the cholinergic and the serotonergic systems may interact in regulating some behavioral functions and that the level of regulations may, in some respects, be dependent upon genetically controlled factors.

3.3.1.4. Conclusions. The experiments summarized in this section indicate that a serotonergic depletion may decrease the efficacy of pharmacological agents enhancing cholinergic neurotransmission in reversing the cognitive consequences of systemic cholinergic blockade. They also suggest that a serotonergic depletion may exacerbate the sensitivity of animals towards the cognitive consequences of anticholinergic drugs given systemically.

3.3.2. *Selective pharmacological approaches.* Using selective pharmacological approaches, essentially three types of serotonergic receptors have been investigated as being potentially involved in a serotonergic modulation of cognitive function sensitive to cholinergic depletions. These receptors are of the 5-HT_{1A}, 5-HT_2 and 5-HT_3 types. The studies have been carried out most often on intact or serotonin-depleted rats, and only a few studies were performed on rats with lesions that disrupted one or the other component of the central cholinergic system. A few studies also considered other 5-HT receptors, such as, for instance, the 5-HT_{1B} receptors.

3.3.2.1. 5-Hydroxytryptamine$_{1A}$ receptors. Riekkinen *et al.*[205] have investigated the effects on water-maze performance of 8-OH-DPAT (30 μg/kg, i.p.), a 5-HT_{1A} receptor agonist, and mecamylamine (2.5 g/kg, i.p.), both these drugs being given either 40 min before a training trial or immediately after a training trial in intact and serotonin-depleted rats

(pCPA). In intact rats, the combined injection of 8-OH-DPAT and mecamylamine impaired only working memory performance when the injection was made before training (post-training injections had no effect). In pCPA-treated rats, the drug-induced impairment was more pronounced than in intact rats. However, it is noteworthy that none of these effects was observed in well trained rats. Lee *et al.*[117] have shown that when it is infused directly after training into the lateral septum, 8-OH-DPAT (5 μg) is able to impair memory retrieval in a passive-avoidance task, whereas the infusion of fluoxetine or zimelidine, two inhibitors of 5-HT re-uptake, has beneficial effects upon memory. Riekkinen[195] has found that both in scopolamine-treated rats and in rats with lesions of the medial septum, an injection of 8-OH-DPAT aggravated the effects of the lesion- or drug-induced cholinergic dysfunction on acquisition (generally assessed with a protocol using a pretraining injection) and consolidation (post-training injection) of a passive-avoidance response. Using an operant delayed matching-to-position task in order to measure short-term working memory performance in rats, Cole *et al.*[51] reported that 8-OH-DPAT and ipsapirone improved the matching accuracy at the respective doses of 0.1 mg/kg and 3 mg/kg (delay: 30 s). Conversely, NAN 190, a 5-HT_{1A} antagonist, impaired performance at the dose of 4 mg/kg. Scopolamine (0.14 mg/kg) induced a delay-dependent impairment which was dose-dependently attenuated (0.1 and 0.3 mg/kg) or aggravated (1 mg/kg) by 8-OH-DPAT. NAN 190 only tended to potentiate the deficit resulting from scopolamine treatment.

Altogether, these data indicate that the activation of central 5-HT_{1A} receptors may exacerbate memory deficits due to drug-induced or lesion-induced cholinergic dysfunctions. The 5-HT_{1A} receptors involved in these deleterious effects on memory are probably post-junctional ones, even though low doses of systemically-given agonists primarily activate somatodentritic autoreceptors such as the ones described on the serotonergic cell bodies in the raphe. Also, there is some recent evidence showing that part of the deleterious effects of 8-OH-DPAT might be mediated by 5-HT_{1A} receptors located in the CA1 region of the hippocampus[42] and that their activation alters spatial discrimination without affecting visual discrimination capabilities.

3.3.2.2. 5-Hydroxytryptamine$_2$ receptors. Sakurai and Wenk[213] trained rats in an operant chamber according to a continuous non-matching-to-sample non-spatial task and investigated the effects of scopolamine (0.1, 0.2 and 0.3 mg/kg, i.p.), ketanserin (14 mg/kg, i.p.) methysergide (15 and 20 mg/kg, i.p.), as well as of scopolamine (0.2 mg/kg, i.p.) combined with methysergide (15 mg/kg, i.p.) treatments. Methysergide and ketanserin block 5-HT_2 receptors. They found scopolamine to impair performance, whereas ketanserin and methysergide had no effect. When given together, scopolamine and methysergide induced a deficit that was larger than after only scopolamine treatment (although the difference was not significant). Using an approach consisting of infusing ketanserin (0.3 or 0.5 μg) or ritanserin (0.3 and 0.6 μg), another 5-HT_2 antagonist, into the lateral septum of rats immediately after training in a passive-avoidance task, Lee *et al.*[117] found both treatments to have no effect on memory. In rats submitted to pCPA treatment, scopolamine (0.1 mg/kg) surprisingly produced a deficit that was smaller than in intact rats. This difference, nevertheless, was no longer observed at a dosage of 0.3 mg/kg. The authors interpret this paradoxical protective effect of pCPA-induced lesions on the deleterious effects of scopolamine as subsequent to an attenuation of the acetylcholine release inhibition due to serotonin depletion. Riekkinen *et al.*[194] reported data suggesting that 5-HT_2 receptors may also be involved in the cognitive effects of concurrent blockade of cholinergic and serotonergic mechanisms. These authors investigated the effects on water-maze performance of scopolamine (0.8 mg/kg, i.p.), mecamylamine (10 mg/kg, i.p.) and methysergide (2.5, 7.5 and 20 mg/kg, i.p.) with the appropriate control injections. They found that scopolamine and mecamylamine impaired water-maze performance. The combined scopolamine and methysergide (7.5 and 20 mg/kg) treatments induced a deficit that was larger than after only scopolamine treatment. However, methysergide did not exacerbate the deficit in mecamylamine-treated rats. Using a passive-avoidance test in mice, Matsuno *et al.*[132] have shown that post-training administration of ritanserin (dose range: 2.5–40 mg/kg, s.c.) or mianserin (dose range: 5–20 mg/kg, s.c.) had no effect on the passive-avoidance retention deficit induced by scopolamine treatment (0.75 mg/kg, i.p.). In another study, Riekkinen[195] reports DOI, a 5-HT_2 agonist, to impair passive-avoidance acquisition (pre-training injection) and consolidation (post-training injection), the latter effect being prevented by ketanserin. However, the co-injection of DOI and a subthreshold dose of scopolamine had no effect. Normile and Altman[161] have investigated the effects of ketanserin in passive-avoidance retention performance of both intact and NBM lesioned rats. They have found the post-training administration of ketanserin (10 mg/kg, i.p.) to facilitate retention in intact rats, but not in lesioned rats, even at a dose of 30 mg/kg. Using aged rats, Normile *et al.*[160] investigated the effects on step-through latency in a passive-avoidance task of physostigmine (dose range: 0.01–10 μg/kg, i.p.), ketanserin (1 mg/kg, i.p.), or both treatments combined. The injections were made immediately after training. While physostigmine improved performance at a dose of at least 0.1 μg/kg, a hundredfold lower dose was sufficient to induce similar beneficial effects when ketanserin was co-injected. In aged rats, cognitive deficits have also been attributed to a combined loss of cholinergic and serotonergic functions (e.g., Ref. 192).

Altogether, these results seem to suggest that the blockade of central 5-HT_2 receptors may have some beneficial cognitive effects, whereas their activation may rather impair cognitive function. However, the possibility that these effects may be mediated, under some conditions and in specific tasks, by a serotonergically determined modification of central cholinergic function does not seem to be as well established for 5-HT_2 receptors as for 5-HT_{1A} receptors (see conclusions below). Additionally, in the studies using ketanserin, a substance that also has a nanomolar affinity for α_1 adrenergic receptors and H_1 histaminergic receptors, it is impossible to completely exclude that the observed effects were mediated over nonserotonergic mechanisms.

3.3.2.3. 5-Hydroxytryptamine$_3$ receptors. Brambilla *et al.*[37] have examined the effects of DAU 6215 (for general pharmacology, see Ref. 206), a selective 5-HT_3 antagonist on passive-avoidance performance in rats that were given scopolamine (0.75 mg/kg, i.p.). Scopolamine alone disrupted the acquisition of a one-trial step-through passive-avoidance response, an effect which was counterbalanced according to a bell-shaped dose–response curve by DAU 6215. Using another 5-HT_3 antagonist in mice, Chugh *et al.*[50] reported that systemic post-training (one-day delay) treatment with tropisetron (previously called ICS 205930; 1–100 μg/kg, i.p.) is not only able to enhance the step through latencies in a passive-avoidance task, but also that, at the dose of 10 μg/kg, it may attenuate the disruptive effects of scopolamine (3 mg/kg, i.p.) on memory retrieval, Costall *et al.*[57] have summarized a series of experiments in which scopolamine-treated adult mice, adult mice with NBM lesions and aged mice which sustained neither lesions nor scopolamine injections, were tested for habituation in a black and white test box system and for working memory in a food-rewarded T-maze alternation task. In both tests, the scopolamine-treated, the lesioned and the aged mice showed comparable deficits. These deficits could be attenuated by ondansetron (10–100 ng/kg, i.p.), zacopride (10–100 ng/kg, i.p.) and tropisetron (10–1000 ng/kg, i.p.), three 5-HT_3 antagonists. In adult scopolamine-treated mice tested for reference memory in a water-maze task, the three 5-HT_3 antagonists were again able to reduce the scopolamine-induced deficit. Also in a water maze used to assess spatial reference memory in rats, Pitsikas *et al.*[175] have found DAU 6215 (10 and 30 μg/kg, i.p.) to attenuate a scopolamine-induced learning deficit (0.2 mg/kg, s.c.), an effect which was also found by Pitsikas and Algeri[174] when oxiracetam was associated with scopolamine treatment. Beneficial effects of 5-HT_3 antagonists were also found in marmosets exposed to scopolamine.[11,41,67] However, 5-HT_3 antagonists were not always found to attenuate the deleterious consequences of cholinergic dysfunctions. For instance, using a five-choice serial reaction time task, Muir *et al.*[150] assessed visual attentional function in NBM-lesioned rats that were exposed to several drug treatments including phsysotigmine, nicotine and ondansetron. These authors reported that the lesion-induced deficit could be attenuated by physostigmine and nicotine, but not by ondansetron (0.3 and 10 ng/kg).

3.3.2.4. Other 5-hydroxytryptamine receptors. Noda *et al.*[159] investigated the role of cholinergic mechanisms in the behavioral effects of a 5-HT_{1B} agonist, RU-24969. They found RU-24969 (2 and 5 mg/kg, i.p.) to induce hyperlocomotion and to reduce spontaneous alternation in a T-maze, both effects being also observed after scopolamine treatment (0.5–2 mg/kg, i.p.) and being counterbalanced by propranolol (5-HT_{1A}/5-HT_{1B} agonist). The authors interpret the behavioral effects of RU-24969 as consecutive to 5-HT_{1B}-induced inhibition of acetylcholine release in brain regions crucial for mnesic processes. The 5-HT_4 receptors (e.g., Ref. 24) have also been shown to modulate cholinergic function in the brain and to have electrophysiological effects which can be blocked by scopolamine (e.g., Ref. 25). However, to our knowledge, no study has been published so far on the cognitive effects of either a selective activation (e.g., BIMU 1 and BIMU 8 are 5-HT_4 agonists) or a selective blockade (e.g., SDZ 205557, DAU 6215 and DAU 6285 are 5-HT_4 antagonists) of this receptor in animals with drug-induced or lesion-induced cholinergic depletions.

3.4. *Summary and conclusions*

The acute (systemic drug treatments), semichronic (pCA and pCPA) or chronic (5,7-DHT) disruption of the central serotonergic function is able to potentiate the cognitive effects of a drug-induced or a lesion-induced central cholinergic dysfunctioning in, at least, the septohippocampal and the basalocortical systems (for summary, see Table 4). In most experiments, the serotonin depletion alone is not sufficient to produce deleterious effects on learning or memory processes.

As regards the septohippocampal system, it was found that following a multitransmitter hippocampal denervation, combined cholinergic and serotonergic grafts produced a level of functional (cognitive) recovery that neither single graft was able to induce. All these data point to the conclusion that interactions between the central serotonergic and cholinergic systems are relevant to cognition. Concurrent manipulations of both systems with drugs specific for given receptor subtypes allowed to setup a better characterization of the pharmacological substrates which may be involved in the cholinergic/serotonergic interactions. In rats with drug-induced cholinergic dysfunctions, the activation of 5-HT_{1A} or 5-HT_{1B} receptors as well as the inhibition of 5-HT_2 receptors was found to exacerbate the deficit due to central muscarinic blockade or to cholinergic lesions.

Conversely, the blockade of 5-HT_3 receptors was

Table 4. Synthetic outline of the main cognitive effects of concurrent manipulations of the serotonergic and cholinergic systems in the rat brain

Cognitive deficit due to	Deficit aggravated by[a]	Deficit attenuated by	Task[Ref.]
Medial septal lesions	5,7-DHT (i.c.v.)	Intrahippocampal co-grafts of raphe and septal cell suspensions	MWM[155,158]
Medial septal lesions	5,7-DHT (i.c.v.)	Intrahippocampal grafts of a raphe cell suspension	MWM[186]
Medial septum lesions	8-OH-DPAT (syst.)		PA[195]
Medial septum lesions	pCPA (syst.)	THA (syst.)	PA,MWM[199]
Fimbria–fornix lesions		Intrahippocampal co-grafts of raphe and septal cell suspensions	MWM[103]
Nucleus basalis lesions	Dorsal raphe lesions		MWM[201]
Nucleus basalis lesions	pCPA (syst.)		PA[100]
Nucleus basalis lesions	pCA (syst.)[b]		CDNMST[130]
Nucleus basalis lesions		Ondansetron (syst.) Zacropide (syst.) Tropisetron (syst.)	BWTB[57]
pCA treatment (syst.)		THA and physostigmine (syst.)	PA[132]
pCPA (syst.) +mecamylamine (syst.)		Nicotine (syst.)	PA[193]
Mecamylamine (syst.)			MWM[197]
8-OH-DPAT (syst.) +mecamylamine (syst.)	pCPA (syst.)		MWM[205]
Scopolamine (syst.)	5,7-DHT (i.c.v.)	Pilocarpine (syst.)	MWM[203]
Scopolamine (syst.)	pCPA (syst.)		PA,MWM[236]
Scopolamine (syst.)	pizotifen (syst.)[c]		StP[239]
Scopolamine (syst.)		FG-7080 (syst.)	RAM,PA[144]
Scopolamine (syst.)	8-OH-DPAT (syst.)		PA[195]
Scopolamine (syst.)	8-OH-DPAT (syst.)[d]	8-OH-DPAT (syst.)[e]	ODMTPT[51]
Scopolamine (syst.)	Methysergide (syst.)		CNMTST[213]
Scopolamine (syst.)	Methysergide (syst.)		MWM[194]
Scopolamine (syst.)		DAU 6215 (syst.)	PA[37]
Scopolamine (syst.)		Tropisetron (syst.)	PA[50]
Scapolamine (syst.)	Alaproclate (syst.)	Pilocarpine (syst.)[f]	MWM[196]
Scopolamine (syst.)		Ondansetron (syst.) Zacropide (syst.) Tropisetron (syst.)	BWTB[57]
Scopolamine (syst.)		DAU 6215 (syst.)	MWM[175]
Scopolamine (syst) +RU-24969 (syst.)		Propranolol (syst.)	SA[159]

For doses and other technical details, see text. BWTB, black and white testbox; CDNMST, cued delayed non-matching-to sample task; CNMTST, continuous non-matching-to-sample task; MWM, Morris water-maze; ODMTPT, operant delayed matching-to-position task; PA, passive avoidance; SA, spontaneous alternation; StP, swim-to-platform task; syst., treatment administered systemically.

[a]Neither of these treatments has perturbating effects upon cognition when used alone; [b]only slows down recovery from nucleus basalis lesions; [c]only at an intermediate dose of 5 mg/kg; [d]at the doses of 0.1 and 0.3 mg/kg; [e]at the dose of 1 mg/kg; [f]pilocarpine reduced the deficit resulting from combined scopolamine and alaproclate treatments.

found to attenuate the cognitive deficits due to central cholinergic disruption.

In vivo and *in vitro*, 5-HT_{1B} agonists attenuate the release of acetylcholine in at least the hippocampus (see sections 2.4.1. and 2.4.2.). Thus, the cognitive perturbations induced by such agonists might, in some respects, be explained by their inhibitory effects on central cholinergic function. *In vivo*, a systemic administration of 5-HT_{1A} or 5-HT_3 agonists exerts facilitatory effects on acetylcholine release in the hippocampus, but neither of these agonists is able to enhance cognitive function in pharmacological or surgical paradigms of cholinergic dysfunction. Also 5-HT_2 antagonists have facilitatory effects towards acetylcholine release in the hippocampus but, in acetylcholine-depleted animals, a systemic administration of such antagonists worsens the cognitive perturbations due to cholinergic depletion. All these findings appear to contradict the predictions that the *in vitro* and/or *in vivo* pharmacological experiments would lead us to make: a substance having facilitatory effects on acetylcholine release should also enhance cognitive functions. Thus, as regards the cognitive alterations which, in acetylcholine-depleted animals, are induced by agonists of 5-HT_{1A} or 5-HT_3 receptors, or antagonists of 5-HT_2 receptors, it seems justified to consider that the drug-induced cognitive alterations cannot be reduced to a simple and direct modulatory influence that the drugs might exert on central cholinergic function. These apparent contradictions between the expectations founded on *in vivo* and *in vitro* pharmacological approaches and the behavioral observations from psychopharmacological experiments strongly suggest that the interaction between serotonergic and cholinergic systems in the brain is very complicate. In addition to the effects of

serotonin on cholinergic function, one has to consider the possibility that serotonin has its own cognitive actions, for instance in modulating functions that may be essential for mnesic processes to occur efficiently (e.g., anxiety, arousal, attention; for a review, see Ref. 224).

Finally, we feel it necessary to particularly stress the importance of more methodological aspects. In the experiments which used serotonin-depleted animals exposed to cholinergic blockade in order to investigate the effects of additional drug treatments (nicotinic and muscarinic agonists or antagonist and drugs selective for different 5-HT receptor subtypes), there is a point which needs to be emphasized and which also applies to some experiments summarized in Section 3.1. In the studies using scopolamine as the agent impairing task performance, the doses ranged from 0.2 to 5 mg/kg. This large range might account for some inconsistencies in the ability of other drugs to reverse or to potentiate the deleterious effects of scopolamine on cognition. One possibility is that when scopolamine is used at a dose inducing submaximal cognitive impairments, concomitant manipulations of the serotonergic system may exacerbate or attenuate a cognitive perturbation. Conversely, when scopolamine is used at doses producing maximal disturbances (e.g., 5 mg/kg), there is no possibility to further exacerbate the deficit by other drugs and the possibility of observing drug-reversal effects might be smaller than in the case of doses inducing submaximal effects. Another point is that in the studies using scopolamine, the scopolamine-induced effects on cognition were interpreted as resulting directly from cholinergic blockade. Thus, as scopolamine-induced deficits may be reversed or attenuated by a number of non-cholinergic agents (e.g., Ref. 64) it may also be questioned whether the cognitive effects of concurrent cholinergic and serotonergic manipulations do not reflect alterations of other neurotransmitter systems. Future experiments should help to resolve these problems, as well as others related to the role of serotonergic/cholinergic interactions in cognition. Since a large majority of the aforementioned studies have used the passive avoidance or the water-maze tasks to evaluate the mnesic impact of concurrent serotonergic and cholinergic dysfunctions, additional information should be gained from experiments using other mnesic tasks. Also, most experiments have been carried out to investigate the cognitive effects of one specific agonist or antagonist in animals with perturbations of central cholinergic function induced by anticholinergic drug treatments or by lesions. A more systematic exploration might be based on protocols which would not only consider the effects of either an agonist or an antagonist of serotonin receptors, but also compare the effects of both types of treatments in the same animals or pay attention to serotonergic receptors which have not yet been taken into account (e.g., 5-HT_4). Another crucial point that clearly deserves further investigations is the identification of the brain locus or system (the neuroanatomical substrate) in which the functional effects of a serotonergic/cholinergic interaction are determined. Regarding the latter point, lesion approaches combined to systemic drug injections, or even local intracerebral drug infusion techniques, represent, in addition to molecular biology approaches, two possibilities that should allow further progress in the near future. It might also be helpful to direct experiments at determining the neurotransmitters involved in the different 'relays' of the polysynaptic loops in which a modification of the serotonergic input may modify the activity of the cholinergic system. Finally, additional experiments should also try to better characterize the type of mnesic process that is sensitive to concurrent serotonergic/cholinergic manipulations. Do such manipulations alter the ability of the animals to pick up information necessary to solve the task in their environment (by changing, for instance, the signal/noise ratio), do they perturbate the consolidation processes (of either or both the target stimulus or the context), do they disturb the retrieval of acquired information or do they impair the ability to appropriately use the mnesic trace?

4. GENERAL CONCLUSIONS

The present review has tried to set up an outline of histological, electrophysiological, pharmacological and behavioral data suggesting that serotonin is able to modulate central cholinergic function and that this modulation may have, in some respects, cognitive implications, particularly as regards learning and memory processes.

To conclude with some caution, a few additional qualifying remarks are necessary. *In vitro* findings show that an application of serotonin as well as that of 5-HT agonists or antagonists to stimulated slices from given brain regions may alter the evoked release of acetylcholine. However, these findings do not necessarily demonstrate that the serotonergic modulation is directly occurring on the cholinergic terminal by means of 5-HT heteroceptors. During an electrically evoked or a potassium-evoked stimulation, all terminals present in the experimental material are stimulated and release part of their neurotransmitter content. Therefore, it cannot be excluded that a serotonin-induced attenuation of acetylcholine release is mediated by a decreased or an increased release of another transmitter (different from serotonin) to which cholinergic terminals would be sensitive without being sensitive to 5-HT. This problem, however, can be circumvented in preparations using synaptosomes. *In vivo*, the problem gets even more complicated as the serotonergic influence on cholinergic function, especially when drug treatments are administered systemically, may involve complex polysynaptic loops, several brain regions and various neurotransmitter systems. Thus, although a change in the serotonergic input may result in a modification of

the cholinergic output, the number of intermediate events and their neurochemical identity are almost always unknown. Here, lesion techniques may be useful tools to explore the structural organization and the functional substrates of such loops. Finally, at the most integrated level of organization, namely that of the organism, the degree of complexity reaches a maximum. At this level, the functional consequences of an interaction do not necessarily suppose two neurotransmitter systems to cooperate or interact directly (e.g., serotonin activating heteroceptors of the cholinergic terminal). The functional consequences may also be determined by the cooperation between various functions working in parallel, each of which being considered as resulting from interactions involving various transmitter systems in the brain. Actually, that serotonin may modulate cognitive functions by a more or less direct influence on cholinergic mechanisms in the brain is a concept that has a heuristic value equivalent to considering that serotonin produces a functional change at the level of the organism (e.g., on attentional processes, on motricity, or on arousal) that, in turn, would allow an enhanced efficiency of the cholinergic contribution to another function of the organism (e.g., learning).

In any case, the literature contains a series of experimental arguments finding their roots in histological, electrophysiological, pharmacological and behavioral research fields and demonstrating or suggesting that, in the mammalian brain, cholinergic function may be under serotonergic modulatory influence and that this modulation may have cognitive implications. However, these arguments should not be regarded as an invitation to share the idea that cognitive processes which involve cholinergic function may be simply explained by additional consideration of serotonergic modulatory mechanisms. Indeed, while the purpose of this review was to provide an overview of the serotonergic modulation of cholinergic and cognitive functions, it must be emphasized that interactions involving neurotransmitter systems other than the serotonergic one have also been dealt with in neuroscience. Each of these interactions is nowadays sufficiently documented and might become the subject of reviews. To briefly mention a few of them, research during the last twenty years has demonstrated the cognitive relevance of cholinergic/noradrenergic interactions,[61,62,63,64,85,98,202,204,212] cholinergic/glutamatergic interactions,[98] GABAergic/cholinergic ones[215] and other ones involving dopamine,[120] opiates (endorphins, enkephalins) or substance P.[64]

Also, as regards the topic of this review, we have deliberately chosen to concentrate exclusively on one side of the cholinergic/serotonergic interaction, namely that serotonin may modulate cholinergic processes in the brain. There is another viewpoint in which acetylcholine is considered as a neurotransmitter that may modulate serotonergic processes. Although this aspect is less well documented than the serotonergic modulation of cholinergic function, there are some recent findings suggesting that, under some conditions, a modification of cholinergic function in the brain may have important consequential effects upon serotonergic function (e.g., Refs 19, 184). Finally, another aspect that was not considered in this review, especially as concerns the interpretation of the concurrent serotonergic/cholinergic lesion-induced dysfunctions followed by systemic treatment with serotonergic drugs, is that serotonin may exert neurotrophic effects on cholinergic neurons mediated by at least 5-HT_{1A} receptors (e.g., Refs 183, 254). Thus, the possibility arises that the cognitive effects attributed to acute drug-induced functional changes also involve structural modifications induced by neurotrophic effects of serotonergic drugs, an interpretation that might apply to the observation reported by Markowska and Wenk[130] in rats with lesions of the NBM (see Section 3.1.2.).

In his introduction to the book edited by Levin, Decker and Butcher,[119] Russell quotes the following sentences written by Moore and Mahler in their 'Introduction to Molecular Psychology':[145] "*We are today close to a critical point in history at which the mind of man by taking thought (and making experiments) will be able to understand the chemical and physical mechanisms responsible for thought itself. Just as molecular biology has provided a chemical basis for heredity and evolution, molecular psychology will elucidate the chemistry of memory, learning, sensation, emotion and finally human consciousness*". It is tempting to add that 30 years later, we are merely coming closer to this critical point without finding a way to reach it. We approach it like an asymptotic function approaches a straight line. Nevertheless, along this path, new (yet experimental) horizons have been opened in terms of treating cognitive correlates of a neurodegenerative disease such as AD. Evidence has emerged suggesting that, in patients with AD, the symptomatic treatment of cognitive dysfunctions should orient towards pharmacological tools recognizing that the disease is a multitransmitter system disease, and not a disease that mainly affects the forebrain cholinergic system (e.g., Refs 5, 13, 79, 86, 89, 129, 168). In that concern, one should question whether paradigms of selective cholinergic lesions (e.g., Refs 17, 30, 87, 157) are really more appropriate animal models of AD than lesion or pharmacological paradigms producing simultaneous alterations in several neurotransmitter systems. Several authors have considered such an approach. Beside Ingram *et al.*[93] who addressed the therapeutical value of combined glutamatergic (*N*-methyl-D-aspartate type) and cholinergic treatments, of more relevance to the present review is the idea raised by Bowen's group[33,74] regarding the multiple neurotransmitter deficits found in the cortex of patients with AD, the possible cognitive implications of these deficits and the novel therapeutical perspectives which ensue therefrom. The authors reviewed a series of arguments

suggesting that in the cortex of AD patients, one major chemoanatomical change beside the cholinergic depletion is the degeneration of cortical glutamatergic, presumably pyramidal, neurons. Conversely, the cortical serotonergic innervation seems to be relatively preserved. Furthermore, when observed, the loss of serotonergic terminals can be compensated for by surviving terminals which increase the 5-HT turnover. It is known that cortical pyramidal neurons are under a hyperpolarizing influence of serotonin that probably involves 5-HT_{1A} receptors. The degeneration of these pyramidal neurons may result in a reduced excitatory glutamatergic cortical input, a reduction which may account for cognitive alterations as can be extrapolated from animal studies (e.g., Ref. 93). The glutamatergic cortical tone may be further reduced subsequently to the degeneration of the basalocortical neurons exerting an excitatory cholinergic modulation, whilst the inhibitory tone exerted by the preserved GABAergic neurons is maintained. Therefore, Francis *et al.*[74] have proposed to consider the use of drugs that would reduce the serotonergic tone (e.g., 5-HT_{1A} or 5-HT_3 antagonists) as an interesting perspective of transmitter-based therapies for the cognitive deficiencies in sufferers of AD.

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REFERENCES

1. Aghajanian G. K., Sprouse J. S., Sheldon P. and Rasmussen K. (1990) Electrophysiology of the central serotonin system: receptor subtypes and transducer mechanisms. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp.93–103. New York.
2. Alonso R. and Soubrié P. (1991) Effects of serotonergic denervation on the density and plasticity of brain muscarinic receptors in the rat. *Synapse* **8,** 30–37.
3. Altman H. J., Normile H. J., Galloway M. P., Ramirez A. and Azmitia E. C. (1990) Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. *Brain Res.* **518,** 61–66.
4. Altman H. J., Stone W. S. and Ogren S. O. (1987) Evidence for a possible functional interaction between serotonergic and cholinergic mechanisms in memory retrieval. *Behav. neural Biol.* **48,** 49–62.
5. Amenta F., Zaccheo D. and Collier W. L. (1991) Neurotransmitters, neuroreceptors and aging. *Mech. Ageing Dev.* **61,** 249–273.
6. Ashby C. R. Jr, Minable Y., Edwards E. and Wang R. Y. (1991) 5-HT_3-like receptors in the rat medial prefrontal cortex: an electrophysiological study. *Brain Res.* **518,** 181–191.
7. Azmitia E. C. and Segal M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. comp. Neurol.* **179,** 641–668.
8. Barnes J. M., Barnes N. M., Champaneria S., Costall B. and Naylor R. J. (1990) Characterization and autoradiographic localisation of 5-HT_3 receptor recognition sites identified with [^{3}H]-(S)-zacopride in the forebrain of the rat. *Neuropharmacology* **29,** 1037–1045.
9. Barnes J. M., Barnes N. M., Costall B., Ironside J. W. and Naylor R. J. (1989) Identification and characterization of 5-hydroxytryptamine 3 recognition sites in human brain tissue. *J. Neurochem.* **53,** 1787–1793.
10. Barnes J. M., Barnes N. M., Costall B. and Tyers M. B. (1989) 5-HT_3 receptors mediate inhibition of acetylcholine release in cortical tissue. *Nature* **338,** 762–763.
11. Barnes J. M., Costall B., Coughlan J., Domeney A. M., Gerrard P. A., Kelly M. E., Naylor R., Onaivi E. S., Tomkins D. M. and Tyers M. B. (1990) The effects of ondansetron, a 5-HT_3 receptor antagonist, on cognition in rodents and primates. *Pharmac. Biochem. Behav.* **35,** 955–962.
12. Barnes J. M., Costall B. and Naylor R. J. (1988) [^{3}H]Zacopride indentifies 5-HT_3 binding sites in rat entorhinal cortex. *Br. J. Pharmac., Suppl.* **94,** 391.
13. Bartus R. T., Dean R. L., Pontecorvo M. J. and Flicker C. (1985) The cholinergic hypothesis: a historical overview, current perspective, and future directions. In *Memory Dysfunctions, Annals of the New York Academy of Sciences, Vol.* 444 (eds Olton D., Gamzu E. and Corkin S.), pp. 332–358. New York.
14. Baumgarten H. G., Björklund A., Lachenmayer L., Nobin A. and Stenevi U. (1971) Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. *Acta physiol. scand., Suppl.* **373,** 1–15.
15. Baumgarten H. G. and Lachenmayer L. (1972) 5,7-Dihydroxytryptamine: improvement in chemical lesioning of indolamine neurons in mammalian brain. *Z. Zellforsch.* **135,** 399–414.
16. Baumgarten H. G., Björklund A., Lachenmayer L. and Nobin A. (1973) Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. *Acta physiol. scand., Suppl.* **391,** 1–19.
17. Bergersweeney J., Heckers S., Mesulam M. M., Wiley R. G., Lappi D. A. and Sharma M. (1994) Effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. *J. Neurosci.* **14,** 4507–4519.
18. Bertorelli R., Amoroso D., Girotti P. and Consolo S. (1992) Effect of tianeptine on the central cholinergic system—involvement of serotonin. *Naunyn-Schmiedeberg's Arch. Pharmac.* **345,** 276–281.
19. Bhattacharya S. K. and Sen A. P. (1992) Effects of muscarinic receptor agonists and antagonists on rat brain serotonergic activity. *J. neural Transm.* **90,** 241–247.

20. Bianchi C., Siniscalchi A. and Beani L. (1986) The influence of 5-hydroxytryptamine on the release of acetylcholine from guinea-pig brain *ex vivo* and *in vivo*. *Neuropharmacology* **25,** 1043–1049.
21. Bianchi C., Siniscalchi A. and Beani L. (1989) Effect of 5-hydroxytryptamine on [^{3}H]-acetylcholine release from guinea-pig striatal slices. *Br. J. Pharmac.* **97,** 213–221.
22. Bianchi C., Siniscalchi A. and Beani L. (1990) 5-HT_{1A} agonists increase and 5-HT_3 agonists decrease acetylcholine efflux from the cerebral cortex of freely-moving guinea-pigs. *Br. J. Pharmac.* **101,** 448–452.
23. Biegon A., Rainbow T. C. and McEwen B. S. (1982) Quantitative autoradiography of serotonin receptors in the rat brain. *Brain Res.* **242,** 197–204.
24. Bockaert J., Fozard J. R., Dumuis A. and Clarke D. E. (1992) The 5-HT_4 receptor: a place in the sun. *Trends pharmac. Sci.* **13,** 141–145.
25. Boddeke H. G. W. M. and Kalkman H. O. (1990) Zacopride and BRL 24924 induce an increase in EEG-energy in rats. *Eur. J. Pharmac.* **101,** 281–284.
26. Boess F. G. and Martin I. L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology* **33,** 275–317.
27. Bolanos-Jiménez F., Manhaes de Castro R. and Fillion G. (1993) Antagonism by citalopram and tianeptine of presynaptic 5-HT_{1B} heteroreceptors inhibiting acetylcholine release. *Eur. J. Pharmac.* **242,** 1–6.
28. Bolanos-Jiménez F., Manhaes de Castro R. and Fillion G. (1994) Effect of chronic antidepressant treatment on 5-HT_{1B} presynaptic heteroreceptors inhibiting acetylcholine release. *Neuropharmacology* **33,** 77–81.
29. Bolanos F. and Fillion G. (1989) Minaprine antagonises the serotonergic inhibitory effect of trifluoromethylphenylpiperazine (TFMPP) on acetylcholine release. *Eur. J. Pharmac.* **168,** 87–92.
30. Book A. A., Wiley R. G. and Schweitzer J. B. (1994) 192 IgG-saporin. 1. Specific lethality for cholinergic neurons in the basal forebrain of the rat. *J. Neuropath. exp. Neurol.* **53,** 95–102.
31. Boschert U., Amara D. A., Segu L. and Hen R. (1994) The mouse 5-hydroxytryptamine$_{1B}$ receptor is localized predominantly on axon terminals. *Neuroscience* **58,** 167–182.
32. Bowen D. M., Allen S. J., Benton S. J., Goodhardt M. J., Haan E. A., Plamer A. M., Sims N. R., Smith C. C. T., Spillane J. A., Esiri M. M., Neary D., Snowdon J. S., Wilcock G. K. and Davison A. N. (1983) Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. *J. Neurochem.* **41,** 266–272.
33. Bowen D., Francis P., Sims N. and Cross A. (1992) Protection from dementia. *Science* **258,** 1422–1423.
34. Bowen D. M., Smith C. B., While P., Flack R. H. A., Carrasco L. H., Gedye J. L., Davison A. N. (1977) Chemical pathology of the organic dementias. *Brain* **100,** 427–453.
35. Bradley P. B. (1987) 5-HT_3 receptors in the brain. *Nature* **330,** 969.
36. Bradley P. B., Handley S. J., Cooper S. J., Key B. J., Barnes N. M. and Coote J. H. (1992) *Serotonin, CNS receptors and Brain Function*, 423 pp. Pergamon, Oxford.
37. Brambilla A., Ghiorzi A., Pitsikas N. and Borsini F. (1993) DAU-6215, a novel 5-HT(3)-receptor antagonist, selectively antagonizes scopolamine-induced deficit in a passive-avoidance task, but not scopolamine-induced hypermotility in rats. *J. Pharmac. Pharmacol.* **45,** 841–843.
38. Butcher L. L. (1992) The cholinergic basal forebrain and its telencephalic targets: interrelations and implications for cognitive function. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 15–26. Birkhaüser, Boston.
39. Butcher L. L. and Woolf N. J. (1984) Histochemical distribution of acetylcholinesterase in the central nervous system: clues to the localization of cholinergic neurons. In *Classical Transmitters and Transmitter Receptors in the CNS* (eds Björklund A., Hökfelt T. and Kuhar M. J.), pp. 1–50. Elsevier, Amsterdam.
40. Butcher S. H., Butcher L. L. and Cho L. (1976) Modulation of neostriatal acetylcholine in the rat by dopamine and 5-hydroxytryptamine afferents. *Life Sci.* **18,** 733–744.
41. Carey G. J., Costall B., Domeney A. M., Gerrard A., Jones D. N. C., Naylor R. J. and Tyers M. B. (1992) Ondansetron and arecoline prevent scopolamine-induced cognitive deficits in the marmoset. *Pharmac. Biochem. Behav.* **42,** 75–83.
42. Carli M., Luschi R., Garofalo P. and Samanin R. (1995) 8-OH-DPAT impairs spatial but not visual learning in a water maze by stimulating 5-HT_{1A} receptors in the hippocampus. *Behav. Brain Res.* **67,** 67–74.
43. Carli M. and Samanin R. (1992) 8-Hydroxy-2-(di-normal-propylamino)tetralin impairs spatial learning in a water maze—role of postsynaptic 5-HT_{1A} receptors. *Br. J. Pharmac.* **105,** 720–726.
44. Cassel J. C., Kelche C., Majchrzak M. and Will B. E. (1992) Factors influencing structure and function of intracerebral grafts in the mammalian brain: a review. *Rest. Neurol. Neurosci.* **4,** 65–96.
45. Cassel J. C., Neufang B., Kelche C., Aiple F., Will B. E., Hertting G. and Jackisch R. (1992) Effects of septal and/or raphe cell suspension grafts on hippocampal choline acetyltransferase activity, high affinity synaptosomal uptake of choline and serotonin, and behavior in rats with extensive septohippocampal lesions. *Brain Res.* **585,** 243–254.
46. Cassel J. C., Neufang B., Kelche C., Jeltsch H., Will B. E., Hertting G. and Jackisch R. (1993) Effects of grafts containing cholinergic and/or serotonergic neurons on cholinergic, serotonergic and noradrenergic markers in the denervated rat hippocampus. *Brain Res.* **604,** 53–63.
47. Chaki S., Muramatsu M. and Otomo S. (1994) Involvement of protein kinase C activation on regulation of acetylcholine release from rat hippocampal slices by minaprine. *Neurochem. Int.* **24,** 37–41.
48. Chalmers D. T. and Watson S. J. (1991) Comparative anatomical distribution of 5-HT_{1A} receptor and mRNA and 5-HT_{1A} binding in rat brain—a combined *in situ* hybridisation/in vitro receptor autoradiographic study. *Brain Res.* **561,** 51–60.
49. Charney D. S., Woods S. W., Krystal J. H. and Heninger G. R. (1990) Serotonin function and human anxiety disorders. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.) pp. 558–573. New York.
50. Chugh Y., Saha N., Sankaranarayanan A. and Datta H. (1991) Enhancement of memory retrieval and attenuation of scopolamine-induced amnesia following administration of 5-HT_3 antagonist ICS 205-930. *Pharmac. Toxicol.* **69,** 105–106.
51. Cole B. J., Jones G. H. and Turner J. D. (1994) 5-HT_{1A} receptor agonists improve the performance of normal and scopolamine-impaired rats in an operant delayed matching to position task. *Psychopharmacology* **116,** 135–142.
52. Collerton D. (1986) Cholinergic function and intellectual decline in Alzheimer's disease. *Neuroscience* **19,** 1–28.
53. Consolo S., Arnaboldi S., Giorgi S., Russi G. and Ladinsky H. (1994) 5-HT_4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. *NeuroReport* **5,** 1230–1232.

54. Consolo S., Bertorelli R., Russi G. and Ladinsky H. (1994) Serotonergic facilitation of acetylcholine release *in vivo* from rat dorsal hippocampus via serotonin 5-HT_3 receptors. *J. Neurochem.* **62,** 2254–2261.
55. Consolo S., Ladinsky H., Forloni G. L., Tirelli A. S. and Garattini S. (1980) Comparison of the effects of the stereoisomers of fenfluramine on the acetylcholine content of rat striatum, hippocampus and nucleus accumbens. *J. Pharmac. Pharmacol.* **32,** 201–203.
56. Consolo S., Wu F. C., Fiorentini F., Ladinsky H. and Vezzani A. (1987) Determination of endogenous acetylcholine release in freely moving rats by transstriatal dialysis coupled to a radioenzymatic assay: effect of drugs. *J. Neurochem.* **48,** 1459–1465.
57. Costall B., Domeney A. M., Kelly M. E. and Naylor R. J. (1992) Influence of 5-HT on cognitive performance. In *Serotonin, CNS receptors and Brain Function* (eds Bradley P. B., Handley S. J., Cooper S. J., Key B. J., Barnes N. M. and Coote J. H.), pp. 147–164. Pergamon, Oxford.
58. Curzon G. (1990) Serotonin and appetite. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 521–531. New York.
59. Davies P. and Maloney A. F. J. (1976) Selective loss of cerebral cholinergic neurons in Alzheimer's disease. *Lancet* **2,** 1403.
60. De Belleroche J. and Gardiner I. M. (1982) Contrasting effects of 5-hydroxytryptamine on the release of dopamine and acetylcholine in the nucleus accumbens of rat. *J. neural Transm.* **55,** 227–242.
61. Decker M. W. (1992) Cholinergic/noradrenergic interactions and memory. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 78–90. Birkhaüser, Boston.
62. Decker M. W. and Gallagher M. (1987) Scopolamine-disruption of radial arm maze performance: modification by noradrenergic depletion. *Brain Res.* **417,** 423–438.
63. Decker M. W. and McGaugh J. L. (1989) Effects of concurrent manipulations of cholinergic and noradrenergic function on learning and retention in mice. *Brain Res.* **477,** 29–37.
64. Decker M. W. and McGaugh J. L. (1991) The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* **7,** 151–168.
65. Dekker A. J. and Thal L. J. (1993) Independent effects of cholinergic and serotonergic lesions on acetylcholine and serotonin release in the neocortex of the rat. *Neurochem. Res.* **18,** 277–283.
66. Dewar K. M., Grondin L., Carli M., Lima L. and Reader T. (1992) [^{3}H]Paroxetine binding and serotonin content of rat cortical areas, hippocampus, neostriatum, ventral mesencephalic tegmentum, and midbrain raphe nuclei region following *p*-chlorophenylalanine and *p*-chloroamphetamine treatment. *J. Neurochem.* **58,** 250–257.
67. Domeney A. M., Costall B., Gerrard P. A., Jones N. D. C., Naylor R. J. and Tyers M. B. (1991) The effects of ondansetron on cognitive performance in the marmoset. *Pharmac. Biochem. Behav.* **38,** 169–175.
68. Dringenberg H. C. and Vanderwolf C. H. (1994) Transcallosal evoked potentials: Behavior-dependent modulation by muscarinic and serotonergic receptors. *Brain Res. Bull.* **34,** 555–562.
69. Dunnett S. B. (1990) Neural transplantation in animal models of dementia. *Eur. J. Neurosci.* **2,** 567–587.
70. Early B., Glennon M., Lally M., Leonard B. E. and Junien J. L. (1995) Autoradiographic distribution of cholinergic muscarinic receptors and serotonin(2) receptors in olfactory bulbectomized (OB) rats after chronic treatment with mianserin and desipramine. *Hum. Psychopharmac. clin. Exp.* **9,** 397–407.
71. Euvrard C., Javoy F., Herbet A. and Glowinski J. (1977) Effect of quipazine, a serotonin-like drug, on striatal cholinergic interneurons. *Eur. J. Pharmac.* **41,** 281–289.
72. Fischette C. T., Nock B. and Renner K. (1987) Effects of 5,7-dihydroxytryptamine on serotonin$_1$ and serotonin$_2$ receptors throughout the rat central nervous system using quantitative autoradiography. *Brain Res.* **421,** 263–279.
73. Fisher A. and Hanin I. (1986) Potential animal models for senile dementia of Alzheimer's type, with emphasis on AF64A-induced cholinotoxicity. *A. Rev. Pharmac. Toxicol.* **26,** 161–181.
74. Francis P. T., Sims N. R., Procter A. W. and Bowen D. M. (1993) Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: investigative and therapeutic perspectives. *J. Neurochem.* **60,** 1589–1604.
75. Freund T. F. (1989) GABAergic septohippocampal neurons contain parvalbumin. *Brain Res.* **478,** 375–381.
76. Gage F. H. and Björklund A. (1986) Compensatory collateral sprouting of aminergic systems in the hippocampal formation following partial deafferentation. In *The Hippocampus, Vol. III* (eds Isaacson R. L. and Pribram K. H.), pp. 33–64. Plenum, New York.
77. Gillet G., Ammor S. and Fillion G. (1985) Serotonin inhibits acetylcholine release from rat striatum slices: evidence for a presynaptic receptor-mediated effect. *J. Neurochem.* **45,** 1687–1691.
78. Gorzalca B. B., Mendelson S. D. and Watson N. V. (1990) Serotonin receptor subtypes and sexual behavior. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 435–446. New York.
79. Gottfries C. G. (1990) Neurochemical aspects on aging and diseases with cognitive impairment. *J. Neurosci. Res.* **27,** 541–547.
80. Guyenet P., Euvrard C., Javoy F., Herbet A. and Glowinski J. (1977) Regional differences in the sensitivity of cholinergic neurons to dopaminergic drugs and quipazine in the rat striatum. *Brain Res.* **136,** 487–500.
81. Hamon M., Gozlan H., El Mestikawy S., Emeritt M. B., Bolanos F. and Schechter L. (1990) The central 5-HT_{1A} receptors: pharmacological, biochemical, functional and regulatory properties. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 114–131. New York.
82. Hanin I. (1990) AF64A-induced cholinergic hypofunction. In *Progress in Brain Research, Vol.* 84 (eds Aquilonius S. M. and Gillberg P. G.), pp. 289–299. Elsevier, Amsterdam.
83. Hanin I, Fisher A., Hörtnagel H., Leventer S. M., Potter P. E. and Walsh T. J. (1987) Ethylcholine arizidinium (AF64A; ECMA) and other potential cholinergic neuron-specific neurotoxins. In *Psychopharmacology: The Third Generation of Progress* (ed Meltzer H. Y.), pp. 341–349. Raven, New York.
84. Harel-Dupas C., Cloëz I. and Fillion G. (1991) The inhibitory effect of trifluoromethylphenylpiperazine on [^{3}H]acetylcholine release in guinea pig hippocampal synaptosomes is mediated by a 5-hydroxytryptamine$_1$ receptor distinct from 1A, 1B and 1C subtypes. *J. Neurochem.* **56,** 221–227.

85. Haroutunian V., Kanof P. D., Tsuboyama G. and Davis K. L. (1990) Restoration of cholinomimetic activity by clonidine in cholinergic plus noradrenergic lesioned rats. *Brain Res.* **507,** 261–266.
86. Haroutunian V., Santucci A. C. and Davis K. L. (1990) Implications of multiple transmitter system lesions for cholinomimetic therapy in Alzheimer's disease. In *Progress in Brain Research, Vol.* 84 (eds Aquilonius S. M. and Gillberg P. G.), pp. 333–346. Elsevier, Amsterdam.
87. Heckers S., Ohtake T., Wiley R. G., Lappi D. A., Geula C. and Mesulam M. M. (1994) Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *J. Neurosci.* **14,** 1271–1289.
88. Hertting G., Zumstein A., Jackisch R., Hoffmann I. and Starke K. (1980) Modulation by endogenous dopamine of the release of acetylcholine in the caudate nucleus of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmac.* **315,** 111–117.
89. Holtum J. R. and Gershon S. (1993) The cholinergic model of dementia of the Alzheimer type: progression from the unitary transmitter concept. *Dementia* **3,** 174–185.
90. Hong Y. and Krnjevic K. (1989) Serotonin blocks the facilitatory action of muscarinic and nicotinic agents in the hippocampus *in vivo*. *Can. J. Physiol. Pharmac.* **67,** 47–53.
91. Hoyer D., Clarke D. E., Fozard J. R., Hartig P. R., Martin G. R., Mylecharane E. J., Saxena P. R. and Humphrey P. P. A. (1994) VII International Union of Pharmacology: classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmac. Rev.* **46,** 57–204.
92. Hoyer D., Schoeffter P., Waeber C. and Palacios J. M. (1990) Serotonin 5-HT_{1D} receptors. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 168–182. New York.
93. Ingram D. K., Spangler E. L., Iijima S., Ikari H., Kuo H., Greig N. H. and London E. D. (1994) Rodent models of memory dysfunction in Alzheimer's disease and normal aging: moving beyond the cholinergic hypothesis. *Life Sci.* **55,** 2037–2049.
94. Izumi J., Washizuka M., Miura N., Hiraga Y. and Ikeda Y. (1994) Hippocampal serotonin 5-HT_{1A} receptor enhances acetylcholine release in conscious rats. *J. Neurochem.* **62,** 1804–1808.
95. Jackson D., Stachowiak M. K., Bruno J. P. and Zigmond M. J. (1988) Inhibition of striatal acetylcholine release by endogenous serotonin. *Brain Res.* **457,** 259–266.
96. Jacobs B. L. and Azmitia E. C. (1992) Structure and function of the brain serotonin system. *Physiol. Rev.* **72,** 165–229.
97. Jacobs B. L., Fornal C. A. and Wilkinson L. O. (1990) Neurophysiological and neurochemical studies of brain serotonergic neurons in behaving animals. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 260–271.
98. Jaffard R., Marighetto A. and Micheau J. (1992) Septal noradrenergic and glutamatergic influences on hippocampal cholinergic activity in relation to spatial learning and memory in mice. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M W. and Butcher L. L.), pp. 103–117. Birkhaüser, Boston.
99. Jäkälä P., Sirviö J., Jolkonen J., Riekkinen P. Jr, Acsady L. and Riekkinen P. (1992) The effects of p-chlorophenylalanine-induced serotonin synthesis inhibition and muscarinic blockade on the performance of rats in a 5-choice serial reaction time task. *Behav. Brain Res.* **51,** 29–40.
100. Jäkälä P., Mazurkiewiscz M., Sirviö J., Riekkinen P. and Riekkinen P. (1993) The behavioral effects of serotonin synthesis inhibition and quisqualic acid-induced lesions of the nucleus basalis magnocellularis in rats. *Gen. Pharmac.* **24,** 1141–1148.
101. Jansen J. H. M. and Andrews J. S. (1994) The effects of serotonergic drugs on short-term spatial memory in rats. *J. Psychopharmac.* **8,** 157–163.
102. Jeltsch H., Cassel J. C., Jackisch R., Neufang B., Greene P. L., Kelche C., Hertting G. and Will B. E. (1994) Lesions of supracallosal and infracallosal hippocampal pathways in the rat: behavioral, neurochemical, and histological effects. *Behav. neural Biol.* **62,** 121–133.
103. Jeltsch H., Cassel J. C., Neufang B., Kelche C., Hertting G., Jackisch R. and Will B. E. (1994) The effects of intrahippocampal raphe and/or septal grafts in rats with fimbria–fornix lesions depend on the origin of the grafted tissue and the behavioural task used. *Neuroscience* **63,** 19–39.
104. Jeltsch H., Cassel J. C., Simler S., Lazarus C., Kelche C., Hertting G., Jackisch R. and Will B. E. (1994) Hippocampal amino acid concentrations after raphe and/or septal cell suspension grafts in rats with fimbria–fornix lesions. *Neuroscience* **63,** 41–45.
105. Jimerson D. C., Lesem M. D., Hegg A. P. and Brewerton T. D. (1990) Serotonin in human eating disorders. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 532–544. New York.
106. Johnson R. M., Inouye G. T., Eglen R. M. and Wong E. H. F. (1993) 5-HT_3 receptor ligands lack modulatory influence on acetylcholine release in rat entorhinal cortex. *Naunyn-Schmiedeberg's Arch. Pharmc.* **347,** 241–247.
107. Jones B. E. and Cuello A. C. (1989) Afferents to the basal forebrain cholinergic cell area from pontomesencephalic—catecholamine, serotonin, and acetylcholine—neurons. *Neuroscience* **31,** 37–61.
108. Jonsson (1984) Chemical lesioning techniques: monoamine neurotoxins. In *Classical Transmitters and Transmitter Receptors in the CNS* (eds Björklund A., Hökfelt T. and Kuhar M. J.) pp. 463–507. Elsevier, Amsterdam.
109. Khateb A., Fort P., Alonso A., Jones B. E. and Mühlethaler M. (1993) Pharmacological and immunohistochemical evidence for serotonergic modulation of cholinergic nucleus basalis neurons. *Eur. J. Neurosci.* **5,** 541–547.
110. Kilpatrick G. J., Jones B. J. and Tyers M. B. (1987) Identification and distribution of 5-HT_3 receptors in rat using radioligand binding. *Nature* **330,** 746–748.
111. Kimura H., McGeer P. L. and Jeng-Hsiung Peng (1984) Choline acetyltransferase-containing neurons in the rat brain. In *Classical Transmitters and Transmitter Receptors in the CNS* (eds Björklund A., Hökfelt T. and Kuhar M. J.), pp. 51–67. Elsevier, Amsterdam.
112. Kiss J., Patel A., Baimbridge K. G. and Freund T. F. (1990) Topographical localization of neurons containing parvalbumin and choline acetyltransferase in the medial septum-diagonal band region of the rat. *Neuroscience* **36,** 61–72.
113. Köhler C., Chan-Palay V. and Steinbusch H. (1982) The distribution and origin of serotonin-containing fibers in the septal area: a combined immunohistochemical and fluorescent retrograde tracing study in the rat. *J. comp. Neurol.* **209,** 91–111.

114. Lahtinen H., Miettinen R., Ylinen A., Halonen T. and Riekkinen P. J. Sr. (1993) Biochemical and morphological changes in the rat hippocampus following fimbria–fornix lesioning. *Expl Neurol.* **121,** 311–318.
115. Lalonde R. and Vikis-Freibergs V. (1985) Manipulations of 5-HT activity and memory in the rat. *Pharmac. Biochem. Behav.* **22,** 377–382.
116. Lawrence J. A., Olverman H. J., Shirakawa K., Kelly J. S. and Butcher S. P. (1993) Binding of 5-HT_{1A} receptor and 5-HT transporter ligands in rat cortex and hippocampus following cholinergic and serotonergic lesions. *Brain Res.* **612,** 326–329.
117 Lee E. H. Y., Lin W. R., Chen H. Y., Shiu W. H. and Liang K. C. (1992) Fluoxetine and 8-OH-DPAT in the lateral septum enhances and impairs retention of an inhibitory avoidance response in rats. *Physiol. Behav.* **51,** 681–688.
118. Leung L. S. and Martin L. A. (1994) Hippocampal theta rhythm in behaving rats following ibotenic acid lesion of the septum. *Hippocampus* **4,** 136–147.
119. Levin E. D., Decker M. W. and Butcher L. L. (1990) *Neurotransmitter Interactions and Cognitive Function*, 362 pp. Birkhaüser, Boston.
120. Levin E. D. and Rose J. E. (1992) The importance of D_1 and D_2 interactions with nicotinic and muscarinic systems for working memory function. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 144–158. Birkhaüser, Boston.
121. Levkovitz Y. and Segal M. (1994) Acetylcholine mediates the effects of fenfluramine on dentate granule cell excitability in the rat. *Eur. J. Pharmac.* **264,** 279–284.
122. Leysen J. E. and Pauwels P. J. (1990) 5-HT_2 receptors, roles and regulation. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 183-193. New York.
123. Lorden J. F., Rickert E. J. and Berry D. W. (1983) Forebrain monoamines and associative learning: I. Latent inhibition and conditioned inhibition. *Behav. Brain Res.* **9,** 181–199.
124. Losier B. J. and Semba K. (1993) Dual projections of single cholinergic and aminergic brainstem neurons to the thalamus and basal forebrain in the rat. *Brain Res.* **604,** 41–52.
125. Lown O. P., Keith A. B., Lloyd S., Sahgal A. and Ferrier I. N. (1992) Cortical serotonin receptors (5-HT_{1A} and 5-HT_2) following serotonergic and cholinergic lesions in rats. *Neurosci. Res. Commun.* **11,** 119–126.
126. Luebke J. I., Greene R. W., Semba K., Kamondi A., McCarley R. W. and Reiner P. B. (1992) Serotonin hyperpolarizes cholinergic low-threshold burst neurons in the rat laterodorsal tegmental nucleus *in vitro*. *Proc. natn. Acad. Sci. U.S.A.* **89,** 743–747.
127. MacDonald E. and Sirviö J. (1993) Neurotoxins as tools in lesioning experiments. In *Natural and Synthetic Neurotoxins* (ed Harvey A.), pp. 1–46. Academic Press, London.
128. Maeda T., Kaneko S. and 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.
129. Mann D. M. A. and Yates P. O. (1986) Neurotransmitter deficits in Alzheimer's disease and in other dementing disorders. *Human Neurobiol.* **5,** 147–158.
130. Markowska A. L. and Wenk G. L. (1991) Serotonin influences the behavioral recovery of rats following nucleus basalis lesions. *Pharmac. Biochem. Behav.* **38,** 731–737.
131. Martin G. R. and Humphrey P. P. A. (1994) Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacology* **33,** 261–273.
132. Matsuno K., Senda T., Matsunaga K., Mita S. and Kaneto H. (1993) Similar ameliorating effects of benzomorphans and 5-HT_2 antagonists on drug-induced impairment of passive avoidance response in mice: comparison with acetylcholinesterase inhibitors. *Psychopharmacology* **112,** 134–141.
133. Maura G., Andrioli G. C., Cavazzani P. and Raiteri M. (1992) 5-Hydroxytryptamine$_3$ receptors sited on cholinergic axon terminals of human cerebral cortex mediate inhibition of acetylcholine release. *J. Neurochem.* **58,** 2334–2337.
134. Maura G., Fedele E. and Raiteri M. (1989) Acetylcholine release from rat hippocampal slices is modulated by 5-hydroxytryptamine. *Eur. J. Pharmac.* **165,** 173–179.
135. Maura G. and Raiteri M. (1986) Cholinergic terminals in rats hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release. *Eur. J. Pharmac.* **129,** 333–337.
136. McCormick D. A. and Wang Z. (1991) Serotonin and noradrenaline excite GABAergic neurons in the guinea-pig and cat nucleus reticularis thalami. *J. Physiol.* **442,** 235–255.
137. McEntee W. J. and Crook T. H. (1991) Serotonin, memory, and the aging brain. *Psychopharmacology* **103,** 143–149.
138. McNaughton N., Azmitia E. C., Williams J. H., Buchan A. and Gray J. A. (1980) Septal elicitation of hippocampal theta rhythm after localized deafferentation of serotonergic fiber. *Brain Res.* **200,** 259–269.
139. Meador K. J., Hendricks N., Nochols M. E., Oberzan R., Moore E. E. and Loring D. W. (1993) Cholinergic and serotonergic effects on memory and EEG activation in humans. *J. clin. exp. Neuropsychol.* **15,** 110–110.
140. Meibach R. C. (1984) Serotonergic receptors. In *Classical Transmitters and Transmitter Receptors in the CNS* (eds Björklund A., Hökfelt T. and Kuhar M. J.), pp. 304–324. Elsevier, Amsterdam.
141. Meltzer H. Y. (1990) Role of serotonin in depression. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* **600** (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 486–500. New York.
142. Middlemiss D. N. and Huston P. H. (1990) The 5-HT_{1B} receptor. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* **600** (ed Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 132–148. New York.
143. Milner T. A. and Veznedaroglu E. (1991) Serotonin-containing terminals synapse on septohippocampal neurons in the rat. *J. Neurosci. Res.* **36,** 260–271.
144. Miura N., Nakata N., Tanaka Y., Hiraga Y., Ikeda Y., Ohata H. and Iwasaki T. (1993) Improving effects of FG-7080, a serotonin reuptake inhibitor, on scopolamine-induced performance deficits of memory tasks in rats. *Jap. J. Pharmac.* **62,** 203–206.
145. Moore W. J. and Mahler H. (1965) Introduction to molecular psychology. *J. chem. Educ.* **42,** 49–60.
146. Morain P., Abraham C., Portevin B. and Denanteuil G. (1994) Biguanide derivatives: agonist pharmacology at 5-hydroxytryptamine type 3 receptors *in vitro*. *Molec. Pharmac.* **46,** 732–742.
147. Morilak D. A. and Ciaranello R. D. (1993) 5-HT_2 receptor immunoreactivity on cholinergic neurons of the pontomesenphalic tegmentum shown by double immunofluorescence. *Brain Res.* **627,** 49–54.

148. Morilak D. A., Garlow S. J. and Ciaranello R. D. (1993) Immunocytochemical localization and description of neurons expressing serotonin$_2$ receptors in the rat brain. *Neuroscience* **54,** 701–717.
149. Morin L. P., Michels K. M. and Moore R. Y. (1990) Serotonin regulation of circadian rhythmicity. In *The Neuropharmacology of Serotonin. Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 418–426. New York.
150. Muir J. L., Everitt B. J. and Robbins T. W. (1995) Reversal of visual attentional dysfunction by physostigmine and nicotine but not by the 5-HT$_3$ receptor antagonis ondansetron. *Psychopharmacology* **118,** 82–92.
151. Muramatsu M., Tamaki-Ohasli J., Usuki C., Araki H. and Aihara H. (1988) Serotonin-2 receptor-mediated regulation of release of acetylcholine by minaprine in cholinergic nerve terminal of hippocampus of rat. *Neurophamacology* **27,** 603–609.
152. Murtha S. J. E. and Pappas B. A. (1994) Neurochemical, histopathological and mnemonic effects of combined lesions of the medial septal and serotonin afferents to the hippocampus. *Brain Res.* **651,** 16–26.
153. Nabeshima T., Yamada K., Hayashi T., Hasegawa T., Ishihara S., Kameyama T., Morimasa T., Kaneyuki T. and Shohmori T. (1994) Changes in muscarinic cholinergic, GABA(A), D-1, and 5-HT(2A) receptor binding, but not in benzodiazepine receptor binding in the brains of aged rats. *Life Sci.* **55,** 1585–1593.
154. Nicoll R. A., Malenka R. C. and Kauer J. A. (1990) Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Psychol. Rev.* **70,** 513–565.
155. Nilsson O. G., Brundin P. and Björklund A. (1990) Amelioration of spatial memory impairment by intrahippocampal grafts of mixed septal and raphe tissue in rats with combined cholinergic and serotonergic denervation of the forebrain. *Brain Res.* **515,** 193–206.
156. Nilsson O. G., Leanza G. and Björklund A. (1992) Acetylcholine release in the hippocampus: regulation by monoaminergic afferents as assessed by *in vivo* microdialysis. *Brain Res.* **584,** 132–140.
157. Nilsson O. G., Leanza G., Rosenblad C., Lappi D. A., Wiley R. G. and Björklund A. (1992) Spatial learning impairments in rats with selective immunolesion of the forebrain cholinergic system. *Neuroreport* **3,** 1005–1008.
158. Nilsson O. G., Strecker R. E., Daszuta A. and Björklund A. (1988) Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat. *Brain Res.* **453,** 235–246.
159. Noda Y., Ochi Y., Shimada E. and Oka M. (1991) Involvement of central cholinergic mechanism in RU-24969-induced behavioral deficits. *Pharmac. Biochem. Behav.* **38,** 441–446.
160. Normile H. J. and Altman H. J. (1992) Effects of combined acetylcholinesterase inhibition and serotonin receptor blockade on age-associated memory impairments in rats. *Neurobiol. Aging* **13,** 735–740.
161. Normile H. J. and Altman H. J. (1992) Serotonin influences on cholinergic function: possible interactions in learning and memory. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 207–227. Birkhaüser, Boston.
162. Normile H. J., Jenden D. J., Kuhn D. M., Wolf W. A. and Altman H. J. (1990) Effects of combined serotonin depletion and lesions of the nucleus basalis magnocellularis on aquisition of complex spatial discrimination task in the rat. *Brain Res.* **536,** 245–250.
163. Ohno M., Yamamoto T. and Watanabe S. (1993) Working memory deficits induced by intrahippocampal administration of 8-OH-DPAT, a 5-HT(1A) receptor agonist, in the rat. *Eur. J. Pharmac.* **234,** 29–34.
164. Ohue T., Koshimura K., Akiyama Y., Ito A., Kido T., Takagi Y. and Miwa S. (1992) Regulation of acetylcholine release *in vivo* from rat hippocampus by monoamines as revealed by novel column-switching HPLC with electrochemical detection. *Brain Res.* **572,** 340–344.
165. Oleskevich S., Descarries L., Watkins K. C., Séguéla P. and Daszuta A. (1991) Ultrastructural features of the serotonin innervation in adult rat hippocampus: an immunocytochemical description in single and serial thin sections. *Neuroscience* **42,** 777–791.
166. Palacios J. M., Waeber C., Hoyer D. and Mengod G. (1990) Distribution of serotonin receptors. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 36–52. New York.
167. Passani M. B., Pugliese A. M., Azzurrini M. and Corradetti R. (1994) Effects of DAU 6215, a novel 5-hydroxytryptamine$_3$ (5-HT$_3$) antagonist on electrophysiological properties of the rat hippocampus. *Br. J. Pharmac.* **112,** 695–703.
168. Pathy M. S. J. (1993) The pharmacological management of cognitive impairment in the demented patient. *Prog. Neuropsychopharmac. Biol. Psychiat.* **17,** 515–524.
169. Pavone F., Fagioli S. and Castellano C. (1993) Effects of oxotremorine on inhibitory avoidance behaviour in two inbred strains of mice: interaction with 5-methoxy-NN-dimethyltryptamine. *Psychopharmacology* **112,** 249–252.
170. Pazos A. and Palacios J. M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.* **346,** 205–230.
171. Peck B. K. and Vanderwolf C. H. (1991) Effects of raphe stimulation on hippocampal and neocortical activity and behaviour. *Brain Res.* **568,** 244–252.
172. Perry E. K., Gibson P. H., Blessed G., Perry R. H. and Tomlinson B. E. (1977) Neurotransmitter enzyme abnormalities in senile dementia. *J. neurol. Sci.* **34,** 247–265.
173. Perry E. K., Perry R. H., Blessed G. and Tomlinson B. E. (1977) Necropsy evidence of cerebral cholinergic deficits in senile dementia. *Lancet* **1,** 189.
174. Pitsikas N. and Algeri S. (1992) Effect of oxiracetam on scopolamine-induced amnesia in the rat in a spatial learning task. *Pharmac. Biochem. Behav.* **43,** 943–951.
175. Pitsikas N., Brambilla A. and Borsini F. (1994) Effect of DAU 6215, a novel 5-HT$_3$ receptor antagonist, on scopolamine-induced amnesia in the rat in a spatial learning task. *Pharmac. Biochem. Behav.* **47,** 95–99.
176. Pompeiano M., Palacios J. M. and Mengod G. (1992) Distribution and cellular localization of mRNA coding for 5-HT$_{1A}$ receptor in the rat brain: correlation with receptor binding. *J. Neurosci.* **12,** 440–453.
177. Quirion R. and Richard J. (1987) Differential effects of selective lesions of cholinergic and dopaminergic neurons on serotonin-type 1 receptors in the rat brain. *Synapse* **1,** 124–130.
178. Quirion R., Richard J. and Dam T. V. (1985) Evidence for the existence of serotonin type-2 receptors on cholinergic terminals in rat cortex. *Brain Res.* **333,** 345–349.
179. Rada P. V., Mark G. P. and Hoebel B. G. (1993) *In vivo* modulation of acetylcholine in the nucleus accumbens of freely moving rats: I. Inhibition by serotonin. *Brain Res.* **619,** 98–104.

180. Raiteri M., Marchi M., Maura G. and Bonanno G. (1989) Presynaptic regulation of acetylcholine release in the CNS. *Cell Biol. int. Rep.* **12,** 1109–1118.
181. Reader T. A. (1989) Neurotoxins that affect central indoleamine neurons. In *Drugs as Tools in Neurotransmitter Research* (eds Boulton A. A., Baker G. B. and Juorio A. V.), pp. 49–102. Humana, Clifton.
182. Reynolds G. P., Arnold L., Rossor M. N., Iversen L. L., Mountjoy C. Q. and Roth M. (1984) Reduced binding of [^{3}H]Ketanserin to cortical 5-HT_2 receptors in senile dementia of the Alzheimer type. *Neurosci. Lett.* **44,** 47–51.
183. Riad M., Emeritt M. B. and Hamon M. (1994) Neurotrophic effects of ipsapirone and other 5-H_{1A} receptor agonists on septal cholinergic neurons in culture. *Devl Brain Res.* **82,** 245–258.
184. Ribeiro E. B., Bettiker R. L., Bogdanov M. and Wurtman R. J. (1993) Effects of systemic nicotine on serotonin release in rat brain. *Brain Res.* **621,** 311–318.
185. Richardson B. P. (1990) Serotonin and nociception. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 511–520. New York.
186. Richter-Levin G., Greenberger V. and Segal M. (1993) Regional specificity of raphe graft-induced recovery of behavioral functions impaired by combined serotonergic/cholinergic lesions. *Expl Neurol.* **121,** 256–260.
187. Richter-Levin G., Greenberger V. and Segal M. (1994) The effects of general and restricted serotonergic lesions on hippocampal electrophysiology and behavior. *Brain Res.* **642,** 111–116.
188. Richter-Levin G. and Segal M. (1989) Spatial performance is severely impaired in rats with combined reduction of serotonergic and cholinergic transmission. *Brain Res.* **477,** 404–407.
189. Richter-Levin G. and Segal M. (1989) Raphe cells grafted into the hippocampus cameliorate spatial memory deficits in rats with combined serotonergic/cholinergic deficiencies. *Brain Res.* **478,** 184–186.
190. Richter-Levin G. and Segal M. (1991) The effects of serotonin depletion and raphe grafts on hippocampal electrophysiology and behavior. *J. Neurosci.* **11,** 1585–1596.
191. Richter-Levin G. and Segal M. (1992) The contribution of the serotonergic innervation of the hippocampus to the effects of combined cholinergic/serotonergic deficits. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 228–239. Birkhaüser, Boston.
192. Richter-Levin G. and Segal M. (1993) Age-related cognitive deficits in rats are associated with a combined loss of cholinergic and serotonergic functions. *Ann. New York Acad. Sci.* **695,** 254–257.
193. Riekkinen M., Sirviö J. and Riekkinen P. Jr (1993) Pharmacological consequences of nicotinergic plus serotonergic manipulations. *Brain Res.* **622,** 139–146.
194. Riekkinen M., Riekkinen P., Sirviö J. and Riekkinen P. Jr. (1992) Effects of combined methysergide and mecamylamine/scopolamine treatment on spatial navigation. *Brain Res.* **585,** 322–326.
195. Riekkinen P. (1994) 5-HT(1A) and muscarinic acetylcholine receptors jointly regulate passive avoidance behavior. *Eur. J. Pharmac.* **262,** 77–90.
196. Riekkinen P. Jr, Jäkälä P., Sirviö J. and Riekkinen P. (1991) The effects of increased serotonergic and decreased cholinergic activities on spatial navigation performance in rats. *Pharmac. Biochem. Behav.* **39,** 25–29.
197. Riekkinen P. Jr, Riekkinen M., Sirviö J. and Riekkinen P. (1992) Effects of concurrent nicotinic antagonist and pCPA treatments on spatial and passive avoidance learning. *Brain Res.* **575,** 247–250.
198. Riekkinen P. Jr, Sirviö J., Miettinen R. and Riekkinen P. (1990) Interaction between raphe dorsalis and nucleus basalis magnocellularis in the regulation of high-voltage spindle activity in the rat neocortex. *Brain Res.* **526,** 31–36.
199. Riekkinen P., Sirviö J. and Riekkinen M. (1994) Serotonin depletion decreases the therapeutic effect of nicotine, but not THA, in medial septal-lesioned rats. *Brain Res.* **662,** 95–102.
200. Riekkinen P. Jr, Sirviö J. and Riekkinen P. (1991) Non-reversal of scopolamine- or age-related EEG changes by ondansetron, methysergide or alaproclate. *Psychopharmacology* **103,** 567–570.
201. Riekkinen P. Jr, Sirviö J. and Riekkinen P. (1990) Interaction between raphe dorsalis and nucleus basalis magnocellularis in spatial learning. *Brain Res.* **527,** 342–435.
202. Riekkinen P. J., Sirviö J., Riekkinen P. Jr, Valjakka A., Jäkälä P., Koivisto E. and Lammintautsa R. (1992) The role of the noradrenergic system in higher cerebral functions—experimental studies about the effects of noradrenergic modulation on electrophysiology and behavior. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 91–102. Birkhaüser, Boston.
203. Riekkinen P. Jr, Sirviö J., Valjakka A., Miettinen R. and Riekkinen P. (1991) Pharmacological consequences of cholinergic plus serotonergic manipulations. *Brain Res.* **552,** 23–26.
204. Riekkinen P. Jr, Sirviö J., Valjakka A., Pitkänen A., Partanen J. and Riekkinen P. (1990) The effects of concurrent manipulations of cholinergic and noradrenergic systems on neocortical EEG and spatial learning. *Behav. neural Biol.* **54,** 204–210.
205. Riekkinen M., Tolonen R. and Riekkinen P. Jr (1994) Interaction between 5-HT(1A) and nicotinic cholinergic receptors in the regulation of water maze navigation behavior. *Brain Res.* **649,** 174–180.
206. Rizzi C. A., Prudentino A. and Giraldo E. (1993) Effects on general behaviour and neurotransmitter functions of a new 5-hydroxytryptamine$_3$ receptor antagonist with potential therapeutic relevance in central nervous system disturbances. *Arzneimittelforschung* **43,** 1033–1041.
207. Robinson S. E. (1983) Effect of specifc serotonergic lesions on cholinergic neurons in the hippocampus, cortex and striatum. *Life Sci.* **32,** 345–353.
208. Robinson T. E., Castaneda E. and Whishaw I. Q. (1993) Effects of cortical serotonin depletion induced by 3,4-methylenedioxymetamphetamine (MDMA) on behavior, before and after additional cholinergic blockade. *Neuropsychopharmacology* **8,** 77–85.
209. Rotter A. (1984) Cholinergic receptors. In *Classical Transmitters and Transmitter Receptors in the CNS* (eds Björklund A., Hökfelt T. and Kuhar M. J.), pp. 273–303. Elsevier, Amsterdam.
210. Russell R. W. (1992) Interactions among neurotransmitters: their importance to the "integrated organism". In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 1–14. Birkhaüser, Boston.
211. Sahgal A. and Keith A. B. (1993) Combined serotonergic-cholinergic lesions do not disrupt memory in rats. *Pharmac. Biochem. Behav.* **45,** 995–1001.

212. Sahgal A., Keith A. B., Lloyd S., Kervin J. M., Perry E. K. and Edwardson J. A. (1990) Memory following cholinergic (NBM) and noradrenergic (DNAB) lesions made singly or in combination: potentiation of disruption by scopolamine. *Pharmac. Biochem. Behav.* **37,** 597–605.
213. Sakurai Y. and Wenk G. L. (1990) The interaction of acetylcholinergic and serotonergic neural systems on performance in a continuous non-matching to sample task. *Brain Res.* **519,** 118–121.
214. Samanin R., Quattrone A., Peri G., Ladinsky H. and Consolo S. (1978) Evidence of an interaction between serotonergic and cholinergic neurons in the corpus striatum and hippocampus of the rat brain. *Brain Res.* **151,** 70–82.
215. Sarter M., Dudchenko P., Moore H., Holley L. A. and Bruno J. P. (1992) Cognition enhancement based on GABA-cholinergic interactions. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher), pp. 329–354. Birkhaüser, Boston.
216. Segal M. (1990) Serotonin attenuates a slow inhibitory postsynaptic potential in rat hippocampal neurons. *Neuroscience* **36,** 631–641.
217. Segal M., Richter-Levin G. and Markram H. (1989) Cholinergic-serotonergic interactions and cognitive functions of the hippocampus. In *The Biology of Memory* (eds Squire L. R. and Lindenlaub E.). 547 pp. FK Schattauer, Stuttgart.
218. Séguéla P., Watkins K. C. and Descarries L. (1989) Ultrastructural relationships of serotonin axon terminals in the cerebral cortex of the adult rat. *J. comp. Neurol.* **289,** 129–142.
219. Semba K, Reiner P. B., McGeer E. G. and Fibiger H. C. (1988) Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. *J. comp. Neurol.* **267,** 433–453.
220. Sijbesma H., Schipper J., Cornelissen J. C. H. M. and de Kloet E. R. (1991) Species differences in the distribution of central 5-HT_1 binding sites: a comparative autoradiographic study between rat and guinea pig. *Brain Res.* **555,** 295–304.
221. Sinden J. D., Hodges H. and Gray J. A. (1995) Neural transplantation and recovery of cognitive function. *Behav. Brain Sci.* **18,** 10–35.
222. Sinhababu A. K. and Borchardt R. T. (1988) Molecular mechanism of biological action of the serotonin neurotoxin 5,7-dihydroxytryptamine. *Neurochem. Int.* **12,** 273–284.
223. Siniscalchi A., Beani L. and Bianchi C. (1990) Different effects of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, on cortical acetylcholine release, electrocortigram and body temperature in guinea pigs and rats. *Eur. J. Pharmac.* **175,** 219–223.
224. Sirviö J., Riekkinen P. Jr, Jäkälä P. and Riekkinen P. (1994) Experimental studies on the role of serotonin in cognition. *Prog. Neurobiol.* **43,** 363–379.
225. Slater P. and Patel S. (1983) Autoradiographic distribution of serotonin$_2$ receptors in the rat brain. *Eur. J. Pharmac.* **92,** 297–298.
226. Smith G. (1988) Animal models of Alzheimer's disease: experimental cholinergic denervation. *Brain Res. Rev.* **13,** 103–118.
227. Smith R. F. (1979) Attenuation of septal lesion-induced shuttlebox facilitation by 5-hydroxytryptophan. *Physiol. Psychol.* **7,** 419–421.
228. Starke K., Göthert M. and Kilbinger H. (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev.* **69,** 864–988.
229. Steckler T. and Sahgal A. (1995) The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. *Behav. Brain Res.* **67,** 165–199.
230. Steinbusch H. W. M. (1984) Serotonin-immunoreactive neurons and their projections in the CNS. In *Classical Transmitters and Transmitter Receptors in the CNS* (eds Björklund A., Hökfelt T. and Kuhar M. J.), pp. 68–125. Elsevier, Amsterdam.
231. Sugita S., Shen K. Z. and North R. A. (1992) 5-Hydroxytryptamine is a fast excitatory transmitter at 5-HT_3 receptors in rat amygdala. *Neuron* **8,** 199–203.
232. Taylor D. P. (1990) Serotonin agents and anxiety. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 545–557. New York.
233. Törk I. (1990) Anatomy of the serotonergic system. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 9–35. New York.
234. Tyers M. B. (1990) 5-HT_3 receptors. In *The Neuropharmacology of Serotonin, Ann. New York Acad. Sci., Vol.* **600** (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 194–205.
235. Tranzer J. P. and Thoenen H. (1967) Ultramorphologische Veränderungen der sympathischen Nervenendingungen der Katze nach Vorbehandlung mit 5- und 6-Hydroxy-Dopamine. *Naunyn-Schmiedebarg's Arch. Pharmac.* **257,** 343.
236. Vanderwolf C. H. (1987) Near-total loss of learning and memory as a result of combined cholinergic and serotonergic blockade in the rat. *Bahav. Brain Res.* **23,** 43–57.
237. Vanderwolf C. H. and Baker G. B. (1986) Evidence that serotonin mediates non-cholinergic neocortical low voltage fast activity, non-cholinergic hippocampal slow activity and contributes to intelligent behavior. *Brain Res.* **374,** 342–356.
238. Vanderwolf C. H., Baker G. B. and Dickson C. (1990) Serotonergic control of cerebral activity and behavior: models of dementia. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 366–383.
239. Vanderwolf C. H. and Penava D. (1992) Potentiation of the effects of antimuscarinic drugs on behavior by serotonin depletion: specifcity and relation to learning and memory. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher L. L.), pp. 257–276. Birkhaüser, Boston.
240. Vertes R. P. (1988) Brainstem afferents to the basal forebrain in the rat. *Neuroscience* **24,** 907–935.
241. Villani F. and Johnston D. (1993) Serotonin inhibits induction of long-term potentiation at commissural synapses in hippocampus. *Brain Res.* **606,** 304–308.
242. Vizi E. S., Harsing L. G. Jr and Zsilla G. (1981) Evidence of the modulatory role of serotonin in acetylcholine release from striatal interneurons. *Brain Res.* **212,** 89–99.
243. Volpe B. T., Hendrix C. S., Park D. H., Towle A. C. and Davis H. P. (1992) Early post-natal administration of 5,7-dihydroxytryptamine destroys 5-HT neurons but does not affect spatial memory. *Brain Res.* **589,** 262–267.
244. Waeber C., Dietle M. M., Hoyer D. and Palacios J. M. (1989) 5-HT_1 receptors in the vertebrate brain. Regional distribution examined by autoradiography. *Naunyn-Schmiedeberg's Arch. Pharmac.* **340,** 486–494.

245. Waeber C., Hoyer D. and Palacios G. M. (1989) 5-hydroxytryptamine$_3$ receptors in the human brain: autoradiographic visualization using [^{3}H]ICS 205-930. *Neuroscience* **31,** 393–400.
246. Wainer B. H. and Mesulam M. M. (1990) Ascending cholinergic pathways in the rat brain. In *Brain Cholinergic Systems* (eds Steriade M. and Biesold D.), pp. 65–119. Oxford University Press, Oxford.
247. Wallis E. D., Overstreet D. H. and Crocker A. D. (1988) Selective breeding for increased cholinergic function: increased serotonergic sensitivity. *Pharmac. Biochem. Behav.* **1,** 345–350.
248. Watling K., Aspley S., Swain C. and Sanders J. (1988) [^{3}H]Quaternised ICS 205-930 labels 5-HT$_3$ receptor binding sites in rat brain. *Eur. J. Pharmac.* **149,** 397–398.
249. Wauquier A. and Dugovic C. (1990) Serotonin and sleep-wakefulness. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 447-459.
250. Wenk G. L. (1984) Pharmacological manipulation of substantia innominata-cortical cholinergic pathway. *Neurosci. Lett.* **51,** 99–103.
251. Wenk G. L. and Engisch K. L. (1986) [^{3}H]Ketanserin (serotonin type-2) binding increases in rat cortex following basal forebrain lesions with ibotenic acid. *J. Neurochem.* **47,** 845–850.
252. Wenk G. L., Hughey D., Boundy V., Kim A., Walker L., Olton D. (1987) Neurotransmitters and memory: role of cholinergic, serotonergic and noradrenergic systems. *Behav. Neurosci.* **101,** 325–332.
253. Whitaker-Azmitia P. M. and Peroutka S. J. (1990) *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600, 718 pp.
254. Whitaker-Azmitia P. M., Shemer A. V., Caruso J., Molino L. and Azmitia E. C. (1990) Role of high affinity serotonin receptors in neuronal growth. In *The Neuropharmacology of Serotonin, Annals of the New York Academy of Sciences, Vol.* 600 (eds Whitaker-Azmitia P. M. and Peroutka J. M.), pp. 315–330. New York.
255. White P., Goodhardt M. J., Keet J. P., Hiley C. R., Carrasco L. H., Williams I. E. I. and Bowen D. M. (1977) Neocortical cholinergic neurons in elderly people. *Lancet* **1,** 668–671.
256. Wilkinson L. O., Middlemiss D. N. and Hutson P. H. (1994) 5-HT$_{1A}$ receptor activation increases hippocampal acetylcholine efflux and motor activity in the guinea pig: agonist efficacy influences functional activity *in vivo*. *J. Pharmac. exp. Ther.* **270,** 656–664.
257. Winter J. C. and Petti D. T. (1987) The effects of 8-hydroxy-2-(di-n-propylamino) tetralin and other serotonergic agonists on performance in a radial maze: a possible role for 5-H$_{1A}$ receptors in memory. *Pharmac. Biochem. Behav.* **27,** 625–628.
258. Wooley D. W. and Van der Hoeven T. (1963) Alteration in learning ability caused by changes in cerebral serotonin and catecholamines. *Science* **139,** 610–611.
259. Zaborszky L. (1992) Synaptic organization of basal forebrain cholinergic projection neurons. In *Neurotransmitter Interactions and Cognitive Function* (eds Levin E. D., Decker M. W. and Butcher), pp. 27–65. Birkhaüser, Boston.
260. Zifa E. and Fillion G. (1992) 5-hydroxytryptamine receptors. *Pharmac. Rev.* **44,** 402–458.
261. Zilles K., Werner L., Qü M., Schleicher A. and Gross G. (1991) Quantitative autoradiography of 11 different transmitter binding sites in the basal forebrain region of the rat—evidence of heterogeneity in distribution patterns. *Neuroscience* **42,** 473–581.

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