Role of serotonin in memory impairment

Marie-Christine Buhot, Stéphanie Martin and Louis Segu

As a result of its presence in various structures of the central nervous system serotonin (5-HT) plays a role in a great variety of behaviours such as food intake, activity rythms, sexual behaviour and emotional states. Despite this lack of functional specialization, the serotonergic system plays a significant role in learning and memory, in particular by interacting with the cholinergic, glutamatergic, dopaminergic or GABAergic systems. Its action is mediated via specific receptors located in crucial brain structures involved in these functions, primarily the septo–hippocampal complex and the nucleus basalis magnocellularis (NBM)–frontal cortex. Converging evidence suggests that the administration of 5-$HT_{2A/2C}$ or 5-HT_4 receptor agonists or 5-HT_{1A} or 5-HT_3 and 5-HT_{1B} receptor antagonists prevents memory impairment and facilitates learning in situations involving a high cognitive demand. In contrast, antagonists for 5-$HT_{2A/2C}$ and 5-HT_4, or agonists for 5-HT_{1A} or 5-HT_3 and 5-HT_{1B} generally have opposite effects. A better understanding of the role played by these and other serotonin receptor subtypes in learning and memory is likely to result from the recent availability of highly specific ligands, such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} receptor antagonists, and new molecular tools, such as gene knock-out mice, especially inducible mice in which a specific genetic alteration can be restricted both temporally and anatomically.

Key words: Alzheimer's disease; behaviour; knock-out mice; learning and memory; mice; pharmacology; rat; serotonin; serotonin receptors.

Ann Med 2000; 32: 210–221.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is widely distributed in the whole brain and plays a role in a great variety of behaviours such as activity rythms, food intake, sexual behaviour and emotional states. Selective serotonin reuptake inhibitors (SSRI), eg fluoxetine, are widely used as antidepressants. This variety suggests a lack of functional specialization. However, there is increasing evidence showing that 5-HT is associated with cognitive functions, especially learning, memory and attentional processes (1, 2). These functions are not independent from each other or from other behavioural levels. For example, there are some connections between anxiety and memory, or between memory and attentional processes. It is thus clear that 5-HT may modulate learning and memory by both direct and indirect ways. If memory functions are mainly and more directly controlled by other neurotransmission systems, such as the cholinergic or the glutamatergic ones, it is presently clear that by interacting with these systems, 5-HT and its various receptors play an important role in learning and memory.

The aim of the present review is to present recent scientific arguments supporting this view, in particular in cases in which these functions are deficient, ie in memory impairment. We will focuse on the functional relationship of the behavioural and neurobiological levels to provide a better understanding of the neuronal mechanisms underlying learning and memory.

From the Laboratoire de Neurosciences Cognitives, Centre National de la Recherche Scientifique, UMR 5106, Université de Bordeaux 1, 33405 Talence Cedex, France.

Correspondence: Marie-Christine Buhot, PhD, CNRS UMR 5807, Université Bordeaux 1, Avenue des Facultés, 33405 Talence Cedex, France. E-mail: buhot@neurocog.u-bordeaux.fr, Fax: +33 0556848743.

Abbreviations and acronyms

5-HT	serotonin, 5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
5,7-DHT	5,7-dihydroxytryptamine
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
ACh	acetylcholine
AD	Alzheimer's disease
CFS	conditioned fear stress
CNS	central nervous system
DA	dopamine
DA-D1	DA receptor 1
DR	dorsal raphe
GABA	γ-aminobutyric acid
GLU	glutamate
MS/DBB	medial septum/diagonal band of Broca
NBM	nucleus basalis magnocellularis
SSRI	selective serotonin reuptake inhibitor
tacrine	9-amino-1,2,3,4-tetrahydroacridine

Main focus will be on the role played by the different 5-HT receptors rather than on the role of 5-HT itself. Besides pharmacological strategies, new molecular approaches, such as the use of knock-out mice for a given receptor, are emerging, which add to our knowledge on the function of 5-HT in memory.

Global strategies

Much of the earlier work on the role of 5-HT in cognition used global strategies, in which the entire 5-HT system was manipulated and the effects of increasing or reducing central 5-HT neurotransmission were observed in various learning models.

Soubrié (3) suggested that the primary function of serotonergic neurotransmission was to inhibit ongoing behaviour. Thus, reducing 5-HT transmission was found to induce impulsivity, ie it made animals less tolerant to the delay of positive reinforcement (4). Decreasing the level of 5-HT in the brain results in a general response disinhibition, increasing thus resistance to extinction (5, 6), ie the inability to disconnect previously formed associations, preventing thus the subject from forming new associations, ie from new learning.

Reducing central 5-HT synthesis through L-tryptophan restriction impairs specifically short-term and long-term memory performance in rats and humans (7, 8). Some studies, however, did not reveal any effect on rat learning performance with such treatment administered either acutely or chronically (9). At the neurobiological level, depletion of 5-HT (by using the tryptophan hydroxylase inhibitor, parachlorophenylalanine, pCPA) during synaptogenesis decreases synaptic density in the adult rat hippocampus thus inducing spatial learning deficits associated with failure of extinction and consequently failure of relearning (10). A recent work demonstrated that depletion in 5-HT also decreases the number of newly generated cells in the rat hippocampus (11).

However, 5-HT depletion following 5,7-dihydroxytryptamine (5,7-DHT) lesioning has been found to induce facilitatory effects on acquisition and performance of various learning tasks in rats, such as spatial discrimination (12), conditional visual discrimination, presumably by reducing proactive interference (13), and temporal discrimination under certain conditions, eg when the difficulty of the task is increased (14). These 5-HT pathways, including projections to the hippocampus and the amygdala, have also been found to be crucially involved in the formation of inhibitory associations in rats (15, 16). If 5-HT is particularly active in behavioural inhibition and if its inactivation facilitates performance in relation to task difficulty, then it is possible that the role of 5-HT in cognitive function could be to modulate the signal-to-noise ratio in the central nervous system (CNS), a crucial function for memory formation.

Using a consistent experimental design, including a combination of different modes of depletion of 5-HT and of learning models, Santucci and co-workers (17) demonstrated that 5-HT depletion does not have a direct effect on learning performance. Excessive 5-HT release on the other hand produces critical impairments, probably via an overactivation of postsynaptic receptors. Fluoxetine, a SSRI, which is supposed to enhance the brain 5-HT level, was found to weaken associative memory in the rat (18), whereas aged rats treated with a low dose of the 5-HT precursor 5-hydroxytryptophan (5-HTP) improved their performance over controls in a spatial memory task (19). Recreational users of 'ectasy' (3,4-methylenedioxymethamphetamine, MDMA) exhibit selective memory impairment (immediate and delayed recall), which is likely to be associated with the long-term neurotoxic effect of MDMA on serotonergic activity (20).

Because of its known role in mood disorders, anxiety and depression, serotonin may be considered a key neurotransmitter in the processing of emotional states, in particular emotional memory. Inoue and coworkers (21) demonstrated a change in 5-HT metabolism in the prefrontal cortex, the nucleus accumbens and the amygdala of rats subjected to the conditioned fear stress (CFS), and, more recently, they observed a reduction of CFS-induced freezing behaviour following treatment with a SSRI (22). In contrast, global 5-HT lesions have been found to induce specific impairments of contextual conditioning in rats while sparing discrete cue-conditioning (23). More specifically, selective lesions of the dorsal raphe (DR) induce impairments of unconditioned (innate) fear, a response

mediated by the DR–PAG (periaqueducal gray) projection, but have facilitatory effects on learned fear, a response mediated by the DR–amygdala and DR–frontal cortex pathways (24). Response to novelty is another way conceptualizing the relationship between memory and emotion, as novelty is an ambiguous characteristic of the environment, inducing either approach (from its cognitive value) or avoidance (from its emotional fear-inducing value). A recent study suggests the involvement of the medial frontal cortex, as a neuroanatomical focus for an interaction between 5-HT and other neurotransmission systems (eg, the dopaminergic and noradrenergic ones), in mediating neuroendocrine and neurochemical changes induced by exposure (or behavioural response) to novelty in an open field (25). It is thus clear that 5-HT plays a role in the balance between memory and emotion.

From a majority of these studies emerge inconsistencies about the mechanism by which 5-HT might be involved in memory functions or dysfunctions (see also (26)). These are probably attributable, in part, to the application of this global experimental strategy which modifies the entire serotonergic system and its interactions with other neurotransmitters. The result of such manipulations cannot affect memory processes specifically without affecting other behaviours, which are themselves regulated by 5-HT through the 'nonspecific' effects on several different types of receptors.

Interactions of the serotonergic and cholinergic systems

Learning and memory deficits appear with ageing and in Alzheimer's disease (AD) and are positively correlated with the decline in cholinergic neurotransmission (27). However, there is increasing evidence showing that age-related cognitive deficits are associated with a combined dysfunctioning of the cholinergic and serotonergic systems (28, 29). There is presently no doubt that the serotonergic and cholinergic systems display important functional interactions in learning and memory (30–32). Simultaneous loss of both acetylcholine (ACh) and 5-HT transmission prolong memory impairment in rats as compared with the effect of the loss of cholinergic neurotransmission alone (33).

A basic explanation is given by converging data showing that 5-HT is involved in the regulation of central cholinergic activity by modulating ACh release in various cerebral structures, ie cholinergic pathways, such as from the medial septum/diagonal band of Broca (MS/DBB) to the hippocampus, or from the nucleus basalis magnocellularis (NBM) to the cerebral cortex and amygdala (see, eg (30, 31, 34, 35)). Both ACh and 5-HT are crucial for maintaining synapses in the hippocampus and are critically involved in the acquisition of spatial memory (36, 37). Combined disruption of muscarinic and serotonergic function induces severe deficits in spatial performance in rats (38, 39) that tacrine (9-amino-1,2,3,4-tetrahydroacridine), an acetylcholinesterase inhibitor considered to be highly efficient in the treatment of AD, is able to alleviate (40). Intrahippocampal co-grafts, rich in cholinergic and serotonergic fetal neurones, have a selective beneficial effect on spatial reference memory impairment induced by extensive lesions of the dorsal septo–hippocampal pathways, however, with no effect on working memory performance (41). Serotonin is able to modulate cholinergic septo–hippocampal neurones (42) both directly as well as indirectly by interacting with GABAergic interneurones that synapse on medial septum cholinergic neurones (43).

The role of the serotonergic system in learning and memory becomes less controversial when the effects of specific drugs binding to specific types or subtypes of receptors are being analysed. The use of intracerebral infusions of drugs to activate or inactivate specific 5-HT receptors furthermore provides a better understanding of the relationhip between the modulation of a particular cerebral structure and defined cognitive functions.

5-HT receptors in memory systems

Each 5-HT receptor type or subtype has a specific regional distribution in the brain (44). In addition to the 5-HT transporter, which is widely distributed in the whole brain and mimicks 5-HT innervation, some specific 5-HT receptors, such as the 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_4, are potentially involved in memory functions, depending their anatomical localization. The possibility that 5-HT receptors play a role in memory functions lies on the evidence that these receptors are present in regions crucial to these functions, such as the hippocampal formation, the frontal cortex, the striatum and related structures.

The 5-HT_{1A} receptor is mainly concentrated in the hippocampus, the septum, the raphe nucleus and, to a lesser extent, in the cortical regions. The 5-$HT_{1B/1D}$ receptor is found in the substantia nigra, the hippocampus, the dorsal subiculum and, to a lesser extent, in the striatum. The 5-HT_2 receptor is present in a high concentration in the cortical areas. The 5-HT_3 receptor is widely distributed in the cortex and in all subfields of the hippocampus. Lastly, the 5-HT_4 receptor is mainly localized in the frontal cortex and hippocampus.

In addition to its anatomical location, the potential role of a particular receptor in learning and memory also depends on its cellular location in the circuitry,

on whether it is located on the cholinergic septo–hippocampal and NBM–frontal cortex pathways, on the glutamatergic pyramidal cells present in the hippocampus, the subiculum, the entorhinal and the frontal cortices, or on the GABAergic interneurones in different regions, suggesting interactive influences between 5-HT and these different neurotransmission systems (Fig 1). Until presently, only 5-HT_{1A}, 5-HT_{1B}, 5-HT_2, 5-HT_3 and 5-HT_4 receptors have been demonstrated to play a role in learning and memory. Their influence on a given neurone will also depend on their linkage to a second messenger system. 5-HT_{1A} and 5-HT_{1B} receptors are negatively linked to adenylyl cyclase while the 5-HT_4 receptor is positively linked to this cyclase. The 5-HT_2 receptor stimulates phospholipase C and the 5-HT_3 receptor is a ligand-gated ion channel. Thus as a result, the activation of the 5-HT_{1A} and 5-HT_{1B} receptors will result in an inhibitory effect, whereas the activation of the 5-HT_2 and 5-HT_4 receptors will have an opposite effect, ie a facilitatory effect.

The 5-HT_{1A} receptors occupy somatodendritic positions, while 5-HT_{1B} are predominantly preterminals. The 5-HT_{1A} autoreceptors in the raphe nucleus inhibit the activity of serotonergic neurones, while 5-HT_{1B} autoreceptors inhibit the release of 5-HT in the different projecting areas, such as the septum, hippocampus, the NBM or the entorhinal and frontal cortices. The 5-HT_{1A} postsynaptic heteroreceptors are predominant on pyramidal neurones of the frontal cortex and the hippocampus that they inhibit when they are stimulated. In contrast, those located on GABAergic interneurones in the hippocampus indirectly facilitate the activity of pyramidal cells in this structure. The 5-HT_{1B} heteroreceptors located on septal terminals inhibit the release of ACh in the hippocampus; however, those located on GABAergic interneurones in the frontal cortex indirectly facilitate ACh release in this region. The 5-HT_2 receptors have a direct positive influence on the pyramidal cells of the frontal cortex. Those located in the NBM–frontal cortex pathway facilitate the cholinergic release in the frontal cortex. However, those located on GABAergic interneurones in the medial septum indirectly inhibit ACh release in the hippocampus. Thus far, the 5-HT_4 receptors have revealed consistent facilitatory in-

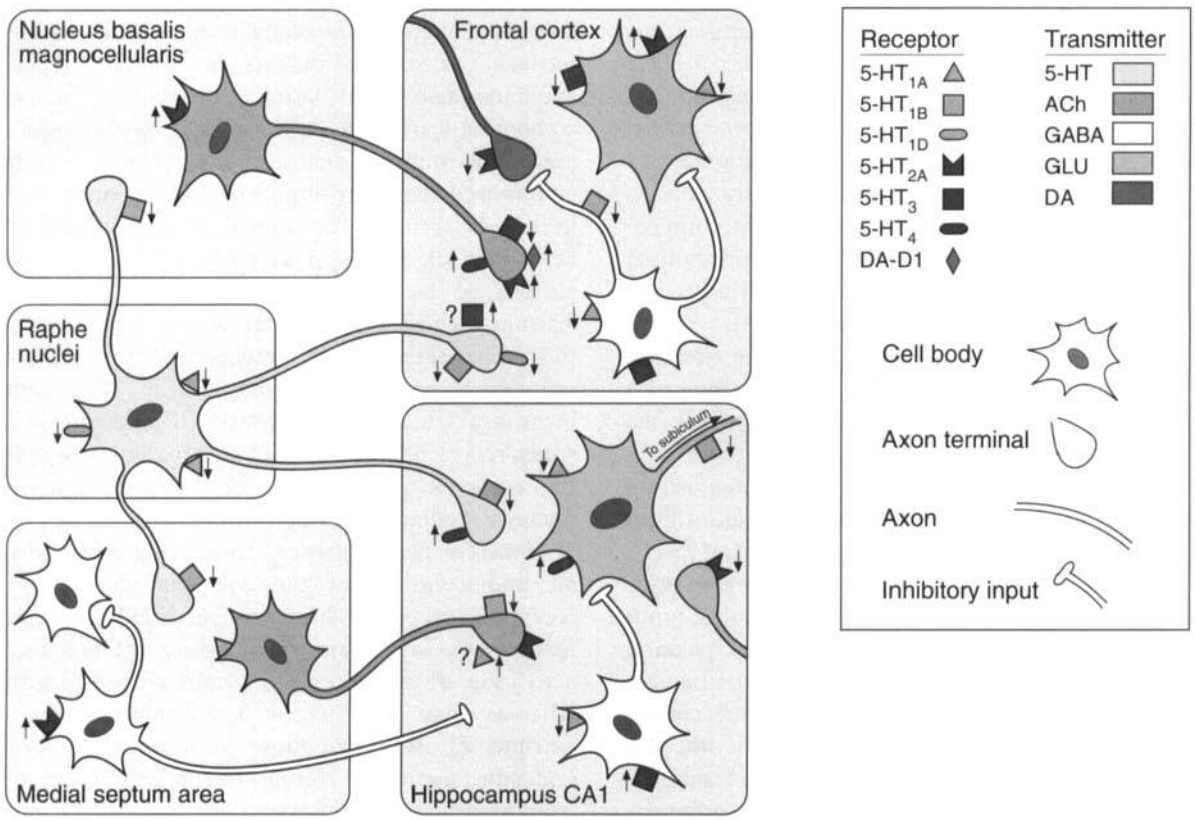

Figure 1. Localization of the different 5-HT receptor subtypes in different neuronal membrane compartments of the septo–hippocampal and basal forebrain–cortical complexes. Schematic representation of data obtained by different authors by using electrophysiology, microscopy and neurotransmitter release methods. The functional consequences of the activation of specific 5-HT receptors is indicated by an upward arrow (↑) for an excitatory effect, and a downward (↓) arrow for an inhibitory effect on the target cell (with ? signifying uncertainties). For definitions, please refer to the list of abbreviations.

fluences. Their activation results in a facilitatory effect on cholinergic release in the frontal cortex in addition to their direct positive influence on hippocampal pyramidal cells.

Thus, depending on the cognitive demand of a given behavioural task, different circuits (or cognitive systems) will be solicited, with a major role played by one of the 5-HT receptors, and even for a given receptor its relative influence on learning and memory processes will depend on its strategical location in a particular circuit.

5-HT receptors in learning and memory impairment

The 5-HT$_{1A}$ receptor

The 5-HT$_{1A}$ receptor is characterized by its high concentration in the limbic system, especially in the hippocampus (Fig 1) as well as in the raphe nucleus, where autoreceptors are also likely to exert an indirect influence on cognitive functions. This receptor subtype interacts with other neurotransmitter systems, such as the noradrenergic, dopaminergic and GABAergic systems (45, 46).

Stimulation of the 5-HT$_{1A}$ receptors has controversial effects on working memory (47, 48). When coadministered intrahippocampally with the muscarinic receptor antagonist scopolamine, NAN-190, a 5-HT$_{1A}$ antagonist, is able to reduce scopolamine-induced working memory impairment (49). However, there is no direct reciprocal interaction as physostigmine (a cholinesterase inhibitor), which is generally able to compensate cholinergic dysfunction, fails to compensate the deficit induced by an intrahippocampal injection of 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), a specific 5-HT$_{1A}$ agonist (50).

8-OH-DPAT dose-dependently impairs the retention of a well-learned maze, as well as the acquisition of a novel configuration of the maze (51). Interestingly, the activation of 5-HT$_{1A}$ receptors combined with subthreshold anticholinergic treatment impairs acquisition of the water maze task, but not its retention, in both normal rats and rats subjected to a central 5-HT depletion, emphasizing thus the important role of postsynaptic targets (52). Both systemic and intrahippocampal injections of 8-OH-DPAT impair acquisition in spatial memory tasks in rats, a deficit attributable to the activation of postsynaptic 5-HT$_{1A}$ receptors. Combining both modes of injection with the use of a 5-HT$_{1A}$ antagonist, WAY-100135, Carli and coworkers demonstrated that the activation of hippocampal 5-HT$_{1A}$ receptors selectively impairs spatial but not visual discrimination (53). In contrast, intradorsal raphe nucleus (DRN) stimulation of 5-HT$_{1A}$ autoreceptors can compensate spatial learning deficits induced by intrahippocampal scopolamine injections (54) that the authors interpret as facilitating the transfer of facilitatory information from the entorhinal cortex to the hippocampus (55). WAY-100135 and WAY-100635 (another more potent 5-HT$_{1A}$ antagonist (56)) are equally able to antagonize this deficit (57, 58). Similarly, WAY-100635 is able to prevent spatial memory impairment induced by blockade of hippocampal N-methyl-D-aspartate (NMDA) receptors (59). More generally, a deficit in learning following fornix transection in the marmoset can be alleviated by WAY-100635 (60).

If inconsistencies are still found in the different works using both systemic and intracerebral routes of drug administration and different target structures such as the raphe nucleus (presynaptic autostructure for 5-HT) versus the hippocampus (postsynaptic structure for 5-HT), the results presented here support a consistent role for 5-HT$_{1A}$ receptors in learning and memory impairment and recovery by the way of their relationships with the cholinergic and the glutamatergic systems in particular.

In the context of emotional memory studies, hippocampal 5-HT$_{1A}$ receptors were found to participate in the development of tolerance to aversive events (61). Buspirone, a 5-HT$_{1A}$ receptor partial agonist, given either systemically or applied locally into the hippocampus, has detrimental effects on all stages of emotional memory, ie acquisition, consolidation and retrieval as studied in rats in different types of avoidance tasks (62). Using a behavioural paradigm combining learned helplessness, fear-conditioning and escape learning, Maier and co-workers (63) have emphasized the contribution of 5-HT$_{1A}$ autoreceptors in these behaviours. An original study showed that the activation of 5-HT$_{1A}$ receptors induces a complex pattern of facilitatory and detrimental effects on learning, which is dependent on the task conditions (64). The authors have interpreted their results in terms of the particular role played by 5-HT$_{1A}$ receptors in arousal, in a manner similar to mild stress. This study represents a particularly interesting example of the complex participation of 5-HT to emotional memory mediated by 5-HT$_{1A}$ receptors.

Considerable progress is thus being made toward an understanding of the role played by 5-HT$_{1A}$ receptors, especially hippocampal 5-HT$_{1A}$ receptors, in memory. In a majority of cases, but not all, the activation of these receptors compromises learning, whereas their inactivation selectively reduces this detrimental effect and those associated with cholinergic dysfunction. A recent research strategy comes from molecular biology with the generation of specific knock-out and transgenic mice. The availability of both constitutive and inducible 5-HT$_{1A}$ receptor knock-out mice will certainly add further critical knowledge regarding the cognitive functions regulated by this receptor subtype (65–67). The learning and

memory abilities of one of these 5-HT_{1A} receptor constitutive knock-out mice are under current research in our laboratory (the mouse from reference (65)).

Some recent neurobiological studies have added to our understanding of the way 5-HT_{1A} receptors may interfere with the septo–hippocampal formation (Fig 1). The activation of median raphe 5-HT_{1A} autoreceptors has been found to produce the hippocampal theta rhythm and a rythmical firing pattern of MS/DBB neurones strongly correlating with this rhythm (68). Moreover, 5-HT_{1A} receptors are expressed in a subpopulation of cholinergic neurones belonging to the MS/DBB complex (69). 5-HT, acting via 5-HT_{1A} receptors, is able on one hand to decrease CA1 pyramidal cell activity directly and on the other hand to disinhibit these cells by acting on interneurones, thus controlling the balance between excitation and inhibition in this region (70). Stimulation of 5-HT_{1A} receptors by BAY x 3702, a novel, high-affinity 5-HT_{1A} agonist, enhances ACh release in the rat cortex and hippocampus, an effect compensated by the specific 5-HT_{1A} receptor antagonist WAY-100635 (71). This effect most probably involves hippocampal 5-HT_{1A} receptors, not autoreceptors (72).

Considering that enhancing ACh activity, in particular in the hippocampus, may have a positive effect on hippocampal-dependent learning abilities, there is some inconsistency between the behavioural level, which globally shows detrimental effects of the activation of (especially hippocampal) 5-HT_{1A} receptors on learning performance, and the neurobiological level, with converging evidence showing increased ACh release following 5-HT_{1A} receptor activation, probably via an indirect mechanism. Further research is needed to elucidate this mechanism.

The 5-HT_{1B} receptor

In contrast to the 5-HT_{1A} receptor, the 5-HT_{1B} receptor is predominantly located on axon terminals (Fig 1). This receptor is present in high concentrations in the hippocampal formation (especially in the dorsal subiculum) and in the substantia nigra.

We demonstrated that the stimulation of hippocampal 5-HT_{1B} receptors by the specific 5-HT_{1B} receptor agonist CP 93129 impairs the performance of rats trained in a radial arm maze by using a procedure that makes it possible to distinguish between the errors of the working memory and those of the reference memory. This impairment affected preferentially reference memory (73). These results might be explained by the specific cellular and subcellular locations of the 5-HT_{1B} receptor in the hippocampal formation (74) (Fig 1). In particular, 5-HT_{1B} heteroreceptors located on hippocampal terminals of septal cholinergic neurones decrease the release of ACh (75), whereas those located on axon terminals of glutamatergic CA1 pyramidal cells (76) control CA1 transmission in the subiculum (77). In contrast, the activation of 5-HT_{1B} receptors has been demonstrated to increase ACh release in the frontal cortex probably by acting on GABAergic interneurones thus stimulating cortical pyramidal cells (78). For a memory model involving primarily the recruitment of the frontal cortex (as working memory or sequential processing), one might expect a facilitatory role of 5-HT_{1B} agonists on memory via an interaction with the cholinergic system.

Thus far, specific 5-HT_{1B} antagonists enabling to study the functions of this receptor have been lacking, but the recent availability of GR127935 allows to consider further functional research. Thus, Meneses and co-workers (79) observed a variable pattern of results following a systemic injection of GR127935, with a low dose decreasing and a high dose increasing consolidation of learning. This effect of GR127935 alone might be partially explained by the fact that this compound displays mixed agonist–antagonist properties and possesses a moderate 5-HT_{2A} receptor-blocking profile.

We recently studied the learning and memory abilities of 5-HT_{1B} receptor knock-out mice (80, 81). In the Morris water maze, the 5-HT_{1B} knock-out mice learned the spatial reference memory task faster than the control (wild-type) mice and were found to display higher flexibility when confronted with a relocated platform. No difference in genotype was found in contextual fear conditioning. In an object exploration task, these knock-out mice displayed higher exploratory activity (82). This result is opposite to that observed previously in rats showing decreased exploratory activity and neophobic reactions following specific stimulation (using CP 93129) of hippocampal 5-HT_{1B} receptors, (83). Independently, it was observed that the 5-HT_{1B} knock-out mice displayed longer periods of paradoxical sleep than the wild-type mice (84). Given the positive influence of paradoxical sleep on memory consolidation (85), this result converges with our findings showing enhanced long-term memory performance in 5-HT_{1B} knock-out mice (82). Furthermore, this facilitatory effect of the 5-HT_{1B} gene deletion on memory is accentuated in aged mutant mice (86).

These results are promising in view of possible therapeutical applications, in particular the use of functionally selective 5-HT_{1B} receptor antagonists with potential utility in the treatment of ageing or AD-induced memory disabilities.

The 5-HT_{2A} and 5-HT_{2C} receptors

The 5-HT_{2A} and 5-HT_{2C} (5-$HT_{2A/2C}$) receptors stimulate phospholipase C. They are of close homology; currently, there are no ligands quite selective for either of these receptor subtypes. They are present in high

concentrations in cortical areas and are thought to mediate attentional rather than memory processes (see (1)). Nevertheless, scarce data are currently available. MDL100907, a 5-HT_{2A} antagonist, which has no effect by itself, is able to selectively abolish the improvement elicited by DOI, a 5-$HT_{2A/2C}$ receptor agonist, on memory consolidation in rats (79). This effect might in part result from the presence of 5-HT_2 receptors in the septo–hippocampal formation as, for example, MDL100907 is able to block the excitatory effect of serotonin on septo–hippocampal neurones via 5-HT_2 receptors probably located on GABAergic neurones belonging to the MS/DBB complex (87). At the systems level, the activation of 5-$HT_{2A/2C}$ receptors induces a facilitatory effect on cholinergic release in the rat frontal cortex (88).

Harvey (89) reviewed different studies addressing the effects of 5-$HT_{2A/2C}$ receptor agonists and antagonists on associative learning as assessed by conditioned avoidance response in the rat and by the nictitating membrane response in the rabbit. These two tasks are highly sensitive to the specific manipulation of 5-$HT_{2A/2C}$ agonists or antagonists. Agonists are consistently observed to enhance learning in both tasks, whereas 5-HT_{1A} receptor agonists have either no effect or retard learning. Interestingly, the author suggests that these drug effects are observed only when the task is difficult and generates a low level of acquisition. The positive effect of 5-$HT_{2A/2C}$ receptor agonists (see also (90)) might, therefore, be particularly efficient in situations in which task difficulty is increased. This suggests the use of 5-$HT_{2A/2C}$ agonists as therapeutic tools for the treatment of severe memory deficits in humans, as in AD and also even in normal ageing.

The 5-HT_{2C} receptor-deficient mutant mouse, generated by Tecott and co-workers, exhibits dysfunctions including weight gain and a high probability of spontaneous death associated with seizures (91). This animal is considered as a robust model for the examination of serotonergic mechanisms in epilepsy, the occurrence of which is frequently associated with the activation of cells in the hippocampus. It was found that this mouse exhibited an abnormal performance in the water maze as well as an impairment in synaptic long-term potentiation restricted to the main input of the hippocampus (perforant path–dentate gyrus) (92). This result elucidates the role of 5-HT, mediated by 5-HT_{2C} receptors, in neuronal plasticities underlying learning and memory functions.

The 5-HT_3 receptor

Among the 5-HT receptors, the 5-HT_3 receptor is a unique one, as it is a ligand-gated ion channel. Since the last decade, 5-HT_3 antagonists have been found to clearly improve learning and memory or to antagonize the effects of anticholinergic or age-induced memory loss in rodents and primates (93), which is a recent discovery for other types of 5-HT receptors. The beneficial effect of 5-HT_3 antagonists is currently enforced by additional arguments. Thus, whereas mCPBG, a 5HT_3 agonist, impairs the retention of an associative learning task in rat, 5-HT_3 antagonists, ondansetron and tropisetron improve it (94). Ondansetron prevents scopolamine-induced impairment of short-term memory retrieval in the mouse (95) as well as intrahippocampal scopolamine-induced impairment in spatial learning in the rat (96). Y-25130, a 5-HT_3 antagonist, also has a similar effect on working memory in the rat. This compound, however, was found ineffective to compensating for memory impairment caused by blockade of glutamatergic transmission (97).

Interestingly, Pitsikas and Borsini (98) demonstrated that ondansetron (on passive avoidance) and tropisetron (on spatial learning) had different efficacies on scopolamine-induced memory impairment. The authors suggested that different subtypes of the 5-HT_3 receptor might explain such a functional dissociation, a hypothesis that emerges from recent studies (99). But it must also be kept in mind that different cerebral structures or circuits preferentially underlie the various tasks. Hence, Arnsten and co-workers (100) observed mixed drug responses obtained with two 5-HT_3 antagonists (ondansetron and SEC-579) in cognitive improvement of aged monkeys in the reversal of a visual discrimination task. In the task some monkeys either markedly improved or impaired, depending probably on the competition for control of cognitive processes between the orbital prefrontal cortex and the inferior temporal cortex. In addition, RS-56812, a novel 5-HT_3 antagonist, was observed to facilitate short-term memory rather than attention in monkeys (101).

The cognitive-enhancing property of ondansetron in aged rats is significant and selective in cognitively impaired animals (as determined by a prescreening procedure) when compared with that of a cholinergic agonist (102). This prescreening procedure might be a particularly sensitive method for detecting those aged subjects who sustain critical memory deficits that can be reversed with drugs. In addition, 5-HT_3 antagonists, in contrast to nicotine, have no effect on acquisition of the water maze task in intact rats, but antagonize both acquisition and retention impairments in rats sustaining a combined lesion of the medial septum and the NBM (103).

Taken together, these data clearly suggest that the efficacy of 5-HT_3 receptor antagonists in compensating for cholinergic dysfunction may be better than that of the cholinergic agonists. However, extrapolation to humans is not so clear, as it was found recently that ondansetron is not able to attenuate the

scopolamine-induced impairment of episodic memory in young healthy volunteers (104). In the context of emotional memory, Yoshioka and co-workers (105) have observed a correlation between CFS-induced freezing behaviour and an increase in the extracellular concentration of 5-HT in the rat prefrontal cortex; both the behavioural and biochemical responses were abolished by 5-HT_3 receptor antagonists.

There is no doubt that the 'cognitive profile' of the recent model of 5-HT_3 receptor knock-out mouse will be of great interest (106).

How are 5-HT_3 receptors involved in the mechanisms underlying learning and memory? The inactivation of 5-HT_3 receptors has been found to increase the frequency of the hippocampal theta rhythm and the magnitude and duration of long-term potentiation in field CA1 of the hippocampus of freely moving rats (107). These neurophysiological effects have been observed to correlate with improved retention in both spatial and olfactory memory tasks, behaviours which require the integrity of the hippocampus (107). It is worth underlining here how fruitful the combination of electrophysiological and behavioural approaches in freely moving animals can be. 5-HT_3 receptors occupy a somatodendritic subcellular position and are widely distributed in the cortex and in all the subfields of the hippocampus (Fig 1). 5-HT_3 receptors have been detected on GABAergic interneurones in the rat hippocampus and cortex (108). As a consequence, systemic administration of ondansetron decreases the firing activity of CA1 hippocampal interneurones, with concomitant increases in the firing rate of pyramidal cells (109). Ramirez and co-workers (110) have demonstrated that in the entorhinal cortex 5-HT_3 receptors (probably located on GABAergic interneurones) tonically inhibit ACh release.

This model of 5-HT_3 receptor-mediated control of an inhibitory influence on cholinergic neurones and modulation of the hippocampal theta rhythm and long-term potentiation may underpin the so-called 'memory-enhancing' property of various 5-HT_3 antagonists. These compounds may be used in potential therapies aimed at correcting memory deficits resulting from cholinergic hypofunction (111).

The 5-HT_4 receptor

The 5-HT_4 receptor is present in the limbic system, particularly in the septo–hippocampal formation (Fig 1). 5-HT_4 receptor agonists stimulate adenylate cyclase, thereby increasing cAMP levels and producing a decreased after-hyperpolarization that may increase neuronal excitability and neurotransmitter release (112). The stimulation of 5-HT_4 receptors by specific agonists increases the release of ACh in the rat frontal cortex, but not in the striatum (112), as well as the extracellular level of 5-HT in the hippocampus (113). Hence, the number of hippocampal 5-HT_4 receptors was found reduced in patients with AD (114).

These basic modifications in neuronal excitability and/or neurotransmitter release by 5-HT_4 ligands in anatomical structures underlying learning and memory suggest an active role for these receptors in these functions. RS67333, a selective 5-HT_4 agonist, prevents the performance deficit induced by atropine in rats on the Morris water maze, an effect reversed by the selective 5-HT_4 antagonist RS67532 (115). RS17017, another 5-HT_4 agonist, was found to enhance dose and delay-dependently matching-to-sample performance in aged and young monkeys (116). Even if the 5-HT_4 receptors do not seem to be tonically activated, the pretraining administration of various 5-HT_4 agonists generally improves memory (115, 117–119), whereas the post-training (consolidation period) administration generally impairs it (119).

On the whole, these experimental arguments suggest a beneficial role for 5-HT_4 receptor agonists in learning and memory dysfunctions.

Conclusions and perspectives

Most of the research analysed in this review comes from laboratories specialized in basic research in that they contribute to our insight on the mechanisms of memory formation and memory dysfunction. The practical applications of this research correspond to a crucial need in the population. Age-induced memory disabilities are considered as 'normal', except in case of premature serious pathologies such as AD. There is no miraculous treatment for these memory deficits. They correlate well with the progressive degradation of the cholinergic system (27), and the preponderant pharmacological strategy has thus far been cholinergic in nature.

However, 5-HT constitutes another possibility as the degradation of the cholinergic system is aggravated by the inhibitory influence of 5-HT on this system via the different specific receptors. Converging evidence indicates that different receptor subtypes potentially interact to contribute to a particular function (120). This is the case with 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 and 5-HT_4 receptor subtypes in hippocampal functions, and probably also with 5-HT_2 and 5-HT_3 receptors in those functions which depend more critically on cortical control. Evidence is emerging regarding the underlying neurobiological mechanisms; however, uncertainties persist (see Fig 1).

Throughout the review it has been suggested that 5-HT plays a selective, and probably crucial, role in situations involving increased cognitive demand. This has been demonstrated following 5-HT depletion, stimulation of 5-$HT_{2A/2C}$ and 5-HT_4 receptors and inactivation of 5-HT_{1A} and 5-HT_3 receptors. The role

of 5-HT in behavioural inhibition may also involve the stimulation of 5-HT_{1B} receptors.

Given the prevalent influence of 5-HT on emotion, it would be valuable to study more thoroughly how 5-HT, via some of its various receptors, is able to control the interaction between memory and emotion. If 5-HT receptors are involved in the cognitive deficits of AD, they also play a significant role in behavioural disorders accompagning this disease, such as anxiety, agitation, hallucination, psychotic delusions and hostility. Some of these disorders are sensitive, for example, to treatment with 5-HT_2 agonists (121).

Through the development of more specific tools, both pharmacology and molecular biology will contribute to a better understanding of the functions of the 5-HT receptor subtypes. These tools will include new selective antagonists and agonists, as well as the ability to target changes in genes at specific times (eg, to bypass the developmental compensatory mechanisms) and at specific anatomical sites (122–124).

In summary, one of the most interesting lines of research to presently deserving to be supported concretely is the study of the role of the interactive relationship between the cholinergic and the serotonergic systems in memory disabilities resulting from normal ageing or linked to devastating neurodegenerative disorders such as AD. Most of the 5-HT receptors interact with the cholinergic system in the hippocampus and the frontal cortex, which are the main neurobiological substrats underlying learning and memory. The most interesting example of this is the 5-HT_{1B} mutant mouse which, lacking a specific 5-HT receptor, displays enhanced learning and memory performances in adulthood, and is also less affected by age-induced memory impairments than its homologous wild-type. If the 5-HT_{1B} receptor (among 14 other types) is revealed to be a particularly efficient target for pharmacological therapy, we can expect a beneficial role for specific 5-HT_{1B} antagonists in delaying age-related memory deficits.

Building up new pharmacological tools aimed at delaying age-induced memory disabilities must be one of the main aims of this new century as our life expectancy increases progressively, partly as a result of the progress in 'organic' medicine. 'Total amnesia is rare, but we face an 'epidemic' of memory loss. At present there are around 18 million people worldwide with Alzheimer's disease, and this figure is predicted to double in the next 25 years' (125).

This work was supported by the Centre National de la Recherche Scientifique and Université Bordeaux. We thank Michel Chaigniau for the illustration.

References

1. **Buhot M-C.** Serotonin receptors in cognitive behaviors. *Curr Opin Neurobiol* 1997; 7: 243–54.
2. **Sirviö J, Riekkinen P Jr, Jäkälä P, Riekkinen PJ.** Experimental studies on the role of serotonin in cognition. *Prog Neurobiol* 1994; 43: 363–79.
3. **Soubrié P.** Reconciling the role of central serotonin neurons in human and animal behaviour. *Behav Brain Sci* 1986; 9: 319–64.
4. **Wogar MA, Bradshaw CM, Szabadi E.** Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on choice between delayed reinforcers. *Psychopharmacology* 1993; 111: 239–43.
5. **Asin KE, Wirtshafter D, Kent EW.** Impaired patterned responding in rats with electrolytic median raphe lesions. *Physiol Behav* 1979; 23: 803–6.
6. **Beninger RJ, Phillips AG.** Possible involvement of serotonin in extinction. *Pharmacol Biochem Behav* 1979; 10: 37–41.
7. **Gonzalez-Burgos I, Perez-Vega MI, Del Angel-Meza AR, Feria-Velasco A.** Effect of tryptophan restriction on short-term memory. *Physiol Behav* 1998; 63: 165–9.
8. **Riedel WJ, Klaassen T, Deutz NE, van Someren A, van Praag HM.** Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacology* 1999; 141: 362–9.
9. **Stancampiano R, Cocco S, Melis F, Cugusi C, Sarais L, Fadda F.** The decrease of serotonin release induced by tryptophan-free amino acid diet does not affect spatial and passive avoidance learning. *Brain Res* 1997; 762: 269–74.
10. **Mazer C, Muneyyirci J, Taheny K, Raio N, Borella A, Whitaker-Azmitia P.** Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits. *Brain Res* 1997; 760: 68–73.
11. **Brezun JM, Daszuta A.** Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* 1999; 89: 999–1002.
12. **Altman HJ, Normile HJ, Galloway MP, Ramirez A, Azmitia EC.** Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. *Brain Res* 1990; 18: 61–6.
13. **Ward BO, Wilkinson LS, Robbins TW, Everitt BJ.** Forebrain serotonin depletion facilitates acquisition and performance of a conditional visual discrimination task in rats. *Behav Brain Res* 1999; 100: 51–65.
14. **Al-Zahrani SSA, Ho M-Y, Velazquez-Martinez DN, Lopez Cabrera M, Bradshaw CM, Szabadi E.** Effect of destruction of the 5-hydroxytryptaminergic pathways on the acquisition of temporal discrimination and memory for duration in a delayed conditional discrimination task. *Psychopharmacology* 1996; 123: 103–10.
15. **Lister S, Pearce JM, Butcher SP, Collard KJ, Foster GA.** Acquisition of conditioned inhibition in rats is impaired by ablation of serotoninergic pathways. *Eur J Neurosci* 1996; 8: 415–23.
16. **Avanzi V, Castilho VM, de Andrade TG, Brandao ML.** Regulation of contextual conditioning by median raphe nucleus. *Brain Res* 1998; 790: 178–84.

17. **Santucci AC, Knott PJ, Haroutunian V.** Excessive serotonin release, not depletion, leads to memory impairments in rats. *Eur J Pharmacol* 1996; 295: 7–17.
18. **Nelson CJ, Jordan WP, Bohan RT.** Daily fluoxetine administration impairs avoidance learning in the rat without altering sensory thresholds. *Prog Neuro-Psychopharmacol Biol Psychiat* 1997; 21: 1043–57.
19. **Richter-Levin G, Segal M.** Serotonin, aging and cognitive functions of the hippocampus. *Rev Neurosci* 1996; 7: 103–13.
20. **Morgan MJ.** Memory deficits associated with recreational use of 'ectasy' (MDMA). *Psychopharmacology* 1999; 141: 30–6.
21. **Inoue T, Tsuchiya K, Koyama T.** Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmacol Biochem Behav* 1994; 49: 911–20.
22. **Inoue T, Tsuchiya K, Koyama T.** Serotonin activation reduces defensive freezing in the conditioned fear paradigm. *Pharmacol Biochem Behav* 1996; 53: 825–31.
23. **Wilkinson LS, Humby T, Robbins TW, Everitt BJ.** Differential effects of forebrain 5-hydroxytryptamine depletions on Pavlovian aversive conditioning to discrete and contextual stimuli in the rat. *Eur J Neurosci* 1995; 7: 2042–52.
24. **Graeff FG, Viana MB, Mora PO.** Opposed regulation by dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T-maze. *Pharmacol Biochem Behav* 1996; 53: 171–7.
25. **Handa RJ, George M, Gordon BH, Campbell DB, Lorens SA.** Responses to novelty stress in female F344 rats: effects of age and d-fenfluramine. *Pharmacol Biochem Behav* 1996; 53: 641–7.
26. **Harrison AA, Everitt BJ, Robbins TW.** Central serotonin depletion facilitates the acquisition and performance of a conditional visual discrimination task in rats. *Behav Brain Res* 1999; 100: 99–112.
27. **Sirviö J.** Strategies that support declining cholinergic neurotransmission in Alzheimer's disease patients. *Gerontology* 1999; 45 Suppl 1: 3–14.
28. **Richter-Levin G, Segal M.** Age-related cognitive deficits in rats are associated with a combined loss of cholinergic and serotonergic functions. *Ann N Y Acad Sci* 1993; 695: 254–7.
29. **Decker MW, McGaugh JL.** The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* 1991; 7: 151–68.
30. **Cassel J-C, Jeltsch H.** Serotonergic modulation of cholinergic function in the central nervous system: cognitive implications. *Neuroscience* 1995; 69: 1–41.
31. **Steckler T, Sahgal A.** The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. *Behav Brain Res* 1995; 67: 165–99.
32. **Ruotsalainen S, Miettinen R, MacDonald E, Riekkinen M, Sirviö J.** The role of the dorsal raphe-serotonergic system and cholinergic receptors in the modulation of working memory. *Neurosci Biobehav Rev* 1998; 22: 21–31.
33. **Markowska AL, Wenk GL.** Serotonin influences the behavioral recovery of rats following nucleus basalis lesions. *Pharmacol Biochem Behav* 1991; 38: 731–7.
34. **Kocsis B, Vertes RP.** Midbrain raphe cell firing and hippocampal theta rhythm in urethane anaesthetized rats. *Neuroreport* 1996; 7: 2867–72.
35. **McQuade R, Sharp T.** Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *J Neurochem* 1997; 69: 791–6.
36. **Matsukawa M, Ogawa M, Nakadate K, Maeshima T, Ichitani Y, Kawai N, et al.** Serotonin and acetylcholine are crucial to maintain hippocampal synapses and memory acquisition in rats. *Neurosci Lett* 1997; 230: 13–6.
37. **Stancampiano R, Cocco S, Cugusi C, Sarais L, Fadda F.** Serotonin and acetylcholine release response in the rat hippocampus during a spatial memory task. *Neuroscience* 1999; 89: 1135–43.
38. **Beiko J, Candusso L, Cain DP.** The effect of nonspatial water maze pretraining in rats subjected to serotonin depletion and muscarinic receptor antagonism: a detailed behavioural assessment of spatial performance. *Behav Brain Res* 1997; 88: 201–11.
39. **Harder JA, Kelly ME, Cheng CH, Costall B.** Combined pCPA and muscarinic antagonist treatment produces a deficit in rat water maze acquisition. *Pharmacol Biochem Behav* 1996; 55: 61–5.
40. **Harder JA, Kelly ME.** The effect of several putative cognition enhancers on a water maze acquisition deficit induced by pCPA + scopolamine combination treatment. *Pharmacol Biochem Behav* 1997; 56: 657–61.
41. **Balse E, Lazarus C, Kelche C, Jeltsch H, Jackisch R, Cassel J-C.** Intrahippocampal grafts containing cholinergic and serotonergic fetal neurons ameliorate spatial reference but not working memory in rats with fimbria-fornix/cingular bundle lesions. *Brain Res Bull* 1999; 49: 263–72.
42. **Milner TA, Veznedaroglu E.** Serotonin-containing terminals synapse on septohippocampal neurons in the rat. *J Neurosci Res* 1993; 36: 260–71.
43. **Farr SA, Uezu K, Flood JF, Morley JE.** Septo-hippocampal drug interactions in post-trial memory processing. *Brain Res* 1999; 847: 221–30.
44. **Hoyer D, Martin GR.** Classification and nomenclature of 5-HT receptors: a comment on current issues. *Behav Brain Res* 1996; 73: 263–8.
45. **Gobert A, Rivet JM, Cistarelli L, Millan MJ.** Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely moving rats by combined blockade of 5-HT1A and 5-HT1B receptors with WAY 100,635 and GR 127,935. *J Neurochem* 1997; 68: 1159–63.
46. **Hajós-Korcsok E, Sharp T.** 8-OH-DPAT-induced release of hippocampal noradrenaline in vivo: evidence for a role of both 5-HT1A and dopamine D1 receptors. *Eur J Pharmacol* 1996; 314: 285–91.
47. **Herremans AHJ, Hijzen TH, Olivier B, Slangen JL.** Serotonergic drug effects on a delayed conditional discrimination task in the rat: involvement of the 5-HT1A receptor in working memory. *J Psychopharmacol* 1995; 9: 242–50.
48. **Warburton EC, Harrison AA, Robbins TW, Everitt BJ.** Contrasting effects of systemic and intracerebral infusions of the 5-HT1A receptor agonist 8-OH-DPAT on spatial short-term working memory in rats. *Behav Brain Res* 1997; 84: 247–58.
49. **Ohno M, Watanabe S.** Blockade of 5-HT1A receptors compensates loss of hippocampal cholinergic neurotransmission involved in working memory of rats. *Brain Res* 1996 736: 180–8.
50. **Ohno M, Kishi A, Watanabe S.** Effect of cholinergic activation by physostigmine on working memory failure caused in rats by pharmacological manipulation of hippocampal glutamatergic and 5-HTergic neurotransmission. *Neurosci Lett* 1996; 217: 21–4.
51. **Kant GJ, Meininger GR, Maughan KR, Wright WL, Robinson TN III, Neely TM.** Effects of the serotonin receptor agonists 8-OH-DPAT and TFMPP on learning as assessed using a novel water maze. *Pharmacol Biochem Behav* 1996; 53: 385–90.
52. **Riekkinen M, Sirviö J, Toivanen T, Riekkinen P Jr.** Combined treatment with a 5-HT1A receptor agonist and a muscarinic acetylcholine receptor antagonist disrupts water maze navigation behavior. *Psychopharmacology* 1995;

122:137–46.
53. **Carli M, Luschi R, Garofalo P, Samanin R.** 8-OH-DPAT impairs spatial but not visual learning in a water maze by stimulating 5-HT1A receptors in the hippocampus. *Behav Brain Res* 1995; 67: 67–74.
54. **Carli M, Bonalumi P, Samanin R.** Stimulation of 5-HT1A receptors in the dorsal raphe reverses the impairment of spatial learning caused by intrahippocampal scopolamine in rats. *Eur J Pharmacol* 1998; 10: 221–30.
55. **Carli M, Balducci C, Millan MJ, Bonalumi P, Samanin R.** S 15535, a benzodioxopiperazine acting as presynaptic agonist and postsynaptic 5-HT1A receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal scopolamine. *Br J Pharmacol* 1999; 128: 1207–14.
56. **Fletcher A, Forster EA, Bill DJ, Brown G, Cliffe IA, Hartley JE, et al.** Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT1A receptor antagonist. *Behav Brain Res* 1996; 73: 337–53.
57. **Carli M, Luschi R, Samanin R.** (S)-WAY 100135, a 5-HT1A receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal scopolamine. *Eur J Pharmacol* 1995; 283: 133–9.
58. **Carli M, Bonalumi P, Samanin R.** WAY 100635, a 5-HT1A receptor antagonist, prevents the impairment of spatial learning caused by intrahippocampal administration of scopolamine or 7-chloro-kynurenic acid. *Brain Res* 1997; 774: 167–74.
59. **Carli M, Silva S, Balducci C, Samanin R.** Way 100635, a 5-HT1A receptor antagonist, prevents the impairment of spatial learning caused by blockade of hippocampal NMDA receptors. *Neuropharmacology* 1999; 38: 1165–73.
60. **Harder JA, Maclean CJ, Alder JT, Francis PT, Ridley RM.** The 5-HT1A antagonist, WAY 100635, ameliorates the cognitive impairment induced by fornix transection in the marmoset. *Psychopharmacology* 1996; 127: 245–54.
61. **Guimares FS, Del Bel EA, Padovan CM, Netto SM, de Almeida RT.** Hippocampal 5-HT receptors and consolidation of stressful memories. *Behav Brain Res* 1993; 58: 133–9.
62. **Liang KC, Tsui KY, Tyan YM, Chiang TC.** Buspirone impaired acquisition and retention in avoidance tasks: involvement of the hippocampus. *Chin J Physiol* 1998; 41: 33–44.
63. **Maier SF, Grahn RE, Watkins LR.** 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear-conditioning and interference with escape produced by exposure to inescapable shock. *Behav Neurosci 1995* 109: 404–12.
64. **Balleine BW, Fletcher N, Dickinson A.** Effect of the 5-HT1A agonist, 8-OH-DPAT on instrumental performance in rats. *Psychopharmacology* 1996; 125: 79–88.
65. **Ramboz S, Oosting R, Ait Amara D, Kung HF, Blier P, Mendelsohn M, et al.** Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* 1998; 95: 14476–81.
66. **Parks CL, Robinson PS, Sibille E, Shenk T, Toth M.** Increased anxiety of mice lacking the serotonin1A receptor. *Proc Natl Acad Sci U S A* 1998; 95: 10734–9.
67. **Heisler LK, Chu H-M, Brennan TJ, Danao JA, Bajwa P, Parsons LH, et al.** Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. *Proc Natl Acad Sci U S A* 1998; 95: 15049–54.
68. **Kinney GG, Kocsis B, Vertes RP.** Medial septal unit firing characteristics following injections of 8-OH-DPAT into the median raphe nucleus. *Brain Res* 1996; 708: 116–22.
69. **Kia HK, Brisorgueil MJ, Daval G, Langlois X, Hamon M, Vergé D.** Serotonin1A receptors are expressed by a subpopulation of cholinergic neurons in the rat medial septum and diagonal band of Broca. A double immunocytochemical study. *Neuroscience* 1996; 74: 143–54.
70. **Schmitz D, Empson RM, Heinemann U.** Serotonin reduces inhibition via 5-HT1A receptors in area CA1 of rat hippocampal slices in vitro. *J Neurosci* 1995 15: 7217–25.
71. **Koyama T, Nakajima Y, Fujii T, Kawashima K.** Enhancement of cortical and hippocampal cholinergic neurotransmission through 5-HT1A receptor-mediated pathways by BAY x 3702 in freely moving rats. *Neurosci Lett* 1999; 265: 33–6.
72. **Nakai K, Fujii T, Fujimoto K, Suzuki T, Kawashima K.** Effect of WAY-100135 on the hippocampal acetylcholine release potentiated by 8-0H-DPAT, a serotonin1A receptor agonist, in normal and p-chlorophenylalanine-treated rats as measured by in vivo microdialysis. *Neurosci Res* 1998; 31: 23–9.
73. **Buhot M-C, Patra SK, Naïli S.** Spatial memory deficits following stimulation of hippocampal 5-HT1B receptors in the rat. *Eur J Pharmacol* 1995; 285: 221–8.
74. **Sari Y, Lefèvre K, Bancila M, Quignon M, Miquel MC, Langlois X, et al.** Light and electron microscopic immunocytochemical visualization of 5-HT1B receptors in the rat brain. *Brain Res* 1997; 760: 281–86.
75. **Cassel J-C, Jeltsch H, Neufang B, Lauth D, Szabo B, Jackisch R.** Downregulation of muscarinic- and 5-HT1B-mediated modulation of [^{3}H]acetylcholine release in hippocampal slices of rats with fimbria-fornix lesions and intrahippocampal grafts of septal origin. *Brain Res* 1995; 704: 153–66.
76. **Aït Amara D, Segu L, Naïli S, Buhot M-C.** Serotonin 1B receptor regulation after dorsal subiculum deafferentation. *Brain Res Bull* 1995; 38: 17–23.
77. **Boeijinga PH, Boddeke HWGM.** Activation of 5-HT1B receptors suppresses low but not high frequency synaptic transmission in the rat subicular cortex in vitro. *Brain Res* 1996; 721: 59–65.
78. **Consolo S, Arnaboldi S, Ramponi S, Nannini L, Ladinsky H, Baldi G.** Endogenous serotonin facilitates in vivo acetylcholine release in rat frontal cortex through 5-HT1B receptors. *J Pharmacol Exp Ther* 1996; 277: 823–30.
79. **Meneses A, Terrón JA, Hong E.** Effects of the 5-HT receptor antagonists GR127935 (5-HT1B/1D) and MDL 100907 (5-HT2A) in the consolidation of learning. *Behav Brain Res* 1997; 89: 217–23.
80. **Saudou F, Aït Amara D, Dierich A, LeMeur M, Ramboz S, Segu L, et al.** Enhanced aggressive behavior in mice lacking 5-HT1B receptor. *Science* 1994; 265: 1875–8.
81. **Ramboz S, Saudou F, Aït Amara D, Belzung C, Segu L, Misslin R, et al.** 5-HT1B receptor knock out – behavioral consequences. *Behav Brain Res* 1996; 73: 305–12.
82. **Malleret G, Hen R, Guillou J-L, Segu L, Buhot M-C.** 5-HT1B receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. *J Neurosci* 1999; 19: 6157–68.
83. **Buhot M-C, Naïli S.** Changes in exploratory activity following stimulation of hippocampal 5-HT1A and 5-HT1B receptors in the rat. *Hippocampus* 1995; 5: 198–208.
84. **Boutrel B, Franc B, Hen R, Hamon M, Adrien J.** Key role of 5-HT1B receptors in the regulation of paradoxical sleep as evidenced in 5-HT1B knock-out mice. *J Neurosci* 1999; 19: 3204–12.
85. **Hennevin E, Hars B, Maho C, Bloch V.** Processing of learned information in paradoxical sleep: relevance for memory. *Behav Brain Res* 1995; 69: 125–35.
86. **Malleret G, Savova M, Hen R, Segu L, Buhot M-C.** 5-HT1B receptor mutation decreases age-related impairment of spatial reference memory performance. *Soc Neurosci Abst* 1999; 25: 2156.
87. **Liu W, Alreja M.** Atypical antipsychotics block the excitatory effects of serotonin in septohippocampal neurons in the rat. *Neuroscience* 1997; 79: 369–82.
88. **Hirano H, Day J, Fibiger HC.** Serotonergic regulation of

acetylcholine release in rat frontal cortex. *J Neurochem* 1995; 65: 1139–45.
89. **Harvey JA.** Serotonergic regulation of associative learning. *Behav Brain Res* 1996; 73: 47–50.
90. **Nowakowska E, Chodera A, Kus K, Rybakowski J.** Some behavioural effects of risperidone in rats: comparison with haloperidol. *Eur Neuropsychopharmacol* 1999; 9: 421–6.
91. **Applegate CD, Tecott LH.** Global increases in seizure susceptibility in mice lacking 5-HT2C receptors: a behavioral analysis. *Exp Neurol* 1998;154: 522–30.
92. **Tecott LH, Logue SF, Wehner JM, Kauer JA.** Perturbed dentate gyrus function in serotonin 5-HT2C receptor mutant mice. *Proc Natl Acad Sci U S A* 1998; 95: 15026–31.
93. **Barnes JM, Costall B, Coughlan J, Domeney AM, Gerrard PA, Kelly ME, et al.** The effects of ondansetron, a 5-HT3 receptor antagonist, on cognition in rodents and primates. *Pharmacol Biochem Behav* 1990; 35: 955–62.
94. **Hong E, Meneses A.** Systemic injection of p-chloroamphetamine eliminates the effect of the 5-HT3 compounds on learning. *Pharmacol Biochem Behav* 1996; 53: 765–9.
95. **Roychoudhury M, Kulkarni SK.** Effects of ondansetron on short-term memory retrieval in mice. *Meth Find Exp Clin Pharmacol* 1997; 19: 43–6.
96. **Carli M, Luschi R, Samanin R.** Dose–related impairment of spatial learning by intrahippocampal scopolamine: antagonism by ondansetron, a 5–HT3 receptor antagonist. *Behav Brain Res* 1997; 82: 185–94.
97. **Ohno M, Watanabe S.** Differential effects of 5-HT3 receptor antagonism on working memory failure due to deficiency of hippocampal cholinergic and glutamatergic transmission in rats. *Brain Res* 1997; 762: 211–5.
98. **Pitsikas N, Borsini F.** Different effects of tropisetron and ondansetron in learning and memory paradigms. *Pharmacol Biochem Behav* 1997; 56: 571–6.
99. **Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG, et al.** The 5-HT3B subunit is a major determinant of serotonin-receptor function. *Nature* 1999; 397: 359–63.
100. **Arnsten AFT, Lin CH, Van Dyck CH, Stanhope KJ.** The effects of 5-HT3 receptor antagonists on cognitive performance in aged monkeys. *Neurobiol Aging* 1997; 18: 21–8.
101. **Terry AVJr, Buccafusco JJ, Prendergast MA, Jackson WJ, Fontana DL, Wong EHF, et al.** The 5-HT3 receptor antagonist, RS-56812, enhances delayed matching performance in monkeys. *Neuroreport* 1996; 8: 49–54.
102. **Fontana DJ, Daniels SE, Henderson C, Eglen RM, Wong EHF.** Ondansetron improves cognitive performance in the Morris water maze spatial navigation task. *Psychopharmacology* 1995; 120: 409–17.
103. **Hodges H, Sowinski P, Turner JJ, Fletcher A.** Comparison of the effects of the 5-HT3 receptor antagonists, WAY-100579 and ondansetron on spatial learning in the water maze in rats with excitotoxic lesions of the forebrain cholinergic projection system. *Psychopharmacology* 1996; 125: 146–61.
104. **Brooks A, Little JT, Martin A, Minichiello MD, Dubbert B, Mack C, et al.** The influence of ondansetron and m-chlorophenylpiperazine on scopolamine-induced cognitive, behavioral, and physiological responses in young healthy controls. *Biol Psychiatry* 1998; 43: 408–16.
105. **Yoshioka M, Marsumoto M, Togashi H, Saito H.** Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. *Pharmacol Biochem Behav* 1995; 51: 515–9.
106. **Guy NTM, Malmberg AB, Basbaum AL, Julius D.** Pain behaviour in 5-HT3 receptor mutant mice. *Soc Neurosci Abstr* 1997, 23: 290.
107. **Staubli U, Xu FB.** Effects of 5-HT-sub-3 receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. *J Neurosci* 1995; 15: 2445–52.
108. **Morales M, Bloom FE.** The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J Neurosci* 1997; 17: 3157–67.
109. **Reznic J, Staubli U.** Effects of 5-HT3 receptor antagonism on hippocampal cellular activity in the freely moving rat. *J Neurophysiol* 1997; 77: 517–21.
110. **Ramirez MJ, Cenarruzabeitia E, Lasheras B, Del Rio J.** Involvement of GABA systems in acetylcholine release induced by 5-HT3 receptor blockade in slices from rat entorhinal cortex. *Brain Res* 1996; 712: 274–80.
111. **Passani MB, Blandina P.** Cognitive implications for H3 and 5-HT3 receptor modulation of cortical cholinergic function: a parallel story. *Meth Find Exp Clin Pharmacol* 1998; 20: 725–33.
112. **Eglen RM, Wong EHF, Dumuis A, Bockaert J.** Central 5-HT4 receptors. *Trends Pharmacol Sci* 1995; 16: 391–8.
113. **Van Ge J, Barnes NM.** 5-HT4 receptor-mediated modulation of 5-HT release in the rat hippocampus in vivo. *Br J Pharmacol* 1996; 117: 1475–80.
114. **Wong EHF, Reynolds GP, Bonhaus DW, Hsu S, Eglen RM.** Characterization of [3H] GR 113808 binding to 5-HT4 receptors in brain tissues from patients with neurodegenerative disorders. *Behav Brain Res* 1996; 73: 249–52.
115. **Fontana DJ, Daniels SE, Wong EHF, Clark RD, Eglen RM.** The effects of novel, selective 5-hydroxytryptamine (5HT)4 receptor ligands in rat spatial navigation. *Neuropharmacology* 1997; 36: 689–96.
116. **Terry AV, Buccafusco JJ, Jackson WJ, Prendergast MA, Fontana DJ, Wong EHF, et al.** Enhanced delayed matching performance in younger and older macaques administered the 5-HT4 receptor agonist, RS 17017. *Psychopharmacology* 1998; 135: 407–15.
117. **Letty S, Child R, Dumuis A, Pantaloni A, Bockaert J, Rondouin G.** 5-HT4 receptors improve social olfactory memory in the rat. *Neuropharmacology* 1997; 36: 681–7.
118. **Marchetti-Gauthier E, Roman FS, Dumuis A, Bockaert J, Soumireu-Mourat B.** BIMU1 increases associative memory in rats by activating 5-HT4 receptors. *Neuropharmacology* 1997; 36: 697–706.
119. **Meneses A, Hong E.** Effects of 5-HT4 receptor agonists and antagonists in learning. *Pharmacol Biochem Behav* 1997; 56: 347–51.
120. **Uphouse L.** Multiple serotonin receptors: too many, not enough, or just the right number? *Neurosci Biobehav Rev* 1997; 21: 679–98.
121. **Stoppe G, Brandt CA, Staedt JH.** Behavioural problems associated with dementia: the role of newer antipsychotics. *Drugs Aging* 1999; 14: 41–54.
122. **Mayford M, Mansuy IM, Muller RU, Kandel ER.** Memory and behavior: a second generation of genetically modified mice. *Curr Biol* 1997; 7: R580–9.
123. **Stark KL, Ghavami A, Segu L, Mayford M, Kandel K, Hen R.** Inducible knockout and tissue-specific rescue of the 5-HT1B receptor. *Soc Neurosci Abstr* 1997; 23: 290.
124. **Mansuy IM, Winder DG, Moallem TM, Osman M, Mayford M, Hawkins RD, et al.** Inducible and reversible gene expression with the rtTA system for the study of memory. *Neuron* 1998; 21: 257–65.
125. **Fletcher L.** Memories are made of this: the genetic basis of memory. *Mol Med Today* 1997; 3: 429–34.