

A role for the 5-HT_{1A}, 5-HT₄ and 5-HT₆ receptors in learning and memory

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The serotonergic system is implicated in the neurobiological control of learning and memory, both in healthy individuals and pathological disorders, although the underlying mechanisms remain elusive. After the cloning and characterization of serotonin, or 5-hydroxytryptamine (5-HT), receptors and the resultant development of selective agonist and antagonist compounds and transgenic receptor-knockout mice, our understanding of the role of various serotonin receptors in learning and memory has improved. 5-HT_{1A}, 5-HT₄ and 5-HT₆ receptors are densely expressed in brain regions innervated by serotonergic projections from the raphe nuclei and are associated with learning and memory. Here, we review the evidence that compounds acting on these receptors can have positive effects on learning and memory, and we discuss the potential mechanisms involved. This information raises the possibility that such compounds could be developed as adjunct therapeutics with existing treatments to improve learning and memory deficits, which are core symptoms of Alzheimer's disease, schizophrenia and depression.

Introduction

Learning and memory is currently a major area of research because not only do these processes underpin normal human behaviour but they are also essential abnormal behavioural components in disorders ranging from addictions, anxiety, depression, schizophrenia and neurodegenerative diseases such as Parkinson's and Alzheimer's disease. There are currently no effective treatments for these learning and memory impairments so there is considerable interest in developing novel therapeutic approaches to treat this aspect; and drugs acting at specific serotonin, or 5-hydroxytryptamine (5-HT), receptors are one such strategy. The search for compounds that either reverse cognitive deficits associated with disease or even improve normal cognitive functioning, the so-called cognitive enhancers (Box 1), is an active area of neuropharmacological research. The serotonergic system innervates specific forebrain areas that are important in the regulation of memory and learning processes (Box 2); this innervation is closely associated with the expression of a range of 5-HT receptors (Figure 1), making serotonin an obvious target for investigation. Important features of the serotonergic system are the extensive nature of the forebrain innervation derived from discrete clusters of neurones situated in the raphe nuclei and the diversity of its receptors, of which there are 14. These receptors include 5-HT_{1A},

5-HT₄ and 5-HT₆, all of which are densely expressed in brain regions associated with learning and memory and have been implicated in various human cognitive disorders. Furthermore, selective agonist and/or antagonist compounds seem to have potent pro-cognitive effects in preclinical behavioural paradigms by acting on these three receptors, which are each located postsynaptically in relation to serotonergic terminals. Binding of these compounds to their receptor also modulates similar neurotransmitter systems, indirectly modifying cholinergic, γ -aminobutyric acid (GABA)ergic and/or glutamatergic pathways thought to control learning and memory, rather than altering 5-HT release. Work over several years has identified the function of specific 5-HT receptors. For example, the 5-HT_{1A} receptor functions both as the somatodendritic inhibitory autoreceptor, involved in the autoregulation of 5-HT neuronal function in the raphe nuclei, in addition to being found at postsynaptic locations in the brain (such as on hippocampal pyramidal and granule cells, where it causes neuronal inhibition; Figure 1). This dual functional distribution makes it important to identify which synaptic location behavioural responses are derived from because responses mediated by the autoreceptor implicate reduced serotonergic neuronal activity and release, whereas postsynaptic receptor activation is associated with increased 5-HT release. The 5-HT₄ receptor is expressed in abundance in hippocampal, cortical and striatal regions and is known to play an important part in the regulation of hippocampal acetylcholine release (Figure 1). The 5-HT₆ receptor is also expressed in abundance in the frontal cortex, hippocampus, amygdala and striatum, where it seems to be

Glossary

Attentional set shifting: discrimination of the salient stimulus category to locate food reward, requiring attention and executive function involving the frontal cortex.

Autoshaping: food-motivated conditioned operant response task, involving associative learning.

Contextual-fear conditioning: conditioned emotional associative memory involving hippocampal and amygdala function in contextual retention trials.

Delayed non-match to sample: working memory task, distinguishing representation of a familiar from a nonfamiliar cue to obtain reward after variable inter-trial intervals.

Morris water maze: escape motivated hippocampal-dependent spatial-memory task.

Novel object recognition: declarative memory task involving innate preference for unfamiliar objects involving the entorhinal and perirhinal cortices and hippocampus.

Olfactory associative learning: olfactory recognition memory task involving orbitofrontal, entorhinal and perirhinal cortices.

Social recognition: short-term working memory in the domain of recognition of familiar and unfamiliar conspecifics.

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Box 1. Cognitive enhancers

Drugs that improve aspects of memory and learning, so-called 'smart drugs', nootropic agents or cognitive enhancers, function through a large variety of distinct pharmacological mechanisms, as briefly discussed here. Such cognitive enhancers are being developed to aid attention span, learning and memory in people with dementia or cognitive impairment, either as a result of the normal ageing processes or due to pathological conditions such as Alzheimer's disease. However, cognitive enhancers might also be used by individuals with no particular learning and memory impairment who wish to enhance cognitive performance by maintenance of wakefulness and/or attention. The first four groups of compounds listed are available on prescription for dementia associated with Alzheimer's or Parkinson's disease, inattentive behaviour in attention deficit hyperactivity disorder (ADHD) or narcolepsy, but most are currently preclinical experimental tools, some examples of which are given here.

- Acetylcholinesterase inhibitors [such as donepezil (Aricept), rivastigmine (Exelon) or galantamine (Razadyne)] are competitive reversible inhibitors of the cholinesterase enzyme responsible for biological inactivation of acetylcholine and, thus, potentiate the action of this neurotransmitter in central pathways involved in cognition. These drugs are licensed for use in mild-to-moderately severe dementia in Alzheimer's disease, in which degeneration of cholinergic pathways involved in learning and memory is a prominent feature.
- Memantine (Ebixa) is a voltage-dependent, moderate-affinity, uncompetitive *N*-methyl-D-aspartic acid (NMDA)-receptor antagonist that is thought to block the effects of pathologically elevated glutamate levels that might lead to neuronal dysfunction and resultant cognitive impairment. It has been licensed for use in moderately severe-to-severe Alzheimer's disease and can be used in clinical trials with patients who have mild-to-severe dementia.
- Psychostimulants – including amphetamine derivatives (D- and L-amphetamine, such as Adderall) or inhibitors of the dopamine- and noradrenaline-reuptake transporter, such as methylphenidate (e.g. Concerta or Ritalin) – are prescribed to treat the inattentive, hyperactive and impulsive symptoms of children and adolescents suffering from ADHD. Methylphenidate might be most effective in young people, enhancing spatial working memory and cognitive flexibility. Another non-stimulant drug licensed for the treatment of ADHD is the selective noradrenaline-reuptake inhibitor atomoxetine (Strattera), which seems to function by altering both noradrenaline and dopamine function. There are also indications that agonists acting at α_2 -adrenoceptors, such as clonidine and guanfacine, improve attention in ADHD patients.
- Modafinil (Provigil) is a non-stimulant, the pharmacological mechanism of action of which has yet to be determined, and is licensed for sleep disorders such as narcolepsy. However, it has also shown promise in the treatment of small-scale Phase I trials for disorders such as ADHD, producing improvements in verbal working memory, planning performance and executive inhibitory control.

- Serotonin-selective reuptake inhibitors (SSRIs), such as fluoxetine (Prozac), citalopram (Ciprallex) and paroxetine (Seroxat), seem to promote neurogenesis (production of new neurones) in areas such as the hippocampus, which might result in improved cognitive performance and contribute to the beneficial effect of SSRIs in the treatment of depression.
- Ampakines such as Aniracetam, a pyrrolidone derivative, function as positive allosteric modulators of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, the expression of which is upregulated in the process of consolidation and long-term potentiation (LTP) and might enhance synaptic plasticity. Initial Phase I clinical trials with the ampakine CX-516, given as an add-on therapy with clozapine, indicate that it has some beneficial action on the negative symptoms and cognitive deficits in schizophrenia, but more studies are necessary.
- D-Serine and D-cycloserine are agents that stimulate the strychnine-insensitive glycine co-agonist site on the NMDA receptor and form another pharmacological approach to enhance NMDA-receptor function that might be hypofunctional in schizophrenia. These agents seem to improve fear conditioning in preclinical models and might moderately improve both negative symptoms and cognitive dysfunction in patients with schizophrenia, but other studies have found contradictory results.
- Tolcapone and entacapone inhibit catechol-O-methyltransferase (COMT; an enzyme involved in the deactivation of the monoamine neurotransmitters dopamine, noradrenaline and adrenaline) and can increase performance in working-memory tasks in rodents and primates. Polymorphisms occurring in the gene encoding COMT have been associated with working-memory deficits, and individuals with the Val/Val and Val/Met genotype of the Val158Met polymorphism might show altered metabolism of dopamine in the prefrontal cortex and so, theoretically, might benefit from COMT inhibitors.
- Other so-called nootropic drugs, for example piracetam, improve memory in animal tests by an unknown mechanism, but have shown only limited effects in human patients. Likewise, there are several 'natural products' that have been reported to produce beneficial effects on cognition, but the evidence lacks robust clinical information. Some of these 'natural' nootropic drugs are plant based, for example *Bacopa monniera*. Others, however are mixtures; for example, Memeron contains galantamine plus L- α -glycerylphosphoryl choline and Synaptine is a combination of piracetam and choline – a scroll through the appropriate websites will come up with a host of examples, although purchasing examples is likely to make you poorer rather than wiser.

Altered expression of 5-HT_{1A}, 5-HT₄ and 5-HT₆ receptors has been implicated in various human cognitive disorders, and selective agonist or antagonist compounds seem to have pro-cognitive effects in preclinical behavioural paradigms, justifying addition of these compounds to the list of potential cognitive enhancers. Phase I and II clinical trials with several compounds in this class are underway.

prevalent on GABAergic neurones and activation indirectly regulates a variety of neurotransmitters, including acetylcholine, glutamate and dopamine, in a brain-region-specific manner, as identified in more detail later.

A wide range of preclinical cognitive paradigms have been developed to assess particular aspects of learning and memory (see Box 2), such as prefrontal-cortex executive function (delayed response and problem-solving tasks), hippocampal-dependent spatial working memory (Morris water maze; see Glossary) or striatal-dependent associative learning. In the assessment of any potential novel therapeutic agent, it is essential that performance is assessed in a variety of these cognitive paradigms to

establish the specificity and full potential utility of these compounds. Therefore, here, we focus largely on preclinical studies that examine the role of three particular 5-HT receptors – 5-HT_{1A}, 5-HT₄ and 5-HT₆ – all of which have been implicated in various human cognitive disorders and for which selective agonist and/or antagonist compounds seem to have potent pro-cognitive effects in preclinical behavioural paradigms.

5-HT_{1A} receptors and learning and memory

It is well established that the 5-HT_{1A} receptor, which (as mentioned) functions as the somatodendritic inhibitory autoreceptor in addition to an inhibitory postsynaptic heteroreceptor, is abundant in areas important for learning

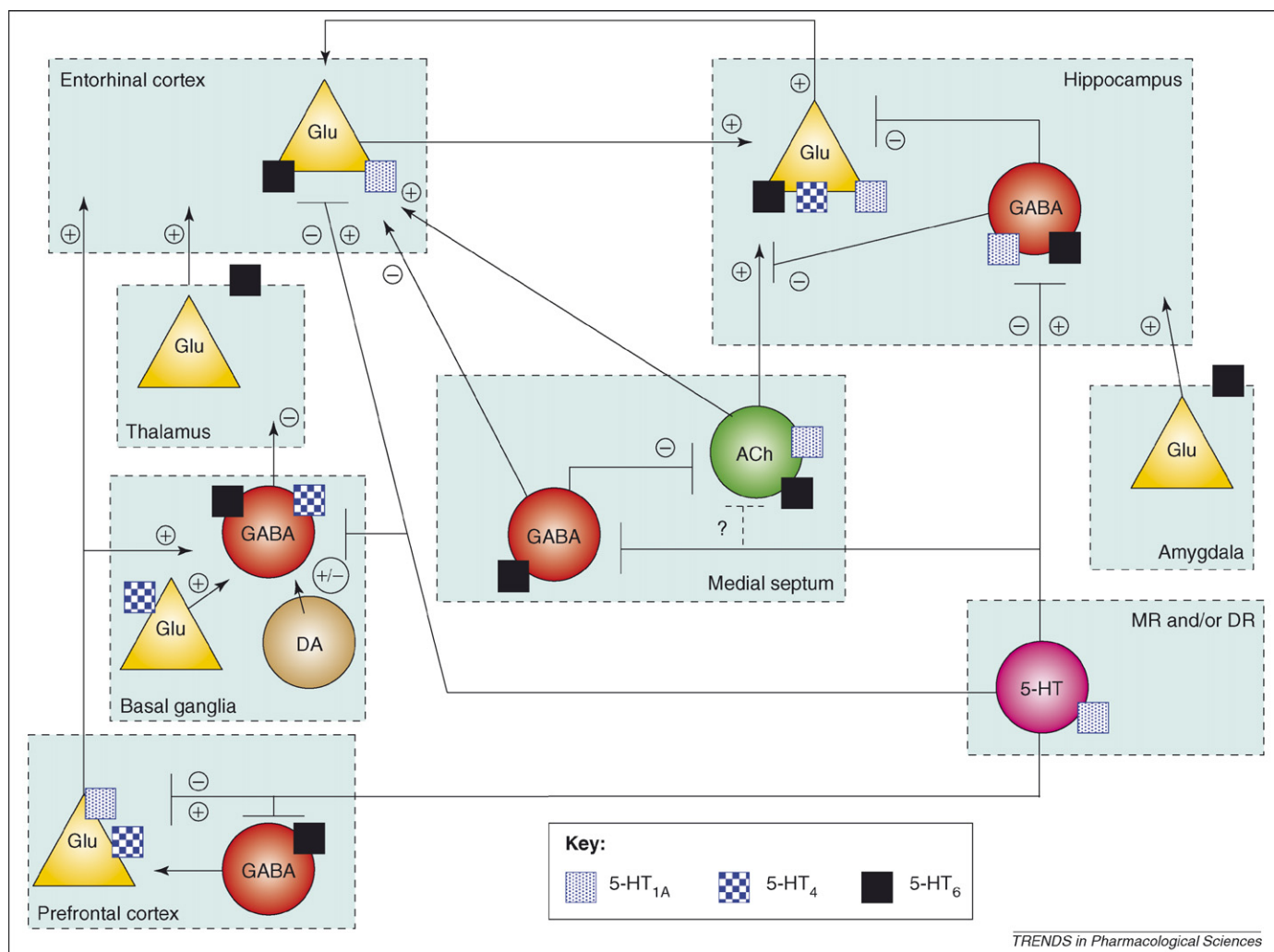


Figure 1. 5-HT receptors and learning and memory. Diagram showing the possible brain regions and neuronal pathways involved in the role of 5-HT_{1A} (lightly filled squares), 5-HT₄ (check-filled squares) and 5-HT₆ (black squares) receptors in the process of learning and memory. Serotonin-containing neurones in the dorsal and/or medial raphe nuclei (DR and MR, respectively) project to the hippocampus, medial septum, basal ganglia and cortex, and this system is thought to form the source of synapses where the serotonergic receptors indicated are expressed in these brain areas. The diagram indicates some of the key pathways thought to be involved in modifying the process of learning and memory. When the location of a particular 5-HT receptor is known, this is indicated by placing it on a specific neurone (i.e. for which the neurotransmitter phenotype is indicated) but this does not imply that both receptors necessarily co-exist on the same neurone in that area. Furthermore, for most sites it is unclear whether activation of one receptor has prevalence over the activation of another subtype. When a receptor is found within a region but the neuronal location has not been confirmed, the regional localization has been indicated. Established pathways are indicated by ⊥ and known pathways connecting regions by ↑. The functional activity of the pathways is shown by + for excitatory and – for inhibitory actions on the target neurones. The three 5-HT receptors discussed are all G protein coupled. 5-HT_{1A} receptors are inhibitory and linked to inhibition of adenylate cyclase, whereas 5-HT₄ and 5-HT₆ are excitatory. Thus, 5-HT pathways that end in the activation of a postsynaptic 5-HT_{1A} receptor are shown as – and those leading to activation of 5-HT₄ or 5-HT₆ receptors as +. The dopamine innervation in the basal ganglia is both excitatory (D₁, direct pathway) and inhibitory (D₂, indirect pathway). Abbreviations: ACh, acetylcholine; DA, dopamine; GABA, γ-aminobutyric acid; Glu, glutamate.

and memory, such as the frontal cortex, hippocampus and septum [1]. Several groups have consistently reported that 5-HT_{1A} receptors are decreased in Alzheimer's disease and through ageing [2,3]. However, recent evidence [4] indicates that, after training (in an autoshaping response task, see Box 2), 5-HT_{1A}-receptor expression is augmented in some brain areas (e.g. septal nucleus, caudate putamen, amygdala, the parietal, temporal and granular retrosplinal cortices, and the raphe nuclei) but decreased [e.g. in the hippocampus (CA1), frontal occipital and cingulate cortices] or unmodified in others compared with untrained controls, indicating that expression might also be a function of memory formation. The distribution of the receptor and the availability of several highly selective antagonists and partial agonists, combined with the role of the receptor as an inhibitory modulator of glutamatergic and cholin-

ergic neurones (both functions identified as important in learning and memory), has led to detailed investigation using animal models.

Studies using the original full 5-HT_{1A} agonist (Table 1), 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), other agonists such as tandospirone, and partial agonists such as buspirone, in rodents have shown a range of responses in cognitive tasks from improvement to impairment. The overall picture, however, is that, although low doses might improve cognitive performance (see later), high doses impair performance using a range of tests (reviewed in Ref. [5]) including spatial learning [6,7] and active and passive avoidance [8,9]. Within the hippocampus, 5-HT_{1A} receptors are present on excitatory pyramidal and granule neurones and the terminals of GABAergic inhibitory interneurones, where they cause

Box 2. Assessment of learning and memory in rodents

Learning is acquisition of new information resulting in a change in behaviour, and memory is the retention of this learned information. These can be subdivided into declarative (facts and events) and non-declarative [skills, habits (procedural) and classical conditioning] memory.

Constructs important in learning and memory

- Attention: selective processing of simultaneous sources of information.
- Working memory: temporary form of information storage of limited capacity that requires rehearsal.
- Short-term learning and memory (recent memory): information is stored but not necessarily retained unless importance is attached.
- Long-term learning and memory: ability to store and retrieve specific information, often associated with reinforcement.
- Social cognitive processing: ability to recognize and discriminate the type of effect in facial or vocal cues.
- Executive function: ability to generate and maintain rules that moderate subsequent behaviour.

Which brain regions are involved in animal behavioural tests of learning and memory?

- The temporal cortex, consisting of the hippocampus, and entorhinal and perirhinal cortices, with input from the association areas of the cerebral cortex and output through the fornix to the hypothalamus, cingulate and frontal cortex via the thalamus. Important in declarative-memory consolidation (delayed non-match to sample or position, radial arm maze, novel object recognition (NOR) and social recognition behaviour). The hippocampus is also important in spatial memory (place cells), which is clearly demonstrated in rodents (Morris water maze, object location and delayed Y-maze task).
- The prefrontal cortex has links to and from temporal and cingulate cortices and is important in working memory, attention and executive function (delayed-response tests, problem solving and planning tasks).
- Striatal regions (caudate nucleus and putamen) have an essential role in the control of voluntary movement and are important in procedural memory and development of behavioural habits (associative learning such as conditioned autoshaping tasks).
- The amygdala has important connections with the hypothalamus, hippocampus and cortex and is strongly associated with aversive learning (active and passive avoidance and cue-associated conditioned emotional response).

Mechanistic processes associated with learning and memory

Long-term potentiation (LTP) is a candidate mechanism by which the brain can store memories by altering the synaptic strength of neuronal connections by a process of synaptic plasticity. A key element in this process is modification of an, as yet, poorly defined intracellular-signalling-pathway cascade involving second messengers (calcium-dependent protein kinases such as calcium-calmodulin-dependent kinase II, tyrosine kinase and protein kinase C) in the early phase with subsequent changes in transcription factors [e.g. cyclic AMP response element binding protein (CREB) and Zif268], protein expression [e.g. brain derived neurotrophic factor (BDNF)] and phosphorylation of key G-protein-coupled receptors such as subunits of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor involved in glutamatergic neurotransmission, in areas such as the hippocampus. This process plays an essential part of the consolidation of information and, thus, its transfer from short- to long-term memory.

hyperpolarization and neuronal inhibition. Knockout mice, in which the 5-HT_{1A} receptor is absent, show impaired hippocampal-dependent learning, such as spatial working memory in the Morris water maze [10], which is consistent with this receptor being important in episodic memory.

The real interest, however, has been the emerging evidence that antagonism of 5-HT_{1A} receptors reverses cognitive impairments induced by a range of pharmacological approaches, such as fornix lesions and *N*-methyl-D-aspartic acid (NMDA)-receptor antagonists [11,12], and not just those induced by 5-HT_{1A}-receptor agonists. This raises the possibility that 5-HT_{1A} antagonists could be of value in the treatment of disorders involving glutamatergic and/or cholinergic dysfunction because 5-HT_{1A} receptors regulate the release of these two neurotransmitters.

For example, an early study [11,12] showed that the glutamatergic antagonist dizocilpine impairs cognitive function in the common marmoset, a non-human primate, using both shape and visual-spatial discrimination tasks. Furthermore, this impairment was attenuated by combining treatment of dizocilpine with that of the 'silent' 5-HT_{1A}-receptor antagonist WAY-100635 [13], an antagonist with no intrinsic activity. The results, however, with WAY-100635 and other 5-HT_{1A} antagonists have been varied, ranging from no effect to either improvement or impairment. There are several factors that might influence the results observed, including the type of cognitive task used, the specificity of the compounds (not just in terms of their action at 5-HT_{1A} receptors but also differential effects at pre and postsynaptic 5-HT_{1A} receptors) and drug bioavailability and brain penetration. Furthermore, agonist studies using compounds such as 8-OH-DPAT might be complicated in their interpretation by the other well-established postsynaptic receptor-mediated behavioural effects elicited by these drugs (stereotype motor effects commonly referred to as the 'serotonin syndrome', consisting of hyperactivity, lateral head weaving, reciprocal forepaw treading and hyperthermia). Recent studies [14,15] have concentrated on trying to address some of these issues by investigating the contribution of sensorimotor disturbances on spatial-learning performance in agonist studies and relating cognitive behavioural effects to receptor occupancy using an antagonist (WAY-101405) with good oral bioavailability and central nervous system (CNS) penetration.

Administration of 8-OH-DPAT into the raphe nuclei, which contain serotonergic neurone cell bodies, causes inhibition of 5-HT neuronal firing via activation of the dendritic inhibitory 5-HT_{1A} autoreceptors in this brain area, which results in decreased 5-HT release from terminal 5-HT-neurone projection areas (Figure 1). When 8-OH-DPAT is injected into the rat medial raphe nucleus, there is improved delayed non-matching to position (Box 2) performance, indicating that reduced 5-HT neuronal activity improves behavioural performance in this paradigm [16]. This dissociation between effects on cognition of stimulating pre (inhibiting 5-HT function) or postsynaptic 5-HT_{1A} receptors has been further demonstrated using a passive avoidance test in which 8-OH-DPAT showed a biphasic dose effect, with low doses (presynaptic specific) causing facilitation and high doses (pre plus postsynaptic receptor activation) causing impairment [14]. The view that impaired function is associated with postsynaptic 5-HT_{1A}-receptor activation is further supported by studies showing that

Table 1. Chemical names of receptor-selective agonist and antagonist compounds

| | Agonists | Antagonists |
|--------------------|--|--|
| 5-HT _{1A} | 8-OH-DPAT : 8-hydroxy-2-(di- <i>n</i> -propylamino)tetralin Buspirone : 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4.5]decane-7,9-dione Tandospirone : 3 α ,4 β ,7 β ,7 $\alpha\alpha$ -hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1H-iso-indole-1,3(2H)dione dihydrogen citrate | NAD-299 : (<i>R</i>)-3- <i>N,N</i> -dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide hydrogen(2 <i>R</i> ,3 <i>R</i>) tartrate monohydrate SRA-333 (Lecozotan) : 4-cyano- <i>N</i> -(2 <i>R</i> -(4-(2,3-dihydrobenzo[1,4]-dioxin-5-yl)-piperazin-1-yl)-propyl)- <i>N</i> -pyridin-2-yl-benzamide HCl WAY-101405 : (<i>R</i>)- <i>N</i> -(2-methyl-(4-indolyl-1-piperazinyl)ethyl)- <i>N</i> -(2-pyridinyl)-cyclohexane carboxamide WAY-100635 : <i>N</i> -(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-2-(2-pyrinyl)cyclohexanecarboxamide trihydrochloride |
| 5-HT ₄ | RS67333 : 1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone RS17017 : 1-(4-amino-5-chloro-2-methoxyphenyl)-5-(piperidin-1-yl)-1-pentanone SL65.0155 : 5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one-monohydrochloride VRX-03011 : 6,7-dihydro-4-hydroxy-7-isopropyl-6-oxo- <i>N</i> -(3-(piperidin-1-yl)propyl)thieno[2,3- <i>b</i>]pyridine-5-carboxamide | SDZ205557 : 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester GR125487 : 1-[2-[(methylsulphonyl)-amino]ethyl]-4-piperidinyl-methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate |
| 5-HT ₆ | E-6801 : 6-chloro- <i>N</i> -(3-(2-(dimethylamino)ethyl)-1H-indol-5-yl)imidazo[2,1- <i>b</i>]thiazole-5-sulfonamide EMD 386088 : 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole R-13c : <i>R</i> -2-chloro- <i>N</i> -(3-[(2 <i>R</i>)-1-methyl-2-pyrrolidinyl]methyl)-1H-indol-5-yl)benzenesulfonamide WAY-181187 : 2-[1-(6-Chloroimidazo[2,1- <i>b</i>]thiazol-5-yl)sulfonyl]-1H-indol-3-yl] ethylamine WAY-208466 : structure not disclosed | BGC20-761 : 5-methoxy-2-phenyl- <i>N,N</i> -dimethyltryptamine LY-483518 : 1-methyl-3-(1-methylpiperidin-4-yl)-1H-indol-5-yl 2,6- <i>ifluorobenzenesulfonate</i> Ro 4368554 : 3-benzenesulfonyl-7-(4-methyl-piperazin-1-yl)1H-indole PRX-07034 : structure not disclosed SB-258585 : [4-methoxy-3-(4-methylpiperazin-1-yl)phenyl] benzene-sulfonamide SB-271046 : 5-chloro- <i>N</i> -(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide SB-399885 : <i>N</i> -[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzene sulfonamide SB-742457 : 3-(phenylsulfonyl)-8-(piperazin-1-yl)quinoline |

impairments in water-maze performance still occur in the 5,7-dihydroxytryptamine (5,7-DHT) lesioned rat [8], in which presynaptic 5-HT nerve terminals have been destroyed. Conversely, selective stimulation of the pre-synaptic 5-HT_{1A} receptor results in improved cognitive function.

There are now many examples, using a range of tests, showing that selective 5-HT_{1A}-receptor antagonists (Table 1), including WAY-10635 and NAD-299, reverse deficits in learning and memory induced by 5-HT_{1A} agonists (for a review, see Ref. [17]). Recently, one group [15] demonstrated that the potent (nanomolar affinity) and selective (100-fold) 'silent' 5-HT_{1A} antagonist WAY-101405, which has both good oral bioavailability and brain penetration, enhanced retention in the novel object recognition (NOR – the ability to distinguish between a novel and a familiar object on the second of a two-trial exploration task) test and reversed deficits in both NOR and contextual-fear conditioning induced by the muscarinic-receptor antagonist scopolamine (Box 2). These behavioural effects were observed using doses that produced ~90% occupancy of the postsynaptic 5-HT_{1A} receptors in the hippocampus. The 'silent' nature of the antagonism was confirmed by showing that WAY-101405 had no effect on basal hippocampal 5-HT, measured by microdialysis, but blocked the reduction in 5-HT overflow produced by 8-OH-DPAT.

Although the evidence points to the cognitive improvements observed with 5-HT_{1A} antagonists being mediated by postsynaptic 5-HT_{1A} receptors, it is still unclear which other neurotransmitters and mechanisms are involved.

Both WAY-100635 [18] and WAY-101405 [15] increase extracellular acetylcholine in the hippocampus, although with WAY-101405 the increase is modest. Alternatively, the action might be through enhancement of glutamate function, which increases after treatment with Lecozotan (also known as SRA-333; Table 1), another selective 5-HT_{1A}-receptor antagonist [19]. The glutamatergic pyramidal cells in the hippocampus are important in cognitive events. Furthermore, hippocampal 5-HT_{1A}-receptor activation disrupts pyramidal glutamate function by causing hyperpolarization [20] (Figure 1) and, as mentioned, the antagonist WAY-100365 attenuates a cognitive impairment induced by NMDA-receptor blockade in the marmoset, using dizocilpine as the antagonist [12]. It is suggested that 5-HT_{1A} antagonists might act through a combination of increasing acetylcholine function and preventing glutamatergic hyperpolarization [14]. In addition, the nitric-oxide donor molsidomine can reverse the impairment of NOR induced by 8-OH-DPAT [21], and there is evidence that nitric oxide reduces cortical and hippocampal 5-HT release [22,23]. In turn, nitric oxide is thought to facilitate glutamate release via retrograde signalling and, by this means, it might modulate long-term potentiation (LTP), a putative cellular mechanism involved in transfer to long-term memory (Box 2), raising the possibility that 5-HT-receptor function might be important in memory consolidation [24]. Interestingly, a recent study has shown constitutive activity of 5-HT_{1A} receptors together with a link between the expression of cAMP-response-element binding (CREB) [25] and other transcription factors. Furthermore, as mentioned, constitutive 5-HT_{1A}-knockout mice show

impaired cognitive function in hippocampal-dependent, spatial-learning paradigms (Box 2) such as the Morris water maze or the Y-maze (Box 2), but not in hippocampal-independent tasks such as spontaneous alternation [10]. This provides a possible link between 5-HT_{1A}-receptor function, second-messenger production and gene expression associated with LTP and memory. The 5-HT_{1A} receptor clearly offers a potential target for the development of drugs that could alleviate the symptoms associated with disorders of learning and memory, including those seen in schizophrenia and Alzheimer's disease where cognitive dysfunction is a major symptom.

5-HT₄ receptors and learning and memory

The distribution of 5-HT₄ receptors is also consistent with an involvement in learning and memory processes. Expression is highest within the basal ganglia and hippocampus (granule cell layer of the dentate gyrus and pyramidal cell layer of the CA fields and subiculum), with moderate levels in the frontal cortex, septum and amygdala, and low levels in the raphe nuclei (Figure 1). Lesion studies indicate that the receptor is present on GABAergic and glutamatergic (but probably not dopaminergic) neurones, and comparison of mRNA distribution and radioligand binding reveals localization on both somato-dendritic and 5-HT terminal regions [26,27]. Limited evidence indicates that 5-HT₄-receptor polymorphisms could predispose to schizophrenia [28] and attention deficit hyperactivity disorder (ADHD) [29], although more interest has focused on neurodegenerative disorders such as Alzheimer's disease, which is associated with decreased 5-HT₄-receptor expression in the hippocampus and prefrontal cortex [30].

Systemic administration of selective 5-HT₄-receptor partial agonists such as RS67333 and RS17017 (Table 1) improves rodent performance in tests of social [31], olfactory associative learning [32] and spatial memory [33], and also improves delayed matching-to-sample in young or aged macaques [34]. These effects can be attributed to modulation of memory acquisition and/or consolidation rather than recall because RS67333 also enhances place and NOR (ability to recall the position of the object and to distinguish between a familiar and novel object, respectively) in young or aged rats when administered before the initial familiarization trial, but has no effect if given before the second or choice trial (i.e. at the time of retrieval) [35]. Several other lines of evidence support the conjecture that 5-HT₄ receptors might be involved in memory consolidation, although findings are not universally consistent. For example, post-acquisition administration of 5-HT₄-receptor agonists impairs performance in a conditioned autoshaping response task [36] (and training alone in this task alters hippocampal, cortical and striatal 5-HT₄-receptor expression [37]) but enhances performance of aged rats in the NOR task [35]. In addition, administration of the 5-HT₄-receptor antagonists SDZ205557 and GR125487 (Table 1) impairs passive avoidance memory [38]. Consolidation of place memory was unaffected by systemic administration of RS67333 [35] but enhanced by localized injection into the nucleus basalis magnocellularis (NBM), which sends cholinergic projections to the neocortex, interestingly,

with higher intra-NBM doses necessary to improve acquisition [39].

Consistent with a regulatory effect on cholinergic neurotransmission, 5-HT₄-receptor agonists reverse scopolamine-induced deficits in a variety of cognitive paradigms [38,40], enhance electrically stimulated [³H]choline efflux from guinea-pig cortical, hippocampal and NBM slices *in vitro* [41] and increase acetylcholine efflux (measured using microdialysis) in the frontal cortex [42] and hippocampus [43] of conscious freely moving rats. These findings indicate that 5-HT₄-receptor agonists might have a role in the treatment of cholinergic dysfunction, such as that associated with Alzheimer's disease. Furthermore, the agonists RS67333 [44], SL65.0155 [45] and VRX-03011 [46] all exhibit synergistic interactions with cholinesterase inhibitors, such as donepezil, in young, aged and scopolamine-impaired animals. Thus, sub-efficacious doses of a 5-HT₄-receptor agonist and cholinesterase inhibitor enhance spatial memory and NOR when given in combination. This raises the possibility that co-administration of a 5-HT₄-receptor agonist with existing treatments for Alzheimer's disease might enable reduction of dose, and therefore side effects, while maintaining therapeutic activity.

It is not yet clear whether the 5-HT₄ receptors that regulate acetylcholine release within the hippocampus are located on cholinergic nerve terminals, cholinergic cell bodies or interneurons (for a review see Ref. [47]). The activity of 5-HT₄-receptor agonists in isolated brain-slice preparations is consistent with a nerve-terminal location, but failure of the same compounds to enhance K⁺-evoked [³H]choline release from hippocampal synaptosomes [41] is not. This issue could, perhaps, be resolved by examining the impact of cholinergic lesions on 5-HT₄-receptor expression, or by using dual-label immunohistochemistry to determine whether 5-HT₄ receptors are located on cholinergic neurones. In addition to stimulating acetylcholine release, 5-HT₄-receptor agonists exert a biphasic effect on GABA efflux from guinea-pig hippocampal slices, with low concentrations augmenting electrically stimulated release and higher concentrations attenuating it [48]; these effects are sensitive to subtype-selective muscarinic-receptor antagonists (M₁ and/or M₃ and M₂ receptors, respectively) and also seem to be secondary to enhanced cholinergic activity, although there is evidence that 5-HT₄ receptors are expressed on GABAergic neurones, at least in the striatum [27]. 5-HT₄ receptors are also expressed on glutamatergic pyramidal cells in the hippocampus [26,27,49] and frontal cortex [50], and considerable interest has focused on the mechanisms by which their activation enhances cognitive processing (Figure 1). 5-HT₄-receptor agonists increase neuronal excitability within the hippocampus (and presumably other brain regions) by reducing the Ca²⁺-evoked K⁺ currents responsible for after-hyperpolarization [49]. This effect has been attributed to inhibition of Ca²⁺-induced Ca²⁺ release from intracellular stores [51] and, consistent with positive coupling of the 5-HT₄ receptor to adenylate cyclase through G_s proteins, seems to involve protein kinase A (PKA) plus additional cAMP-dependent PKA-independent signalling pathways [52] that are thought to be involved in LTP. Accumulating evidence indicates that 5-HT₄-receptor agonists modulate

synaptic plasticity within the hippocampus and amygdala by augmenting LTP, attenuating depotentiation and altering patterns of long-term depression [32,43,53,54]. These effects persist in hippocampal tissue from transgenic mice overexpressing the amyloid β peptide [55] and, taken together, these findings further strengthen the case for a role of 5-HT₄-receptor compounds in managing the symptoms of Alzheimer's disease.

It is extremely interesting to note that, in addition to providing symptomatic relief, 5-HT₄-receptor agonists have the potential to modify the pathogenesis of Alzheimer's disease. In transfected Chinese hamster ovary cells and a human neuroblastoma cell line, 5-HT₄-receptor activation not only stimulates secretion of the soluble non-amyloidogenic form of the amyloid precursor protein (sAPP α), which has neuroprotective, neurotrophic and cognitive enhancing effects, but also decreases extracellular accumulation of amyloid β [56–58]. This effect is mediated via cAMP-dependent PKA-independent signalling pathways that seem to involve activation of Rac, a small GTPase of the Rho family, by a cAMP, Epac1, Rap1 and STEF (SIF and Tiam1-like exchange factor) cascade [59,60]. 5-HT₄-receptor-agonist-induced increases in sAPP α levels *in vivo* have been demonstrated recently in the cortex and hippocampus of healthy young adult mice, in addition to the cortex of transgenic APP-overexpressing mice [61]. 5-HT₄ receptors are, therefore, an exciting potential target for the treatment of Alzheimer's disease symptomatology and pathology.

5-HT₆ receptors and learning and memory

The 5-HT₆ receptor is expressed almost exclusively within the CNS, offering the possibility that pro-cognitive compounds operating through this mechanism could have only a few peripheral side effects. Particularly high levels of 5-HT₆-receptor mRNA occur in the striatum, nucleus accumbens and olfactory tubercles and in limbic and forebrain regions, including the hippocampus and cortex. Neither hippocampal nor striatal 5-HT₆ mRNA is altered by the 5-HT neurotoxin 5,7-DHT, indicating the receptors are located postsynaptic to serotonergic neurones and not on serotonergic nerve terminals [62]. Dual-labelled immunohistochemical studies [63] and lesioning cholinergic neurones with 192-IgG-saporin [64] both indicate that there are few 5-HT₆ receptors on cholinergic neurones. Conversely, this receptor seems to be co-localized extensively with glutamic acid decarboxylase, which is present in GABAergic neurones (Figure 1) [63]. Indeed, within the striatum 5-HT₆-receptor mRNA is expressed extensively on GABAergic striato-pallidal neurones and striato-nigral neurones [65], and increasing striatal receptor expression using a gene-targeting approach impairs acquisition of an instrumental learning task, which is reversed by the 5-HT₆-receptor antagonist SB-258585 [66]. Although the 5-HT₆ receptor is thought to be involved in anxiety and depression [67], and eating disorders [68,69], the strongest preclinical evidence is for a role in learning and memory [63,70,71]. For instance, the Cys267Thr polymorphism of the receptor has been associated in some studies both with Alzheimer's disease [72] and the positive response to clozapine in drug-resistant schizophrenics [73]. Furthermore,

receptor expression is reduced in the prefrontal cortex of Alzheimer's patients [74].

Antisense oligonucleotides directed against the 5-HT₆ receptor given by injection into the lateral cerebral ventricles prolonged retention of the correct location of the hidden platform in the Morris water maze, an effect that was mirrored in subsequent studies giving systemic administration of selective antagonists [75], providing the first indirect preclinical evidence for a role of this receptor in learning and memory. Indeed, on acute injection, antagonists improve retention primarily without affecting acquisition in spatial-learning tasks such as the water maze [75,76]. By contrast, longer-term (for >1 week) administration to either young adult [77,78] or 20-month-old rats also improves acquisition. The acute effect might be mediated by potentiation of cholinergic mechanisms in the hippocampus, whereas the longer-term action on acquisition could involve neuronal dendrite proliferation, as implied by the increase in the neural cell-adhesion molecule polysialylation state [79,80], and increased expression of brain-derived neurotrophic factor (BDNF) and the immediate early gene (IEG), activity-regulated, cytoskeletal-associated protein (Arc) [81] in cortical and hippocampal neurones, all of which are produced by antagonists.

In NOR, 5-HT₆-receptor antagonists delay natural forgetting and reverse impairments produced by the muscarinic-receptor antagonist scopolamine (used to reduce cholinergic neuronal mechanisms involved in memory) [76,78,82–84]. 5-HT₆-receptor antagonists are most effective when administered just before or immediately after the familiarization trial, indicating that they improve consolidation [82]. Furthermore, pretreatment with the NMDA-receptor antagonist MK801 prevents 5-HT₆-antagonist-induced improvement in NOR, which is consistent with activation of glutamatergic mechanisms involved in consolidation. In an autoshaping conditioned-response task, 5-HT₆-receptor antagonists also seem to enhance consolidation [85], a process that might involve alteration in 5-HT₆-receptor expression in the basal ganglia [86]. However, microdialysis experiments in several brain regions do not to support a direct effect of antagonists on 5-HT release [87]; therefore, it has been speculated that this might involve an indirect effect. Interestingly, changes in 5-HT₆-receptor mRNA expression in the olfactory tubercle and dentate gyrus have been linked to novelty-seeking behaviour in a rat model of this trait behaviour [79], which could also contribute to altered performance in this task. In a social-recognition paradigm (Box 2) monitoring the extent that an adult rat can recall interaction with a previously encountered juvenile, the antagonists BGC20-761 (5-methoxy-2-phenyl-*N,N*-dimethyltryptamine) [84] and Ro 4368554 [76] also reverse a scopolamine-induced amnesia. Because discrete injection of SB-271046 into the frontal cortex (but not the striatum or NBM) reversed a delay-induced reduction in social recognition, it seems that cortical 5-HT₆ receptors might have a pivotal role in this pro-cognitive effect [88]. A similar potentiation of cholinergic neuronal function probably accounts for the reversal of scopolamine-induced passive-avoidance-learning deficits caused by antagonists

in young adult [76,89] and 20-month-old rats [77]. Once again, the antagonists do little in this paradigm when they are given alone. In a fear-potentiated startle paradigm, produced by exposure to an acoustic startle after shock pairing of an auditory and visual cue, the antagonist Ro 4368554 also reversed a scopolamine deficit [90]. In this case, blockade of 5-HT₆ receptors in the amygdala might be involved because the response was conditioned to the accompanying light cue and not simply elicited by association of the shock with the context, which is primarily hippocampal dependent.

Most groups have examined the effect of antagonist compounds in normal healthy rats rather than in animal models of human cognitive disorders (such as rats reared in social isolation from weaning or treated by chronic administration of phencyclidine), but the latter is clearly necessary to fully evaluate their potential clinical utility and is beginning to appear in the literature. In the attentional set-shifting task [91] (Box 2), which could have therapeutic relevance to cognitive inflexibility seen in schizophrenia, administration of SB-399885-T for eight days reduced the number of trials to complete the extra-dimensional (ED) shift (correct response requiring attention to a different stimulus category, i.e. odour instead of media). Because 5-HT₆-receptor antagonists elevate dopamine [87] and acetylcholine levels [89] in the rat medial prefrontal cortex (an area crucial for performance of the ED shift [92]), changes in these neurotransmitters might account for this particular pro-cognitive effect.

Recently, the structure of selective agonists with nanomolar affinity for the 5-HT₆ receptor (Table 1), for example *R*-2-chloro-*N*-(3-[(2*R*)-1-methyl-2-pyrrolidinyl)methyl]-1*H*-indol-5-yl)benzenesulfonamide (also known as *R*-13c), 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole, WAY-466 (also known as WAY-208466), WAY-181187, 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole (or EMD 386088) and 6-chloro-*N*-(3-(2-(dimethylamino)ethyl)-1*H*-indol-5-yl)imidazo[2,1-*b*]thiazole-5-sulfonamide (or E-6801) have been reported. Both WAY-181187 and WAY-208466 increase GABA efflux in the rat frontal cortex, hippocampus, striatum and amygdala [93], which is consistent with the suggested disinhibition of cholinergic and glutamatergic activity of 5-HT₆ antagonists. However, the agonists decrease cortical and striatal 5-HT and dopamine release, and inhibit hippocampal potassium-induced glutamate release [93,94], all of which seem to be attenuated by the antagonist SB-271046. These neurochemical findings indicate that 5-HT₆-receptor activation might impair cognition. Yet, the agonists *R*-13c [95] and E-6801 [96] both improve NOR after a four-hour inter-trial interval to simulate natural forgetting of the objects. In agreement with this finding, acute injection of the agonist WAY-181187 enhanced the ED shift in the attentional set-shifting paradigm [97]. By contrast, in the social-recognition paradigm both systemic administration and discrete injection of WAY-181187 into the frontal cortex attenuated social recognition, which could be prevented by 5-HT₆ antagonists [88].

How 5-HT₆-receptor agonists and antagonists could both have pro-cognitive effects is currently unclear. One proposal is that an agonist could activate 5-HT₆ receptors

located directly on glutamatergic and/or cholinergic neurones that receive little serotonergic input under normal conditions, whereas antagonists attenuate tonic serotonergic input to upstream inhibitory GABAergic neurones, which reduces the inhibition of glutamate and/or acetylcholine release [63] (Figure 1). The 5-HT₆ receptor seems to be functionally coupled to adenylyl cyclase *in vitro* in cell lines, but it has been difficult to demonstrate this conclusively *in vivo*. It is also noteworthy that, in humans, the 5-HT₆ receptor seems to couple to Fyn-tyrosine kinase, which is expressed in neurones [98], and, in rats, it might open K⁺ channels in striatal cholinergic interneurones [99], so it will be interesting to see whether agonists and antagonists have differential effects on these two signalling pathways.

Concluding remarks

Serotonin-containing neurones in the dorsal and/or medial raphe nuclei (DR and/or MR) project to the hippocampus, medial septum and prefrontal cortex. The activity of serotonergic neurones in the raphe nuclei is under autoinhibitory control mediated by 5-HT_{1A} receptors. Selective activation of these presynaptic 5HT_{1A} receptors leads to reduced serotonergic function and facilitation of cognition. Conversely, activation of the inhibitory postsynaptic 5-HT_{1A} receptors in the hippocampus and septal regions disrupts glutamatergic and/or cholinergic activity causing impaired cognition, so drugs that block the effects of serotonin at these receptors (5-HT_{1A} antagonists) improve cognition by increasing acetylcholine release and preventing glutamatergic hyperpolarization.

5-HT₄ receptors located in the hippocampus, septum, basal ganglia and prefrontal cortex seem to be associated with GABAergic, glutamatergic and cholinergic neurotransmission. 5-HT₄-receptor activation increases cholinergic function and improves cognition. 5-HT₄ receptors are also found on glutamatergic pyramidal cells in the hippocampus and frontal cortex, where their activation increases neuronal excitability. In addition, 5-HT₄-receptor agonists might alter secretion of the soluble non-amyloidogenic form of sAPP α , which has neuroprotective, neurotrophic and cognitive enhancing effects that could be beneficial in the treatment of Alzheimer's disease.

5-HT₆ receptors are expressed in the hippocampus and basal ganglia, located on GABAergic neurones, which, when activated, increase inhibitory GABA function and consequently decrease cholinergic and/or glutamatergic neuronal activity. Acute treatment with 5-HT₆-receptor antagonists improves consolidation and retention of information by preventing 5-HT₆-mediated inhibition of cholinergic function. However, with longer-term 5-HT₆-receptor-antagonist treatment there are also improvements in acquisition, which could involve dendrite proliferation. 5-HT₆-receptor agonists might also improve cognition, and one suggestion for this unexpected finding is that there are also 5-HT₆ receptors located directly on cholinergic and/or glutamatergic neurones that, when activated, produce a direct increase in both glutamate and cholinergic function.

The multiple CNS sites and several distinct neurotransmitter systems affected by the 5-HT_{1A}, 5-HT₄ and 5-HT₆ receptors might provide the opportunity for compounds

acting at these receptors to provide a unique approach to the treatment of cognitive dysfunction. However, so far, the data available is primarily from preclinical studies. One challenge for the pharmaceutical industry is to decide whether agonists or antagonist compounds are likely to provide the most effective therapeutic strategy to treat human cognitive dysfunction. Another major future challenge to cognitive research is the characterization and validation of preclinical cognitive paradigms that have predictive reliability and are of translational relevance to human learning and memory disorders. To the best of our knowledge, Lecozotan [a 5-HT_{1A} antagonist; Wyeth (<http://www.wyeth.co.uk>)] and Xaliproden [a nerve-growth-factor agonist and 5-HT_{1A} agonist; Sanofi-Aventis (<http://en.sanofi-aventis.com>)] are in Phase II clinical trials for Alzheimer's disease, and PRX-00023 [a 5-HT_{1A} agonist; Epix Pharmaceuticals (<http://www.epixpharma.com>)] is still in Phase II for depression. 5-HT₄-receptor partial agonists have already been used to treat gastrointestinal disorders, and brain-penetrant partial agonists SL65.0155 and PRX-03140 have entered Phase II clinical trials for Alzheimer's disease. To our knowledge, eight 5-HT₆-receptor compounds [seven antagonists and one agonist, most recently the competitive antagonist SUVN-502; Suven Life Sciences (<http://www.suven.com>)] have entered clinical trials, six (both agonists and antagonists) for cognitive dysfunction in Alzheimer's dementia and/or schizophrenia [69]. Two 5-HT₆ antagonists have reached Phase II (LY-483518 and SB-742457) and a third compound (PRX-07034) might enter such trials for cognitive dysfunction soon. The result of these trials is eagerly awaited to ascertain whether earlier preclinical evaluations gave a reliable prediction of their therapeutic potential.

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