5-HT_6 Receptor Antagonists as Novel Cognitive Enhancing Agents for Alzheimer's Disease

Neil Upton, Tsu Tshen Chuang, Ann J. Hunter, and David J. Virley

GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, United Kingdom

Summary: Alzheimer's disease (AD) is a devastating neurological condition characterized by a progressive decline in cognitive performance accompanied by behavioral and psychological syndromes, such as depression and psychosis. The neurochemical correlates of these clinical manifestations now appear to involve dysfunctions of multiple neurotransmitter pathways. Because of the extensive serotonergic denervation that has been observed in the AD brain and the important role played by serotonin (5-HT) in both cognition and behavioral control, this neurotransmitter system has become a focus of concerted research efforts to identify new treatments for AD. 5-HT exerts its diverse physiological and pharmacological effects through actions on multiple receptor subtypes. One of the newest members of this family is the 5-HT_6 receptor, a subtype localized almost exclusively in the CNS, predominating in brain regions associated with cognition and behavior. With the subsequent development of selective 5-HT_6 receptor antagonists, preclinical studies in rodents and primates have elucidated the function of this receptor subtype in more detail. It is increasingly clear that blockade of 5-HT_6 receptors leads to an improvement of cognitive performance in a wide variety of learning and memory paradigms and also results in anxiolytic and antidepressant-like activity. These actions are largely underpinned by enhancements of cholinergic, glutamatergic, noradrenergic, and dopaminergic neurotransmission, together with learning-associated neuronal remodeling. A preliminary report that the cognitive enhancing properties of a 5-HT_6 receptor antagonist (namely, SB-742457) extends into AD sufferers further highlights the therapeutic promise of this mechanistic approach. **Key Words:** 5-HT_6 receptor antagonists, Alzheimer's disease, cognition, learning, memory, behavioral symptoms, glutamate, dopamine, acetylcholine, norepinephrine, synaptic plasticity, SB-742457, neural cell adhesion molecule.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disorder and poses the biggest unmet medical need in neurology, with more than 26 million sufferers today worldwide—a statistic expected to quadruple by the year 2050 as an unfortunate by-product of increasing life expectancy.[1] Clinically, AD is characterized by progressive cognitive decline associated with impairment in activities of daily living and behavioral symptoms such as depression, agitation, psychosis, and aggression that can vary in severity according to the stage of the disease.

The causes of AD are still rather poorly understood, and it is now considered probable that AD is of heterogenous origin, with various etiologies leading to the hallmark plaque and tangle pathology and profound neuronal loss. At the neurochemical level, some 20 to 30 years ago AD was thought to be primarily a disorder of cortical cholinergic innervation—the so-called cholinergic hypothesis of aging and AD.[2] Given the complexity and diversity of the cognitive and behavioral abnormalities observed in AD, however, it is highly likely that dysfunction of multiple neurotransmitter systems is at play. The observation that cholinesterase inhibitors, agents designed specifically to boost residual cholinergic transmission, are at best only partially effective in treating the clinical manifestations of AD, certainly supports this view.[3] More recently, deficits in the serotonergic, GABAergic, noradrenergic, dopaminergic, and glutamatergic pathways have all been described to track, to a greater or lesser extent, with cognitive and/or behavioral changes in AD (see Ramirez et al.[4] for a brief overview).

From a therapeutic perspective, the serotonergic system appears to be a particularly attractive target, because it has been implicated not only in cognitive processes but also in depression, psychosis, and aggression.[4] Within this particular neurotransmitter pathway, the 5-HT_6 receptor class of serotonin receptors has received the most

Address correspondence and reprint requests to: Neil Upton, Ph.D., GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK. E-mail: Neil_Upton@gsk.com.

Table 1. *Comparison of Pharmacological and Pharmacokinetic Profiles of Key 5-HT$_6$ Receptor Antagonist Tools*

Characteristic	Ro 04-6790	SB-271046	SB-399885	Ro 4368554	SB-742457
Binding affinity, pK_i	7.3 (h); 7.4 (r)	8.9 (h); 9.0 (r)	9.1 (h); 8.8 (r)	9.4 (h)	9.6 (h); 9.6 (r)*
Selectivity[†]	≥100-fold	≥200-fold	≥200-fold	≥100-fold	≥100-fold*[‡]
ED_{50} in rat *ex vivo* binding assay,[§] mg/kg p.o.		11	2	7.8 (i.p.)	0.3*
Effective dose range in cognition models, mg/kg p.o. or i.p.	10–30 (i.p.)	10–30 (p.o.); 0.3–19 (i.p.)	1–30 (p.o.)	100 (p.o.); 1–30 (i.p.)	0.5–4.4 (p.o.)
Oral bioavailability, %		82 (r); 82 (m)	52 (r)		76 (r)*
Brain:blood ratio in rat	<0.01:1	~0.05:1	~0.15:1		~0.5:1*
Half-life $T_{½}$ in rat, hours		4.8; 7.8*	2.7	1.4	3.0*

Data compiled from multiple sources.[5,16–21,30,31,35–38,40–42]
h = human; i.p. = intraperitoneal administration; m = monkey; p.o. = oral administration; r = rat.
*Unpublished data obtained using similar methodology to that used for equivalent studies with SB-271046 and SB-399885.
[†]Unless stated otherwise, ≥100 fold selective versus 23–85 other receptors, enzymes, and ion channels.
[‡]5-HT$_{2A}$ pK_i = 8.0 (human).
[§]Assessed using [^{125}I]SB-258585 binding in striatum.

attention and interest in recent years, in line with the development of potent and selective tool molecules with which to probe its function. This review highlights the now compelling evidence-base showing that selective blockade of 5-HT$_6$ receptors results in a preclinical profile consistent with the potential to attenuate both the cognitive and the behavioral abnormalities of AD via modulation of multiple neurotransmitters and also synaptic plasticity. A first report that a 5-HT$_6$ receptor antagonist, SB-742457,[5] is of clinical benefit in AD patients (see http://www.gsk.com/investors/presentations/2007/neurosciences-seminar-dec07/jackie-hunter.pdf) provides further evidence of the therapeutic potential of this approach.

5-HT$_6$ RECEPTOR DISCOVERY, DISTRIBUTION AND GENETICS

The 5-HT$_6$ receptor is one of the most recent additions to the serotonin receptor family having been discovered by independent groups using molecular cloning approaches as recently as 1993.[6,7] It is a 7 transmembrane receptor positively coupled to the Gs protein and thus activates cAMP.[6,7] Several techniques have been used to assess the distribution of the 5-HT$_6$ receptor, which has proven to be almost exclusively localized within the CNS, being particularly abundant in regions that play key roles in modulating cognitive processes and neurotransmitter release. Using the highly selective 5-HT$_6$ receptor antagonist radioligand [^{125}I]SB-258585, autoradiographic binding studies in the rat brain have demonstrated a high level of specific binding within striatum, nucleus accumbens, islands of Calleja, and olfactory tubercule.[8] More moderate levels of specific binding were shown within several regions of the hippocampal formation and layers of the cerebral cortex. These results are in general accord with mRNA and immunolabeling localization studies.[9,10] The lack of receptors in the periphery corresponds to an absence of unwanted peripheral side effects for 5-HT$_6$ receptor antagonists, as observed to date in both preclinical and clinical studies.

The pattern of expression of 5-HT$_6$ receptors is remarkably similar between human and rat,[11] as is the potency of 5-HT$_6$ receptor antagonists (Table 1), suggesting the potential value of this particular rodent species for translational studies. This is in contrast to the case for mouse, where brain levels of 5-HT$_6$ receptors are much lower than those found in human or rat, and where the affinity of 5-HT$_6$ receptor antagonists is also reduced compared with other species.[12] This is somewhat unfortunate, given that many experimental models designed to mimic various aspects of AD pathology have been developed using transgenic mice.

During their work on cloning the human 5-HT$_6$ receptor gene, Kohen et al.[13] identified a silent polymorphism at base pair 267 (C267T). Although this polymorphism does not produce any consequential change in the amino acid sequence of the protein, its discovery led to an explosion in genetic association studies investigating potential correlation with several CNS disorders. In brief, although there is evidence linking this polymorphism in several syndromes that affect cognition, including dementia and schizophrenia, these findings have not always been replicated in additional cohorts or ethnic groups, and their significance remains to be established (see Woolley et al.[11] and Mitchell and Neumaier[14]).

DEVELOPMENT OF SELECTIVE TOOLS TO PROBE 5-HT$_6$ RECEPTOR FUNCTION

Once the 5-HT$_6$ receptor was discovered, a battery of compounds were soon assessed for affinity at this new

subtype. Several molecules that were already in widespread clinical use as either antipsychotic (e.g., clozapine[15]) or antidepressant (e.g., amitryptiline[6]) agents were found to have high affinity for 5-HT_6 receptors, but none proved to be selective. Initial studies to explore the functional role of the 5-HT_6 receptor subtype *in vivo* relied on the use of antisense oligonucleotides that had to be administered directly into the brain. In a relatively short time, a number of potent and selective small molecular weight 5-HT_6 receptor antagonists became available, the first examples of which were Ro 04-6790 and Ro 63-0563,[16] quickly followed by the structurally distinct molecule SB-271046.[17,18] Although all three compounds have proven to be extremely useful investigative tools, they are less than ideal for *in vivo* studies, mainly due to their poor ability to penetrate into the CNS after systemic dosing. Sustained discovery efforts have managed to synthesize orally active molecules with significantly enhanced brain penetrance characteristics while maintaining the excellent potency and selectivity of the earlier 5-HT_6 antagonist molecules. Examples in this category include SB-399885,[19] Ro 4368554,[20] and one of the most recently described compounds, SB-742457 (Table 1).[5] This last molecule is of particular interest as the first example within this mechanistic class reported to show efficacy in AD patients.

In terms of defining the current understanding of the potential utility of 5-HT_6 receptor antagonists in AD, much of the pivotal data has been derived from a small subset of the available compounds. The key pharmacological and pharmacokinetic properties of these leading tool molecules are summarized in Table 1, and the data from the now extensive behavioral profiling, mechanistic studies and recent clinical trials are described in detail in the next sections. The information available on other 5-HT_6 receptor antagonists, including those still believed to be in clinical development for cognitive disorders (Table 2), is more limited, but is remarkably confirmatory (see Schreiber et al.[21]).

In contrast to the success in generating selective 5-HT_6 receptor antagonists, selective agonists have proven to be much more elusive and to date have had less of an impact on defining the role of 5-HT_6 receptors, especially *in vivo*. The most notable exceptions are WAY-466 and WAY-181187, two compounds that have recently been used in both cognitive and neurochemical studies.[22–24]

COGNITIVE ENHANCING AND BEHAVIORAL PROFILES OF 5-HT_6 RECEPTOR ANTAGONISTS

The first studies investigating the potential role of 5-HT_6 receptors *in vivo* were conducted using intracerebroventricularly administered antisense oligonucleotides in rats to induce receptor knockdown.[25] The reduced cortical 5-HT_6 receptor level was correlated with increased yawning and stretching behavior, which was almost completely abrogated by atropine, a nonselective antagonist of muscarinic receptors. This observation was confirmed and extended by a follow-up study in which the enhanced stretching behavior induced by the 5-HT_6 receptor antagonist Ro 04-6790 was blocked by the muscarinic antagonists atropine and scopolamine.[26] These findings indicate that the endogenous 5-HT_6 receptors are under tonic activation to suppress cholinergic neurotransmission. Because acetylcholine (ACh) was known to be directly involved in a range of cognitive and behavioral functions, including memory, anxiety, arousal, attention, and fatigue,[27,28] a potential therapeutic role for 5-HT_6 receptor antagonists in cognitive and psychiatric diseases became apparent.

This view gained further momentum when the 5-HT_6 receptor antisense oligonucleotides were demonstrated to improve spatial learning and memory in the Morris water maze test in normal rats.[29,30] Additional work with Ro-04-6790 (10 and 30 mg/kg, i.p.) confirmed the influence of 5-HT_6 receptor blockade on cognitive processes relevant to the retention of information, but not on learning acquisition, in this paradigm.[30] SB-271046 (10 mg/kg, p.o.), was also shown to enhance retention but not acquisition in young unimpaired rats, using the Morris water maze,[31] suggesting that chronic administration may improve long-term retention of the task rather than directly modulate the learning process per se. However, a number of independent groups have attempted to replicate these findings with both Ro-04-6790 and SB-271046 in the Morris water maze without success.[32,33] Different experimental protocols may contribute to these discrepancies, and further work is required to improve the validity and reliability of these results.

Alternative cognitive paradigms have been used to expand on previous reports documenting procognitive effects, using a wide range of 5-HT_6 receptor antagonists and a variety of tests and conditions that probe a number of cognitive domains (i.e., encoding, consolidation and storage, and retrieval). One such model, the object recognition test, has been used by a number of groups to ascertain 5-HT_6 receptor antagonist effects across a range of experimental manipulations (i.e., using delay or pharmacologically induced deficits in normal rats and naturally occurring deficits in aged rats) to provide confirmation of cognitive enhancement. This test is thought to engage the perirhinal and postrhinal cortex, where object processing is considered to be mediated and which has reciprocal connections with the hippocampus to provide an integration of event-related memories.[34]

Both Ro-04-6790 (10 mg/kg, i.p.) and SB-271046 (10 mg/kg, i.p.) increased novel object recognition in normal adult rats after acute and chronic (lead-in) administration protocols,[35] in which the trial interval between the sam-

Table 2. *5-HT$_6$ Receptor Antagonists in Clinical Development for Cognitive Disorders*

Compound (Company)	Study; Indication	Clinical Data and Status
SB-742457 (GSK)	Phase II; AD	In several phase I studies, SB-742457 was found to be well tolerated, with a safety profile similar to placebo. Terminal half-life was >24 h; at a dose of 35 mg, 5-HT$_6$ receptor occupancy in the brain was >80%. Two phase II trials have now been completed.
SAM-531 (Wyeth)	Phase II; AD	Four separate phase I safety, PK, and PD studies have been completed and SAM-531 has progressed to the next stage of development. A phase II trial in 78 patients with mild-to-moderate AD is ongoing to assess the safety, PK, and PD of multiple ascending fixed doses. Studies are also reportedly underway to evaluate the PD effects of SAM-531 on sleep and quantitative wake EEG in healthy subjects.
SGS-518 (Lundbeck/Lilly)	Phase II; schizophrenia	In phase I studies, SGS-518 was well tolerated in both a dose-ranging and a multidose cohort. Encouragingly, in a small trial involving 20 schizophrenia patients stable on antipsychotic medication SGS-518 produced a dose-proportionate improvement in cognition as determined using the Brief Assessment of Cognition in Schizophrenia scale. This effect reached significance at the highest dose tested (240 mg), and no dose-limiting adverse effects were apparent.
PRX-07034 (EPIX)	Phase I; AD and obesity	A number of phase I trials have been completed with PRX-07034. Most significant has been a multiple ascending-dose study in which PRX-07034 was administered once daily for 28 days to 33 obese, but otherwise healthy, adults. The compound was generally well tolerated at up to 600 mg and at this dose level produced a significant improvement in several performance measures within a cognitive screening battery (CogScreen; developed by G.G. Kay). PRX-07034 has been selected by the Treatment Units for Research on Neurocognition and Schizophrenia for a future phase II trial.
SYN-114 (Synosia/Roche)	Phase I; AD	An initial phase I trial with SYN-114 has been completed, but to date no clinical data have been reported.
SUVN-502 (Suven)	Phase I; AD and obesity	Phase I studies with SUVN-502 are underway.

Information on the general design and purpose of clinical trials was obtained from the U.S. National Institutes of Health http://www.clinicaltrials.govwebsite. Results data were obtained from press releases on the website of the sponsoring company. AD = Alzheimer's disease; PD = pharmacodynamic; PK = pharmacokinetic.

ple and discrimination test was 4 h (the resulting differential between novel and familiar object exploration is typically abolished). Effects on consolidation and storage, rather than encoding or retrieval per se, were inferred from experiments in which both compounds were administered before, or immediately after, the sample trial. Pretreatment with the NMDA receptor antagonist MK-801 prevented the cognitive enhancing effect of Ro-04-6790 on delay-induced deficits in object recognition, suggesting that 5-HT$_6$ antagonists may enhance consolidation and storage processes through increasing central glutamatergic neurotransmission.[35]

Another study examined the effects of a longer intertrial interval (e.g., 48 h) between the sample and discrimination phases, and showed significant dose-dependent improvements in novel object recognition with SB-271046 (0.3–10 mg/kg, i.p.).[36] Furthermore, SB-271046 (0.3–3.0 mg/kg, i.p.) has also been shown to dose-dependently reverse a 5-h novel object recognition memory deficit in aged F344 rats,[36] suggesting that 5-HT$_6$ receptor blockade confers cognitive benefit within an aged background. Procognitive effects with SB-399885 (10 mg/kg, p.o., unpublished observation) and Ro-4368554 (3 and 10 mg/kg, i.p.)[21] have been demonstrated in the object recognition paradigm, using adult rats, suggesting that this test is very sensitive to the cognitive enhancing effects of 5-HT$_6$ receptor blockade. Furthermore, when the nonselective muscarinic antagonist scopolamine was used to disrupt cognitive performance on the object recognition test in rats, by reducing central cholinergic neurotransmission, robust improvements were also generally reported for 5-HT$_6$ antagonists. Thus, the reversal of a scopolamine-induced object recognition deficit has been shown for Ro-04-6790 (10 mg/kg, i.p.),[37] SB-399885 (10 mg/kg, p.o.),[19] Ro-4368554 (3 and 10 mg/kg, i.p.)[21,38]

and SB-742457 (1.5 mg/kg, p.o.),[5] and has formed a benchmark cognitive test for more recently discovered 5-HT_6 receptor antagonist compounds (e.g., SUVN-507).[39] Other tests sensitive to the behavioral and cognitive impairments induced by scopolamine (e.g., passive avoidance, autoshaping, social recognition, contextual fear-conditioning, Morris water maze) have also confirmed a procognitive profile with the majority of the aforementioned 5-HT_6 receptor antagonists, depending on the test conditions used and doses selected (see review by Mitchell and Neumaier[14]).

In studies modeling an age-induced impairment on cognitive performance in rats using the Morris water maze, a number of 5-HT_6 receptor antagonists have demonstrated significant reversal. SB-271046 (10–20 mg/kg, p.o., and 1–6 mg/kg, i.p.),[36,40] SB-399885 (10 mg/kg, p.o.),[19] and SB-742457 (1.5 mg/kg, p.o.)[5] have all shown consistent improvements in both acquisition and retention, suggesting that in aged rats this test is sensitive to the cognitive enhancing properties of several 5-HT_6 receptor antagonists. However, Ro-4368554 (1–10 mg/kg, p.o.) did not improve the performance of aged rats in the Morris water maze.[41] Notably, a potential additive or synergistic effect of SB-271046 (1.0 mg/kg, i.p.) with donepezil (0.3 mg/kg, i.p.) has been demonstrated using this paradigm,[36] suggesting that there maybe some utility in combining 5-HT_6 receptor antagonists with cholinesterase inhibitors to improve cognitive performance or to increase therapeutic responsiveness in AD.

More recently, the 5-HT_6 receptor agonist WAY-181187 has been shown to impair social recognition in rats, a profile commonly provoked by scopolamine.[24] When suboptimal doses of WAY-181187 and scopolamine were combined in this social memory paradigm, there was a synergistic shift toward a deficit, suggesting that tonic activation of the 5-HT_6 receptor can disrupt cognitive processes linked to reductions in cholinergic tone. Furthermore, SB-271046 (20 mg/kg, i.p.) abolished both the WAY-181187-induced and the scopolamine-induced social recognition deficit, further reinforcing the notion that 5-HT_6 receptor blockade improves cognitive function by modulating central cholinergic tone. The site of action for these studies was confirmed as being within the frontal cortex (using bilateral direct injections of WAY-181187 and SB-271046),[24] a key area involved with modulating cognitive function in this social recognition paradigm and one in which 5-HT_6 receptor localization has been reported. SB-399885 (1–30 mg/kg, p.o.) has also been shown to improve performance in an autoshaping and an attentional set shifting task in rats, which suggests a role for 5-HT_6 receptor blockade in enhancing cognitive flexibility.[42,43] These findings may provide some basis for the potential treatment of social cognition and attentional deficits demonstrated in AD, schizophrenia, and other CNS disorders. A number of 5-HT_6 receptor antagonists are in development for cognitive disorders associated with schizophrenia (e.g., SGS-518) (Table 2).

In terms of translating the cognitive enhancing properties of 5-HT_6 antagonists from rodents to nonhuman primates, a limited number of studies have been conducted to assess this mechanism in monkeys. For example, SB-271046 (10 and 30 mg/kg, p.o.) was shown to improve correct responses in a delayed matching-to-sample test in a small number of aged rhesus monkeys with established delay-dependent impairments (unpublished data). Furthermore, SB-271046 (3 and 10 mg/kg, p.o.) was shown to significantly reverse MK-801-induced impairments on both perceptual visual discrimination and visuospatial conditional discrimination tasks (FIG. 1), suggesting a modulatory effect on central glutamatergic pathways within the cortex and hippocampus. These results indicate that 5-HT_6 receptor blockade can enhance cognitive function in a primate species, and emerging data show that this observation also extends to humans.

A number of 5-HT_6 receptor antagonists, including several compounds still in development, have successfully undergone phase I clinical studies in healthy volunteers. In all cases, excellent safety and tolerability profiles have been observed after single or repeated doses, indicating no apparent liabilities for the 5-HT_6 receptor antagonist approach at this stage (Table 2). For the most advanced compound, SB-742457, two phase II trials have recently been completed in subjects with mild-to-moderate AD. The first was reportedly a dose-ranging trial comparing SB-742457 with placebo, and the second was an exploratory study with SB-742457 and donepezil arms. Overall, these studies have demonstrated that SB-742457 is well tolerated in AD patients, with a safety profile comparable to placebo. Regarding therapeutic efficacy, SB-742457 produced an improvement in both cognitive and global function in AD as assessed by ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive subscale) and CIBIC+ (Clinician's Interview-Based Impression of Change-plus Caregiver Input), respectively. The effect size measured at the dose of 35 mg SB-742457 was similar to that of donepezil (see http://www.gsk.com/investors/presentations/2007/neurosciences-seminar-dec07/jackie-hunter.pdf). These preliminary data represent the first demonstration of symptomatic benefit in AD for a 5-HT_6 receptor antagonist.

Regarding other behavioral symptoms associated with AD, such as anxiety and depression, there is evidence to suggest that 5-HT_6 receptor antagonists have both anxiolytic and antidepressant properties in preclinical models. For example, SB-399885 has been shown to be active in the Vogel conflict (1–3 mg/kg, i.p.) and the elevated-plus maze (0.3–3 mg/kg, i.p.) models of anxiety and in the forced swim test (10 mg/kg, i.p.) model of

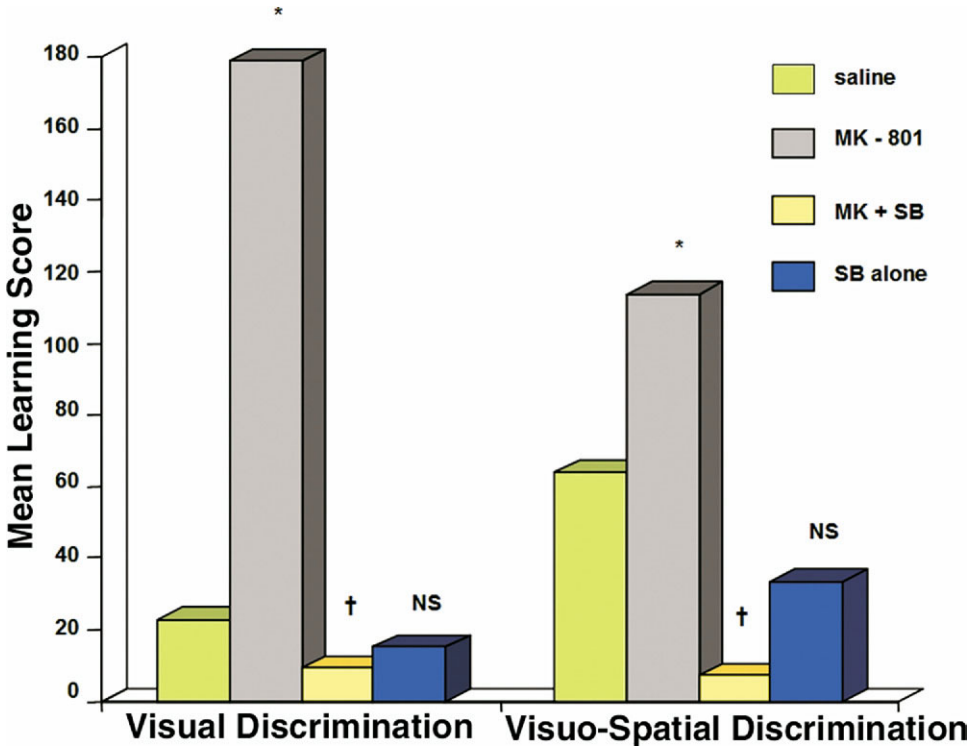

FIG. 1. Reversal of MK-801-induced deficits in perceptual visual discrimination and visuospatial conditional discrimination tasks by SB-271046 in the common marmoset. MK-801 (0.015 mg/kg intramuscularly) produced a significant impairment in performance of both the perceptual visual discrimination (cortical–dependent) and visuospatial (hippocampal-dependent) tests, relative to saline-control administration (*). SB-271046 (3 and 10 mg/kg, p.o.; data shown for highest dose only) completely reversed (†) these deficits back to a saline-control level of performance. The learning score refers to the number of trials required before the 90% correct criterion was met (i.e., 27 correct responses over 30 consecutive trials). The higher the learning score, the more trials needed to learn the task and thus the more difficult the marmoset found the task. The methodology used in this study was comparable to that reported previously.[95,96] NS, nonsignificant compared with saline-controls. Data courtesy of Josie Harder, Bradford University.

depression in rats.[44] The magnitudes of effects demonstrated were similar to those of reference standards (i.e., diazepam and imipramine for anxiety and depression, respectively). In addition, the same research group demonstrated an antidepressant profile in the rat forced swim test after the combined administration of subactive doses of SB-399855 (3 mg/kg, i.p.) with either desipramine (norepinephrine [NE] reuptake inhibitor) or bupropion (dopamine [DA] reuptake inhibitor), but not citalopram (5-HT reuptake inhibitor).[45] This evidence suggests that 5-HT$_6$ blockade may enhance central noradrenergic and dopaminergic tone within pathways relevant to antidepressant activity. A more recent study has confirmed that intrahippocampal administration of a 5-HT$_6$ antagonist (SB-258585) produces an anxiolytic and antidepressant profile in both Vogel conflict and forced swim tests, respectively, and suggests that the hippocampus, where 5-HT$_6$ receptors are indeed present, plays a key role in mediating these behaviors.[46] Such evidence suggests that 5-HT$_6$ receptor antagonists may improve both the cognitive and behavioral symptoms associated with AD by modulating multiple central neurotransmitter systems.

A number of studies have also pointed toward potential food intake and body weight reductions with 5-HT$_6$ receptor antagonists, suggesting potential therapeutic application in obesity. For example, early studies demonstrated effects of 5-HT$_6$ receptor-directed antisense oligonucleotides injected intracerebroventricularly on reducing food consumption and body weight gain.[30] Reductions in body weight gain were also reported for Ro-04-6790 (30 mg/kg, i.p.).[30] Subsequent reports confirmed effects on food intake and body weight with the following 5-HT$_6$ antagonists: SB-271046,[11] SUVN-503 and SUVN-504,[47] E-6837,[48] and BVT compounds.[49] As a result of these preclinical rodent studies, several lead compounds are currently in development for obesity (i.e., SUVN-504, E-6837, PRX-07034, and BVT-74316). SB-742457, however, did not induce any significant body weight or food intake reductions at cognitive enhancing doses in preclinical species tested to date (unpublished data).

Overall, largely consistent effects have been demonstrated with 5-HT$_6$ receptor antagonists in preclinical models of cognition, indicative of enhancement of learning and memory related to the consolidation and storage of information. These properties appear to be underpinned, at least in part, by modulation of cholinergic and glutamatergic tone within reciprocal pathways between the cortex and hippocampus. This putative mechanism,

together with effects on noradrenergic and dopaminergic pathways, may also be relevant in the treatment of behavioral symptoms associated with AD, such as anxiety and depression. This therapeutic promise now appears to have translated to AD patients, based on the outcome of the first reported clinical trials with a 5-HT_6 receptor antagonist in this population. Recent rodent data suggest that there may be some synergistic or additive cognitive benefit in combining a 5-HT_6 receptor antagonist with a cholinesterase inhibitor, highlighting promise of a new paradigm for symptomatic treatment approaches for AD.

5-HT_6 RECEPTOR ANTAGONISTS MODULATE MULTIPLE NEUROTRANSMITTER PATHWAYS

Several microdialysis studies with selective 5-HT_6 receptor antagonists have provided direct evidence for the ability of 5-HT_6 receptor blockade to elevate cholinergic neurotransmission in different brain regions of freely moving adult rats, including the prefrontal cortex (4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl) phenylamine[50] and SB-399885[19]) and the dorsal hippocampus (SB-271046[51]). This neurochemical effect is corroborated by the ability of 5-HT_6 receptor antagonists to reverse learning and memory deficits induced by cholinergic antagonists.

Nonetheless, the procognitive properties of 5-HT_6 receptor antagonists are unlikely to be attributable solely to their effect on the cholinergic system. In addition to stimulating cholinergic neurotransmission, SB-271046 also induces the release of the excitatory neurotransmitter glutamate in the dorsal hippocampus and frontal cortex, as well as aspartate in the frontal cortex.[52,53] Excitatory neurotransmission plays an important role in long-term potentiation, learning, and memory, as exemplified by the observed impairment of cognitive functions by blocking glutamate neurotransmission in a range of behavioral paradigms and by the loss of glutamatergic neurons in postmortem brain of AD sufferers.[54,55] The glutamate-enhancing effect of 5-HT_6 receptor antagonism may therefore provide an additional mechanism for improving cognition. From a therapeutic point of view, it is of note that the induction of glutamate and ACh are independent processes,[53] so conceptually the demise of cholinergic neurons should not compromise the cognitive enhancing properties of 5-HT_6 receptor antagonists as mediated *via* elevation of glutamate release. 5-HT_6 receptor antagonists additionally stimulate the release of DA and NE in the prelimbic–infralimbic subregion of the medial prefrontal cortex.[56] This brain region is abundantly innervated by catecholaminergic neurons, and is associated with cognitive function, temporal organization of behavior, and behavioral flexibility.[57,58]

Although the expression profile of 5-HT_6 receptors in the brain has been extensively studied, the cellular localization and the neuronal circuitries that determine the neurochemical effects of these receptors are less well explored. Lesioning studies with the serotonergic neurotoxin 5,7-dihydroxytryptamine have shown that 5-HT_6 receptors are located postsynaptically to serotonergic neurons and do not serve a role as a serotonergic autoreceptor.[9] This is in line with the lack of effect of 5-HT_6 receptor antagonism on the extracellular levels of 5-HT in several brain regions.[53,56] On the other hand, although 5-HT_6 receptor antagonists elevate the release of ACh, glutamate, NE, and DA, there has so far been no evidence for the expression of 5-HT_6 receptors on any of these neurons based on immunohistochemical, microdialysis, and neurochemical lesioning studies.[8,11,52,53,59,60] Thus, the induction of these neurotransmitters is expected to be indirect.

Significantly, several reports now provide converging evidence that 5-HT_6 receptors are expressed on GABAergic neurons. First, immunohistochemical data show the presence of the receptors on medium spiny neurons in the striatum,[61] consistent with localization to GABA neurons. This is supported by the co-staining of 5-HT_6 receptors and the GABAergic neuronal marker glutamic acid decarboxylase 67 (GAD67) in several brain regions of the rat, including the hippocampus, cortex, basal ganglia, thalamus, and cerebellum.[11] Second, functional confirmation is provided by microdialysis studies on freely moving rats, showing increases in extracellular levels of GABA in the frontal cortex and dorsal hippocampus upon the administration of the 5-HT_6 receptor agonist WAY-466, as well as a reduction in the induced release of glutamate from an *in vitro* hippocampal culture.[22]

In summary, cumulative data support a model of neural circuitry in which tonic activation of 5-HT_6 receptors expressed on GABAergic neurons leads to the induction of GABA release and in turn the inhibition of downstream cholinergic and glutamatergic neurons. Blockade of 5-HT_6 receptors effectively removes this tonic GABAergic inhibition of downstream neurons, resulting in enhanced neurotransmission of at least ACh and glutamate. As yet, there are no direct data linking the effects of 5-HT_6 receptors on GABA and the consequent release of DA and NE. A single study has suggested that the induction of dopamine release by 5-HT_6 receptor antagonists in the striatum may be through the modulation of ACh,[16] but again the absence of 5-HT_6 receptors on cholinergic neurons precludes them from being the initial target cells. There does not, as yet, appear to be any obvious downside to this unique neurochemical profile, in that 5-HT_6 receptor antagonists have been generally well tolerated in all of the human and preclinical studies reported to date. The ability of 5-HT_6 receptor antagonists to inhibit GABAergic function must be subtle: it is sufficient to produce cognitive enhancement, but does

not reach levels that can lead to unwanted stimulant effects such as anxiety or enhanced seizure liability. Indeed, 5-HT$_6$ receptor antagonists are anxiolytic, and in some cases anticonvulsant.[18]

ADDITIONAL MECHANISMS OF ACTION

In addition to the regulation of neurotransmission, other mechanisms may contribute to the therapeutic potential of 5-HT$_6$ receptor antagonists in AD, including the induction of structural plasticity and regulation of Fyn kinase.

Structural plasticity refers to the physical flexibility of synapses, which enables the neuronal networks to adapt to and to process the constant flow of information in the brain. Increased structural plasticity is associated with improved learning and memory functions. A key mediator of interneuronal contacts is neural cell adhesion molecule (NCAM), which is expressed on the cell surface of perisynaptic neuronal processes.[62,63] Although it is necessary for the induction of long-term potentiation and some forms of cognition,[64–66] the plastic nature is achieved by the post-translational polysialylation of NCAM through the attachment of large homopolymers of α2,8 polysialic acid (PSA) to form PSA-NCAM. PSA provides antiadhesive properties that reduce the binding affinity between NCAM on neuronal processes,[67,68] thus promoting structural plasticity. Enzymatic removal of hippocampal PSA eliminates the induction and maintenance of hippocampal long-term potentiation,[69,70] impairs spatial learning,[70,71] and synaptogenesis on NCAM-expressing neurons.[72] The *in vivo* deletion of polysialyltransferase-1, an enzyme that attaches PSA to NCAM, can also lead to impairment of learning and memory.[73]

In both rat and human hippocampus, PSA-NCAM is predominantly expressed in the subgranular zone of the dentate gyrus and along the mossy fibers.[74–76] This anatomical localization is consistent with the identification of the subgranular zone as a neural stem cell niche where new neurons are produced throughout life and extend their dendrites to form the mossy fibers that extend to the CA3 region. Young neurons express high levels of PSA-NCAM, have greater degrees of structural plasticity, exhibit enhanced long-term potentiation, and appear to be preferentially inserted into neural circuits of memory.[77–80] These findings led to the connotation that the upregulation of PSA-NCAM in the hippocampus is positively correlated with cognitive function. This is exemplified by several studies in different rat models of learning and memory, such as spatial learning in the Morris water maze and fear-conditioning.[71,81,82]

The 5-HT$_6$ receptor antagonists SB-271046,[83,84] SB-399885,[84] and SB-742457[5] have all been shown to augment the expression levels of PSA-NCAM in the dentate gyrus of the rat. The effect of the compounds on behaviorally naïve animals has been observed even after chronic dosing for 40 days, indicating the lack of desensitization of this induction effect. Furthermore, the acute or chronic predosing of SB-271046 to rats undergoing training in a Morris water maze paradigm significantly increased the level of PSA-NCAM above that induced by training alone in the water maze.[83] This is in line with the ability of SB-271046, SB-399885, and SB-742457 to significantly improve learning and memory in the aged rat Morris water maze task.[5,19,84] These data demonstrate the ability of 5-HT$_6$ receptor antagonists to induce the expression of PSA-NCAM, a vital mediator of neural plasticity and cognition, in a brain region that is critical for learning and memory. Notably, a PSA-NCAM mimotope (a cyclic 12-amino acid peptide that can conformationally mimic antigenic specificity) designated PR 21C is currently undergoing preclinical development by Pharmaxon (Marseilles, France) aiming for target indications of mild cognitive impairment and Alzheimer's disease (http://www.pharmaxon.com/#42). Figure 2 provides a hypothetical model linking the effects of 5-HT$_6$ receptor antagonists on cognition, glutamate release and PSA-NCAM expression.

A single recent report on the physical and functional association of 5-HT$_6$ receptors with Fyn kinase has raised an intriguing, though speculative, new angle on 5-HT$_6$ receptor antagonism. Activation of 5-HT$_6$ receptors leads to phosphorylation of tyrosine 420 on Fyn, an activating phosphorylation, as well as Fyn-dependent activation of Erk1/2.[85] This novel finding could have far-reaching implications in AD, because of the potentially deleterious effects of active Fyn in β-amyloid toxicity,[86] synaptic and cognitive impairments,[87] and phosphorylation of tau.[88] It can be hypothesized that the activation of Fyn by 5-HT$_6$ receptors would exacerbate these pathological consequences; in this way, antagonism of the 5-HT$_6$ receptor may be protective. On the other hand, active Fyn supports excitatory synaptic transmission by mediating the membrane recruitment of NMDA receptors in the postsynaptic density,[89] while mice deficient in Fyn exhibit defective long-term potentiation, impaired special memory, and increased fearfulness.[90,91] Thus, the potential therapeutic relevance of the 5-HT$_6$ receptor–Fyn pathway remains to be clarified.

CONCLUSIONS

Alzheimer's disease is one of the most important medical, social, and economic problems confronting contemporary society. Although cholinesterase inhibitors and, introduced more recently, memantine (a reported modulator of glutamatergic function[92]) represent an advance in the treatment of AD, they are by no means panaceas. For example, a review of the available data on the use of donepezil for dementia due to

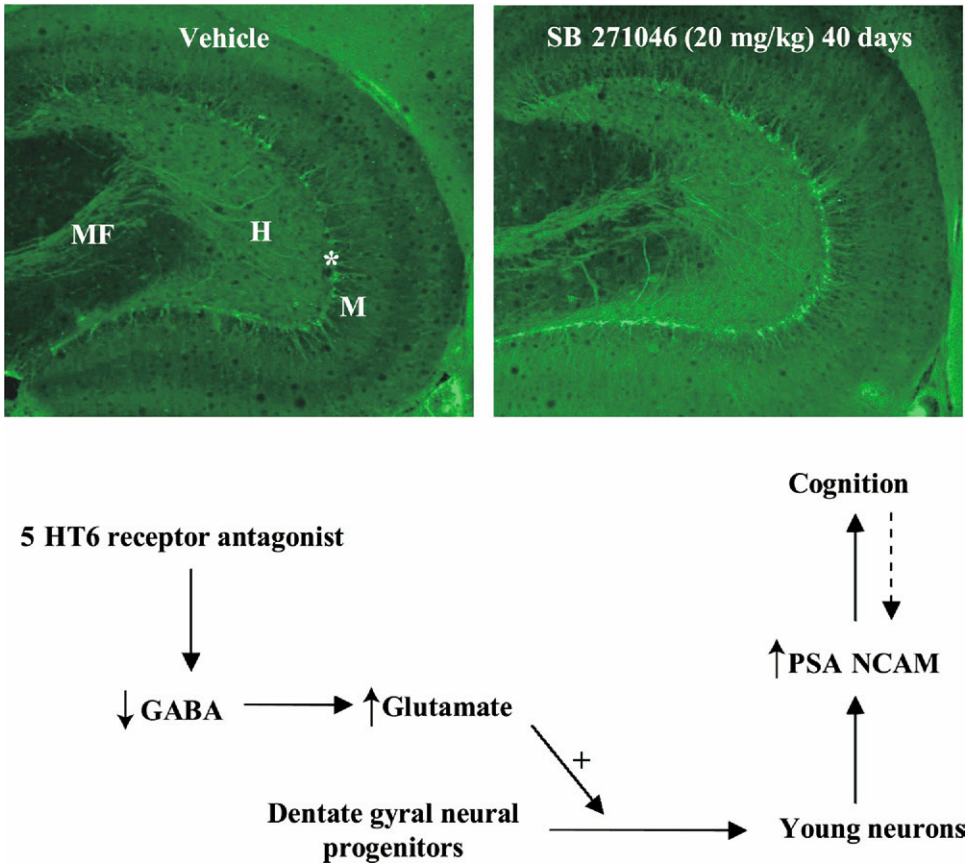

FIG. 2. Regulation of PSA-NCAM expression in the dentate gyrus by 5-HT_6 receptor antagonists. *Top:* Induction of PSA-NCAM expression on granule neurons in the dentate gyrus of rats treated for 40 days with SB-271046. PSA-NCAM immunofluorescence is localized to the neuronal cell body in the subgranular/granular layers (*) and dendritic processes that extend into the molecular layer (M) of the dentate gyrus. PSA-NCAM immunofluorescence is also expressed on the mossy fiber (MF) axons in the hilus region (H), although to a lesser extent. After treatment with SB-271046, a striking increase in the number of neurons in the subgranular/granule layers expressing PSA-NCAM immunofluorescence was observed. Images prepared by A.G. Foley and courtesy of C.M. Regan, Department of Pharmacology, The Conway Institute, University College Dublin. *Bottom:* A hypothetical model for the induction of PSA-NCAM expression. 5-HT_6 receptor antagonists reduce the tonic activation of GABA release,[22] thus suppressing the inhibitory tone on glutamatergic neurons and resulting in elevated glutamate release in the hippocampus. Glutamate promotes the neuronal differentiation of neural progenitor cells, a process that entails the transitory formation of immature young neurons, which express elevated levels of PSA-NCAM.[75] The effect of glutamate on the neural progenitors appears to specifically affect only the differentiation process, without increasing their proliferation rate,[97] which is in accord with the lack of any effect by SB-271046 on the proliferation index in the dentate gyrus.[84] The increased level of PSA-NCAM contributes to improved cognition, and may form a positive feedback loop with cognitive activities, given that brain activity associated with learning can induce PSA-NCAM expression.[82,98,99]

AD concluded that treatment effects are small and not always apparent in clinical practice.[93] Unwanted side effects (largely peripheral in nature) can also be problematic. Thus, there is still a clear need to develop novel approaches to the symptomatic relief of AD that differentiate from current treatment options either in improved efficacy and tolerability profiles, responder rates, or overall clinical benefit.

Because neurochemical losses or imbalances in AD are not restricted only to the cholinergic or glutamatergic systems, it is expected that, to deliver more effective therapies in the future, agents that modulate several neurotransmitter pathways will be required. Indeed, clinical data have already shown that patients with moderate to severe AD who received donepezil (the most widely used ChEI) coadministered with memantine had significantly greater cognitive improvement than did those given donepezil alone.[94] This finding is in keeping with the view that it is the balance of effect on pairs of neurotransmitters that will ultimately be important in determining therapeutic outcome.[4]

At present, many compounds that alter the function of varied neurotransmitters are being developed with AD as a target indication. Of these, the 5-HT_6 receptor antagonists appear to hold much potential as new therapies, because in preclinical studies they are clearly able to modulate multiple neurotransmitter systems and by so doing enhance cognition and attenuate anxiety and depression-like behaviors. It is even more encouraging that this initial promise has now translated into the first demonstration of clinical efficacy of a 5-HT_6 receptor antagonist (SB-742457) in AD patients, and that cognitive

enhancement was attained at doses of SB-742457 that were generally well tolerated.

The outcome of continued studies in AD (and also potentially other disorders characterized by cognitive deficits, such as schizophrenia and Parkinson's disease) with SB-742457 and alternative 5-HT$_6$ receptor antagonists currently at earlier stages of development is therefore eagerly awaited. Only then will it be known whether 5-HT$_6$ receptor antagonists are truly more advantageous than existing therapies, or than the many other mechanistic classes of symptomatic approaches presently under clinical evaluation, but at the very least there is substantive reason to remain optimistic at this stage.

Acknowledgments: We would like to thank the SB-742457 Project team (especially Jo Hunter, Gareth Maher-Edwards, and Ruth Dixon) for providing the clinical trials data on this compound, Josie Harder for kindly allowing us to report her nonhuman primate study with SB-271046 and MK-801, Andrew Foley and Ciaran Regan for providing their PSA-NCAM data, and Chris Ioannou for his comprehensive review of current literature and company news in the ever-progressing 5-HT$_6$ field.

REFERENCES

1. Brookmeyer R, Johnson E, Zeigler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimer's Dement 2007;3:186–191.
2. Terry AV Jr, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 2003;306:821–827.
3. Courtney C, Farrell D, Gray R, et al.; AD2000 Collaborative Group. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. Lancet 2004;363:2105–2115.
4. Ramirez MJ, Aisa B, Garcia-Alloza M, Gil-Bea FJ, Marcos B. Involvement of the serotonergic system in cognitive and behavioral symptoms of Alzheimer's disease. Curr Psychiatry Rev 2005; 1:337–343.
5. Chuang ATT, Foley A, Pugh PL, et al. 5-HT$_6$ receptor antagonist SB-742457 as a novel cognitive enhancing agent for Alzheimer's disease. Alzheimers Dement 2006;2:S631–S632 (abstract).
6. Monsma FJ Jr, Shen Y, Ward RP, Hamblin MW, Sibley DR. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol 1993;43: 320–327.
7. Ruat M, Traiffort E, Arrang J-M, et al. A novel rat serotonin (5-HT$_6$) receptor: molecular cloning, localization, and stimulation of cAMP accumulation. Biochem Biophys Res Commun 1993; 193:268–276.
8. Roberts JC, Reavill C, East S, et al. The distribution of 5-HT$_6$ receptors in rat brain: an autoradiographic radioligand binding study using the radiolabelled 5-HT$_6$ receptor antagonist [^{125}I]SB-258585. Brain Res 2002;934:49–57.
9. Gérard C, el Mestikawy S, Lebrand C, et al. Quantitative RT-PCR distribution of serotonin 5-HT$_6$ receptor mRNA in the central nervous system of control or 5,7–dihydroxytryptamine-treated rats. Synapse 1996;23:164–173.
10. Hamon M, Doucet E, Lefèvre K, et al. Antibodies and antisense oligonucleotides for probing the distribution and putative functions of central 5-HT$_6$ receptors. Neuropsychopharmacology 1999;21 Suppl 2:68S–76S.
11. Woolley ML, Marsden CA, Fone KCF. 5-HT$_6$ receptors. Curr Drug Targets CNS Neurol Disord 2004;3:59–79.
12. Hirst WD, Abrahamsen B, Blaney FE, et al. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 2003;64: 1295–1308.
13. Kohen R, Metcalf MA, Khan N, et al. Cloning, characterization, and chromosomal localization of a human 5-HT$_6$ serotonin receptor. J Neurochem 1996;66:47–56.
14. Mitchell ES, Neumaier JF. 5-HT$_6$ receptors: a novel target for cognitive enhancement. Pharmacol Ther 2005;108:320–333.
15. Roth BL, Craigo SC, Choudray MS, et al. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther 1994;268: 1403–1410.
16. Sleight AJ, Boess FG, Bös M, Levet-Trafit B, Riemer C, Bourson A. Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT$_6$ receptors. Br J Pharmacol 1998;124:556–562.
17. Bromidge SM, Brown AM, Clarke SE, et al. 5-Chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide (SB-271046): a potent, selective and orally bioavailable 5-HT$_6$ receptor antagonist. J Med Chem 1999;42:202–205.
18. Routledge C, Bromidge SM, Moss SF, et al. Characterization of SB-271046: a potent, selective, orally active 5-HT$_6$ receptor antagonist. Br J Pharmacol 2000;130:1606–1612.
19. Hirst WD, Stean TO, Rogers DC, et al. SB-399885 is a potent, selective 5-HT$_6$ receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol 2006;553:109–119.
20. Bonhaus DW, Martin R, Brothers J, et al. Ro4368554, a high affinity, selective, CNS penetrating 5-HT$_6$ receptor antagonist. Soc Neurosci Abstr 2002;884.5 (abstract).
21. Schreiber R, Vivian J, Hedley L, et al. Effects of the novel 5-HT$_6$ receptor antagonist Ro4368554 in rat models for cognition and sensorimotor gating. Eur Neuropsychopharmacol 2007;17:277–288.
22. Schechter LE, Smith DL, Zhang GM, et al. Pharmacological profile of a novel and selective 5-HT$_6$ receptor agonist. Fundam Clin Pharmacol 2004;18(Suppl 1):135–151:SAT-P 7.5 (abstract).
23. Schechter LE, Lin Q, Smith DL, et al. Neuropharmacological profile of novel and selective 5-HT$_6$ receptor agonists: WAY-181187 and WAY-208466. Neuropsychopharmacology 2008;33:1323–1335.
24. Loiseau F, Dekeyne A, Millan MJ. Pro-cognitive effects of 5-HT$_6$ receptor antagonists in the social recognition procedure in rats: implication of the frontal cortex. Psychopharmacology (Berl) 2008;196:93–104.
25. Bourson A, Borroni E, Austin RH, Monsma FJ Jr, Sleight AJ. Determination of the role of the 5-HT$_6$ receptor in the rat brain: a study using antisense oligonucleotides. J Pharmacol Exp Ther 1995;274:173–180.
26. Bentley JC, Bourson A, Boess FG, et al. Investigation of stretching behaviour induced by the selective 5-HT$_6$ receptor antagonist, Ro 04-6790, in rats. Br J Pharmacol 1999;126:1537–1542.
27. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry 1999;66:137–147.
28. Perry E, Walker M, Grace J, Perry R. Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends Neurosci 1999;22:273–280.
29. Bentley JC, Sleight AJ, Marsden CA, Fone KCF. 5-HT$_6$ antisense oligonucleotide i.c.v. affects rat performance in the water maze and feeding. J Psychopharmacol 1997;11 (3 Suppl):A64 (abstract).
30. Woolley ML, Bentley JC, Sleight AJ, Marsden CA, Fone KC. A role for 5-HT$_6$ receptors in retention of spatial learning in the Morris water maze. Neuropharmacology 2001;41:210–219.
31. Rogers DC, Hagan JJ. 5-HT$_6$ receptor antagonists enhance retention of a water maze task in the rat. Psychopharmacology (Berl) 2001;158:114–119.
32. Russell MGN, Dias R. Memories are made of this (perhaps): a review of serotonin 5-HT$_6$ receptor ligands and their biological functions. Curr Top Med Chem 2002;2:643–654.
33. Lindner MD, Hodges DB Jr, Hogan JB, et al. An assessment of the

effects of serotonin 6 (5-HT_6) receptor antagonists in rodent models of learning. J Pharmacol Exp Ther 2003;307:682–691.
34. Bussey TJ, Muir JL, Aggleton JP. Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. J Neurosci 1999;19:495–502.
35. King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC. 5-HT_6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation: an effect sensitive to NMDA receptor antagonism. Neuropharmacology 2004;47:195–204.
36. Callahan PM, Ilch CP, Rowe WB, Tehim A. Characterization of the selective 5-HT_6 receptor antagonist SB-271046 in behavioral models of cognition. Soc Neurosci Abstr 2004;776.19 (abstract).
37. Woolley ML, Marsden CA, Sleight AJ, Fone KC. Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT_6 receptor antagonist, Ro 04-6790. Psychopharmacology (Berl) 2003;170:358–367.
38. Lieben CK, Blokland A, Sik A, Sung E, van Nieuwenhuizen P, Schreiber R. The selective 5-HT_6 receptor antagonist Ro4368554 restores memory performance in cholinergic and serotonergic models of memory deficiency in the rat. Neuropsychopharmacology 2005;30:2169–2179.
39. Nirogi RV, Vishwakarma SL, Abraham R, Jayarajan P, Goyal RK, Shirsath VS. SUVN-507 reverses scopolamine induced amnesia in Morris water maze and novel object recognition task. Soc Neurosci Abstr 2006;162.12 (abstract).
40. Foley AG, Murphy KJ, Hirst WD, et al. The 5-HT_6 receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. Neuropsychopharmacology 2004;29:93–100.
41. Szczepanski KV, Vivian JA, Dorsch K, et al. Procognitive effects of the 5-HT_6 receptor antagonist Ro4368554 in rats. Soc Neurosci Abstr 2002;290.2 (abstract).
42. Perez-García G, Meneses A. Oral administration of the 5-HT_6 receptor antagonists SB-357134 and SB-399885 improves memory formation in an autoshaping learning task. Pharmacol Biochem Behav 5;81:673–682.
43. Hatcher PD, Brown VJ, Tait DS, et al. 5-HT_6 receptor antagonists improve performance in an attentional set shifting task in rats. Psychopharmacology (Berl) 2005;181:253–259.
44. Wesołowska A, Nikiforuk A. Effects of the brain-penetrant and selective 5-HT_6 receptor antagonist SB-399885 in animal models of anxiety and depression. Neuropharmacology 2007;52:1274–1283.
45. Nikiforuk A, Wesołowska A, Popik P. Effect of combined administration of the selective serotonin 6 receptor antagonist SB-399885 and antidepressants in the forced swim test in rats. Soc Neurosci Abstr 2007;709.7 (abstract).
46. Wesołowska A, Nikiforuk A, Stachowicz K. Anxiolytic-like and antidepressant-like effects produced by the selective 5-HT_6 receptor antagonist SB-258585 after intrahippocampal administration to rats. Behav Pharmacol 2007;18:439–446.
47. Pendharkar VV, Vishwakarma SL, Patel AS, Shirsath VS, Kambhampati SR, Nirogi RVS. Effect of selective 5-HT_6 receptor antagonists on food-intake and body weight gain. Soc Neurosci Abstr 2005;533.7 (abstract).
48. Jackson HC, Cheetham SC, Dickinson K, et al. Effects of chronic administration and withdrawal of the novel 5-HT_6 ligand, E-6837, in a rat model of dietary-induced obesity. Soc Neurosci Abstr 2006;62.2 (abstract).
49. Svartengren J, Axelsson-Lendin P, Multan U, et al. Characterization of a new selective BVT serotonin 5-HT_6 receptor antagonist in animal models for feeding and body weight regulation. Soc Neurosci Abstr 2007;629.11 (abstract).
50. Riemer C, Borroni E, Levet-Trafit B, et al. Influence of the 5-HT_6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT_6 receptor antagonist. J Med Chem 2003;46:1273–1276.
51. Zhang MY, Hughes ZA, Kerns EH, Lin Q, Beyer CE. Development of a liquid chromatography/tandem mass spectrometry method for the quantitation of acetylcholine and related neurotransmitters in brain microdialysis samples. J Pharm Biomed Anal 2007;44:586–593.
52. Dawson LA, Nguyen HQ, Li P. In vivo effects of the 5-HT_6 antagonist SB-271046 on striatal and frontal cortex extracellular concentrations of noradrenaline, dopamine, 5-HT, glutamate and aspartate. Br J Pharmacol 2000;130:23–26.
53. Dawson LA, Nguyen HQ, Li P. The 5-HT_6 receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology 2001;25:662–668.
54. Francis PT, Sims NR, Procter AW, Bowen DM. Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: investigative and therapeutic perspectives. J Neurochem 1993;60:1589–1604.
55. Palmer AM. Neurochemical studies of Alzheimer's disease. Neurodegeneration 1996;5:381–391.
56. Lacroix LP, Dawson LA, Hagan JJ, Heidbreder CA. 5-HT_6 receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. Synapse 2004;51:158–164.
57. Kolb B. Functions of the frontal cortex of the rat: a comparative review. Brain Res 1984;320:65–98.
58. Passetti F, Chudasama Y, Robbins TW. The frontal cortex of the rat and visual attentional performance: dissociable functions of distinct medial prefrontal subregions. Cereb Cortex 2002;12:1254–1268.
59. Dawson LA, Li P. Effects of 5-HT_6 receptor blockade on the neurochemical outcome of antidepressant treatment in the frontal cortex of the rat. J Neural Transm 2003;110:577–590.
60. Marcos B, Gil-Bea FJ, Hirst WD, García-Alloza M, Ramírez MJ. Lack of localization of 5-HT_6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT_6 receptor-mediated acetylcholine release. Eur J Neurosci 2006;24:1299–1306.
61. Gerard C, Martres MP, Lefèvre K, et al. Immuno-localization of serotonin 5-HT_6 receptor-like material in the rat central nervous system. Brain Res 1997;746:207–219.
62. Persohn E, Schachner M. Immunoelectron microscopic localization of the neural cell adhesion molecules L1 and N-CAM during postnatal development of the mouse cerebellum. J Cell Biol 1987;105:569–576.
63. Schuster T, Krug M, Stalder M, Hackel N, Gerardy-Schahn R, Schachner M. Immunoelectron microscopic localization of the neural recognition molecules L1, NCAM, and its isoform NCAM180, the NCAM-associated polysialic acid, beta1 integrin and the extracellular matrix molecule tenascin-R in synapses of the adult rat hippocampus. J Neurobiol 2001;49:142–158.
64. Doyle E, Nolan PM, Bell R, Regan CM. Intraventricular infusions of anti-neural cell adhesion molecules in a discrete posttraining period impair consolidation of a passive avoidance response in the rat. J Neurochem 1992;59:1570–1573.
65. Scholey AB, Rose SP, Zamani MR, Bock E, Schachner M. A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein synthesis six hours following passive avoidance training of the young chick. Neuroscience 1993;55:499–509.
66. Lüthl A, Laurent JP, Figurov A, Muller D, Schachner M. Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. Nature 1994,372:777–779.
67. Sadoul R, Hirn M, Deagostini-Bazin H, Rougon G, Goridis C. Adult and embryonic mouse neural cell adhesion molecules have different binding properties. Nature 1983;304:347–349.
68. Moran N, Bock E. Characterization of the kinetics of neural cell adhesion molecule homophilic binding. FEBS Lett 1988;242:121–124.
69. Muller D, Wang C, Skibo G, et al. PSA-NCAM is required for activity-induced synaptic plasticity. Neuron 1996;7:413–422.
70. Becker CG, Artola A, Gerardy-Schahn R, Becker T, Welzl H, Schachner M. The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. J Neurosci Res 1996;45:143–152.
71. Venero C, Herrero AI, Touyarot K, et al. Hippocampal up-regulation of NCAM expression and polysialylation plays a key role on spatial memory. Eur J Neurosci 2006;23:1585–1595.

72. Diyatev A, Dityateva G, Sytnyk V, et al. Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. J Neurosci 2004;24:9372–9382.
73. Markram K, Gerardy-Schahn R, Sandi C. Selective learning and memory impairments in mice deficient for polysialylated NCAM in adulthood. Neuroscience 2007;144:788–796.
74. Seki T, Arai Y. Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. J Neurosci 1993;13:2351–2358.
75. Seki T, Arai Y. Different polysialic acid-neural cell adhesion molecule expression patterns in distinct types of mossy fiber boutons in the adult hippocampus. J Comp Neurol 1999;410:115–125.
76. Ní Dhúill CM, Fox GB, Pittock SJ, O'Connell AW, Murphy KJ, Regan CM. Polysialylated neural cell adhesion molecule expression in the dentate gyrus of the human hippocampal formation from infancy to old age. J Neurosci Res 1999;55:99–106.
77. Ge S, Yang CH, Hsu KS, Ming GL, Song H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron 2007;54:559–566.
78. Zhao C, Teng EM, Summers RG Jr, Ming GL, Gage FH. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci 2006;26:3–11.
79. Kee N, Teixeira CM, Wang AH, Frankland PW. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nat Neurosci 2007;10:355–362.
80. Kitabatake Y, Sailor KA, Ming GL, Song H. Adult neurogenesis and hippocampal memory function: new cells, more plasticity, new memories? Neurosurg Clin N Am 2007;18:105–113.
81. Senkov O, Sun M, Weinhold B, Gerardy-Schahn R, Schachner M, Dityatev A. Polysialylated neural cell adhesion molecule is involved in induction of long-term potentiation and memory acquisition and consolidation in a fear-conditioning paradigm. J Neurosci 2006;26:10888–10898.
82. Lopez-Fernandez MA, Montaron MF, Varea E, et al. Upregulation of polysialylated neural cell adhesion molecule in the dorsal hippocampus after contextual fear conditioning is involved in long-term memory formation. J Neurosci 2007;27:4552–4561.
83. Johnson CN, Roland A, Upton N. New symptomatic strategies in Alzheimer's disease. Drug Discov Today Ther Strateg 2004;1:13–20.
84. Foley AG, Hirst WD, Gallagher HS, et al. The 5-HT$_6$ receptor antagonists SB-271046 and SB-399885 potentiate NCAM PSA immunolabeling of dentate granule cells, but not neurogenesis, in the hippocampal formation of mature Wistar rats. Neuropharmacology 2008;54:1166–1174.
85. Yun HM, Kim S, Kim HJ, et al. The novel cellular mechanism of human 5-HT$_6$ receptor through an interaction with Fyn. J Biol Chem 2007;282:5496–5505.
86. Lambert MP, Barlow AK, Chromy BA, et al. Diffusible, nonfibrillar ligands derived from Aβ_{1-42} are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A 1998;95:6448–6453.
87. Chin J, Palop JJ, Puoliväli J, et al. Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease. J Neurosci 2005;25:9694–9703.
88. Lee G, Thangavel R, Sharma VM, et al. Phosphorylation of tau by fyn: implications for Alzheimer's disease. J Neurosci 2004;24:2304–2312.
89. Dunah AW, Sirianni AC, Fienberg AA, Bastia E, Schwarzschild MA, Standaert DG. Dopamine D1-dependent trafficking of striatal *N*-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. Mol Pharmacol 2004;65:121–129.
90. Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. Science 1992;258:1903–1910.
91. Miyakawa T, Yagi T, Watanabe S, Niki H. Increased fearfulness of Fyn tyrosine kinase deficient mice. Mol Brain Res 1994;27:179–182.
92. Danysz W, Parsons CG. The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer's disease: preclinical evidence. Int J Geriatr Psychiatry 2003;18 Suppl 1:S23–S32.
93. Birks J, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. Cochrane Database Syst Rev 2006;1:CD001190.
94. Tariot PN, Farlow MR, Grossberg GT, Graham SM, McDonald S, Gergel I. Memantine treatment in patients with moderate to severe Alzheimer's disease already receiving donepezil. JAMA 2004;291:317–324.
95. Harder JA, Ridley RM. The 5-HT$_{1A}$ antagonist, WAY 100 635, alleviates cognitive impairments induced by dizocilpine (MK-801) in monkeys. Neuropharmacology 2000;39:547–552.
96. Schechter LE, Smith DL, Rosenzweig-Lipson S, et al. Lecozotan (SRA-333): a selective serotonin 1A receptor antagonist that enhances the stimulated release of glutamate and acetylcholine in the hippocampus and possesses cognitive-enhancing properties. J Pharmacol Exp Ther 2005;314:1274–1289.
97. Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC. Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron 2004;42:535–552.
98. Murphy KJ, O'Connell AW, Regan CM. Repetitive and transient increases in hippocampal neural cell adhesion molecule polysialylation state following multitrial spatial training. J Neurochem 1996;67:1268–1274.
99. Fox GB, O'Connell AW, Murphy KJ, Regan CM. Memory consolidation induces a transient and time-dependent increase in the frequency of neural cell adhesion molecule polysialylated cells in the adult rat hippocampus. J Neurochem 1995;65:2796–2799.