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DUAL-ACTING AGENTS FOR IMPROVING COGNITION AND REAL-WORLD FUNCTION IN ALZHEIMER'S DISEASE: FOCUS ON 5-HT6 AND D3 RECEPTORS AS HUBS

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Abstract

To date, there are no interventions that impede the inexorable progression of Alzheimer's disease (AD), and currently-available drugs cholinesterase (AChE) inhibitors and the N-Methyl-D-Aspartate receptor antagonist, memantine, offer only modest symptomatic benefit. Moreover, a range of mechanistically-diverse agents (glutamatergic, histaminergic, monoaminergic, cholinergic) have disappointed in clinical trials, alone and/or in association with AChE inhibitors. This includes serotonin (5-HT) receptor-6 antagonists, despite compelling preclinical observations in rodents and primates suggesting a positive influence on cognition. The emphasis has so far been on *high* selectivity. However, for a multi-factorial disorder like idiopathic AD, 5-HT6 antagonists possessing additional pharmacological actions might be more effective, by analogy to "multi-target" antipsychotics. Based on this notion, drug discovery programmes have coupled 5-HT6 blockade to 5-HT4 agonism and inhibition of AchE. Further, combined 5-HT6/dopamine D3 receptor (D3) antagonists are of especial interest since D3 blockade mirrors 5-HT6 antagonism in exerting broad-based pro-cognitive properties in animals. Moreover, 5-HT6 and dopamine D3 antagonists promote neurocognition and social cognition *via* both distinctive *and* convergent actions expressed mainly in frontal cortex, including suppression of mTOR over-activation and reinforcement of cholinergic and glutamatergic transmission. In addition, 5-HT6 blockade affords potential anti-anxiety, anti-depressive and anti-epileptic properties, and antagonising 5-HT6 receptors may be associated with neuroprotective ("disease-modifying") properties. Finally D3 antagonism may counter psychotic episodes and D3 receptors themselves offer a promising hub for multi-target agents. The present article reviews the status of "R and D" into multi-target 5-HT6 and D3 ligands for improved treatment of AD and other neurodegenerative disorders of aging.

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Introduction: Alzheimer's disease and its current treatment

AD, which accounts for some 60 to 70 % of cases of dementia, is characterised by a bewildering suite of risk factors, a panoply of cognitive and psychiatric symptoms, and a diversity of mutually-reinforcing pathophysiological changes, with heterogeneity amongst patients translating into differential responsiveness to treatments (**Figure 1**) (Deardorff and Grossberg, 2019; Sala Frigerio and De Strooper, 2017; Joe and Ringman, 2019; Scheltens et al, 2016). Clinical diagnosis occurs only decades after the emergence of cellular pathology, but improvements in the imaging of β-amyloid and Tau, in the detection of these neurotoxic proteins in biofluids, and in the application of biomarkers like circulating miRNAs, polygenic scores, environmental risk factors and electroencephalography offer prospects for pinpointing vulnerable subjects and hence more precocious intervention (Chasioti et al, 2019; Cummings, 2019; Eid et al, 2019; Gaubert et al, 2019; Leuzy et al; 2019; Millan, 2017; Molinuevo et al, 2018). Despite enormous efforts to develop drugs that block the accumulation and aggregation of β-amyloid, clinical trials have not yet led to authorisation, although there still remain some irons in the fire (Aducanumab data have been resuscitated and this antibody against β-amyloid, which markedly reduces its levels in the brain and provides some cognitive benefit, will be proposed to the FDA in Spring 2020) (Howard and Liu, 2019; Sevigny et al, 2016). Further, efforts are continuing to impede the onset and progression of AD by interfering with the aberrant processing, folding and spread of tau, countering neuroinflammation and restoring cerebral energetics (Boland et al, 2018; Cunnane et al, in press; Paouri and Georgopoulos, 2019; Ryu et al, 2019; Scheltens et al, 2016; Veitch et al, 2019). In parallel, the adoption of life-style changes embracing Mediterranean and related diets, regular exercise and improved sleep may be related to the *decreasing* incidence of AD, a contention supported by the relative success of the "FINGER" trial, with improved control of cardiovascular risks factors also helping curtail the development of AD (Ngandu et al, 2015; Park et al, 2019; Saito et al, 2019).

It is crucial to better understand the pathological mechanisms driving AD, other forms of dementia and related neurodegenerative diseases of aging, and to advance disease-modifying medication for course-alteration (Sala Frigerio and De Strooper, 2017; Scheltens et al, 2016; Veitch et al, 2019). Nonetheless, notwithstanding a litany of failures in the past, it is important not to forego efforts to improve *symptoms* in patients at various stages of the disorder from Mild Cognitive Impairment (MCI) to late stage AD *via* mechanisms that do *not* necessarily attack the pathological foundations of the disorder (Alam et al, 2017; Birks and Harvey, 2018; Dou et al, 2018; Joe and Ringman, 2019; Kandiah et al, 2017; McShane et al, 2019; Parsons et al, 1999; Scheltens et al, 2016). Indeed, it would seem ethically questionable to abandon those tens of millions of subjects already suffering from AD yet unlikely to benefit from future progress in disease-modifying therapy. This is particularly true since the few agents currently authorised to treat AD target only *two* mechanisms of action: inhibition of AChE (donezepil, galantamine and rivastigmine) and low affinity antagonism of N-Methyl-D-Aspartate (NMDA) receptors (memantine). Moreover, these medications manifest only modest efficacy for restoration of cognitive performance and global function, with little improvement in neuropsychiatric symptoms (Alam et al, 2017; Birks and Harvey, 2018; Francis et al, 2012; Joe and Ringman, 2019; McShane et al, 2019; Parsons et al, 1999). The failure to generate innovative agents

for treating the symptoms of AD is starkly displayed in **Figure 2** in comparison to the steady stream of mechanistically-diverse drugs (and other classes of intervention) for treating major depression, although this is not to claim that the management of depression is anywhere near ideal, in particular as regards the problems of resistance and partial responsivity (Millan, 2006; Millan et al, 2015 ; Wilkinson and Sanacora, 2018).

As regards the improved control of symptoms in AD, a strategy beyond the purely neurocognitive would be advisable, including social cognition which is also strongly affected in AD, neuropsychiatric symptoms like aggression, confusion, anxiety and depression, disrupted sleep patterns, anomalous circadian rhythms, motor anomalies and poor nutrition and energy balance (Christidi et al, 2018; Deardorff and Grossberg 2019; Henry et al; 2016; McArdle et al, 2017; McClam et al, 2015; Peralta and Cuesta; 2017; Porceli et al, 2019; Ryu et al, 2019; Wise et al, 2019). The influence of interventions should best be monitored using improved readouts as compared to those conventionally employed to date. In this respect, both for AD and for its earlier, preclinical manifestations, one might hope for the emergence of revised rating scales, a mixture of self- and carer-effected quantification of treatment impact, novel readouts and the insightful use of digital real-world monitoring and other nascent technologies (Gold et al, 2018; Kueper et al, 2018; Piau et al, 2019; Porceli et al, 2018; Veitch et al, 2019).

Finding broadly-effective, course-altering and symptomatic treatments for AD will not be an easy task and, in addition to their exploitation upon a favourable life-style and energetic background, interventions with multiple and *complementary* mechanisms of action may have the greatest chance of success in view of the complexity and diversity of the disorder. Indeed, an excessive penchant for high selectivity may have compromised the effectiveness of many potentially useful targets for altering the course of AD – not to mention other complex CNS disorders. One single mechanism of action (MOA) may not be sufficient to reach the threshold for clinically-demonstrable efficacy, explaining why certain agents have not proven successful in the clinic despite encouraging preclinical observations (see below) (de Freitas Silva et al, 2019; Millan, 2006; Sahoo et al, 2018; Zhang et al, 2019).

Available drugs for treating cognitive deficits in AD: limited efficacy and therapeutic range

As shown in **Figure** 2, only four agents are currently authorised for treating the cognitive impairment of AD. Three of these are inhibitors of AChE (donepezil, galantamine and rivastigmine) (Alam et al, 2017; Joe and Ringman, 2019; Kandiah et al, 2017; Parsons et al, 1999; Tricco et al, 2018). Though AChE inhibitors should recuit several classes of post-synaptic nicotinic and muscarinic receptor, their respective contributions to relief of cognitive disruption in AD remains unknow (Verma et al, 2018). Acetylcholine levels are also regulated by butyrylcholinesterase (mainly localised in glia and white matter), so the additional actions of rivastigmine at this enzyme may reinforce its ability to protect ACh from degradation as well as, speculatively, impeding disease progression, though such advantages have yet to be proven in the clinic (Birks and Harvey, 2018; Darvesh, 2016; Joe and Ringman, 2019; Kandiah et al, 2017). In addition, galantamine purportedly possesses antagonist properties at nicotinic receptors, but the clinical relevance of this potential action has been strongly questioned (Kowal et al, 2018).

Memantine, authorised for the treatment of moderate to severe AD, is a fast on/off kinetic, voltage-dependent, non-competitive antagonist ("channel blocker") of moderate affinity at NMDA receptors (Alam et al, 2017; Francis et al, 2012; McShane et al, 2018; Parsons et al, 2013; Parsons et al, 1999). Interestingly, memantine has a number of other low affinity interactions at various ion channels and other sites (Parsons et al,1999), but it is unclear to what extent they contribute to its clinical influence on cognitive function. Finally, possibly reflecting convergent recruitment of "glutamatergic" and cholinergic mechanisms, memantine and AChE inhibitors are often administered in association, and a fixed combination of memantine and donepezil ("Namzaric") has been introduced in the US (Francis et al, 2012; Kennedy et al, 2018; Matsunaga et al, 2014; Parsons et al, 2013; Schmidt et al, 2015)

Astonishingly, or shockingly perhaps, since 2000 and the introduction of memantine, no further agent has been authorized for treatment of the cognitive deficits of AD. This dearth of new treatments is a serious issue for patients and carers alike in view of the only modest clinical benefits of currently available agents : this penury also contrasts starkly with the abundance of novel therapies for countering major depression (*vide supra*) (Figure 2). Moreover, in distinction to psychosocial-cognitive-behavioural and stimulation therapies, despite some encouraging progress, no "alternative" treatments are as yet formally available to improve cognitive dysfunction in AD (Arendash et al, 2019; Chang et al, 2018). Moreover, despite extensive efforts and considerable progress in understanding the neural underpinnings of AD, strategies for interrupting its relentless progress have not yet been identified.

The need to counter cognitive impairment and other neuropsychiatric symptoms of AD

The above observations underpin the importance of continuing to seek novel approaches for relieving the cognitive impairment and other symptoms of AD. Indeed, for patients already diagnosed with AD, it is unlikely that they will benefit from any imminent or future disease-modifying therapies that impede the onset or progression of AD (Joe and Ringman, 2019; Lanctot et al, 2017; Stahl, 2018). A key point here is the need to look beyond classic learning and memory deficits in attempting to restore other domains of impairment, in particular social cognition (Christidi et al, 2018; Porcelli et al, 2019). Furthermore, the motor and highly disruptive "neuropsychiatic" symptoms of AD have not been sufficiently explored for potential therapeutic improvement. They include impairments in sleep and circadian rhythms, anxiodepressive states that respond poorly to existing agents, aggressiveness and irritability (Deardorff and Grossberg 2019; Lanctot et al, 2017; McClam et al, 2018; Peralta and Cuesta; 2017; Wise et al, 2018). Another frequent complication of AD is psychosis, often associated with agitation: this is especially problematic since antipsychotic use is risky and discouraged in elderly patients with AD, although risperidone is currently the best-supported option (Creese et al, 2018; Deardorff and Grossberg, 2019; Lanctot et al, 2017).

Ideally, one would devise agents capable of improving multiple domains of dysfunction, although it is unclear whether they can be achieved with any single MOA.

Poor efficacy of highly selective agents for treating cognitive deficits in AD

Notwithstanding an impressive suite of preclinical observations and solid conceptual foundations, a broad array of highly-selective agents have failed to attain efficacy sufficient for authorisation for the improvement of cognitive performance in AD. These include agonists at specific subtypes of nicotinic and muscarinic receptor (Verma et al, 2018), positive allosteric modulators (PAMs) of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Bernard et al, 2019), antagonists (or inverse agonists) at histamine H3 receptors (Kubo et al, 2015), 5-HT4 receptor agonists (Lalut et al, 2016) and inhibitors of Phosphodiesterase 4A (Prickaerts et al, 2017). (We will come to 5-HT6 antagonists below). Quite apart from a *putative* lack of target relevance, reasons underlying the lack of success are complex and lie beyond the compass of this review (Cummings, 2019; Drummond and Wisnieski, 2017; Khoury et al, 2018; Molinuevo et al, 2018; Mullane and Williams, 2019; Neuner et al, 2019; Sabbagh et al, 2019). We may nonetheless one evoke such general issues as:

- Inadequate experimental validation, notably a paucity of studies of the influence of agents in animal models for AD, whereby their ability to "normalize" disrupted cognitive performance is established, preferably under long-term administration and employing a variety of behavioural and neurochemical readouts. This is of greater relevance that shifts in "baseline" cognition or reversals of delay-and pharmacologically induced disruption of performance but, of course, such investigations are far more onerous, and not realistic for early screening.

- Following up the previous point, poor translation of drug target, with insufficient data linking results from animal tests of cognition, electrophysiology, neurochemistry and endocrine status etc to observations in patients with AD;

- Failure to prove appropriate target engagement in patients (in certain cases owing to a lack of radioligands allowing *in vivo* imaging in humans) at doses employed clinically;
  Drawbacks of classic rating scales and procedures like the omnipresent and *de rigeur* yet far-from-perfect "ADAS-cog";

- The loss of a robust signal for one specific cognitive domain in overall assessments of cognitive impact;

- Substantial inter-individual variability amongst poorly stratified populations of AD patient;

- Alterations in clinical procedures, drug dose and schedule, and outcome measure etc following a successful Phase II upon shifting to the determinant Phase III.

More specific concerns include rapid receptor desensitization for targets like nicotinic and AMPA receptors, and a low therapeutic window between doses required for a robust therapeutic effect compared to those triggering adverse effects, notably for AMPA modulators and Phosphodiesterase inhibitors (Coombs et al, 2019; Joe and Ringman, 2019; Prickaerts et al, 2017; Verma et al, 2018). It is also possible that outcome in measures of cognitive performance is modified by (a concomitant influence of agents on) poorly-controlled factors like the energetic status of the brain, diurnal rhythms and sleep, and the gut microbiota (Cunnane et al, in press; Kowalski and Mulak, 2019; Ryu et al, 2019; Van Erum et al, 2019).

Nonetheless, a more prosaic answer as to why so many promising agents have failed to exert robust, broad-based and clinically significant improvement of cognitive deficits in large populations of patients may be their exceptional selectivity for one specific class of target. Paradoxically, then, it is precisely this high degree of specificity that represents the Achilles' heel. This notion is of quite general relevance to the control of complex disorders - AIDS being the most familiar example - and is particularly relevant to AD in view of its multi-faceted and progressive pathophysiology (de Freitas Silva et al, 2019; Millan, 2006; Sahoo et al, 2018; Zhang et al, 2019). Furthermore, this notion is just as relevant to efforts to develop agents that interfere with disease processes driving AD as to those designed to alleviate its symptoms. There is a good degree of irony here in view of the almost blind obsession with selectivity that has typified many (in particular, « High Throughput Screening and so-called Rational Drug Discovery ») programmes of compound selection and characterisation; even to the point of eliminating a-la-robot potentially useful, unexpected properties of novel compounds rather than reinforcing and intelligently valorizing them (Millan, 2008). Perhaps the most peculiar aspect of all this is the dubious conclusion that the target is "wrong" or "doesn't work" following disappointing clinical trials with ligands of very high selectivity, a bit like concluding that the wheels on a car without a steering wheel "don't work" because it won't go in the right direction (Abbott and Dolgin, 2016; Bespalov et al, 2016; Millan, 2006, 2008). Rather, it may be that target would be more effectively exploitable in association with a *complementary* MOA for reinforcing efficacy while expanding the therapeutic window (side-effects should not be additive for two mechanisms of actions sharing pro-cognitive properties) (Millan, 2006).

The concept of multi-target strategies for AD, a multi-factorial disorder

The above-evoked issues underpin the notion that the association of two agents possessing complementary MOAs, or a single agent possessing itself two or more MOAs, may be more effective than highly selective compounds in the management of AD and other complex disorders (Cummings et al, 2019; de Dreitas Silva et al; 2018; Millan, 2006; Umar and Hoda, 2019; Van der Schyf et al, 2006; Zhang et al; 2019). Such agents have been dubbed selectively non-selective (maintaining sites providing efficacy while dialling out those triggering side-effects), multi-modal, multi-functional, multi-target directed and multi-target amongst other appellations but we will stick to the latter term herein in view of its simplicity. Moreover, "functional" might be misconstrued as alluding to the roles of specific *chemical* elements, "multi-modal" implies association of medication with other types of therapy like cognitive-behavioural and "selectively non-selective" is a bit confusing for the non-initiated.

Irrespective of the appellation, the basic idea is that acting *via* two or more complementary MOAs should reinforce and broaden therapeutic efficacy in AD against cognitive impairment and other types of symptom. These MOAs might in theory be up and downstream, such as the coupling of G protein coupled receptor ligand with 1), a Phosphodiesterase inhibitor to boost alterations in AMP and/or cGMP or 2), a suppressor of mammalian target of rapamycin (mTOR) (implicated in cognitive processes, see below) (Bockaert and Marin, 2015; Millan et al, 2008; Prickaerts et al, 2017). Alternatively, one might imagine

agents that converge onto common neural substrates thought to promote cognition: here, cholinergic pathways and glutamatergic neurotransmission come to mind. Another possibility would be to act *via* independent cellular and/or neurotransmitter systems distributed across various cerebral structures impacted in AD in order to broaden and deepen therapeutic efficacy. In this case, one might hope for additional therapeutic benefits over and above relief of cognitive impairment, a notion advocated and explored below.

As illustrated in **Figure 3**, the frontal cortex, a projection site for many ascending pathways controlling cognition and mood is a site where multi-target agents designed to counter cognitive impairment and neuropsychiatric symptoms in AD could well interact (Millan et al, 2016; Sahoo et al, 2018; Zhang et al, 2019).

Influence of 5-HT6 receptors upon cognition: preclinical studies and clinical trials in AD patients

5-HT6 receptors, which are located postsynaptic to serotonergic neurons, are almost exclusively expressed in the CNS, supporting the notion that they can be modulated, stimulated or blocked while minimizing adverse, target-related side-effects (Charnay and Leger, 2010; Fone, 2008; Helboe et al, 2015; Hornung, 2003). The highest expression of receptor protein and mRNA in rats and man occurs in areas like the striatum, hippocampus and frontal cortex supporting a potential influence on cognition and mood (Chaumont-Dubel et al, 2019; Helboe et al, 2015; Millan et al, 2008). In the hippocampus and frontal cortex, 5-HT6 receptors appear to be largely located on neuronal dendrites, and, somewhat unusually for a G-protein coupled receptor, on primary neuronal cilia of glutamatergic and GABAergic neurones (Brailov et al, 2000; Fone, 2008; Helboe et al, 2015; Hu et al, 2017) as well as some cholinergic neurones. Cilial location is interesting since these are hubs for integration of cellular signaling, and they may be implicated in the pathogenesis of AD (Armato et al, 2013; Brodsky et al, 2017; Chaumont-Dubel et al, 2019; Hu et al, 2019; Lesiak et al, 2018). GABAergic interneurons are themselves inhibitory to glutamatergic and other pathways controlling cognition (Millan et al, 2012 Kann, 2016; Zott et al 2018).

One peculiarity of 5-HT6 receptors is their constitutive activity: that is, "spontaneous", ligand-independent signaling in the absence of agonists *via*, for example, Gs and adenylyl cyclase (Chaumont-Dubel et al, 2019; Duhr et al, 2014; Dupuis et al, 2008; Millan et al 2008). This is seen at both recombinant and native populations of 5-HT6 receptor in neurons, and can be influenced by various protein partners, though it is not entirely clear under which conditions (if any) cerebral populations of 5-HT6 receptor would be completely deprived of 5-HT (Chaumont-Dubel et al, 2019; Deraredj Nadim et al, 2017). Based on current evidence, it appears that agents acting either as neutral antagonists (no intrinsic actions themselves while blocking the actions of both agonists and inverse agonists) or as inverse agonists are active in tests of pro-cognitive activity. However, the precise relationship between negative efficacy and improved cognition is unclear - especially for human 5-HT6 receptors under pathological conditions. This should be borne in mind in the comments below where, mainly for clarity and by convention, we refer to 5-HT6 receptor "antagonists".

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A wealth of preclinical data (Codony, 2011; De Jong and Mork, 2017; King et al, 2008; Ramirez, 2013), has shown that selective 5-HT6 receptor antagonists improve cognition in rats, mice and primates in a large array of behavioural paradigms: spatial learning and memory, associative learning and memory, autoshaping, attentional set-shifting, novel object discrimination and social recognition (Eskenazi et al, 2015; Fone, 2008; Hu et al, 2017; Loiseau et al, 2008; Ramirez, 2013; Woods et al, 2012; Woolley et al, 2001). They are effective in both young and old animals, they reverse GABAergic-, cholinergic- and glutamatergic-induced cognitive impairment (Aparicio-Nava et al, 2019; King et al, 2004; Loiseau et al, 2008; Woods et al, 2012) and, in microdialysis studies, they enhance acetylcholine and glutamate release in the frontal cortex and hippocampus (Codony, 2012; De Jong and Mork, 2017; King et al, 2008; Mork et al, 2017; Ramirez, 2013). Interestingly, 5-HT6 receptor antagonists may, at least in part, modify cognitive function by altering the morphology and function of hippocampal neuronal primary cilia (Chaumont-Dubel et al, 2019). Supporting this notion, "transgenic" Amyloid Precursor Protein (APP)/Presenilin 1 mice overexpressing β-amyloid show cognitive impairment in water maze, Y-maze, and fear-conditioning tasks, and 7 days 5-HT6 antagonist treatment both recovered cognitive function and normalized cilia length (Hu et al, 2017). Together with findings that 5-HT6 antagonists like SB-271046 reverse a palette of age-related cognitive defects in aged mice and rats (Callaghan et al, 2012; Da Silva Costa et al, 2009, 2011; Foley et al, 2004; Hirst et al, 2006), these observation are of particular relevance to their use in AD. 5-HT6 antagonists also improve learning and memory deficits in neurodevelopmental models for cognitive dysfunction, such as isolation-reared rats (Fone, 2008). In other animal models, 5-HT6 receptor antagonists act synergistically with AChE inhibitors to promote cognition, observations that prompted clinical studies of their adjunctive utilization in AD (De Jong and Mork, 2017; Khoury et al, 2018). As a final remark, care should be taken when considering the pharmacological actions of 5-HT6 ligands in mice since several agents (like SB-258585 and Ro 04-6790) have lower affinity for mouse *vs* as compared to rat and human receptors: this is largely due to differences at residues 188 and 290 in transmembrane regions 5 and 6 of the 5-HT6 receptor (Hirst et al, 2003).

Over ten years after discovery of selective antagonists, the first 5-HT6 receptors agonists (such as WAY-181187 and E6801) emerged and, surprisingly, several groups found them to mimic antagonists in enhancing cognitive function in tasks of novel object recognition (Kendall et al, 2011), spontaneous alternation (Rychtyk, 2019), attentional set-shifting (Nikiforuk et al, 2013) and conditioned fear-motivated learning (Woods et al, 2012). Conversely, other labs did *not* find agonist-induced memory enhancement (Amodeo et al, 2018; Fone, 2008): indeed, WAY-181187 attenuated social recognition (Loiseau et al, 2008). Further, while 5-HT6 agonists SB-271046-reversibly elicited increases in levels of mRNA encoding cytoskeleton-associated protein (Arc) in hippocampus, their elevation in frontal cortex was *reproduced* by SB-271046 rather than attenuated (de Foubert et al, 2007).

Several reasons may account for the apparent anomaly that agonists mimic some pro-cognitive effects of antagonists (Fone, 2008; De Jong and Mork, 2017). One hypothesis is that antagonists modulate function exclusively in pathways with a high serotonergic tone. In addition, agonists and antagonists may act *via* distinct signaling pathways. Another possibility is that 5-HT6 agonists and antagonists alter the activity of *different*, spatially discrete populations of 5-HT6 receptor. For example, agonists might directly activate 5-HT6 receptors on cholinergic and/or glutamatergic neurones to promote ACh and glutamate release, whereas

antagonists likely inhibit receptors on GABAergic interneurones to *indirectly* enhance the release of these neurotransmitters (Fone, 2008; Mork et al, 2017; De Jong and Mork, 2017). Finally, the relationship of 5-HT6 receptor activity to cognitive function could show a bell-shaped relationship, modified in opposite directions by agonists compared to antagonists. These competing (but not mutually exclusive) explanations await further experimental evaluation.

As regards cellular substrates underlying the pro-cognitive actions of 5-HT6 receptor antagonists, they are promiscuous in coupling to a large variety of signalling pathways (Chaumont-Dubel et al, 2019; Codony et al, 2011; Deraredj Nadim et al, 2016; Millan et al, 2008). 5-HT6 receptors are coupled *via* Gs to generation of cAMP and activate adenylyl cyclase in a Gs-dependent manner, leading to recruitment of protein kinase A and cAMP-response binding element-1 ("CREB"), a prototypical lynch-pin of neuroplasticity. Blockade of CREB in GABAergic neurons might be involved in the effects of 5-HT6 antagonists, but the role of CREB in the control of cognition by 5-HT6 receptors is unlikely to be confined to this action (Codony et al, 2011; Deraredj Nadim et al, 2016; Millan et al, 2008; Teng et al, 2019). More recently 5-HT6 receptors were shown to interact with cyclin-dependent kinase 5 (Cdk5) which regulates neurite outgrowth: alterations in Cdk5 activity in the hippocampus conceivably mediate changes in cilial morphology and hippocampal-dependent learning and memory (Chaumont-Dubel et al, 2019; Dayer et al, 2015; Duhr et al, 2014; Hu et al, 2019). Additional partners of 5-HT6 receptors include Jun activation domain-binding protein-1 (Jab1) and the light chain 1 subunit of microtubule associated protein 1B (MAP1B) (Chaumont-Dubel et al, 2019; Kim et al, 2014; Liu et al, 2019; Yun et al, 2010). Their role in the control of cognition remains unclear, but one specific protein partner of 5-HT6 receptors has been strongly implicated in the regulation of neuronal plasticity and cognition, mTOR (Bockaert and Marin, 2015; Mueed et al, 2018). In the frontal cortex, a physical interaction of the C-terminal domain of the 5-HT6 receptor with the mTOR complex causes its phosphorylation-activation to impair cognition in the novel object recognition and social recognition tasks: these actions are prevented by the mTOR antagonist, rapamycin and by selective 5-HT6 receptor antagonist (Fone et al, 208; Loiseau et al, 2008; Meffre et al, 2012). mTOR activity is augmented in neonatal phencyclidine and isolation-rearing models of cognitive impairment, and the deficits are attenuated both by rapamycin and by 5-HT6 receptor antagonists (Meffre et al, 2012). Furthermore, Dietary Restriction was suggested to promote hippocampal Long-Term Potentiation and memory by *negative* modulation of 5-HT6 receptor mediated signaling (Teng et al, 2019). This action involved a role for mTOR as well as a NMDA receptor mediated induction of hippocampal pools of Brain-Derived Neurotrophic Factor (Teng et al, 2019). Regulation of hippocampal Brain-Derived Neurotrophic Factor expression may more generally be involved in the influence of both antagonists and agonists upon cognition and depressed mood (de Foubert et al, 2007; Rychtyk et al, 2019). Other mechanisms implicated in the pro-cognitive effects of 5-HT6 receptor antagonists include the induction of neural cell adhesion molecule polysialylation in the entorhinal and perirhinal (Foley et al, 2008).

Despite this array of cellular mechanisms, it is unlikely that they represent the full picture of how 5-HT6 receptors influence cognition. Further research is warranted to elucidate the molecular substrates underlying the pro-cognitive actions of 5-HT6 antagonists, which may well differ between specific classes of neuron. This would likely provide insights into potentially innovative classes of multi-target agent and their clinical exploitation. In addition, as mentioned above, the precise significance of negative ligand efficacy and cognitive performance remains to be clarified as regards the actions of agonists and antagonists.

Regrettably, despite the impressive array of experimental data suggesting that 5-HT6 antagonists exert pro-cognitive effects, clinical trials in AD - and schizophrenia - have not demonstrated sufficient therapeutic benefit to justify authorization, either alone or in association with AChE inhibitors (Atri et al, 2018; Maher-Edwards et al, 2015; Matsunaga et al, 2019; Wilkinson et al, 2014). A plethora of 5-HT6 antagonists, including SB-742457 (intepirdine), SAM-760, A-964324, AVN-322, masupirdine, SYN-120, idalorpidine have, then, unfortunately failed to reach patients (De Jong and Mork, 2017; Ferrero et al, 2017; Khoury et al, 2018; Matsunaga et al, 2019; Wicke et al, 2015). This must surely vie with the collapse of Neurokinin1 antagonist programmes for depression (Millan et al, 2015; Rupniak and Kramer, 2017) as one of the most frustrating and unforeseen disappointments in modern psychopharmacological drug development. The reasons underlying the inability to concretize promising preclinical and Phase II data with 5-HT6 antagonists are multifarious, discussed in the papers herewith cited, and embrace several issues underlying the more general failure of putative pro-cognitive agents to achieve therapeutic success in AD (see above). Most specifically, and in accordance with the core message of this paper, the potential utility of 5-HT6 receptor antagonism for improving cognition in AD and other disorders may require exploitation in association with *additional* MOAs, either in combination or, preferentially, integrated into a single molecule.

5-HT6 receptors as a foundation for multi-target therapies

As summarized above, enrichment of 5-HT6 receptors in the cortex, hippocampus, basal ganglia and other regions, together with their influence on glutamatergic, cholinergic and GABAergic transmission, provides a neuronal framework for the broad-based influence of antagonists on cognition and mood. On the other hand, despite encouraging Phase II readouts, phase III investigations with highly *selective* 5-HT6 antagonists have been disappointing. However, even discounting the many issues that have plagued trials of pro-cognitive agents in AD (*vide supra*), this is not to say that 5-HT6 sites are irrelevant to the treatment of AD (Abbott and Dolgin, 2016; Millan, 2006; Bespalov et al, 2016). This would be a case of false logic in the same manner as declaring that 5-HT2A receptors are irrelevant to the pathogenesis and management of schizophrenia on the basis that highly selective antagonists were not themselves effective in clinical trials alone: rather 5-HT2A receptor blockade is an advantageous component of the multi-target profile of agents that likewise block dopamine D2 and D3 receptors (Meltzer, 2012).

Accordingly, it would be of interest to associate 5-HT6 receptor blockade with other MOAs that favour the relief of cognitive dysfunction. In theory, this might be realised by either drug combinations or multi-target agents (Cummings et al, 2019 ; Millan, 2014; Sahoo et al; 2018). However, the latter strategy predominates, partly since polypharmacology is a particular concern in the elderly (Millan, 2014; Ravona-Springer and Davison, 2014). Further, no selective 5-HT6 antagonists are currently on the market rendering their adjunctive use impossible. In fact, "atypical antipsychotics" like clozapine and olanzapine possess significant antagonist properties at 5-HT6 receptors yet, as discussed elsewhere, their blockade of muscarinic, histamine-1 and α1-adrenoceptors as well as D2 receptors interferes with any putative, pro-cognitive actions (Meltzer, 2012; Millan et al, 2012, 2016). This point underpins the need to focus on compounds displaying

5-HT6 receptor antagonist properties plus one or two additional, well-defined and useful attributes, rather than generating ligands that indiscriminately interact with many receptor types. In practical terms, the most realistic approach is to design and search for dual-acting agents, while monitoring and amplifying rather than ignoring or discarding other beneficial pharmacological activities that may crop up unexpectedly. In any event, the avoidance of interactions triggering side-effects and directly interrupting pro-cognitive processes is obviously important.

As pointed out above, several clinical trials for AD were performed in patients treated with AChE inhibitors ("on top of"), most significantly those with idalorpidine where no arm with the drug alone was included in the Phase III study (Atri et al, 2018; Ferrero et al, 2017; Khoury et al, 2018; Matsunaga et al, 2019). Retrospectively it is easy to carp, but lack of information on the drug alone complicates interpretation of the failure to reach the primary outcome. It also raises the question of whether intrinsic, procognitive actions of 5-HT6 antagonists alone might paradoxically have been *blunted* rather than enhanced due to long-term AChE inhibition (De Jong and Mork, 2017; Kennedy et al, 2018). While a pharmacokinetic interaction is unlikely and easily controlled for, there may have been pharmacological interference such as nicotinic receptor desensitization (see above). This is speculative, but the coupling of 5-HT6 antagonism to AChE inhibition does not appear particularly attractive for multi-target ligands. Moreover, in developing "5-HT6 plus" ligands, an important goal is not merely to provide more robust control of cognitive deficits but also, if possible, to counter the neuropsychiatric symptoms of AD (*vide supra*). The notion of generating multi-target 5-HT6 ligands with additional *disease-modifying* properties is also briefly outlined below although, compared to other classes of potential therapy, 5-HT6 antagonism as a starting point does not appear especially auspicious for course-alteration.

Thus, the emphasis here is on 5-HT6 antagonism as a pivotal activity for improving the *symptoms* of AD - and possibly those of other neurodegenerative disorders. Relief of symptoms has, in view of clinical failures and a hugely-expanded range of options for attacking core pathophysiology, taken a back-seat in recent years (Scheltens, 2016; Stahl, 2018). However, to reiterate, for already-diagnosed patients with emerging and established dysfunction, alleviation of their symptoms is understandably their primary preoccupation, so maintaining "R and D" in this domain is more than justified.

Two examples of potential routes towards multi-target 5-HT6 ligands are shown in **Figures 4 and 5**. As mentioned above, and exemplified here for social cognition, the mTOR inhibitor, rapamycin, blocks the "amnesic" actions of the 5-HT6 agonist, WAY181,187 and, more significantly it potentiates the pro-ecognitive effects of the antagonist, SB258,585. This observation suggests scope for facilitating the improvement of cognition by 5-HT6 blockade, potentially in association with mTOR inhibition. It has been previously shown that glutamatergic neurotransmission and NMDA/Glycine B receptors are involved in the pro-cognitive properties of 5-HT6 antagonists (Codony et al, 2011; Fone et al, 2008), and the NMDA channel blocker, dizocilpine, duly prevented the actions of the 5-HT6 antagonist, SB271,046, in the social recognition procedure. In addition, and again pointing to a multi-target 5-HT6 plus mechanisms for restoring cognition in AD, the Glycine B co-agonist, D-Serine (Fossat et al, 2012; Ivanov and Mothet, 2019) enhanced the efficacy of SB271,046.

These findings, while clearly requiring extension, support the notion of multi-target 5-HT6 blockade for AD. Amongst a range of dual-acting 5-HT6 antagonist-based options currently under exploration, for reasons outlined below, the focus herein is on agents with complementary antagonist properties at D3 receptors. However, prior to a detailed consideration of this strategy, the following paragraphs summarize progress with several other classes of multi-target 5-HT6 ligand.

5-HT6 receptor blockade: fusion with 5-HT4 agonist, AChE inhibitory and other properties

In view of the opportunities for exploiting 5-HT6 receptors as a fulcrum for the elaboration of multi-target agents, it is surprising that so little work has been undertaken. For example, one could imagine the association of 5-HT6 receptor blockade with PAM properties at either AMPA receptors or specific subtypes of nicotinic receptor, or their combination with of histamine H3 receptor blockade REF. Further, as indicated above, rapamycin potentiated the pro-cognitive properties of 5-HT6 antagonists in rats, so mixed 5-HT6 antagonists/mTOR inhibitors are conceivable (Chaumont-Dubel et al 2019; Meffre et al, 2012). Quite apart from the entrenched resistance on logistical grounds of most drug-discovery managers to challenging multi-target (*vs* comparatively straightforward, selective) drug discovery projects, the dearth of multi-target 5-HT6 antagonists probably reflects the original conviction that *selective* 5-HT6 antagonists would be clinically successful: there were few or no drug discovery programmes run in parallel with a view to finding 5-HT6 antagonists with additional pharmacological properties. Ironically, the realisation that the clinical efficacy of *selective* 5-HT6 antagonists is limited is now proving equally discouraging: it is being assumed, based on the *lack* of therapeutic efficacy of selective antagonists, that 5-HT6 receptors are irrelevant (see above)! Thus, the handful of mechanisms outlined below represents only a sub-set of those in theory available for exploration. The focus is on concepts and pharmacological profile: readers are referred to the original papers cited below and to reviews for discussion (by experts) of the underlying chemistry (Zhang et al, 2019). In fact, the search for multi-target 5-HT6 ligands advanced in parallel with efforts to find both selective and multi-target 5-HT4 agonists and, in both cases, an association with AChE inhibition has attracted attention - despite reservations expressed above.

One early study cobbled together pharmacophores for both AChE and 5-HT6 receptors to generate a centrally-active derivative of tacrine possessing well-balanced activities of around 10 - 30 nM both for 5-HT6 receptors and for AChE and butyrylcholinesterase (Wieckowska et al, 2018). Subsequently, the same group reported a compound ("12") that possessed marked antagonist activity at 5-HT6 receptors (K_i/K_b, 18/132 nM) and which also inhibited AChE and butyrylcholinesterase at comparable concentrations (IC_{50} values, 14 and 22 nM, respectively). Interestingly, it was also active, albeit at higher concentrations (1.27 uM), in a (thioflavin) assay assessing inhibition of β-amyloid1-42 aggregation. Compound 12 showed reasonable blood-brain barrier penetration and - together with agents lacking a potentially hepatotoxic tacrine structure - it is an interesting starting point for further investigation of novel classes of multi-target 5-HT6 ligand (Wieckowska et al, 2018). While there remain concerns about potential cardiotoxic effects, the

therapeutic use of 5-HT4 agonists for treating constipation suggests that clinically safe ligands are feasible (Lalut et al, 2017).

In parallel, originating in the chance discovery that the "selective" 5-HT4 partial agonist, RS67333 interacts with modest affinity with AChE, other groups have searched for 5-HT4R ligands that even more potently inhibit the activity of AChE, as exemplified by donecopride, a hydrid of RS67333 and donepezil (Lecoutey et al, 2014; Legay et al, 2015; Rochais et al, 2015). Donecopride has robust affinity for both 5-HT4 receptors (6.6 nM) and AChE (16 nM). Reflecting its 5-HT4 agonist properties, it promoted the non-amyloidogenic processing of APP (11 nM) leading to preferential release of soluble APPα and decreased formation of β-amyloid1-42. Further, donecopride was active in a novel object recognition test *in vivo* (Lecoutey et al, 2014; Rochais et al, 2015). It would be interesting to learn more about donecopride and related agents (Hughes et al, 2016; Legay et al, 2015).

It is hardly astonishing that efforts have been made to merge these strategies, and the first description of a combined 5-HT4 (5.3 nM) and 5-HT6 receptor ligand (219 nM) was made by Yahiaoui et al, in 2016. However, the highlighted ligand, "7m", left considerable room for improvement. It had a substantial (40-fold) margin between these activities, it was also a potent ligand at 5-HT2A (330 nM) and 5-HT2C receptors (160 nM), and its ostensible "anti-amnesic" activity in a model of spontaneous alteration disrupted by scopolamine was, not to put too fine a point on it, marginal. Much more encouragingly, the same group reported the discovery of ligands possessing "triple" activity at 5-HT$_4$ receptors, 5-HT6 receptors and AChE. One of these "pleiotropic" compounds ("10"), moderated the deficits in performance elicited by scopolamine in a rat model of working memory (Hatat et al, 2019). Another line of study has focused on multi-target ligands that interact both with 5-HT6 receptors and with butyrylcholinesterase and that, in addition, possess anti-oxidant properties, in the hope of adding neuroprotective to pro-cognitive properties (Marcinkowska et al, 2019). Further work to attain compounds with well-balanced activities at these sites and functional activity *in vivo* would be desirable.

Very recently, another class of multi-target 5-HT6 receptor ligand has been described: putative, pro-cognitive agents interacting with both 5-HT6 and 5-HT2A receptors. Not unnaturally, these agents are primarily intended for the treatment of psychosis (Staron et al, 2019) but their potential relevance to AD is worth keeping in mind in view of a favourable influence upon sleep and psychosis by virtue of antagonist (inverse agonist) properties at 5-HT2A receptors (Ballard et al, 2018; Millan, 2006; Monti, 2010). Moreover, in a paper that appears to have been largely overlooked, an imaging study reported that the "highly selective" 5-HT6 antagonist, SB742,547, dose-dependently occupied 5-HT2A receptors in the frontal cortex over a dose range only slightly higher than that seeing 5-HT6 receptors in the hippocampus: intriguingly, doses that blocked both 5-HT6 *and* 5-HT2A receptors correlated to those putatively promoting cognition (Parker et al, 2012). While supporting interest in dual-acting 5-HT6 and 5-HT2A receptor antagonists for opposing cognitive impairment in AD, this paper raises questions about the design and interpretation of the clinical effects of « highly selective" 5-HT6 antagonists in AD (see above).

Finally, in another line of investigation that similarly straddles therapy for both psychosis/schizophrenia and AD, agents that associate 5-HT6 antagonism with partial agonist properties at

D2 receptors have been designed for the potential treatment of the behavioural symptoms and psychosis that characterise dementia, as well as the accompanying cognitive impairment (Kolakszowski et al, 2015). The up-fronted ligand,"47" (5-HT6, 1.0 nM and D2, 6.3 nM) displayed antidepressant and anxiolytic properties in *aged* rats, but putative pro-cognitive actions have not as yet been described. Furthermore, equipotent activity was seen at a number of other sites including α1-adrenoceptors and 5-HT1A as well as D4 receptors. While such actions are not necessarily deleterious, they hardly conform to the profile of a combined 5-HT6 antagonist/D2 partial agonist *per se*, a profile remaining to be described. In fact, compound 47 possessed high affinity for hD3 receptors , neatly anticipating a consideration of combined 5-HT6/D3 receptor ligands below.

However, prior to this discussion, it is important to consider why D3 receptors appear so attractive as candidate targets for integration with 5-HT6 antagonist properties.

D3 receptor antagonists mimic 5-HT6 antagonists in enhancing cognitive performance in a suite of preclinical procedures

By analogy to 5-HT6 receptor antagonists, there is a rich body of evidence suggesting that D3 receptor blockade is associated with improved cognitive performance (Leggio et al, 2016; Millan et al, 2012; Nakajima et al, 2013), with the perhaps decisive difference that D3 receptor agonists (in distinction to 5-HT6 agonists) act oppositely in consistently *interfering* with cognitive performance.

As regards D2 (and D1) receptors, their activation favours cognitive processes, whereas blockade exerts a deleterious influence - potent D2 receptor antagonism likely interferes with any potentially beneficial influence of second-generation antipsychotics upon cognition in schizophrenia (Leggio et al, 2016; Millan et al, 2012; Watson et al, 2012). Conversely, stimulation of D3 receptors has the opposite effect, being linked to a negative impact on cognition, whereas D3 receptor antagonists enhance cognitive performance. This distinctive role of D3 receptors, which parallels that of 5-HT6 receptors, is underscored by a broad-based body of data from mice genetically deprived of D3R and with selective D3 *vs* D2 antagonists. As reviewed elsewhere (Leggio et al, 2016; Millan and Brocco, 2008; Millan et al, 2010; Nakajima et al, 2013 ; Sokoloff, 2013), pro-cognitive actions of D3 antagonists are expressed:

- in mice, rats and primates, upon single and repeated administration, in tests incorporating measures of attention, working memory, long-term memory and executive function, as well as procedural memory and conditioned learning, and in procedures monitoring social cognition, in particular social recognition (*op cit*);
- upon direct introduction of D3 ligands into specific brain regions, principally the frontal cortex, but with potential roles for other cortical zones (like the cingulate cortex) as well as the nucleus accumbens, midbrain and hippocampus (Chang et al, 2020; Choi et al, 2010; Cole et al, 2012; Glickstein et al, 2005; Guma et al, 2019; Loiseau and Millan, 2009; Papp et al, 2019; Watson et al, 2012).

- in subjects displaying ageing-related memory deficits, and in interaction with proteins implicated in the cognitive deficits of AD and other neurodegenerative disorders (D'Amico et al, 2013; Leggio et al, 2016; Micale et al, 2010; Nakajima et al, 2013; Xing et al, 2010).
- in animal models of CNS disorders like psychosis, depression and PD, and in procedures incorporating conditions of fear, chronic stress and ovarian hormone deprivation (Millan and Brocco, 2008; Nakajima et al, 2013; Leggio et al, 2016; Papp et al, 2017).

As regards the neural and cellular substrates implicated in the positive influence of D3 receptor blockade upon cognition, they remain to be fully clarified and lack of certainty concerning their precise cellular localization in frontal cortex complicates matters. In any event, there is neurochemical and functional evidence that, by analogy to 5-HT6 receptors, D3 receptor stimulation may (over)activate mTOR in the FCX and other structures and hence interfere with cognitive processing (Cavalleri et al, 2018 ; Collo et al, 2014): this possibly reflects a direct, physical interaction of D3 receptors with cytoplasmic mTOR (Marin, P pers. comm.). Indeed, likewise mimicking 5-HT6 receptors, rapamycin blunts the amnesic actions of D3 agonists, notably in tests of social cognition and novel object recognition (Watson, D and Dekeyne, A, unpub obs) (Leggio et al, 2016 ; Nakajima et al, 2013). It should be noted that, dependent on the cellular environment and duration of activation, D3 receptors may also *inhibit* mTOR (Barroso-Chinea et al, 2019). While no link between the latter findings and cognition has as yet been made, this observation underpins the notion that mTOR behaves something like a "Rheostat" with a set-point defining an optimal level of activity guaranteeing cognitive processes - both under *and* over activation may be unfavourable; this holds true for D3 as well as 5-HT6 receptors - and presumably other upstream mechanisms (Bockaert and Marin, 2015; Teng et al, 2019). Roles for D3 receptor-Gi/o coupled cascades converging onto CREB, Mitogen Activated Protein Kinase and/or Glycogen Synthase Kinase3β are also potentially implicated in the reinforcement of cognition by D3 antagonists (Chang et al, 2020; Collo et al, 2014; Cussac et al, 1999; Jiao et al, 2007; Millan et al, 2008a; Nakajima et al, 2013; Newman-Tancredi et al, 1998).

At the level of neurotransmitters, D3 antagonists promote the release of ACh in the frontal cortex, and may potentiate D-Serine gating of NMDA receptors, while synchronising circuits by virtue of their influence on GABA receptors (Millan et al, 2000, 2007, 2008b, 2016; Swant et al, 2008; Sokoloff et al, 2013; Watson et al, 2012). Induction of ACh release mirrors the effects of 5-HT6 antagonists, and reinforcement of signalling at NMDA receptors would be synergistic to enhancement of glutamatergic neurotransmission by 5-HT6 antagonists (see above). On the other hand, in contrast to 5-HT6 blockade, D3R antagonists do *not* affect extracellular levels of dopamine and noradrenaline in frontal cortex (De Jong and Mork, 2017; Millan et al, 2000; 2008b, 2016). There are, thus, both distinctive *and* convergent mechanisms for pro-cognitive properties for D3R compared to 5-HT6R antagonists.

From the above remarks, there is clearly a powerful body of evidence supporting the potential relevance of D3 receptor blockade to improvement of cognition in AD and other forms of dementia as well as Mild Cognitive Impairment and age-related cognitive deficits in general. Furthermore, there are many data more generally linking alterations in dopaminergic neurotransmission, and especially D2 and D3 receptors, with

AD (Pan et al, 2019). An important caveat, shared with 5-HT6 antagonists, should be mentioned, however. Data from dedicated animal models for AD are awaited. This reflects the fact that, by analogy to 5-HT6 receptors, the majority of interest in D3 antagonists was initially directed towards their use in schizophrenia with AD crystallising as a valid therapeutic objective comparatively late in their lifetime of drug discovery and characterisation (de Bruin and Kruise, 2015; Gross et al, 2013; Miramai et al, 2018, Sokoloff et al, 2013; Millan et al, 2012, 2016; Watson et al, 2011).

Not to ignore this point, dual acting 5-HT6/D3 antagonists are evidently of considerable interest as potential agents for symptomatic control of AD. Other potential classes of multi-target D3 ligand, though not the central theme of this paper, are also briefly outlined below.

The concept of dual-acting 5-HT6/D3 antagonists: experimental support

Based on the above and other observations, it can be conjectured that co-joint blockade of 5-HT6 and D3 receptors may cooperatively promote cortically-integrated neurocognition and social cognition (**Figure 6**). Further, while D3R antagonism might moderate the occurrence of psychotic episodes, 5-HT6 receptor blockade may afford complementary anxio-depressive properties (Grychowska et al, 2016; Hirano et al, 2009; Wesolowska, 2010). Evidence for anti-epileptic properties of 5-HT6 antagonists is also of pertinence since the incidence of epilepsy and seizures is elevated in elderly patients with AD and other forms of dementia (Cretin, 2018; Lyou et al, 2019; Wang et al, 2015).

In this light, it is interesting to look at some experimental data where selective D3 and 5-HT6 antagonists have been combined in order to evaluate their co-joint influence upon extracellular levels of ACh in the frontal cortex and upon social recognition. As shown in **Figure 7,** (Millan et al, 2000, 2007; Mork et al, 2017), conjoint administration of SB271,046 (5-HT6 antagonist) and S33084 (D3 antagonist) exerts a more robust increase in extracellular levels of ACh in the frontal cortex of freely-moving rats than either drug alone. Considerable further study would be needed to determine whether this is a merely additive or in fact "synergistic" interaction. Ideally, such work should be undertaken in animal models for AD. Notwithstanding obvious limitations, the data are promising and are mirrored by studies of the co-administration of 5-HT6 and D3 antagonists in a model of social recognition. Both classes of compound elicit dose-dependent and robust procognitive actions in these models (Loiseau et al, 2008; Loiseau and Millan, 2009) and, as shown in **Figure 8**, low, *subactive* doses given together collectively displayed procognitive properties. Again, the key word here is preliminary, and it would be of particular interest to evaluate how concurrent blockade of 5-HT6 and D3 receptors might influence cognitive deficits in mouse models of AD employing multiple readouts embracing, say, executive performance and working memory. Furthermore, exactly how 5-HT6 and D3 receptors interact to promote ACh release and cognition remains to be elucidated, but one possibility is a cojoint blunting of mTOR signalling (see above).

Another intriguing question as yet to be addressed is whether there may be a physical interaction between 5-HT6 and D3 receptors that would be relevant to their control of cognition. One obvious place to look would be cerebral structures involved in the control of cognition where they are both enriched, notably

the FCX, though perhaps also in the limbic system involved in the emotional dimension of cognition, and even the cerebellum which plays a more prominent role in cognition than generally realized (Fone, 2008; Leggio et al, 2016; Li and Kuzhikanathil, 2012; Loiseau et al, 2008; Millan et al, 2012; Watson et al, 2012; Wagner and Luo, 2020). At the cellular level, both 5-HT6 and D3 receptors are localized on GABAergic neurons controlling the activity of cortical and cortico-subcortical networks, so this would be an interesting class of neuron to examine (Fone et al, 2008; Helboe et al, 2015 Leggio et al, 2019; Li and Kuzhikanathil, 2012; Millan et al, 2012; Swant et al 2008). Currently, despite the ability of 5-HT6 and D3 receptors to associate with other proteins (that influence cognition), and the propensity of D3 receptors to form heterodimers with other types of G-protein coupled receptor, there does not appear to be any concrete (cellular or proteomic) support for the existence of 5-HT6/D3 heterodimers (Chaumont-Dubel et al, 2019; Deraredj Nadim et al, 2016; Hounsou et al, 2019; Leggio et al, 2019; Maggio et al, 2009; Marin et al, 2012; Missale et al, 2010). Of course, they may only pop up upon systematically being sought for.

Further work would be desirable and several questions remain, yet the above observations provide support for the concept that the fusion of 5-HT6 and D3 antagonist properties into a single distinctive structure may yield agents with a more powerful influence on impaired cognition in AD than selective compounds alone. Underpinning interest in such dual-acting agents, 5-HT6 and D3 receptor antagonists are distinctive in view of the virtual lack of on-target-driven, undesirable actions: in any case, shared positive actions should allow for an increase in therapeutic window *vs* putative undesirable effects triggered separately.

Identification and characterisation of mixed 5-HT6/D3 receptor antagonists

The reasoning and observations outlined above have prompted several groups to search for dual-acting 5-HT6 /D3 antagonists.

The first ligands described that possess robust affinities for both 5-HT6 and D3 sites were produced by GSK in the wake of a whole sequence of highly selective and well-characterised 5-HT6 antagonists - including SB271,046 and SB285,585. One example of such a mixed ligand is SB737,050 (**Figure 9**) (Dupuis et al, 2008; Glennon et al, 2010). However, SB737,500 and related ligands still show high affinity for D2 sites, and at that time were oriented towards schizophrenia. Moreover, they do not seem to be very active *in vivo* and have not been pursued, possibly for strategic reasons that have little to do with pharmacology and therapeutics.

In a later series of studies, high affinity 5-HT6 antagonists served as a starting point for structural modifications designed to introduce moieties that preferentially favoured interaction with D3 over D2 sites. (Saavedra et al, 2017). The compounds generated displayed fairly decent affinity for 5-HT6 sites and encouraging D3 over D2 selectivity. However, as exemplified by compound 13, selectivity was limited, ligands were of only modest potency and - since they were racemic - it was not deemed useful to confirm that they behaved as genuine antagonists at 5-HT6 and D3 sites. Rather, chemistry was pursued resulting in further sets of ligands that present more robust affinities at 5-HT6 and D3 sites and more pronounced selective

for D3 over D2 sites. One of these compounds has been described previously, "45", and is shown in Figure 9 whereas another, S48475, is still under wraps for Intellectual Property reasons - its structure will be released in a primary article in the not too distant future (Ortuno, 2016). In any event, S48475 serves to illustrate a compound that behaves as an equi-potent antagonist at both h5-HT6 (pKi, 8.6) and hD3 (pKi, 8.5) vs hD2 (pKi, 6.8) sites and which possesses pro-cognitive properties *in vivo* in a test of social cognition disrupted by Scopolamine (**Figure 10)**. Further, S48475 showed anxiolytic properties in a Vogel Conflict Text, presumably reflecting its 5-HT6 antagonist properties (Figure 10). Finally, reflecting its preferential blockade of D3 over D2 receptors (25-fold higher affinity for the former), S48475 blunted amphetamine-induced locomotion without eliciting catalepsy (unpub obs), suggesting that it may be useful for counteracting psychosis in AD. S48475 and related compounds display low affinity for other classes of serotonin and dopamine receptor (Saavedra et al, 2018; unpub obs). Work is continuing on such ligands in order to identify solid clinical candidates, but the core point here is that it is indeed possible to build brain-penetrant and functionally-active ligands that possess balanced and potent activity at 5-HT6 and D3 receptors yet much lower affinity for D2 receptors.

More recently, the neutral 5-HT6 antagonist, "CPPQ", was exploited as a novel template for the generation of mixed 5-HT6 and D3 antagonists in an independent laboratory (Grychowska et al, 2019). PZ-1643, the leading representative of this set of compounds, is depicted in Figure 9. It shows good and similar affinity for 5-HT6 and D3 receptors, behaving as an antagonist at D3 receptors as determined employing cAMP (adenylyl cyclase) and other types of cellular assay. Interestingly, it behaves as a neutral antagonist at (constitutively active) 5-HT6 sites in contrast to several selective agents which manifest inverse agonist properties (Dupuis et al, 2008; Grychowska et al, 2019). Affinities at other receptors examined, like 5-HT1A and 5-HT2A, were low. Moreover, this agent blocked the disruption by phencyclidine of novel objection recognition in rats (Grychowska et al, 2019). Intriguingly, further, it possessed neuroprotective properties in an astrocytic model of neurotoxicity *in vitro*, although the underlying mechanisms and relevance to degeneration in AD remain to be clarified. In any event, PZ-1643 and similar compounds provide a promising template for the development of novel classes of ligand possessing 5-HT6 and antagonist D3 properties, which are of potential utility for improving both the cognitive and neuropsychiatric symptoms of AD and other neurodegenerative disorders of aging.

Other potential classes of dual-acting D3 antagonist

The focus here is on 5-HT6 receptors as a point of departure for the design of novel classes of multi-target agent, but D3 receptors could themselves be exploited as a point of departure. Curiously, there does not appear to be much in the way of chemical activity around this objective in the framework of AD and other neurodegenerative disorders of aging (Cortes et al, 2016; Leggio et al, 2016 ; Miramai et al, 2015; Sokoloff, 2017). Nonetheless, as an incitement for such efforts, there is some - albeit very preliminary - evidence that the procognitive actions of D3R antagonists can be potentiated by other classes of agent.

Thus, as shown in **Figure 11,** the pro-cognitive actions of S33084 in the novel object recognition procedure were potentiated by the metabotropic glutamatergic receptor (mGluR)-2 PAM, LY379,268 (Ikichi et al, 2013). These are only *initial* observations requiring consolidation and extension but it is interesting to note that a mGluR2 PAM similarly potentiated the actions of muscarinic M1 and M5 agonists as regards their ability to blunt the deleterious influence of the NMDA receptor channel blocker, dizocilpine, in tests of novel object recognition as well as delayed alternation (Cieslik et al, 2018). Supporting the notion of combining pharmacological activities, tested alone, LY379,268 - which is thought to moderate excessive hippocampal circuit activity in animal models of AD – did not restore cognitive function in mice overexpressing β-amyloid (Hascup et al, 2019). To reiterate the point made above, it is a pity that D3 antagonists have not (to our knowledge) been evaluated in this and other animal models for AD: this would be desirable both alone and in association with mGluR2 PAMs, 5-HT6 antagonists and other classes of potentially useful therapeutic ligand.

We have also found preliminary indications that selective D1 agonists - prototypical pro-cognitive agents difficult to exploit clinically (Floresco, 2013; Jiao et al, 2007; Kim et al, 2015) - and D3 antagonists reveal additive pro-cognitive actions in the social recognition test (Figure 11). It is not yet known how this interaction operates, but it may be of relevance that, mimicking D3R antagonists, D1R agonists stimulate the release of ACh in frontal cortex (Di Cara et al, 2007).

These initial observations, the broad range of options available, and the fact that D3 receptor pharmacophores can be integrated with structures interacting with sites like 5-HT6 receptors support the design of multi-target D3 antagonists for countering the cognitive impairment of AD and other disorders: for example, Parkinson's disease, where D3 receptor blockade itself favours the relief of motor dysfunction (Mela et al, 2010; Visanji et al 2009).

Potential disease-modifying properties of dual-acting agents?

As pointed out at the inception of this article, the strategies articulated herein are principally directed towards the improved symptomatic control of AD. Nonetheless, a word on a potential impact on the course of the disease itself is warranted.

This question has often been raised whether long-term treatment with agents possessing AChE inhibitory properties may be associated with "neuroprotective properties" and a slowing of disease progression. Despite some indications of reduced mortality in retrospective analyses for specific AChE inhibitors like donepezil or galantamine, unequivocal evidence that clinically-used doses of AChE inhibitors slow or prevent the evolution of AD in prospective trials has *not* been acquired (Bhattacharjee et al, 2019; Tricco et al 2018). As regards 5-HT4 agonists, in view of their above-mentioned ability to impede the generation of β-amyloid from APP in experimental models (Lalut et al 2017), it would be particularly worth exploring this facet for combined 5-HT4/AChE and 5-HT6/5-HT4 ligands. Curiously enough, mirroring the pro-cognitive actions of 5-HT6 antagonists *and* agonists, there has been a report that they both counter the neurotoxic actions of β-amyloid fragments in neuronal cultures (Bokare et al, 2017). However, apart from a

generalised suppression of Radical Oxygen Species, no MOA could be advanced. This remains, then an isolated observation but, surprisingly again, both a 5-HT6 antagonist *and* an agonist were found to induce hippocampal expression of Brain-Derived Neurotrophic Factor. Like induction of hippocampal neurogenesis, this action might be favourable under conditions of a degenerating hippocampus but it is not a cipher for a "neuroprotective action" *per se* and the more obvious link is to cognition (Codnony et al, 2011; Rychtyk et al, 2019). Nonetheless, it is of interest that epileptic seizures induce 5-HT6 expression in the hippocampus, and 5-HT6 antagonists block both the accompanying disruption of cognition as well as neuronal apoptosis (Liu et al, 2019). Finally, inasmuch as this article has forwarded dual-acting 5-HT6/D3 ligands as agents for relieve of cognitive dysfunction in AD, it is worth noting that there is evidence for induction of hippocampus neurogenesis by blockade of D3 receptors (Egeland et al, 2012).

On a rather different tack, 5-HT6 receptors lie upstream of and stimulate mTOR, Cyclin-dependent Kinase 5 and Fyn kinases that play a role in Tau hyperphosphorylation (Chaumont-Dubel et al, 2019; Caccamo et al, 2013; Codony et al, 2011; Di Domenico et al, 2018; Meffre et al, 2012; Millan, 2017; Yun et al, 2007). Accordingly, while not neglecting cellular compartmentalization, it is not impossible that 5-HT6 receptor blockade palliates Tau-driven pathophysiological mechanisms driving AD. Such agents would correspond to the previously-evoked notion of "hybrid" agents which, by virtue of their MOA, express comparatively rapid symptomatic effects that presage (and are neurally linked to) longer-term disease-modifying actions that impede the progression of the disorder (Millan et al, 2016).

Overall, despite some tantalising hints, the foundations for potential disease-modifying properties of dual-acting 5-HT6/D3 ligands and other classes of 5-HT6 receptor-based agent are shaky, but they justify focussed evaluation in both cellular and *in vivo* models for AD, as well as Parkinson disease and other classes of neurodegenerative disorder.

General Discussion and future prospects

Several more general points arising from the above discussion merit brief commentary.

First, the development and characterisation of novel classes of multi-target agent for treating AD articulated around 5-HT6 and D3 receptors is still in its infancy and much work remains to be performed for their further validation, refinement and characterisation. Moreover, the classes of ligand presented herein are only a small subset of those that could, at least in theory, be designed and developed for improving the lives of people suffering from AD (**Figure 12**). As regards future clinical development, it will be important to sidestep past problems, errors and constraints (see above) encountered with selective 5-HT6 antagonists. For example, we can highlight the crucial importance of establishing target engagement in patients at doses evaluated in clinical trials of efficacy. The availability of Positive Emission Tomography (PET) ligands for characterising drug interactions with D6 and D3 receptors in animals is of particular importance in this regard (Matuskey et al, 2016; Parker et al, 2012, 2015; Radhakrishnan et al, 2018; Tateno et al, 2018).

Second, genetics, imaging, biofluid and other classes of biomarker are greatly progressing the stratification of AD and the identification of target subpopulations patients best amenable to be improved by specific classes of therapeutic agent (Molinuevo et al, 2019). Expanding the repertoire of procedures for assessing cognitive and functional status in AD, from novel measures of cognitive function to remote digital monitoring should also prove informative (Gold et al, 2018; Kueper et al, 2018; Piau et al, 2019; Porcelli et al, 2019)

Third, logically enough, the present discussion focused on 5-HT6 antagonist properties since the rational is compelling and this approach has been pursued clinically. Nonetheless, as pointed out above, many "antagonists" behave as inverse agonists in cellular models, and the relevance of inverse agonist properties to the improvement of cognitive performance (or lack of effect) remains to be deciphered. It is also worth considering what might be the utility of multi-target 5-HT6 *agonists* in light of above-discussed data suggesting that they could also, reflecting recruitment of neural circuits contrasting to those engaged by antagonists, promote cognition. An interesting parallel here is provided by α7-nicotinic agonists (Verma et al, 2018). Most of the focus has been on PAMs, but they rapidly desensitize and *negative* allosteric modulators might palliate the β-amyloid driven hippocampal network (glutamatergic) hyperexcitability/hyperactivity that characterises early phases of AD (Zott et al, 2018) - though a very recent paper on conjoint blockade of α7 and α4β2 receptors questions this idea (Sun et al, 2019).

Fourth, while the present article has focussed on AD, we should evoke a potential role for 5-HT6/D3 antagonists for the amelioration of cognitive function and neuropsychiatric symptoms in other classes of neurodegenerative disorder of ageing. In addition, the potential evaluation of dual-acting 5-HT6/D3 antagonists in the control of the cognitive and other symptoms of schizophrenia - where neither class of agent alone may be sufficiently active - alone is justified (De Bruin and Kruse, 2016; Gross et al, 2013; Huang et al, 2015; Millan and Brocco, 2008; Nikiforuk, 2014; Takeuchi et al, 2019; Watson et al, 2011; Kotani et al, 2016). Interestingly, studies of receptor occupancy indicate that the antipsychotics, cariprazine (a partial D3 agonist) and blonanserin, preferentially occupy D3 over D2 receptors *in vivo* (despite similar affinities), consistent with a beneficial influence on cognition, though this remains to be clinically proven (Baba et al, 2015; Girgis et al, 2019; Tateno et al, 2018). In addition to schizophrenia, the potential use of 5-HT6/D3 antagonists for the improvement of neurocognitive performance, attention and social integration in autism and attention deficit hyperactivity disorder deserves consideration (Barth et al, 2013; Chang et al, 2020).

Finally, improved medication for controlling the cognitive, neuropsychiatric and other symptoms of AD and for impeding its progression would clearly be of immense importance. Nonetheless, mirroring the notion of multi-target agents, it is unlikely that any single drug would provide an ideal solution. The association of several classes of medication with other appropriate non-pharmacotherapeutic interventions will likely prove essential for the broad-based and effective treatment of the majority of people suffering from AD (Ngandu et al, 2016; Saito et al, 2019; Van den Brink et al, 2019).

Concluding comments

To conclude, it is important to forward multi-target strategies for symptomatic control of Mild Cognitive Impairment, AD, dementia and other neurodegenerative disorders of aging in parallel with increasingly intense efforts to find disease-modifying treatment. This currently stalled pursuit of therapies to alleviate the cognitive and other deficits of AD is important since, despite the impressive progress in knowledge of the pathology of AD, no panacea to stop its progression in the tens of millions of patients already hit by idiopathic AD is yet in the offing. It is certain that a whole arsenal of mechanistically-diverse, symptomatic and course-altering medications will be needed, in harmony with lifestyle changes and other types of intervention, to provide a broad-based palette of options for palliating the onset and impact of AD and other neurodegenerative disorders of aging. These efforts are being increasingly oriented around the concept of "Personalized" and "Precision" Medicine to prevent and treat AD. They should not, however, distract from the no less urgent imperative to address more universal risk factors for aging, brain and mental health such as pervasive metabolic and infectious disorders, climate change, environmental degradation, loss of biodiversity, poverty, inequality and urban decay which not infrequently strike the most vulnerable and less well-off communities first (Eid et al, 2019; Millan et al, 2015).

Acknowledgements

Figure legends

Figure 1 An overview of the core features of Alzheimer disease.

The figure summarizes the major clinical symptoms of late onset, sporadic, non-familial AD, together with a broad spectrum of risk factors. There is considerable heterogeneity between patients, and the precise pattern of clinical deficits and neural anomalies evolves over time for an individual patient. The central image is of Auguste D, the first case of AD described by Alois Alzheimer in Frankfurt in 1901. As indicated, AD represents around 60-70% cases of dementia, with vascular dementias, dementia with Lewy bodies and other forms comprising the remaining patients. Some 5-10% cases of AD are autosomal dominant and attributable to disruption of genes encoding Presenilin (PSEN) 1 and PSEN2 or APP.

Figure 2 A schematic depiction of the paucity of agents for improving symptoms of AD compared to the rich panoply of options for major depression, itself a risk factor for AD.

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A shown in the upper arrow, there has been a continuous supply of novel and mechanistically-distinct agents to treat major depression culminating most recently in esketamine for Treatment-Resistant Depression and brexanolone for Post-Menstrual Depression - which is not to ignore their (for some people/organisations prohibitively) high costs. In addition, cognitive-behavioural-psychosocial therapies are available as well as electroconvulsive therapy and various modes of brain stimulation. Conversely, only two classes of medication are available for AD, in addition (in the US) to an association of Donepezil and Memantine (Namzaric). Classes of medication; 1), Tricyclic; 2), Irreversible Monoamine Reuptake Inhibitor; 3), Preferential DA reuptake inhibitor; 4), Selective 5-HT reuptake inhibitor, 5), Glutamatergic modulator; 6), Reversible Monoamine Reuptake Inhibitor; 7), 5-HT/Noradrenaline reuptake inhibitor; 8), 5-HT$_{2A/C}$/α2-adrenoceptor antagonist; 9), Preferential Noradrenaline reuptake inhibitor; 10), Melatonin agonist/5-HT$_{2C}$ antagonist; 11), 5-HT$_{1A}$ partial agonist/5-HT reuptake inhibitor; 12), Multi-target 5-HT receptor ligand/5-HT reuptake inhibitor; 13), Non-competitive blocker of NMDA receptors and 14), Allosteric modulator of GABA$_A$ receptors. I), AChE inhibitor and II), Rapid kinetic, moderate potency, non-competitive NMDA receptor antagonist.

Figure 3 A schematic depiction of the convergence of serotonin and other classes of neurotransmitter modulating cognition in the frontal cortex.

The classical neurotransmitters depicted, as well as others, interact in the control of cognitive processes and other functions like mood and motor behaviour by virtue of cortico-cortical and cortico-subcortical circuits. Although the major focus to date has been on agents that target *individual* mechanisms rather than the interplay amongst them, this provides a template for association of serotoninergic (5-HT6) and dopaminergic (D3) mechanisms as discussed herein (Figure 4). Note that interactions also occur in inter-connected regions like the basal ganglia and hippocampus.

Figure 4 Influence of the mTOR inhibitor, rapamycin, on the amnesic and pro-cognitive properties of 5-HT6 agonists and antagonists, respectively in the social recognition test in rats.

As described previously (Meffre et al, 2012; Watson et al, 2012), T2-T1 represents the difference in exploration time of a juvenile by an adult rat in two 5 minutes sessions separated by 120 mins: after this period, in vehicle-treated adults, the younger rat has been forgotten so there is little difference in T2 and T1. Accordingly, an increase in this difference (high negative T2-T1 value) suggests improved retention of social recognition. Left panel: Rapamycin blocks the amnesic actions of the 5-HT6 agonist in the social recognition test in rats. The closed asterisk shows significance of the amnesic effect of WAY181,187 and the open asterisk depicts the significance of the blocking action of rapamycin against WAY181,187. Right panel:

Rapamycin facilitates the pro-cognitive actions of a sub-active dose of the 5-HT6 antagonist, SB258,585. Closed asterisks show the significance of the potentiating action of rapamycin (Rap/SB) vs Veh/SB and Rap/Veh respectively. Data were analysed by ANOVA followed by Newman-Keul's test (*P< 0.05).

Figure 5 Interaction of the NMDA antagonist, dizocilpine, and of the NMDA/Glycine B agonist, D-Serine, with the 5-HT6 antagonist, SB271,046 in the social recognition test in rats.

See legend to Figure 4 for explanation of the methodology and the significance of T2-T1 (Watson et al, 2012). Left panel: Dizocilpine blocks the pro-cognitive properties of SB271,046. Right panel: D-Serine potentiates the pro-cognitive properties of a sub-active dose of SB271,046. Closed asterisks show the significance of the blocking action of dizocilpine action *vs* SB271,046 (Diz/SB vs Veh/SB) and of the potentiating actions of D-Serine for SB271,046 (D-Ser/SB vs Veh/SB), respectively. Data were analysed by ANOVA followed by Newman-Keul's test (*P< 0.05).

Figure 6 A conceptual framework for the association of 5-HT6 and D3 antagonist properties for the more effective symptomatic treatment of AD.

The notion of association is principally for a single agent that possess dual 5-HT6 and D3>D2 antagonist properties but it could also apply to the combination of selective agents. As outlined in the text, 5-HT6 and D3 antagonist properties may promote cognition by both common (such as increased release of ACh and suppression of overactive mTOR) and independent mechanisms. While blockade of 5-HT6 receptors has been associated with anxiolytic and anti-depressive properties, antagonism of D3 receptors may be useful in the alleviation of psychosis.

Figure 7 Additive increases in extracellular levels of acetylcholine in dialysates of frontal cortex of freely-moving rats by administration of S33084 and SB271,046, selective antagonists at D3 and 5-HT6 receptors, respectively.

Extracellular levels of acetylcholine were determined in the absence of cholinesterase inhibitors in the frontal cortex of freely-moving rats by HPLC coupled to electrochemical detection, as described previously, and expressed in relation to basal values (2.2 +- 0.4 pg/20ul, defined as 100%) (Millan et al, 2007). The arrows indicate the time of injection of D3 and 5-HT6 antagonists, respectively. The closed asterisks indicate the significance of differences for Veh/S33084 and Veh/SB271,046 vs Veh/Veh values, and the hashed sign indicates the significance of differences between S33084/SB271,046 and Veh/SB271,046 values. Data were analysed by ANOVA followed by Newman-Keul's test (*P< 0.05).

Figure 8 Additive improvements in social recognition by administration of S33084 and SB271,046 or SB258,585, selective antagonists at D3 and 5-HT6 receptors, respectively.

See legend to Figure 4 for explanation of the methodology and the significance of T2-T1 (Watson et al, 2012). Both D3 and 5-HT6 antagonists promote social cognition in a dose-dependent manner in this procedure (Loiseau et al, 2008; Loiseau and Millan, 2009), but here were evaluated separately and together at sub-effective doses. The asterisks indicate statistical significance of differences between S33084/SB271,046 vs veh/SB271,046 and of S33084/SB228,585 vs veh/SB258,585 values. Data were analysed by ANOVA followed by Newman-Keul's test (*P< 0.05).

Figure 9 Chemical structures of several published agents possessing dual antagonist properties at D3 and 5-HT6 receptors.

SB737,050 has high affinity for 5-HT6 receptors and balanced affinity at D3 and D2 receptors: it has served as a useful starting point for generating dual-acting 5-HT6/D3 agents. Compounds 13b and the more potent 45 have more balanced affinities for 5-HT6 and D3 *versus* D2 receptors.

Figure 10 Functional profile of an agent, S48475, possessing high affinity for both 5-HT6 and D3 *vs* D2 receptors.

Upper panels: Antagonist properties of the mixed 5-HT6/D3>D2 receptor antagonist, S48475, in cellular procedures for actions at h5-HT6, D3 and D2 receptors - it was inactive alone (not shown). Left lower panel, pro-cognitive actions of S48475 in a test of social recognition (blockade of disruption by the muscarinic antagonist, scopolamine (1.25 mg/kg)) in rats. Right lower panel: anxiolytic properties in the Vogel conflict test - disinhibition of punished responding in rats. Data were analysed by ANOVA followed by Newman-Keul's test (*P< 0.05).

Figure 11 Additive actions in tests of cognitive function of the selective dopamine D3 antagonist, S33084, given together with the mGluR2 PAM, LY379,268, or with the dopamine D1 agonist, SKF 81297.

Top Panel. As described previously (Watson et al, 2012), the D2 score reflects the increase in performance in the novel object recognition test in a second *vs* first exposure to the object. The open asterisks indicate the significance of differences between Veh/S33084 and Veh/Veh values, and between LY379268/S33084 and LY379268/Veh values. The closed asterisk indicates the significance of differences between LY379268/S33084 and Veh/S33084 vales. Data were analysed by ANOVA followed by Newman-Keul's test (*P< 0.05). Bottom Panel. See legend to Figure 4 for explanation of T2-T1. Both D3 antagonists and D1 agonists promote social cognition in a dose-dependent manner in the social recognition procedure but were

here evaluated separately and together at sub-effective doses (Di Cara et al, 2007; Millan et al, 2007). The closed asterisk indicates the significance of differences between SKF81297/S33084 and Veh/SKF81297 values. Data were analysed by ANOVA followed by Newman-Keul's test (*$P< 0.05$).

Figure 12 A schematic illustration of potential classes of dual-acting ligand articulated around 5-HT6 and D3 receptors as hubs.

Agonist, agonist and ant, antagonist. The interlinked targets could be mutually modulated by dual-acting agents, but the notion of drugs that interact with more than two of these sites should also be considered. The "pyramid" is non-exhaustive, based on available pharmacological studies and/or chemical structures and could be expanded to additional classes of mechanisms that potentially promote cognition and oppose neuropsychiatric symptoms in AD and neurodegenerative disorders of aging.

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