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Central 5-HT receptors and their function; present and future

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Abstract

Since our review of central 5-HT receptors and their function twenty years ago, no new 5-HT receptors have been discovered and there is little evidence that this situation will change in the near future. Nevertheless, over this time significant progress has been made in our understanding of the properties of these receptors and in the clinical translation of this information, and some of these developments are highlighted herein. Such highlights include extensive mapping of 5-HT receptors in both animal and human brain, culminating in readily-accessible brain atlases of 5-HT receptor distribution, as well as emerging data on how 5-HT receptors are distributed within complex neural circuits. Also, a range of important pharmacological and genetic tools have been developed that allow selective 5-HT receptor manipulation, in cells through to whole organism models. Moreover, unexpected complexity in 5-HT receptor function has been identified including agonist-dependent signalling that goes beyond the pharmacology of canonical 5-HT receptor signalling pathways set down in the 1980s and 1990s. This new knowledge of 5-HT signalling has been extended by the discovery of combined signalling of 5-HT and co-released neurotransmitters, especially glutamate. Another important advance has been the progression of a large number of 5-HT ligands through to experimental medicine studies and clinical trials, and some such agents have already become prescribed therapeutic drugs. Much more needs to be discovered and understood by 5-HT neuropharmacologists, not least how the diverse signalling effects of so many 5-HT receptor types interact with complex neural circuits to generate neurophysiological changes which ultimately lead to altered cognitions and behaviour.

1. Introduction

Following the initial identification of two types of 5-HT receptor in peripheral tissue sixty years ago, 'D' and 'M' 5-HT receptors (Gaddum & Picarelli, 1957), a combination of pharmacological and then molecular biological investigations led to the discovery of an unexpected diversity of 5-HT receptor types. An excellent account of the history of 5-HT receptor discovery and classification is given by Bockaert and colleagues (Bockaert *et al.*, 2010). The current consensus is that the brain expresses 7 types of 5-HT receptors (5-HT_{1-7}) comprising a total of 14 subtypes (Figure 1) although 2 of these receptors, 5-ht_{1e} and 5-ht_{5b}, retain lower case appellation and are still classed as gene products since they have not yet been linked to a functional response in native cells/tissues. All 5-HT receptor subtypes are found in both brain and peripheral tissues with the exception of 5-ht_{1e}, 5-HT_{2C} and 5-HT_6 receptors, for which there is limited expression outside the CNS. Also, multiple isoforms of certain 5-HT receptors (notably 5-HT_{2C}, 5-HT_3, 5-HT_4 and 5-HT_7 receptors) have been reported, but the pharmacological and functional significance of this diversity remains uncertain. This receptor diversity is comparable to that of the most complex neurotransmitter systems including glutamate and purines. Despite this complexity, a wide range of selective agonists, antagonists and radioligands are now available to target specific 5-HT receptor subtypes (summarized in Table 1) and probe their localisation, signalling properties, and CNS function.

In the human genome, the 5-HT receptor subtypes are coded by 17 genes, with 12 encoding metabotropic 5-HT receptors and 5 encoding subunits for ionotropic 5-HT_3 receptors (an additional sequence in the human genome corresponds to the 5-ht_{5b} receptor in some other species, although the presence of stop codons in the human gene prevents full length protein expression). The genes encoding 5-HT receptors (and the 5-HT transporter) demonstrate polymorphisms in coding and non-coding regions, which are often common in the human population. However, despite many reports of associations between specific 5-HT receptor gene polymorphisms and particular neuropsychiatric disorders, as with the vast majority of such gene-associations, effect sizes are typically modest and findings await replication in large scale datasets. This work is now expanding to incorporate findings of epigenetic regulation of 5-HT receptors which might provide a

clearer picture of how natural variation in 5-HT receptor genes links to CNS disorder. Consequently, the importance of variability in 5-HT receptor (and 5-HT transporter) genes as mental health risk factors currently remains uncertain, and detailed analysis and review is warranted.

2. 5-HT receptor signalling

2.1. Metabotropic 5-HT receptors: canonical signalling

Thirteen of the 14 5-HT receptor subtypes are classical metabotropic G-protein coupled receptors which couple to canonical signalling pathways, that elicit the expected second messenger cascades (Table 1). The 5-HT_1 receptor family, comprises 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1e}, and 5-HT_{1F} subtypes (5-HT_{1C} subsequently being re-designated 2-HT_{2C}; (Humphrey *et al.*, 1993), and these are G_i-coupled and inhibit adenylate cyclase and decrease cAMP formation (Masson *et al.*, 2012). These receptors also indirectly open G-protein-gated inwardly rectifying potassium channels (GIRKs) to hyperpolarise neurons, and inhibit the opening of voltage-gated calcium channels (eg. (Montalbano *et al.*, 2015). The 5-HT_5 receptor family, 5-HT_{5A} and 5-ht_{5b} (the full-length protein of the latter receptor is not expressed in humans), is also G_i-coupled, and 5-HT_{5A} receptor-mediated hyperpolarization was identified in cortical neurons (Goodfellow *et al.*, 2012).

In comparison, the 5-HT_2 receptor family, which comprises 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor subtypes, is $G_{q/11}$-coupled and activates phospholipase C leading to increased formation of inositol trisphosphate and diacylglycerol, and the triggering of their downstream signalling events. 5-HT_2 receptor stimulation also results in neuronal excitation in a variety of brain regions, likely involving the closure of potassium channels and depolarisation. A 5-HT_2 receptor-mediated increase in excitatory postsynaptic currents is also often observed in *in vitro* preparations, which is thought to be mediated either directly or indirectly through increased release of glutamate (Lamb & Aghajanian, 2006; Marek, 2010). The remaining metabotropic 5-HT receptors, 5-HT_4, 5-HT_6, and 5-HT_7 receptors, are all excitatory with effects mediated through G_s-coupling, adenylate cyclase activation and increased cAMP formation. At the electrophysiological level, 5-HT_4 receptor

activation in neurons is associated with a slow membrane depolarization mediated by a reduction of a slow after-hyperpolarization potential, and facilitation of L-type calcium channels through protein kinase A activation (Andrade & Chaput, 1991; Birnstiel & Beck, 1995). Similarly, the 5-HT_6 receptor is reported to exert excitatory effects on specific neuron types (striatal cholinergic interneurons, hippocampal GABA interneurons) in recordings from brain slices (West *et al.*, 2009; Tassone *et al.*, 2010). Additionally, the 5-HT_7 receptor is associated with a direct depolarizing or excitatory effect, also likely mediated by actions on slow after-hyperpolarization and L-type calcium channels (Bacon & Beck, 2000; Andrade, 2006).

2.2. Metabotropic 5-HT receptors: non-canonical signalling

An intriguing aspect of metabotropic 5-HT receptor function is the discovery that they elicit non-canonical signals that can be either mediated by a host of alternative G-proteins, or be G-protein independent. This G-protein independent signalling often involves β-arrestins which, as signal transduction scaffolds, bring elements of other signaling pathways such as the mitogen-activated protein kinases including extracellular signal-regulated kinase (ERK), into close proximity. Moreover, there is growing evidence that different 5-HT receptor agonists interact with the same 5-HT receptor but preferentially activate specific intracellular signalling pathways, a property referred to as 'functional selectivity' or 'biased agonism'. A summary of the non-canonical signaling and biased agonism capabilities of different 5-HT receptor subtypes is provided in Table 2.

There is convincing evidence that the 5-HT_{1A} receptor signals not only via G_i-coupled adenylate cyclase but also a variety of non-canonical pathways (Masson *et al.*, 2012; Albert & Vahid-Ansari, 2019). This includes the ERK signalling pathway which mediates a wide range of cellular responses ranging from structural changes to different forms of synaptic plasticity, as well as signalling via small G-proteins (eg. Rho A). This signalling diversity may in part connect with evidence that 5-HT_{1A} receptors couple to a diversity of G-proteins depending on their localisation within the brain (Mannoury la Cour *et al.*, 2006). It might also help explain long-standing evidence of presynaptic versus postsynaptic differences in 5-HT_{1A} receptor reserve and efficiency of G-protein coupling. This knowledge is currently being exploited to identify 5-HT_{1A} receptor biased

agonists that preferentially direct receptor signalling to particular intracellular pathways in a brain region-specific manner (Sniecikowska *et al.*, 2019). A number of 5-HT_{1A} receptor biased agonists are currently under investigation as potential novel CNS therapies as discussed later. Evidence that 5-HT_{1B} receptors are capable of non-canonical signaling via β-arrestin and other signaling pathways is also reported and reviewed elsewhere (Millan *et al.*, 2008; Liu *et al.*, 2019).

It is now recognised that 5-HT_2 receptor signalling (the 'D' receptor identified by Gaddum and Picarelli) also goes beyond the canonical $G_{q/11}$-coupled phospholipase C signalling pathway. Non-canonical signalling pathways associated with the 5-HT_{2A} receptor include phospholipase A_2, the ERK pathway, and the β-arrestin coupled *Src*/Akt cascade as recently reviewed (Bockaert *et al.*, 2010; Halberstadt, 2015; McCorvy & Roth, 2015; Maroteaux *et al.*, 2016). Both 5-HT_{2B} and 5-HT_{2C} receptors are reported to interact with a similar group of non-canonical signalling pathways (Labasque *et al.*, 2008), and contrasting agonist efficacies for different 5-HT_{2C} signaling pathways are reported (Berg *et al.*, 1998; Berg *et al.*, 2008; Millan *et al.*, 2008; Cheng *et al.*, 2016). Furthermore, recent studies of 5-HT_{2B} receptor evoked G-protein and β-arrestin signalling reported evidence of agonist-dependent signals (eg. LSD, ergotamine), and then used the crystal structure of the 5-HT_{2B} receptor to identify with high resolution, thereby providing details of the structural basis for this biased signalling and a rational basis for the discovery of 5-HT_{2B} receptor agonists without cardiac side-effects (Wacker *et al.*, 2013; McCorvy *et al.*, 2018b). Evidence of 5-HT_{2A} receptor biased agonism has relevance to the psychotropic effects of 5-HT_{2A} receptor agonists, most of which are hallucinogenic but apparently not all such as lisuride (Gonzalez-Maeso *et al.*, 2007). Thus, there is the interesting possibility of identifying 5-HT_{2A} receptor agonists without hallucinogenic properties and also, potentially, without effects on peripheral tissues such as vascular and other smooth muscle.

In addition to 5-HT_1 and 5-HT_2 receptors, the capacity for both canonical and non-canonical signalling is evident for other metabotropic 5-HT receptors, as detailed for 5-HT_4, 5-HT_6 and 5-HT_7 receptors in other reviews (Bockaert *et al.*, 2010; Masson *et al.*, 2012; Blattner *et al.*, 2019; Chaumont-Dubel *et al.*, 2019). These signaling pathways includes; ERK for 5-HT_4 receptors, ERK,

mTOR and Cdk5 for 5-HT_6 receptors, and ERK and small G-proteins for 5-HT_7 receptors. These data suggest that biased agonism is also likely to emerge as a property of these receptors. Indeed, evidence of agonist-dependent signalling at 5-HT_4 receptors (RS 67333, prucalopride) and 5-HT_7 receptors has recently been reported (Gaven *et al.*, 2013; Kim *et al.*, 2018; McCorvy *et al.*, 2018a).

It presently remains to be established how much of this 5-HT receptor signalling diversity is expressed in native neurons (as opposed to transfected cell cultures), and there is a need for validation in neurons and brain regions in which the 5-HT receptors are naturally expressed. Nevertheless, these developments raise the exciting prospect of designing drugs (biased agonists) to target a particular 5-HT receptor and elicit one or a small number of the many possible signalling pathways coupled to that same receptor. Thus, agonists with selectivity for evoking specific signaling effects via a particular receptor may offer therapeutic benefits over agonists that elicit multiple signaling effects via that receptor (eg. non-hallucingenic 5-HT_{2A} receptor agonists, cardiac side effect-free 5-HT_{2B} receptor agonists). Linking specific 5-HT receptor-induced signalling effects to specific functional/behavioural effects will be challenging and require not only biased agonists but also cell- and animal-based models expressing 5-HT receptors with modified signalling capabilities. One approach would be the knock-in of mutated 5-HT receptors which have limited capacity to signal via a particular pathway, as recently reported for the 5-HT_{2B} receptor (McCorvy *et al.*, 2018b).

Another intriguing aspect of this potential for the triggering of multiple pathways by individual 5-HT receptors, is its possible physiological purpose. This differential signaling may translate into different behavioural and cognitive effects (see above), and might also reflect a capacity for signalling over different temporal scales (eg. short-term synaptic effects of ion channel modulation versus enduring effects of gene expression) and spatial scales (neuron type, brain region), and at different stages of development. In regard to the latter, it is notable that certain signalling pathways (eg. Rho A, mTOR, Cdk5) linked to particular 5-HT receptors (eg. 5-HT_{1A}, 5-HT_6, 5-HT_7) mediate changes in neuronal architecture (neurite, synapse and neural circuit formation) and placement (neuronal migration). These signalling effects are likely to be associated with the long recognized

neurotrophic and neurodevelopmental role of 5-HT (Daubert & Condron, 2010; Teissier *et al.*, 2017).

Aside from canonical and non-canonical pathways, further complexity in metabotropic 5-HT receptor signalling arises from the large number of protein partners that have been found to influence cellular localization and trafficking of these receptors, and also alter receptor function through homo- or heterodimerization with other GPCRs, as noted in recent reviews (Hannon & Hoyer, 2008; Bockaert *et al.*, 2010; McCorvy & Roth, 2015; Chaumont-Dubel *et al.*, 2019). Alternative RNA splicing and editing is reported to generate multiple isoforms of certain metabotropic 5-HT receptors (especially 5-HT_{2C}, 5-HT_4 and 5-HT_7 receptors). Although the pharmacological significance of this diversity remains uncertain, it provides another potential route for differential targeting of 5-HT receptor function. Finally, there are increasing reports of allosteric modulators of 5-HT metabotropic receptors, as in recent examples for 5-HT_{2B} and 5-HT_{2C} receptors (Singh *et al.*, 2019; Wild *et al.*, 2019), and siRNA approaches for targeting 5-HT receptors have promise as noted shown for knockdown of the 5-HT_{1A} receptor (Bortolozzi *et al.*, 2012). Although the latter fields are currently in their infancy, they may provide further avenues for 5-HT drug discovery.

2.3. Ionotropic 5-HT receptor signalling

As a complement to the slow signalling elicited by metabotropic 5-HT receptors, the ionotropic 5-HT_3 receptor mediates rapid, excitatory synaptic transmission. 5-HT_3 receptors belong to the Cys-loop family of ligand-gated ion channels, which also includes nicotinic acetylcholine receptors and $GABA_A$ receptors. They are composed of five subunits (5-HT_3A-E) surrounding a central ion-conducting pore that is permeable to sodium, potassium, and calcium ions (Barnes *et al.*, 2009). All 5-HT_3 receptor subunits are expressed in the CNS and, while there is uncertainty regarding the composition of the native 5-HT_3 receptor(s) in the brain, pentameric co-assemblies of 5-HT_3A and 5-HT_3B subunits is a common occurrence (for reviews see (Niesler, 2011; Thompson & Lummis, 2013). Heteromeric assemblies of 5-HT_3AB receptors differ in single channel conductance, calcium permeability, 5-HT concentration-response curves (potency and degree of cooperativity),

desensitization rate, current-voltage relationships, and also sensitivity to certain agents including general anaesthetics (Thompson & Lummis, 2013). To date, inclusion of the 5-HT_3A subunit appears to be a requisite for 5-HT_3 receptor function.

A number of high affinity 5-HT_3 receptor agonists have been identified, with meta-chlorophenyl-biguanide being one of the most selective and potent, and there are many selective, high affinity competitive and non-competitive antagonists (Fakhfouri *et al.*, 2019). Allosteric modulators of the 5-HT_3 receptor and action via the pharmacological mechanism coined 'cryptic orthosteric modulation', are emerging as a new pharmacological phenomenon to add to the possibilities for 5-HT_3 receptor manipulation for therapeutic benefit (e.g. (Trattnig *et al.*, 2012; Newman *et al.*, 2013; Powell *et al.*, 2016).

Because of the restricted distribution of 5-HT_3 receptors in the CNS, fast synaptic transmission is commonly assumed to be a property of only a minority of 5-HT synapses, with transmission at the majority of 5-HT synapses being slow and neuromodulatory. However, in an exciting development it has been discovered that across species, up to 70-80% of 5-HT neurons co-localise the vesicular glutamate transporter VGLUT3 (Gras *et al.*, 2002; Hioki *et al.*, 2010; Vigneault *et al.*, 2015), implying co-release of 5-HT and glutamate from 5-HT neuron terminals via a mechanism that is currently uncertain (Figure 2). Interestingly, combined neurophysiological and optogenetic analysis of the synaptic effects of optically activated 5-HT neurons has revealed the presence of both fast, glutamate-mediated ionotropic responses and slow 5-HT-mediated metabotropic responses, often co-existing on the same postsynaptic neuron (Liu *et al.*, 2014; Kapoor *et al.*, 2016; Sengupta *et al.*, 2017). Although not yet tested, these observations raise the intriguing possibility that co-released glutamate plays a role in the therapeutic effect of 5-HT targeted drugs such as SSRIs. For example, it has been speculated that delayed onset of antidepressant action of SSRI treatment could be explained by an initial autoreceptor-mediated decreased in the glutamate component of the dual 5-HT-glutamate signalling (Fischer *et al.*, 2014).

3. Expression pattern of 5-HT receptors in the brain

A combination of approaches including receptor autoradiography, *in situ* hybridisation and immunocytochemistry has revealed the distribution of 5-HT receptors binding sites and mRNA expression in the brain (Mengod *et al.*, 2006; Mengod *et al.*, 2010; Palacios, 2016). To add to these approaches, the anatomical and cellular detail of 5-HT receptor mapping is now advancing through the generation of transgenic mice engineered to express a fluorescent or coloured reporter genes under the control of specific 5-HT receptor promoters, for instance as in the case of 5-HT_{2A} receptor (Weber & Andrade, 2010). Collectively, evidence suggests that the pattern of 5-HT receptor distribution is largely similar across vertebrate species. However, notable exceptions to this rule include the 5-ht_{1e} receptor not being expressed in rodents, the full length 5-ht_{5b} receptor not being expressed in humans, and forebrain 5-HT_3 and 5-HT_6 receptors being differentially expressed between rodents and humans.

A remarkable collection of brain atlases is currently under construction that are aiming to map the expression of a multitude of neurotransmitter receptor genes (and multiple other key genes) in the adult as well as developing mouse brain, with comparative atlases for the non-human primate and human brain (https://portal.brain-map.org). Presently, the mouse atlas provides a comprehensive map of the gene expression pattern of the majority of 5-HT receptors as well as the 5-HT transporter. Aside from these gene expression atlases, a high-resolution PET atlas of the binding sites for four 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_4 receptors) as well as the 5-HT transporter was recently described for the human brain (Beliveau *et al.*, 2017).

It is clear from mapping studies such as those noted above, that each 5-HT receptor subtype has a unique distribution pattern in the brain although one that often overlaps with that of other 5-HT receptor subtypes. These differential distribution patterns suggest that different 5-HT receptor subtypes are likely associated with distinct CNS functions amenable to manipulation using 5-HT receptor subtype selective pharmacological agents.

3.1 Presynaptic 5-HT receptors: 5-HT neuron feedback control

It is well established that two 5-HT receptors, 5-HT_{1A} and 5-HT_{1B} are expressed by 5-HT neurons and function as autoreceptors. 5-HT_{1A} receptors are located on the soma and dendrites of 5-HT neurons (Verge *et al.*, 1985; Riad *et al.*, 2000), and inhibit the firing of 5-HT neurons and decrease 5-HT release when activated (e.g. (Vandermaelen & Aghajanian, 1983; Sharp *et al.*, 1989; Adell & Artigas, 1998). On the other hand, 5-HT_{1B} receptors are expressed at the soma and then trafficked to the terminals where they exert inhibitory control over 5-HT release (Gothert & Weinheimer, 1979; Mounsey *et al.*, 1982; Engel *et al.*, 1986; Fink & Gothert, 2007).

Other 5-HT receptor types are reported to be expressed by midbrain 5-HT neurons, in particular 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_{5A} (Erlander *et al.*, 1993; Bruinvels *et al.*, 1994; Bonaventure *et al.*, 1998; Belmer & Maroteaux, 2019; Okaty *et al.*, 2019) but the expression level of these receptors is relatively low with the exception of 5-HT_{2C}. However, in the latter case the receptor appears to be primarily located on raphe GABAergic interneurons and not 5-HT neurons (Sharp *et al.*, 2007). Nevertheless, feedback control of 5-HT neurons likely extends beyond 5-HT_1 autoreceptors to include 5-HT receptors located on postsynaptic neurons rather than 5-HT neurons themselves. For example, evidence supports a role for postsynaptic 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors in the inhibitory control of 5-HT neurons, whereas 5-HT_4 and 5-HT_6 receptors are excitatory in this regard (Lucas *et al.*, 2005; Sharp *et al.*, 2007; Sharp, 2010; Brouard *et al.*, 2015). Many of these feedback pathways appear to be localised on cortical pathways projecting back to the midbrain raphe nuclei (5-HT_{1A}, 5-HT_{2A}, 5-HT_4, 5-HT_6 receptors) although contributions from habenula inputs to the raphe (5-HT_{2C} receptors) are also implicated (Sharp *et al.*, 2007). In addition, there are numerous interactions between 5-HT receptors and other neurotransmitter systems, including noradrenaline (eg Done and Sharp, 1992), which modulate 5-HT neurons.

The physiological importance of these postsynaptic feedback pathways remains uncertain and awaits the development of genetic tools for their selective manipulation. These postsynaptic 5-HT feedback systems do, however, offer novel ways to pharmacologically target 5-HT neurons. An example of an important clinical area where knowledge of 5-HT feedback control might have therapeutic application is mood disorder, and specifically antidepressant augmentation for

treatment-resistant depression. There is good preclinical evidence that pharmacological blockade of presynaptic 5-HT_{1A} and 5-HT_{1B} receptors (Gartside *et al.*, 1995; Sharp *et al.*, 1997), and also postsynaptic 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_3 receptors (Boothman *et al.*, 2006; Cremers *et al.*, 2007; Diaz *et al.*, 2012; Betry *et al.*, 2015), will augment the 5-HT enhancing properties of SSRIs. Drugs with combined 5-HT receptor and 5-HT-transporter blocking actions continue to be sought after for their antidepressant potential (Millan, 2009; Artigas *et al.*, 2018), with vortioxetine having now reached the market (see later).

3.2 Postsynaptic 5-HT receptors

All 5-HT receptors, including 5-HT_{1A} and 5-HT_{1B} receptors, are located postsynaptically (ie expressed by non-5-HT neurons) with each 5-HT receptor subtype having its own distinct expression pattern. Even 5-HT receptors from the same family have different CNS distributions (eg 5-HT_{2A} versus 5-HT_{2C}, 5-HT_{1A} versus 5-HT_{1B}). Some 5-HT receptors have high levels of expression (5-HT_{1A} and 5-HT_{2A}) whereas others are much less abundant (5-HT_{1D}, 5-ht_{1e}, 5-HT_{1F}, 5-HT_{2B} and 5-HT_{5A}). At the macro-level, 5-HT receptors are widely distributed, with specific brain regions having distinct complements of 5-HT receptor subtypes: for instance cortical and limbic areas - 5-HT_{1A}, 5-$HT_{2A/C}$, 5-HT_3, 5-HT_4, 5-HT_6; basal ganglia - 5-HT_{1B}, 5-$HT_{2A/C}$, 5-HT_4, 5-HT_6; mesolimbic pathways - 5-HT_{1B}, 5-$HT_{2A/C}$, 5-HT_4 receptors; hypothalamus (eg. paraventricular and arcuate nuclei) - 5-HT_{2C}; suprachiasmatic nucleus - 5-HT_7; trigeminal nucleus - 5-$HT_{1B/D/F}$; dorsal vagal complex (encompassing the area postrema and nucleus tractus solitarus, and dorsal motor nucleus of the vagus nerve) - 5-HT_3.

3.3 Cellular localisation

The majority of data favour 5-HT receptors being principally expressed by neurons; evidence that they are expressed by non-neural cells is often conflicting with the possible exception of cells of the cerebrovasculature, especially as in the case of the 5-HT_{1B} receptor (Parsons, 1991; Riad *et al.*, 1998). In particular, the literature describing the expression of 5-HT receptor subtypes by glial cells is controversial and incomplete. As an example, mRNA of multiple 5-HT receptors subtypes, including 5-HT_{1A} and 5-HT_{2A} receptors were detected in rat cultured astrocytes, but none the

receptors tested were functionally coupled (Hirst *et al.*, 1998). Moreover, it is uncertain whether the receptor expression in cultured astrocytes parallels expression in astrocytes in the brain. Thus, in the case of the 5-HT_{1A} receptor, whilst there are reports that 5-HT_{1A} receptor-like immunoreactivity is present in both neurons and a large proportion of CNS astrocytes (Whitaker-Azmitia *et al.*, 1993), other groups have shown that immunoreactivity is restricted to neurons, except in the spinal cord where weak staining may correspond to astrocytes/glial cells (Gerard *et al.*, 1994; Kia *et al.*, 1996). Similarly, immunocytochemical studies demonstrate that the 5-HT_{2A} receptor is predominantly expressed in neurons but not glial cells (Morilak *et al.*, 1993). An interesting possibility is that the pattern of non-neuronal expression of 5-HT receptors is different in the developing brain, or changes in response to pathology but currently there are little data addressing these points.

Typically there is a very good match between the distribution of 5-HT receptor mRNA and protein (Mengod *et al.*, 2010), suggesting that most 5-HT receptors are not trafficked along the axon but reside preferentially at the level of the soma and dendrites. Notable exceptions are 5-HT_{1B} and 5-HT_3 receptors which are trafficked along axons to nerve terminals to modulate neurotransmitter release, and this may also be the case for 5-HT_{2A} receptors (Lamb & Aghajanian, 2006).

Given the differential expression patterns of the 5-HT receptors, all fourteen 5-HT receptor subtypes are not likely expressed by a single neuron. Rather, evidence from double- and triple-labelling *in situ* hybridisation studies support the idea that a single postsynaptic synapse may express a limited combination of perhaps 2-3 different 5-HT receptor subtypes (Mengod *et al.*, 2010). This idea is in keeping with findings from recent studies combining whole-cell patch clamp recordings with single cell transcriptome profiling. For example, analysis of the 5-HT receptor mRNA content of neurons of the bed nucleus of the stria terminalis revealed that individual neurons could be subdivided according to the prominent expression of 2-3 distinct 5-HT receptor transcripts in largely excitatory/inhibitory combinations (eg. 5-HT_{1A}/5-HT_7, 5-HT_3/5-HT_7, 5-HT_{1B}/5-HT_4, 5-HT_{1A}/5-HT_{1B}/5-HT_{2A}, and 5-HT_{1A}/5-HT_{2A} receptors) that matched electrophysiological observations (Hazra *et al.*, 2012). A similar analysis of the 5-HT receptor mRNA in the preoptic

nucleus revealed two types of neurons that predominantly expressed a combination of inhibitory (5-HT_{1A}) and excitatory (5-HT_{2C}, 5-HT_4, 5-HT_7) 5-HT receptors whereas others expressed excitatory 5-HT receptors alone, again in keeping with electrophysiological findings (Sangare *et al.*, 2016). This evidence of 5-HT receptor expression conferring both excitatory and inhibitory signalling effects of 5-HT at the single cell level is born out more generally in electrophysiological experiments recording the effects of either bath application of 5-HT or electrical and optogenetic activation of 5-HT neurons (Andrade, 2006; Andrade & Beck, 2010; Sengupta *et al.*, 2017).

It is clear that expression of specific 5-HT receptors is not restricted to a particular neuron type. For example, within the cerebral cortex, hippocampus and amygdala, 5-HT_{1A} and 5-HT_{2A} receptors are expressed both on pyramidal neurons (glutamatergic) as well as major classes of inhibitory (GABAergic) interneurons (in particular those expressing parvalbumin). Whilst cortical 5-HT_3 receptors mark a specific population of GABA interneurons with distinct chemical, morphological and anatomical properties (Lee *et al.*, 2010), these receptors are also expressed by pyramidal neurons, particularly in humans (Brady *et al.*, 2007). Contrary to early evidence from immunocytochemistry studies that 5-HT_6 receptors were located specifically on cortical GABA interneurons, more recent *in situ* hybridization findings using are 5-HT_6 selective probes demonstrate that the receptor is preferentially expressed by pyramidal cells in cerebral cortex, although expression in a small population of interneurons was detected (Helboe *et al.*, 2015).

As an example of the complexity of the neuronal interactions of 5-HT, Figure 3 illustrates the present understanding of the distribution of 5-HT receptor subtypes within the microcircuitry of the basolateral nucleus in the amygdala, a key neural substrate of emotional learning (Bocchio *et al.*, 2016). Quite how this arrangement of multiple 5-HT receptor subtypes distributed across many cells types plays out at a neural network level is unknown. In this regard, it is important to consider the localization of specific 5-HT receptor subtypes on defined neuron types. Thus, a 5-HT receptor leading to membrane depolarization will have a different effect on network depending on whether it is located on an excitatory pyramidal neuron or an inhibitory interneuron. It is speculated that in the amygdala 5-HT may play an important role in the recruitment (5-HT_{2A} receptor-mediated) of

parvalbumin positive interneurons that are thought to synchronize large numbers of pyramidal neurons, and through this shape neural network synchrony and potentially facilitate fear learning (Bocchio *et al.*, 2016).

It is noteworthy that at the ultrastructural level, electron microscopy studies show that all metabotropic 5-HT receptors visualised thus far are located extrasynaptically, which has reinforced the idea that 5-HT signals via volume transmission (Descarries *et al.*, 2006). However, synaptic localisation of 5-HT receptors cannot be excluded as these are challenging studies which are often limited by the properties and specificity of available antibodies. Moreover, it is beyond doubt that 5-HT is present in vesicles that are synaptic as well as non-synaptic. As noted above, an additional complexity in signalling at 5-HT synapses is co-transmission, specifically involving ionotropic receptor signaling via co-released glutamate emerging (Sengupta *et al.*, 2017).

Looking forward, important insights into how 5-HT influences cerebral microcircuits are likely to come from the application of 5-HT-targeted optogenetic, chemogenetic and cell-specific knockout/knock-in strategies, large scale recordings of 5-HT-sensitive neural networks, as well as the development of computational modelling of 5-HT interactions with cortical microcircuitry that has already commenced (Joshi et al, 2020). Recent transgenic mouse studies demonstrating that behavioural responses of SSRI treatment are dependent on the expression of specific 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{5A}) by specific populations of hippocampal interneurons (parvalbumin and CCK) and pyramidal cells, provide examples of the power of such approaches (Samuels *et al.*, 2015; Medrihan *et al.*, 2017; Sagi *et al.*, 2019) and emphasise the importance of neuron type-specific pharmacological targeting. Neuronal network dysfunction is increasingly recognized as a symptom neuropsychiatric disorder and may provide new entry points for intervention. Once these complex interactions of 5-HT are better understood at the defined neural microcircuit level, then a more rational basis for CNS 5-HT drug discovery in the neuropsychiatric disorder field is likely to follow.

4. Effects of 5-HT receptors on behaviour and cognition: towards new drug therapies

The availability of selective pharmacological and genetic tools to manipulate 5-HT receptors, combined with a knowledge of the signalling properties of these receptors and their brain distribution, has helped establish a comprehensive knowledge of the behavioural and cognitive effects of 5-HT receptor manipulation (summarized in Table 3). This then has provided the rationale for the discovery and development of 5-HT ligands aimed at various neuropsychiatric disorders ranging from migraine and eating disorder through to depression and schizophrenia. Some of these agents have reached experimental medicine studies and clinical trials, and some have progressed all the way to therapeutic application. This subject is addressed in detail in a recent publication (Barnes et al, 2020), but salient points are covered below.

4.1 5-HT_1 receptor family

5-HT_{1A} receptors: Across a range of species, 5 HT_{1A} receptor agonists evoke a range of behavioural and physiological effects including altered motor function (especially aspects of the 5-HT behavioural syndrome), body temperature, and neuroendocrine activity. Moreover, there is an abundance of preclinical and clinical evidence that 5-HT_{1A} receptor agonists have antidepressant and anxiolytic properties, and influence a range of cognitive domains relevant to symptoms of schizophrenia (for review see (Traber & Glaser, 1987; Handley, 1995; Newman-Tancredi, 2010). The many and diverse behavioural effects of 5-HT_{1A} receptor agonists are likely to reflect an action at 5 HT_{1A} receptors in multiple forebrain and midbrain sites; for example, 5-HT_{1A} autoreceptor activation has been linked to anxiolytic effects whereas activation of postsynaptic 5-HT_{1A} receptors is associated with antidepressant effects (see (Richardson-Jones *et al.*, 2010; Richardson-Jones *et al.*, 2011; McCreary & Newman-Tancredi, 2015; Samuels *et al.*, 2016).

In support of this differential impact of pre- versus postsynaptic 5-HT_{1A} receptors on anxiety and depression mechanisms, it is reported that mice with knockdown of the presynaptic 5-HT_{1A} autoreceptor have a high anxiety phenotype whereas mice with knockdown of the postsynaptic 5-HT_{1A} receptor have a depressive-like phenotype (Richardson-Jones *et al.*, 2011). Interestingly these phenotypes are recapitulated by 5-HT_{1A} receptor suppression during a critical period in early

life and not blockade of 5-HT_{1A} receptors in adult mice (Richardson-Jones *et al.*, 2011; Garcia-Garcia *et al.*, 2014; Garcia-Garcia *et al.*, 2016). This suggests that the phenotypes are developmental in origin; that is loss of pre- or postsynaptic 5-HT_{1A} receptor function during early life disrupts neural circuit formation leading to altered anxiety/depression behaviour in adulthood. A similar picture has emerged regarding the high anxiety phenotype of 5-HT transporter mutant mice (Ansorge *et al.*, 2004; Rebello *et al.*, 2014). Consideration of developmental mechanisms is clearly important when interpreting the behavioural phenotype of genetically engineered mice with altered 5-HT receptor expression, as noted previously (Gingrich *et al.*, 2003; Berger & Tecott, 2006; O'Leary & Cryan, 2010).

This functional importance of 5-HT_{1A} receptors has generated much research into the therapeutic potential of 5-HT_{1A} receptor ligands. In particular, in the 1990's the 5-HT_{1A} agonist buspirone was approved for the clinical use as an anxiolytic agent, and the structurally related azapirones gepirone and tandospirone, demonstrated efficacy as antidepressant agents in clinical trials. The targeting of 5-HT_{1A} autoreceptors to speed up and augment antidepressant action generated considerable interest, and antidepressant augmentation was detected in clinical trials with the 5-HT_{1A}/β-adrenoceptor ligand pindolol (Whale *et al.*, 2010) although not the novel 5-HT_{1A} receptor antagonist DU-125530 (Scorza *et al.*, 2012). Moreover, the latter concept triggered the development of the novel antidepressants vilazodone (Dawson & Watson, 2009) and vortioxetine (Sanchez *et al.*, 2015), which have combined high affinity for the 5-HT uptake transporter and 5-HT_{1A} receptor (and multiple other 5-HT receptors in the case of vortioxetine - see later). Whereas additional studies are required to confirm that vilazodone has safety and efficacy over and above SSRI and other antidepressant alternatives (Stuivenga *et al.*, 2019), vortioxetine continues to show promise as a valid option for depressed patients who have not been effectively treated with first-line pharmacotherapies (Thase *et al.*, 2017).

The recent findings of canonical and non-canonical signalling properties of the 5-HT_{1A} receptor noted above, raise the possibility of developing biased 5-HT_{1A} receptor agonists to evoke a restricted range of selected behavioural effects. Indeed, a number of such agents are in

development for a range of CNS disorders. For example, NLX-112 (formerly named F13640) is considered a potential treatment of Parkinson's disease patients suffering from L-DOPA-induced dyskinesia (Newman-Tancredi *et al.*, 2018). NLX-112 is a 'full' agonist but shows preferential activation of ERK signaling, and activates 5-HT_{1A} autoreceptors to inhibit 5-HT cell firing. Here the thinking is that the unwanted motor effects of L-DOPA are mediated by the ectopic synthesis and release of dopamine from 5-HT neurons, and that the symptoms can be ameliorated by inhibition of 5-HT neuronal activity (Fisher *et al.*, 2020). Another biased 5-HT_{1A} receptor agonist NLX-101 (F15599), is reported to preferentially activate postsynaptic 5-HT_{1A} receptors and show antidepressant, pro-cognitive and neuroprotective properties in animal models (Becker *et al.*, 2016; Sniecikowska *et al.*, 2019; Vidal *et al.*, 2019; Aguiar *et al.*, 2020).

5-HT_{1B} receptors. Both preclinical and clinical studies, emphasise a role for 5-HT_{1B} ligands in the management of depression and anxiety (see (Ruf & Bhagwagar, 2009; Fakhoury, 2016) as well as aggression and impulse control (see (Nautiyal *et al.*, 2015). Moreover, the role of pre- versus postsynaptic 5-HT_{1B} receptors in these behaviours is being addressed utilising tissue-specific and time-dependent conditional 5-HT_{1B} receptor mutant mice. A number of selective 5-HT_{1B} receptor antagonists are available including SB224289 (Gaster *et al.*, 1998), however, progression with this receptor has been hampered to some extent by the lack of 5-HT_{1B} receptor agonists with sufficient selectivity or brain penetration.

Evidence suggests that 5-HT_{1B} receptor antagonists are anxiolytic and antidepressant in animal models (Fakhoury, 2016), which is consistent with reports that mice with a global 5-HT_{1B} receptor knockout have an anxiolytic and antidepressant-like phenotype (Mayorga *et al.*, 2001; Jones & Lucki, 2005; Bechtholt *et al.*, 2008). Interestingly, it was reported recently that the latter phenotype is recapitulated in genetic mice with loss of 5-HT_{1B} autoreceptors but not those with loss of postsynaptic 5-HT_{1B} receptors (Nautiyal *et al.*, 2016). Moreover, this phenotype was recapitulated by knockout of 5-HT_{1B} autoreceptors in adulthood and not during early postnatal life, making it unlikely that a developmental mechanism is involved (Nautiyal *et al.*, 2016). Collectively, these data suggest that drugs that selectively block 5-HT_{1B} autoreceptors may be useful for the treatment

of anxiety and depression. Currently there are no drugs with this property although developments with selective 5-HT_{1A} autoreceptor ligands (see above) offer the prospect for similar agents targeting 5-HT_{1B} autoreceptors.

Another interesting effect of global 5-HT_{1B} receptor knockout is increased aggression and impulsivity, which is in keeping with earlier reports that certain 5-HT_{1B} receptor agonists ('serenics') have anti-aggressive properties (Olivier *et al.*, 1995). The recently available conditional 5-HT_{1B} receptor knockout mice has allowed further examination of the role of pre- versus postsynaptic 5-HT_{1B} receptors in aggression and impulsivity. Interestingly, recent studies using the conditional 5-HT_{1B} receptor knockout mice found that heightened aggression and impulsivity was linked to loss postsynaptic 5-HT_{1B} receptors and not 5-HT_{1B} autoreceptors (Nautiyal *et al.*, 2015). Furthermore, it was observed that the impulsivity phenotype did not involve a developmental mechanism (i.e. it was not recapitulated by early life knockout), although the elevated aggression phenotype did have early life origins. These data raise the interesting possibility that drugs which selectively target the postsynaptic versus presynaptic 5-HT_{1B} receptors may be therapeutically effective in the control of heightened impulsivity.

5-$HT_{1D/e/F}$ receptors. As yet no behavioural response can be confidently ascribed to activation of 5-HT_{1D}, 5-ht_{1e} or 5-HT_{1F} receptors. Progress in this regard has been limited by the lack of drug tools with sufficient selectivity or brain penetration, and also doubts regarding the functional importance of these receptors given their low levels of expression. However, the 5-HT_{1D} receptor antagonist BRL15572 (Price *et al.*, 1997) has been important since in combination with the 5-HT_{1B} receptor antagonist SB224289, it allows the discrimination of 5-HT_{1B} or 5-HT_{1D} receptor-mediated responses. Nevertheless, the significance of these receptors to neuropharmacology ought not be underestimated in the sense that triptans (5-$HT_{1B/D}$ receptor agonists) are currently considered the gold standard for acute treatment of migraine (with the relative importance of 5-HT_{1B} versus 5-HT_{1D} remaining uncertain). Although triptans are not efficacious in all patients, and are contraindicated in certain patient groups (eg. coronary artery disease), 5-HT_{1F} receptor agonists are emerging as a potential migraine treatment alternative. In particular, the selective 5-HT_{1F} receptor agonist

lasmiditan (also known as COL-144 and LY573144; (Nelson *et al.*, 2010), which lacks the vasoconstriction property of triptans, is effective in phase 3 clinical trials (Goadsby *et al.*, 2019), and it's global general release as an antimigraine agent looks imminent. Although the mechanism of lasmiditan is not yet established the drug is brain penetrant and involvement of central 5-HT_{1F} receptors seems a likely (potentially via inhibition of the trigeminocervical complex).

4.2 5-HT_2 receptor family

The behavioural effects of 5-HT_2 receptor agonists in rodents are many, ranging from changes in both unconditioned (e.g. head-twitches, increased motor activity, hypophagia, hyperthermia) and conditioned responses (e.g. punished responding, drug discrimination), as reviewed previously (Koek *et al.*, 1992; Barnes & Sharp, 1999; Halberstadt, 2015). It has become possible to identify the role of the different 5-HT_2 receptor subtypes in these behaviours with some degree of confidence, through the availability of highly selective antagonists (for each of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors) and agonists (especially 5-HT_{2C}) as well as mutant mice with the different receptors selectively knocked out (Heisler *et al.*, 2007b; Halberstadt, 2015; Di Giovanni & De Deurwaerdere, 2016).

5-HT_{2A} receptors: There is strong evidence from the use of selective pharmacological and genetic tools that the 5-HT_{2A} receptor is a major contributor to multiple functional responses in animals including 5-HT agonist induced head-twitches, drug discrimination cues, hyperthermia and changes in exploratory behaviour (Halberstadt, 2015). Furthermore, much evidence suggests that the 5-HT_{2A} receptor is the mediator of the hallucinogenic effects of psychedelic drugs in humans. For example, 5-HT_{2A} receptor agonist-induced head-twitches have strong predictive value in this regard (Halberstadt *et al.*, 2020). Despite its simplicity, the 5-HT_{2A} receptor agonist-induced head-twitch response is well characterized using both pharmacological and genetic approaches; the response is likely mediated via the phosphoinositide signaling pathway, and arises from 5-HT_{2A} receptor activation in the forebrain and the prefrontal cortex in particular (Gonzalez-Maeso *et al.*, 2007; Halberstadt, 2015; Antoniadou *et al.*, 2018).

In the 1960's psychedelic drugs like LSD and psilocybin were widely used by psychotherapists as an aid to treat depression as well as anxiety-related disorders and addictions, and results were generally encouraging. In the last few years there has been renewed interest in the use of these agents for therapeutic purposes. Psilocybin in particular has been subject to investigation in a range of human psychopharmacological and brain imaging studies, which have contributed proof-of-principle and dose/safety data for clinical trials (Nutt, 2016; Nutt *et al.*, 2020). Recent clinical data demonstrate that a single dose of psilocybin (combined with psychological support) causes a sustained lowering of depression and anxiety ratings in cancer patients (Griffiths *et al.*, 2016; Ross *et al.*, 2016; Goldberg *et al.*, 2020) and treatment resistant-depression (Carhart-Harris *et al.*, 2016). Moreover, a recent PET study in human volunteers using the novel 5-HT_{2A} agonist radioligand [^{11}C]-Cimbi-36 found that psychotropic effects of psilocybin was closely associated with 5-HT_{2A} receptor occupancy (Madsen *et al.*, 2019), emphasizing the importance of this receptor in the psychotropic effects of psilocybin. Evidence that hallucinogenic 5-HT_{2A} receptor agonists alter markers of synaptic plasticity in animals provides a rational molecular/cellular account of the antidepressant effect of these agents (Vollenweider & Kometer, 2010).

The potent 5-HT_{2A} receptor antagonist properties of antipsychotics typified by clozapine has been linked to an advantageous side effect profile and improved efficacy (Meltzer & Massey, 2011). Moreover, high affinity for the 5-HT_{2A} receptor is feature of the newer antipsychotics such as aripiprazole and brexpiprazole, although high affinity for the 5-HT_{1A} receptor (and other receptors) may play an important role in their actions (McCreary & Newman-Tancredi, 2015). This said, whilst recent meta-analyses suggest that antipsychotics vary in their efficacy and adverse effects (Huhn *et al.*, 2019), currently there is no simple pharmacological account of the individual differences. Certainly, 5-HT_{2A} receptor blockade is unlikely to be sufficient by itself to generate a useful antipsychotic effect due to the lack of antipsychotic effect of the selective 5-HT_{2A} receptor antagonist, MDL 100,907 (volinaserin; (de Paulis, 2001). However, recent evidence that the 5-HT_{2A} receptor inverse agonist pimavanserin has antipsychotic actions in relevant animal models (Vanover *et al.*, 2006) and in certain patient populations (Fox, 2014), emphasizes the value of 5-HT_{2A} receptor modulation in the treatment of psychosis. In this regard, the interesting interaction

between 5-HT_{2A} receptors and mGlu2 glutamate receptors is noteworthy as this offers interesting avenues for the future development of drugs with combined 5-HT_{2A} receptor antagonist - mGlu2 agonist properties (Shah & Gonzalez-Maeso, 2019).

Recent reports that 5-HT_{2A} receptor antagonists augment the effect of 5-HT uptake inhibitors in preclinical models (Marek *et al.*, 2003; Marek *et al.*, 2005; Boothman *et al.*, 2006), may be associated with evidence that drugs with 5-HT_{2A} receptor antagonist properties are helpful as augmenting agents in treatment resistant depression (Marek *et al.*, 2003). Although the mechanism behind this effect of 5-HT_{2A} receptor blockade is not certain, it may link to evidence of an inhibitory 5-HT_{2A} receptor-mediated feedback on 5-HT neurons (Sharp *et al.*, 2007).

Interestingly, the 5-HT_{2A} receptor has also been associated with impulse control; thus, selective 5-HT_{2A} receptor antagonists consistently decrease impulsivity in animal models (Winstanley *et al.*, 2004; Winstanley, 2011; Barkus *et al.*, 2018) and this is supported by psychopharmacological studies in humans using the non-selective 5-HT_{2A} receptor antagonist quetiapine (Rock *et al.*, 2013). In this regard it is noteworthy that lithium and the repurposed lithium-mimetic ebselen are both reported to attenuate 5-HT_{2A} receptor function, likely via inhibition of the phosphoinositide pathway (Singh *et al.*, 2013; Antoniadou *et al.*, 2018). Moreover, ebselen reduced impulsivity in animal as well as human paradigms (Masaki *et al.*, 2016; Barkus *et al.*, 2018). This raises the possibility that 5-HT_{2A} receptor antagonists/inverse agonists as well as novel lithium-like agents such as ebselen may have utility in the control of disorders of impulse control (see also following section on 5-HT_{2C} receptors).

5-HT_{2B} receptors: Evidence of relatively low 5-HT_{2B} receptor expression in the brain and the lack of selective 5-HT_{2B} receptor ligands has hampered progress in establishing the CNS function of these receptors. Interestingly, 5-HT_{2B} knockout mice have striking phenotypes including deficits in sensorimotor gating, social interaction, attention, learning and memory, as well as elevated impulsivity and altered sleep patterns, some of which (sleep, sensorimotor gating) can be phenocopied by 5-HT_{2B} receptor blockade using the selective 5-HT_{2B} receptor antagonist

RS127445 (Pitychoutis *et al.*, 2015). However, 5-HT_{2B} knockout is also associated with severe cardiac abnormalities and embryonic and postnatal lethality, which might lead to neurodevelopment issues in surviving animals that could confound the interpretation of these adult CNS phenotypes (as noted above). Although studies involving conditional 5-HT_{2B} knockout strategies and further pharmacological phenocopying are awaited, these data raise interesting possibilities regarding the functional role of CNS 5-HT_{2B} receptors. Recently discovered interactions between 5-HT_{2B} receptors and raphe 5-HT neurons (Belmer & Maroteaux, 2019) as well as the dopamine system (Devroye *et al.*, 2018), emphasizing a possible role of these receptors in the treatment of depression, are noteworthy in this regard.

5-HT_{2B} receptor agonism in the periphery is associated with unwanted and potentially harmful adverse effects including pulmonary hypertension, and a number of therapeutic agents with 5-HT_{2B} receptor agonist properties (eg. fenfluramine, pergolide, cabergoline) have been withdrawn (Hutcheson *et al.*, 2011). Nevertheless, recent data showing biased agonism at the 5-HT_{2B} receptor (Wacker *et al.*, 2013) offers the prospect of future 5-HT_{2B} receptor agonists devoid of these systemic adverse effects.

5-HT_{2C} receptors: Early neuropharmacological studies using non-selective agents associated 5-HT_{2C} receptor activation with various behavioural and physiological effects in rodents such as hypolocomotion, hypophagia, anxiety, penile erection and hyperthermia, as reviewed earlier (Barnes & Sharp, 1999). With the introduction of more selective 5-HT_{2C} receptor agonists (eg. CP-809101, lorcaserin) and antagonists (eg. SB242084) and 5-HT_{2C} knock out mice, the list 5-HT_{2C} receptor functions has expanded to include compulsive drug- and food-seeking, central control of energy homeostasis, oral dyskinesia, wakefulness and control seizure threshold (Di Giovanni & De Deurwaerdere, 2016).

The link between 5-HT_{2C} receptors and addictive behaviours associated with food and psychostimulant drugs has been under intense investigation (Higgins & Fletcher, 2015; Howell & Cunningham, 2015). It is clear from rodent studies that 5-HT_{2C} receptor activation reduce palatable

food consumption and metabolism, likely via an action on hypothalamic nuclei that promote satiety and regulate energy balance pathways (Heisler *et al.*, 2006; Heisler *et al.*, 2007a; Lam *et al.*, 2008). These agents also disrupt dopamine-mediated addiction mechanisms that lead up to compulsive drug use such as the positive reinforcement, behavioural sensitization, and reinstatement properties of psychostimulants. Interestingly, separate studies have revealed a link between 5-HT_{2C} receptors and impulse control; thus 5-HT_{2C} receptor agonists have been found to reduce premature responding whereas 5-HT_{2C} receptor antagonists have the opposite effect (Higgins *et al.*, 2003; Winstanley *et al.*, 2004; Fletcher *et al.*, 2007; Higgins *et al.*, 2016). Given that impulsivity may influence many aspects of compulsive drug- and food-seeking behaviour, it is speculated that 5-HT_{2C} receptors may modulate addiction indirectly via its effects on impulsive behavior (Higgins *et al.*, 2017).

As a consequence of these discoveries in preclinical studies, the selective 5-HT_{2C} receptor agonist lorcaserin advanced into clinical trial and has been approved for the treatment of obesity (when combined with lifestyle modification). Although the overall effect size of lorcaserin appears to be modest, individual variation in response indicates that patient stratification could be used to optimize treatment outcome in the future (Higgins *et al.*, 2020). These clinical studies in obesity combined with accumulating evidence that lorcaserin is safe and tolerated by patients, may pave the way for further studies into the therapeutic potential of lorcaserin in other clinical indications.

Earlier studies reported opposing functional interactions between 5-HT_{2C} and 5-HT_{2A} receptors (Berendsen & Broekkamp, 1990), and more recent data are in keeping with this. For example, both 5-HT_{2A} receptor antagonists and 5-HT_{2C} receptor agonists lower impulsivity in rodent models (Winstanley, 2011). This interaction could explain why non-selective 5-$HT_{2A/2C}$ receptor ligands and even 5-HT augmenting agents such as SSRIs have generally poor efficacy in drug addiction models, where impulsivity mechanisms have recognised importance. Consequently, it has been proposed that an optimal way to control addictive behaviour may be a 5-HT drug that combines in the same molecule, agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor (Anastasio *et al.*, 2015; Higgins & Fletcher, 2015). Additional approaches for future

investigation could include 5-HT_{2C} receptor agonists with biased agonist properties or positive allosteric modulators of the 5-HT_{2C} receptor.

4.3 5-HT_3 receptors

Outside of the well-established role of 5-HT_3 receptors in the control of emesis (likely central as well as peripheral mechanism), 5-HT_3 receptors have been linked to multiple behavioural effects, ranging from changes in anxiety and cognition to altered pain processing and sensitivity to addictive drugs. Most of the early evidence for this comes from studies of the behavioural effects of 5-HT_3 receptor ligands (mostly antagonists) in animal models; these and more recent findings are extensively reviewed elsewhere (Costall & Naylor, 1992; Bentley & Barnes, 1995; Barnes & Sharp, 1999; Walstab *et al.*, 2010; Gupta *et al.*, 2016). However, many of the behavioural effects of selective 5-HT_3 receptor antagonists observed in preclinical investigations are not confirmed by studies of these agents in clinical populations (Bentley & Barnes, 1995; Walstab *et al.*, 2010).

The apparent failure for the translation of these preclinical findings could in part be explained by sub-optimal clinical dosing, since a bell-shaped dose-response curves are often observed for 5-HT_3 receptor antagonists, particularly in relation to CNS effects. Furthermore the differential expression of forebrain 5-HT_3 receptors when comparing species (e.g. rat versus human; see (Parker *et al.*, 1996) may underlie some species differences in the behavioural consequences following 5-HT_3 receptor antagonism. Nevertheless, the involvement of 5-HT_3 receptors in some of these behaviours receives support from more recent studies of mutant mice with altered 5-HT_3A receptor expression.

In particular, support for a role for 5-HT_3 receptors in depression/anxiety-related behaviours, learning and memory, and pain processing comes from the phenotypic analysis of transgenic mice with 5-HT_3A receptor knockout or overexpression (Harrell & Allan, 2003; Kelley *et al.*, 2003; Berger & Tecott, 2006). Interpretation of the phenotypes of these mice is complicated by findings that, i) they are not always phenocopied by pharmacological agents, ii) they are dependent on the mouse background strain, and iii) over-expression and deletion of the 5-HT_3A receptor sometimes

produce similar effects (for review see (Berger & Tecott, 2006; O'Leary & Cryan, 2010). However, in the case of a putative anxiolytic effect of 5-HT_3 receptor blockade, it is noteworthy that the 5-HT_3 receptor antagonist alosetron was reported to reduce emotional responding in patients with irritable bowel syndrome (Mayer *et al.*, 2002). This effect was associated with reduced activity in limbic regions including amygdala as measured by PET imaging, suggesting a potential anxiolytic mechanism. This said peripheral mechanisms may have contributed, including effects of 5-HT_3 receptor antagonism on enteric neurons (Mayer *et al.*, 2002).

More recent evidence associating 5-HT_3 receptors with emotional behaviours and cognition comes from findings with vortioxetine (see also above), which has atypical pharmacology at the 5-HT_3 receptor with initial agonist action followed by long-term unsurmountable receptor inhibition (Ladefoged *et al.*, 2018) combined with affinity for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_7 and 5-HT transporter binding sites. Vortioxetine has significant antidepressant and pro-cognitive activity in both rodent models and clinical trials (Mork *et al.*, 2012; Sanchez *et al.*, 2015) and is currently marketed as an antidepressant with cognition enhancing properties. In spite of vortioxetine's polymodal pharmacology, blockade of 5-HT_3 receptors is thought to play a prominent role in its mechanism of action, particularly in relation to cognition. Thus, in rodents vortioxetine preferentially occupies 5-HT_3 receptors and the 5-HT transporter at low doses, and 5-HT_3 receptor blockade alone can produce cognition-enhancing effects similar to vortioxetine (Mork *et al.*, 2012; Sanchez *et al.*, 2015). A current mechanistic explanation of these findings is that blockade of 5-HT_3 receptors on specific populations of cortical GABA interneurons contributes to vortioxetine's action (Riga *et al.*, 2016).

4.4 5-HT_4 receptors

Earlier preclinical studies detected cognition-enhancing as well as anxiolytic effects of 5-HT_4 receptor agonists as reviewed previously (Barnes & Sharp, 1999), and these observations have proved consistent in later work (Bockaert *et al.*, 2011; Claeysen *et al.*, 2015; Hagena & Manahan-Vaughan, 2016). Stemming in part from studies of 5-HT_4 knockout mice (Compan *et al.*, 2004), a role of 5-HT_4 receptors in feeding behaviour also now seems clear, with 5-HT_4 receptor agonists

and antagonists have hypo- and hyper-phagic properties, respectively (Jean *et al.*, 2007; Bockaert *et al.*, 2011).

Pro-cognitive effects of 5-HT_4 receptor agonists (eg. RS673333, BIMU 1/BIMU 8) have been described across a range of species and in a variety of experimental paradigms that model different aspects of short- and long-term memory, many of them dependent on hippocampus where 5-HT_4 receptors are reasonably abundant (Hagena & Manahan-Vaughan, 2016). These agents also reverse the cognitive deficits induced by factors such as ageing, pharmacological interventions (e.g. muscarinic antagonists) and Alzheimer's disease-like pathology. The cellular mechanisms underlying the pro-cognitive effects of 5-HT_4 receptor agonists are not certain but may include increased release of 5-HT and acetylcholine, induction of synaptic plasticity, increased synaptic spine formation and altered hippocampal network properties (Boddeke & Kalkman, 1990; Ge & Barnes, 1996; Claeysen *et al.*, 2015; Hagena & Manahan-Vaughan, 2016). 5-HT_4 receptor agonists such as RS673333 also promote amyloid precursor protein cleavage, which has led to speculation that these agents may be useful in the management of Alzheimer's disease (Claeysen *et al.*, 2015). In this regard, it is of interest that the novel compound donecopride which has combined 5-HT_4 receptor agonist and acetylcholinesterase inhibitor properties, was recently reported to have cognition enhancing and neuroprotective properties in a mouse model of Alzheimer's disease (Rochais *et al.*, 2019).

5-HT_4 receptor agonists evoke rapid antidepressant effects in animal models (Lucas *et al.*, 2007) (Vidal *et al.*, 2014; Samuels *et al.*, 2016), and there is preclinical evidence that 5-HT_4 receptor activation plays an important role in the action of SSRIs (Mendez-David *et al.*, 2014) and may be helpful in SSRI augmentation (Licht *et al.*, 2010b). Antidepressant administration causes adaptive changes in 5-HT_4 receptor expression and function (Licht *et al.*, 2010a). Moreover, in a recent PET study using the 5-HT_4 radioligand ^{11}C-SB207145, reported that reduced striatal 5-HT_4 receptors was associated with a family history of depression (Madsen *et al.*, 2014). The mechanism involved in the antidepressant effects of 5-HT_4 receptor agonists in uncertain but a contributory factor may

be activation of a positive feedback control of midbrain 5-HT neurons via 5-HT_4 receptor located in the frontal cortex or hippocampus (Ge & Barnes, 1996; Lucas *et al.*, 2005; Licht *et al.*, 2010b).

Aside from rodent studies, there is an earlier report that the 5-HT_4 receptor agonist RS 17017 improved the cognitive performance in non-human primates (Terry *et al.*, 1998). More recently in a study of healthy human volunteers, a single dose of the 5-HT_4 receptor agonist prucalopride was found to have pro-cognitive effects in a battery of learning and memory tests, although the drug was not active in models of emotional processing that have antidepressant predictive capability (Murphy *et al.*, 2019). Although exploratory in nature, the latter study reported relatively mild adverse effects of prucalopride, in particular headache, nausea and diarrhoea, and consistent with that expected of agents with 5-HT_4 receptor agonist actions. Further studies are required to ascertain the general tolerability of 5-HT_4 receptor agonists, and the extent to which adverse effects might hamper the clinical development of these agents.

4.5 5-HT_5 receptors

Both 5-HT_{5A} and 5-ht_{5b} receptor subtypes have been detected in the CNS of multiple species, but the full length 5-ht_{5b} receptor is not expressed in humans (interruption of coding sequence by stop codons), thereby negating the usefulness of this receptor as a therapeutic target (Nelson, 2004). Due to the lack of suitably selective drug tools there is currently limited information on the CNS functions of the 5-HT_{5A} receptor. A number of drugs with 5-HT_{5A} receptor antagonist activity have been investigated in rodents for anxiolytic, antidepressant and antipsychotic activity, but to date no consistent behavioural effect of 5-HT_{5A} blockade has been identified (Kassai *et al.*, 2012), although a possible link to memory has been noted (Gonzalez *et al.*, 2013). However, these studies should be treated cautiously because they rely on the use of the 5-HT_{5A} receptor antagonist SB-699551; while this agent possesses high affinity for the human and guinea pig 5-HT_{5A} binding sites (~pKi 8.3), its affinity for these sites in the rat and mouse is 2 orders of magnitude lower (pKi 6.3; (Thomas *et al.*, 2006). It is reported that 5-HT_{5A} receptor knockout mice display increased exploratory activity without altered anxiety levels (Grailhe *et al.*, 1999). A drawback of the latter study is that in the absence of supporting data from phenocopying experiments using 5-HT_{5A}

receptor antagonists, it remains uncertain whether these behaviours are driven by the absence of 5-HT_{5A} receptor in the adult brain.

Intriguingly, in 5-HT_{5A} receptor knockout mice the locomotor activation induced by LSD was blunted, suggesting that 5-HT_{5A} receptor activation might contribute to the psychotropic effects of psychedelic agents (Grailhe *et al.*, 1999), and therefore that 5-HT_{5A} antagonists may be antipsychotic. In accordance with this latter finding, a recent study reported that in rats the LSD discriminatory cue was attenuated, albeit partially, by pretreatment with SB-699551-A (Popik *et al.*, 2019) although note the above caveat about this agent. Another recent study associated 5-HT_{5A} receptor function with antidepressant action. Specifically, using a cell-specific 5-HT_{5A} receptor knockout strategy, it was found that the activity of 5-HT_{5A} receptors in hippocampal (dentate gyrus) parvalbumin interneurons was required for behavioural effects of chronic SSRI treatment (Sagi *et al.*, 2019).

4.6 5-HT_6 receptors

The cognitive and behavioural functions of the 5-HT_6 receptor were somewhat obscure until the discovery of selective 5-HT_6 receptor ligands, although the latter development is complicated by important species differences in the pharmacological profile of the 5-HT_6 receptor which is much closer between human and rat, than human and mouse (Setola & Roth, 2003). Moreover, levels of 5-HT_6 receptor expression are also lower in mouse compared to rats and humans (Hirst *et al.*, 2003). Although earlier 5-HT_6 receptor ligands lacked selectivity and brain penetrability, these problems are largely overcome by more recently developed compounds, including the agonists WAY181187 and WAY208466 (Schechter *et al.*, 2008), and antagonists LuAE58054 (idalopirdine), SB258585, and SB399885 (Hirst *et al.*, 2003; Hirst *et al.*, 2006; Arnt *et al.*, 2010). The use of such compounds in rat behavioural models has identified a role for the receptor in a range of CNS functions and learning and memory (Mitchell & Neumaier, 2005; de Jong & Mork, 2017), as well as feeding, addiction and seizure control (Karila *et al.*, 2015; Brodsky *et al.*, 2016; Chaumont-Dubel *et al.*, 2019). In particular, there are consistent findings that 5-HT_6 receptor antagonists enhance learning and memory in preclinical models, ranging from novel object and social recognition to

spatial memory tasks (de Jong & Mork, 2017). These effects are observed in naive animals as well as in animals with memory deficits, for example induced by pharmacological means such as cholinergic antagonists.

The mechanism underlying the pro-cognitive effects of 5-HT_6 receptor blockade is uncertain. An interaction with the cholinergic system is supported by evidence that treatment with the 5-HT_6 receptor antagonist idalopirdine augments the cognition enhancing effects of an acetylcholinesterase inhibitor in both animal models and patients with Alzheimer's disease (Wilkinson *et al.*, 2014; de Jong & Mork, 2017; Kucinski *et al.*, 2017). Moreover, there is neurophysiological and neurochemical evidence that this drug combination has a synergistic effect on cholinergic function (Herrik *et al.*, 2016). However, recent data show that 5-HT_6 receptors are not expressed by cholinergic neurons (Helboe *et al.*, 2015), which would invoke an indirect mechanism. A mechanism involving increased information processing through cortico-striatal circuits was recently proposed (Kucinski *et al.*, 2017).

The evidence that 5-HT_6 receptor antagonists are cognition-enhancing led to their clinical trial in Alzheimer's disease. To date however, despite encouraging findings in early studies, large scale clinical trials with idalopirdine, have not detected a significant therapeutic effect (Atri *et al.*, 2018; Khoury *et al.*, 2018; Matsunaga *et al.*, 2019), and negative findings have also been reported for other 5-HT_6 receptor antagonists (Bennett, 2018). The explanation for the lack of translation from preclinical studies is not clear but is likely to be related to multiple factors, ranging from insufficient receptor occupancy to patient heterogeneity or simply poor predictive validity of the animal models used. Investigation into the potential utility of these compounds in the treatment of cognitive impairment associated with other disorders including schizophrenia is ongoing.

A complication in understanding the pro-cognitive effects of 5-HT_6 receptor antagonists is the paradox that 5-HT_6 receptor agonists are also reported to have pro-cognitive effects in some, albeit not all, animal models (e.g. (Schechter *et al.*, 2008; Burnham *et al.*, 2010; Kendall *et al.*, 2011; Meneses *et al.*, 2011). Part of the explanation could lay in the cognitive domain being measured,

and to date few studies have systematically compared selective 5-HT_6 receptor agonists and antagonists in the same model. There also remains the intriguing possibility that the full answer lies in the pharmacology of 5-HT_6 receptor ligands, and that their categorization in terms of partial agonist, inverse agonist and even biased agonist is not yet complete (e.g. (Romero *et al.*, 2006). Nevertheless, 5-HT_6 receptor partial or inverse agonists are potential candidates for future trials in Alzheimer's disease and other disorders in which cognitive deficits are a feature.

4.7 5-HT_7 receptors

Analogous to the 5-HT_6 receptor, the behavioural and cognitive functions of the 5-HT_7 receptor are now beginning to be revealed through important advances in 5-HT_7 receptor pharmacology with selective agonists (AS-19, LP-211, E-55888) as well antagonists (SB-258719, SB-269970) being available, as well as a 5-HT_7 receptor knockout mouse (for review see (Blattner *et al.*, 2019). Earlier studies finding that 8-OH-DPAT was hypothermic concluded that 5-HT_{1A} receptor were involved before this drug was also recognized as a 5-HT_7 receptor agonist. It is now apparent that 5-HT_7 receptor agonists such as are hypothermic in various species, and that both 5-HT_{1A} and 5-HT_7 receptors were involved in the effect of 8-OH-DPAT (Hagan *et al.*, 2000; Brenchat *et al.*, 2012). Evidence suggests that the 5-HT_7 receptor is associated with not just temperature control but a wide variety of CNS processes including regulation of circadian rhythms, mood, learning and memory, seizure threshold and pain processing as well as mechanisms of addiction. This literature is extensively reviewed elsewhere (Hedlund, 2009; Hauser *et al.*, 2014; Blattner *et al.*, 2019), and only highlights are noted here.

Much preclinical evidence implicates the 5-HT_7 receptor in mechanisms of learning and memory. For example, 5-HT_7 receptor KO mice display specific impairments in various models of spatial learning, and also demonstrate deficits in long term potentiation (Roberts *et al.*, 2004). Moreover, selective 5-HT_7 receptor antagonists such as SB-269970 reverse cognitive deficits induced pharmacologically, in particular by NMDA antagonists (Meneses, 2004), and 5-HT_7 receptor blockade might contribute to the pro-cognitive actions of antipsychotics such as lurasidone

(Horisawa *et al.*, 2013). Evidence also supports a role for the 5-HT_7 receptor in seizure control. For instance, both antagonists SB-258719 and SB-269970 demonstrate anticonvulsant properties in rodent epilepsy models, whereas the agonist AS-19 was proconvulsant (Graf *et al.*, 2004; Yang *et al.*, 2012).

The 5-HT_7 receptor modulation of circadian rhythms was established in early studies showing that the circadian phase shift of suprachiasmatic nucleus neurons induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT was mediated by 5-HT_7 and not 5-HT_{1A} receptors (Lovenberg *et al.*, 1993). On the basis of these findings, studies have explored the role of the 5-HT_7 receptor in sleep regulation. Evidence from rodent studies shows that 5-HT_7 receptor blockade, for example using SB-269970, suppresses rapid eye movement (REM) sleep, and similar observations have been made using the 5-HT_7 receptor antagonist JNJ-18038683 in healthy human volunteers (Hedlund, 2009; Bonaventure *et al.*, 2012). However, in these studies neither pharmacological blockade nor genetic knockout of the 5-HT_7 receptor significantly altered the sleep-wake state, suggesting that the therapeutic targeting of 5-HT_7 receptors antagonists for sleep disorder *per se* may not be fruitful.

In accordance with the link between 5-HT_7 receptors and circadian rhythm regulation, both 5-HT_7 receptor knockout and 5-HT_7 receptor blockade are consistently reported to have antidepressant effects in a range of models in rats and mice, and also augment antidepressant effects (Hedlund, 2009; Blattner *et al.*, 2019). In support of the rationale for the use of 5-HT_7 receptor antagonists in the treatment of depression, in animal models the antidepressant efficacy of a number of drugs such as desipramine and reboxetine, and certain antipsychotic agents including amisulpiride, was found to be 5-HT_7 receptor-dependent (Blattner *et al.*, 2019). Moreover, other antidepressant and antipsychotic drugs (tricyclics, lurasidone, aripiprazole, etc) in clinical use have 5-HT_7 receptor antagonist properties.

This encouraging preclinical data led to a clinical trial of JNJ-18038683, in major depression (Bonaventure *et al.*, 2012). Although the latter study found that JNJ-18038683 was well-tolerated, it

was inconclusive regarding the potential efficacy of the drug in depression due to a failure of the positive control. As yet, there appears to be no further reports of clinical trials of 5-HT_7 compounds in major depression. Interestingly, the Bonaventure study used REM as a biomarker of successful target engagement. Attempts to develop 5-HT_7 PET radioligands are making progress, and the availability of a ligand for human use looks imminent (L'Estrade *et al.*, 2019). The emergence of a suitable 5-HT_7 PET radioligand will be important for establishing occupancy of 5-HT_7 receptor ligands prior to their use in experimental medicine studies and clinical trials. Overall, the full therapeutic potential of the 5-HT_7 receptor remains uncertain. Whilst the majority of the research thus far in this area has focused on antagonists, selective brain penetrant 5-HT_7 receptor agonists are now becoming available as pharmacological tools (Di Pilato *et al.*, 2014) to help further define the function of the 5-HT_7 receptor and open up new therapeutic avenues.

5. Concluding remarks

Our review of 5-HT receptors in 1999 closed on the note that the clinical benefits of discoveries in basic 5-HT neuropharmacology had yet to be fully realized. Since then important therapeutic progress has been made through the use of 5-HT drugs with notable examples of prescribed drugs for the treatment of mood disorder (5-HTT/5-HT_{1A},/5-HT_{1B}/5-HT_3/5-HT_7; vortioxetine), schizophrenia and psychosis (5-HT_{2A}, 5-HT_{1A}; aripirazole, brexpiprazole, pimvanserin), migraine (5-HT_{1F}; lasmiditan) and eating disorder (5-HT_{2C}; lorcaserin), although there have also been disappointments (eg. 5-HT_6; idalopirdine). It is apparent from these advances that success in the clinical has come about from drugs that target specific 5-HT receptors as well as drugs that target multiple 5-HT (and other) receptors; equally, neither approach guarantees success. Looking forward, groundwork has been laid for the potential therapeutic application of 5-HT drugs (targeting one or many 5-HT receptors) in these and many other areas including impulse control (5-HT_{2A}, 5-HT_{2C}), cognitive decline (5-HT_4, 5-HT_6) and depression and anxiety (5-HT_{2A}, 5-HT_4, 5-HT_7).

It is also not difficult to imagine that clinical advances will eventually come from further investigation of the complexity in 5-HT signaling that is now being revealed, including agonist-dependent signalling of metabotropic 5-HT receptors, 5-HT receptor-interacting proteins, and 5-

HT/glutamate co-release. Perhaps more challenging for the future will be to understand better how 5-HT neurons interact with key neural circuits to generate changes in cognition and behaviour, but this will be facilitated by new genetic tools and technologies that are fast becoming available. Finally, the translation of these and other discoveries in basic 5-HT neuropharmacology will be facilitated by developments in 5-HT PET radioligand tracers and other molecular imaging modalities, not just to establish the level of target engagement by drugs but also for use as tools to investigate the role of 5-HT and 5-HT release mechanisms in the neurobiology of neuropsychiatric disorder.

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Table 1. Summary of selective 5-HT receptor ligands and radioligands. Examples of PET radioligands with proven utility in humans are given. See text and references therein for further details. Readers are specifically directed to comprehensive reviews of 5-HT receptor pharmacology by Andrades *et al*, 2019 and Barnes *et al*, 2020.

5-HT receptor	Agonist	Antagonist	Radioligand for binding studies	PET radioligand
5-HT_{1A}	8-OH-DPAT NLX-101 (F15599)	WAY100635 NAD-299 (robalzotan)	^{3}H-WAY100635 ^{3}H-8-OH-DPAT	^{11}C-WAY100635
5-HT_{1B}	CP94253	SB224289 SB236057	^{3}H-GR 125,743 ^{3}H-N-methyl-AZ10419369	^{11}C-AZ10419369 ^{18}F-AZ10419096
5-HT_{1D}	PNU109291	BRL15572 SB714786	^{3}H-eletriptan ^{3}H-sumatriptan	
5-ht_{1e}	BRL-54443		^{3}H-5-HT	
5-HT_{1F}	Lasmiditan LY334370		^{3}H-LY334370	
5-HT_{2A}	DOI	MDL100907 Pimavanserin	^{3}H-ketanserin ^{3}H-fananserin	^{11}C-Cimbi-36 ^{11}C-MDL 100,907
5-HT_{2B}	BW723C86 Ro 60-0175	RS127445 LY266097	^{3}H-LSD	
5-HT_{2C}	Lorcaserin WAY163909	SB242084 RS-102221	^{3}H-mesulergine	
5-HT_{3}	*m*-chlorophenyl-biguanide SR57227A	Granisetron (s)-Zacopride	^{3}H-GR65630 ^{3}H-(s)-Zacopride	

5-HT_4	RS67333 BIMU 8	SB204070 GR113808	[^{3}H]-GR113808 [^{125}I]-SB207710	^{11}C-SB207145
5-HT_{5A}	LSD	SB699551 ASP5736	^{125}I-LSD	
5-ht_{5b}			^{125}I-LSD	
5-HT_6	WAY181187 WAY208466	SB399885 SB271046	[^{125}I]-SB258585	[^{11}C]-GSK215083
5-HT_7	E-55888 AS-19	SB269970 SB258719	[^{3}H]-SB269970	

Table 2. Canonical and non-canonical signalling, and biased agonism at 5-HT receptors. Examples of non-canonical signalling are given; see text and references therein for further details. Abbreviations; Akt - serine/threonine protein kinase, AC - adenylate cyclase, Cdk5 - Cyclin-dependent kinase, ERK - extracellular-signal-regulated kinase, mTOR - mammalian target of rapamycin (serine/threonine protein kinase), PKA - protein kinase A, PLC - phospholipase C, PLA_2 - phospholipase A_2, PLD - phospholipase D.

5-HT receptor	Canonical signalling	Non-canonical signalling	Evidence of biased agonism
$5\text{-}HT_{1A}$	$G_{i/o}$, AC, PKA	ERK, Small G-proteins	Yes (NLX-112, NLX-101, Sniecikowska et al 2019)
$5\text{-}HT_{1B}$	$G_{i/o}$, AC, PKA	ERK, Small G-proteins	
$5\text{-}HT_{1D}$	$G_{i/o}$, AC, PKA		
$5\text{-}ht_{1e}$	$G_{i/o}$, AC, PKA		
$5\text{-}HT_{1F}$	$G_{i/o}$, AC, PKA		
$5\text{-}HT_{2A}$	$G_{q/11}$, PLC	ERK, PLA_2, PLD, Src/Akt	Yes (LSD, lisuride; Gonzalez-Maeso et al, 2007)
$5\text{-}HT_{2B}$	$G_{q/11}$, PLC	ERK, PLA_2	Yes (LSD, ergotamine; McCorvy et al, 2018b)
$5\text{-}HT_{2C}$	$G_{q/11}$, PLC	ERK, PLA_2, PLD	Yes (DOI; Berg et al, 1998: Compound (+)-7e; Chen et al, 2016)
$5\text{-}HT_3$	Cation channel		

5-HT_4	G_s, AC, PKA	ERK	Yes (RS 67333, prucalopride; Gaven et al 2013)
5-HT_{5A}	$G_{i/o}$		
5-ht_{5b}	$G_{i/o}$		
5-HT_6	G_s, AC, PKA	ERK, mTOR, Cdk5	
5-HT_7	G_s, AC, PKA	ERK, Small G-proteins	Yes (Compound 1g; Kim et al 2018: Compound 2; McCorvy et al 2018a)

Table 3. Summary of some of the main behavioural and cognitive effects currently associated with pharmacological manipulation of 5-HT receptor subtypes in animal models, in comparison with examples of findings in clinical studies (where data are available). See text for further details and references.

5-HT receptor	Pharmacological manipulation	Effects in rodent models	Effects in clinical trials
5-HT_{1A}	Agonist	Antidepressant	Azapirones (eg. buspirone) - antidepressant
		Anxiolytic	Azapirones (eg. buspirone) - anxiolytic
		Procognitive	Lurasidone, aripiprazole and brexpiprazole - antipsychotic (non-selective agents, possible involvement in 5-HT_{2A} as well as other receptors)
		Sexual enhancement	Flibanserin - sexual dysfunction (non-selective, possible 5-HT_{2A} involvement)
		DOPA dyskinesia	
		Analgesia	
		Hyperphagia	
	Antagonist	Neuroprotection	
		Antidepressant	Pindolol - antidepressant augmentation (but note lack of effect of 5-HT_{1A} antagonist DU-125530; Scorza et al, 2012)
5-HT_{1B}	Agonist	Aggression reducing	
		Impulsivity lowering	
	Antagonist	Antidepressant	
		Anxiolytic	
$5\text{-HT}_{1B/D}$	Agonist	Antimigraine	Triptans - antimigraine

5-HT_{1F}	Agonist	Antimigraine	LY334370 - antimigraine Lasmiditan - antimigraine
5-HT_{2A}	Agonist	Psychotomimetic	LSD, psilocybin etc - hallucinogen
		Antidepressant, plasticity enhancing	Psilocybin - antidepressant
	Antagonist	Antipsychotic	Pimvanserin – antipsychotic (but note lack of effect of 5-HT_{2A} antagonist MDL100907; De Paulis, 2001)
		Impulsivity lowering	Quetiapine - impulsivity lowering
		Antidepressant augmentation	
		Sleep	
5-HT_{2B}	Agonist	Antidepressant augmentation	
	Antagonist	Sleep	
		Sensorimotor gating	
5-HT_{2C}	Agonist	Appetite suppression	Lorcaserin - anti-obesity
		Reduced drug seeking behaviour	
		Impulsivity lowering	
		Anxiogenic	
		Anticonvulsant	
	Antagonist	Antidepressant augmentation	
		Anxiolytic	
5-HT_{3}	Antagonist	Antiemetic	Palonosetron, granisetron etc - antiemetics
			Vortioxetine - antidepressant

		Procognitive	with cognition-enhancing properties (possible involvement of 5-$HT_{1A/7}$ receptors)
		Anxiolytic	Alosetron, ondansetron - reduced emotional component of diarrhoea-predominant irritable bowel syndrome with possible central mechanism (Mayer et al 2002)
		Analgesic	
5-HT_4	Agonist	Procognitive	Prucalopride – cognition-enhancing
		Antidepressant	
	Antagonist	Appetite suppression	
5-HT_{5A}	Agonist	Psychotropic (hallucinogenic)?	
		Antidepressant?	
	Antagonist	Procognitive?	
5-HT_6	Agonist	Procognitive	
	Antagonist	Procognitive	Lack of effect of idalopirdine and other selective 5-HT_6 antagonists in Alzheimer's disease
		Appetite suppression	
5-HT_7	Agonist	Circadian rhythm modulation	
	Antagonist	Antidepressant	Lack of antidepressant effect of selective 5-HT_7 antagonist JNJ-18038683 (Bonaventure et al, 2012)
		Sleep	Increased REM suppression by JNJ-18038683 (Bonaventure et al, 2012)
		Procognitive	
		Anticonvulsant	
		Analgesic	

Figure legends

Figure 1

Summary of history of 5-HT receptor identification and classification using pharmacological and molecular biological (receptor cloning and sequencing) approaches. Dates are approximate. Figure redrawn from Bockaert et al (2010) where further details can be found.

Figure 2

Possible mechanisms of 5-HT-glutamate co-release and signalling. 5-HT and glutamate may be a) co-packaged in the same synaptic vesicles, b) packaged in distinct synaptic vesicles or c) packaged in distinct synaptic vesicles in separate nerve terminals. In the illustration, both 5-HT and glutamate receptors are located on the same neuron, but neurons may preferentially express receptors for only one of the two transmitters and therefore signal to distinct neuronal circuits. Further complexity is likely through signalling via ionotropic and metabotropic 5-HT and glutamate receptors. Figure taken from (Tritsch *et al.*, 2016) and reworked.

Figure 3

Illustration of 5-HT receptor expression in microcircuitry of the amygdala (basolateral nucleus). a) Green fluorescent protein in the mouse amygdaloid complex expressed in anterograde-tracer labelled 5-HT axons arising from the dorsal raphe nucleus (DRN). Key abbreviations: BA, basal amygdala; BLp, basolateral amygdala posterior portion; BLv, basolateral amygdala ventral portion; BM, basomedial amygdala. b) 5-HT input from the (DRN) innervates pyramidal neurons (PN), and GABA interneurons expressing parvalbumin (PV), somatostatin (SOM), vasoactive intestinal peptide (VIP), cholecystokinin (CCK), neuropeptide Y (NPY) or 5-HT_{3A} receptor subunits. Note the combination of excitatory and inhibitory, metabotropic and ionotropic 5-HT receptors. c) A key interaction may be the excitatory 5-HT input (5-HT_{2A} receptor-mediated) onto PV neurons (green)

which generate synchrony in the network of PNs (black). Figure redrawn from Bocchio et al (2016) where further details can be found.