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Abstract:	BACKGROUND The serotonergic system is a key component of physiological brain function and is essential for neurological proper activity. Numerous neuropsychiatric disorders are associated with deregulation of the serotonergic system. Accordingly, many pharmacological treatments are focused on modulation of this system. Whilst providing a promising line of therapeutic moderation, these approaches may be complicated due to the presence of alternative splicing events for key genes in this pathway. Alternative splicing is a co-transcriptional process by which different mRNA transcripts can be produced from the same gene. These different isoforms may have diverse activities and functions and their relative balance is often critical for the maintenance of homeostasis. Alternative splicing greatly increases the production of proteins, augmenting cell plasticity and provides an important control point for regulation of gene expression. AIM The objective of this narrative review is to discuss the potential impact of alternative splicing of different components of the serotonergic system and speculate on their involvement in several neuropsychiatric disorders. CONCLUSIONS The specific role of each isoform in disease and their relative activities in the signalling pathways involved is yet to be determined. We need to gain a better understanding the basis of alternative isoforms of the serotonergic system in order to fully understand their impact and be able to develop new effective pharmacological isoform-specific targets.

Alternative splicing on serotonergic system: implications in neuropsychiatric disorders

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Abstract

BACKGROUND The serotonergic system is a key component of physiological brain function and is essential for neurological proper activity. Numerous neuropsychiatric disorders are associated with deregulation of the serotonergic system. Accordingly, many pharmacological treatments are focused on modulation of this system. Whilst providing a promising line of therapeutic moderation, these approaches may be complicated due to the presence of alternative splicing events for key genes in this pathway. Alternative splicing is a co-transcriptional process by which different mRNA transcripts can be produced from the same gene. These different isoforms may have diverse activities and functions and their relative balance is often critical for the maintenance of homeostasis. Alternative splicing greatly increases the production of proteins, augmenting cell plasticity and provides an important control point for regulation of gene expression. AIM The objective of this narrative review is to discuss the potential impact of alternative splicing of different components of the serotonergic system and speculate on their involvement in several neuropsychiatric disorders. CONCLUSIONS The specific role of each isoform in disease and their relative activities in the signalling pathways involved is yet to be determined. We need to gain a better understanding the basis of alternative isoforms of the serotonergic system in order to fully understand their impact and be able to develop new effective pharmacological isoform-specific targets.

Keywords: Alternative splicing, serotonin, serotonergic system, neuropsychiatric disorders

Serotonin is one of the most important neurotransmitters that influence mental health. The development of selective serotonin reuptake inhibitors (SSRIs) illustrates the importance of the serotonergic system in the treatment of mental disorders. However, treatment with SSRIs for 5 to 8 weeks is required for remission, which only occurs in 30% of patients (Akil et al., 2018). The precise role of the serotonergic system in neuropsychiatric disorders remains elusive, even after decades of intensive research, falling in some cases to yield effective therapeutic management. Part of the explanation for this may be that therapeutic moderation of genes in this system may be complicated by the presence of alternative isoforms of key genes, which may influence treatment response. Differentially-expressed isoforms may be generated by alternative splicing. This process is a key regulator of gene expression, increasing transcriptomic and proteomic diversity and influencing cellular plasticity (Su et al., 2018). The existence of multiple isoforms for many components of the serotoninergic system greatly increases the complexity of the system. Thus, the role of alternative splicing and the impact of multiple isoforms of genes in the serotonergic system on neuropsychiatric disorders remains almost unexplored. The objective of this narrative review is to curate the available literature and produce a definitive assessment of current knowledge and assess the importance that these isoforms may have in the pathogenesis and treatment of numerous neuropsychiatric disorders. In this review, we discuss about the importance of alternative splicing and their impact on serotonergic system and function of their components as tryptophan hydroxylase-2, serotonin transporter, monoamine oxidase A and serotonin receptors.

Alternative Splicing

The correct regulation of gene expression is fundamental for the control of serotonergic function and is achieved through mechanisms such as alternative splicing. This is a co-transcriptional process, which allows the generation of multiple forms of mRNA transcript from a single coding unit and is emerging as an important control point for gene expression. In this process, exons (or even introns) can be either included or excluded from precursor-mRNA resulting in multiple mature mRNA variants (Kelemen et al., 2013) which if translated, result in different isoforms which may have antagonistic functions or differential temporal and/or spatial expression patterns, yielding enormous plasticity and adaptability to the cells (Wang et al., 2015).

This process is a crucial mechanism for gene regulation and for generating transcriptomic diversity. Recent estimates indicate that the expression of over 95% of human multi-exon genes involves alternative splicing (Black, 2003). Splicing is carried out by the spliceosome, a massive structure in which five small nuclear ribonucleoprotein particles and a large number of auxiliary proteins cooperate to accurately recognize the splice sites and catalyse the two steps of the splicing reaction (Wahl et al., 2009) (Figure 1). There are numerous modes of alternative splicing, but the most common is exon skipping. In this mode, a particular exon may be included in mRNA under some conditions or in particular tissues and omitted from the mRNA in others. Changes in exon exclusion, intron retention or the use of alternative splice sites have also been reported which can alter protein structure, localization, regulation or function (Kelemen et al., 2013). The final outcome of alternative splicing is mainly the translation of related but distinct protein variants, encoded by the same gene, but differing in sequence and therefore potentially in their biomolecular and cellular properties (Bindereif, 2015). Alternative splicing of

mRNA can also act as a direct regulator of gene expression, by the inclusion of poison exons which include premature stop codons, which are substrates for degradation by nonsense-mediated decay (McGlincy and Smith, 2008).

DNA methylation was originally thought to only affect transcription; however, emerging evidence shows that it can also regulate alternative splicing (Zhu et al., 2018). Exons, and especially splice sites, have higher levels of DNA methylation, and the splicing of about 22% of alternative exons is regulated by DNA methylation (Gelfman and Ast, 2013). Different mechanisms convey DNA methylation information into the regulation of alternative splicing; the modulation of the elongation rate of RNA polymerase II, and the formation of a protein bridge by heterochromatin protein 1 that recruits splicing factors onto transcribed alternative exons (Lev Maor et al., 2015).

Regulation of alternative splicing is an intricate process whereby multiple *cis*- and trans-acting components work in a co-ordinated fashion, guide the functional coupling between transcription and splicing. Additional molecular features, such as chromatin structure, DNA methylation, RNA structure and alternative transcription initiation and termination, collaborate with these basic components to generate the transcriptomic diversity due to alternative mRNA processing. Tissue-specific RNA binding proteins and microRNAs can also coordinate and regulate alternative splicing patterns (Grabowski, 2011), regulating the balanced production of isoforms according to cell needs.

Alternative splicing is a major mechanism used to generate proteomic diversity in the brain. Proteins affected by alternative splicing may have unaltered function, altered function, or no function at all. Splicing that generates non-functional isoforms have a

significant impact on susceptibility to and development of a range of diseases. Therefore, alternative splicing is known to be involved in the regulation of normal physiological functions as well as in numerous pathologies.

Serotonergic system

The serotonergic system plays an essential role in the physiological functions of the central nervous system and the dysregulation of serotonin homeostasis is implicated in many neuropsychiatric disorders as anxiety, migraine or depression (Gingrich and Hen, 2001). The serotonergic system is the target of numerous pharmacological treatments; triptans, tricyclic antidepressants, agonists and antagonists of serotonin receptors or selective serotonin reuptake inhibitors (SSRIs) are frequently utilised in the treatment of neuropsychiatric disorders. Understanding the regulation of different components of serotonergic system is therefore critical for insight into the diagnosis and treatments of neuropsychiatric disorders.

Serotonin or 5-hydroxytryptamine (5-HT) is a critical monoamine neurotransmitter that plays a crucial role in the control of several brain function as mood, sleep or appetite (Pratelli and Pasqualetti, 2018) and is also related to measures of cognitive function, including memory and learning (Cowen and Sherwood, 2013). Raphe nuclei neurons are the principal source of 5-HT in the brain, where 5-HT is synthetized from the amino acid L-tryptophan, by the concerted action of two enzymes: tryptophan hydroxylase (TPH) and aromatic amino acid decarboxylase (AAAD). Once released, 5-HT triggers its regulatory effects by binding specific 5-HT receptors. The activity of 5-HT also depends on its extracellular availability, which is mainly modulated by the specific serotonin transporter (SERT) which removes

secreted 5-HT from extracellular medium when it is no longer required. Finally, internalized 5-HT can be enzymatically degraded by mono-amino oxidases (MAO) (Charnay and Leger, 2010). All these components together form the serotonergic system which is active throughout the body but has critical functions at the intestinal and central nervous levels. Neuronal serotonergic system is composed by an 'ON' system of production (represented by serotonergic neurons which express TPH2) and an 'OFF' system (represented by the SERT that uptakes 5-HT, expressed in the same neurons). The activity of 'ON' and 'OFF' systems determines the 5-HT levels, and therefore, 'ON' and 'OFF' balance regulates serotonergic effects. Finally, a great diversity of receptors drives the biological activity of the serotonergic signals through seven different receptor classes of serotonin receptors, classified as from 5-HTR1 to 5-HTR7. 5-HTR3 is a ligand gated ion channel, while the rest of the receptors belong to the G-protein coupled receptor family (Nichols and Nichols, 2008). As a result of the above, the effects of 5-HT are wide, and sometimes divergent.

The regulation of serotonergic components expression is fundamental for homeostasis. A better understanding of the expression, activity and regulation of the serotonergic system is critical for the development of new therapies for neuropsychiatric disorders. Alternative splicing has been described for genes responsible for the synthesis, uptake and degradation of serotonin as well as for serotonin receptors; some alternatively expressed isoforms are known to impact neuropsychiatric pathologies or resistance to treatments (Table 1).

In this review we summarize the knowledge about alternative mRNA processing patterns of genes in the serotonergic system (tryptophan hydroxylase-2, serotonin transporter, monoamine oxidase A and serotonin receptors) and discuss their implications for neuropsychiatric disorders. We conclude that deregulated alternative

splicing of the serotonergic system is implicated in the aetiology of neuropsychiatric diseases and in some cases may underpin resistance to treatment.

Tryptophan hydroxylase-2

Tryptophan hydroxylase-2 (TPH2) is the rate-limiting enzyme in brain 5-HT synthesis, and is a candidate gene for disruption of brain serotonergic homeostasis in neuropsychiatric disorders (Chen and Miller, 2013; Waider et al., 2011). Decreased 5-HT level have also been associated with vulnerability to suicidal behaviour; a complex trait influenced by genetic and environmental risk factors (Menon and Kattimani, 2015). The physiological impact of early life events on genes involved in stress response and the serotonergic system is thought to be mediated by epigenetic processes (Booij et al., 2013; Turecki et al., 2012). This may lead to neurobiological changes that contribute to developmental, emotional, cognitive and behavioural phenotypes, and consequently increase the risk for suicidal behaviour. *TPH2* expression is increased in the dorsal raphe nucleus of suicidal individuals with depression (Bach-Mizrachi et al., 2008; Chen et al., 2017) and may act to compensate low 5-HT levels. Increased *TPH2* mRNA expression has also been reported in response to early life stressful events in rodents (Hale et al., 2011), suggesting that adverse environmental stimuli may influence serotonin homeostasis. Although *TPH2* overexpression may represent a compensatory mechanism for low serotonin levels (Bach-Mizrachi et al., 2008), it is known that *TPH2* mRNAs undergo complex post-transcriptional processing (alternative splicing and RNA editing) to increase the variety of functionally different protein isoforms (Abumaria et al., 2008; Grohmann et al., 2010).

Except for a few examples where functional characterization has been undertaken (Abumaria et al., 2008; Grohmann et al., 2010), the majority of human *TPH2* genetic variants remain to be physiologically uncharacterized. *TPH2* encodes two alternatively spliced variants, denoted *TPH2a* and *TPH2b*, where TPH2b has higher activity than TPH2a. Both splice variants undergo dynamic RNA-editing in a mutually exclusive manner, suggesting a complex fine-tuning of central nervous system 5-HT biosynthesis at the level of the RNA transcript (Grohmann et al., 2010). A truncated TPH2 protein (TPH2-TR) generated by alternative splicing and lacking enzyme activity has also been reported (Zhang et al., 2011). *TPH2* intron 7 is highly polymorphic, containing several variants affecting the 3' splice site which may alter splicing efficiency (Kloiber et al., 2010). Most of the identified SNPs in *TPH2* are located in the introns or promoter region and probably act by altering *TPH2* transcription or disruption of splicing by alteration of cis-acting spicing regulatory elements.

Genetic analyses have indicated potential associations between variants in the *TPH2* gene and neuropsychiatric disorders such as depression (Gao et al., 2012), bipolar disorder (Khanzada et al., 2017), suicidal behaviour (Pompili et al., 2017), autism (Egawa et al., 2013) and attention-deficit/hyperactivity disorder (Ottenhof et al., 2018). However, other studies in different cohorts report a lack of association with these disorders (Geissler et al., 2017; Pan et al., 2019). Discrepancies in such studies are difficult to reconcile, but not unexpected given the different cohorts used, the requirement for large sample numbers and limitations in diagnostic criteria. The specific involvement of TPH2a and TPH2b isoforms in these disorders remains unexplored and may be differentially impacted by genetic variation.

Serotonin transporter

Serotonin signalling is also regulated by the activity of the serotonin transporter (SERT, encoded by *SLC6A4* gene) which uptakes extracellular 5-HT into the neurons (Chen et al., 2004). Selective serotonin-reuptake inhibitors (SSRIs) are common therapies for a variety of affective and anxiety disorders and are the most effective and used antidepressant drugs. Unfortunately, a large percentage of patients do not respond to initial therapy and a similarly large fraction experiences side effects (Vaswani et al., 2003). In the presence of SSRIs, 5-HT remains in the extracellular space longer, as SSRIs inhibit SERT activity, allowing prolonged activation of 5-HT receptors. Moreover, as SERT protein is identical in the brain and the gut, systemic SSRIs also affect 5-HT signalling in the gut causing adverse gastrointestinal reactions (Grover and Camilleri, 2013).

The key physiological roles played by serotonin throughout the brain support the hypothesis that variations of SERT activity and/or expression might lead to changes in serotonergic signalling. Common variation in the promoter region of the *SLC6A4* gene is associated with altered functional expression of SERT. A well-defined 44 base pair insertion/deletion polymorphism in this region leads to reduced expression of transporter. Several reports have identified associations between the presence of the variant and psychiatric conditions, including stress-associated depression (Peitl et al., 2017), alcohol dependence (Twitchell et al., 2001) or neuroticism (Greenberg et al., 2000; Twitchell et al., 2001). Altered SERT function could also play a key role in the pathogenesis of many neuropsychiatric disorders such as bipolar disorder (Chou et al., 2016), depression (Lira et al., 2003), autism (Tanaka et al., 2018), eating disorders (Tauscher et al., 2001) or anxiety (Maron et al., 2004), among others. Evidence suggests gene-by-environment interactions could be fundamental

for SERT expression and their consequent implications in diseases (Caspi et al., 2003; Karg et al., 2011). Individuals who have been subject to child abuse demonstrate altered DNA methylation which may influence the expression of SERT spliced variants (Vijayendran et al., 2012). Altered CpG methylation within the promoter of *SLC6A4* has been associated with early or recent exposure to psychosocial stress, and a number of neuropsychiatric disorders demonstrate an imbalance of SERT isoforms (Palma-Gudiel and Fananas, 2017). This indicates that stress-induced DNA methylation changes may impact the alternative splicing patterns of *SLC6A4*.

The *SLC6A4* gene encodes several variants of exon-1 (1A, 1B and 1C). Alternative splicing yields two mRNA species comprising exons 1A+2 (SERT-1A) and 1A+1B+2 (SERT-1AB). The transcription of both mRNAs is controlled by a promoter containing highly polymorphic sequences (Ozsarac et al., 2002) which has the potential to yield multiple SERT isoforms. A study of human intestine has revealed the existence of three SERT mRNA species (SERT-1A, SERT-1AB and SERT-1C) (Gill et al., 2008). The distinct transcriptional start site and alternate promoters suggest that intestinal SERT could potentially be differentially regulated compared with brain SERT. This raises the possibility of site-specific therapeutics for SERT regulation in the treatment of multiple disorders which may have efficacy without the associated side effects. Spliced SERT isoforms may also impact 5-HT availability during SSRIs treatment and alter drug efficacy and risk of adverse reactions.

It is also important to note that SERT may undergo other post-translational changes which could impact their activity. Studies using *in vivo* and *in vitro* model systems have demonstrated that post-translational modifications, including phosphorylation, glycosylation, serotonylation, and disulfide bond formation, all of which favourably

influence SERT conformation and allow the transporter to function most efficiently (Cooper et al., 2019).

Monoamino Oxidase A Enzyme

Once 5-HT is taken up by SERT, 5-HT can be degraded by MAO-A (monoamine oxidase A). This enzyme plays a vital role in the deamination of dietary monoamines and neurotransmitters as 5-HT (Gaweska and Fitzpatrick, 2011). Abnormal MAO-A activity has been reported in several neuropsychiatric disorders, including schizophrenia (Sun et al., 2012), depression (Rivera et al., 2009) or Alzheimer's disease (Takehashi et al., 2002) and MAO inhibitors are used as an effective treatment for depression (Thomas et al., 2015). Moreover, some MAO-A polymorphisms have been related with aggressive behaviour (Frau et al., 2019; Xiang et al., 2019).

MAO-A can be alternative spliced, creating a less efficient isoform (Biondo, 2017). The spliced isoform (MAO-A short) excludes the exon 14 generating a frameshift mutation that results in a premature stop codon in exon 15. This variant encodes for a truncated protein in the transmembrane domain which loses the enzymatic activity.

Although serotonin is metabolized by MAO-A, the predominant enzyme in the dorsal raphe nucleus is MAO-B (Arai et al., 1997). Other functional MAOs exist but may act on other neurotransmitters as phenylethylamine, dopamine or benzylamine (Gaweska and Fitzpatrick, 2011). Increased *MAO-B* mRNA levels have been related to Parkinson disease and dementia (Mallajosyula et al., 2008) and similarly to MAO-A, some spliced isoforms for MAO-B have been also described (Jakubauskiene et al., 2012).

MAOs have been implicated in the pathogenesis of Alzheimer's disease, and may influence the formation of amyloid plaques and neurofibrillary tangles resulting in cognitive impairment. Several studies have indicated that MAO inhibitors might improve cognitive deficits and reverse amyloid Aβ pathology. Thus, MAO inhibitors may have promise as future therapeutic agents for Alzheimer´s disease (Cai, 2014). A better understanding of MAO alterative spliced isoforms would be necessary to realise this aim.

Serotonin Receptors

Eighteen different genes encode serotonin receptor family members (Molderings, 2012). Whilst five genes belonging to the 5-HTR1 class and two from the 5-HTR5 class do not demonstrate alternative splicing, alternative isoforms have been reported for. 5-HTR2 (De Lucchini et al., 2001), 5-HTR3 (Bruss et al., 2000), 5-HTR4 (Bender et al., 2000), 5-HTR6 (Olsen et al., 1999) and 5-HTR7 (Gellynck et al., 2008) in various organisms, including humans.

The 5-HT_{1A} receptor is the most widespread of all 5-HT receptors and its expression is related with anxiety and major depression (Garcia-Garcia et al., 2014). In the central nervous system, the 5-HT_{1A} receptor is expressed in the cerebral cortex, hippocampus, amygdala and raphe nuclei in higher density, while low amounts also are found in the basal ganglia and the thalamus. The 5-HT1A gene, originally thought to be intronless, is now known to undergo alternatively splicing in its 3′-UTR region, yielding two novel splice variants (Le Francois et al., 2018). This results in the removal of a miR135 binding site, which stabilises 5-HT1A RNA and increases 5-HT_{1A} expression. The spliced variants are extremely stable compared with the

unspliced version that is rapidly degraded, consistent with destabilization induced by miR-135 (Issler et al., 2014). The spliced 5-HT_{1A} variants were also seen to be reduced in individuals with major depression in a genotype-dependent manner (Albert et al., 2019).

Serotonin receptor 2 (5-HT_2)

The 5-HT_2 receptor subfamily comprises a group of excitatory, primarily postsynaptic, G-protein-coupled receptors, which bring about their effects via stimulation of phospholipase C. This subfamily contains three receptors (2A, 2B and 2C) which are functionally linked to promote release of intracellular Ca^{2+}. Their pharmacological significance is substantial due to both the clinical importance and complex pharmacological features of these receptors.

Receptor 2A (5-HT_{2A}) is abundantly expressed on pyramidal cells and interneurons in the prefrontal cortex, where it regulates the balance between excitatory and inhibitory responses (Puig and Gulledge, 2011). Many studies have demonstrated that serotonin signalling from dorsal raphe to the prefrontal cortex are involved in cognitive behaviour, with 5-HT_{2A} being crucial for serotonergic signalling (Zhang and Stackman, 2015). In addition, 5-HT_{2A} is also highly expressed in limbic neurocircuitry and has been strongly implicated in the regulation of anxiety-like behaviour (Ghasemi et al., 2018; Weisstaub et al., 2006).

The *HTR2A* gene expresses up to 10 distinct sense-encoded exons generated through alternative splicing, generating at least 8 protein isoforms of *HTR2A* (Ruble et al., 2016). Another *HTR2A* isoform exists, which includes an 118bp insertion that produces a premature stop codon, resulting in a truncated and inactivated protein

(Guest et al., 2000). Although this variant lacks the structural domains involved with ligand and intracellular signalling, it could regulate the function of native serotonin receptors on their synthesis, protein-ligand interactions or intracellular trafficking. Patients with schizophrenia exhibit reduced cortical 5-HT_{2A} activity, but it is as yet unclear whether this reduction might result from medication (Abi-Dargham, 2007). Many schizophrenia patients carry a genetic variant (rs6314) which has potential to alter the alternative splicing pattern of *HTR2A* gene (Blasi et al., 2013). As SERT and TPH2, 5-HT_{2A} can also be regulated by DNA methylation, which has been shown to affect the balance of isoforms in schizophrenia (Cheah et al., 2017). An imbalance of *HTR2A* isoforms has also been suggested to impair working memory and attenuate improvement after olanzapine (5-HT_{2A} antagonist) treatment (Blasi et al., 2013).

The physiological role of the serotonin receptor 2B (5-HT_{2B}) is not yet fully understood. Several animal studies have suggested that 5-HT_{2B} receptor mediates the embryonic morphogenesis (Nebigil et al., 2000) and its activation causes anxiety and reduced grooming in mice (Duxon et al., 1997). It has also been described as a pharmacological candidate gene for early-onset obsessive-compulsive disorder (Kim et al., 2000; de Leeuw and Westenberg, 2008). The *HTR2B* gene has 4 spliced regions extending at least 100 base pairs beyond each exon–intron boundary. The isoforms are currently poorly characterised (Kim et al., 2000), but an alternative isoform has been reported (Bonhaus et al., 1995). Potential associations have been suggested between the 5-HT_{2B} receptor and migraine that may explain the efficacy of methysergide and cyproheptadine (5-HT_{2B} antagonists) for migraine prophylaxis (Segelcke and Messlinger, 2017).

The serotonin receptor 2C (5-HT_{2C}) controls key physiological functions, such as food intake, anxiety, sleep and motor neuron activity (Heisler et al., 2007; Monti, 2011). Deregulation of 5-HT_{2C} receptors activity has been described in depression (Martin et al., 2014), schizophrenia (Castensson et al., 2005), suicidal behaviour (Gurevich et al., 2002), and spinal cord injury (Murray et al., 2010) in humans as well as in mouse models of obesity (Schellekens et al., 2012). Many antipsychotic drugs used to treat depression, anxiety, and schizophrenic disorders are known to interact with the 5-HT_{2C} receptors, which may contributes to the drug efficacy (Chagraoui et al., 2016; Martin et al., 2014). The *HTR2C* gene is mainly regulated by pre-mRNA processing. The *HTR2C* gene generates at least 33 mRNA isoforms encoding 25 proteins through alternative splicing and RNA editing (Stamm et al., 2017), which has effects on constitutive activity as well as alternative splicing. A truncated isoform (5-HT_{2C}_tr) has also been reported with an attenuate activity through heterodimerization (Martin et al., 2013; Zhang et al., 2016). Increased levels of 5-HT_{2C}_tr isoform have also been reported in the hypothalamus of mice with Prader-Willi Syndrome (PWS), an imprinting disorder resulting in altered serotonin and satiety responses (Garfield et al., 2016). Editing of the *HTR2C* gene is highly dynamic and changes under both physiological and pathological challenge, such as water maze learning (Du et al., 2007), obesity (Schellekens et al., 2012) or spinal cord injury (Di Narzo et al., 2014). This has potential to generate 5-HT_{2C} isoforms with different signalling properties. Moreover, it evidence suggests that the balance of 5-HT2c isoforms is critical in neuronal differentiation; changing the predominant isoform upon neuronal commitment, favours production of full-length receptor isoforms with higher activity (Bratkovic et al., 2018).

Serotonin receptor 3 (5-HT$_3$)

5-HT$_3$ is a well-known 5-HT receptor in the gut where is involved in the regulation of intestinal motility (De Ponti and Tonini, 2001), nausea and vomiting (Endo et al., 2000). 5-HT$_3$ is also present in central nervous system, specifically in the hippocampus, amygdala and spinal cord (Chameau and van Hooft, 2006), but its exact role is still not fully understood. The treatment with 5-HT$_3$ antagonists in animal models has shown psychotropic effects and improvement of cognitive function (Costall and Naylor, 2004).

5-HT$_3$ belongs to the Cys-loop superfamily of ligand-gated ion channel, and therefore differs structurally and functionally from other 5-HT receptors which are G protein-coupled receptors. A functional channel may be composed by five identical 5-HT$_{3A}$ subunits (monopentameric) or a mixture of 5-HT$_{3A}$ and one of 5-HT$_{3B}$ (heteropentameric). The existence of two alternative promoters in the *HTR3B* gene that codes for the B-subunit have been reported. The alternative promoters demonstrate tissue specificity, as the canonical transcript could be detected in gut, while the alternative transcript was only detected in brain (Tzvetkov et al., 2007). Similarly, the *HTR3A* gene is expressed as two splice isoforms (short and long variants) which are differentially regulated in vivo. The additional six amino acids in the long form may change the structure in such a way as to prevent or allow access of appropriate enzymes, resulting in differential phosphorylation levels (Hubbard et al., 2000). 5-HT$_3$ antagonists have been extensively used to treat chemotherapy-induced emesis and diarrhoea-predominant irritable bowel syndrome and have a significantly slowing effect on gastrointestinal transit. However, there are few side effects related to the use of 5-HT$_3$ antagonists; the most common are headache and dizziness. Moreover, 5-HT3 antagonists seem to be a feature of new antidepressant

drugs such as vortioxetine. As 5-HT_3 isoforms seem to be tissue-specific, the development of isoform-specific antagonists may help ameliorate side effects.

Serotonin receptor 4 (5-HT_4)

5-HT_4 is involved in cognitive function and memory consolidation. This receptor serves as a potential target for the development of therapeutic agents implicated in neurological disorders including Alzheimer's disease (Maillet et al., 2004), anorexia nervosa (Jean et al., 2007), anxiety (Bockaert et al., 2004) and depression (Lucas et al., 2007). The absence of 5-HT_4 also modulates depression- and anxiety-responses in mice (Amigo et al., 2016) and SSRI treatment under pathological depression appear to be critically dependent on 5-HT_4 (Mendez-David et al., 2014).

At least 11 human 5-HT_4 splice variants have been reported to date (Rebholz et al., 2018). The *HTR4* gene undergoes alternative splicing at its C-terminus to produce 4 variants; A and B are abundantly expressed in brain, whilst C and D are enteric-specific (Liu et al., 2009). Isoforms A and B show differences in ligand binding, signal transduction and pharmacological patterns, and differential response to drugs (Pindon et al., 2002). A study has also identified 4 new variants in the N-terminus of the *HTR4* gene (Azim et al., 2012) in mouse brain designated as T1, T2l, T2s, and T3. All variants differ in their first two exons making a unique N-termini for HTR4 variants, giving them different properties in terms of acetylation, N-glycosylation, phosphorylation and their consequent functionally repercussions. Given the variety of *HTR4* isoforms, the pharmacological characterisation of the spliced variants is necessary to understand the implicated mechanisms and develop adequate effective

treatments (Brattelid et al., 2004). To date, no specific isoform has been linked to neuropsychiatric disorders; however, interactions cannot be discarded.

Serotonin receptor 6 (5-HT$_6$)

5-HT$_6$ is found in the limbic and extrapyramidal areas of the brain, supporting the suggestion that this receptor may be involved in the mechanism of action of antipsychotics (Morozova et al., 2017). Pharmacological studies have demonstrated that atypical antipsychotic drugs have high affinity for this receptor and its mRNA expression is altered in schizophrenia patients, suggesting it may hold promise as a potential therapeutic target for schizophrenia (East et al., 2002). Some 5-HT$_6$ antagonists have shown efficacy in animal models for cognitive impairment in multiple cognitive domains relevant for schizophrenia (de Bruin and Kruse, 2015). Moreover, during the last decade, 5-HT$_6$ receptor has received increasing attention and become a promising target for improving cognition. Some 5-HT$_6$-targeted compounds have been suggested as powerful drug candidates for the treatment of Alzheimer's disease (Ramirez, 2013).

To date, only one spliced isoform has been described for *HTR6*, generated by a 289 bp deletion. This isoform encodes a receptor which possesses only the first three transmembrane domains and exhibits a different expression pattern, being detected only in caudate and substantia nigra, while the canonical transcript was located in cortex, hippocampus, cerebellum, thalamus, caudate and substantia nigra (Olsen et al., 1999). The spliced isoform is expressed in the cell membrane; however its ability to fold properly and form the correct ligand binding site seems unlikely. Tissue specific regulation of alternatively spliced transcripts may provide a by which

specialized cells can generate different proteins in response to environmental challenges (Porazinski and Ladomery, 2018). More studies are needed to develop a therapeutic therapy based in 5-HT_6 isoforms.

Apart from 5-HT_6 spliced isoform, this receptor could play a role in regulating alternative splicing of other important genes. In fact, a recent study has described the interaction between 5-HT_6 and Nova-1, a brain-enriched splicing regulator of proteins involved in synapse formation or synaptic transmission, including inhibitory GABA receptors. In particular, the overexpression of 5-HT_6 reduces the splicing activity of Nova-1, and contrast, overexpression of Nova-1 weakens the activity and stability of 5-HT_6 via promoting proteasomal degradation (Kim et al., 2019).

Serotonin receptor 7 (5-HT_7)

The 5-HT_7 receptor is one of the most recently identified members of the serotonin receptor family. The physiological role for the 5-HT_7 receptor within the central nervous has been clearly established in regulation of circadian rhythm (Glass et al., 2003) and in thermoregulation (Hedlund et al., 2004) as well as in learning and memory (Meneses, 2014). Other biological functions including moderation of the effects of atypical neuroleptics (Manfra et al., 2015) and antidepressants (Sarkisyan et al., 2010) or participation in pain and inflammatory pathways (Rocha-Gonzalez et al., 2005) have also been related to 5-HT_7 receptor activity. Selective 5-HT_7 receptor ligands may therefore have potential therapeutic applications for the treatment of pain and migraine, schizophrenia, anxiety, cognitive disturbances and inflammation. However, it is critical to know in depth the differences of 5-HT_7 isoforms and develop isoforms-specific ligands.

Four *HTR7* splice variants, (a, b, c, and d) have been described in human and rat tissues. These variants differ in their carboxyl terminal as a consequence of alternative splicing (Guthrie et al., 2005). Two of these, (a and b) are conserved in rat and human. An additional form, 5-HT_{7c} is expressed only in rat tissues, whereas *HTR7d* is expressed only in humans. All of the isoforms appear to be functionally active and have similar agonist binding characteristics (Krobert and Levy, 2002), but the distribution of expression of the receptor isoforms is different in several brain regions and peripheral tissues (Krobert et al., 2001). Differences in pharmacology have also been described; The HTR7d isoform exhibits receptor trafficking that is distinct from HTR7a or HTR7b, whereas human HTR7d receptors display agonist-independent internalization with internalization noted even in the presence of antagonist (Guthrie et al., 2005). Improved characterisation of HTR7 isoforms is required to fully explore their implications in neuropsychiatric diseases.

Conclusions

Transcriptome profiling in human tissues has greatly increased our appreciation of the diversity of RNA isoforms, revealing that alternative splicing is a key mechanism for gene regulation. Alternative splicing creates transcriptomic and proteomic diversity and cellular plasticity and plays a critical role in the development of many diseases. Identification of specific isoforms that are dysregulated in diseases raise the possibility developing tailored therapeutics which could be successfully harnessed in the clinic (Havens et al., 2013).

Alternative splicing plays a key role in regulating the activity of the serotonergic system, increasing the complexity of the system, with the presence of tissue specific

isoforms and diversity in phosphorylation levels and intracellular trafficking. As the Figure 2 shows, the 5-HT levels are controlled by the activity of TPH2, SERT and MAO-A with at least, 7 different isoforms from the three genes. The combination of those spliced isoforms would generate numerous scenarios where 5-HT levels could be differently regulated. In addition to those 5-HT controlling-isoforms, alternative splicing increases hugely the variety of 5-HT receptors, being a clear example the 33 spliced isoforms for 5-HT_{2C}. The numerous spliced variant receptors present differences in their activity, expression, regulation, structure and sensitivities to ligands. Hence the need of in-depth characterization of spliced isoforms and development of isoform-specific targets are required for a better treatment of neuropsychiatric disorders.

The serotonergic system has been implicated in numerous neuropsychiatric disorders and the existence of different serotonergic isoforms could therefore play a critical role in susceptibility, disease development or the incidence of side effects, as well as representing future potential therapeutic targets. Recent studies plead for therapeutic approaches on alternative splicing (Harries, 2019; Lipscombe and Lopez Soto, 2019).

This is exemplified by the *HTR2C* gene, comprises a good candidate for RNA therapy for multiple neuropsychiatric disorders. The ratio between truncated and canonical receptors could be manipulated through antisense oligonucleotides (AONs), allowing selective modulation of 5-HT_{2C} receptor activity. The production of an attenuated splice variant may allow regulation the activity of the 5-HT_{2C} receptor. This could prove useful for future treatment of disorders such as hyperphagia, as demonstrated by the promising results reported for Prader–Willi syndrome (Zhang et al., 2016). A more in-depth characterization of spliced isoforms from genes

underpinning the serotonergic system and assessment of their involvement in human pathology is merited. It is also important to note, that much of our knowledge about the function of splice isoforms comes from cell culture techniques; validation *in vivo* is therefore an important pre-requisite for clinical targeting important candidate genes. Serotonergic components are, in many cases, coding by large genes multiple introns and exons. The limited exploration of the splicing events in the serotonergic system genes probably does not capture the extent of spliced isoforms and a fine characterization of serotonergic isoforms is critically needed. The exact role of each isoform in disease and their relative activities in the signalling pathways involved is also yet to be determined in order to fully understand their impact. Although it has been shown that some splice variants have different sensitivities to ligands, it has only been demonstrated with a small number of agonists. It remains a considerable challenge to identify and develop splice variant-selective drugs.

Therefore, we need to gain a better understanding the basis of alternative isoforms of the serotonergic system to develop new effective pharmacological isoform-specific targets. The advanced knowledge of spliced isoforms will also enable us to adapt the best treatment for each patient to the different pathologies related to neuropsychiatric disorders linked to the serotonergic system.

Declaration of interest

The authors report no competing interests.

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Figure 1: Schematic representation of alternative splicing process. The diagram illustrates how alternative splicing allows the production of different variants of the same protein from the same gene. Starting from a single gene (DNA coding sequence), the spliceosome (represented by 4 green units) can include or excluded the intron1 in a co-transcriptional process, creating two different mRNAs that will be traduced in two similar proteins (isoforms) but with different function properties.

Figure 2: Alternative splicing of serotonergic system. 5-HT levels are controlled by the activity of TPH2, SERT and MAO-A. TPH2 has three spiced variants: TPH2a, TPH2b and TPH2-TR. The TPH2a variant presents a reduced activity compared with TPH2b. On the contrary, TPH2-TR is a truncated isoform with a lack of activity. SERT has three spliced variants (SERT-1A, SERT-1AB, SERT-1C) which are differentially polyadenylated and differ in their translational regulations. MAO-A is alternative spliced, creating a less efficient isoform (MAO-A short). Alternative splicing of these three genes could seriously impact on 5-HT levels playing a critical role in neuropsychiatric disorders. Regarding 5-HT receptors, there are numerous spliced variants presenting differences in their activity, expression, regulation, structure and sensitivities to ligands.

Table1: Serotonergic system components, alternative spliced isoforms and their implication in neuropsychiatric disorders. Genetic information obtained by NCBI and Genetics Home Reference (NIH).

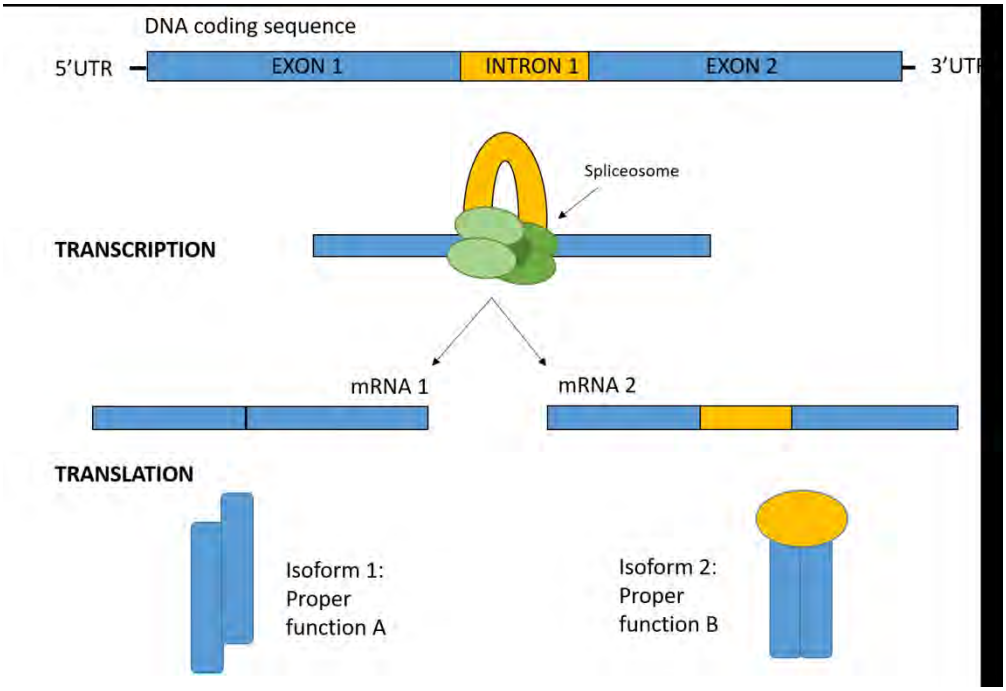
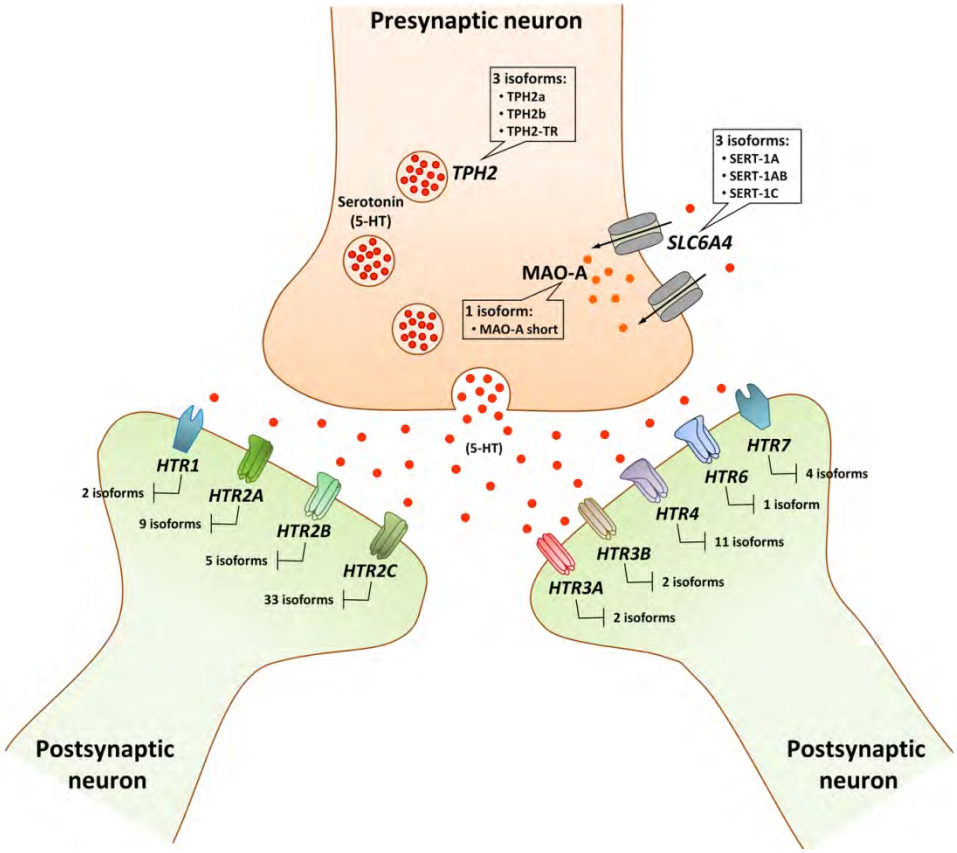


Figure 1

266x181mm (150 x 150 DPI)



Figue 2

119x104mm (300 x 300 DPI)

Table 1: Serotonergic components and their implication on neuropsychiatric pathologies

Name	Gene	Location	Intron/exon	Spliced Isoforms	References	Related Neuropsychiatric Pathologies
Tryptophan hydroxylase-2	TPH2	12q21.1	10 introns 11 exons	3	Grohmann et al., 2010 Zhang et al., 2011	Depression (Gao et al., 2012) , Bipolar Disorder (Khanzada et al., 2017), ADHD (Ottenhof et al., 2018), Suicidal behaviour (Pompili et al., 2017), Autism (Egawa et al., 2013)
Serotonin transporter	SLC6A4	17q11.2	13 introns 14 exons	3	Ozsarac et al., 2002 (Gill et al., 2008)	Bipolar Disorder (Chou et al., 2016), Depression (Peitl et al., 2017), Autism (Tanaka et al., 2018), Neuroticism, Alcohol dependence (Twitchell et al., 2001), Anxiety (Maron et al., 2004), Eating disorders (Tauscher et al., 2001)
Monoamine oxidase A	MAOA	Xp11.3	14 introns 15 exons	1	Biondo, 2017	Schizophrenia (Sun et al., 2012), Depression (Thomas et al., 2015), Alzheimer´s disease (Cai, 2014), Aggressive behaviour (Frau et al., 2019)
Serotonin receptor 1A	HTR1A	5q63.26	1 exon	2	Le Francois et al., 2018	Anxiety, Major Depression (Garcia-Garcia et al., 2014)
Serotonin receptor 2	HTR2A	13q14.2	3 introns 4 exons	9	Ruble et al., 2016 Guest et al., 2000	Schizophrenia (Abi-Dargham, 2007), Cognition (Blasi et al., 2013), Depression, Alcohol dependence, Anxiety (Ghasemi et al., 2018)
	HTR2B	2q37.1	3 introns 4 exons	5	Kim et al., 2000	Obsessive-compulsive disorder, Anxiety (de Leeuw and Westenberg, 2008), Migraine (Segelcke and Messlinger, 2017)
	HTR2C	Xq23	5 introns 6 exons	33	Stamm et al., 2017 Zhang et al., 2016	Depression (Martin et al., 2014), Anxiety (Monti, 2011),, Obesity (Garfield et al., 2016), Schizophrenia (Castensson et al., 2005),Suicidal behaviour (Chagraoui et al., 2016)
Serotonin receptor 3	HTR3A	11q23.	7 introns 8 exons	2	Hubbard et al., 2000	Cognition Disturbances (Costall and Naylor, 2004), Analgesia, Schizophrenia, Bipolar disorder (Chameau and van Hooft, 2006)
	HTR3B	11q23.2	8 introns 9 exons	2	Tzvetkov et al., 2007	Schizophrenia, Depression, Addiction, Obsessive-compulsive disorder(Chameau and van Hooft, 2006)
Serotonin receptor 4	HTR4	5q32	7 introns 8 exons	11	Rebholz et al., 2018	Alzheimer's disease (Maillet et al., 2004), Anxiety (Bockaert et al., 2004), Anorexia nervosa (Jean et al., 2007), Depression

						(Amigo et al., 2016)
Serotonin receptor 6	HTR6	1p36.13	2 introns 3 exons	1	Olsen et al., 1999	Schizophrenia (Morozova et al., 2017), Depression, Anxiety, Cognitive disturbances (de Bruin and Kruse, 2015), Alzheimer´s disease (Ramirez, 2013)
Serotonin receptor 7	HTR7	10q21.31	3 introns 4 exons	4	Guthrie et al., 2005 Krobert and Levy, 2002	Analgesia, Migraine, Schizophrenia (Manfra et al., 2015) , Anxiety, Inflammation (Rocha-Gonzalez et al., 2005), Cognitive disturbances (Meneses, 2014)