Curr Pharm Des. Author manuscript; available in PMC 2015 July 24.

Published in final edited form as:
Curr Pharm Des. 2014 ; 20(23): 3738–3750.

Transcriptional Dys-regulation in Anxiety and Major Depression: 5-HT1A Gene Promoter Architecture as a Therapeutic Opportunity

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Abstract

The etiology of major depression remains unclear, but reduced activity of the serotonin (5-HT) system remains implicated and treatments that increase 5-HT neurotransmission can ameliorate depressive symptoms. 5-HT1A receptors are critical regulators of the 5-HT system. They are expressed as both presynaptic autoreceptors that negatively regulate 5-HT neurons, and as post-synaptic heteroreceptors on non-serotonergic neurons in the hippocampus, cortex, and limbic system that are critical to mediate the antidepressant actions of 5-HT. Thus, 5-HT1A auto- and heteroreceptors have opposite actions on serotonergic neurotransmission. Because most 5-HT1A ligands target both auto- and heteroreceptors their efficacy has been limited, resulting in weak or unclear responses. We propose that by understanding the transcriptional regulation of the 5-HT1A receptor it may be possible to regulate its expression differentially in raphe and projection regions. Here we review the transcriptional architecture of the 5-HT1A gene (HTR1A) with a focus on specific DNA elements and transcription factors that have been shown to regulate 5-HT1A receptor expression in the brain. Association studies with the functional HTR1A promoter polymorphism rs6295 suggest a new model for the role of the 5-HT1A receptor in susceptibility to depression involving early deficits in cognitive, fear and stress reactivity as stressors that may ultimately lead to depression. We present evidence that by targeting specific transcription factors it may be possible to oppositely regulate 5-HT1A auto- and heteroreceptor expression, synergistically increasing serotonergic neurotransmission for the treatment of depression.

Keywords

Serotonin; repressor; enhancer; antidepressant; raphe; autoreceptor

SEROTONIN AND MAJOR DEPRESSION

Depression continues to grow as a major health challenge with a lifetime prevalence for major depressive disorder (MDD) estimated at 16 % [1, 2] (Box 1). In developed countries, MDD currently accounts for the second highest lifetime burden of disease, and is predicted

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CONFLICT OF INTEREST
The authors confirm that this article content has no conflicts of interest.

to be highest by 2030 [3–7]. Reduced serotonin (5-HT) neurotransmission has been implicated in MDD and related disorders such as anxiety, obsessive-compulsive disorders, and bulimia, which show improvement when treated with 5-HT-specific reuptake inhibitors (SSRIs) such as fluoxetine (Prozac) [8–19] (Box 2). Most antidepressants, including SSRIs, monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), are thought to act in part by enhancing 5-HT neurotransmission. Oppositely, acute tryptophan depletion rapidly reduces 5-HT synthesis and can induce a relapse in 50–80% of depressed patients [20–22]. Hence the 5-HT system is implicated in susceptibility and treatment of MDD. However, current treatment strategies require several weeks for clinical improvement, and are not always effective. For example, in the STAR*D studies, only about 50% of patients responded to the initial SSRI treatment, which achieved a remission rate of only 30% [23–28]. Even with adjunctive or alternate therapy only a remission rate of 40% could be achieved. In addition, due to the long 3–6 week latency required to assess responsiveness to therapy, many patients remain ineffectively treated, with a concomitant risk of suicide that is 15% in depressed subjects [29, 30].

Box 1

Depression, a major health challenge

- Depression is a chronic condition of high incidence (15% lifetime prevalence); by comparison lifetime prevalence for schizophrenia is 1%.
- Depression is associated with a high risk of suicide and decreased quality of life and direct health-care costs at Cdn $4.7 billion.
- Depression is ranked 2nd among medical conditions with the greatest worldwide disease burden and 1st by 2030 (World Health Organization).
- Depression is diagnosed by psychiatric tests; no genetic markers are currently available. This leads to under-diagnosis and under-medication, and increased likelihood of suicide completion.
- Gene-environment interactions are thought to predispose to Depression.

Box 2

Anxiety and MDD are associated with decreased 5-HT

- Acute tryptophan depletion triggers relapses in recovered depressed subjects; triggers depressed phenotype in normals
- Antidepressants target increase in 5-HT (and NE) systems, esp. SSRI
- PET studies of depressed, postmortem studies of depressed suicide victims show decreases in 5-HT synthesis and in 5-HT heteroreceptors in cortex and hippocampus of depressed patients.
- SSRIs are effective for many mood disorders: bipolar, OCD, anxiety; bulimia, PMDD, PPD, etc., suggesting a role for 5-HT in many mood disorders.

- Animal loss-of-function models (5-HT1A−/−, 5-HT (Pet-1, TPH2), 5-HTT-knockout) indicate roles for 5-HT in anxiety models.
- Case-control association studies with functional 5-HTT/5-HT1A gene polymorphisms associate hypofunction of 5-HT with depression

However, SSRI require 2–3 weeks to demonstrate effect; why? Adaptive changes!

Understanding of the etiology of MDD remains incomplete, and both genetic and environmental factors are believed to contribute to an increased predisposition to depression [19, 31–33]. Studies of hereditary transmission suggest a genetic component of up to 50% [34], however linkage analyses have been difficult as depression is likely to result from a combination of many genetic factors of small effect sizes [35, 36]. Adding to the problem of identifying genetic risk factors for depression is the heterogeneity of categories of depression that make it difficult to assess the role of specific factors or genes in this illness. Major depression is diagnosed by psychiatric tests that pool very different behavioral phenotypes [37]. For example, one can be diagnosed as having MDD based on criteria that are often opposite such as too much or too little sleep, hyper- or hypophagia, suicidal thoughts or anhedonia, anxiety or depressed thoughts, etc. Given that at least any 5 of 9 criteria need to be met, two patients diagnosed with MDD may not have a single symptom in common apart from sad mood. As a result, association studies in major depression have produced a great deal of variability and there is a lack of effective genetic markers for depression, or indeed any other mental illness. Genome-wide association studies have uncovered a number of intriguing susceptibility genes such as ion channels (CACNA1C) [38] [39], synaptic proteins [40], and transcription factors (e.g., Sp4 [41, 42]) that associate with major depression. However, these polymorphisms have very small effect sizes, limiting their usefulness as genetic markers [43]. Similarly, genome-wide associations for SSRI response have yielded several candidate polymorphisms, but replicating these results has been challenging [44]. Better phenotypic analysis of depression subtypes could greatly enhance the likelihood of identifying useful genetic markers. For example, a recent meta-analysis indicates an association of the functional 5-HT1A gene promoter polymorphism (rs6295) with major depression [45], but this association appears to be stronger in depressed patients with a comorbid anxiety disorder [46]. Unexpectedly, the only genome-wide linkage of the 5-HT1A gene is to type 1 diabetes [47], perhaps reflecting better classification criteria compared to mental illness. Further analysis of large cohorts in terms of subgroups of depression phenotypes may lead to stronger genetic associations than seen with major depression as a whole [48]. Given the above limitations in classifying mental disorders and validating risk alleles, we have focused on the roles of transcription factors in regulating the tone of the 5-HT system, with a particular focus on regulators of the HTR1A promoter (Box 3).

Box 3

Strategy for Identification of Novel Transcriptional Targets for Anxiety and Depression

- Identify gene regulatory elements of key targets for serotonin system: e.g., autoreceptor genes (eg. 5-HT1A; DRD2 genes)
- Clone critical regulators (Freud 1/2-CC2D1A/B family) and identify new gene targets, mutations, and regulation for these “master switches”
- Identify novel functional promoter polymorphisms that affect the expression of depression genes: HTR1A C(-1019)G (rs6295)
- Identify the DNA binding proteins affected by these polymorphisms (Deaf1, HES)
- Identify in vivo functions of HTR1A DNA regulators (Deaf1/HES/PET1) using animal models (eg. Deaf1 −/−; Hes1 −/−; Pet-1 −/− mice)
- Target therapeutics to activate/induce or inhibit/repress specific transcription factors

THE SEROTONIN SYSTEM AND 5-HT1A RECEPTORS

The serotonin system originates from a small set of 2×10^4 neurons (0.2 billionth of the 10^{11} neurons in the human central nervous system) [49, 50] that are located in the raphe nuclei of the midbrain. These neurons express the neuronal form of tryptophan hydroxylase (TPH2) for the synthesis of 5-HT [51, 52]. Although small in number, 5-HT neurons send axons that project throughout the central nervous system, including to the cortex, hippocampus, septum, amygdala, hypothalamus and spinal cord, to regulate mood, stress responses, autonomic and hormonal functions [49, 50, 53–56]. Serotonin action is terminated by reuptake via the 5-HT transporter (5-HTT), and subsequent degradation by monoamine oxidase. Among the 14 different mammalian 5-HT receptors, the 5-HT1A receptor is one of the most abundant, being expressed as a postsynaptic heteroreceptor in cortex, limbic areas (septum, hippocampus, amygdala), hypothalamus and other areas, where it is implicated in a diversity of physiological, cognitive and affective functions [57–62]. In addition, the 5-HT1A receptor is expressed presynaptically on the cell body and dendrites of 5-HT neurons in the raphe nuclei, where it functions as an inhibitory autoreceptor [63, 64], acting as a key negative regulator of the activity of serotonergic neurons by mediating inhibitory feedback of 5-HT release [65–70]. The inhibitory effects of both the 5-HT1A auto- and heteroreceptors occur through activation of G-protein inward rectifying potassium channels [53] to reduce neuronal firing rate, inhibition of voltage-gated calcium channels to reduce calcium entry, and inhibition of adenylyl cyclase. In raphe cells, they also inhibit extracellular regulated kinase activation [71].

5-HT1A auto- and heteroreceptors

Since the 5-HT1A autoreceptor inhibits serotonergic activity, while the heteroreceptors mediate 5-HT action, global inhibition of the 5-HT1A receptor has opposing effects on the serotonin system. While knockout of the 5-HT1A heteroreceptor results in a depressed phenotype, specific repression of the 5-HT1A autoreceptor leads to increased serotonergic activity, a depression-resistant phenotype, and enhanced response to antidepressants [72, 73]. Because most 5-HT1A ligands target both auto- and heteroreceptors their efficacy is limited. However, some 5-HT1A partial agonists, such as pindolol, induce selective desensitization of 5-HT1A autoreceptors which may accelerate antidepressant action [74] (Fig. 1). Antidepressants such as SSRIs rapidly enter the brain yet, as mentioned above, they require at least 2–3 weeks of treatment for clinical efficacy. Acute treatment with antidepressants results in a recurrent activation of 5-HT1A autoreceptors, reducing 5-HT neuron firing and compensating for antidepressant-induced increases in 5-HT [75–77]. During chronic treatment with SSRIs, the 5-HT1A autoreceptor becomes desensitized whereas post-synaptic 5-HT receptors remain sensitive, resulting in enhanced 5-HT neurotransmission. The >2-week time period required for 5-HT1A autoreceptor desensitization is more prolonged than agonist-induced receptor phosphorylation (sec), internalization (sec-min) and even down-regulation (estimated at 2–3 days *in vivo* [73, 78]) and may involve decreased receptor synthesis. Recent Positron emission tomography (PET) imaging studies indicate that 5-HT1A autoreceptor density is reduced following chronic SSRI treatment [79], suggesting that long-term adaptive mechanisms such as transcriptional regulation could be involved [80] (BOX 2). Based on these findings and data from post-mortem studies showing a specific increase in 5-HT1A autoreceptor binding in depressed suicides [81], we hypothesized that transcriptional dys-regulation driven by genetic, epigenetic and environmental alterations combine to reduce serotonergic activity, predisposing individuals to depression and suicide. Thus we have developed a strategy for identification of key regulators in the serotonin system focusing on the HTR1A promoter (Box 3).

Animal models of 5-HT1A receptor function

Mouse genetic and behavioural models have provided valuable insights into the role of 5-HT1A receptors in depression and anxiety BOX 2. Several groups have shown that knockout of the 5-HT1A receptor gene in mice results in increased anxiety-like behaviours [82–84]. In addition, 5-HT1A-null mice fail to respond to chronic SSRI treatment, implicating a key role for 5-HT1A receptors in mediating antidepressant action [85]. Conversely, mice that over-express the 5-HT1A receptor display reduced anxiety behaviours [86]. Similarly, mice with enhanced hippocampal 5-HT1A-G i2 signaling also display reduced anxiety [87]. In 5-HT1A knockout mice, rescue of 5-HT1A receptor expression in early postnatal forebrain restores a normal anxiety phenotype, whereas pharmacological blockade of the 5-HT1A receptor during postnatal days 13–34 induces an anxiety phenotype that emerges in the adult. These findings indicate that post-synaptic 5-HT1A receptors play a critical role during early postnatal development in the establishment of anxiety behaviour [78, 88]. However, hyperactivity of the 5-HT system induced by specific knockout of 5-HT1A autoreceptors increases anxiety, suggesting that additional 5-HT receptors, such as the 5-HT2A receptor [89], may also regulate anxiety. On the other hand, mice with a 30% increase in 5-HT1A

autoreceptors in adulthood display reduced 5-HT neuron firing, reduced 5-HT release, increased depressive behaviours, and resistance to SSRI treatment, but no change in anxiety [73]. Thus, an increase in post-synaptic forebrain 5-HT1A receptors during postnatal development reduced anxiety, while an increase in presynaptic 5-HT1A autoreceptors in the adult mouse induces a depression-like phenotype and resistance to antidepressants. Similar changes in 5-HT1A auto- and heteroreceptors during human development may be induced by the C(-1019)G polymorphism and could lead to anxiety or depression phenotypes, but this remains to be assessed.

5-HT1A receptor dys-regulation in anxiety and depression

In humans, altered levels of 5-HT1A receptors have been reported in postmortem and PET imaging studies of 5-HT1A receptor levels in depression (Fig. 1). In depression, increased levels of 5-HT1A autoreceptors have been reported [81], while in some studies, a decrease in the number of 5-HT neurons and projections, as well as 5-HT1A autoreceptors, has been observed in post-mortem tissues from depressed suicides [90, 91]. Fewer 5-HT neurons may reflect a developmental dys-regulation that would reduce 5-HT1A autoreceptor levels but also decrease 5-HT neurotransmission, which has been associated with depression. In PET imaging studies of MDD [92], bipolar disorder [93], and temporal lobe epilepsy patients with depression [94], 5-HT1A binding potential was increased mainly in the raphe nuclei. In MDD patients, this increase in 5-HT1A autoreceptor levels persists following remission [95], suggesting that it represents a trait of MDD. In contrast, early PET imaging studies suggested a reduction in 5-HT1A autoreceptors in depression [96]; however, technical considerations argue for the opposite change in these studies [92, 97]. Post-synaptically, in the hippocampus and dorsolateral prefrontal cortex (DLPFC), decreases in 5-HT1A RNA and 5-HT1A signaling have been observed in depressed suicides [98, 99]. PET imaging studies of human subjects with bipolar disorder, MDD or panic disorder have shown a decrease in 5-HT1A heteroreceptor levels, particularly in DLPFC [100–102]. Reduced expression of cortical 5-HT1A heteroreceptors and increased 5-HT1A autoreceptor levels would combine to reduce 5-HT neurotransmission in these disorders.

The alterations in 5-HT1A receptor levels observed in depressed patients may have significant functional consequences. The 50% increase in 5-HT1A autoreceptor binding potential in depressed subjects [92] is similar to the 30% increase in 5-HT1A autoreceptor levels in mice that resulted in an increased susceptibility to stress-induced depression and resistance to SSRI treatment [73]. Mice with increased 5-HT1A autoreceptor levels displayed reduced basal firing of the 5-HT neurons and robust electrophysiological responses to a 5-HT1A agonist, indicating enhanced inhibition of serotonergic tone (Fig. 1). Thus, the long-term transcriptional regulation of the 5-HT1A receptor appears critical to set the tone of serotonergic activity and responsiveness to SSRI treatment [103].

5-HT1A promoter polymorphisms and mental illness

We have previously hypothesized that alterations in transcriptional activity of the 5-HT1A promoter conferred by sequence variations could predispose to MDD, and may also affect the clinical response to anti-depressants that target the serotonin system [104]. Several HTR1A polymorphisms have been identified, but many are too rare to assess their

association with mental illness [105–109]. In a preliminary report we identified a common C(-1019)G 5-HT1A polymorphism [110] that is identical to the site reported as C(-1018)G [108]. Based on the consensus 5- HT1A promoter sequence, the designation C(-1019)G has been retained, and the SNP database designation is rs6295. We showed that this change was associated with MDD, and subsequently found that the G-allele and GG-genotype is also associated with completed suicide [111]. We also demonstrated that the polymorphism is functional and leads to up-regulation of 5-HT1A promoter activity, particularly in RN46A cells: 5-HT neurons that express 5-HT1A autoreceptors (Fig. 2). An increase in 5-HT1A autoreceptor binding potential is associated with the rs6295 5-HT1A promoter polymorphism in human MDD subjects [112], consistent with a genetic basis for increased 5-HT1A autoreceptors in depression. These studies provided the first evidence that a functional promoter polymorphism that alters transcription in neuronal cells could be associated with MDD.

Since our initial study, several independent studies have shown that the G(-1019) and GG(-1019) genotype of the rs6295 HTR1A polymorphism are associated with MDD [113, 114]. A recent meta-analysis supports the association of this polymorphism (rs6295), as well as a more upstream HTR1A promoter polymorphism (rs878567), with MDD [45, 115]. Another meta-analysis indicates schizophrenia [116]. Recent studies indicate that the GG genotype is associated with increased amygdala reactivity to fearful faces [117, 118], which correlates with increased presynaptic 5-HT1A autoreceptor expression [119]. Interestingly, in bipolar depression the GG genotype was associated with smaller amygdala volume, suggesting a developmental effect [120]. The 5-HT1A receptor gene has been associated with panic disorder [121], in which the G-allele was associated with increased panic symptom severity [122], again suggesting increased fear or stress responsiveness. In normal subjects the GG genotype was associated with an impaired glucocorticoid response to social stress, similar to that associated with early life stress [123]. Together, these results suggest that the G-allele confers increased stress reactivity and reduced stress coping that may predispose individuals to depression (Fig. 3). Consistent with a genotype-stress relationship, bipolar disorder patients with the GG genotype report fewer stressful events prior to hospitalization, indicating an increased susceptibility to stress for triggering depressive episodes [124]. Similarly, a study in a Chinese population showed that the rs6295 polymorphism was associated with negative life events in 20–29 year-olds with MDD [125]. Consistent with a role for stress interacting with the HTR1A rs6295 genotype, in elderly patients who suffered a fall, the G-allele is associated with increased depressive symptoms [126]. These studies suggest that increased stress reactivity in later life may be conferred by the rs6295 polymorphism that could precipitate depression symptoms. Similarly, while the G-allele was not associated with suicide attempts, there was a trend for its association with stressful life events in suicide attempters [127]. In subjects with personality disorders, the G-allele was associated with emotional-dramatic behavior [128]. In normal subjects the GG-genotype has been found to confer an increase in impulsivity [129], as well as with neuroticism [130], although the latter has not been replicated [131]. However, no association with anxiety or depression personality traits was detected in a large study of healthy subjects [132], suggesting that these associations may be most robust in patients compared to normal subjects. Alternately, more sensitive measures of depressive or anxious behaviour may show

association in normal subjects. For example, normal subjects with the GG-genotype showed reactions associated with increased negative emotionality in a reward-punishment paradigm [133]. Recent studies indicate that the presence of the G-allele in normal subjects associated with specific impairments in cognitive ability, including error and attentional processing [134, 135]. These findings in normal subjects suggest that the rs6295 polymorphism generates impairments in emotional and cognitive processing that lead to an inability to handle stressful situations, leading to an increased susceptibility to depression and anxiety (Fig. 3). Consistent with this model, recent multivariate Bayesian modeling of rs6295 association studies have suggested that an association with impulsivity may be intermediary to its association with MDD [136].

We also found that the HTR1A GG-genotype was associated with a reduced response to antidepressant treatment [137, 138], which has been confirmed in several studies [113, 114]. However, this remains controversial, as one study in Japanese subjects identified a greater response to SSRIs in carriers of the G-allele [139]. This may reflect a difference in ethnicity in which the G-allele is infrequent in oriental populations, or may suggest the presence of additional polymorphisms, which could potentiate the effects of rs6295 [140]. Recently, the presence of the GG(-1019) genotype was found to associate with a lack of rapid response to SSRI treatment in panic disorder patients compared to patients with the C(-1019) allele [141]. Interestingly, a report shows that the CC-genotype also confers a better response to transcranial magnetic stimulation [142], suggesting that modulation of the 5-HT1A receptor may also participate in treatments that target cortical processes, perhaps via induced expression of the 5-HT1A receptor in cortex and amygdala [143]. The GG-genotype has also been associated with weaker negative symptom improvement in schizophrenia patients treated with atypical antipsychotics [144, 145]. Taken together these studies indicate that the G-allele, which is associated with increased 5-HT1A autoreceptor levels, also associates with reduced responses to therapies that modify the 5-HT system.

5-HT1A RECEPTOR PROMOTER ARCHITECTURE

Basal promoter structure

As part of a strategy to normalize transcription of the HTR1A gene, it is important to identify discrete regulators of its transcription as potential therapeutic targets (Box 3). The 5-HT1A receptor gene (HTR1A) upstream promoter-enhancer region has been intensively studied and is regulated by several transcription factors. Although promoter regions are divergent in sequence, the proximal 5′ upstream 5-HT1A promoter region has the greatest conservation between humans and the mouse or rat, which provide useful genetic and behavioral models of anxiety and depression (Fig. 4). Within the first 715-bp, the sequences are CpG-rich and display over 70% nucleotide identity including multiple conserved MAZ and Sp1 sites (MAZ I–IV) that drive basal expression [146, 147]. These “housekeeping” gene enhancers drive strong expression in all cell types regardless of whether they endogenously express 5-HT1A receptors. Both TATA-dependent (in rat) and –independent (mouse, human) transcriptional initiation are observed, but MAZ/Sp1 regulation is conserved [146–148]. Interestingly, a possible human splice variant including a short sequence in the 5′-untranslated region (Fig. 4) has been identified from the EST database,

but its prevalence is unclear. However, the coding region of HTR1A is intronless, yielding a receptor protein of the same amino acid sequence in all tissues.

Inducible DNA elements

DNA elements of fundamental importance are often conserved across multiple species, especially human, rat and mouse HTR1A promoter sequences (Fig. 4). Two conserved NFκB sites (−79 bp; −350 bp) have been implicated in mitogen-induced up-regulation of the 5-HT1A receptor in immune cells (B-cells, T-cells, neutrophils and macrophages) [149, 150]. The 5-HT1A receptor is also negatively regulated by glucocorticoids, which can act indirectly through NFκB or Sp1 sites located on the promoter [147, 151], a mechanism that may be more important in immune cells than in neurons. Further upstream a novel negative glucocorticoid response element (nGRE), containing two GRE half-sites separated by six nucleotides rather than three as for a positive GRE [152], is conserved in mammalian HTR1A genes (Fig. 5A). This nGRE mediates repression by both glucocorticoid and mineralocorticoid receptors, of which the latter are ten-fold more sensitive to glucocorticoids and are highly expressed in the hippocampus. Thus, hippocampal 5-HT1A receptors are exquisitely glucocorticoid- and stress-sensitive, and implicated in stress-induced reduction of 5-HT neurotransmission in hippocampus [153].

Raphe-specific Pet-1 enhancer

Pet-1 is a transcription factor that is expressed exclusively in raphe nuclei and is required for differentiation of serotonin neurons and expression of serotonergic genes [154–156]. Several conserved Pet-1 sites are present in the 5-HT1A promoter (Fig. 4), but the upstream site (−1400 bp) shows the greatest conservation (Fig. 5B) and is the most critical for raphe-specific expression of the 5-HT1A autoreceptor [156]. Due to its exclusive expression in 5-HT neurons in the brain, inhibiting Pet-1 may be useful to reduce 5-HT1A autoreceptor levels (Box 4), but since Pet-1 also affects TPH and 5-HTT levels, inhibiting Pet-1 could undesirably reduce 5-HT levels [155]. Consistent with this, the Pet-1 knockout mice display one fifth of wild-type brain 5-HT content, as well as an increased aggressivity and anxiety phenotype [154].

Box 4

Transcriptional regulation of the 5-HT1A autoreceptor as a therapeutic opportunity

- 5-HT1A autoreceptors negatively regulate tone of 5-HT system and appear to be upregulated in major depression
- Increased 5-HT1A autoreceptors prevent response to 5-HT selective antidepressants; blockade or desensitization of 5-HT1A autoreceptors accelerate or permit response to antidepressants
- The functional HTR1A C(-1019)G (rs6295) promoter polymorphisms is associated with increased 5-HT1A autoreceptor expression, MDD, and resistance or relapse to 5-HT selective antidepressants

- DNA binding proteins inhibited by HTR1A rs6295 polymorphism (Deaf1, HES) repress 5-HT1A autoreceptor transcription in 5-HT neuronal cells
- In vivo loss-of-function mouse models of HTR1A DNA regulators (eg. Deaf1 −/−; Hes1 −/−; Pet-1 −/− mice) display increased levels of 5-HT1A autoreceptor expression
- Target therapeutics to activate/induce repressors of 5-HT1A autoreceptors (e.g., Deaf1, Hes1/5, Freud-1, REST)

Upstream repressor elements

Upstream of the basal promoter, we identified a region with strong repressor activity in human and rat HTR1A genes that completely silenced transcription in non-neuronal cells, but also partially repressed transcription in neuronal cells [148, 157]. This suggested that the repressor region plays a key role in regulating the basal expression of 5-HT1A receptors in neurons. Three overlapping repressor elements are functional and conserved (88% nucleotide identity to rat/mouse, Fig. 5C) [158]. These include two copies (5′ and 3′) of a novel dual repressor element (**DRE, 31-bp**) and an **RE-1** (neuronal repressor REST binding site) [158]. The DRE is composed of two conserved elements (Fig. 5C): 5′-repressor element (FRE, 14-bp) active in 5-HT1A-positive neurons, and 3′-RE (TRE, 12-bp) active in 5-HT1A-negative cells [157]. Mutational inactivation of the FRE in the rat 5-HT1A promoter results in ten-fold induction in raphe serotonergic cells but not non-neuronal cells [157]. This indicates that FRE is a powerful repressor of 5-HT1A transcription in 5-HT-expressing neurons. By yeast one-hybrid screening we identified Freud-1 (**FRE U**nder **D**ual repression binding protein)/CC2D1A, a novel protein that binds FRE and represses the 5-HT1A gene in neurons [158, 159]. Freud-2/CC2D1B is second repressor homologous to Freud-1 that binds to the 5-HT1A-TRE [160, 161]. Freud-1 and Freud-2 are powerful regulators of basal 5-HT1A receptor expression in neuronal cells, however their roles in the regulation of 5-HT1A receptor expression *in vivo* remain to be demonstrated. Freud-1 is strongly expressed in the raphe nuclei, suggesting that it may play an important role in regulation of the 5-HT1A autoreceptor [159], while Freud-2 is sparsely expressed in the raphe [160]. Thus, it may be possible to target Freud-1 to modify 5-HT1A autoreceptor expression (Box 4). In prefrontal cortex or hippocampus, both Freud-1 and Freud-2 are strongly expressed and colocalize with 5-HT1A receptors, suggesting that both regulate post-synaptic 5-HT1A receptor expression [160, 162]. Importantly, levels of Freud-1 and Freud-2 are altered in depression in a region-specific manner [163], suggesting that differential regulation of these transcription factors modifies 5-HT1A auto- vs. heteroreceptor expression.

Polymorphic palindrome element

In searching for DNA elements that may be important in regulating the human 5-HT1A receptor, we hypothesized that a mutation in the repressor region of the promoter could up-regulate 5-HT1A autoreceptor expression and may correlate with depression. By amplifying a 718-bp segment of the human 5-HT1A repressor region we found the novel C(-1019)G polymorphism (designated rs6295), which has been associated with several clinical

populations (see above). The C-G change impairs the binding of nuclear proteins from raphe cells to a palindrome DNA element (Fig. 5D, E) located at the polymorphism [111]. Using yeast one-hybrid cloning, we cloned Deformed epidermal autoregulatory factor 1 (Deaf1) and Hairy and enhancer of split 5 (HES5), which bind and repress at the C-allele but not the G-allele of the HTR1A promoter. Since Deaf1 but not HES5 is co-expressed with 5-HT1A receptors in the adult brain, we have mainly focused on Deaf1. Both Deaf1 and HES5 repress expression at the HTR1A promoter in raphe cells, and hence the G-allele was predicted to increase 5-HT1A autoreceptor expression. Intriguingly, in some non-serotonergic neuronal cells that express 5-HT1A receptors, Deaf1 displayed opposite activity to enhance rather than repress HTR1A transcription, while the G-allele reduced HTR1A transcription [164]. The role of Deaf1 in regulation of the 5-HT1A receptor *in vivo* was addressed using Deaf1 −/− mice, and we found a 50% increase in 5-HT1A RNA in raphe, but a 30% decrease in prefrontal cortex [165]. Even more striking changes in 5-HT1A receptor expression were observed using dual immunofluorescence to identify 5-HT1A autoreceptors in TPH-positive 5-HT neurons. Thus, the lack of Deaf1 modifies 5-HT1A auto- and heteroreceptor expression as observed in studies of MDD and anxiety disorders (see above). The role of Deaf1 to oppositely regulate 5-HT1A auto- and heteroreceptor expression suggests that agents that activate or induce Deaf1 expression may be useful in correcting altered expression of 5-HT1A receptors in MDD or anxiety.

We also examined whether a Deaf1 element is present in the mouse 5-HT1A promoter as observed in the human HTR1A gene [165]. We defined Deaf1 elements as containing at least one minimal TCG (or reverse CGA) sequence, and located within a palindrome region (Fig. 5D, E). As shown in the alignment of human, mouse and rat HTR1A genes, unlike the conserved DNA elements identified above, the Deaf1 element was not highly conserved and did not align well with the human Deaf1 element containing the polymorphism, which is present in a region of relatively low nucleotide similarity (Fig. 4). Instead both rat and mouse 5-HT1A genes had potential Deaf1 sites located further downstream, but not aligned with each other. In addition we identified a second potential mouse Deaf1 element located close to the human polymorphism site, however this site was not functional [165]. In comparing the Deaf1 sites of different primates, there is complete conservation of the reverse CGAA Deaf1 site (CGA in macaques), as well as the overall sequence of the palindrome (Fig. 5D). However, most other mammalian species lack the TCG sequence in this region, although a relatively highly conserved Deaf1 site cluster is found further downstream. These results argue that the presence of this Deaf1 site is a late evolutionary change that is specific to primates. The polymorphism appears to be specific to humans, as we did not detect it in macaque or cynomolgus monkeys (unpublished data). Thus, had the criteria of sequence similarity been applied, the importance of the palindrome region in regulation of 5-HT1A receptor expression would not have been detected. In evolutionarily “younger” regions of high variation, it may be that common functional polymorphisms are more likely to appear.

Genetic polymorphisms, such as the C(-1019)G polymorphism, can modify the transcriptional activity of the promoter over the lifetime by affecting the binding of different transcription factors. Thus, the G(-1019) allele blocks Deaf1 binding through life, but also prevents the binding of Hes proteins, which in combination with Pet-1, control the timing of

induction of 5-HT1A autoreceptor expression during differentiation of 5-HT neurons [156]. Thus the G-allele leads to a lifelong alteration in 5-HT1A autoreceptor expression to set the tone of the serotonin system, and affect the development of cognitive and emotional reactivity, thereby increasing the risk of depression or other psychopathology (Fig. 3). It is possible that by activating repressors like Freud-1 or Deaf1 it may be possible to suppress the expression of 5-HT1A autoreceptors sufficiently to mediate antidepressant activity, as even a 30% reduction may be sufficient [73] (Box 4). However, this strategy may not be as effective for anxiety-related behaviors which appear to develop in the early postnatal period, and may only respond to epigenetic strategies that can reverse environment-driven changes that set adult stress reactivity [166, 167]. The targeting of transcription factors during a period of transcriptional plasticity may lead to global re-establishment of the set point for homeostatic regulation of neurotransmitter activity.

CONCLUSIONS

As discussed above, transcriptional regulation of the 5-HT1A receptor could be an attractive target for novel therapeutic approaches (Box 4, 5). Both anxiety and MDD involve reduced activity of the serotonin system that could be driven in part by over-expression of 5-HT1A autoreceptors and/or reduced expression of 5-HT1A heteroreceptors. However, effective treatment of these disorders would require inhibition or down-regulation of 5-HT1A autoreceptors (Box 4), but activation or up-regulation of cortical and hippocampal 5-HT1A heteroreceptors (Box 5). To date, ligand-based therapeutics such as buspirone or pindolol have had limited success in treating anxiety or MDD, in part because they regulate both 5-HT1A auto- and heteroreceptors in the same way. Recently however some promising compounds may have selective activity at either 5-HT1A autoreceptors or 5-HT1A heteroreceptors, and thus may be more effective as anti-depressant or anti-anxiety compounds. Evidence from *in vitro* and recent *in vivo* studies indicates that some of the transcriptional regulators of the 5-HT1A receptor may have high selectivity for 5-HT1A auto- vs. heteroreceptors. For example, activation of Deaf1, Hes1/Hes5 or Freud-1/CC2D1A would suppress 5-HT1A autoreceptor expression. However, Deaf1 alone has the interesting property of enhancing cortical 5-HT1A heteroreceptor expression, while Freud-1/CC2D1A globally represses 5-HT1A auto- and heteroreceptors. Therefore by activating Deaf1, a dual benefit could be possible: namely reducing 5-HT1A autoreceptor expression, while increasing 5-HT1A heteroreceptor expression. However, these transcription factors could have additional gene targets, which need to be considered. In the case of Deaf1, it appears to be regulating a select type of immune response that may not lead to adverse effects. Nevertheless, targeting 5-HT1A autoreceptors selectively should enhance responsiveness to SSRIs, and mediate more rapid response and recovery from anxiety and MDD.

Box 5

Transcriptional regulation of the 5-HT1A heteroreceptor as a therapeutic opportunity

- Reductions of 5-HT1A heteroreceptors or their activity are observed in cortical regions in anxiety and MDD

- In animal models, loss or blockade of forebrain 5-HT1A heteroreceptors in development increases anxiety and prevents response to 5-HT selective antidepressants
- The functional HTR1A C(-1019)G (rs6295) promoter polymorphism is associated with increased stress response and anxiety phenotypes
- DNA binding proteins inhibited by HTR1A rs6295 polymorphism (Deaf1) enhances 5-HT1A heteroreceptor transcription in non-5-HT neuronal cells
- In vivo loss-of-function of HTR1A regulator Deaf1 reduces levels of 5-HT1A heteroreceptor expression in frontal cortex
- Target therapeutics to activate/induce enhancers of 5-HT1A heteroreceptors (e.g., Deaf1) or inhibit repressors (e.g. Freud-2)

Acknowledgments

PRA was supported by funding from the Canadian Institutes of Health Research, Ontario Mental Health Foundation, and Heart and Stroke Foundation Centre for Stroke Recovery. LMF was supported by postdoctoral funding from the National Science and Engineering Research Council of Canada.

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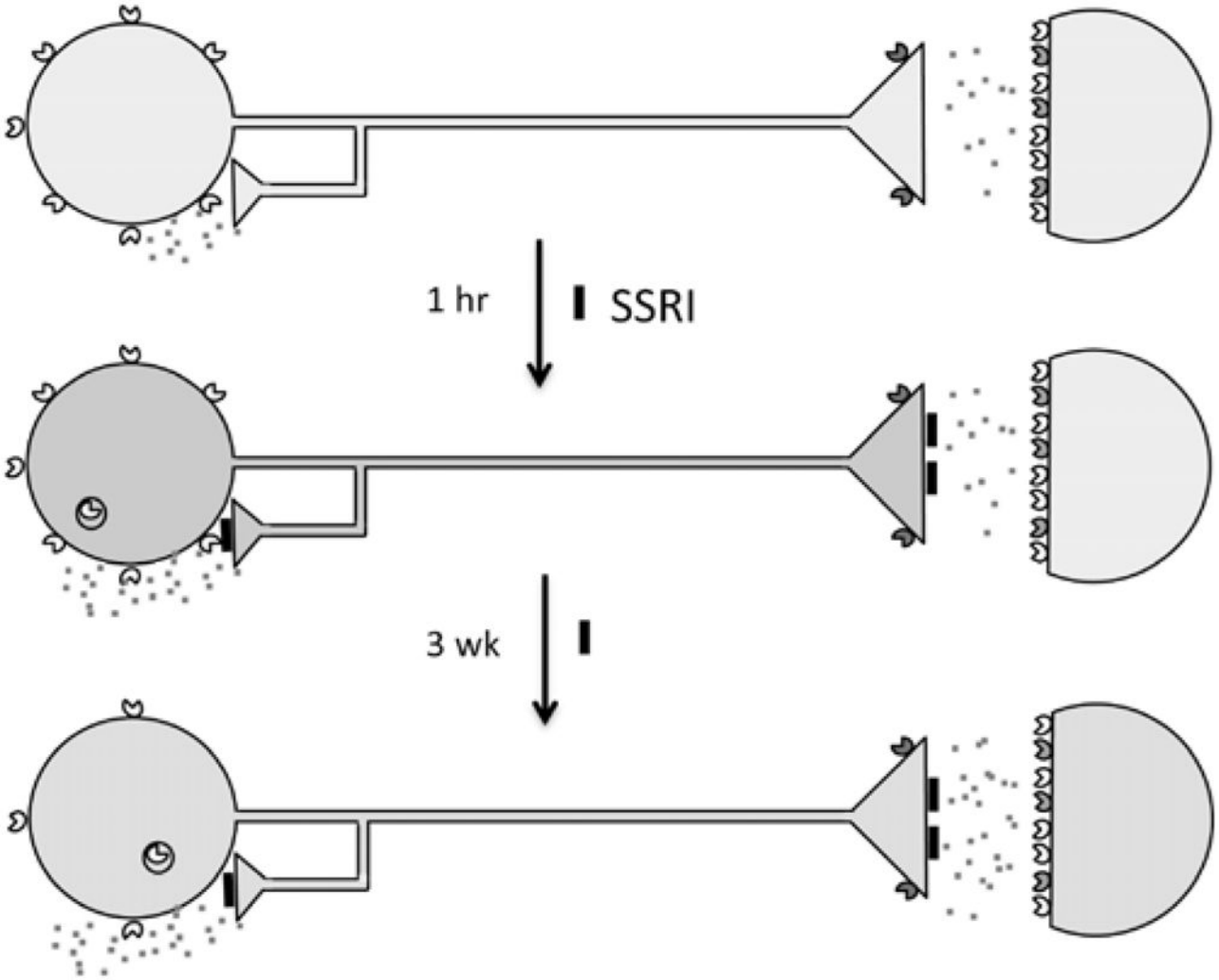

Fig. 1. Adaptive changes in 5-HT neurons upon antidepressant treatment

In major depression, 5-HT neurons are thought to have reduced neurotransmission (pale blue). 5-HT1A autoreceptors (yellow carets) are expressed presynaptically on 5-HT neuron cell bodies and dendrites (at left); 5-HT1B receptors (green carets) are presynaptic at the nerve terminal; 5-HT1A heteroreceptors and other 5-HT receptors are expressed postsynaptically (colored carets). The 5-HT1A receptor couples to Gi/Go protein to inhibit adenylyl cyclase (AC), open potassium channels (K+) to hyperpolarize membrane potential and reduce firing rate. Acutely, SSRI antidepressants inhibit (black blocks) the 5-HT transporter increasing synaptic 5-HT (red specks), leading to activation of 5-HT1A autoreceptors which inhibit 5-HT neuronal firing (blue), and transiently internalize. After 3 weeks of SSRI treatment, 5-HT1A autoreceptors desensitize and receptor levels are reduced, dis-inhibiting 5-HT neuronal firing and enhancing 5-HT neurotransmission (green).

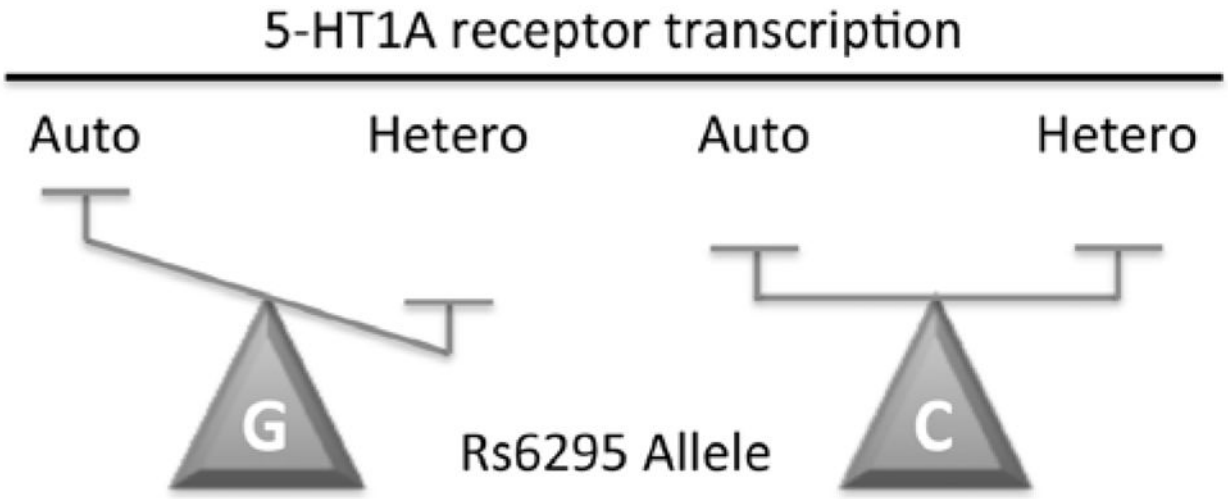

Fig. 2. Effect of C(-1019)G polymorphism rs6295 on 5-HT1A receptor expression

A simplistic model of the C(-1019)G change (labeled C or G), which prevents Deaf1 from recognizing its element on the 5-HT1A promoter, is presented. In 5-HT neuronal cells, Deaf1 represses 5-HT1A receptor transcription, but in certain non-serotonergic neurons it oppositely enhances 5-HT1A expression. An upregulation of 5-HT1A autoreceptor (Auto) expression in raphe neurons is predicted for the G(-1019) allele based on loss of Deaf1-mediated repression presynaptically. Conversely, in some non-serotonergic neurons a reduction in 5-HT1A heteroreceptor (Hetero) expression is predicted based on the loss of Deaf1 enhancer activity.

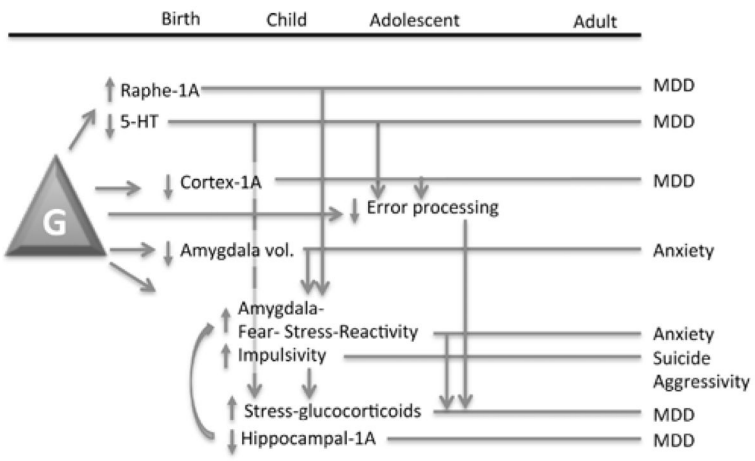

Fig. 3. Lifetime associations with the HTR1A genotype

Presented is a figurative summary of association data obtained for the GG genotype (G) of the HTR1A rs6295 polymorphism and how these changes might present and interact over the course of the lifetime. Arrows indicate associations of changes with each other, as well as feed-forward consequences of the changes. Bars indicate the lifetime trajectory of the indicated changes contributing to the indicated psychopathology. Note that the GG genotype may attenuate a number of cognitive and emotional processing capabilities that could ultimately lead to psychopathology.

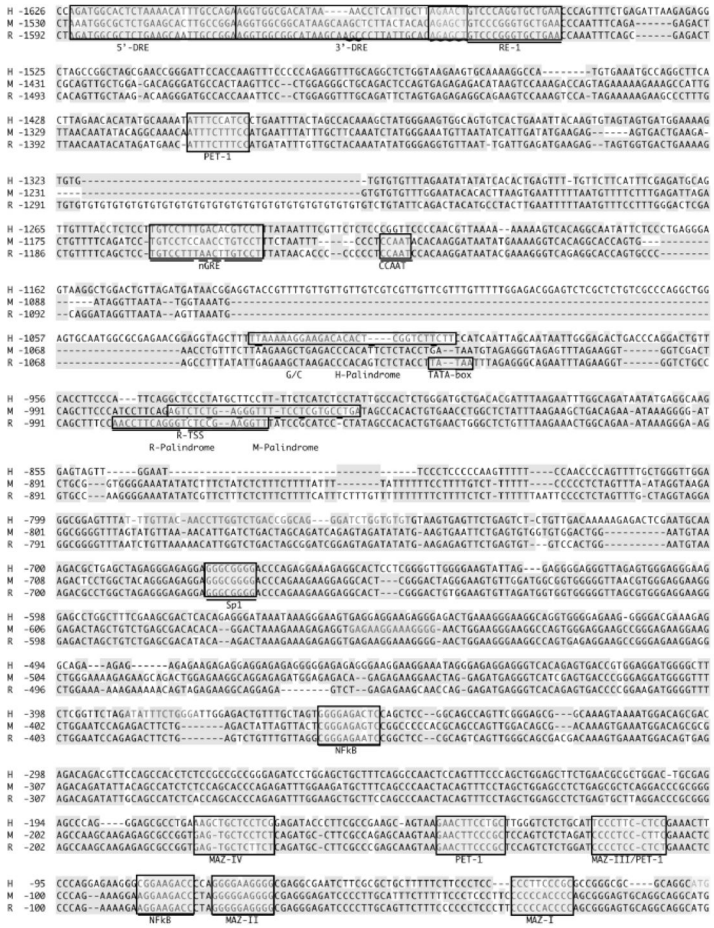

Fig. 4. HTR1A promoter alignment

Nucleotide alignment of the human (H), mouse (M) and rat (R) 5-HT1A promoter sequence is shown ending at the translational initiation ATG codon (yellow). Sequence counts are shown at right, matches are in blue, and gaps shown by dashes. A potential alternately spliced non-coding exon is shown in red. Boxes depict the proximal promoter elements (Sp1, MAZ), NFkB sites, negative glucocorticoid receptor element (nGRE), raphe-specific Pet-1 elements (PET-1), and the repressor elements for Freud-1 and Freud-2 (DRE) and REST/NRSF (RE-1); palindrome Deaf1 elements (Palindrome) for human (H), mouse M) and rat R), TATA-box and CCAAT box. Highlighted are the human C(-1019)G polymorphism (C/G) and the rat transcription start site (R-TSS). The % nucleotide identity (human vs. mouse) over the initial 800 bp was 73%. Alignment was obtained from the Ensembl database.

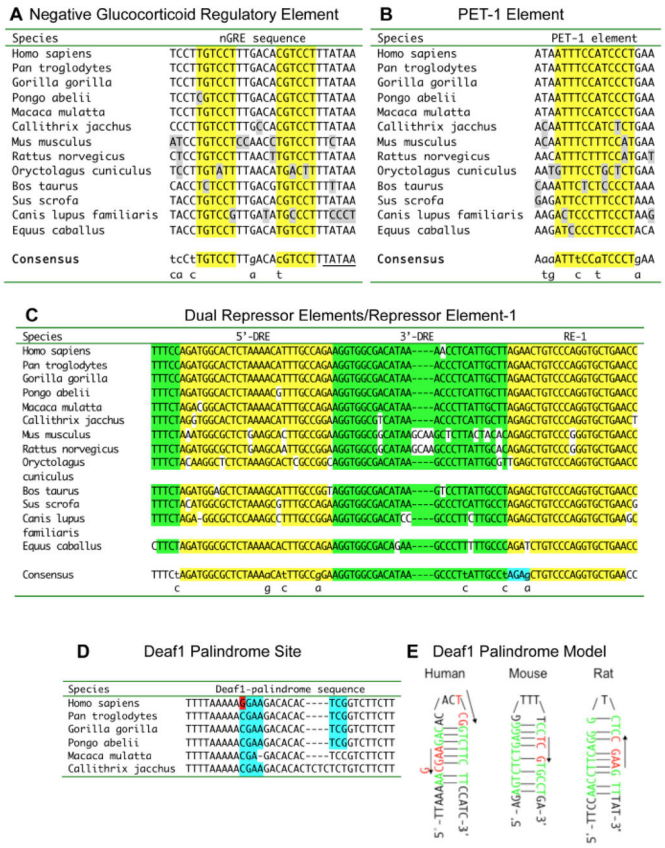

Fig. 5. Conserved HTR1A DNA elements

The alignment of DNA elements from mammalian 5-HT1A receptor gene sequences is shown with elements of interest highlighted; below is the consensus sequence shared in three or more species; mismatches from the consensus are shown in grey.

A. Negative Glucocorticoid Regulatory Element (nGRE). The GRE half-site sequence is highlighted and a 3′-flanking TATA-box sequence of unknown function is also shown. B. PET-1 element. C. Dual repressor elements (DRE)/repressor element-1 (RE-1). The 5′DRE and RE-1 are highlighted in yellow; 3′-DRE and conserved upstream sequence in green; overlap in blue; and mismatches in white. D. Deaf1 palindrome site. Shown are conserved sequences in primate species; the minimal Deaf1 recognition sequence is in blue; the site of the rs6295 polymorphism (G-allele shown) is in red. E. Deaf1 palindrome model. Deaf1 binding sites in human, mouse and rat 5-HT1A promoters are shown as “hybridized” to highlight imperfect palindrome sequences shown in green; the consensus Deaf1 minimal TCG (forward) or CGA (reverse) is shown in red. The location of the G(-1019) rs6295 site in the human 5-HT1A palindrome is indicated.