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Review

Transcriptional regulation of the 5-HT$_{1A}$ receptor: implications for mental illness

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The serotonin-1A (5-HT$_{1A}$) receptor is an abundant post-synaptic 5-HT receptor (heteroreceptor) implicated in regulation of mood, emotion and stress responses and is the major somatodendritic autoreceptor that negatively regulates 5-HT neuronal activity. Based on animal models, an integrated model for opposing roles of pre- and post-synaptic 5-HT$_{1A}$ receptors in anxiety and depression phenotypes and response to antidepressants is proposed. Understanding differential transcriptional regulation of pre- versus post-synaptic 5-HT$_{1A}$ receptors could provide better tools for their selective regulation. This review examines the transcription factors that regulate brain region-specific basal and stress-induced expression of the 5-HT$_{1A}$ receptor gene (*Htr1a*). A functional polymorphism, rs6295 in the *Htr1a* promoter region, blocks the function of specific repressors Hes1, Hes5 and Deaf1, resulting in increased 5-HT$_{1A}$ autoreceptor expression in animal models and humans. Its association with altered 5-HT$_{1A}$ expression, depression, anxiety and antidepressant response are related to genotype frequency in different populations, sample homogeneity, disease outcome measures and severity. Preliminary evidence from gene × environment studies suggests the potential for synergistic interaction of stress-mediated repression of 5-HT$_{1A}$ heteroreceptors, and rs6295-induced upregulation of 5-HT$_{1A}$ autoreceptors. Targeted therapeutics to inhibit 5-HT$_{1A}$ autoreceptor expression and induce 5-HT$_{1A}$ heteroreceptor expression may ameliorate treatment of anxiety and major depression.

Keywords: serotonin; transcription; receptor; raphe; anxiety; depression

1. INTRODUCTION

The 5-HT$_{1A}$ receptor has been increasingly associated with alterations in mood and emotion and has opposing functions as a pre-synaptic somatodendritic autoreceptor and a post-synaptic heteroreceptor. The 5-HT$_{1A}$ autoreceptor mediates negative feedback inhibition on 5-HT neurons, while the 5-HT$_{1A}$ heteroreceptors mediate 5-HT actions on target neurons. We focus on the transcriptional mechanisms and polymorphic changes that regulate pre- versus post-synaptic 5-HT$_{1A}$ receptors, and how this differential regulation could be used to understand the etiology and improve the treatment of mental illnesses.

2. 5-HT$_{1A}$ AUTORECEPTORS AS BRAKES FOR 5-HT NEUROTRANSMISSION

The concept of the 'autoreceptor' as a receptor that regulates (usually inhibits) the release of its own neurotransmitter goes back to the 1960s, originally described by Carlsson and colleagues [1,2] for the dopamine system. The key observations that these receptors regulate release of their own neurotransmitters came from evidence that by inhibiting autoreceptors using pharmacological blockers such as haloperidol or chlorpromazine, dopamine release and turnover was greatly augmented. Oppositely, agonists such as apomorphine suppressed basal dopamine release. These key observations were replicated in the noradrenergic and serotonergic systems, and now the concept of autoreceptors has been generalized to include a number of other systems, including histaminergic, glutaminergic, cholinergic and other major neurotransmitter systems [3].

For the serotonin (5-hydroxytryptamine, 5-HT) system, the presence of autoreceptors was indicated by evidence that non-selective agonist LSD or 5-HT itself reduced 5-HT release, while receptor antagonists like methiothepin increased 5-HT release [4]. Aghajanian's group showed that autoregulation of firing was mediated by 5-HT receptors on the 5-HT neurons [5–7]. Using the selective agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), Hamon's group identified the 5-HT$_{1A}$ receptor as the major autoreceptor on the cell body and dendrites of 5-HT neurons of the raphe nuclei [8–11]. The 5-HT$_{1A}$ autoreceptor was then shown to mediate 5-HT auto-inhibition [7,12]. A consistent observation has been that reduction or ablation of 5-HT$_{1A}$ autoreceptors leads to increased 5-HT neurotransmission [13–17], while over-expression of 5-HT$_{1A}$ autoreceptors reduces 5-HT neurotransmission [18–20]. While 5-HT$_{1A}$ antagonists do not greatly affect basal firing, they consistently reverse inhibition of firing by 5-HT$_{1A}$ agonist or specific reuptake inhibitor (SSRI) treatment, suggesting that the basal level of 5-HT under recording conditions may be insufficient to see effects. In 5-HT$_{1A}$ knockout

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animals, an elevated basal raphe firing may result from a chronic absence of 5-HT$_{1A}$ receptors. By contrast, the 5-HT$_{1B}$ subtype is the key autoreceptor on presynaptic 5-HT nerve terminals regulating 5-HT release [21,22]. Recently, targeting of the 5-HT$_{1A}$ autoreceptor to soma and dendrites has been shown to be mediated by interaction of the receptor C-terminal tail with Yif1B [23,24]. 5-HT$_{1A}$-mediated autoinhibition involves 5-HT$_{1A}$-induced hyperpolarization of raphe neurons that is mediated by coupling of pertussis toxin-sensitive Gi proteins to activation of inward-rectifying potassium (GIRK) channels [25–28], possibly GIRK2 channels [29], which are co-expressed with 5-HT$_{1A}$ receptors in raphe and post-synaptic regions [30]. In addition, 5-HT$_{1A}$ heteroreceptors mediate an indirect negative feedback pathway by inhibition of pyramidal cortical neurons that project to raphe 5-HT neurons [31]. Oppositely, 5-HT$_{2A}$ and 5-HT$_4$ receptors mediate positive feedback of 5-HT neurons via prefrontal cortex and hippocampus projections, respectively [31,32]. Together, the direct and indirect 5-HT$_{1A}$-mediated feedback mechanisms negatively regulate the activity of the 5-HT system.

Release of 5-HT$_{1A}$-mediated autoinhibition by receptor desensitization appears to play a key role in the efficacy of antidepressant treatments, especially 5-HT-SSRIs [13,33–35]. Acute treatment with SSRI leads to a local increase in 5-HT levels in the raphe nuclei [36,37], activating 5-HT$_{1A}$-mediated autoinhibition to inhibit firing of 5-HT neurons. With chronic SSRI treatment, 5-HT$_{1A}$ autoreceptors (but not heteroreceptors) desensitize, restoring raphe firing to enhance 5-HT release. By contrast, sensitization of 5-HT$_{1A}$ heteroreceptors is observed following chronic antidepressant treatment, although the mechanisms involved remain unclear [38–43].

Activation of 5-HT$_{1A}$ heteroreceptors plays a prominent role in the antidepressant and neurogenic actions of SSRIs [44–46]. Several possible mechanisms have been implicated in 5-HT$_{1A}$ autoreceptor desensitization [42] including uncoupling from G-proteins [41,47–50], receptor internalization [51], G-protein inactivation [52] and reduction in 5-HT$_{1A}$ autoreceptors [38,53]. In addition, coupling to the GIRK is reduced upon chronic fluoxetine treatment [54], one mechanism that may disinhibit raphe firing and allow for enhanced 5-HT neurotransmission. However, these mechanisms of rapid desensitization do not account for the chronic treatment required for antidepressant effects, and are also rapidly reversible [51]. The above studies were done in normal animals, while in animal models of depression chronic antidepressant treatment leads to a downregulation of 5-HT$_{1A}$ autoreceptor RNA or binding sites in the raphe nuclei [55–57] (figure 1). Similarly, in human depression in elderly subjects a reduction in 5-HT$_{1A}$ autoreceptors was correlated with an increased response to SSRI treatment [58], while another study found that in patients treated with SSRIs, increased 5-HT$_{1A}$ autoreceptor availability correlated with more severe depression [59]. Thus, while rapid desensitization of 5-HT$_{1A}$ autoreceptors occurs, transcriptional downregulation of 5-HT$_{1A}$ autoreceptors may play a role in the long-term adaptive changes in response to chronic antidepressant treatment in depressed subjects. Consistent with this, mice engineered to repress 5-HT$_{1A}$ autoreceptor expression in adults by only 30 per cent responded to chronic SSRI treatment within days, while wild-type mice failed to respond to a three-week treatment [19]. Since partial or complete repression of 5-HT$_{1A}$ autoreceptors enhances 5-HT neuronal activity and 5-HT release in target tissues [19,20,60], these studies clearly indicate the level of 5-HT$_{1A}$ autoreceptors serves as a gate for response to chronic SSRI treatment [61].

Alterations in 5-HT$_{1A}$ receptor expression result in anxiety- and depression-like behaviours in animal models. Knockout of the 5-HT$_{1A}$ receptor gene leads to increased anxiety behaviour in at least three different mouse strains [62–64]. Specific repression of 5-HT$_{1A}$ autoreceptors also increases anxiety, suggesting that their loss leads to increased activation of other post-synaptic 5-HT receptors [20]. Pharmacological blockade of 5-HT$_{1A}$ receptors in early post-natal development also elicits an anxiety phenotype [65], while early post-natal expression of forebrain 5-HT$_{1A}$ receptors rescues the anxiety phenotype of 5-HT$_{1A}$-null mice [66]. Similarly, transient over-expression of 5-HT$_{1A}$ receptors reduces anxiety in mice [67]. Selective repression of 5-HT$_{1A}$ heteroreceptors leads to depression-like behaviour, consistent with a role for post-synaptic 5-HT$_{1A}$ receptors in depression [20]. In agreement with this, enhancement of 5-HT$_{1A}$-Gi2 signalling reduced depression-like behaviours, presumably via 5-HT$_{1A}$ heteroreceptor signalling [46]. Furthermore, 5-HT$_{1A}$ heteroreceptors appear to be obligatory for response to chronic SSRI treatment since 5-HT$_{1A}$ knockout mice lack behavioural and neurogenic response to SSRI [68]. Thus, 5-HT$_{1A}$ heteroreceptors appear critical for both the development of the depression phenotype as well as the antidepressant response to chronic SSRI treatment. Conversely, the 5-HT$_{1A}$ autoreceptor negatively regulates the activity of 5-HT neurons, and restrains the development of the anxiety phenotype as well as reducing and delaying response to SSRI treatment in mouse models (figure 1).

3. TRANSCRIPTIONAL REGULATION OF 5-HT$_{1A}$ AUTORECEPTORS VERSUS HETERORECEPTORS

The earlier-mentioned results indicate the importance of the 5-HT$_{1A}$ autoreceptor as a brake for 5-HT neurotransmission *in vivo*, suggesting that regulators of the *Htr1a* gene might affect basal 5-HT neurotransmission and susceptibility to depression or anxiety disorders (figure 1). The *Htr1a* gene lacks introns in its coding region and is strongly expressed in specific brain regions, but almost not at all in non-neuronal tissues [69,70]. The *Htr1a* gene contains a GC-rich proximal promoter region containing DNA elements for several ubiquitous transcription factors, including Myc-associated zinc finger protein (MAZ), Sp1 and NFκB that drive its expression in all cell types examined [71–73]. By contrast, the *Htr1a* promoter also contains several Pet-1 sites recognized by the raphe-specific enhancer, Pet-1, which primarily enhances 5-HT$_{1A}$ autoreceptor expression [74]. Knockout of Pet-1 leads to reduced

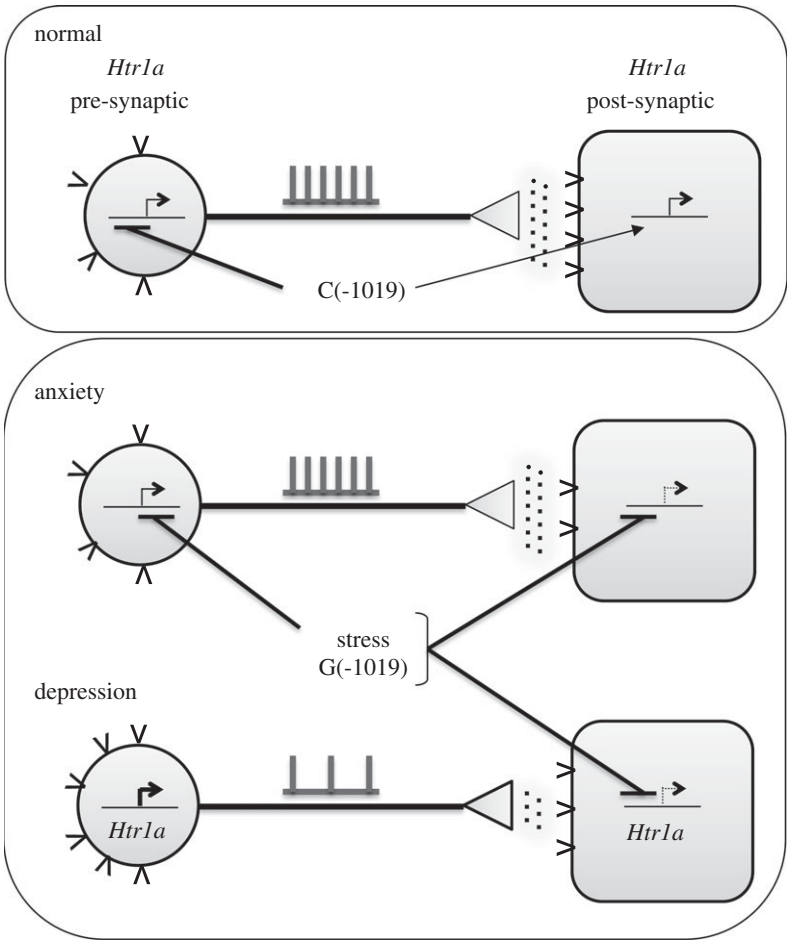

Figure 1. Model for *Htr1a* dysregulation in human anxiety and depression. Shown is a model of differential *Htr1a* regulation in pre-synaptic raphe 5-HT neurons projecting to post-synaptic neurons in target tissues involved in mood and affect, such as prefrontal cortex, hippocampus, amygdala or hypothalamus. Pre-synaptic 5-HT$_{1A}$ autoreceptors (v) negatively regulate 5-HT firing neuronal activity and regulate the release of 5-HT (dots) and activation of post-synaptic 5-HT$_{1A}$ heteroreceptors. In normal subjects, the set point for raphe firing frequency is determined in part by the density of 5-HT$_{1A}$ autoreceptors that is restrained by repression at the rs6295 C(-1019) allele (bar); 5-HT$_{1A}$ heteroreceptors, especially in cortex appear to be increased by enhancer activity at the C(-1019) allele (arrow). In anxiety, the combination of genetic (rs6295 G(-1019) allele) and environmental (life stress) glucocorticoid-mediated repression of the 5-HT$_{1A}$ promoter leads to a stronger decrease in 5-HT$_{1A}$ heteroreceptors in glucocorticoid-sensitive tissues, such as hippocampus and prefrontal cortex, and to a lesser extent the raphe nucleus; anxiety subjects appear to compensate for the de-repression of 5-HT$_{1A}$ autoreceptors in the raphe. In depression, the combination of G(-1019) allele and stress promote reductions in post-synaptic 5-HT$_{1A}$ heteroreceptors; however, pre-synaptic 5-HT$_{1A}$ autoreceptors become upregulated due to the lack of Deaf1 repression at the G(-1019) allele, leading to reduced 5-HT release, and compensatory partial upregulation of 5-HT$_{1A}$ heteroreceptors. In this model, antidepressants act mainly at heteroreceptors or other post-synaptic processes for anti-anxiety actions, while they also act to desensitize pre-synaptic sites to mediate antidepressant actions.

expression of 5-HT$_{1A}$ autoreceptors, as well as a general reduction in serotonergic differentiation markers [74,75]. Thus, Pet-1 is a key positive regulator of 5-HT$_{1A}$ autoreceptor expression.

To restrict *Htr1a* expression to neuronal cells, a series of repressor elements located upstream of the promoter coordinately silence the gene [73]. These include the RE-1 site for neural restrictive factor (REST/NRSF) [76] and a powerful dual repressor element that is regulated by a pair of conserved repressors, Freud-1/CC2D1A and Freud-2/CC2D1B [77–79]. Unlike REST, which silences neuronal genes mainly in non-neuronal cells, Freud-1 and Freud-2 also repress 5-HT$_{1A}$ expression in neuronal cells [76]. Reduction of Freud-1 or Freud-2 expression using antisense or siRNA increases expression of neuronal 5-HT$_{1A}$ receptors [78–81]. Together, these repressors silence the *Htr1a* gene in non-neuronal cells, but reversibly regulate its expression in 5-HT$_{1A}$-positive neuronal cells. In raphe cells, Freud-1 is co-expressed with 5-HT$_{1A}$ autoreceptors and represses the *Htr1a* gene, while in target regions both Freud-1 and Freud-2 are expressed, and they both repress *Htr1a* expression in non-serotonergic neuronal cells. Thus, Freud-1 is implicated in 5-HT$_{1A}$ autoreceptor expression, while both Freud-1 and Freud-2 regulate 5-HT$_{1A}$ heteroreceptor expression.

Another region implicated in 5-HT$_{1A}$ autoreceptor regulation is the C(-1019)G (rs6295) *Htr1a* promoter polymorphism located within a palindrome (inverted repeat) sequence that is recognized by transcription factors NUDR/Deaf1 and Hes proteins Hes1 and Hes5 [82,83]. In raphe cells, Deaf1 and Hes repress *Htr1a* and the polymorphic change prevents their binding and repression, upregulating 5-HT$_{1A}$ autoreceptor

expression. Hes1 and Hes5 are restricted to neuronal progenitors and silenced upon neuronal differentiation [84]. Knockout of Hes1 results in premature and expanded expression of midbrain 5-HT$_{1A}$ receptor RNA, suggesting a role for Hes1 to restrict 5-HT$_{1A}$ receptor expression to serotonergic neurons [83]. Recent results indicate that knockout of Deaf1 results in a 50 per cent increase in 5-HT$_{1A}$ autoreceptor expression in dorsal and medial raphe nuclei [85]. Thus, by disrupting repression both by Hes1/5 and by Deaf1, the G(-1019) allele is expected to increase 5-HT$_{1A}$ autoreceptor expression, reducing serotonergic activity and increasing the risk of depression (figure 1).

It is important to note that all of these transcriptional mechanisms interact to regulate 5-HT$_{1A}$ autoreceptor expression. For example, reduction in calcium levels by 5-HT$_{1A}$ autoreceptor signalling could relieve calcium-dependent inactivation of Freud-1 [78], leading to agonist-induced downregulation of *Htr1a* transcription. Oppositely, along with a trend for an increase in 5-HT$_{1A}$ RNA in raphe tissue from depressed versus control female subjects, REST and Deaf-1 RNA were also increased, suggesting a compensatory mechanism to normalize 5-HT$_{1A}$ autoreceptor expression [86]. Thus, transcriptional upregulation of the 5-HT$_{1A}$ autoreceptor in depression could be blunted by altered regulation of these key repressors in raphe cells.

Differential transcriptional regulation of 5-HT$_{1A}$ autoreceptors versus heteroreceptors is partly dictated by developmental and regional distribution of *Htr1a* transcriptional factors and alterations in regulators such as glucocorticoids. Thus, Pet-1 is raphe-specific, while Freud-2 is not detected in raphe cells and thus specifically regulates 5-HT$_{1A}$ heteroreceptors. Similarly, high- and low-affinity glucocorticoid receptors (mineralocorticoid receptor, MR, and glucocorticoid receptor, GR, respectively) are enriched in hippocampus compared with raphe and are critical for stress- or glucocorticoid-induced downregulation of hippocampal 5-HT$_{1A}$ receptors [40,71,87–92]. Consistent with the importance of negative regulation of 5-HT$_{1A}$ heteroreceptors by glucocorticoids, an inverse correlation between glucocorticoid levels and hippocampal and amygdala but not raphe 5-HT$_{1A}$ receptor levels is seen in anxiety disorder patients [93]. With elevated glucocorticoid conditions, such as in chronic mild stress or sleep deprivation [94,95], GR appears to repress 5-HT$_{1A}$ autoreceptors [96]. Glucocorticoids can also uncouple 5-HT$_{1A}$ autoreceptors by reducing GIRK2 RNA levels [29]. Paradoxically, over-expression of MR or GR in the mouse forebrain increases 5-HT$_{1A}$ heteroreceptor expression (possibly via suppression of glucocorticoids), which was associated with an anti-anxiety/anti-depressed phenotype and increased SSRI responsiveness, respectively [97,98]. Thus, chronic life stress may dysregulate the 5-HT system by reducing 5-HT$_{1A}$ heteroreceptor expression and increase susceptibility to mental illness (figure 1).

4. IMPLICATIONS OF 5-HT$_{1A}$ AUTORECEPTOR DYSREGULATION FOR MENTAL ILLNESS

Several lines of evidence suggest that depression and anxiety disorders in humans are associated with alterations in 5-HT$_{1A}$ receptor expression. An increase in 5-HT$_{1A}$ autoreceptor expression has been reported in the rostral raphe region of post-mortem tissue from a depressed suicide victim compared with control subjects, but with reduced 5-HT$_{1A}$ autoreceptor levels in caudal raphe regions [99,100]. Positron emission tomography (PET) imaging studies in living depression patients using the 5-HT$_{1A}$ antagonist [^{11}C]WAY100635 also show a prominent 50 per cent increase in 5-HT$_{1A}$ autoreceptors in antidepressant-free or naive depressed subjects [101–103], as well as a twofold increase in male bipolar depression patients [104]. An upregulation of 5-HT$_{1A}$ autoreceptors is likely to reduce serotonergic neurotransmission, as associated with human depression and suicide (figure 1).

With regard to 5-HT$_{1A}$ heteroreceptor expression, post-mortem studies have generally shown reduced 5-HT$_{1A}$ receptor expression in several regions of the frontal cortex of depressed suicide victims [105,106]. However, in depressed suicide tissue some cortical regions display any increase in 5-HT$_{1A}$ RNA, such as in the frontopolar cortex compared with a decrease in orbital frontal cortex suggesting that dysregulation of 5-HT$_{1A}$ heteroreceptors is region-specific [107,108]. Such region-specific changes in 5-HT$_{1A}$ receptor expression have not been observed in PET imaging studies, but rather global decreases or increases have been observed, which may reflect the limited special resolution of imaging studies [109]. In depression, decreases in 5-HT$_{1A}$ heteroreceptors are more pronounced than for autoreceptors [110–113], which do not change or increase. In panic disorder, there is a reduction in cortical 5-HT$_{1A}$ heteroreceptors that is normalized by treatment [114,115]. Similarly, in social anxiety disorder, reductions in 5-HT$_{1A}$ binding in amygdala and anterior cingulate cortex were most prominent, with some decrease in raphe [116]. Reduced cortical 5-HT$_{1A}$ receptors were correlated with anxiety behaviour in normal subjects [117,118]. These results are consistent with animal studies that indicate a key role for 5-HT$_{1A}$ heteroreceptors in anxiety-like behaviours in mice [20].

Discrepancies in PET imaging results from different groups may be accounted for by methodological differences in reference tissue [101,119], or by confounds such as limited resolution (e.g. for raphe sub-regions), medication status or ligand competition with receptor-bound 5-HT *in vivo*. With regard to ligand competition, since PET ligand [^{11}C]WAY100635 is a high-affinity 5-HT$_{1A}$ antagonist, it detects total 5-HT$_{1A}$ receptors, not distinguishing between coupled or uncoupled receptors and is not readily displaced by 5-HT. Treatment of rats with fenfluramine to release synaptic 5-HT reduced [^{11}C]WAY100635 only in hippocampus [120] but reduced [18F]MPPF (a lower-affinity antagonist) binding in several brain areas [121]. The recent development of a labelled 5-HT$_{1A}$ agonist may provide a sensitive measure of increase in functional 5-HT$_{1A}$ autoreceptors in depression [122].

Taken together, results from human and animal studies are consistent with a model in which anxiety disorder involves a reduction in 5-HT$_{1A}$ heteroreceptors in limbic areas, such as hippocampus, amygdala and prefrontal cortex, with a lesser decrease in 5-HT$_{1A}$ autoreceptors (figure 1). A reduction or

inactivation of 5-HT$_{1A}$ autoreceptors is correlated with increased amygdala activation typical of anxiety phenotypes and is thought to be due to increased 5-HT release [123]. On the other hand, depression appears to be driven by reduced 5-HT neurotransmission, in part due to an increase in pre-synaptic 5-HT$_{1A}$ autoreceptors which inhibits the release of 5-HT. As well, depression is associated with reduced levels of 5-HT$_{1A}$ heteroreceptors, particularly in the hippocampus and prefrontal cortex, that may be induced in part by chronic stress [124]. These results suggest that strategies that target preferentially post-synaptic 5-HT$_{1A}$ heteroreceptors may have greater effects in anxiety [125], while strategies that both augment 5-HT release and enhance post-synaptic 5-HT$_{1A}$ signalling would be more effective to treat depression. Consistent with this idea, chronic SSRI treatment of anxiety subjects selectively reduced post-synaptic 5-HT$_{1A}$ receptor levels in hippocampus and prefrontal cortex [126]. To date, 5-HT$_{1A}$ ligands, such as buspirone, have lacked selectivity, targeting both pre- and post-synaptic 5-HT$_{1A}$ receptors [127,128], and display limited efficacy for treatment of anxiety or depression. Because autoreceptors and heteroreceptors have opposing actions on serotonergic neurotransmission, these compounds are of limited benefit. Yet, in combination with SSRI, buspirone augments the antidepressant response due to preferential desensitization of 5-HT$_{1A}$ autoreceptors [129,130]. Recently, compounds with selectivity for post-synaptic receptors have been developed [131] that may demonstrate increased benefit for treatment of anxiety or depression.

It is important to emphasize that multiple mechanisms in addition to 5-HT$_{1A}$ autoreceptor levels regulate 5-HT neurotransmission and could contribute to depression and anxiety. For example, a reduction in TPH2 gene expression or activity [132], or reduced differentiation of 5-HT neurons as seen in Pet-1-deficient mice reduces 5-HT levels [74,75]. Hence, changes in 5-HT$_{1A}$ autoreceptor levels may be secondary to or enhanced by alterations in 5-HT levels. Furthermore, regional diversity of the raphe nuclei has been suggested, with a Pet-1-insensitive population of 5-HT neurons regulating anxiety behaviour [133]: differential regulation of 5-HT$_{1A}$ receptors within these populations may predispose to anxiety versus depression. Thus, certain populations of 5-HT neurons may display similar levels of 5-HT$_{1A}$ autoreceptors, while others may be affected by the rs6295 polymorphism, stress or other factors. The diversity of mechanisms regulating 5-HT neurotransmission is likely to underlie in part the heterogeneity of results in 5-HT$_{1A}$ receptor levels in depression. Nevertheless, therapeutic strategies that target 5-HT$_{1A}$ autoreceptors could be of benefit by resetting the level of 5-HT neurotransmission (figure 1).

5. ASSOCIATION OF rs6295 WITH ALTERED 5-HT$_{1A}$ RECEPTOR EXPRESSION IN HUMANS

As described earlier, the G(-1019) allele of rs6295 would be expected to cause an upregulation of 5-HT$_{1A}$ autoreceptor expression by preventing Hes1 or Deaf1 repression, but may induce selective reductions in post-synaptic 5-HT$_{1A}$ receptors in specific brain regions due to blocking Deaf1 enhancer activity [134]. Recent PET imaging studies in human-depressed patients show an association of rs6295 with an increase in raphe 5-HT$_{1A}$ binding potential. A significant association was observed in unmedicated or antidepressant-naive depressed patients [102]. In this cohort, the rs6295 risk allele and genotype also associated with depression. In a replication study, the level of 5-HT$_{1A}$ receptor binding potential correlated with the genetic load, increasing from CC-CG-GG [101], which also correlated with reduced response to antidepressant treatment. In bipolar depression, the rs6295 genotype also tended to associate with increased raphe 5-HT$_{1A}$ binding [104]. A similar trend of increased 5-HT$_{1A}$ autoreceptor levels was seen using a different 5-HT$_{1A}$ ligand in two female depression patients with the GG genotype [135]. By contrast, in normal subjects, there was a trend for an association of increased 5-HT$_{1A}$ autoreceptor binding potential associated with the GG genotype, but this was not statistically significant [136]. The finding of a more robust increase in 5-HT$_{1A}$ autoreceptor levels with the GG genotype in depressed compared with normal subjects suggests that depressed subjects may not compensate efficiently for the dysregulation conferred by the G(-1019) allele.

Increase in 5-HT$_{1A}$ autoreceptors due to the rs6295 genotype may be augmented by a reduction in synaptic 5-HT release in depression and be reversed by SSRI antidepressants that increase synaptic 5-HT levels. Consistent with this, 5-HT$_{1A}$ autoreceptor levels negatively correlate with levels of the plasmalemmal 5-HT transporter (SERT) in PET studies [137,138], and in post-mortem studies [108,139]. Interestingly, increased 5-HT$_{1A}$ autoreceptor binding is associated with reduced response to antidepressants [102,140], which could reflect greater autoreceptor-mediated inhibition of 5-HT. Treatment of anxiety disorder patients with antidepressants appears to normalize the imbalance between increased 5-HT$_{1A}$ autoreceptor levels and decreased 5-HT$_{1A}$ heteroreceptors [141]. In addition, the level of 5-HT$_{1A}$ autoreceptors is altered by additional factors. For example, the level of 5-HT$_{1A}$ autoreceptor binding potential varies with the oestrous cycle in females [142], which may account for the increased predisposition of females to depression. Despite these variables and the small numbers of patients that can be studied by PET imaging, these data provide important evidence that the rs6295 polymorphism is functional in humans and leads to alterations in 5-HT$_{1A}$ receptor levels in depression.

6. ASSOCIATION STUDIES OF *Htr1a* POLYMORPHISMS IN DEPRESSION- AND ANXIETY-RELATED DISORDERS

Since the initial report of an association of rs6295 with major depression and completed suicide [82], several studies have replicated these results [143]. A meta-analysis confirmed the association of the G-allele of rs6295 with depression, and found an especially strong association in Asian depression [144]. This could be due to the much lower frequency of the G-allele in Asian populations (10–20%) compared with 40–50% in Caucasian subjects. Thus, a twofold

enrichment of the G-allele with depression may be observed in Asians, but a much smaller effect would be present in Caucasians. For example, in a study of a Caucasian Utah cohort with over 300 depressed and 300 control subjects, the G-allele was significantly enriched in depression by only 1.1-fold; the G/G genotype was 1.36-fold enriched [145]. Since these studies, several new association studies with the G-allele of rs6295 have been published for depression [101,146–150], negative emotionality [151], anxiety [150,152], eating disorder [153], bipolar depression [104,147], alcohol withdrawal symptoms [154] and suicide [155]. One study suggests that the G-allele may be most strongly associated with depression with co-morbid anxiety [150], consistent with the importance of dysregulated *Htr1a* expression in both disorders. In support of this, the G-allele was associated with reduced amygdala activation in normal subjects [156]. Interestingly, in panic disorder and depressed patients the G-allele associated with increased amygdala activation but reduced right prefrontal cortex activation [157,158], suggesting altered fear circuitry [124]. In addition, recent findings have associated the G(-1019) allele with reductions in cognitive functioning in mismatch, attentional and error monitoring paradigms [159–162]. Importantly, several studies of response to chronic SSRI treatment have found an association with reduced response of rs6295 alone or with other *Htr1a* polymorphisms [61,143,163–167]. Interestingly, the G-allele is associated with a reduced effect on negative symptoms of atypical antipsychotics that have partial agonist activity at 5-HT$_{1A}$ receptors [168,169]. Thus, the G-allele appears to both confer risk for affective disorders and resistance to antidepressant and antipsychotic treatments that target the 5-HT system.

Not all studies have identified the association of rs6295 with depression or psychological symptoms [170–172]. In these cases, the allele frequency was close to 50 per cent; hence, the expected effect size would be very small as argued already. In addition, stronger associations may be expected by use of ethnically homogenous populations [145], or examination of robust phenotypes, such as current depression or completed suicide rather than personality traits or suicidal thoughts, as done in the above-mentioned studies. A stronger association of rs6295 with current depression compared with depression traits is consistent with the idea that normal subjects may be able to compensate for 5-HT$_{1A}$ dysregulation in the presence of the G(-1019) allele, as suggested by PET imaging studies (see above). Most importantly, investigation of the association of rs6295 with a specific depression subtype (e.g. depression with co-morbid anxiety [150]) or specific endophenotypes (e.g. limbic activation, amygdala volume), appears to provide stronger associations in very small depression cohorts [124,157,173]. However, these studies need independent replication. The challenge remains to uncover reliable biomarkers and endophenotypes to distinguish different forms of depression and anxiety. By understanding the actions of specific functional polymorphisms, such as rs6295, it may be possible to sub-categorize different types of depression and rationally design optimal treatment strategies.

Based on studies of the 5-HT transporter long polymorphic repeat (5-HTTLPR), a well-studied promotor polymorphism [174], the role of early or late life stress in increasing vulnerability to depression or anxiety of the rs6295 polymorphism has also been examined. Unlike the 5-HTTLPR, early life stress did not appear to interact with the *Htr1a* genotype. The homozygous G(-1019) genotype was associated with panic disorder [152], but there was no interaction with early life stress. Similarly, no environment effect was seen in children with attention-deficit hyperactivity disorder (ADHD) for the association of the G-allele on emotional or anxiety behaviour [175]. Rather, recent stress may interact more strongly with rs6295 than early life stress in predisposing to depression [148]. Similarly, recent stress events, but not early life events, interacted with the *Htr1a* G-allele in susceptibility to complete suicide [155]. Similarly, bipolar depressed patients were more likely to be hospitalized after recent stress if they had the G/G *Htr1a* genotype [176]. In animal models, early life stress leads to region-specific alterations in 5-HT$_{1A}$ receptor levels [177,178] and appears to interact with late life stress to induce deficits in 5-HT$_{1A}$ receptor signalling [179]. Thus, stress-induced dysregulation of 5-HT$_{1A}$ receptor expression may be exacerbated by the presence of the G-allele, leading to increased predisposition to mental illness. Interestingly, the G-allele is associated with a blunted cortisol response to acute stress [180] and increased stress susceptibility [181], further suggesting a role for altered regulation of the 5-HT$_{1A}$ autoreceptor in impaired stress response in depressed patients [182]. In agreement with this, mice with an increase in 5-HT$_{1A}$ autoreceptors display impaired stress responses [19]. However, the specific interaction between 5-HT$_{1A}$ genotype and stress on 5-HT$_{1A}$ receptor expression and behavioural outcomes remains to be tested.

Genome-wide association studies have failed to confirm association with candidate genes [183], in part, because not all candidate gene polymorphisms were examined. However, specific genotype analysis for rs6295 identified an association of the G-allele with more severe depression symptoms and reduced response to citalopram in a subgroup of the STAR*D sample [167]. In the larger sample, rs6295 was not examined, but other 5-HT$_{1A}$ polymorphisms were associated with antidepressant response. Interestingly, preliminary studies suggest that the C-allele may be associated with risk of illnes for premenstrual dysphoria [184] or ADHD [185]. In a separate ADHD cohort, the G-allele was associated with decreased anxiety-fear disorders [175]. These findings need replication, but may suggest that hyperactivity of the 5-HT system due to fewer 5-HT$_{1A}$ autoreceptors could predispose to certain disorders such as ADHD.

7. CONCLUSION: A MODEL FOR 5-HT$_{1A}$ RECEPTOR DYSREGULATION IN AFFECTIVE DISORDERS

The results from studies in animal models and in human depression and anxiety suggest that altered 5-HT$_{1A}$ receptor expression leads to impaired serotonergic function and predisposes to depression and anxiety disorders

(figure 1). The G-allele of rs6295 5-HT$_{1A}$ promoter polymorphism or loss of Deaf1 function is associated with 5-HT$_{1A}$ autoreceptor upregulation *in vivo* [85,101], and inhibition of 5-HT neuronal firing frequency to reduce 5-HT release [19]. In addition, the C(-1019)G polymorphism reduces 5-HT$_{1A}$ heteroreceptor expression in a cell-specific manner [134], with loss of Deaf1 reducing 5-HT$_{1A}$ receptor levels in prefrontal cortex [85]. Other *Htr1a* polymorphisms in linkage disequilibrium with rs6295 have yet to be functionally characterized and may augment pre- or post-synaptic functions of the C(-1019)G change. Finally, stressful life environment, especially recent stress appears to enhance the susceptibility to depression or suicide that is conferred by the G(-1019) allele. Glucocorticoid-mediated downregulation of hippocampal 5-HT$_{1A}$ heteroreceptor expression could synergize with genotype-driven reductions. Presynaptically, glucocorticoid-induced repression may be particularly important in anxiety, whereas in depression, a blunted cortisol response and lack of stress sensitivity could reduce the effect of cortisol. In anxiety, 5-HT$_{1A}$ autoreceptor expression may be unaffected or reduced due to glucocorticoid-induced repression, while post-synaptic 5-HT$_{1A}$ heteroreceptors would be strongly reduced by both cortisol and rs6295 genotype. On the other hand, in depression, a G-allele-driven increase in 5-HT$_{1A}$ autoreceptor expression would mediate a reduction in 5-HT neuronal activity that predisposes to a depression phenotype [19,20].

Based on the different and sometimes opposing roles of pre- and post-synaptic 5-HT$_{1A}$ receptors in 5-HT regulation and behaviour, we propose that selective pharmacological manipulation of 5-HT$_{1A}$ autoreceptors or heteroceptors might provide a way to improve the treatment of depression and anxiety. Potential approaches to selectively target the 5-HT$_{1A}$ autoreceptor could include targeting its greater autoreceptor reserve [186], Gi3-selective signalling [187,188], or desensitization with biased ligands [189]; targeting its differentially regulation by transcription factors, such as Deaf1 or Freud1 [61]; or by enhancing the use of siRNA-based ligands to downregulate its expression [60]. Recently, intranasal administration of a chemical conjugate of an SSRI to 5-HT$_{1A}$ siRNA was shown to selectively reduce 5-HT$_{1A}$ autoreceptor expression and exert a rapid antidepressant effect, suggesting a novel clinical approach for antidepressant treatment [60]. A combination of selective 5-HT$_{1A}$ autoreceptor inactivation and SSRI treatment should lead to more effective and rapidly acting antidepressant treatment strategies.

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