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Genetics of emotional regulation: the role of the serotonin transporter in neural function

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Identifying biological mechanisms through which genes lead to individual differences in emotional behavior is paramount to our understanding of how such differences confer risk for neuropsychiatric illness. The emergence of techniques such as *in vivo* imaging of brain function in humans and genetic engineering in rodents has provided important new insights into the impact of serotonin (5-HT), a key modulator of emotional behavior, on neural systems subserving anxiety and depression. A major finding has been the discovery of genetic variation in a crucial regulatory molecule within the 5-HT system, the 5HT transporter (5-HTT), and its influence on emotional traits. The study of the 5-HTT provides a new foundation for understanding the neurobiological and genetic basis of emotional regulation and affective illness.

Introduction

The nature of individual differences in human behavior and such complex emergent phenomena as character, temperament and personality remains a fundamental question in neuropsychology. In parallel, there is a growing emphasis on identifying specific biological pathways that contribute to complex cognitive and emotional behaviors; an endeavor central to our understanding of how individual differences in these behaviors emerge and how such differences confer relative vulnerability (and resilience) to psychiatric disease. One approach is to understand behavior at the genetic level. Genes can impact all levels of biology and, in the context of behavioral pathologies, not only transcend phenomenological diagnosis, but represent ultimate mechanisms of disease. As such, understanding the genetic basis of neuropsychiatric disease can serve to identify at-risk individuals as well as target the development of new treatments to salient biological pathways.

Of course, most human behaviors cannot be explained by genes alone. Indeed, it is possible that genetic factors play a relatively minor role in determining inter-individual differences in many complex behaviors, including emotion, in the absence of appropriate environmental conditions. Nonetheless, it is becoming increasingly clear that even common variations in genetic sequence that impact gene function contribute significant, measurable variance to these more complex phenomena. Recent advances in both molecular genetics and non-invasive functional neuroimaging have begun to provide the tools necessary to explore these and other behaviorally relevant biological mechanisms. In this review, we highlight recent findings from one particularly fruitful avenue of research focused on understanding the impact of genetically driven variation in serotonin (5-hydroxytryptamine; 5-HT) function on the development and integrity of neural systems which support emotional behaviors and which are implicated in the pathophysiology of mood disorders.

Consistent with a long-standing hypothesis positing a major role for 5-HT in the modulation of emotional states [1], abnormalities in 5-HT function are found in patients with mood and anxiety disorders [2] and the 5-HT system is a primary target for drugs that are efficacious in treating these disorders [3]. In humans and rodents, corticolimbic neural circuits that mediate emotional behaviors and which are implicated in the

Glossary

Epistasis: The nonadditive interaction between genes at different loci effecting a phenotype (i.e., the effect of one gene is dependent on that of another).
Transgenic and gene targeting: The insertion of DNA either randomly (transgenesis) or into a specific site (gene targeting) in the genome to alter the function of a molecule of interest.
Hardy–Weinberg equilibrium: When allele and genotype frequencies are stable over time (generations) in a given population under certain conditions.
***In vivo* chronoamperometry:** A method for assessing the clearance of endogenous 5-HT from the extracellular space via quantification of changes in the current evoked by oxidation of 5-HT over time.
***In vivo* microdialysis:** A technique to measure levels of endogenous 5-HT in the extracellular space by sampling from extracellular fluid extracted via a dialysis fiber implanted into the brain.
Meta-analyses: Statistical approach whereby data from published independent studies are pooled to assess the general pattern between variables of interest.
Polymorphism: A gene sequence variant present at >1% in a population.
Voxel-based structural MRI: Automated technique for the identification of structural changes in grey matter, white matter and cerebrospinal fluid measures derived from structural MRI data.

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pathophysiology of emotional disorders are densely innervated by 5-HT neurons and exhibit rich expression of 5-HT receptors [4]. Supporting the functional importance of this system to affect regulation, a corpus of data demonstrates that experimentally induced alterations in these 5-HT pathways produce changes in emotion-related behaviors [5].

Genetic variation in the serotonin transporter

5-HT activity in the brain, like that of the other monoamine neurotransmitters, is regulated by a sodium-chloride-dependent transporter located in the plasma membrane of the cell [6]. Following release of 5-HT, the pre-synaptically located 5-HTT returns 5-HT to the cell for recycling or metabolic degradation. Under normal physiological conditions, the reuptake mechanism of the 5-HTT is the principle means for actively clearing 5-HT from the extracellular space. Thus, the 5-HTT plays an essential role in determining the duration and intensity of the 5-HT communication with its receptors on postsynaptic targets such as those located in limbic regions mediating emotion, and with presynaptic receptors that exert inhibitory control over the 5-HT neuron itself (Figure 1).

Given the pivotal role of the 5-HTT in controlling 5-HT neurotransmission, the finding that the function of this molecule is itself under genetic regulation is of great interest. Hints that abnormalities in emotional regulation might be related to 5-HTT dysfunction came from evidence that drug-free patients with mood and anxiety disorders exhibited significant reductions in brain 5-HTT binding relative to normals [7,8]. Then almost a decade ago, Lesch, Murphy and colleagues described a relatively common genetic polymorphism (see Glossary) in the human 5-HTT gene (*SLC6A4*) located on chromosome 17q11.1-q12 [9]. Having one or two copies of the short, 'S,' allelic form of this polymorphism is associated with significantly lesser

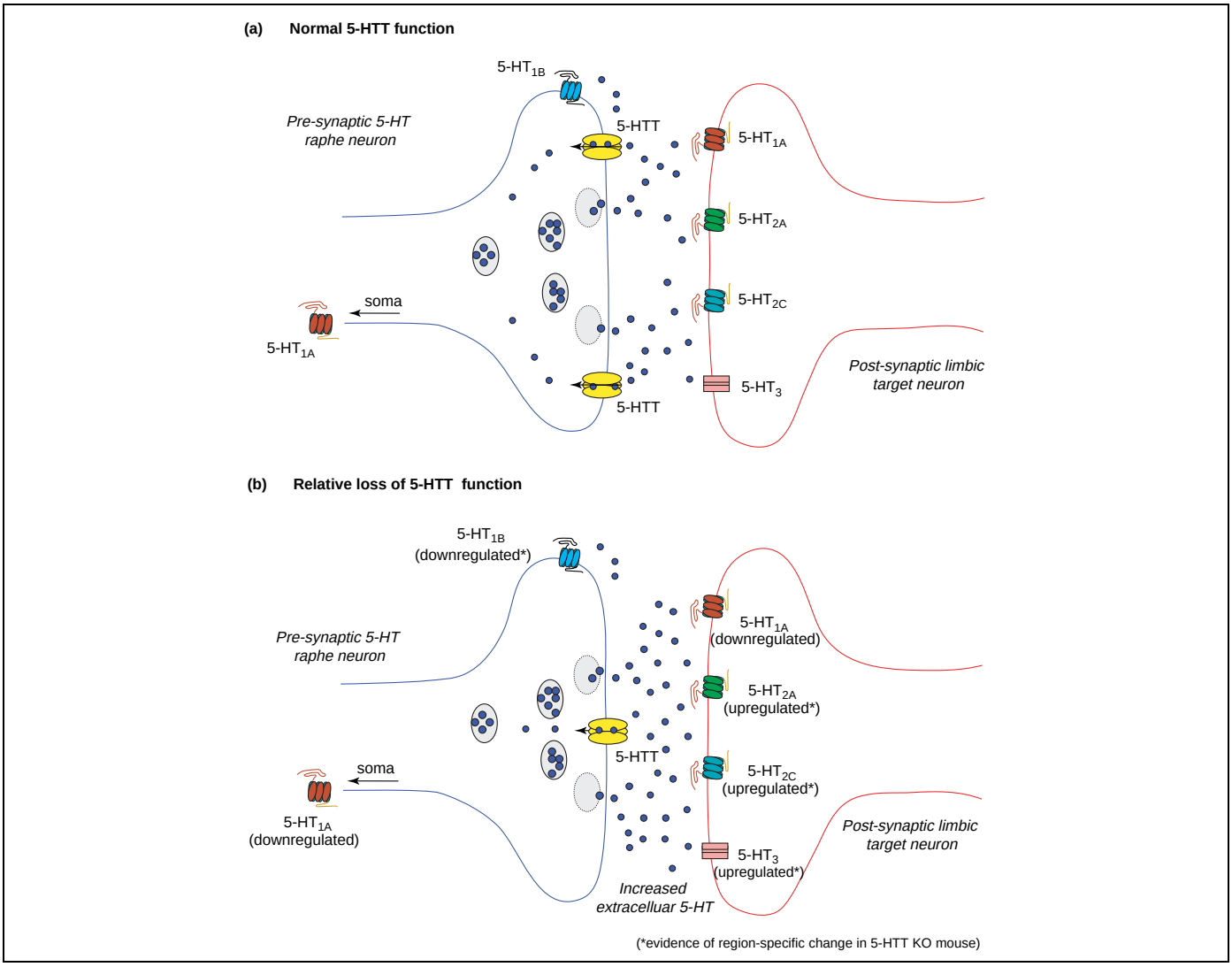

Figure 1. Putative effects of 5-HTT gene variation on human 5-HT neurotransmission based on findings from 5-HTT knockout mice. **(a)** Following release of 5-HT, 5-HTT actively returns 5-HT to the presynaptic neuron and thereby determines the duration and intensity of 5-HT communication with its receptors on postsynaptic targets located in limbic regions mediating emotion. **(b)** A low-expressing ('S allele') form of the human 5-HTT gene has been associated with relatively lesser 5-HTT mRNA transcription and 5-HTT binding, and reduced platelet 5-HT reuptake [9–11], as well as reduced 5-HT1A receptor binding in brain [61]. In mice genetically engineered without a functioning 5-HTT, loss of 5-HTT gene function increases extracellular levels of 5-HT and leads to brain region-specific reductions in 5-HT1A and 5-HT1B receptor binding and increases in 5-HT2A, 5-HT2C and 5-HT3 receptor mRNA levels and/or ligand binding [15–17,24,60]. Although the net effect of these complex changes is not fully understood, they might contribute to alterations in emotional processing associated with a relative loss of 5-HTT function in S allele carriers.

Box 1. Genetic variation in the 5-HTT and effects on 5-HT function

The 5-HTT-linked polymorphic region (5-HTTLPR) is a variable repeat sequence in the promoter region of the gene that encodes two allelic forms: a short (S) variant comprising 14 copies of a 20–23 base pair irregular repeat unit, and a long (L) variant comprising 16 copies. In populations of European ancestry, the frequency of the S allele is ~0.40, and the genotype frequencies are in Hardy–Weinberg equilibrium (see Glossary) (LL=0.36, LS=0.48, SS=0.16) (although these relative allele frequencies vary substantially across different populations [71]).

Although there are several examples of polymorphisms that are of potential interest to psychiatry but whose precise functionality is undetermined [72], there is good evidence that the 5-HTTLPR modifies the function of the 5-HTT. Using cultured lymphoblast cell lines or platelets as an assay for effects of the polymorphism in brain, the S allele has been associated with significantly lower concentrations of 5-HTT mRNA and nearly twofold lesser 5-HT re-uptake in comparison with preparations homozygous for the L allele [11,73]. In the brain itself, analysis of post-mortem tissue revealed lesser 5-HTT binding in individuals carrying the S allele in the region of the dorsal raphe nuclei, which contain the major 5-HT-producing neurons and the highest density of 5-HTT [74]. A similar finding was obtained in living brain using single-photon-emission computed tomography (SPECT) imaging of binding to the 5-HTT (and dopamine transporter) radioligand, β-CIT [11].

These findings indicate less 5-HTT transcription, expression and function in S allele carriers than in L allele homozygotes. However, not all studies have been able to detect clear effects of the 5-HTTLPR on 5-HTT availability (see [75]). There are several factors that could contribute to these discrepancies across studies, including differences in population stratification, sex, age, imaging methodology, and the specific brain regions examined. There is also evidence that additional regulatory variation within the 5-HTT gene influences transcription and biases the ability to detect effects of the 5-HTTLPR. For example, Goldman and colleagues have recently demonstrated that a single nucleotide polymorphism (SNP) located in the L allele determines whether the allele acts like an L or an S allele in terms of effects on 5-HTT mRNA [76]. Additional sources of epistasis (see Glossary) within the 5-HTT gene have been described [77–79], and it is likely that still more will be identified. A better understanding of these and other (including non-5-HTT sources of epistasis) genetic factors controlling 5-HTT function will be essential to our ability to understand and predict the effects of 5-HTT gene variation on emotional phenotypes.

5-HTT binding in brain [10], and lower 5-HTT mRNA expression and 5-HT uptake in lymphoblasts relative to having two copies of the long, 'L,' allelic variant [9,11]. However, given growing evidence of the complexity of the 5-HTT gene beyond this dichotomy, together with the inherent limitations of studying genetic effects on brain function in humans, it has proven difficult to precisely elucidate the effects of the S allele on 5-HT function (see Box 1).

In this context, the emergence of transgenic and gene targeting techniques has allowed for the generation of mutant mouse models that have been integral to understanding genetic effects on the 5-HT system [12,13]. Rodents do not have an orthologue of the 5-HTTLPR polymorphism. However, by deleting a critical region of the gene via homologous recombination, mice have been engineered with a functionally inactive 5-HTT (5-HTT knockout, KO) [14]. Studies in these mice using *in vivo* chronoamperometry and microdialysis (see Glossary) have shown that loss of 5-HTT gene function results in impaired clearance and marked elevation of extracellular levels of 5-HT in forebrain regions including the hippocampus and frontal cortex [15,16] (Figure 1). This primary effect is associated with a complex array of adaptive changes in 5-HT synthesis and metabolism and, as discussed below, in the expression and function of various 5-HT receptors [17]. Whether comparable changes occur in individuals carrying the S allele is unclear and inherently more difficult to demonstrate in humans. Indeed, it is important to bear in mind that the 5-HTT KO mouse provides a simplified model system of 5-HTT gene variation with limitations inherent in any animal analogue of complex human brain functions and behavioral processes [12]. Notwithstanding, findings from 5-HTT KO studies provide insight into the possible neurochemical phenotype of S allele carriers, such as increased extracellular 5-HT concentrations and altered 5-HT receptor densities/function, and thereby provide a unique basis for understanding the emotional abnormalities associated with alterations in 5-HTT gene function. This approach will be yet further strengthened by the identification and development of novel rodent models of 5-HTT gene variation, such as the naturally occurring 5-HTT gene variants across mouse and rat strains.

The effects of 5-HTT gene variation on anxiety

At the time of the discovery of the 5-HTTLPR and of its potential effects on human brain 5-HT function, Lesch and colleagues [11] reported that individuals carrying the S allele displayed higher levels of trait anxiety and particularly 'neuroticism' and 'harm avoidance' than LL homozygotes. The 5-HTT polymorphism accounted for 3–4% of the inter-individual variance and 7–9% of the genetic variance within the normal range of anxiety in a healthy, rather than clinical, population. In other words, the effect of the polymorphism was to bias towards increased anxiety rather than deterministically cause extreme emotional disturbances. An association between the S allele and various measures of heightened fear and anxiety in normal populations has been replicated by several studies (see [18]). Notably, three independent meta-analyses (see Glossary) have demonstrated a significant association between the S allele and increased neuroticism or harm avoidance [19–21]. However, other studies, including an exceptionally large study of >85 000 subjects, have failed to find an association [22]. A degree of inconsistency appears to be inherent in genetic association studies (see Box 1), and might partly reflect the genetically complex (polygenic) nature of 'anxiety,' with a single gene variant such as the 5-HTTLPR contributing only a small amount of the overall inter-individual variance within the milieu of other genetic (see Box 2) and environmental influences [23].

In this context, 5-HTT KO mice provide a unique means to study the effect of loss of 5-HTT gene function on anxiety-related behaviors under genetically and environmentally controlled conditions. Interestingly, 5-HTT KO mice exhibit evidence of increased anxiety-like

Box 2. Gene–gene effects on emotion

The effect of functional variation in a single gene such as the 5-HTT on a complex emotional trait will be modified, not only by environmental factors, as discussed in the main text, but also by other genetic factors (ancestral origins in man, background strain in mouse). In this context, genetic background strain has been shown to exert a major influence on the manifestation of anxiety- and depression-related phenotypes in 5-HTT KO mice [24,48]. Along similar lines, contributions of multiple genetic polymorphisms acting in concert or opposition in the context of unique environmental challenges will ultimately account for the majority of variance in a given emotion-related neural or behavioral phenotype.

There is emerging evidence that allelic variants identified in various 5-HT genes, particularly those representing crucial bottlenecks in 5-HT synthesis, reuptake and metabolism can exert alterations in 5-HT neurotransmission and consequently, the functional integrity of affective brain circuits. For example, a promoter polymorphism in the human MAOA gene, responsible for the intracellular catabolism of 5-HT to 5-HIAA, has been associated with altered transcriptional activity and heightened levels of aggression and impulsivity in men [80]. In addition, a polymorphism in the human gene for tryptophan hydroxylase (TPH), the rate-limiting enzyme responsible for catalyzing the oxygenation of tryptophan to 5-hydroxytryptophan, has been associated with increased risk of suicide, impulsivity, aggression and alcoholism [81]. These findings have been recently extended based on findings in mice that there exists a second TPH gene (*TPH2*) exclusively responsible for regulating TPH expression and 5-HT synthesis in the murine brain [82]. Brown and colleagues demonstrated the first *in vivo* significance of human TPH2 (*hTPH2*) in brain by establishing an association between a frequent regulatory SNP in *hTPH2* and increased amygdala reactivity [83]. Notably, the region of the amygdala exhibiting an *hTPH2* effect significantly overlaps with that associated with the 5-HTTLPR S allele, raising the possibility that variability in the functional reactivity of the amygdala might reflect the cumulative impact of genetically driven variation in multiple 5-HT subsystems. Elucidating the nature of such complex gene–gene interactions is a crucial question for future research in this field and will require a major undertaking of efforts using both neuroimaging and mutant mouse approaches.

behavior on various tests that are validated for their sensitivity to drugs that affect anxiety in humans, such as the elevated plus-maze and light/dark exploration test [24,25]. Indeed, demonstrating an impressive consistency of this phenotype not always found in mutant mouse models [26], this anxiety-like phenotype has been replicated in a separately generated 5-HTT KO mouse model [27]. In addition, recent data show that a third line of mutants lacking the C-terminus of the 5-HTT also exhibit heightened anxiety-like behavior [28]. Thus, the effects of genetic inactivation of the 5-HTT on anxiety-like behavior in mice are robust and provide an independent line of evidence supporting a link between the low-expressing 5-HTT gene variant with anxiety in humans.

The effects of 5-HTT gene variation on stress-reactivity

Emerging evidence from human and animal studies indicates that relative loss of 5-HTT gene function not only biases towards increased anxiety but exerts a negative influence on the capacity to cope with stress. Although there is compelling evidence that stress increases risk for emotional disorders and does so in a manner suggestive of a strong genetic influence, specific genes involved this process have been difficult to identify [29,30]. However, in an influential study by Caspi and colleagues, individuals with the S allele were found to be more likely to suffer from major depression, but crucially, only if they had also suffered multiple traumatic life events, such as childhood abuse or neglect, the loss of a job or a divorce [31]. The finding that individuals with the S allele are at greater risk for depression following stress than are L allele homozygotes has been replicated by other groups [32,33] (but see [34,35]). Indeed, the available evidence suggests that the S allele might increase sensitivity to the depressogenic effects of a variety of environmental insults ranging from broad indices of stressful living [36,37], to specific neurochemical challenges such as tryptophan depletion [38] (see Table 1). In addition, once depression is manifest clinically, the S allele appears to be associated with more severe symptomatology and poorer prognosis (including a weak or even adverse response to antidepressant drugs) [39–42].

The hypothesis that variation in 5-HTT gene function increases vulnerability to environmental stress again finds strong support from research using animal models. Rhesus macaques carrying an orthologue of the 5-HTTLPR S allele have been found to exhibit exaggerated behavioral and neuroendocrine responses to stress and abnormalities in 5-HT metabolism, but only when reared in a stressful environment [43,44]. 5-HTT KO mice show significant behavioral, neuroendocrine and

Table 1. Relative loss of 5-HTT gene function is associated with increased vulnerability to environmental stress across species

Environmental stressor	Endpoint measure
Low-expressing form of the human 5-HTT gene	
Multiple traumatic life events	Increased incidence of depression [31–33]
Childhood abuse or neglect	Increased incidence of depression [31,32]
Tryptophan depletion	Increased relapse to depression [38]
Unemployment and low socio-economic status	Increased 'mental distress' and decreased 5-HT responsivity [36,37]
Low-expressing form of the Rhesus macaque 5-HTT gene	
Early life maternal separation	Exaggerated behavioral and neuroendocrine response to stress [43,44]
Mutant mice lacking a functional 5-HTT	
Exposure to rat predator	Exaggerated catecholamine responses [46]
Intraperitoneal injection of saline	Exaggerated neuroendocrine response [45]
Exposure to cat odor	Increased anxiety-like response [47]

Table 2. Imaging genetics studies of 5-HTTLPR effects on amygdala reactivity to emotionally provocative stimuli

Sample demographics (reference)	Challenge stimuli	5-HTTLPR effect on amygdala reactivity
American volunteers (N=28) [50]	Angry and fearful faces	S carriers>LL
American volunteers (N=93) [18]	Angry and fearful faces	S carriers>LL
German volunteers (N=29) [53]	Aversive, pleasant, and neutral pictures	S carriers>LL
Italian volunteers (N=28) [58]	Angry and fearful faces	S carriers>LL
American volunteers (N=41) [55]	Negative, positive, and neutral words	S carriers>LL
American volunteers (N=55) [60]	Angry and fearful faces	S carriers>LL
German patients with panic disorder (N=20) [57]	Angry, fearful, happy and neutral faces	S carriers>LL
Dutch patients with social phobia (N=17) [58]	Public speaking task during O15 PET	S carriers>LL

catecholamine responses to relatively mild stressors that are not sufficiently potent to affect wild-type controls, such as saline injection or exposure to a live rat or the odor of a cat [45–47] (see Table 1).

'Depression-related behavior' is also increased in 5-HTT KO mice in well-validated tasks including the passive avoidance and forced swim tests [48,49]. Interestingly, given the strong interaction between the S allele and stress-load described above, 5-HTT KO mice on at least one particular genetic background (C57BL/6J) only exhibit increased depression-related behavior following repeated exposure to the uncontrollable stress of the forced swim and tail suspension procedures ([28], Wellman *et al.*, unpublished). These converging lines of evidence support the hypothesis that relative loss of 5-HTT gene function leads to increased emotional reactivity and sensitivity to environmental stress across species.

5-HTT gene variation and amygdala reactivity to environmental stimuli

Recent studies have begun to shed light on how the relationship between the 5-HTTLPR S allele and stress-reactivity is mediated at the level of the neural pathways regulating emotion. Using the sensitivity of noninvasive neuroimaging such as functional magnetic resonance imaging (fMRI), Hariri and colleagues assessed neural activation in a relatively small (N=14) number of S allele carriers during perceptual processing of fearful and angry human facial expressions [50]. This simple perceptual task is known reliably to engage the amygdala [51], a brain region believed to be central to the neural circuitry mediating emotional arousal and vigilance across species [52]. During the task, S allele carriers exhibited nearly fivefold greater amygdala activity than L homozygotes, a difference accounting for ~20% of the total variance in the amygdala response to fearful and angry faces [50]. Moreover, the effects of the 5-HTTLPR S allele did not extend to the response of the dorsolateral prefrontal cortex during a separate working memory challenge, arguing against a general neural hyperexcitability associated with the variant.

Amygdala hyper-reactivity to emotionally provocative stimuli in comparison with emotionally neutral stimuli in S allele carriers has been reported in four separate cohorts of healthy subjects [53–56] as well as panic disorder patients [57] and social phobics [58] (see Table 2). In addition, Hariri *et al.* replicated their original finding in a large, independent cohort of volunteers (N=92) that were carefully screened to exclude a history of psychiatric illness or treatment [18] (Figure 2). This latter study also revealed that the S allele effect on amygdala hyper-

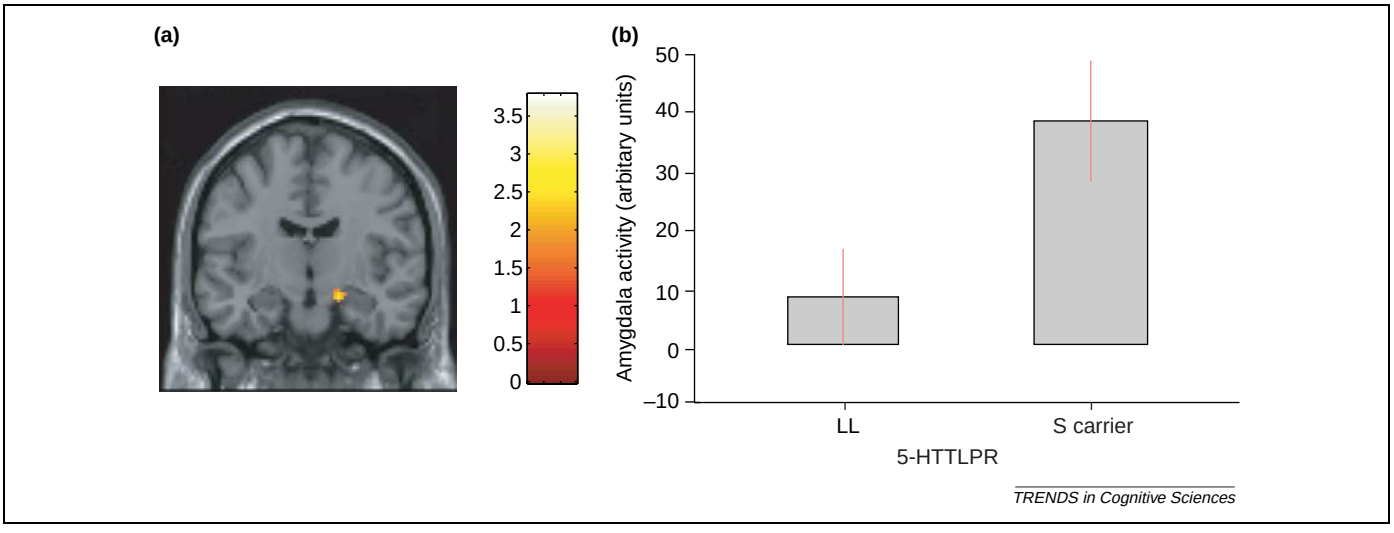

Figure 2. The low-expressing (S allele) 5-HTT gene variant is associated with greater amygdala reactivity in response to emotionally provocative stimuli. **(a)** Illustration of the greater mean right amygdala activity in S allele carriers than in L allele homozygotes (S carriers>LL). Reproduced with permission from [18]. **(b)** Activity of this same right amygdala region in single-subjects from both genotype groups (S carriers versus LL). Note, sample size in most studies has limited the analyses of genotype effects to S carriers (SS and LS) versus L homozygotes (LL). However, both the initial in vitro studies of the 5-HTTLPR [9,11], and a subsequent in vivo study [18] indicate that the S allele has a dominant effect on gene expression and amygdala activity and, thus, support this more general classification scheme.

reactivity was not affected by allele load (i.e. having either one or two S alleles) or by sex. It is also important to emphasize that the 5-HTTLPR S allele effects on amygdala reactivity in all but two of these studies were found in samples of healthy volunteers with no history of affective or other psychiatric disorders. Thus, as noted earlier in the context of 5-HTTLPR effects on trait anxiety, the S allele influence on relative amygdala hyper-reactivity is not in and of itself a predictor of emotional dysfunction.

These findings identify a possible neuroanatomical locus for the negative affect bias associated with the S allele. However, the precise molecular and cellular basis of 5-HTTLPR effects on amygdala reactivity remains to be determined. As schematized in Figure 1, a simple model would predict that putative increases in extracelluar 5-HT in S allele carriers would cause increased activation of presynaptic autoreceptors (5-HT_{1A}, 5-HT_{1B}) on 5-HT neurons, as well as postsynaptic 5-HT receptors (e.g. 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3) on target neurons in regions including the amygdala. However, the consequences of these changes for 5-HT neurotransmission are likely to be highly complex, in part owing to adaptive changes in 5-HT homeostasis resulting from the constitutive reduction in 5-HTT function. Indeed, studies in 5-HTT KO mice have shown that the expression and function of various 5-HT receptors is markedly altered by the gene mutation.

Of particular relevance to the amygdala hyper-reactivity associated with the S allele, 5-HTT KO mice exhibit a significant downregulation of 5-HT_{1A} receptors and a corresponding upregulation of 5-HT_{2C} receptors in the amygdala [24,59]. Because 5-HT_{1A} receptors exert inhibitory effects on postsynaptic neurons whereas 5-HT_{2C} receptors are excitatory, these changes could conceivably shift the balance of 5-HT effects in the amygdala towards hyper-excitability. In addition, 5-HTT KO mice also exhibit a marked downregulation of 5-HT_{1A} autoreceptor function on dorsal raphe neurons, which reduces the ability of the 5-HT neurons to self-regulate [17,60]. A loss of inhibitory control of 5-HT firing and release could possibly serve to further exacerbate the excitatory-bias of 5-HT effects in the amygdala during emotional provocation.

These predictions remain to be tested empirically in 5-HTT KO mice. It also remains to be determined whether or not such functional alterations occur in S allele carriers. However, demonstrating that at least one important change is also seen in S allele carriers, a recent positron emission tomography (PET) study found that 5-HT_{1A} receptor binding is significantly reduced in several brain regions, including the amygdala, dorsal raphe nucleus and prefrontal cortex [61] (but see [62]).

5-HTT gene variation and the neural circuitry of affect regulation

Although the available evidence implicates the amygdala as a key locus underlying the emotional phenotype associated with the S allele, this region is only one component within a distributed and interconnected system of cortical and subcortical pathways regulating affect. In this context, a growing literature from studies in humans and animals points to the importance of a circuit linking the prefrontal cortex (PFC) and amygdala in the modulation of emotion and in the pathophysiology of affective illness [63,64]. Recent data indicate that 5-HTT gene variation is associated with functional alterations in this circuit.

Using voxel-based structural MRI (see Glossary) Pezawas and colleagues found that gray matter volume in a specific region of the PFC previously implicated in affect regulation, the perigenual anterior cingulate cortex (pACC) [63,65], was significantly reduced in S-allele carriers as compared with L-allele homozygotes [66]. Moreover, fMRI analysis of relative activation of the pACC and amygdala during presentation of angry and fearful faces revealed a relatively weaker functional coupling between these regions in the S allele carriers. One interpretation of these data is that the integrity of this key cortico-amygdala pathway is compromised in S allele carriers, resulting in a loss of functional integration and inhibitory regulation of amygdala mediated behavioral and physiologic arousal to environmental provocation (Figure 3). In support of this hypothesis, this study found that although regional functional or structural measures of the pACC or amygdala alone were poorly predictive of an S allele effect on emotional behavior [18,50], indices of functional coupling between the pACC-amygdala accounted for almost 30% of the variance in anxious temperament [66].

Further evidence that alterations in PFC-amygdala connectivity contributes to emotional abnormalities in S allele carriers was recently provided by Heinz and colleagues [53] and replicated by Pezawas *et al.* [66]. This study found that a region of the PFC more dorsal and rostral to the pACC was over-activated in S allele carriers viewing emotionally provocative scenes. In turn, activation of this ventromedial PFC region was positively correlated with amygdala excitability, suggesting that increased activity in this region of the PFC reflected a compensatory effort to regulate the exaggerated amygdala responses of S allele carriers.

The current paucity of data on the comparative anatomy and function of the human versus rodent PFC [67] means that parallels between phenotypic changes in the PFC of S allele carriers and 5-HTT KO mice must be made cautiously. It is certainly noteworthy however, that 5-HTT KO mice exhibit evidence of possible compensatory alterations in a region of the PFC considered to be homologous to that found to be over-activated in S allele carriers. Specifically, pyramidal neurons in the infralimbic cortex of the ventromedial PFC exhibit increased dendritic growth in 5-HTT KO mice as compared with wild-type controls (Wellman *et al.*, unpublished). Moreover, suggesting that these changes may have functional correlates, 5-HTT KO mice exhibit impairment in the recall of fear extinction (Wellman *et al.*, unpublished), a form of emotional regulation that is modulated by a pathway connecting the ventromedial PFC and amygdala in rodents and humans [68,69]. Abnormal PFC function could also contribute to the impaired stress-coping in these KO mice. A recent study by Amat and colleagues

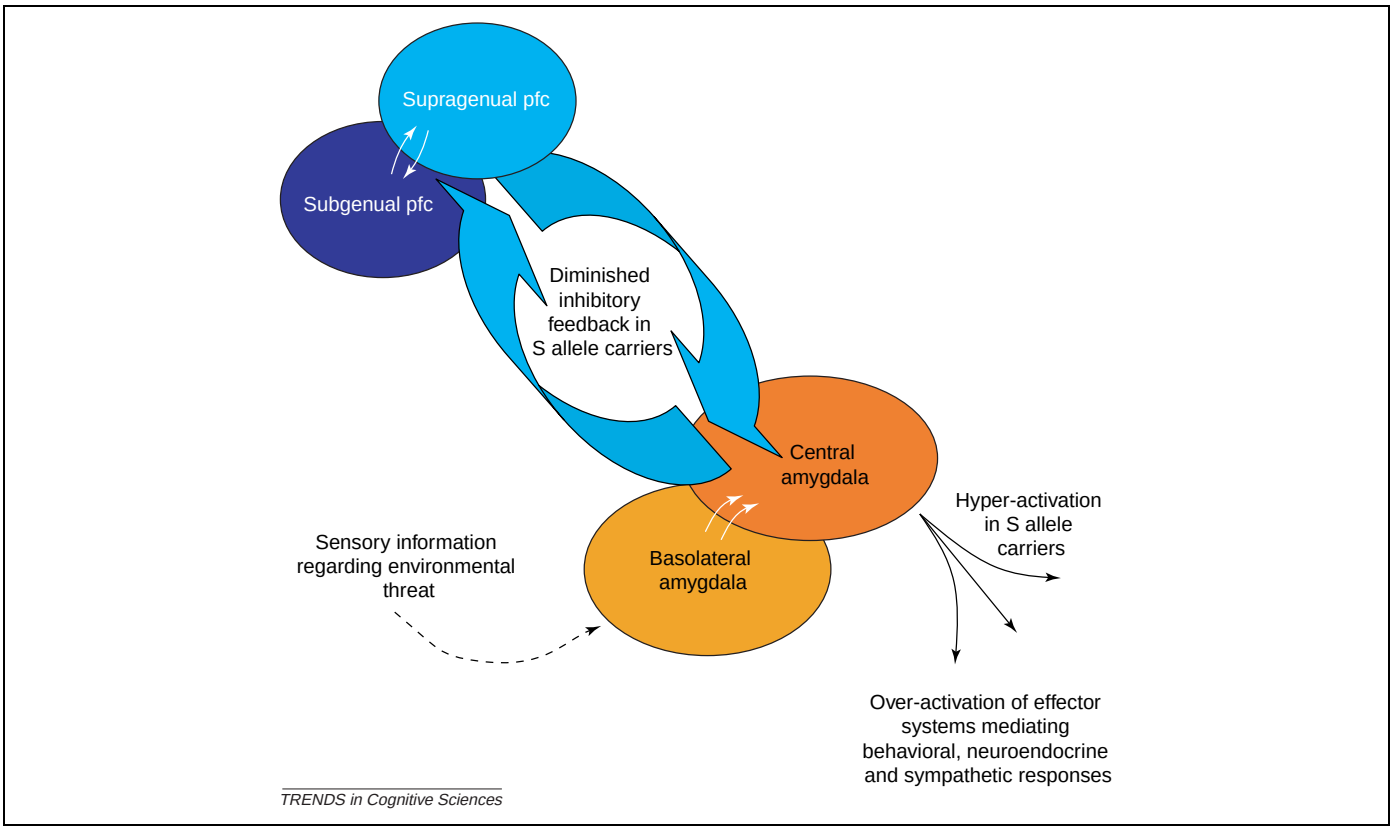

Figure 3. A working model of how 5-HTT gene variation alters the neural circuitry regulating emotion. A low-expressing ('S allele') form of the human 5-HTT gene is associated with a relative exaggeration in the response of the amygdala to anxiety-provoking stimuli [18,50,53–58]. S allele carriers further exhibit diminished communication between the amygdala and components of the pACC, the sub- and supragenual ACC [66], which might result in a reduced capacity for integration of arousal in the planning and execution of complex behaviors and responses to the environment. This deficit in processing emotional information may in turn manifest at the behavioral level as increased negative emotionality and stress vulnerability. Abnormalities in functional connectivity between the prefrontal cortex and amygdala during emotional processing have been independently reported in S allele carriers [53,66] and mutant mice lacking the 5-HTT (Wellman et al., unpublished). Thus, the integrity of this key cortico-amygdala pathway appears to be affected by genetic variation in 5-HTT function.

demonstrated that inactivation of the ventromedial PFC produced learned helplessness behavior in rats exposed to uncontrollable stress [70].

Taken together, the available data from human and mouse studies provide strong evidence for alterations in the wiring of corticolimbic pathways as a result of 5-HTT gene function. These findings propose a model in which relative loss of 5-HTT gene function alters a distributed, dynamically interacting brain system that not only mediates emotional reactivity (via amygdala) but also regulates and integrates this reactivity (via prefrontal cortex). Precisely how this system is compromised by a relative loss of 5-HTT gene function (see Box 3) and whether it extends to other brains regions implicated in

Box 3. Do the effects of 5-HTT gene variation on emotion originate in development?

That a genetically driven relative loss of 5-HTT function is associated with increased risk for anxiety and depression appears counter-intuitive to the anxiolytic and antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) [3]. These drugs act as 5-HTT blockers and, like the 5-HTTLPR S allele, are predicted to increase extracelluar 5-HT availability [84] (see also Figure 1, main text). However, the clinical effects of SSRIs only manifest after chronic treatment and are associated with molecular and neural alterations downstream of their primary action on the 5-HTT [85]. Even if the therapeutic basis of the effects of SSRIs was fully understood, it is unlikely that the effects of pharmacological 5-HTT antagonism would be synonymous with a genetically driven loss of 5-HTT function.

One major difference between genetic and pharmacological influences on the 5-HTT is that a constitutive alteration in 5-HTT function resulting from a gene variation is apparent during development as well as adulthood. There is therefore the potential for alterations in the development of the neural systems subserving emotion as a result of a relative loss of 5-HTT function in S allele carriers and in 5-HTT KO mice [86]. In this context, there is evidence that the loss of 5-HTT gene function in mice during early development disrupts the cytoarchitecture and function of cortical regions [87,88]. There is also growing evidence from studies in rodents that disruption to 5-HT function during critical periods of ontogeny produces lasting abnormalities in emotion. For example, null mutation of either the 5-HT_{1A} receptor or Pet-1, a transcription factor guiding development of the 5-HT system, produces increased anxiety-like behavior in adulthood (for reviews, see [13,89]). In addition, a recent study found that postnatal treatment with the SSRI fluoxetine causes abnormalities on mouse tasks of emotional behavior [27].

Whether the emotional abnormalities [11,31] and corticolimbic dysfunction [18,50,53–58,66] seen in S allele carriers also has its origins in development is currently unknown. Notwithstanding, the available evidence supports a working hypothesis in which a genetically driven relative loss of 5-HTT function might compromise the development of neural circuits necessary for effectively regulating negative affect and stress later in life. If substantiated, this model could have significant implications for devising early diagnostic and preventative treatment of emotional disorders in childhood.

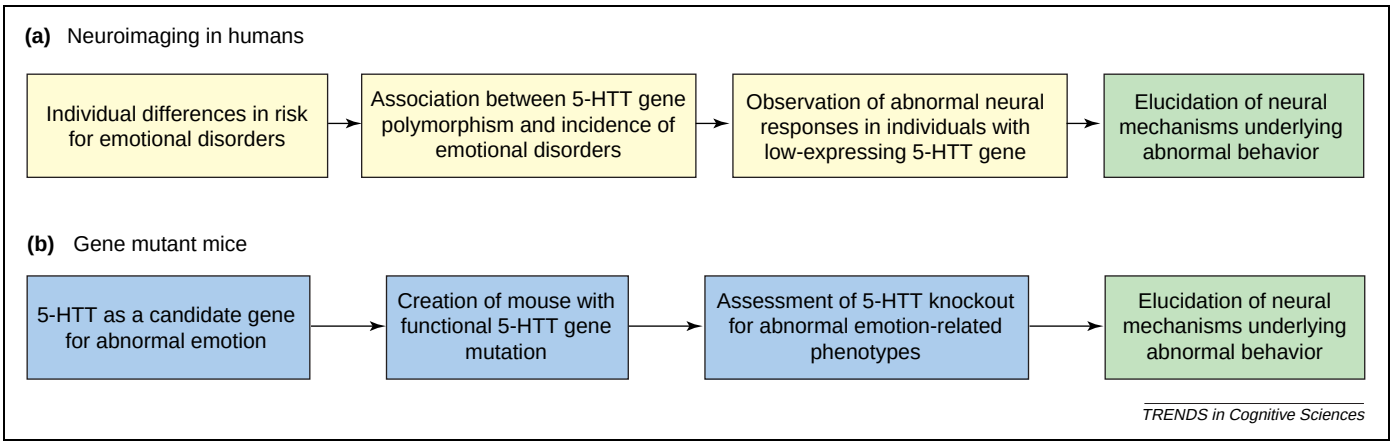

Figure 4. Parallel, complementary approaches to understanding the role of 5-HTT gene variation in influencing emotional regulation using neuroimaging and mutant mice. **(a)** Human genetic studies identified the S allele of the 5-HTTLPR as a factor associated with increased negative emotionality traits and risk for emotional disorders. Functional neuroimaging pinpointed the amygdala as a brain region with exaggerated reactivity to emotionally provocative stimuli in S allele carriers, paving the way for further research to elucidate the precise neural mechanisms underlying the behavioral abnormalities associated with this gene variant. **(b)** The identification of the 5-HTT as a candidate gene for abnormal emotion led to the creation of mice with a functional null gene (knockout) mutation in the 5-HTT. Phenotyping of 5-HTT knockout mice using well-validated tests demonstrated abnormal emotion-related behaviors, supporting the utility of these mice as a model for elucidating the neural mechanisms underlying the effects of 5-HTT gene variation on emotion.

affect regulation (e.g. hippocampus, orbitofrontal cortex) remains to be determined. Notwithstanding, the failure of this circuitry effectively to process environmental threat and adaptively cope with persistent stress provides a possible neural substrate for the emotional abnormalities and increased disease vulnerability associated with genetic variation in the 5-HTT.

Summary

A growing literature from a variety of approaches ranging from noninvasive human neuroimaging studies to gene mutation studies in mice has identified an influence of the gene variation in the 5-HTT in the regulation of emotion. Collectively, these studies have demonstrated that genetically mediated changes in 5-HTT function affect both the structure and function of key corticolimbic pathways regulating the brain's capacity for effectively dealing with stress. Recent evidence suggests that these neural changes contribute to the emergence of individual differences in affect and temperament that are associated with 5-HTT gene variation. With sufficient stress on the system, such heritable differences in corticolimbic reactivity could significantly impact vulnerability to affective illness.

These findings not only identify a promising major candidate gene in psychiatry, they also speak to a fundamental concept regarding how we think about the role of genes in shaping behavior and how we study their ability to influence risk for disease. Moreover, the study of the 5-HTT illustrates how through close dialogue and convergence of experimental approaches, the neurobiological underpinnings of complex behavior and disease could be revealed more rapidly (Figure 4). Clearly, much work still needs to be done to test these models linking genes, brain and bevaior rigorously. Indeed, our understanding of the putative role of 5-HTT gene variation influencing individual differences in emotional regulation and disease risk remains at an early stage.

As behavioral neuroscience advances in the post-genomic era, it becomes increasingly incumbent on investigators from diverse disciplines using divergent methodologies to work together in a reciprocal and mutually informative fashion in the pursuit of knowledge.

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