Targeting the murine serotonin transporter: insights into human neurobiology

Dennis L. Murphy and Klaus-Peter Lesch‡*

Abstract | Mutations resulting in reduced or completely abrogated serotonin-transporter (SERT) function in mice have led to the identification of more than 50 different phenotypic changes, ranging from increased anxiety and stress-related behaviours to gut dysfunction, bone weakness and late-onset obesity with metabolic syndrome. These multiple effects, which can be amplified by gene–environment and gene–gene interactions, are primarily attributable to altered intracellular and extracellular serotonin concentrations during development and adulthood. Much of the human data relating to altered expression of the gene that encodes SERT are based on genetic-association findings or correlations and are therefore not as robust as the experimental mouse results. Nevertheless, SERT-function-modifying gene variants in humans apparently produce many phenotypes that are similar to those that manifest themselves in mice.

Single nucleotide polymorphism
A type of genetic variation within a DNA sequence. It occurs when a single nucleotide (for example, thymine) replaces one of the other three nucleotides (for example, cytosine).

**Laboratory of Clinical Science, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892, USA. ‡Molecular and Clinical Psychobiology, Department of Psychiatry and Psychotherapy, University of Würzburg, 97080 Würzburg, Germany.*
e-mails: DennisMurphy@mail.nih.gov; kplesch@mail.uni-wuerzburg.de
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More than twelve different human behavioural traits and whole-body medical disorders are reported to be associated with serotonin transporter (SERT) gene (*SLC6A4*) variation. Reduced transporter expression and function resulting from variation in the gene's major transcriptional control region (the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR)) is associated with anxiety- and depression-related personality traits[1]. Similarly, the 5-HTTLPR and other regulatory and structural variations (in the non-coding and coding regions, respectively) seem to have a role in neuropsychiatric conditions such as bipolar disorder, depression, anxiety disorders (especially obsessive–compulsive disorder), suicide, eating disorders, substance-abuse disorders, autism, attention-deficit/hyperactivity disorder and neurodegenerative disorders[2–5]. In addition, therapeutic responses and side effects following treatment with selective serotonin-reuptake inhibitors (SSRIs) have been found to be associated with *SLC6A4* variants[6]. Other disorders with *SLC6A4* associations include myocardial infarction, pulmonary hypertension, irritable bowel syndrome and sudden infant death syndrome (SIDS)[4]. Although each of these findings has been replicated at least once or supported by recent meta-analyses[4,7–9], uncertainties remain about the biological bases for the associations[3,4].

Human *SLC6A4* maps to chromosome 17q11.2 and is composed of 14 exons that span ~40 kb (FIG. 1a). The sequence of the transcript predicts a protein made up of 630 amino acids with 12 transmembrane domains. Alternative promoters, differential splicing involving exons 1A, 1B and 1C, and 3′-untranslated-region variability resulting in multiple mRNA species are likely to regulate expression of the gene in humans (FIG. 1a). The transcriptional activity of *SLC6A4* is modulated by a variation in the length of the 5-HTTLPR together with two single nucleotide polymorphisms (SNPs) in this region, rs25531 and rs25532, all of which are located upstream of the transcription start site[1,2] (FIG. 1a). Additional variants at the *SLC6A4* locus include a variable number of tandem repeats (VNTR) polymorphism in functional intron 2 and several other SNPs that change the structure or function of the transporter protein[1,10–12]. Most of these SNPs are rare, but the rs25531 polymorphism has a minor-allele frequency of 9–15% in Caucasians and 24% in African Americans and interacts with the 5-HTTLPR to affect *SLC6A4* transcription (FIG. 1c)[2,13]. Several of the less-common SNPs and their haplotypes are associated with behavioural phenotypes or disorders, including obsessive–compulsive disorder and autism[2,10–12,14–16].

To explore the question of what genetic disorders might be attributable to life-long *SLC6A4*

dysfunction, mice with reduced or completely abrogated SERT protein following targeted disruption of *Slc6a4* were generated[17], revealing a remarkable phenotypic pleiotropy (BOX 1; FIG. 2). Additional genetic engineering tools were used in subsequent investigations of *Slc6a4*, and produced consequences that were largely consistent with those of the original studies of the constitutive-knockout mice[18-21] (FIG. 2). Many of these effects can now be understood on the basis of specific developmental, neurochemical, receptor-signalling and other molecular consequences of *Slc6a4* inactivation. In this Review we describe the remarkable extent of pleiotropy in these mice and discuss the underlying mechanisms. We also provide some thoughts on the relevance of these observations to human and non-human primate neurobiology, evolution, behaviour, gene–environment interactions and neuropsychiatric disorders.

Phenotypes of *Slc6a4*-mutant mice

Anxiety-like behaviours. Various approaches have been used to experimentally alter *Slc6a4* expression and SERT function in mice, including the constitutive *Slc6a4* knockout reviewed here (FIG. 2). The 5-HTTLPR short variant, which results in lower expression of *SLC6A4*, is strongly associated with anxiety-related, harm-avoidant and negative personality traits in humans[1,3,7,8,22]. *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice were therefore predicted to exhibit increased anxiety-like behaviours. This was indeed found to be the case, according to multiple measures, in both male and female *Slc6a4*$^{-/-}$ mice with different genetic backgrounds[23–25]. Gender differences and an intermediate phenotype in heterozygote mice have also been observed[18–20,24–26]. After mild postnatal foot-shock stress experiences[27] or exposure to predator odours[28], anxiety-like behaviours are intensified in *Slc6a4*$^{-/-}$ mice but not in wild-type mice. Latent anxiety-like behaviours

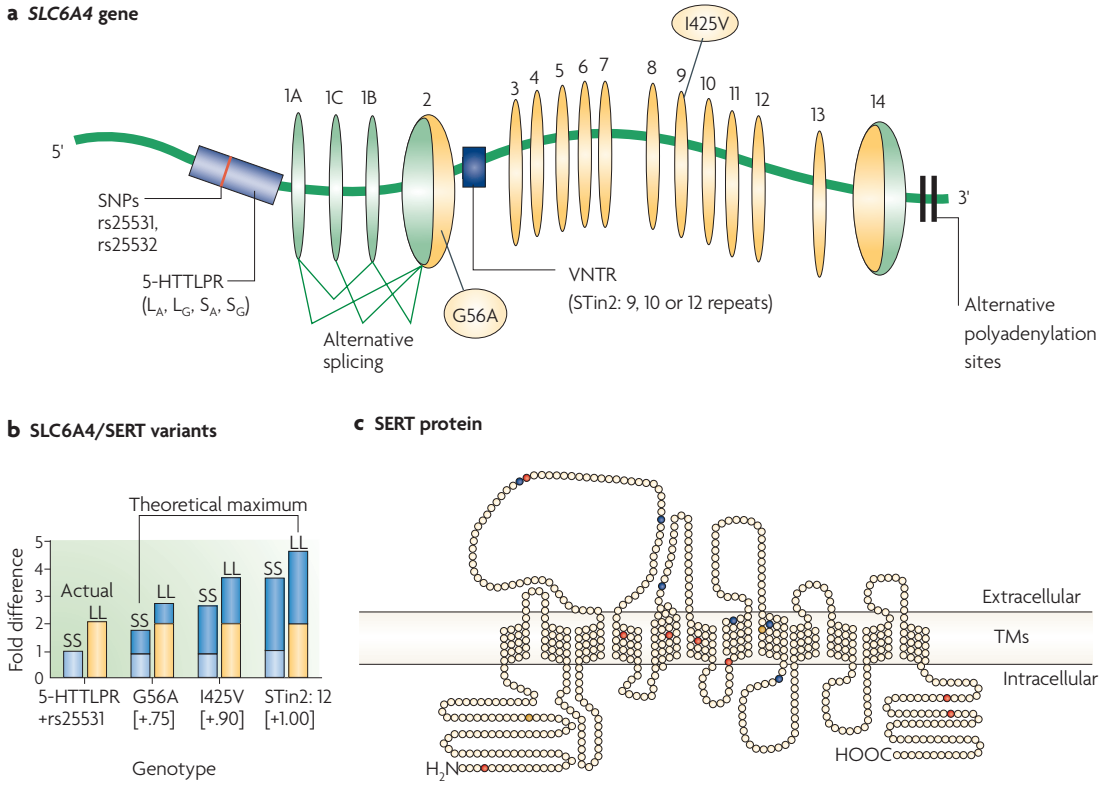


Figure 1 | **Organization of the human serotonin transporter (SERT) gene (*SLC6A4*). a** | The structure of the *SLC6A4* gene, including the sites of the major functional variants: the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR), the variable number of tandem repeats (VNTR) (between 9 and 12 repeats can be found in intron 2 (STin2)) and the single nucleotide polymorphisms (SNPs). **b** | Relative potential additive SERT expression and function for the major *SLC6A4* polymorphisms. There is a theoretical 4.65-fold difference in function between individuals with a combination of the less active allele of each variant and individuals with the most active variants[1,2,4,10,11]. The left-most two bars bars depict the actual differences in SERT expression and function that were measured in human lymphoblasts from large numbers of individuals, with the light-blue bar representing the 5-HTTLPR short/short (SS) + rs25531 genotype and the yellow bar representing the 5-HTTLPR long/long (LL) + rs25531 genotype; the six bars on the right combine this information with the functional consequences of the additional variants (G56A, I425V and STin2) that are predicted from *in vitro* measurements (dark-blue segments). **c** | The SERT protein, with its 12 transmembrane (TM) segments, its extracellular loops and its intracellular amino- and carboxy-terminal tails. SNPs that change amino acids are denoted by red circles (except the functionally validated G56A and I425V SNPs, which are coloured yellow); those in blue are synonymous. Panels **a** and **c** modified, with permission, from REF. 4 © (2004) American Society for Pharmacology and Experimental Therapeutics. Data in part **b** from REFS 1,2,4,10,11.

Epigenetics
Structural modifications of chromosomal regions that alter gene activity without changing the nucleotide sequence.

Extinction recall
A unique learning and recall process, mostly studied with fear conditioning, that requires the alteration of stimulus–response associations such that the organism ceases to respond to a previously rewarded stimulus.

Box 1 | **Pleiotropy and evolution**

Difficulties in unravelling genetically complex disorders, especially those that involve the brain, have been attributed to clinical and genetic heterogeneity, variable expressivity (penetrance), epistasis, imprinting, epigenetics and pleiotropy. Pleiotropy refers to the situation in which multiple traits arise from mutations in a single gene. However, there are remarkably few extended descriptions of pleiotropy available for humans, although pleiotropy has been well documented in non-human primates, flies and yeast. There are also few cases in which the evolutionary molecular and functional pathways that lead to pleiotropic traits or related disease models have been successfully disentangled. Exceptions include the effects of the *dunce* mutation in *Drosophila*[1] and the changes that are observed after targeted disruption of serotonin receptors[2], factors regulating cell growth and differentiation (such as transforming growth factor-β, $p27^{kip1}$ and CRKL), signalling molecules (such as the G protein $G_{\alpha o}$) and transcription factors (such as Pitx2) in mice[122,123]. What is striking about the *Slc6a4* knockout is its minimal impact on anatomical development. By contrast, in the other examples there is a relative lack of evident associated behavioural, neurological and other neurobiological abnormalities. When evolutionary selection occurs on the basis of improved fitness, the general conception has been that the naturally selected mutational variant of the gene relates to a single phenotype that is benefitted. The presence of more than one trait that is influenced by a gene product does not pose a problem, as long as all traits are equally benefitted. It is not yet clear whether this is the situation for the consequences of spontaneous human *SLC6A4* variation or for engineered murine *Slc6a4* variation. Alternate possibilities are that the phenotypes do not affect fitness (termed neutral pleiotropy), have opposing and equal contributions (antagonistic pleiotropy) or have consequences only after sexual maturity is reached[124,125].

Some population biologists favour the view that evolutionary adaptations result from substitutions of single genes, with large effects[126,127]. However, more common views of phenotypic divergence favour very small changes in many genes[124,128]. In the face of this enormous pleiotropy in the *Slc6a4*-modified mouse, an obvious question is why there is only one serotonin transporter. *SLC6A4*'s amino-acid coding structure is highly conserved[4], and the divergence of even the most closely related transporters (for dopamine and noradrenaline) appears to pre-date vertebrate speciation, suggesting that these transporters are at least as far from human serotonin transporter (SERT) in evolution as *Drosophila melanogaster* or *Caenorhabditis elegans* SERT[4].

What has constrained the evolution of SERT? A few theorists have discussed how trait variation would be evolutionarily constrained by pleiotropy[124,129]. Thus, although any two traits can be complimentary, some traits have been found to be or have been considered to be antagonistic, and are therefore subject to selection pressures that act on each one separately. In other words, the sheer number of consequences that arise from any functional genetic variation in SERT or similar molecules would be expected to decrease overall fitness and thus reduce the survival of the new genetic variant.

have also been reported in $Slc6a4^{+/-}$ mice that experience poor maternal care; brain-derived neurotrophic factor (BDNF) has been identified as the molecular substrate of the epigenetic programming that causes these effects[29]. Repeated stressful experiences in adult $Slc6a4^{-/-}$ mice lead to deficits in extinction recall following fear conditioning and to depression-like behaviour[30]. These mice also develop changes in the dendritic morphology and spine density of pyramidal neurons in their infralimbic cortex and basolateral amygdala[30]. Finally, mice treated with the SSRI fluoxetine during early development also exhibit increased anxiety-like behaviour as well as other related behaviours that include avoidance of open-field exposure[18,31].

When SERT-deficient mice are interbred with mice that lack one copy of the BDNF gene, the anxiety-like behaviours and tissue serotonin depletion intensify, in keeping with data that show that BDNF is required for the development and maintenance of the brain's serotonin system[32]. Interestingly, a marked reduction in anxiety-like behaviours was found in transgenic mice generated using a human yeast artificial chromosome (YAC) construct that causes a two- to threefold overexpression of SERT and reduced extracellular serotonin concentrations. This appears to confirm a direct relationship between serotonin availability and anxiety-related behaviours[21].

The anxiety-like behaviour in the $Slc6a4^{+/-}$ and $Slc6a4^{-/-}$ mice can be normalized by the serotonin 1A receptor ($5\text{-}HT_{1A}$ receptor) antagonist WAY 100635, suggesting that the postsynaptic $5\text{-}HT_{1A}$ receptor is a participant in these anxiety-like behaviours[24].

Somatosensory cortex and the whisker barrel pathway. Cytoarchitectonic changes are found in the barrelfield layer IV cortex of $Slc6a4^{-/-}$ and $Slc6a4^{+/-}$ mice, and gene dose-dependent reductions in the density of the barrel images has also been observed[33,34]. Similar changes are also found in mice that lack the gene that codes for monoamine oxidase A (MAOA), an enzyme that is involved in the metabolism of serotonin[34]. To evaluate whether these morphological changes were of functional importance, responses to whisker stimulation in cortical barrelfields were investigated using local cerebral glucose-utilization measurements[35]. In wild-type mice, unilateral stimulation of whiskers leads to a significant glucose-utilization response in the contralateral somatosensory cortex. The magnitude of this response is significantly reduced in $Slc6a4^{-/-}$ mice, and this reduction is also observed in other components of the trigeminal pathway and other somatosensory pathways that connect to the barrelfields[35].

Rescue of cortical barrel architecture abnormalities in $Slc6a4^{-/-}$ and $Slc6a4^{+/-}$ mice can be accomplished by treatment with the serotonin-synthesis inhibitor parachlorophenylalanine (PCPA) in a narrow developmental time window between postnatal day 1 and postnatal day 2 (REF. 33). Partial genetic rescue also occurs following inactivation, in this cortical area, of *Htr1b*, which encodes the serotonin 1B receptor[34] ($5\text{-}HT_{1B}$ receptor).

Neuroendocrine and sympathoadrenal responses to stress. $Slc6a4^{-/-}$ and $Slc6a4^{+/-}$ mice have reduced basal plasma corticosterone levels but respond to acute stress with greater than normal levels of adrenocorticotropic hormone (ACTH) and oxytocin release[36–39]. These exaggerated ACTH responses are further increased in $Slc6a4^{-/-}$ mice that have been interbred with $Bdnf^{+/-}$ mice[40]. ACTH elevations in response to the placement of mice on the elevated plus maze, which increases stress, are also exaggerated in $Slc6a4^{-/-}$ mice[41]. As well, elevated plasma corticosterone levels are found in $Slc6a4^{-/-}$ mice in response to chronic mild stress[42].

Hypothalamo–pituitary and adrenomedullary responses to restraint stress in conscious mice have also been examined[43]. At baseline, adrenal and pituitary serotonin concentrations in $Slc6a4^{-/-}$ mice are markedly lower than in littermate controls. Restraint stress increases plasma levels of catecholamines, ACTH and corticosterone in all genotypes, but these responses are exaggerated in $Slc6a4^{-/-}$ mice. The responses are associated with significant reductions in levels of adrenaline,

Genomic imprinting
For most autosomal genes expression occurs from either allele, whereas a small proportion (< 1 %) of genes are imprinted, meaning that expression occurs from only one allele. Which of the two alleles is expressed is dependent on the parental origin.

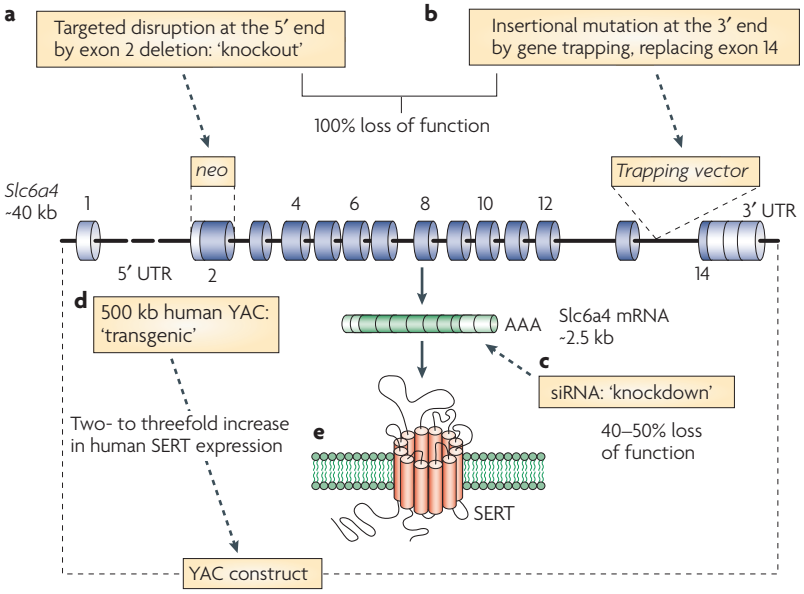


Figure 2 | **Molecular manipulations that alter the expression and function of serotonin transporter (SERT) in mice. a–d** | The central part of the schematic is a linear depiction of the 14 exons and the interconnecting introns of *Slc6a4*. The sites and strategies of the four major genetic modifications that have been performed, along with the resulting effects on SERT expression and function, are illustrated and summarized in the text boxes. **a** | For the constitutive knockout, two similar targeting constructs directed towards exon 2 of *Slc6a4* (the deleted region was replaced with a *neo* cassette) have been used to disrupt the gene, both resulting in a complete abrogation of brain SERT protein and labelled-ligand binding to SERT (that is, an abrogation of SERT binding sites)[17,18]. **b** | In addition, a reduction or complete abrogation of SERT has been achieved using a gene-trap approach that replaced exon 14 with a non-functional sequence[20]. **c** | Short-interfering RNA (siRNA) has also been used to partially reduce (knockdown) SERT levels[19]. **d** | Finally, a human yeast artificial chromosome (YAC) construct that resulted in a two- to threefold overexpression of SERT and a reduction in extracellular serotonin concentrations has also been created[21]. **e** | Also shown is one theoretical model of how SERT components might insert into the lipid bilayer membrane to yield cross-membrane facilitated transport.

noradrenaline and serotonin in the adrenal glands, and pituitary tissue ACTH is also significantly reduced[43]. These differences suggest that one function of SERT is to restrain adrenomedullary activation in response to severe stress[44]. The usual increase in tyrosine-hydroxylase transcription and adrenomedullary angiotensin-II-receptor expression that follows adrenal adrenaline and noradrenaline release in response to restraint and other forms of stress does not occur in *Slc6a4*$^{-/-}$ mice[45]. Thus, exaggerated adrenomedullary responses seem to be an autonomic correlate of the anxiety-like behaviours and exaggerated hypothalamo–pituitary responses that occur in *Slc6a4*$^{-/-}$ mice[43–45].

Aggression. *Slc6a4*$^{-/-}$ mice are less aggressive than control mice in the isolated-resident/intruder test[46]. Additionally, *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice fail to shorten the latency time to first attack in a second intruder-encounter experiment, unlike wild-type mice. This confirms a role for altered SERT levels in aggressive behaviours and indicates that *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice have altered emotional learning in comparison with wild-type mice. The findings might also be related to other social-interaction deficits in the *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice and suggest that these mice might be a useful model of some aspects of social anxiety and autism[46,47]. Reports of altered aggression in mice with a deletion of one *Bdnf* allele suggest that studies of *Slc6a4* × *Bdnf* double-mutant mice might provide further evidence of specific epistasis that is similar to that which is observed for anxiety-like behaviours and neuroendocrine responses in these mice[40,48,49].

Sleep, brain excitability, body temperature and gut motility. *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice have substantially increased rapid eye movement (REM) sleep time[50]. Furthermore, brain excitability (as reflected in their susceptibility to pentylenetetrazole-induced seizures) is reduced in these mice[51], whereas baseline body temperature is increased[52]. Gut physiological function is also abnormal in *Slc6a4*$^{-/-}$ mice, which have increased colonic motility and other symptoms that resemble the symptoms of human irritable bowel syndrome[53]. These changes have also been found to accompany the serotonin-precursor (5-hydroxytryptophan; 5-HTP)-induced serotonin syndrome[54].

Sensory, bladder and vascular function. Sensory function is reduced in *Slc6a4*$^{-/-}$ mice, as reflected by their reduced spinal reflexes and reduced responses to mildly painful thermal or nerve-crush injuries[49,55]. Likewise, bladder responses to stretching are reduced in female *Slc6a4*$^{-/-}$ mice[56]. However, basic neurological and motor testing reveals no overt changes in other reflexes, including the eye-blink, whisker and righting reflexes[46].

Both blood plasma and platelets are devoid of serotonin in *Slc6a4*$^{-/-}$ mice[53,57]. Furthermore, most peripheral organs have markedly depleted serotonin levels, confirming that the normal presence of serotonin in most peripheral tissues is dependent on SERT[58]. The elevated blood pressure that normally develops in response to reduced oxygen availability at simulated high altitudes is reduced or absent in *Slc6a4*$^{-/-}$ mice. This might provide an explanation for the protection against the development of human pulmonary hypertension that is apparently afforded by the lesser-expressing short allele and short/short genotype of the 5-HTTLPR[4,57]. Also, *Slc6a4*$^{-/-}$ mice display lower left cardiac ventricular weight relative to body weight and develop cardiac fibrosis as well as valvulopathy[57,59]. Thus, serotonin and SERT have a role not only in visceral function but also in cardiovascular and respiratory function.

Bone and muscle strength, and agility. The ability to cling to a wire-mesh screen (a test of strength) and to maintain a grasp on a rotating metal rod is reduced in *Slc6a4*$^{-/-}$ mice[46]. Likewise, bone weight, thickness and structural resistance to fracture *in vitro* are also significantly reduced[60,61]. One plausible basis for these impairments is the lifetime reduction in physical activity and exercise that results from reduced horizontal, vertical and risk-assessment behaviours (including standing and surveying and sniffing the environment) in SERT-deficient mice[46,62]. There do not appear to have been any

Barrels
Cylindrical columns of neurons that are found in layer IV of the rodent neocortex. Each barrel receives sensory input from a single whisker follicle and the topographical organization of the barrels corresponds precisely to the arrangement of whisker follicles on the face.

Isolated-resident/intruder test
A test for social interaction and offensive aggressive behaviour in rodents. An unfamiliar mouse (the 'intruder') is introduced into the cage of a mouse that has been kept isolated in its 'resident' cage for several months.

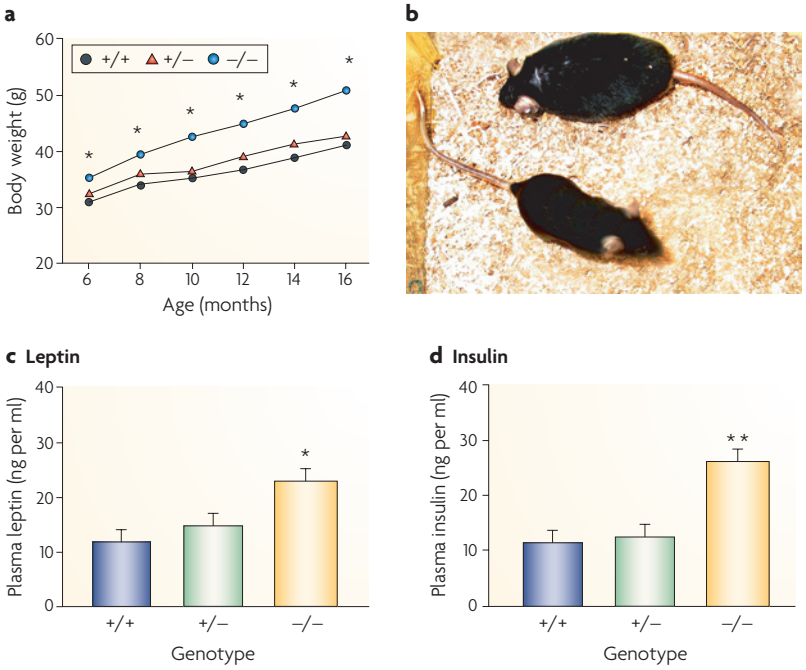


Figure 3 | **Association of age-dependent obesity with increased plasma insulin and leptin in *Slc6a4*-mutant mice. a** | Body weight as a function of age in *Slc6a4*$^{-/-}$, *Slc6a4*$^{+/-}$ and control mice. Across all ages the *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice had greater body weight than the control mice. **b** | An obese *Slc6a4*$^{-/-}$ mouse (top) and a control mouse (bottom). **c**,**d** | Plasma leptin and insulin concentrations are higher in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice than in control mice. *, significant change; **, highly significant change. Data from REF. 62.

comparable evaluations of the effect of human *SLC6A4*-variant associations on physical fitness or strength. However, similar results would be predicted from the observations in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice, particularly because reductions in bone mineral density are found in humans receiving SSRI treatment[63,64].

Body weight, locomotor activity and obesity. An obesity phenotype emerges in *Slc6a4*$^{-/-}$ mice at approximately 3 months of age and becomes more exaggerated throughout life[62,65] (FIG. 3a,b). Obesity is associated with increased plasma levels of insulin and leptin[62] (FIG. 3c,d). Obese adult *Slc6a4*$^{-/-}$ mice also exhibit hyperglycaemia (despite normal glucose clearance) and reduced basal corticosterone levels. Plasma triglycerides and cholesterol are also elevated in *Slc6a4*$^{-/-}$ mice, and preliminary data indicate the presence of insulin resistance, as reflected by the smaller-than-normal glucose reductions that occur following insulin administration (Holmes, A. and D.L.M., unpublished observations). Daily food consumption is normal in *Slc6a4*$^{-/-}$ mice, indicating that the obesity is not simply dietary in origin[46]. Indeed, daily home-cage locomotor activity (measured concomitantly with food consumption) is lower in both pre-obese and obese *Slc6a4*$^{-/-}$ mice[26,46]. These observations contrast with the normal motor activity that is observed in other laboratory assessments, including habituation to the apparatus[17,66].

This obesity phenotype resembles that of type 2 diabetes with obesity — 'metabolic syndrome' — in humans. The lack of hyperphagia and lack of differential response to a high-fat diet, together with normal metabolic function, suggest that reduced serotonin signalling affects the development of obesity through reduced motor activity — a 'couch potato' syndrome. However, a confirmed basis for the locomotor-activity deficit is unknown at present, as are the potential contributions of some endocrine and other metabolic factors[67,68].

Responses to drugs. Many pharmacologic agents that act through SERT (including 3,4-methylenedioxymethamphetamine (MDMA), 2′-NH_2-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP) and SSRIs) or through serotonin receptors (including 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), RU24969 and 2,5-dimethoxy-4-iodoamphetamine (DOI)) have reduced efficacy in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice, reflecting the reduced availability or downregulated function of these drug targets[17,37,46,51,69,70]. MDMA self-administration is absent in *Slc6a4*$^{-/-}$ mice, and alcohol preference and ingestion are reduced (although alcohol's sedative effects are increased)[71–74]. By contrast, cocaine preference is enhanced in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice[75]. However, in *Slc6a4*-mutant mice that have been interbred with mice that lack the dopamine transporter, cocaine preference is abolished[66,75]. Behavioral changes and large changes in body temperature that result from treatment with 5-HT alone or 5-HT and monoamine oxidase inhibitors occur as components of exaggerated serotonin syndrome in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice. These behavioural changes encompass a wide range of cognitive, autonomic and somatic features[76,77]. They suggest that humans carrying the lesser-expressing 5-HTTLPR short variant could be at a higher risk for developing the human serotonin syndrome[77].

Slc6a4-mutant-phenotype mechanisms

The basis of SERT pleiotropy. An overview of the multiple phenotypes that are observed in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice is provided in FIG. 4, together with a comprehensive summary of the directions of the experimental differences that are seen in these mice compared with wild-type mice. One evident basis for these multiple changes is the anatomy of the serotonin system, which is best considered as four interacting subsystems (FIG. 5b). Of these, the serotonin subsystem in the CNS has been the most comprehensively studied (FIG. 5a). Cell bodies in the raphe nuclei provide modulatory serotonergic input to multiple neuronal circuits in the brain through the midbrain and upper pons cell groups, plus input to the spinal cord from its brainstem caudal cell groups (FIG. 5a). In the periphery, a partially separate serotonergic enteric neural system (ENS) exists within the gut[78,79] and interacts with other CNS and peripheral serotonergic mechanisms (FIG. 5b). Furthermore, several other tissues without any prominent serotonergic innervations (such as lung, heart, blood-vessel and pancreatic tissue, as well as platelets) contain functional SERT. These tissues normally take up and store serotonin in vesicles, and can release it in response to local stimuli. Thus, they can be considered to be a peripheral serotonergic system. A neuroendocrine serotonergic

Epistasis
A characteristic of the interactions between two or more genetic loci. Negative epistasis occurs when the combined phenotypic effect of two or more loci is less than the sum of the effects at individual loci, whereas positive epistasis occurs when the combined effect of the two or more loci is greater than the sum at individual loci.

Rapid eye movement (REM) sleep
The period of sleep during which dreaming is thought to occur. REM sleep is characterized by increased brain-wave activity, bursts of rapid eye movement, accelerated respiration and heart rate, and muscle relaxation to the point of paralysis.

system responds to serotonin release with diverse signalling through hormones such as prolactin, ACTH, corticosterone and oxytocin.

The primary functional basis for the multiple phenotypic changes that occur in *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice is a profound alteration in serotonin neurochemistry and regulatory responses[17,58]. These abnormalities act in somewhat different ways during development (when serotonin has neurotrophic and morphogenic effects[80]) and in adulthood (when the continuing excess of extracellular serotonin seems most important). Thus, this pleiotropy (BOX 1) can be considered to result from several distinct or overlapping pathways. Furthermore, there are possible differences in pathway formation that lead to different pleiotropic consequences based on developmental timelines: for example, the same reductions in SERT function might lead to independent and different consequences in the brain (for example, anxiety-like behaviours) and in the gut (for example, hypermotility) — this would be 'true' pleiotropy. Alternatively, the consequences of SERT dysfunction in the brain might cause or contribute to gut hypermotility directly, possibly through increased anxiety. Less likely, but conceivably, gut dysfunction might cause or contribute to brain and behaviour dysfunction,

Biochemical features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
SERT binding sites	↓	↓
Serotonin uptake	↓	↓
Serotonin clearance	↓	↓
Serotonin content in brain and periphery	↓	NS
Extracellular fluid serotonin	↑	↑
Serotonin synthesis	↑	NS
5-HT_{1A} receptor sites and mRNA	↓	↓
5-HT_{1B} receptors	↓	↓
5-HT_{2A} and 5-HT_{2C} receptors	Δ	NS
5-HT_3 receptors	↑	Δ
Adenosine receptors	↑	NT
OCT3 mRNA	↑	NS
Glucose	↑	NS
Leptin	↑	NT
Cholesterol	↑	NT
Triglycerides	↑	NT

Behavioural features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Anxiety	↑	↑
Learned fear	↑	NS
Learned helplessness (forced-swim and tail-suspension tests)	↑	NS
Aggression	↓	↓
Acoustic startle response	↑	↑
Exploratory activity	↓	NS
Rotorod agility	↓	NS
Wire hang	↓	↓

Anatomical features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Somatosensory and visual cortex	Δ	Δ
Infralimbic cortex (dendrite morphology changes)	Δ	Δ
Apoptosis in neonatal brain	↓	NS
Cell density in neonatal cortex	↑	NT
Pyramidal neuron spine density in amygdala	↑	NT

Physiological features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Stress responses (ACTH, corticosterone, epinephrine, temperature and motor responses)	↑	↑
Gut motility (diarrhoea, constipation)	↑	NT
Body weight	↑	NS
Glucose tolerance	↓	NT
Insulin sensitivity	↓	NT
Brain glucose utilization	↓	NT
Bone mass and strength	↓	NT
Nociception (nerve injury and thermal)	↓	NT
Bladder function	↓	NS
Hypoxia-induced pulmonary hypertension	↓	NT
Raphe serotonin neuron firing rate	↓	↓
REM sleep	↑	↑
EEG power spectra, 'bursting'	Δ	NS

Pharmacological features	*Slc6a4*$^{-/-}$	*Slc6a4*$^{+/-}$
Effect of SSRIs (inescapable stress and 5-HT clearance)	↓	Δ
Effect of ipsapirone (serotonin synthesis and DRN firing rate)	↓	NS
Effect of RU24969 (motor function)	↓	NS
Effect of CP 93129 (serotonin clearance)	↓	NS
Effect of DOI (head twitch)	↓	↓
Effect of pentylenetetrazole (seizure)	↓	NS
Effect of MDMA (motor function and self-administration)	↓	↓
Effect of 2′-NH_2-MPTP (motor function and temperature)	↓	NS
Effect of alcohol (10-day intake)	↓	NS
Effect of 8-OH-DPAT (temperature and neuronal activity)	↓	↓
Effect of cocaine (preference)	↑	↑
Effect of alcohol (motor function)	↑	NT

***Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice: overview of major phenotypes**

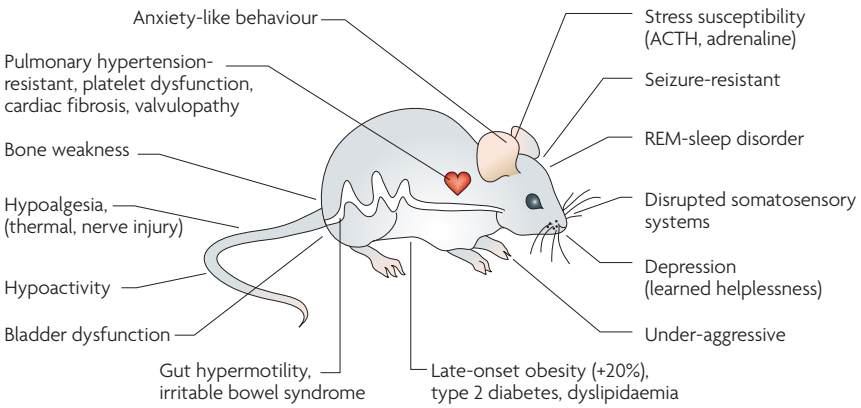


Figure 4 | **Overview of the major pleiotropic central and peripheral phenotypes discovered in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice.** Explicit, specific experimental results are summarized using symbols to indicate increases (↑), decreases (↓) or other types of significant qualitative changes (Δ) in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice compared with control mice. For a more comprehensive evaluation and review of pharmacological features, see REF. 51. 2′-NH_2-MPTP, 2′-NH_2-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-dipropylamino-tetralin; ACTH, adrenocorticotropic hormone; DOI, 2,5-dimethoxy-4-iodoamphetamine; DRN, dorsal raphe nucleus; EEG, electroencephalogram; MDMA, 3,4-methylenedioxymethamphetamine; NS, changes were not significant; NT, not tested; OCT3, organic cation transporter 3; REM, rapid eye movement; SERT, serotonin transporter; SSRI, selective serotonin-reuptake inhibitor.

Serotonin syndrome
An uncommon but potentially life-threatening adverse drug reaction that is caused by excess serotonergic activity at CNS and peripheral serotonin receptors. It can result from intentional self-poisoning, therapeutic drug use or inadvertent interactions between drugs such as SSRIs and monoamine oxidase inhibitors. It can also occur spontaneously in a mild form in *Slc6a4*$^{-/-}$ mice, and *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice are more susceptible to developing the syndrome following the administration of relatively low doses of many drugs.

including anxiety. Of course, different mechanisms might also coexist and interact. Likewise, such interactive mechanisms, possibly involving allelic variation of SERT function, have also been postulated in multiple studies of humans, as illustrated by one recent twin study that directly examined the basis for the well-documented co-occurrence of SERT-variation-related mood disorders and coronary artery disease[81,82].

Neurochemical characteristics. Basal extracellular fluid serotonin concentrations are markedly increased in the striatum and cortex of *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice[83]. Serotonin clearance in the CA3 region of the hippocampus is prolonged in *Slc6a4*$^{-/-}$ mice, to the point where its removal cannot be distinguished from diffusion alone, and is prolonged to an intermediate extent in *Slc6a4*$^{+/-}$ mice[73,83]. These markedly increased extracellular serotonin concentrations thus amplify and extend the duration of serotonin signalling at serotonin receptors, significantly altering the functional state of the entire serotonin system.

In contrast to the extracellular fluid levels, serotonin brain-tissue concentrations are decreased by 40–60% in *Slc6a4*$^{-/-}$ mice; furthermore, serotonin concentrations are reduced to <10% of normal in most peripheral tissues where serotonin is not synthesized[17,38,43,58]. In all of these tissues, and in others that express SERT, the reductions in tissue serotonin content seem to be a direct result of a failure of this specific SERT-mediated uptake system, with a lack of or inadequate compensation by other transporters[84,85].

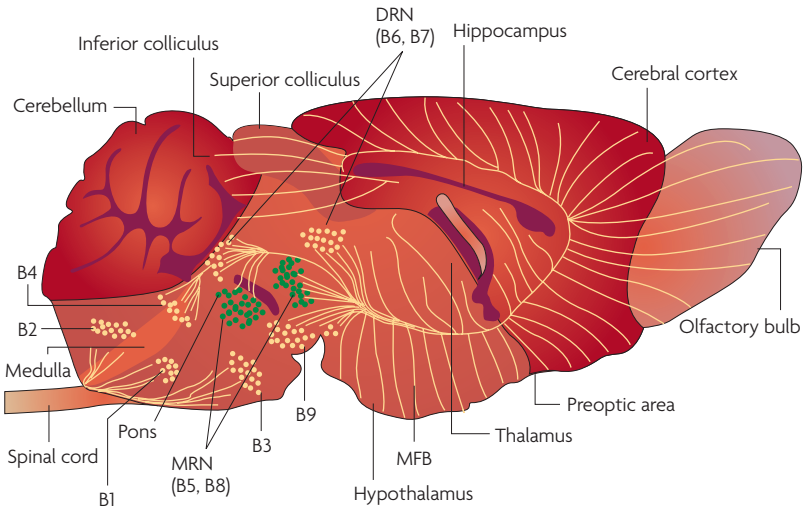
As a consequence of the deficient recycling of serotonin by its transporter, serotonin synthesis and turnover are increased across brain regions in *Slc6a4*$^{-/-}$ mice, with the greatest increase in female mice[58]. Dopaminergic neurons in the substantia nigra accumulate excess serotonin in *Slc6a4*$^{-/-}$ mice through the dopamine transporter[84]. Furthermore, expression of the organic cation transporters OCT1 and OCT3, which are also low-affinity transporters of monoamines, is increased[53,84–86], indicative of some partial but inefficient attempt at compensation through heterologous transporters.

Slc6a4$^{+/-}$ mice have fewer specific SERT binding sites (sites to which a labelled ligand for SERT binds), decreased serotonin clearance and elevated extracellular serotonin levels; however, they have unchanged tissue serotonin concentrations in the brain and in the periphery and have unchanged brain serotonin synthesis and turnover[17,58,73,83]. Thus the loss of one *Slc6a4* allele leads to a decrease in transporter function, but a single copy of *Slc6a4* is adequate to maintain overall tissue serotonin homeostasis.

The spontaneous firing rate of serotonergic neurons in the dorsal raphe of anesthesized *Slc6a4*$^{-/-}$ mice is ~35% of that of wild-type control mice, with intermediate values (~60%) in *Slc6a4*$^{+/-}$ mice[69]. In addition, the time required for CA3 hippocampal neurons to return to normal firing rates following microiontophoretic application of serotonin is prolonged. This probably represents the failure of the rapid clearance of serotonin, as has been documented in the CA3 hippocampal region[69,73]. As expected, the SSRI paroxetine further increased the serotonin-induced prolongation of hippocampal neuron recovery time in *Slc6a4*$^{+/-}$ mice[69].

5-HT$_{1A}$ receptors in the brainstem raphe area are substantially (>60%) decreased in *Slc6a4*$^{-/-}$ female mice, with somewhat smaller reductions in males and intermediate values in *Slc6a4*$^{+/-}$ mice. Female but not male *Slc6a4*$^{-/-}$ mice also show modest reductions in 5-HT$_{1A}$ receptors in the hypothalamus and in some areas of the amygdala and septum, but show no changes in the cortex or hippocampus[36,37,87,88]. Thus, *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice display a downregulation of 5-HT$_{1A}$ receptors at presynaptic somatodendritic sites, accompanied by insensitivity of these receptors and of 5-HT$_{1A}$-mediated neuroendocrine responses. This is similar to the changes that are observed in rodents chronically treated with SSRIs.

a Central serotonergic system



b Peripheral serotonergic system

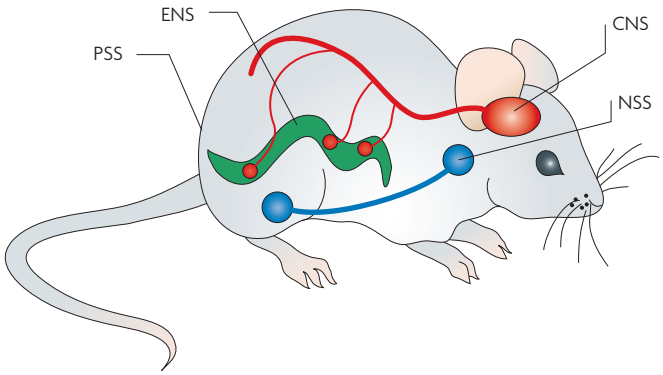


Figure 5 | **Central and peripheral serotonergic systems. a** | CNS serotonin neuron cell-body groups in the nine raphe nuclei, B1–B9. The more caudal nuclei (B1–B3) in the medulla project axons to the spinal cord and the periphery, whereas the more rostral raphe nuclei contain the principal dorsal raphe groups (B6 and B7; depicted in yellow) and the median raphe groups (B5 and B8; depicted in green), which project to different but overlapping brain areas. **b** | Serotonin also functions in the enteric nervous system (ENS), the hypothalamo–pituitary–adrenocortical (HPA) system, the adrenomedullary neuroendocrine serotonin system (NSS) and the peripheral serotonin system (PSS), which includes the lungs, the heart, the blood vessels, the pancreas and platelets[36,37,43,78,130,131]. DRN, dorsal raphe nucleus; MFB, medial frontal bundle; MRN, median raphe nucleus.

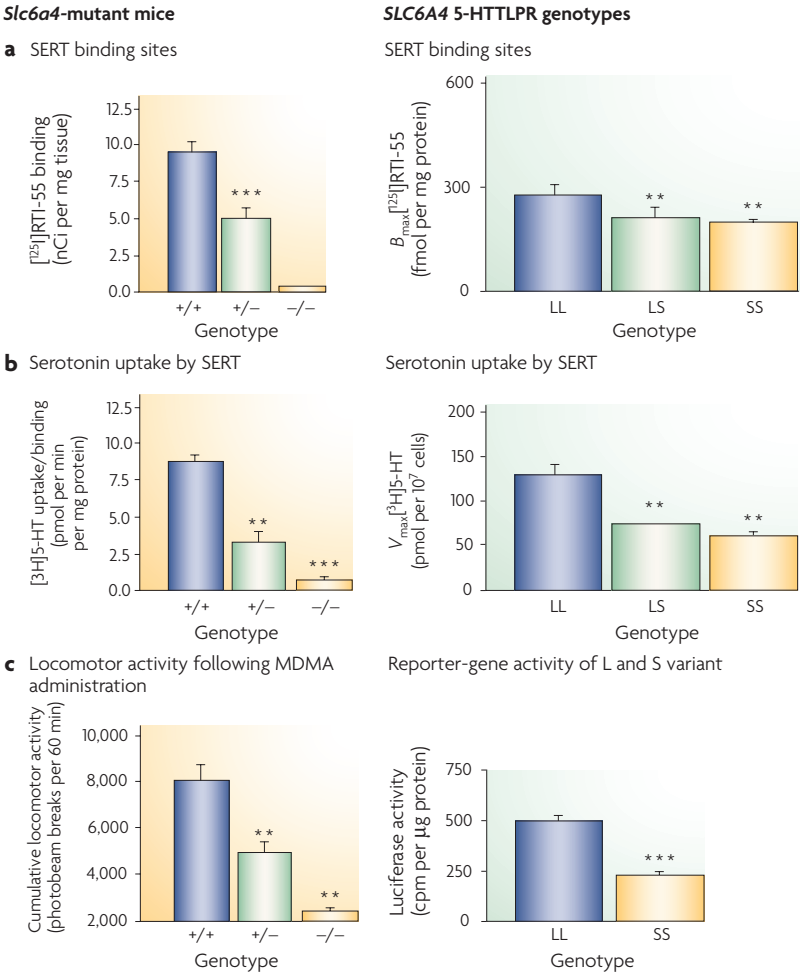


Figure 6 | **Comparison of serotonin-transporter (SERT) expression and function in *Slc6a4*-mutant mice and in humans with different *SLC6A4* 5-HTTLPR genotypes.** Differential reductions in SERT binding sites (sites to which a labelled ligand for SERT binds) (**a**), serotonin transport (**b**) and function (**c**; as reflected in MDMA-elicited locomotor activity (in the mice) or as measured by reporter-gene activity in lymphoblasts (in the humans)) in *Slc6a4*$^{-/-}$, *Slc6a4*$^{+/-}$ and control mice and in humans with different 5-HTTLPR genotypes of *SLC6A4* (long/long (LL), long/short (LS) or short/short (SS)).**, significant change; ***, highly significant change. *SLC6A4* data modified, with permission, from REF. 1 © (1996) American Association for the Advancement of Science. *Slc6a4* data modified, with permission, from REF. 17 © (1998) American Society for Pharmacology and Experimental Therapeutics.

5-HT$_{2A}$ receptors are decreased in the striatum, claustrum and cortex, but increased in the septum and hypothalamus of *Slc6a4*$^{-/-}$ mice[38,89]. By contrast, 5-HT$_{2C}$ receptors are increased in the amygdala and choroid plexus of *Slc6a4*$^{-/-}$ mice, but unchanged in other regions[38]. Chronic SSRI treatment also increases 5-HT$_{2C}$-receptor expression, but does not usually affect 5-HT$_{2A}$-receptor levels[90].

These relatively modest changes in receptor numbers are accompanied by much greater changes in response to selective receptor agonists. For example, changes in hippocampal neuron firing rates, temperature and other responses to 8-OH-DPAT and ipsapirone (5-HT$_{1A}$ agonists), altered locomotor-behaviour responses to RU 24969, and responses to DOI (a 5-HT$_{2A/2C}$ agonist with hallucinogenic effects) are essentially absent in *Slc6a4*$^{-/-}$ mice, with intermediate changes in *Slc6a4*$^{+/-}$ mice on some measures[40,86,91]. These diminished receptor-mediated responses are not attributable to abnormalities in G-protein coupling, but recent evidence indicates that signalling in the 5-HT$_{2A/2C}$–phospholipase A/arachidonic acid pathway is markedly reduced in the cortex, striatum and substantia nigra of *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice[36,38,69,70,91].

Relevance for murine and human disease

As described above, serotonin functions as both a short-range neurotransmitter and a long-range signalling modulator, with multiple effects on whole-organism functions across many species[4,92,93]. This extensive pleiotropy is clearly demonstrated in this Review of the multiple mouse phenotypes that result from a single gene alteration (of *Slc6a4*). Short-range serotonin signals (within nanometer distances inside synapses) activate pre- and postsynaptic serotonin receptors[94]. Long-range signals are mediated by hormone systems both locally, within tissues that express SERT (such as the adrenal gland[43,44]), and through changes in plasma hormone levels. Additionally, blood serotonin, which circulates within platelets and is released into microcirculatory beds of capillaries and arterioles, can regulate regional blood flow[95]; thus, the absence of serotonin in the platelets and blood of *Slc6a4*$^{-/-}$ mice might contribute to the widespread reductions in brain glucose utilization that are observed[35].

Many of the phenotypes that have been discovered in this SERT-targeted mouse have also been glimpsed in studies that examined the association of human diseases or traits with particular human *SLC6A4* variants, such as those involving the 5-HTTLPR (including the SNPs rs25531 and rs25532 within it), the intron-2 VNTR, and the I425V, I425L and G56A variants in *SLC6A4* coding regions. The higher-functioning alleles of these variants, if their potential additive influence (FIG. 1b) were verified by analytical techniques such as those used in studies of mice[83], could confer a four- to fivefold greater serotonin transport capacity than that of the lower-functioning alleles. Furthermore, as substitution of SERT P339L for SERT 339L yields an ~80% reduction in serotonin uptake[11], a theoretical range of differences in serotonin transport capacities across individuals could be as great as 15- to 20-fold if combinations of common and rare *SLC6A4* acted together in influencing SERT expression and function.

Humans with the 5-HTTLPR short/short genotype closely resemble *Slc6a4*$^{+/-}$ mice with regards to levels of SERT expression and function (FIG. 6). These similarities allow predictive appraisals of phenotypes across species[3,4,26,39,48,51]. Thus, it is no surprise to find that anxiety- and depression-related personality traits and affective disorders[1,8,96], alcohol and other drug dependencies[97], sleep and temperature disorders[50,98], irritable bowel syndrome (IBS)[53,99], pulmonary hypertension and chronic obstructive pulmonary disease[57,100] are associated with *SLC6A4* variants in humans and other species, especially when interactions between *SLC6A4* and life stress are taken into account[9,22,101,102]. Some of the human disorders that have been found to be associated with *SLC6A4* have

Spinal reflexes
Reflex actions that occur relatively quickly because the sensory neurons do not pass directly into the brain; rather, they synapse in the spinal cord. This characteristic bypasses the delay of routing signals through the brain, although the brain does receive sensory input while the reflex action occurs.

not yet been investigated in *Slc6a4*$^{-/-}$ and *Slc6a4*$^{+/-}$ mice, for example, SIDS and myocardial infarction[103,104]. Likewise, some of the *Slc6a4*$^{-/-}$ mouse phenotypes, including the obese and type-2-diabetic phenotypes, have yet to be studied for *SLC6A4*-variant frequency distortions in cohorts of human patients; this would seem to be a high priority for investigation.

A genetic contribution to temperament and behavioural traits, including anxiety, dominance and alcohol and drug preferences, has been established in humans and several other species, probably reflecting selective forces among our ancestors. Recent research efforts in this area have therefore been focused on non-human primates, especially rhesus macaques, and are currently proceeding towards the elaboration of an interdisciplinary perspective that will blend behavioural genetics and evolutionary psychology, as well as cognitive and social neuroscience[105]. In this non-human-primate model, environmental influences might be less complex and thus less likely to confound associations between behaviour and genes. Maternal-separation studies in rhesus macaques have demonstrated that genes and environment interact to produce effects on the association of central serotonin turnover and behavioural traits, including stress reactivity and alcohol preference and dependence, with a repeat-length variation that is orthologous to the human 5-HTTLPR[106,107]. This is in keeping with the notion that the 5-HTTLPR might influence the risk for affective and other behavioural disorders through gene–environment interactions, although the molecular and neural mechanisms that underlie the interplay of genes and environmental adversity constituting disease risk remain incompletely understood[9,22,101,102,108].

Finally, gene–gene interactions have been studied in models in which *Slc6a4*-mutant mice have been interbred with mice that lacked either one or both copies of the dopamine transporter gene (*Slc6a3*), the noradrenaline-transporter gene (*Slc6a2*), the MAOA gene (*Maoa*), the 5-HT$_{1B}$-receptor gene (*Htr1b*) or the BDNF gene (*Bdnf*), with consequent amplified, reduced or qualitatively different new phenotypes resulting[23,34,48,66,75,109]. These studies of 'experimental epistasis' complement those in which the *Slc6a4* knockout was placed on congenic C57BL/6J or 129S6 background strains to yield more subtle SERT-related phenotype differences[23].

Evolutionary aspects of SERT biology

This Review of the consequences of an engineered change in one murine gene, *Slc6a4*, documents over 50 phenotypic alterations. This seemingly marked interference with nature could be considered only an artefactual curiosity in light of its production by several types of drastic genetic engineering[17,18,20,21]. However, the findings in fact provide a partial delineation of all of the genetically influenced serotonergic traits that are regulated by SERT in mice. Additionally they provide a glimpse and, maybe, a preview of what is being discovered in humans with *SLC6A4* variants. Most of these murine traits are altered by the loss of a single *Slc6a4* allele — the changes often being intermediate to those that are produced by full knockout of the gene. Furthermore, the attendant changes in expression levels (~50% decrease) and function of murine SERT are similar to those that result from 5-HTTLPR variation and related polymorphisms in human and rhesus *SLC6A4* (FIG. 6). This indicates that pleiotropy with some similarities to that found in mice is likely to exist not only in humans and non-human primates but also in other species, including other rodents, flies and worms that have known spontaneous or targeted genetic variation of the serotonergic pathway[106,110–112]. Indeed, as discussed above, many conditions that are clearly analogous to those that arise in the *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice have been reported in studies of human medical disorders.

It is evident that some of the consequences of this engineered *Slc6a4* mutation are the result of changes that occur downstream in the serotonergic systems[17,51,70,73,83]. However, other consequences, although generally relatable to altered availability of serotonin and reduced or absent SERT, seem to be qualitatively distinct traits. For instance, it is hard to immediately relate bone, heart, lung, bladder and gut structural and functional changes to alterations in aggression or the startle reflex, or to preferences for alcohol, MDMA and cocaine — although a psychosomatocist might give it a try. Thus, multiple mechanisms, some going beyond those that have already been documented[17,69,70,73,83], are predicted by this pleiotropy.

From the most global point of view, the preponderance of changes resulting from the engineered reduction in SERT can be considered deleterious. Differences in SERT expression and function have not yet been studied across mouse species to specifically evaluate how they alter global fitness, and it is clear that more is now known about *SLC6A4* variants in humans and non-human primates than about murine *Slc6a4* mutational consequences in the real world.

Although the mouse *Slc6a4* and the human *SLC6A4* possess a similar set of three splice variants[113,114], other rodents and many non-human primates possess a more basic regulatory region. Furthermore, humans seem to have multiple and more complex regulatory elements that are capable of providing both a broader range of and a more finely tunable regulatory capacity for SERT expression and function, as reflected in the 5-HTTLPR variants, the rs25531 and rs25532 SNPs and the intron-2 VNTR, as well as the rarer functional elements (for example, the I425V variant)[4]. One could speculate that this more finely tuned regulatory capacity of SERT is another example of evolution-based development, like primate and human brain development, serving to enhance fitness to succeed in subsequent eons; however, more data are needed to confirm this.

The coding region of *SLC6A4* has changed over evolutionary time[4], but the non-coding regulatory regions of the gene have not yet been carefully evaluated for such changes beyond the few examples mentioned in this review[16]. Similarly, no attempt has yet been made to quantitate SERT expression and function in relation to fitness in more complex and thus relatively 'higher' organisms, for example, chimpanzees and rhesus macaques. SERT is also present in 'simple' organisms

that have only a neural net as a brain[115], as well as in insects, worms and many other vertebrates[4], but little is known about the relative functional capacity of SERT across these species. The crystal structure of a bacterial leucine transporter was recently elucidated[116] and has been used as a predictive model for the structure of SERT[117–120]. However, although a coherent and extensive tabulation of neurotransmitter sodium symporter (NSS) families that include the SLC group has become available[121], an experimental evolutionary-biology-based study of these bacterial amino-acid transporters has not yet been conducted.

In conclusion, a regulatory variation in the human gene that encodes SERT, the master controller in the fine-tuning of serotonin signalling, is not only associated with anxiety-related traits but also modifies the risk for a wide range of disorders. However, much of the human data to date is based on genetic-association studies alone. Mutations that result in reduced or absent SERT function in mice have led to the identification of more than 50 different phenotypic changes, ranging from increased anxiety and stress-related behaviours to gut dysfunction, bone weakness and late-onset obesity with metabolic syndrome. Although the effects are not as robust as those in the experimental mice, SERT-function-modifying gene variants in humans influence many of the same phenotypes. The need to blend behavioural genetics and evolutionary biology, as well as cognitive and social neuroscience, now defines the future challenges for the biosocial sciences in terms of the evolving genetic architecture of emotional behaviour, social interaction and disease in humans.

1. Lesch, K.-P. *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527–1531 (1996).
This paper provided the first demonstration that a functional-repeat length variation in the transcriptional control region of the *SLC6A4* gene is associated with anxiety-, depression-, and aggression-related personality traits. The authors also suggest a hypothesis for the SERT-specific genetic susceptibility of affective illnesses and related disorders.
2. Hu, X. Z. *et al.* Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am. J. Hum. Genet.* **78**, 815–826 (2006).
3. Lesch, K. P. & Murphy, D. L. in *Membrane Transport Diseases: Molecular Basis of Inherited Transport Defects* (eds Broer, S. & Wagner, C. A.) 349–364 (Kluwer Academic/Plenum, New York, 2003).
4. Murphy, D. L., Lerner, A., Rudnick, G. & Lesch, K. P. Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol. Interv.* **4**, 109–123 (2004).
5. Greenberg, B. D. *et al.* Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *Am. J. Med. Genet.* **96**, 202–216 (2000).
6. Hu, X. Z. *et al.* Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. *Arch. Gen. Psychiatry* **64**, 783–792 (2007).
7. Anguelova, M., Benkelfat, C. & Turecki, G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: II. Suicidal behavior. *Mol. Psychiatry* **8**, 646–653 (2003).
8. Sen, S., Burmeister, M. & Ghosh, D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **127**, 85–89 (2004).
9. Uher, R. & McGuffin, P. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. *Mol. Psychiatry* 14 Aug 2007 (doi:10.1038/sj.mp.4002067).
10. Kilic, F., Murphy, D. L. & Rudnick, G. A human serotonin transporter mutation causes constitutive activation of transport activity. *Mol. Pharmacol.* **64**, 440–446 (2003).
11. Prasad, H. C. *et al.* Human serotonin transporter variants display altered sensitivity to protein kinase G and p38 mitogen-activated protein kinase. *Proc. Natl Acad. Sci. USA* **102**, 11545–11550 (2005).
12. Sutcliffe, J. S. *et al.* Allelic heterogeneity at the serotonin transporter locus (*SLC6A4*) confers susceptibility to autism and rigid-compulsive behaviors. *Am. J. Hum. Genet.* **77**, 265–279 (2005).
13. Wendland, J. R., Martin, B. J., Kruse, M. R., Lesch, K.-P. & Murphy, D. L. Simultaneous genotyping of four functional loci of human *SLC6A4*, with a reappraisal of *5-HTTLPR* and rs25531. *Mol. Psychiatry* **11**, 224–226 (2006).
14. Ozaki, N. *et al.* Serotonin transporter missense mutation associated with a complex neuropsychiatric phenotype. *Mol. Psychiatry* **8**, 895 & 933–936 (2003).
This paper and reference 10 identified a functional coding-region variation in the *SLC6A4* gene that segregates with a complex serotonin-related phenotype in several families.
15. Wendland, J. R., Kruse, M. R., Cromer, K. C. & Murphy, D. L. A large case-control study of common functional *SLC6A4* and *BDNF* variants in obsessive-compulsive disorder. *Neuropsychopharmacology* **32**, 2543–2551 (2007).
16. Wendland, J. R. *et al.* A novel, putative gain-of-function haplotype at *SLC6A4* associates with obsessive-compulsive disorder. *Hum. Mol. Genet.* 30 Nov 2007 (doi:10.1093/hmg/ddm343).
17. Bengel, D. *et al.* Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. *Mol. Pharmacol.* **53**, 649–655 (1998).
This study, which used *SLC6A4*-knockout mice, was the first to characterize the relationship between SERT-dependent alterations in brain serotonin homeostasis and behaviour.
18. Ansorge, M. S., Zhou, M., Lira, A., Hen, R. & Gingrich, J. A. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* **306**, 879–881 (2004).
This work provided evidence that transient pharmacological inhibition of SERT during early development produced abnormal emotional behaviours in adult mice and thus mimicked the behavioural phenotype of *Slc6a4*-mutant mice. The results suggest that a developmental mechanism explains how low SERT function increases vulnerability to psychiatric disorders.
19. Thakker, D. R. *et al.* siRNA-mediated knockdown of the serotonin transporter in the adult mouse brain. *Mol. Psychiatry* **10**, 782–789 (2005).
20. Zhao, S. *et al.* Insertion mutation at the C-terminus of the serotonin transporter disrupts brain serotonin function and emotion-related behaviors in mice. *Neuroscience* **140**, 321–334 (2006).
21. Jennings, K. A. *et al.* Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. *J. Neurosci.* **26**, 8955–8964 (2006).
22. Kendler, K. S., Kuhn, J. W., Vittum, J., Prescott, C. A. & Riley, B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch. Gen. Psychiatry* **62**, 529–535 (2005).
23. Holmes, A., Li, Q., Murphy, D. L., Gold, E. & Crawley, J. N. Abnormal anxiety-related behavior in serotonin transporter null mutant mice: the influence of genetic background. *Genes Brain Behav.* **2**, 365–380 (2003).
24. Holmes, A., Yang, R. J., Lesch, K. P., Crawley, J. N. & Murphy, D. L. Mice lacking the serotonin transporter exhibit 5-HT_{1A} receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology* **28**, 2077–2088 (2003).
25. Lira, A. *et al.* Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. *Biol. Psychiatry* **54**, 960–971 (2003).
26. Kalueff, A. V., Ren-Patterson, R. F. & Murphy, D. L. The developing use of heterozygous mutant mouse models in brain monoamine transporter research. *Trends Pharmacol. Sci.* **28**,122–127 (2007).
27. Carroll, J. C. *et al.* Effects of mild early life stress on abnormal emotion-related behaviors in 5-HTT knockout mice. *Behav. Genet.* **37**, 214–222 (2007).
28. Adamec, R., Burton, P., Blundell, J., Murphy, D. L. & Holmes, A. Vulnerability to mild predator stress in serotonin transporter knockout mice. *Behav. Brain Res.* **170**, 126–140 (2006).
29. Carola, V. *et al.* Identifying molecular substrates in a mouse model of the 5-HTT × environment risk factor for anxiety and depression. *Biol. Psychiatry* 17 Oct 2007 (doi: 10.1016/j.biopsych.2007.08.013).
30. Wellman, C. L. *et al.* Impaired stress-coping and fear extinction and abnormal corticolimbic morphology in serotonin transporter knock-out mice. *J. Neurosci.* **27**, 684–691 (2007).
31. Maciag, D. *et al.* Neonatal antidepressant exposure has lasting effects on behavior and serotonin circuitry. *Neuropsychopharmacology* **31**, 47–57 (2006).
32. Mamounas, L. A. *et al.* BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain. *J. Neurosci.* **20**, 771–782 (2000).
33. Persico, A. M. *et al.* Barrel pattern formation requires serotonin uptake by thalamocortical afferents, and not vesicular monoamine release. *J. Neurosci.* **21**, 6862–6873 (2001).
This paper and reference 34 provided the first neuroanatomical demonstration of neurodevelopmental alterations in brain development as a function of SERT expression.
34. Salichon, N. *et al.* Excessive activation of serotonin (5-HT) 1B receptors disrupts the formation of sensory maps in monoamine oxidase A and 5-HT transporter knock-out mice. *J. Neurosci.* **21**, 884–896 (2001).
35. Esaki, T. *et al.* Developmental disruption of serotonin transporter function impairs cerebral responses to whisker stimulation in mice. *Proc. Natl Acad. Sci. USA* **102**, 5582–5587 (2005).
36. Li, Q., Wichems, C., Heils, A., Lesch, K. P. & Murphy, D. L. Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT_{1A}) in 5-HT transporter knock-out mice: gender and brain region differences. *J. Neurosci.* **20**, 7888–7895 (2000).
37. Li, Q. *et al.* Reduction of 5-hydroxytryptamine (5-$HT)_{1A}$-mediated temperature and neuroendocrine responses and 5-HT_{1A} binding sites in 5-HT transporter knockout mice. *J. Pharmacol. Exp. Ther.* **291**, 999–1007 (1999).
38. Li, Q. *et al.* Brain region-specific alterations of 5-HT_{2A} and 5-HT_{2C} receptors in serotonin transporter knockout mice. *J. Neurochem.* **84**, 1256–1265 (2003).
39. Murphy, D. L. *et al.* Genetic perspectives on the serotonin transporter. *Brain Res. Bull.* **56**, 487–494 (2001).

40. Ren-Patterson, R. F. *et al.* Gender-dependent modulation of brain monoamines and anxiety-like behaviors in mice with genetic serotonin transporter and BDNF deficiencies. *Cell. Mol. Neurobiol.* **26**, 775–780 (2006).
41. Li, Q. Cellular and molecular alterations in mice with deficient and reduced serotonin transporters. *Mol. Neurobiol.* **34**, 51–66 (2006).
42. Lanfumey, L., Mannoury La Cour, C., Froger, N. & Hamon, M. 5-HT-HPA interactions in two models of transgenic mice relevant to major depression. *Neurochem. Res.* **25**, 1199–1206 (2000).
43. Tjurmina, O. A., Armando, I., Saavedra, J. M., Goldstein, D. S. & Murphy, D. L. Exaggerated adrenomedullary response to immobilization in mice with targeted disruption of the serotonin transporter gene. *Endocrinology* **143**, 4520–4526 (2002).
44. Tjurmina, O. A., Armando, I., Saavedra, J. M., Li, Q. & Murphy, D. L. Life-long serotonin reuptake deficiency results in complex alterations in adrenomedullary responses to stress. *Ann. NY Acad. Sci.* **1018**, 99–104 (2004).
45. Armando, I., Tjurmina, O. A., Li, Q., Murphy, D. L. & Saavedra, J. M. The serotonin transporter is required for stress-evoked increases in adrenal catecholamine synthesis and angiotensin II AT_2 receptor expression. *Neuroendocrinology* **78**, 217–225 (2003).
46. Holmes, A., Murphy, D. L. & Crawley, J. N. Reduced aggression in mice lacking the serotonin transporter. *Psychopharmacology (Berl.)* **161**, 160–167 (2002).
47. Kalueff, A. V., Wheaton, M. & Murphy, D. L. What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. *Behav. Brain Res.* **179**,1–18 (2007).
48. Murphy, D. L. *et al.* Experimental gene interaction studies with SERT mutant mice as models for human polygenic and epistatic traits and disorders. *Genes Brain Behav.* **2**, 350–364 (2003).
49. Ren-Patterson, R. F. *et al.* Loss of brain-derived neurotrophic factor gene allele exacerbates brain monoamine deficiencies and increases stress abnormalities of serotonin transporter knockout mice. *J. Neurosci. Res.* **79**, 756–771 (2005).
50. Wisor, J. P. *et al.* Altered rapid eye movement sleep timing in serotonin transporter knockout mice. *Neuroreport* **14**, 233–238 (2003).
51. Fox, M. A. *et al.* A pharmacological analysis of mice with a targeted disruption of the serotonin transporter. *Psychopharmacology* **195**, 147–166 (2007).
52. Li, Q. *et al.* Medial hypothalamic 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors regulate neuroendocrine responses to stress and exploratory locomotor activity: application of recombinant adenovirus containing 5-HT_{1A} sequences. *J. Neurosci.* **24**, 10868–10877 (2004).
53. Chen, J. J. *et al.* Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity serotonin transporter: abnormal intestinal motility and the expression of cation transporters. *J. Neurosci.* **21**, 6348–6361 (2001).
54. Fox, M. A., Jensen, C. L. & Murphy, D. L. Mediation of exaggerated serotonin syndrome-like behaviors and temperature responses in serotonin transporter knockout mice by 5-HT_{1A} and 5-HT_7 serotonin receptors: a possible model and mechanism for differential human vulnerability to the serotonin syndrome. *American College of Neuropsychopharmacology Annual Meeting (Abstr.)* **60**, S221–S222 (2006).
55. Vogel, C. *et al.* Absence of thermal hyperalgesia in serotonin transporter-deficient mice. *J. Neurosci.* **23**, 708–715 (2003).
This study carried out a systematic assessment of pain sensitivity in *Slc6a4*-mutant mice.
56. Cornelissen, L. L., Brooks, D. P. & Wibberley, A. Female, but not male, serotonin reuptake transporter (5-HTT) knockout mice exhibit bladder instability. *Auton. Neurosci.* **122**, 107–110 (2005).
57. Eddahibi, S. *et al.* Attenuated hypoxic pulmonary hypertension in mice lacking the 5-hydroxytryptamine transporter gene. *J. Clin. Invest.* **105**, 1555–1562 (2000).
58. Kim, D. K. *et al.* Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. *Neuropharmacology* **49**, 798–810 (2005).
This paper, along with references 69, 73 and 83, provided the most comprehensive evaluation of the radical changes in serotonin homeostasis that occur in *Slc6a4*$^{+/-}$ and *Slc6a4*$^{-/-}$ mice.
59. Mekontso-Dessap, A. *et al.* Deficiency of the 5-hydroxytryptamine transporter gene leads to cardiac fibrosis and valvulopathy in mice. *Circulation* **113**, 81–89 (2006).
60. Warden, S. J., Bliziotes, M. M., Wiren, K. M., Eshleman, A. J. & Turner, C. H. Neural regulation of bone and the skeletal effects of serotonin (5-hydroxytryptamine). *Mol. Cell Endocrinol.* **242**, 1–9 (2005).
61. Bliziotes, M., Gunness, M., Eshleman, A. & Wiren, K. The role of dopamine and serotonin in regulating bone mass and strength: studies on dopamine and serotonin transporter null mice. *J. Musculoskelet. Neuronal Interact.* **2**, 291–295 (2002).
62. Holmes, A. *et al.* Adult-onset obesity and neurovegetative abnormalities in serotonin transporter null mutant mice. *American College of Neuropsychopharmacology (Abstr.)* **29904** (2002).
63. Diem, S. J. *et al.* Use of antidepressants and rates of hip bone loss in older women: the study of osteoporotic fractures. *Arch. Intern. Med.* **167**, 1240–1245 (2007).
64. Haney, E. M. *et al.* Association of low bone mineral density with selective serotonin reuptake inhibitor use by older men. *Arch. Intern. Med.* **167**, 1246–1251 (2007).
65. Warden, S. J., Robling, A. G., Sanders, M. S., Bliziotes, M. M. & Turner, C. H. Inhibition of the serotonin (5-hydroxytryptamine) transporter reduces bone accrual during growth. *Endocrinology* **146**, 685–693 (2005).
66. Sora, I. *et al.* Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc. Natl Acad. Sci. USA* **98**, 5300–5305 (2001).
67. Tecott, L. H. *et al.* Eating disorder and epilepsy in mice lacking 5-HT^{2C} serotonin receptors. *Nature* **374**, 542–546 (1995).
68. Bouwknecht, J. A. *et al.* Male and female 5-HT_{1B} receptor knockout mice have higher body weights than wildtypes. *Physiol. Behav.* **74**, 507–516 (2001).
69. Gobbi, G., Murphy, D. L., Lesch, K.-P. & Blier, P. Modifications of the serotoninergic system in mice lacking serotonin transporters. An *in vivo* electrophysiological study. *J. Pharmacol. Exp. Ther.* **296**, 987–995 (2001).
70. Qu, Y., Villacreses, N., Murphy, D. L. & Rapoport, S. I. $5\text{-HT}_{2A/2C}$ receptor signaling via phospholipase A_2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. *Psychopharmacology (Berl.)* **180**, 12–20 (2005).
71. Kelai, S. *et al.* Alcohol intake after serotonin transporter inactivation in mice. *Alcohol Alcohol.* **38**, 386–389 (2003).
72. Trigo, J. M. *et al.* 3,4-methylenedioxymethamphetamine self-administration is abolished in serotonin transporter knockout mice. *Biol. Psychiatry* **62**, 669–679 (2007).
73. Montanez, S., Owens, W. A., Gould, G. G., Murphy, D. L. & Daws, L. C. Exaggerated effect of fluvoxamine in heterozygote serotonin transporter knockout mice. *J. Neurochem.* **86**, 210–219 (2003).
74. Boyce-Rustay, J. M. *et al.* Ethanol-related behaviors in serotonin transporter knockout mice. *Alcohol Clin. Exp. Res.* **30**, 1957–1965 (2006).
75. Sora, I. *et al.* Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc. Natl Acad. Sci. USA* **95**, 7699–7704 (1998).
This paper, together with reference 66, used double-knockout mice to provide the first evidence that cocaine reward and dependence is mediated by both the dopamine transporter and SERT.
76. Fox, M. A., Huang, S.-J., Tolliver, T. J. & Murphy, D.L. Enhanced increases in serotonin underlie exaggerated serotonin syndrome behaviors in serotonin transporter (SERT) knockout mice. *Neuropsychopharmacology* (in the press).
77. Fox, M. A., Jensen, C. L., Gallagher, P. S. & Murphy, D. L. Receptor mediation of exaggerated responses to serotonin-enhancing drugs in serotonin transporter (SERT)-deficient mice. *Neuropharmacology* **53**, 643–656 (2007).
78. Gershon, M. D., Payette, R. F. & Rothman, T. P. Development of the enteric nervous system. *Fed. Proc.* **42**, 1620–1625 (1983).
79. Gershon, M. D., Chalazonitis, A. & Rothman, T. P. From neural crest to bowel: development of the enteric nervous system. *J. Neurobiol.* **24**, 199–214 (1993).
80. Gaspar, P., Cases, O. & Maroteaux, L. The developmental role of serotonin: news from mouse molecular genetics. *Nature Rev. Neurosci.* **4**, 1002–1012 (2003).
This paper provides a comprehensive overview of the role of serotonin in the modulation of different developmental processes, such as neurogenesis, apoptosis, axon branching and dendritogenesis.
81. McCaffery, J. M. *et al.* Common genetic vulnerability to depressive symptoms and coronary artery disease: a review and development of candidate genes related to inflammation and serotonin. *Psychosom. Med.* **68**, 187–200 (2006).
82. Scherrer, J. F. *et al.* A twin study of depression symptoms, hypertension, and heart disease in middle-aged men. *Psychosom. Med.* **65**, 548–557 (2003).
83. Mathews, T. A. *et al.* Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. *J. Neurosci. Methods* **140**, 169–181 (2004).
84. Zhou, F. C., Lesch, K. P. & Murphy, D. L. Serotonin uptake into dopamine neurons via dopamine transporters: a compensatory alternative. *Brain Res.* **942**, 109–119 (2002).
85. Schmitt, A. *et al.* Organic cation transporter capable of transporting serotonin is up-regulated in serotonin transporter-deficient mice. *J. Neurosci. Res.* **71**, 701–709 (2003).
86. Lesch, K. P. & Mossner, R. Inactivation of 5HT transport in mice: modeling altered 5HT homeostasis implicated in emotional dysfunction, affective disorders, and somatic syndromes. *Handb. Exp. Pharmacol.*, 417–456 (2006).
87. Fabre, V. *et al.* Altered expression and functions of serotonin 5-HT_{1A} and 5-HT_{1B} receptors in knock-out mice lacking the 5-HT transporter. *Eur. J. Neurosci.* **12**, 2299–2310 (2000).
88. Bouali, S. *et al.* Sex hormone-dependent desensitization of 5-HT_{1A} autoreceptors in knockout mice deficient in the 5-HT transporter. *Eur. J. Neurosci.* **18**, 2203–2212 (2003).
89. Rioux, A. *et al.* Adaptive changes of serotonin 5-HT_{2A} receptors in mice lacking the serotonin transporter. *Neurosci. Lett.* **262**, 113–116 (1999).
90. Laakso, A., Palvimaki, E. P., Kuoppamaki, M., Syvalahti, E. & Hietala, J. Chronic citalopram and fluoxetine treatments upregulate 5-HT_{2C} receptors in the rat choroid plexus. *Neuropsychopharmacology* **15**, 143–151 (1996).
91. Holmes, A., Yang, R. J., Murphy, D. L. & Crawley, J. N. Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. *Neuropsychopharmacology* **27**, 914–923 (2002).
92. Lucki, I. The spectrum of behaviors influenced by serotonin. *Biol. Psychiatry* **44**, 151–162 (1998).
93. Weiger, W. A. Serotonergic modulation of behaviour: a phylogenetic overview. *Biol. Rev. Camb. Philos. Soc.* **72**, 61–95 (1997).
94. Barnes, N. M. & Sharp, T. A review of central 5-HT receptors and their function. *Neuropharmacology* **38**, 1083–1152 (1999).
95. Cohen, Z., Bonvento, G., Lacombe, P. & Hamel, E. Serotonin in the regulation of brain microcirculation. *Prog. Neurobiol.* **50**, 335–362 (1996).
96. Munafo, M. R., Clark, T. G., Roberts, K. H. & Johnstone, E. C. Neuroticism mediates the association of the serotonin transporter gene with lifetime major depression. *Neuropsychobiology* **53**, 1–8 (2006).
97. Lesch, K. P. Alcohol dependence and gene x environment interaction in emotion regulation: is serotonin the link? *Eur. J. Pharmacol.* **526**, 113–124 (2005).
98. Rausch, J. L. *et al.* Depressed patients have higher body temperature: 5-HT transporter long promoter region effects. *Neuropsychobiology* **47**, 120–127 (2003).
99. Yeo, A. *et al.* Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. *Gut* **53**, 1452–1458 (2004).
100. Eddahibi, S. *et al.* Polymorphism of the serotonin transporter gene and pulmonary hypertension in chronic obstructive pulmonary disease. *Circulation* **108**, 1839–1844 (2003).
101. Caspi, A. *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389 (2003).
This paper, together with strong replications (references 9 and 22), proposed that the effect of *SLC6A4*-gene variation on depression risk in humans interacts with psychosocial stress. It also confirmed the findings in non-human-primate and rodent models of the existence of a neurodevelopmentally critical period of early postnatal life during which this interaction occurs.

102. Canli, T. *et al.* Neural correlates of epigenesis. *Proc. Natl Acad. Sci. USA* **103**, 16033–16038 (2006).
This study, together with reference 108, used high-resolution functional MRI to demonstrate that serotonin transport efficiency is important for brain processes involving neural systems that control affective, cognitive and motor processes. The authors also showed that life stress differentially moderates the functional connectivity of the amygdala and hippocampus with a wide network of other brain regions in individuals with different *SLC6A4* genotypes.
103. Nakatani, D. *et al.* Influence of serotonin transporter gene polymorphism on depressive symptoms and new cardiac events after acute myocardial infarction. *Am. Heart J.* **150**, 652–658 (2005).
104. Fumeron, F. *et al.* Serotonin transporter gene polymorphism and myocardial infarction: Etude Cas-Temoins de l'Infarctus du Myocarde (ECTIM). *Circulation* **105**, 2943–2945 (2002).
105. Canli, T. & Lesch, K. P. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nature Neurosci.* **10**, 1103–1109 (2007).
This paper contains a hypothesis that attempts to account for a role of SERT at the interface of emotionality and the social brain. The authors suggest that serotonergic signalling pathways integrate elementary tasks of sensory processing, cognition, emotion regulation and motor activity and posit that the potential impact on social cognition transcends the boundaries of behavioural genetics to embrace biosocial science and create a new social neurogenetics of behaviour.
106. Barr, C. S. *et al.* The utility of the non-human primate; model for studying gene by environment interactions in behavioral research. *Genes Brain Behav.* **2**, 336–340 (2003).
107. Bennett, A. J. *et al.* Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol. Psychiatry* **7**, 118–122 (2002).
By taking advantage of a non-human-primate maternal-separation paradigm, this paper provided the first evidence for a *SLC6A4*-gene–environment interaction that results in persistent alteration of brain serotonin function.
108. Canli, T. *et al.* Beyond affect: a role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. *Proc. Natl Acad. Sci. USA* **102**, 12224–12229 (2005).
109. Shen, H. W. *et al.* Regional differences in extracellular dopamine and serotonin assessed by *in vivo* microdialysis in mice lacking dopamine and/or serotonin transporters. *Neuropsychopharmacology* **29**, 1790–1799 (2004).
110. Bethea, C. L., Gundlah, C. & Mirkes, S. J. Ovarian steroid action in the serotonin neural system of macaques. *Novartis Found. Symp.* **230**, 112–130 (2000).
111. Homberg, J. *et al.* Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. *Neuroscience* **146**, 1662–1676 (2007).
112. Homberg, J. R. *et al.* Serotonin transporter deficiency in rats improves inhibitory control but not behavioural flexibility. *Eur. J. Neurosci.* **26**, 2066–2073 (2007).
113. Sakai, K. *et al.* Novel variants of murine serotonin transporter mRNA and the promoter activity of its upstream site. *Neurosci. Lett.* **342**, 175–178 (2003).
114. Ozsarac, N., Santha, E. & Hoffman, B. J. Alternative non-coding exons support serotonin transporter mRNA expression in the brain and gut. *J. Neurochem.* **82**, 336–344 (2002).
115. Buznikov, G. A., Peterson, R. E., Nikitina, L. A., Bezuglov, V. V. & Lauder, J. M. The pre-nervous serotonergic system of developing sea urchin embryos and larvae: pharmacologic and immunocytochemical evidence. *Neurochem. Res.* **30**, 825–837 (2005).
116. Yamashita, A., Singh, S. K., Kawate, T., Jin, Y. & Gouaux, E. Crystal structure of a bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters. *Nature* **437**, 215–223 (2005).
117. Henry, L. K., Defelice, L. J. & Blakely, R. D. Getting the message across: a recent transporter structure shows the way. *Neuron* **49**, 791–796 (2006).
This paper contains a hypothesis based on data from reference 116 that helps explain the biochemical and mutagenesis studies performed with related mammalian neurotransmitter transporters. The authors suggest a model for how coupling arises between ions and substrates to permit efficient neurotransmitter clearance.
118. Ravna, A. W., Jaronczyk, M. & Sylte, I. A homology model of SERT based on the $LeuT_{Aa}$ template. *Bioorg. Med. Chem. Lett.* **16**, 5594–5597 (2006).
119. Zhang, Y. W. & Rudnick, G. The cytoplasmic substrate permeation pathway of serotonin transporter. *J. Biol. Chem.* **281**, 36213–36220 (2006).
120. Singh, S. K., Yamashita, A. & Gouaux, E. Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. *Nature* **448**, 952–956 (2007).
121. Saier, M. H. Jr. Tracing pathways of transport protein evolution. *Mol. Microbiol.* **48**, 1145–1156 (2003).
122. Gage, P. J., Suh, H. & Camper, S. A. Dosage requirement of *Pitx2* for development of multiple organs. *Development* **126**, 4643–4651 (1999).
123. Nakayama, K. *et al.* Mice lacking $p27^{Kip1}$ display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* **85**, 707–720 (1996).
124. Fisher, R. A. *The Genetical Theory of Natural Selection* (Oxford Univ. Press, Oxford, 1930).
125. Otto, S. P. Two steps forward, one step back: the pleiotropic effects of favoured alleles. *Proc. Biol. Sci.* **271**, 705–714 (2004).
126. Gould, S. J. Is a new and general theory of evolution emerging? *Paleobiology* **6**, 119–130 (1980).
127. Turner, J. R. G. Fisher's evolutionary faith and the challenge of mimicry. *Oxf. Surv. Biol.* **2**, 159–196 (1985).
128. Huxley, J. *Evolution, the Modern Synthesis* (George Allen & Unwin, London, 1942).
129. Lande, R. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* **94**, 203–215 (1980).
130. Fuxe, K. *et al.* Chronic antidepressant treatment and central 5-HT synapses. *Neuropharmacology* **22**, 389–400 (1983).
131. Hillarp, N. A., Fuxe, K. & Dahlstrom, A. Demonstration and mapping of central neurons containing dopamine, noradrenaline, and 5-hydroxytryptamine and their reactions to psychopharmaca. *Pharmacol. Rev.* **18**, 727–741 (1966).

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
5-HT_{1A} receptor | 5-HT_{2A} receptor | 5-HT_{1B} receptor | 5-HT_{2C} receptor | BDNF | MAOA | *SLC6A4* | *Slc6a4* |

FURTHER INFORMATION
Dennis L. Murphy's homepage: http://intramural.nimh.nih.gov/lcs/home.html
Klaus-Peter Lesch's homepage: http://www.psychobiologie.uni-wuerzburg.de
Tabulation of the neurotransmitter sodium symporter (NSS) family: http://www.biology.ucsd.edu/~msaier/transport/
Discussion of reference 1 by K.P.L.: http://www.in-cites.com/papers/KPLesch.html

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