THE DEVELOPMENTAL ROLE OF SEROTONIN: NEWS FROM MOUSE MOLECULAR GENETICS

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New genetic models that target the serotonin system show that transient alterations in serotonin homeostasis cause permanent changes to adult behaviour and modify the fine wiring of brain connections. These findings have revived a long-standing interest in the developmental role of serotonin. Molecular genetic approaches are now showing us that different serotonin receptors, acting at different developmental stages, modulate different developmental processes such as neurogenesis, apoptosis, axon branching and dendritogenesis. Our understanding of the specification of the serotonergic phenotype is improving. In addition, studies have revealed that serotonergic traits are dissociable, as there are populations of neurons that contain serotonin but do not synthesize it.

There is increasing evidence that neurotransmitters are used as developmental signals, which modulate the construction and plasticity of brain circuits. Serotonin (5-hydroxytryptamine or 5-HT) was the first neurotransmitter for which a developmental role was suspected. Serotonergic neurons are among the earliest neurons to be generated, and 5-HT is released by growing axons before conventional synapses are established. Pharmacological studies initially showed that 5-HT can modulate a number of developmental events, including cell division, neuronal migration, cell differentiation and synaptogenesis[1–5]. It therefore came as a surprise that targeted deletions of 5-HT receptors or of genes involved in 5-HT metabolism in mice caused no gross abnormalities of brain development (TABLE 1), or at least not the marked alterations that had been expected on the basis of the previous pharmacological analyses. One of the reasons for this discrepancy is that there is a large variety of 5-HT receptors, and that each receptor might have a limited set of actions during specific periods in development.

Genetic studies in mice provided new ways to understand how the serotonergic phenotype is specified during development, and how, in turn, 5-HT modulates the construction of brain circuits. Among several unexpected discoveries was the finding that, during development, a number of neurons transiently express partial serotonergic phenotypes — they can store and release 5-HT, but they cannot synthesize it. Furthermore, a number of 5-HT receptors show early and dynamic expression during development, and genetic studies in mice helped us to understand how the overactivation or invalidation of certain 5-HT receptor subtypes causes permanent alterations in the maturation of selected brain circuits. So, even though the effects of an excess or a decrease of 5-HT are not as striking as was initially expected and cannot be detected by simple inspection of the brain, they definitely exist. The consequences of these miswiring problems are not trivial in terms of adult behaviour and raise the possibility that early changes in 5-HT homeostasis are involved in the physiopathology of psychiatric diseases, such as anxiety disorders, drug addiction and autism[6–8].

In this review we will consider how mouse genetics have helped us to understand how the serotonergic phenotype is specified early in development, and why some neurons express transient serotonergic features. Finally we will comment on a few recent examples of knockout mice that allow us to see how alterations in the levels of 5-HT or deletion of 5-HT receptors during specific developmental times modify the formation of brain circuits.

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Table 1 | Mouse mutant phenotypes

5-HT target molecules	Adult phenotype of knockout mice	Developmental defects and critical period	References
5-HT$_{1A}$ receptor	Increased anxiety, decreased exploration; reduced effects of antidepressants; altered sleep patterns	Adult neurogenesis; dendritic maturation (hippocampus) (first 3 weeks of postnatal life)	8,60,128–130
5-HT$_{1B}$ receptor	Increased aggression; increased exploration; increased response to cocaine; altered sleep patterns	Axon connection defects (retinotectal)	90,110,131
5-HT$_{2A}$ receptor	Decreased response to hallucinogens; enteric nervous system reactivity	Dendritic maturation (phrenic motor neurons)	64,132
5-HT$_{2B}$ receptor	Dilated cardiomyopathy	Cardiovascular and enteric neuron embryogenesis (cell survival)	105,133
5-HT$_{2C}$ receptor	Feeding behaviour; late-onset obesity; audiogenic seizures; altered LTP in hippocampus	Synaptic plasticity	101,134,135
5-HT$_{3A}$ receptor	Reduced pain behaviour	Not known	136
5-HT$_{4}$ receptor	Altered response to stress; hypersensitivity to seizures	Not known	137
5-HT$_{5A}$ receptor	Increased exploration	Not known	138
5-HT$_{7}$ receptor	Abnormal thermoregulation	Not known	139
Sert	Altered 5-HT homeostasis; modified responses to drugs of abuse	Axon connection defects (retinal, thalamic, barrel field); decreased apoptosis	66,68,140, 141
Maoa	Increased aggression; increased fear conditionning; reduced exploration; altered beam walking	Axon connection defects (retinal, thalamic, barrel field) (first postnatal week); dendritic exuberance (medulla); neuropeptide expression (hypothalamus)	27,37,61, 62,64,65
Maob	Increased reactivity to stress	Not known	142
Vmat2	Lethal; altered feeding/ growth	Increased apoptosis in telencephalon; altered migration	28,67, 69–71
Pet1	Increased aggression; increased anxiety	Not known	22
Gata3	Altered exploration	Not known	19

5-HT, 5-hydroxytryptamine, serotonin.

Specification of 5-HT neurons

The neurons that produce 5-HT are located in a restricted zone of the brainstem. Most are found in the raphe nuclei, on the midline of the rhombencephalon, with a smaller number in the reticular formation. The 5-HT-containing neurons are known as the B1–B9 cell groups[9] and they cluster as two main groups: the caudal division (B1–B5, corresponding to the raphe pallidus, magnus, obscurus and pontis), and the rostral division (B6–B9, corresponding to the dorsal and median raphe nuclei)[10,11] (FIG. 1a). The total number of serotonergic neurons is small — around 20,000 neurons in the rat[12] — compared with the total number of neurons in the central nervous system — some 10^{10}. But serotonergic neurons provide a relatively dense innervation to all the brain areas and the spinal cord, by way of an extensive and diffuse collateralization of their axons.

Serotonergic neurons are generated early in development, on embryonic days (E)10 to 12 in the mouse, and during the first month of gestation in primates[13]. One day after their generation, raphe neurons can synthesize 5-HT and begin to extend profuse axon tracts: the caudal group projects into the spinal cord and the rostral group into the forebrain. The full maturation of the axon terminal network requires more time and is achieved only after birth in rodents[10].

The molecular mechanisms by which neural precursors become serotonergic are beginning to be elucidated[14]. First, the region of the neural tube in which the 5-HT precursor neurons will be produced is defined. This involves the combined action of several secreted positional markers that diffuse from their zone of production, creating a three-dimensional space that is permissive for the specification of 5-HT precursors. In coculture analyses, Sonic hedgehog (Shh), Fgf4 and Fgf8, which are produced by the notochord, the primitive streak and the mid-hindbrain junction,respectively, act together to specify 5-HT precursor cells[15] (FIG. 1b). This scheme is also supported by *in vivo* genetic studies in mice. The role of the boundary between the midbrain and hindbrain as an organizing centre (MHO) has recently been demonstrated in transgenic mice in which the MHO was displaced[16]. When the MHO was moved caudally, the serotonergic neurons were displaced caudal to the new boundary, and the rostral territories were converted into dopamine neurons. There was a corresponding reduction in the rostrocaudal extent of the 5-HT cell groups. Conversely, when the MHO was shifted rostrally, the locus of production of the serotonergic neurons moved in the rostral direction[16].

The role of Shh signalling was demonstrated by the production of a transgenic mouse line in which a constitutively active form of Smoothened, an Shh receptor, was produced. In these mice, the 5-HT precursors were dorsalized, and serotonergic neurons were misplaced into the cerebellum[17]. This indicated a role of the Shh receptor in the dorsoventral control of the 5-HT phenotype. Another transcription factor, Nkx2.2, which is downstream of the Shh receptors, was recently implicated in the generation of the caudal 5-HT cell groups[18], probably in conjunction with Gata3 (REF. 19).

Once the position of the precursors is defined, other transcription factors are required to establish the serotonergic neurochemical phenotype — that is, to induce the enzymatic machinery that is necessary for the production and metabolism of 5-HT (FIG. 1c). These transcription factors are expressed in postmitotic cells, and comprise a Lim homeodomain gene *Lmx1*, and a transcription factor Pet1. Pet1 has a unique expression pattern: it is strictly limited to the raphe nuclei, and appears one day before the serotonergic neurons can be identified. This factor could directly activate the transcription of the genes that define the 5-HT phenotype: TRYPTOPHAN HYDROXYLASE (*Tph*), the AROMATIC AMINO ACID DECARBOXYLASE (*Addc*), the 5-HT TRANSPORTER (*Sert*) and the VESICULAR MONOAMINE TRANSPORTER (*Vmat*)[20,21].

TRYPTOPHAN HYDROXYLASE (Tph1, Tph2). The rate-limiting enzyme for serotonin (5-HT) synthesis. Tph converts tryptophan into 5-hydroxytryptophan. The essential amino acid tryptophan is taken up into the cells by a nonspecific amino-acid transporter. Two Tph genes have been identified in mammals, *Tph1* and *Tph2*, and are expressed in the periphery and in the raphe nuclei, respectively.

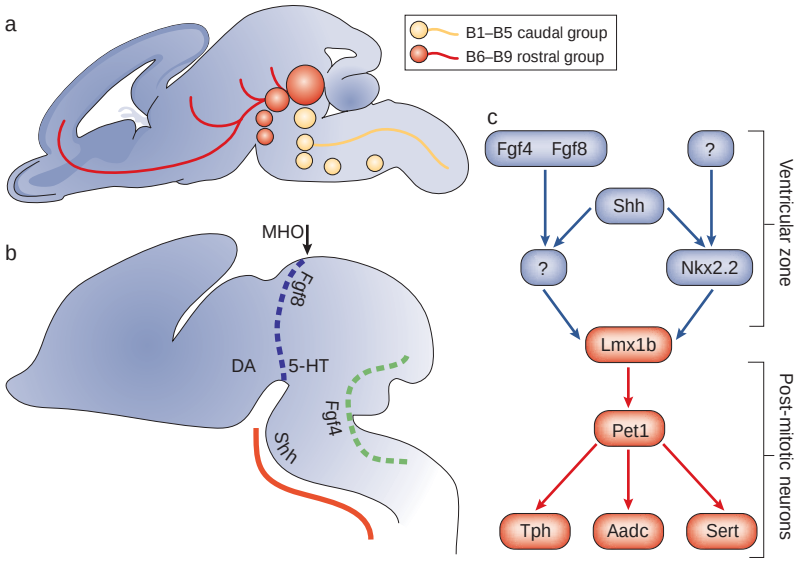

Figure 1 | **Serotonergic specification. a** | Organization of the serotonergic neurons in the brainstem. The serotonergic neurons comprise nine cell groups (B1–B9). The caudal serotonergic cell groups, B1–B5 (yellow), project to the brainstem and spinal cord, whereas the rostral cell groups, B6–B9 (red), send diffuse terminal arbours to the diencephalon and telencephalon. **b** | Specification of serotonin (5-hydroxytryptamine, 5-HT) precursor neurons in the neural tube is determined by the combination of secreted factors. Fibroblast growth factor 8 (Fgf8) is secreted by cells at the boundary between the midbrain and hindrain, in the midbrain–hindbrain organizing centre (MHO); fibroblast growth factor 4 (Fgf4) is produced by the primitive streak located dorsally and laterally in the neural tube; and Sonic hedgehog (Shh) is produced by the notochord in the ventral midline[15,16]. DA, dopamine. **c** | Transcriptional network controlling the 5-HT phenotype in the raphe nucleus. The factors labelled in blue specify the neuroblasts in the ventricular zone, orienting them towards a serotonergic phenotype. Fgf4, Fgf8, Shh[15] and possibly other factors activate the expression of Nkx2.2 in the caudal brainstem[18]. It is likely that a similar, as yet undetermined factor has a similar role in the rostral brainstem. The factors labelled in red induce terminal differentiation of the specified neuroblasts into functional serotonergic neurons. Two factors, Lmx1b and Pet1, are involved. They are expressed in post-mitotic neurons[20,127]. The transcription factor Pet1 directly activates the transcription of genes that are involved in the synthesis (*Tph* and *Aadc*) and the uptake (*Sert*) of 5-HT[20,22].

In mice lacking Pet1, most of the serotonergic neurons in the raphe nuclei fail to differentiate, and the remaining neurons show reduced expression of Tph and Sert[22].

As the raphe neurons begin to differentiate, 5-HT is released and could have a trophic autocrine effect. In cultures of raphe neurons, 5-HT amplifies its own synthesis and increases axon outgrowth[23–25]. Conversely, 5-HT could inhibit the differentiation of other neural precursors into serotonergic neurons: this was shown in explant preparations of the spinal cord, where the suppression of serotonergic inputs induced the appearance of new serotonergic interneurons[26]. Curiously, however, *in vivo* genetic models with massive depletion of, or increases in, brain 5-HT have failed to reveal significant modifications in the anatomical layout and projections of the central serotonergic neurons[27,28].

5-HT is also produced in the periphery: in the pineal gland, the enterochromaffin cells of the gut, the neuroepithelial bodies of the lung, and the parafollicular cells of the thyroid. This constitutes a more widely distributed population of 5-HT-producing cells, although their absolute number remains relatively small. It has been shown recently that different forms of Tph, the biosynthetic enzyme of 5-HT, are expressed in the periphery and in the central nervous system (CNS). Tph (Tph1), which was cloned from pinealocytes, was long thought to be the only Tph isoform, although its expression was acknowledged to be weaker in the raphe nuclei than in the pineal gland[29]. The explanation for this apparent weakness of expression is the existence of a second Tph isoform (Tph2), which is highly homologous to Tph1, but which is the only isoform expressed in the raphe neurons[30]. This difference between the biosynthetic enzymes used to produce 5-HT in the CNS and the periphery could reflect differences in the mechanisms of 5-HT cell specification. Indeed, the transcriptional network that specifies the 5-HT phenotype in neural crest derivatives (enteric and parafollicular cells) involves glial cell-derived neurotrophic factor (Gdnf) and Mash1 (REF. 31), but not the genes that were implicated in the raphe nuclei[21].

Neurons that transiently capture 5-HT

In addition to these serotonergic neurons, a number of cells in the central and peripheral nervous systems show a transient serotonergic phenotype during development. However, this is limited to certain aspects of the serotonergic phenotype: the cells show high-affinity 5-HT uptake and vesicular storage of 5-HT, but do not synthesize 5-HT.

Uptake of 5-HT was first observed in non-neuronal regions such as the heart, the cranial mesenchyme[32,33] and the notochord[34] during early development. At these early embryonic stages, before the genesis of the raphe neurons (at E11), 5-HT has a maternal origin. Later in embryogenesis, several days after the generation of the raphe neurons, 5-HT accumulation can be detected in several categories of neurons, in the thalamus, limbic cortex, hypothalamus, retina and superior olivary nucleus[35–37] (FIG. 2a), with different timing in the various regions. The uptake of 5-HT in these neurons is explained by transient expression of Sert, the high-affinity transporter of 5-HT, with, in most regions, matching expression of Vmat2, the transporter that packages 5-HT into synaptic vesicles[35,38,39]. However, these neurons express neither Tph or Aadc, which are required for 5-HT synthesis, nor the catabolic enzymes MONOAMINE OXIDASE A and B (Maoa and Maob)[40]. So, the presence of functional Sert and Vmat2 proteins in these non-monoaminergic neurons allows them to capture 5-HT that is released or leaks out of neighbouring 5-HT-producing axons from the raphe nuclei (FIG. 2b).

What could be the purpose of this heterologous uptake? One hypothesis is that 5-HT could be used by the neurons as a borrowed transmitter. The evidence for this is indirect and is essentially based on morphological observations showing amine accumulation in synaptic boutons and in small dense-core synaptic vesicles[41,42], pointing to the possibility that the amine could be released at thalamocortical synapses. If this hypothesis is correct, 5-HT uptake and release from the thalamic and cortical neurons would allow these neurons to produce pulses of 5-HT release in phase with incoming neural activity. Another possibility is that uptake creates a morphogenetic gradient of 5-HT between the neurons that produce 5-HT and those that actively pump it.

AROMATIC AMINO ACID DECARBOXYLASE (AADC). Converts 5-hydroxytryptophan into 5-HT. This enzyme is not specific to the serotonergic system as it is also used to decarboxylate dopamine and histidine.

SEROTONIN (5-HT) TRANSPORTER (Sert). This plasma membrane transporter allows a highly efficient capture of 5-HT into cells (affinity 10^{-7} M). It belongs to the family of Na^+- and Cl^--coupled transporters, with 12 transmembrane domains. Energy for this active uptake is generated by the Na^+-K^+ ATPase. Only one gene has been identified. Inhibitors of Sert, SSRIs, are widely prescribed as antidepressants.

Finally, this uptake could be a way to clear 5-HT away from the extracellular space, at a developmental point when the serotonergic fibre network is not fully mature.

The trafficking of 5-HT in the periphery offers examples that can support each of these hypotheses. In mature organisms, a comparable 'dissociated serotonergic phenotype' is found in certain cells (FIG. 2b). Platelets are a prototypical example: they express Sert and Vmat, allowing them to capture 5-HT from the bloodstream and to store it in dense-core vesicles[43]. In platelets, 5-HT is used as a borrowed transmitter that is released when the platelets are activated, for instance after damage to blood vessels. In other cell types, such as intestinal crypt cells, endothelial cells of the pulmonary vessels and thyroid follicular cells, the function of heterologous 5-HT uptake has been viewed as that of a clearance pathway, which could inactivate the 5-HT that is produced and released by neighbouring cells[44,45].

Whatever its precise function, this uptake is highly conserved. Neurons that transiently take up 5-HT during development have been described across many phyla, from lobsters[46] to humans[47]. The types of neuron that transiently capture 5-HT during development have some common characteristics that set them aside from the usual monoaminergic neurons. First, almost all of these neurons use glutamate as their main transmitter, even during the periods when they take up 5-HT. Second, they often belong to highly topographically organized sensory systems such as primary sensory afferents (olfactory and trigeminal nerves retinal ganglion cells) or sensory relay thalamic neurons that form the auditory, visual and somatosensory maps[39,48]. The 5-HT accumulation in these sensory thalamocortical terminals accounts for the dense 5-HT innervation that is seen in the primary sensory areas and more strikingly in the barrel field during early postnatal life[49–51]. The structural organization of these sensory relay neurons is the antithesis of that displayed by the raphe neurons. 5-HT-producing neurons of the raphe nuclei have diffuse, extensive axon terminal arbours, whereas 5-HT-capturing neurons have narrow, restricted terminal arbours. Interestingly, as discussed later, the control of 5-HT levels is important for the construction of the focused axonal arbours of the sensory neurons.

Not much is known about the transcriptional regulation of the transient Sert expression in the non-aminergic neurons. The regulatory mechanisms probably differ from those uncovered in the raphe nuclei, as none of the genes that regulate the 5-HT phenotype in the raphe nuclei have been found in the zones that transiently express Sert[21]. *In vitro* experiments have established that the temporal regulation of the transient expression patterns is cell autonomous: in dissociated thalamic cultures, the onset and time of extinction of the transient *Sert* gene expression is similar to that observed *in vivo*[52]. *In vivo*, sensory deprivation has no effect on the transient expression of Sert and Vmat2 in the thalamus[38,53], indicating that afferent neural activity is not important in this dynamic genetic control. However, neural activity and 5-HT levels could modulate the trafficking of Sert at the plasma membrane[52,54]. On the other hand, hormones, such as thyroid hormones, could control the transient expression of Sert[55].

It would be interesting to determine whether the transient 'monoamine uptake phenotype' can be reactivated under certain conditions in the mature brain and be implicated in neural plasticity. Interestingly, the expression of Sert persists in the dentate gyrus of the hippocampus[38,53], an area of the brain that maintains a life-long capacity for neuronal renewal. The rostral subventricular zone is another region of the brain in which adult neurogenesis occurs and in which expression of Vmat2 continues to be visible in the mature brain (O.C. and P.G., unpublished observations). The function of 5-HT uptake in these neurons could be particularly important for the control of adult neurogenesis[56–60].

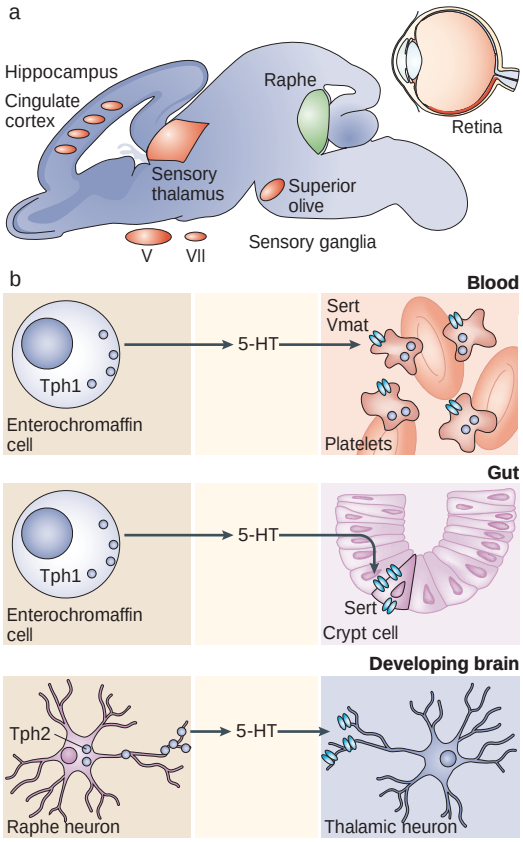

Figure 2 | **Uptake of serotonin (5-HT) in mature cells and developing neurons. a** | Localization of neurons that transiently express Sert during development. The raphe neurons (green) express Sert shortly after neurogenesis (embyonic day (E) 11) and continue this expression throughout life. In addition, Sert is transiently expressed — from E15 to postnatal day (P) 10 — in other areas (red): the sensory thalamic neurons, neurons in the anterior cingulate cortex, the hippocampus, the superior olive, sensory ganglia neurons and retinal ganglion cells. **b** | Circulation of 5-HT between cells that produce 5-HT and those that capture it. In the blood, circulating 5-HT derives essentially from the enterochromaffin cells. Plasma 5-HT is captured and concentrated by platelets, which release it on stimulation[43]. In the gut, enterochromaffin cells release 5-HT that is captured by the crypt cells, which metabolize the amine[45]. In the developing brain, 5-HT is produced by raphe neurons and is captured by thalamic axons, which store it in synaptic vesicles[35].

VESICULAR MONOAMINE TRANSPORTER (Vmat2, Vmat1). A synaptic vesicle protein that transports monoamines (5-HT, dopamine, noradrenaline and histamine) from the cytoplasm into vesicles, using a proton gradient. Two isoforms have been cloned, Vmat2 in the CNS, and Vmat1 in the periphery. Inhibitors of Vmat have been used as amine depleting agents for the treatment of hypertension and can cause depression as a side effect.

MONOAMINE OXIDASES (Maoa, Maob). These are enzymes located in the outer mitochondrial membrane that de-aminate biogenic amines. Maoa is the most active subtype in 5-HT metabolism. Inhibitors of Maoa are potent antidepressants.

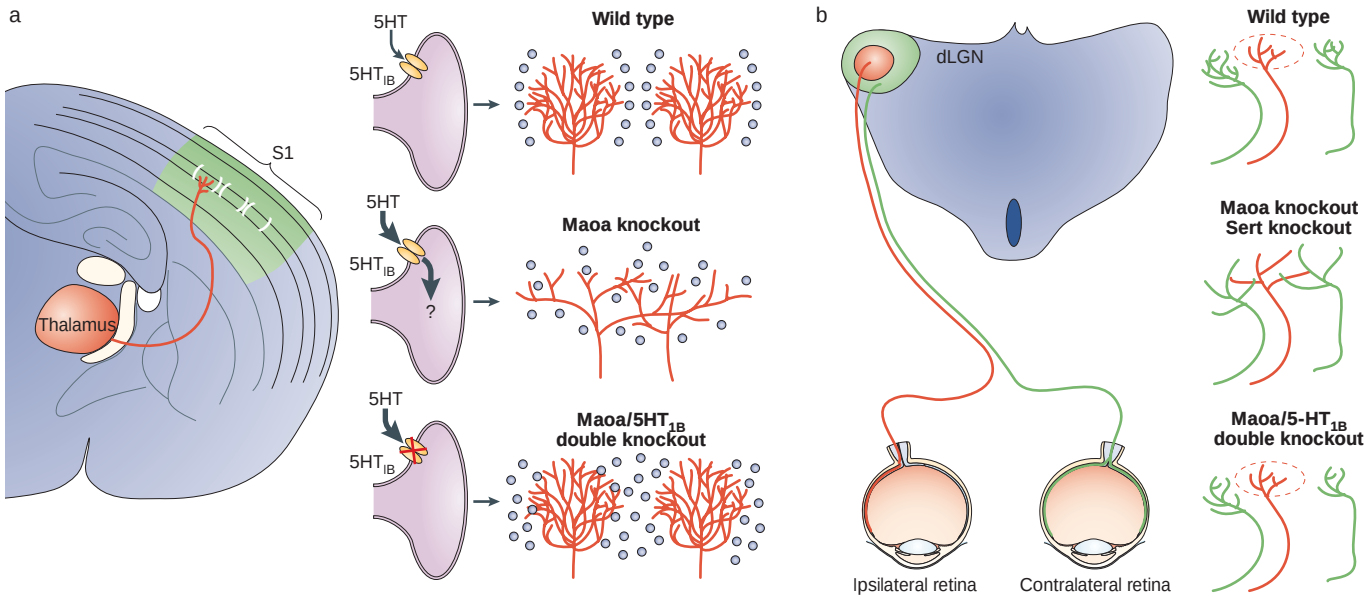

Figure 3 | **Effects of excessive stimulation of the 5-HT$_{1B}$ receptor on the refinement of axonal arbours. a** | In the mouse somatosensory cortex, thalamic axons carrying sensory afferents from one whisker form clusters in layer IV that are called barrels. This organization emerges over the first postnatal days by axon collateral remodelling[62]. During this period, thalamic axon terminals express the 5-HT$_{1B}$ receptor[86]. In Maoa- or Sert-knockout mice, in which 5-HT levels are increased, the thalamic clusters do not form[61,66,67]. Rather, the axon branches retain immature characteristics, with a decreased number of branches[62]. When the 5-HT$_{1B}$ receptor is also invalidated, in double knockout mice, axonal branching is restored and the thalamocortical clusters form normally[62,66]. **b** | The mouse retinothalamic projections. Retinal axons from each eye are organized in disjunctive territories with ipsilateral axons (red) forming a central cluster that is surrounded by axon terminals from the contralateral retina (green). This organization emerges during the first postnatal week by axonal arbour remodelling. Retinal ganglion cells express the 5-HT$_{1B}$ receptor presynaptically[88]. In Maoa-knockout and Sert-knockout mice, the segregation of both types of axons is lost[37,90], and the segregation is restored in mutants that also lack the 5-HT$_{1B}$ receptor[66].

Disturbances of 5-HT homeostasis

The most straightforward way to understand the function of the transient expression of genes that control 5-HT levels during development has been to investigate the effects of invalidation of these genes. Two genetic models, Maoa-knockout and Sert-knockout mice, have been particularly useful in this respect.

Invalidation of the gene that encodes the main enzyme responsible for 5-HT degradation, Maoa, causes a ninefold increase in the level of 5-HT in the brain during the first postnatal week[27]. The effects of Maoa deletion are compensated for later in life by the other monoamine-degrading enzyme, Maob. So, in the knockouts, the effects of the Maoa loss-of-function are maximal during early postnatal life. During this period, 5-HT accumulation was found in all of the neurons that transiently express Sert[36,37], and in the somatosensory and the visual systems the effects of this 5-HT increase were clear-cut. The most striking abnormality was the lack of barrels in the somatosensory cortex[61]. Barrels are the morphological substratum of the somatosensory cortical map (FIG. 3a): each barrel receives sensory afferents from one whisker through specific thalamic afferents, which normally form discrete clusters in cortical layer IV. Increased brain levels of 5-HT during the first postnatal week disrupt the clustering and segregation of the thalamocortical fibres that normally occurs during this time[62,63]. Similar abnormalities were found in the visual system. The retinal axons from both eyes initially overlap in their central targets, the superior colliculus and the lateral geniculate nucleus, and segregate into eye-specific domains during the first postnatal week (FIG. 3b); the normal segregation of these retinal axons was altered in Maoa-knockout mice[37]. Other developmental alterations in Maoa-knockout mice concerned the control of locomotor and respiratory rhythms in neonates. This could be linked to the abnormal maturation of the neural networks that subserve respiratory control[64]. Other subtle developmental defects were noted; for instance, the proportion of neurons expressing vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) in the suprachiasmatic nucleus was significantly increased in Maoa-knockout mice, indicating that 5-HT might participate in this neurochemical differentiation[65].

In Sert-knockout mice, 5-HT continues to be released but cannot be removed from the synaptic cleft. This causes abnormal development of the thalamocortical and retinal axons that is similar to that observed in Maoa-knockout mice[37,66,67]. This similarity indicated that an excessive build-up of 5-HT in the extracellular space is responsible for the developmental changes. A genetic approach showed that excessive and/or continuous stimulation of 5-HT receptors on the thalamocortical and retinotectal axons was the main culprit (see section on 5-HT$_{1B}$ receptors). Other neurons could benefit from this increased stimulation of 5-HT receptors, as indicated by the reduction in the amount of developmental cell death in the telencephalon of Sert-knockout mice[68].

It has been harder to develop genetic models in which the effects of 5-HT depletion could be analysed, but they are now forthcoming. Vmat2-knockout mice are unable to package monoamines into synaptic vesicles, and as a result, all monoamines are rapidly degraded; the consequence is a severe depletion of 5-HT, dopamine and noradrenaline[28,69,70]. Crossing Vmat2-knockout mice and Maoa-knockout mice produced a mouse strain in which 5-HT was selectively increased, whereas dopamine and noradrenaline remained depleted[67,1]. Comparison of these mouse strains allowed us to conclude that monoamines are not required for the initial wiring of the brain[28] or for the formation of thalamocortical connections[67,71], but that they probably modulate late neuronal maturation processes such as cortical cell migration and developmental cell death[68,72]. Unfortunately, Vmat2-knockout mice do not survive long after birth, preventing a full analysis of the consequences of monoamine depletion for fine aspects of brain wiring. Furthermore, it is probable that, during intra-uterine life, monoamines originating from the mother compensate for the deficiency. Pharmacologically depleting 5-HT by administering drugs to the mother (or directly to embryos) during gestation produced more severe changes such as alterations in neurogenesis[5,73], migration[74] and dendritic maturation[3,5,75]. Recently, a knockout of the peripheral isoform of Tph has been generated. In this Tph1 mutant, 5-HT levels are normal in the brain and reduced in the periphery, causing a progressive cardiopathy that leads to heart failure[76]. The analysis of the newly generated genetic models in which the specification of the 5-HT phenotype is deficient will undoubtedly produce interesting insights into the consequences of embryonic and postnatal depletion of amines. In Pet1-knockout mice, which have 80% depletion of 5-HT in the CNS, the general structure of the brain seems to be normal. However, these mice have marked behavioural changes, such as heightened anxiety and aggressive behaviour[22], and it is possible that a closer analysis of the fine brain structure or wiring patterns will reveal developmental abnormalities underlying these behavioural changes.

A variety of 5-HT receptors in development

The rich diversity of 5-HT receptors creates many ways by which 5-HT could affect developing cells, from fast neurotransmission through channel receptor opening, to short- or long-term neuromodulation through intracellular transduction cascades. Fifteen genes that encode 5-HT receptors have been cloned in the mammalian brain[77,78]. Two of these genes encode 5-HT-gated ion channel receptors (5-HT_{3A} and 5-HT_{3B}) and the other thirteen encode G-protein-coupled receptors. These receptors are categorized into four groups according to their second messenger coupling pathways: the 5-HT_1 receptors, which are coupled to G_i proteins (5-HT_{1A}, 5-$HT_{1B–C}$ and 5-$HT_{1D–F}$); the 5-HT_2 receptors, which are coupled to G_q proteins (5-$HT_{2A–C}$); the 5-HT_4, 5-HT_6 and 5-HT_7 receptors, which are coupled to G_s proteins; and the 5-HT_5 receptors (5-HT_{5A} and 5-HT_{5B}), which resemble the previous group but whose transduction cascade is not entirely clear. Furthermore, post-genomic modifications, such as alternative mRNA splicing or mRNA editing, create at least 20 additional 5-HT receptors with different binding affinities and physiological functions[77].

This abundance explains why we are still far from understanding how each receptor, alone or in combination with others, influences brain development. Even the precise cellular localization of each of these receptors during brain development is not well known. However, localization studies show that members of each of the main 5-HT receptor classes are expressed early in embryonic life and are dynamically regulated during pre- or postnatal development. The cellular developmental mechanisms that are triggered by some of these receptors are beginning to be more clearly understood in a few cases, which are considered here.

5-HT_{1A} receptors are expressed early in embryonic life[79], mainly in the raphe nucleus and hippocampus, and are transiently expressed in spinal motor neurons and the cerebellum after birth[80,81]. They have been implicated in various developmental effects *in vitro*[4]. *In vivo* studies indicate that the most striking effects concern the control of adult neurogenesis and of dendritic maturation in the hippocampus. Activation of 5-HT_{1A} receptors stimulates neurogenesis in the dentate gyrus and in the subventricular zone[57,82], two areas where there is life-long production of neuroblasts. This pro-neurogenic effect has been suggested to be a strong determinant of the action of antidepressants such as fluoxetine[59], which inhibits 5-HT uptake and prolongs the action of amine at the synapse. In 5-HT_{1A}-knockout mice the actions of antidepressants on neurogenesis and on behaviour are both abolished[60], further strengthening the link between these phenomena. Using a genetic strategy, the same groups showed that the rescue of 5-HT_{1A} function in the telencephalon is sufficient to rescue the behavioural phenotype, indicating that the lack of 5-HT_{1A} receptors in the hippocampus, but not in the raphe nucleus, is responsible for these changes[8]. However, it remains to be determined whether the neural progenitors themselves express the receptor or whether 5-HT_{1A} receptors exert an indirect trophic effect through glial cells[4], because no mitogenic action of 5-HT_{1A} receptors could be seen in primary neuronal cultures[83]. The other clear effect of 5-HT_{1A} receptor activation was a stimulation of dendritic differentiation: early postnatal depletion of 5-HT reduced the length of dendrites and the number of dendritic spines of hippocampal neurons, an effect that was shown to depend on 5-HT_{1A}receptors[84].

5-HT_{1B} receptors have an early and dynamic expression profile, indicating that they are important during development. 5-HT_{1B} receptors are expressed in the raphe nucleus, the striatum, the cerebellum and the retinal ganglion cells[85]. In addition, they are transiently expressed in all the sensory thalamic relay nuclei during early postnatal development[86,87]. In all of these neurons, 5-HT_{1B} receptors are localized presynaptically on axon terminals and modulate the release of glutamate in relation to incoming neural activity[87,88]. A double knockout strategy demonstrated the role of this receptor in the developmental changes that are induced by increased

Box 1 | Serotonin (5-HT) and long-term plasticity in invertebrates

Invertebrates were the first organisms in which a developmental role of 5-HT was shown[111]; they also provide simplified models in which the cellular and physiological effects of 5-HT can be most readily teased out and are best understood. The most elaborate understanding of how 5-HT participates in structural plastic changes stems from studies of two aquatic molluscs, *Aplysia* and *Helisoma*.

In the pond snail *Helisoma*, 5-HT induces a collapse of growing axons. This response results from the activation of cyclic AMP, which directly activates cyclic-nucleotide-gated sodium channels, leading to neuronal depolarization, calcium entry and a calcium-calmodulin-dependent inhibition of growth cones[112]. In the growth cone, 5-HT affects the behaviour of the motile filopodia and contributes to the redistribution of actin filamin bundles[113].

In *Aplysia*, the gill and siphon withdrawal reflex has been an invaluable model for understanding the mechanisms of neural plasticity[114,115]. A single application of 5-HT induces short-term synaptic facilitation, which is rapid and does not require new macromolecular synthesis. Conversely, repeated application of 5-HT (five applications over 1.5 hours) causes long-term synaptic facilitation that lasts for more than one day, and that requires translation and transcriptional changes[116]. This long-term plasticity involves the growth of new synaptic connections between the sensory and motor neurons[117]. The 5-HT-induced long-term potentiation involves activity-dependent activation of the cAMP signalling pathway[118], cAMP-response element (CRE)-binding protein (CREB) activation and consequent transcriptional changes. Modifications in the trafficking of cell surface receptors were also observed, with a downregulation of the cell adhesion molecule apCAM (CAM, *Aplysia* Fasciclin II) on the surface of the growing sensory axons[119]. This is thought to contribute to the defasciculation of axons, their increased outgrowth and the formation of new synaptic contacts. These mechanisms of 5-HT-induced long-term synaptic plasticity in *Aplysia* could have some distant equivalent in the sensory systems of mammals, where 5-HT modulates activity-dependent remodelling of axonal arbours by acting on a cAMP-coupled presynaptic 5-HT_1 receptor.

brain levels of 5-HT. Maoa-knockout and Sert-knockout mice have an altered patterning of thalamocortical axons in the barrel field and an abnormal segregation of retinal afferents in the lateral geniculate nucleus; these abnormalities were corrected by the additional invalidation of the 5-HT_{1B} receptor (FIG. 3a,b), despite the continued excess of 5-HT[66]. Single axon reconstructions of the thalamocortical axons in these mutants showed that the 5-HT_{1B} receptor could selectively influence the production and retraction of collateral axon branches on thalamic axons[62]. This is consistent with *in vitro* observations of the effects of 5-HT_{1B} agonists on axon growth[89]. Interestingly, both a lack of and excessive stimulation of the 5-HT_{1B} receptors can influence the development of axonal arbours[90]. The role of the 5-HT_{1B} receptor might be similar to that of the presynaptic 5-HT receptor that was identified on *Aplysia* sensory neurons, where it modulates synaptic strength and axon growth (BOX 1). In mammals, however, unlike invertebrates, the 5-HT_{1B} receptor inhibits cyclic AMP production and calcium entry in axon terminals[77,91]. By doing so, it could also be an important modulator of presynaptic plasticity mechanisms[87].

The 5-HT_{2A} receptor is one of the most widely expressed 5-HT receptors in the brain. It is expressed late in development[92,93], indicating that it is involved in late maturation processes. Several studies have indicated that 5-HT_{2A} receptors are important in neuronal differentiation and dendritic maturation[4,83]. At present, there has been no clear genetic confirmation of these effects, as 5-HT_{2A}-knockout mice do not have substantial CNS abnormalities. Conversely, excess activation of 5-HT_{2A} receptors might be implicated in the altered dendritic maturation of phrenic motor neurons in Maoa-knockout mice: the exuberant dendritic development of these neurons was normalized by administering 5-HT_{2A} antagonists during postnatal development[64]. 5-HT_{2A} receptors are also involved in modulating the expression of brain-derived neurotrophic factor (Bdnf) in the neocortex and hippocampus[94], which could in turn modulate several late developmental processes. This effect could, for instance, underlie the anti-apoptotic effect of 5-HT_2 agonists *in vitro*[95].

Another 5-HT_2 receptor subtype, the 5-HT_{2C} receptor, has been implicated in late developmental events. In kittens, the 5-HT_{2C} receptor has a characteristic columnar expression in layer IV of the visual cortex during the critical period for ocular dominance plasticity[96]. 5-HT_{2C} agonists modulate synaptic plasticity during this period[97] and the disruption of 5-HT transmission *in vivo* alters ocular dominance plasticity[98,99]. Similar observations were recently made in the rodent visual cortex, implicating 5-HT_{2C} receptors in developmental synaptic plasticity of the visual cortex[100]. Mice that lack 5-HT_{2C} receptors have deficits in long-term potentiation in the hippocampus[101], further supporting a role for this receptor in synaptic plasticity.

The 5-HT_{2B} receptor is the only 5-HT receptor for which actions have been identified during early embryonic development. It is expressed in proliferating cells in the heart and in neural crest-derived progenitors, at the earliest stages of development (E9). Activation of 5-HT_{2B} receptors enhances cell proliferation, increases cell migration and reduces apoptosis. The mitogenic action involves direct transactivation of the receptor for a growth factor, Pdgf, and involves extracellular signal-regulated kinase (ERK)-dependent pathways that act on cell-cycle proteins such as cyclin E and D[102]. Related pathways might be implicated in the specification of cell fate: 5-HT_{2B} receptors modulate the neural crest derivatives that give rise to the enteric neurons[103]. The anti-apoptotic effect of 5-HT_{2B} seems to involve the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, and the mitochondrial adenine nucleotide translocator through NF-κB[104]. Some 5-HT_{2B}-knockout mice show a mid-gestation lethality because of cardiac malformations, and the surviving pups exhibit severe ventricular hypoplasia[105]. Neural tube closure defects were also observed in a mixed genetic background, although these effects were lost in a pure 129PAS background.

This incomplete overview, limited to five of the fifteen 5-HT receptors, emphasizes that 5-HT has different target receptors at different times during development and in different tissues. For instance, at the earliest embryonic stages corresponding to active neurogenesis, the 5-HT_{2B} receptor seems to be important for the control of neurogenesis, cell specification and cell survival. At later developmental periods, this control could be mediated by other receptors such as 5-HT_{1A} and/or 5-$HT_{2A/2C}$ receptors, according to the brain regions. The type of developmental effect could also depend on the subcellular localization of the receptor in the soma

Box 2 | Serotonin (5-HT) and psychiatric diseases

5-HT is an important factor in psychiatric diseases, and many compounds that target the 5-HT system are used as psychotherapeutic agents. Serotonin-specific reuptake inhibitors (SSRIs) are the most efficient antidepressants, and are widely used for the treatment of depression, obsessive compulsive disorders and anorexia nervosa. Antagonists of 5-$HT_{2A/2C}$ receptors are used as antipsychotics, whereas 5-HT_{1A} receptor antagonists are used as anxiolytics. On the negative side, a number of 5-HT receptor agonists, the most famous of which is LSD (lysergic acid diethylamide), can induce hallucinations, thereby mimicking the symptoms of schizophrenia.

There is mounting evidence that some of the long-term therapeutic effects of 5-HT agonists could involve developmental mechanisms. Antidepressants have been shown to promote neurogenesis in the adult hippocampus[57,58,82] and to increase the production of neurotrophins in the cerebral cortex[94]. Newly generated dentate gyrus neurons require several weeks to integrate into the hippocampal circuits, which could account for the delay that is necessary before antidepressants begin to act. Recent observations in 5-HT_{1A}-knockout mice strengthen this assumption[60].

Despite the broad therapeutic effects of 5-HT-targeted molecules, no primary disorder of the 5-HT system has been found among the psychiatric disorders for which these drugs are administered[120]. However, genetic variations in 5-HT-linked genes, such as the SERT promoter (L-polymorphic repeats), could contribute to the risk of developing anxiety and mood-related disorders[121,122]. Furthermore, the hypothesis that 5-HT-related developmental changes could contribute to abnormal brain function remains appealing. Support for this stems from studies in infantile autism and mild mental retardation. Increased blood levels of 5-HT have been repeatedly found in a subpopulation of autistic patients[123]. Recent imaging studies indicate that the hyperserotonaemia in autistic children could result from disrupted developmental control of 5-HT production. A transient period of high brain 5-HT synthesis is observed in children under the age of five, after which 5-HT levels decrease to adult values in normal but not in autistic children[124]. Autism is, however, a heterogeneous disease that can result from many causes, genetic or environmental. Single family cases can provide interesting clues in this respect: a point mutation of the gene encoding MAOA was found in a Dutch family where the affected males displayed a syndrome that combined mild mental retardation and aggressive antisocial behaviour[125]. The mutation caused a loss of MAOA function: blood levels of 5-HT were increased and the normal metabolite of 5-HT, 5-HIAA, was absent. Although no other mutations of this gene have been described, a polymorphism of the *MAOA* gene has been implicated in the cycle of violence in maltreated children[126], emphasizing the possible importance of serotonin control in the reaction of individuals to stressful situations. It is likely that increasing knowledge of the genes and mechanisms that control the transient expression of 5-HT-related molecules, as well as the terminal differentiation of 5-HT neurons, will offer new target genes to identify genetic variants or polymorphisms that control 5-HT homeostasis and that might be implicated in the genesis of psychiatric disorders.

or axon of the neuron. So, 5-HT_{1A} and 5-HT_{1B} receptors share similar regulatory cascades but are localized in different parts of the neuronal membrane: the 5-HT_{1A} receptors are somatodendritic, whereas 5-HT_{1B} receptors are localized on axons and terminals[106]. Accordingly, the activation of 5-HT_{1A} receptors modulates dendritic growth, whereas 5-HT_{1B} receptors modulate axonal elaboration. There could be a similar differential localization in dendritic and axonal domains in the case of the 5-HT_{2A} and 5-HT_{2C} receptors[107,108]. A better understanding of the developmental effects of 5-HT in the future will therefore be tied to a more complete knowledge of the cellular biology of the 5-HT receptors, their cellular trafficking and their transduction pathways. These are the steps that are now essential to understand the interactions of 5-HT with other developmental signals that instruct the development of the brain.

Late developmental changes

Most of the mice in which knockouts have targeted the 5-HT system exhibit severe to mild behavioural alterations[7,109] (TABLE 1). Such mutants often show an altered response to stressors, reflecting increased (5-HT_{1A}-knockout mice) or decreased (5-HT_{2C}- and 5-HT_{5A}-knockout mice) levels of anxiety. Increased aggression among males is also often seen in mouse models in which 5-HT brain levels are increased[27], 5-HT production is reduced[22] or a 5-HT receptor subtype is lacking[110]. Eating disorders, altered sleep patterns, altered exploration and modified intake of drugs of abuse, such as cocaine, are also often seen in these genetic models. Such abnormalities emphasize the possible links between altered functioning of the serotonergic systems and human psychiatric disorders (BOX 2).

These behavioural changes are observed in adults and could be related to the acute effects of the 5-HT receptors. Alternatively, some of them could reflect mis-wiring of brain connections during development. The latter possibility has been investigated in a limited number of mutants. In Maoa-knockout mice, a pharmacological approach was taken to rescue the metabolic abnormality at different developmental times. This showed that the critical period for causing abnormal brain wiring in the somatosensory cortex was the first postnatal week, and that Maoa inhibition during embryonic life or in adults caused no visible effects[61,63]. Some of the abnormal behaviours of the Maoa-knockout mice, such as open-field exploration and beam walking, were also rescued when 5-HT levels were controlled during early postnatal life[66], indicating that they are caused by developmental alterations and not by the altered metabolism of 5-HT in adults. In 5-HT_{1A}-knockout mice, 5-HT_{1A} receptor function was rescued by molecular genetic techniques at different times during development, showing that invalidation of the receptor during infancy and not during adulthood is necessary to induce altered anxiety-prone behaviours[8].

These two cases underline the importance of early postnatal development for the maximal effects of altered 5-HT homeostasis. This developmental period corresponds to a phase of axonal and dendritic remodelling and synaptic elaboration, which therefore seem to be favoured developmental targets of these 5-HT receptors.

Conclusion

Although mice with targeted deletions of 5-HT receptors or of genes involved with 5-HT metabolism have no gross abnormalities of brain development, molecular genetic studies in mice have provided insights into the specification of the serotonergic phenotype during development and into the modulation of brain wiring by 5-HT. Transient serotonergic phenotypes, including storage and release of 5-HT, are well established, and 5-HT receptors have been shown to have an early and dynamic expression during development. The gain or loss of function of certain 5-HT receptors at critical periods in development causes permanent changes in the formation of selected neural connections. The most clear cut effects are the role of 5-HT_{1A} receptors in the

maturation of the hippocampus, and the role of 5-HT_{1B} receptors in the remodelling of sensory neuron axons. As shown in mice with massive depletions of 5-HT, the amine does not seem to be essential for the overall wiring of the brain, but adequate genetic models inducing a total depletion of 5-HT are still lacking. Continued research in this field is required to determine how alterations in the levels of 5-HT or 5-HT receptors during specific developmental times modify the formation of brain circuits and influence behaviour.

Mouse genetics and molecular biology have led us to consider that the developmental action of 5-HT cannot be viewed in a single monolithic manner. It can be decomposed into a multitude of small individual effects that differ according to the period in development, the type of neuron, the type of receptor and the subcellular localization of the receptors. This allows a single molecule to affect a large number of developmental processes, and also allows the actions of 5-HT to produce potent modifications in brain development, although they do not seem to be required for single developmental events.

New perspectives in this field will be provided by analysing how the 5-HT receptors interact with other developmental cues, such as axon guidance molecules and trophic factors. The use of temporal and spatial conditional mutants is ongoing and should generate a more accurate understanding of the molecular pathways that are involved and their impact on behavioural changes later in life. The knowledge that is derived from these mouse models should continue to provide useful insights into the pathophysiology of psychiatric disorders, such as autism, anxiety disorders and drug addiction.

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Competing interests statement

The authors declare that they have no competing financial interests.

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