Chapter 5

# Sculpting Cerebral Cortex with Serotonin in Rodent and Primate

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Additional information is available at the end of the chapter

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**Abstract**

The mammalian cerebral cortex is critical for sensory and motor integrations and, for higher-order cognitive functions. The construction of mammalian cortical circuits involves the coordinated interplay between cellular processes such as proliferation, migration and differentiation of neural and glial cell subtypes followed by accurate connectivity evolving in complexity in primates. Alteration in cortical development may induce the emergence of various pathological traits and behaviours. Among the large array of factors that regulate the assembly of cortical circuits, serotonin (5-HT) plays important role as a developmental signal that impacts on a broad diversity of cellular processes. 5-HT plays distinct roles during specific sensitive periods and is produced from various sources depending on the perinatal stage. Its roles are mediated by more than fourteen 5-HT receptors that are all G-protein coupled receptors except the ionotropic 5-HT type 3A receptor ($5\text{-}HT_{3A}$) mediating rapid neuronal activation. Importantly, 5-HT metabolism and signalling are influenced by numerous epigenetic and genetic factors, including nutrition and gut microbiota, perinatal stress, infection and inflammation. In this review, we will recapitulate some evidences showing that dysregulation of 5-HT homeostasis and $5\text{-}HT_{3A}$ signalling impairs distinct steps of cortical circuit formation leading to the predisposition of the onset of various psychiatric diseases.

**Keywords:** development, human, monoamine, plasticity, 5-HT3 receptor

## 1. Introduction

The functions of the mammalian cerebral cortex are processed through the activation of multipartite neural networks composed of excitatory glutamatergic pyramidal neurons, local modulatory interneurons that release γ-aminobutyric acid (GABA), neuropeptides and vasoactive substances [1–5] and by 'glial cells' that do far more than just feeding neurones and scavenging debris [6, 7]. Developmental perturbations impacting the maturation of cortical circuits can trigger neuropsychiatric disorders [8–10]. Sensitive periods or windows of vulnerability have been demonstrated in various processes in particular for the rodent sensory systems as well as in the modulation of complex behaviours.

Mammalian cortical circuit formation is the result of a series of sequential events that take place mainly during embryonic and early post-natal development [11–14]. These events include the proliferation, migration and differentiation of neurons and 'glial cells' that are largely governed by genetic programs but are also sensitive to environmental factors. Such extrinsic signals are extremely diverse (including guidance cues, growth factors, cell adhesion molecules) and among them the monoamine serotonin (5-HT) has emerged as an important regulator of neural circuit formation [15, 16].

In mammals, cortical 5-HT arises from multiples sources depending on the developmental stage. At the onset of cortical development, 5-HT is of maternal and placental origin [17–19]. Later, by embryonic day 16 (E16 in mice) [15, 16, 20] and by gestational week 16 (GW16 in human) [13, 14], serotoninergic afferents invade the cerebral cortex and contribute to provide 5-HT locally. Not surprisingly, like in non-mammalian species, serotonin modulates neuronal proliferation, migration and differentiation. In addition, 5-HT is implicated in the emergence of many neuropsychiatric disorders, including mental retardation, autism, depression and anxiety [10, 15, 21–26]. Importantly, 5-HT signalling is influenced by numerous epigenetic and genetic factors, including nutrition and gut microbiota [27, 28], perinatal stress [29–31], infection and inflammation [32–35], 5-HT metabolism and storage [15, 36–38], pharmacological compounds such as selective serotonin reuptake inhibitors [38–40] and genetic alterations [41–44].

Our aim is to give a comprehensive overview on the possible roles of 5-HT receptor signalling and 5-HT homeostasis on the development of the cerebral cortex in rodent and primate with a specific emphasis on human. In this framework, we will highlight more particularly recent studies that have revealed new molecular targets of early-life 5-HT in the construction of cortical circuits; in particular, the ionotropic 5-HT type 3A receptor (5-$HT_{3A}$). We will also review recent clinical studies suggesting that altered 5-HT homeostasis or signalling could participate in the emergence of human psychiatric disease, in particular of mood and anxiety disorders.

*In the following section, we will describe the general structure of the mammalian cerebral cortex focusing on rodent and then presenting the specificities observed in primate/human. Then we will describe the major steps of the development of the mammalian cerebral cortex that is governed by a series of sequential events including proliferation, migration and differentiation of neurons and glial cells. When numerous developmental similarities are observed very precociously in rodent versus primate, significant specificities arose later in development in primate especially in human.*

## 2. Structure and development of the mammalian cerebral cortex

### 2.1. Neuronal components and glial components

The mammalian cerebral cortex comprises of six lamina (layers), each containing specific combination of neurons and 'glial cells'. Cortical excitability is coordinated by the interplay of excitatory pyramidal neurons and inhibitory interneurons. Pyramidal cells, which make up the majority of all neurons in the adult cortex (80% in rodent cortex), are projection neurons that send axons to other areas inside or outside the cortex providing output excitatory drive by releasing glutamate [2]. Inhibitory neurons project locally, release the neurotransmitter GABA and refine cortical excitability. Although GABAergic interneurons are less abundant, they have crucial roles in the development and organization of cortical networks that underlie a wide range of cortical and mental functions [8, 45, 46]. They are extremely diverse, differing in shape, electrophysiological properties and in the combination of neuropeptides and calcium-binding proteins that they express in addition to GABA [1, 47]. To facilitate the description of GABAergic neurons, a consortium of experts has suggested using a unified nomenclature [4, 5]. Thus, one can distinguish four major and highly distinct classes of GABAergic neurons in the mammalian cerebral cortex (**Figure 1A**). First, fast-spiking interneurons expressing parvalbumin (PV) that gate incoming sensory information [48, 49]. Second, adapting Martinotti cells expressing somatostatin (SOM) that control dendritic information through local feedback inhibition [50]. Third, adapting bipolar interneurons expressing mainly the vasoactive intestinal peptide (VIP) and calretinin (CR) that preferentially target other interneurons and receive direct input from the thalamus [20, 51, 52]. Fourth, adapting neurogliaform interneurons expressing vasoactive substances, notably the neuropeptide Y (NPY) and/or nitric oxide (NO) that are responsible for the slow GABAergic inhibition of pyramidal cells and interneurons and vasomotion [53–56].

Although these different types of interneurons have been identified in the primate or human cerebral cortex, their diversity largely surpasses what is observed in rodent [12]. Interestingly, unique to human cerebral cortex, bipolar/von Economo neurons are present in layer V of the anterior cingulate and fronto-insular cortices expressing VMAT2 [57, 58]. Their possible involvement suggested in neuropsychiatric disorders needs to be further investigated [59]. In human and primate, the neuronal composition of the cerebral cortex is less homogeneous between areas with a higher level of arealisation than in rodent. Interestingly, the density of small interneurons appears very high in associative areas [60].

Besides neurons, mature 'glial cells' have been shown to exert roles that are extremely more complex than previously thought. Astrocytes are the largest glial population in the mammalian brain and are well-known to 'feed neurons' by transforming glucose into lactate that neurons can directly use as 'carburant', to scavenge debris and to regulate neural transmission and ionic homeostasis of the brain [61, 62]. Microglial cells play a role of sentinels of inflammatory state of the brain. In addition to these roles, astrocytes and microglial cells participate in regulating cell proliferation, neuronal migration and plasticity (for review, see Refs. [6, 61, 63]). Oligodendrocytes myelinate axons and increase their conduction velocity (they will not be further described in this chapter).

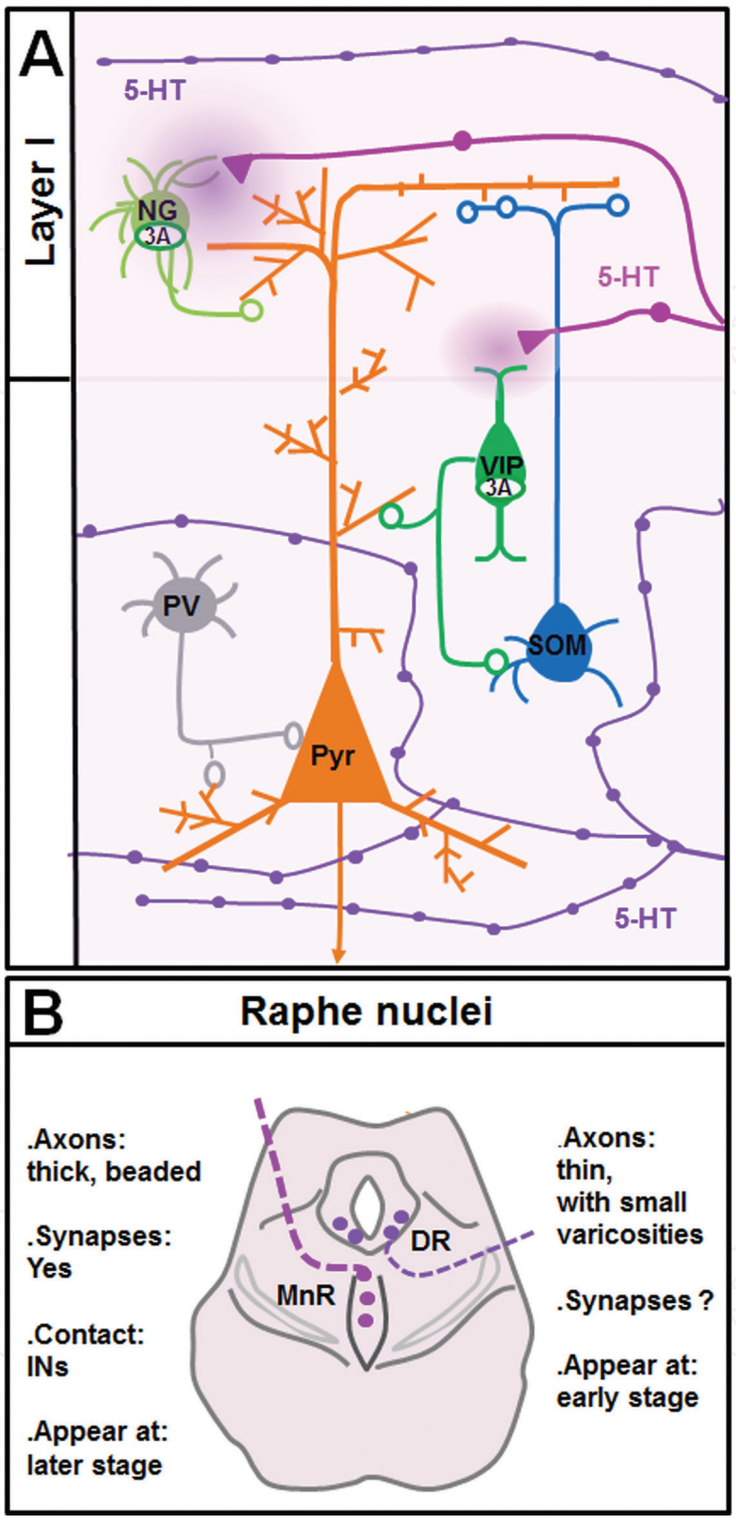


**Figure 1.** Structure of the rodent cerebral cortex and relation with serotoninergic afferents. A, The four main classes of interneurons (NG: neurogliaform, PV: parvalbumin+, VIP: vasoactive intestine peptide+, SOM: somatostatin+) and their relationship with a typical pyramidal glutamatergic neuron (adapted from [64]). B, Serotoninergic afferents arising from the median raphe (MnR) are thin, diffuse and display small varicosities. Serotoninergic afferents arising from the dorsal raphe (DR) are thick, beaded, preferentially located in superficial layer and make true synaptic contacts with small interneurons expressing VIP and with NG interneurons expressing the 5-HT receptor type 3A (3A). Adapted from [65]. 5-HT: serotonin.

### 2.2. Development of the rodent cerebral cortex

The cerebral cortex develops from neuroepithelial germinal cells of the telencephalic pallium and subpallium that massively proliferate by E11-E12 in mice and GW5-6 in human, to form the cerebral vesicles [66]. At this stage, microglial cells—of extracerebral origin—have already started to invade the telencephalon (from E9.5 in rodent [67] and GW5 in human [63]) before blood vessels start to penetrate and ramify in the telencephalon [68]. They will both participate in regulating neurogenesis [69]. The first generated neurons, Cajal-Retzius (C-R) cells and subplate cells (SP; from E10 in mice, GW5-7 in human), constitute transient and heterogeneous populations of cells that originate from both pallial and subpallial territories and form the preplate (PP; Boulder Committee; [66, 70, 71]). SP and reelin-secreting C-R cells provide positioning cues and instructions to developing cortical neurons and afferents [71–74]. The cortical plate, is formed from E13-E17 in mice and GW7-20 in human by post-mitotic excitatory pyramidal neurons migrated along radial glial (RG) fibres in an inside out gradient of development from layer VIa to layer II [13]. At the beginning of cortical plate formation (E13-E14 in mice), pyramidal cells are generated from radial glial cells (RGC), whereas later (E15-E17 in mice), they mainly originate from intermediate progenitor cells (IPC) or basal progenitors deriving from RGC cells [75, 76] (**Figure 2**).

The primate/human cortical neurogenesis is far more complex than that of rodent involving more germinal zones and a larger number of cell types [77, 78]. In particular, beside the early RGC in the VZ, a novel class of radial cells, the outer RG (oRG), located in the outer subventricular zone (SVZ) could be responsible for the increasing number of excitatory neurons and the formation of gyration in primate. The second stage of human cortical development (GW18-20) corresponds to the genesis of the supragranular layers that likely expand from the oRG [14] (**Figure 2A**).

In rodent, the cortical GABAergic interneurons are generated outside the cortical VZ, in the subpallium: mainly in the medial ganglionic eminence (MGE) (E11-E14 in mice) and the caudal ganglionic eminence (CGE) (E14-E17 in mice) [11, 20, 52]. These regions are specified through a combination of distinct transcription factors and morphogenes that produce different classes of interneurons [80]. The ventral and the dorsal parts of the MGE expressing the homeobox transcription factor Lhx6 generate fast-spiking/PV+ and adapting/SOM+ interneurons [81–85]. The CGE, a region that expresses the transcription factor Gsh2, COUP-TFII but lacks the transcription factors Nkx2.1, Nkx6.2 and Lhx6 [80, 86, 87], generates VIP+, CR+, NPY+ and nNOS+ interneurons [20, 52, 85, 88]. Once produced, interneurons are targeted towards specific brain regions, including cortex, depending on the transcription factors and guidance cues they express [87, 89]. They initially follow parallel migratory streams, first in the IZ and MZ and later on along the SVZ, before they switch their migratory mode and incorporate into the developing CP through radial migration (see **Figure 2B**). In mice, cortical migration is almost completed by P4, and is followed by cortical expansion. However, during the first two post-natal weeks and decreasing with age the SVZ retains the capacity to produce CR+ interneurons contributing to the pools of GABAergic neurons mainly populating lower cortical layers and cingulate cortex [90–92]. These events are recapitulated in **Figure 3A** and **B**.

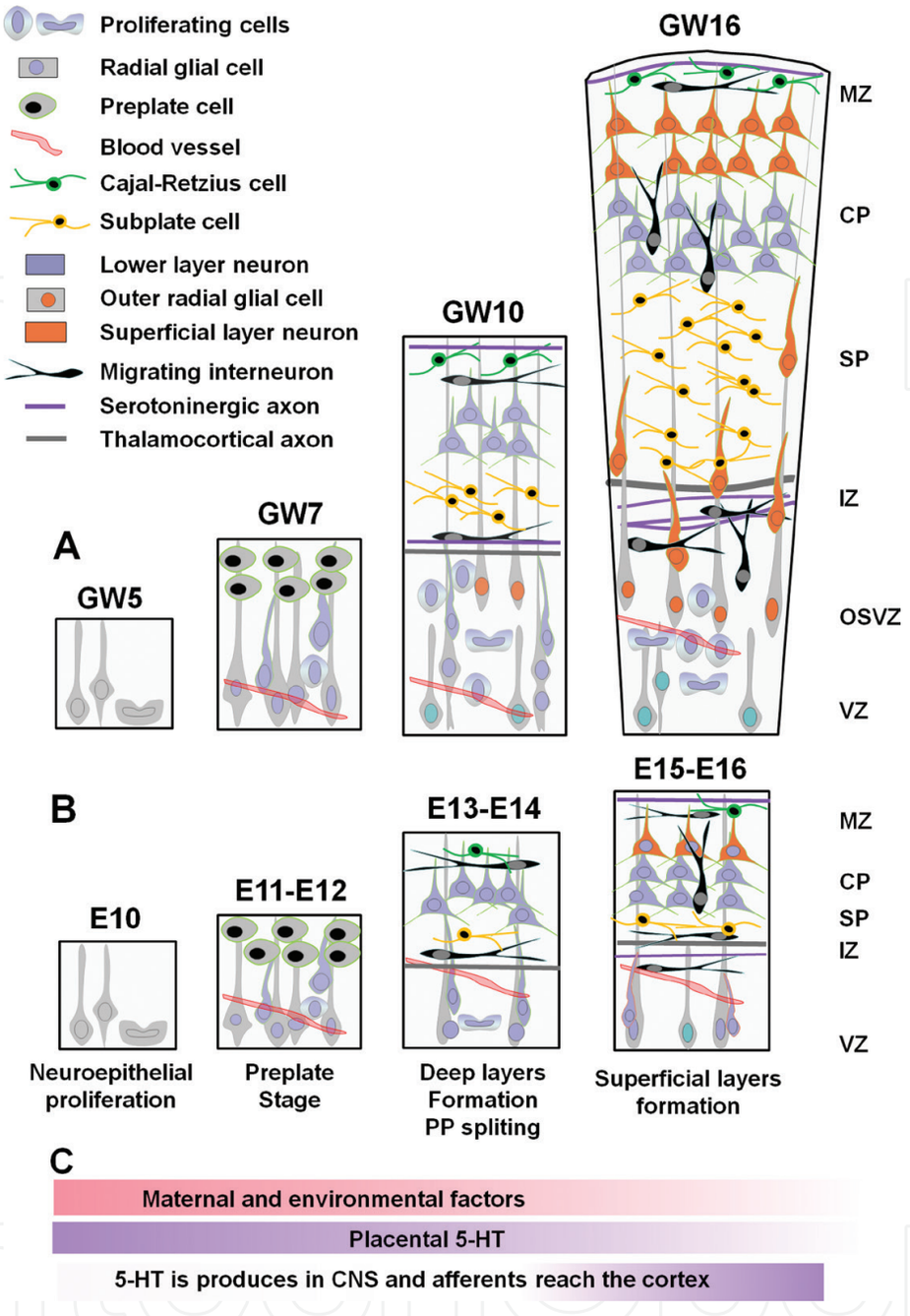


**Figure 2.** Early stages of development of the human (A) and mouse (B) cerebral cortex in relation with 5-HT afferents. A-B, Both in human and rodent intense proliferation of neuroepithelium and the formation of the preplate (PP) take place around (E10; GW5) and (E11-E12; GW6-7) respectively. By E13-E14 in mice and GW8-10 in human, PP is split by the migration of the first pyramidal neurons. Cajal-Retzius cells (C-R) will remain in the marginal zone (MZ) while subplate neurons (SP) will be positioned below the cortical plate (CP). In addition, in human around GW10, another source of progenitors arises: the outer radial glial (oRG) cells that do not maintain contacts with the apical surface. Monoaminergic axons and thalamocortical axons (TC) are already found in the MZ and in the intermediate zone (IZ) and, in the IZ respectively. By E15-E16 in mice most glutamatergic neurons are generated, 5-HT axons and TC run in the MZ and IZ and in the IZ respectively. By GW16 in human, SP occupy a large proportion of the cortical anlage and oRG are still producing a high amount of neurons. Interneurons migrating first tangentially to the pial surface and later radially to it, incorporating CP. C, Bars indicate the time at which different factors (maternal and environmental; 5-HT of placental origin, 5-HT produced by the embryo itself) could affect the development of the mouse embryo. A, is adapted from [20] and B is adapted from [13, 14, 79].

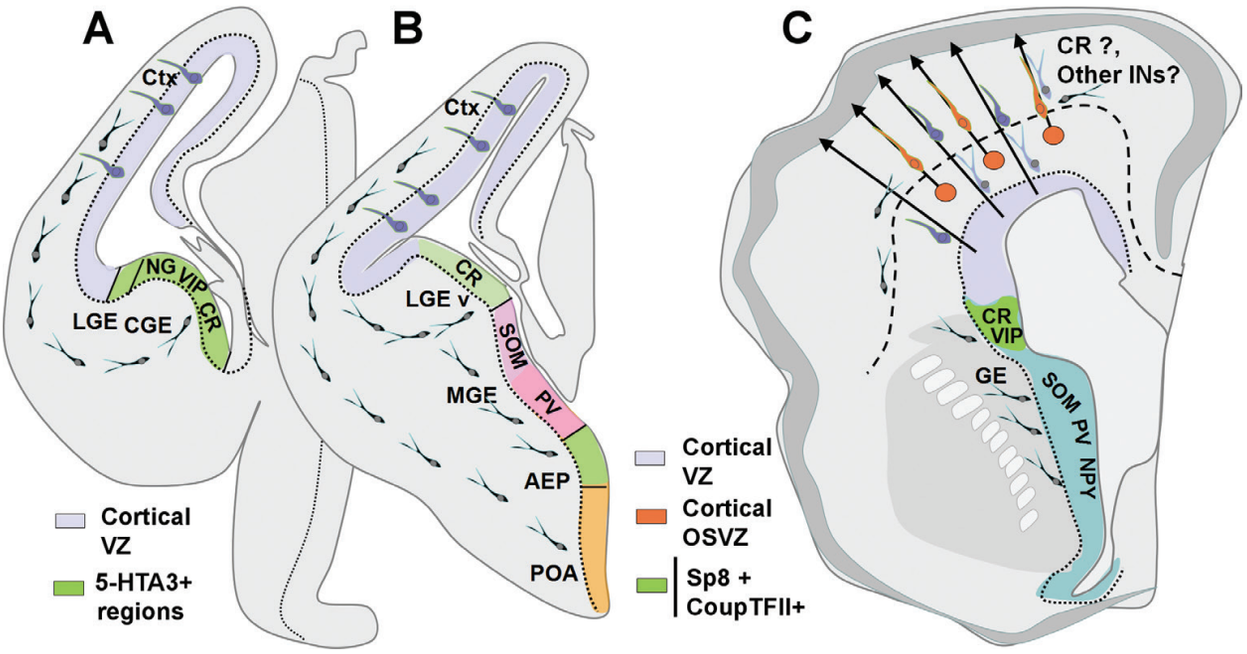


**Figure 3.** Presumptive genesis of cortical GABAergic neurons in the rodent and human/primate embryos and fetuses. (A and B) In rodent, PV+ and SOM+ interneurons (INs) are generated first from the medial ganglionic eminence (MGE) located in the anterior telencephalon. CR+, VIP+ and neurogliaform INs are generated mainly in the caudal GE (CGE) and in the lateral GE (LGE) located in the basal ganglia and to a lesser extent in the anterior entopeduncular area (AEP) and in the pre-optic area (POA). (C) In non-human primate and in human, the picture is less clear. However transcription factors expression suggest that the GE produce a large part of GABAergic neurons. By contrast to rodent brain numerous, INs may be generated in the cortical anlage. Panel C is adapted from Ref. [12]. CR: calretinin, NG: neurogliaform, PV: parvalbumin, SOM: somatostatin and VIP: vasoactive intestine peptide.

In non-human and human primate, the origin of the very heterogenous GABAergic interneurons is not so clear. Recently, studies have shown that in non-human primate, interneurons use a similar coding of transcription factors as in rodents and largely originate from the ganglionic eminences [93] (**Figure 3C**). However, a substantial proportion of them is likely to be generated in the pallium from the VZ and the SVZ [12, 94–96] (**Figure 3C**). Recently, migration of subclasses of human cortical interneurons has been reported to continue after birth [97].

### 2.3. Specificities of the human and primate cerebral cortex

As already mentioned, the first generated neurons, C-R and SP cells are located respectively in the presumptive Layer 1 and the SP zone of the human cortical anlage [66, 98, 99]. Specific to human, the SP zone is the largest transient compartment of the fetal neocortical anlage, about four times thicker than the cortical plate around midgestation [66, 100]. In humans and non-human primate, most SP neurons generated in the ventricular zone initially migrate radially, together with prospective layer VI neurons and secondarily get widespread into the expanding SP zone around midgestation [101]. Interestingly, at this stage, dispersion of SP cells in the extended SP zone is concomitant with the invasion of monoaminergic [102], thalamocortical and corticocortical axons in the cortical anlage [103]. SP zone begins slowly to disappear towards the end of gestation and during the early post-natal period. Finally, many

subplate neurons survive postnatally and transform into interstitial neurons of the subcortical white matter of the adolescent and adult brain [104]. GABA+ interstitial neurons express CB and CR [105]. Subcortical interstitial neurons in the white matter, which have been associated with a variety of neurological and psychiatric disorders of infant and adults, need to be further investigated [105, 106]. Comparison of the rodent/human cortical development could be obtained by comparing **Figure 2A** with **B** and **Figure 3A** and **B** with **C**.

Microglial cells take part in normal establishment and maturation of neuronal circuitry during development [107]. In human, amoeboid microglial cells infiltrate the brain via the choroid plexus, the meninges and the ventricles around GW4,5, progressively colonize the cerebral wall from GW7 and became ramified [108, 109]. Passing through walls from GW10 on. Interestingly, amoeboid microglial cells cluster in a band at the limit of the CP/IZ-SP zone at GW9-13 where early synaptogenesis takes place in the cerebral anlage [110]. They also clustered in major axonal crossroads in the corpus callosum at GW16 and in the coronal radiata at GW19-24 [63]. Interestingly, this last fibres tract area is the target of white matter injury observed in inflammatory process of premature infant in cerebral palsy [111]. Similarly, a cluster of microglia/macrophages is detected in the cingulum bundle in the perinatal rat models of hypoxia and growth restriction developed by Verney and collaborators [112–114].

In mammals, the numerous cortical astrocytes are reported to be mainly generated not only from radial glial cells but also from other cell types that are not clearly elucidated such as progenitors in the SVZ [62]. Human astrocytes are far more complex in diversity and size, and the ratio of glia to neuron is higher when compared to rodent [115]. The protoplasmic and fibrous astrocytes appeared in waves in the cortical anlage [115], begin to differentiate around midgestation and co-expression between vimentin and GFAP is observed [116]. Functional

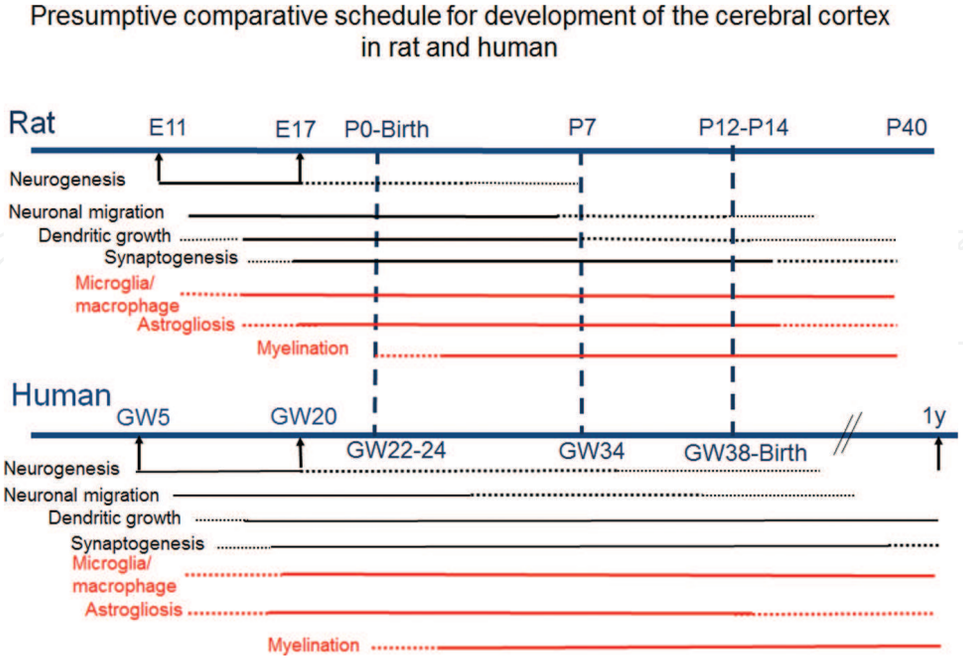


**Figure 4.** Presumptive comparative schedule for development of the cerebral cortex in rat and human.

astrocytes evolve in parallel with the maturation of the vascular endothelial cells involved in blood-brain barrier (BBB) formation [68, 117]. During development, monocarboxylates including lactate represent a major source of energy for the developing neurons [118]. The expression of monocarboxylate transporters such as MCT1 confirms the functionality of astrocytes in the energy trafficking occurring in the human visual cortex from GW19 [119].

Here, we provide a schematic drawing (**Figure 4**) comparing the schedule for the different key events occurring during the cortical development in human and in rat.

*Serotonin is provided to the developing mammalian cerebral cortex via many sources. Numerous studies, cited in the section below, have described this in rodent but only sparse data are available in primate especially in human.*

## 3. Sources of serotonin to the mammalian cortex

### 3.1. Serotonin synthesis and degradation

Serotonin is synthesized from the essential amino acid tryptophan. In the blood stream, tryptophan is linked to serum albumin but a proportion that decreases with age is free to cross the BBB (10% at post-natal day 12 when BBB is thought fully functional [120]). Tryptophan is then transported, accumulated in 5-HT-producing cells and hydroxylated by the tryptophan hydroxylase enzymes (Tph). Tryptophan hydroxylase type 2 (Tph2) is expressed in serotoninergic neurons of the raphe nuclei and myenteric neurons [121, 122], while Tph1 is expressed in the pineal gland, in the placenta and in various peripheral tissues [18, 19, 122, 123]. 5-hydroxytryptophan is then further decarboxylated into 5-HT by the aromatic amino acid decarboxylase (AADC). The availability of tryptophan to synthesise 5-HT depends on the inflammatory status of the organism. In case of inflammation, indoleamine 2,3-dioxygenase (IDO) is generated, which can lead to 5-HT depletion in the organism [35].

5-HT is catabolized by monoamine oxidases A or B (MAOA or MAOB [124, 125]). MAOA has higher affinity for 5-HT than MAOB and is strongly co-expressed with MAOB between E12 to P7 in rodent serotoninergic neurons [126]. After P7, the expression of MAOB is largely predominant in 5-HT+ neurons [126]. MAOs are also expressed by many non-aminergic structures, in particular the placenta and in a subpopulation of VZ-SVZ cells ([126, 127] and our unpublished results) where they may regulate the amount of 5-HT locally. Interestingly, MAOs expression and protein synthesis are tightly regulated and have been shown to be sensitive to environmental factors such as inflammation and ischaemia-like conditions [34].

During embryonic development, the telencephalon receives 5-HT arising from multiple sources that are mainly of extra-embryonic or maternal origin at the beginning of gestation. Later, they progressively arise from different embryonic regions. Below, we will briefly recapitulate the sources of serotonin provided to the embryonic telencephalon in relation with cortical development.

### 3.2. Development of the serotoninergic neurons and projections

In mammals, brainstem serotoninergic neurons are subdivided into 9 groups (B1–B9) forming a caudal and a rostral division. The rostral division (B5–B9; including the dorsal (B6, B7) and median raphe nuclei (B5, B8)) projects to the forebrain [65, 128, 129] (**Figure 1B**). Since these initial descriptions, recent mapping of 5-HT projections have been performed in mice revealing a higher level of refinement in the projections of raphe clusters towards specific targets [130]. Such level of analysis is lacking in primate and human.

In mice, the rostral division differentiates by E10-E11 (E12-E15 in rats); dorsal and median raphe send axons that reach the cortico-striatal junction by E14 in mice before entering the cortical anlage as two tangential streams, one above and the other below the CP [131, 132]. In the MZ, C-R cells and serotoninergic axons are in close apposition and make transient synaptic contacts [133, 134]. Below the CP, 5-HT afferents are mainly restricted to the IZ and the SP [131]. By E16-E17 in mice, thalamocortical axons (TCAs) invade the cortical anlage and are in close apposition with 5-HT axons running in the IZ. At the end of corticogenesis, 5-HT axons gradually arborize, sending numerous branches into the CP [131].

By P21, serotoninergic axons become evenly distributed in the different cortical territories showing their mature pattern of innervation [128]. Dorsal raphe axons are generally thin with pleiotropic varicosities that preferentially arborize in cortical layers IV and V. By contrast, median raphe axons show large spherical varicosities, form true chemical synapses, preferentially arborize in layer I and lower white matter, and contact interneurons containing VIP and cholecystokinin (CCK) [64, 65, 135] (**Figure 1**). Thus, 5-HT could be released along the entire axonal network through volume transmission or in synaptic clefts.

Anatomical studies have described the primate raphe nuclei and the serotonergic cortical innervation at mature stages [136–138], but only a few studies have reported their development. In Rhesus monkey, the genesis of raphe neurons was detected in the first quarter of gestation (E28-E45, birth: E165) [139] and 5HT+ fibres were reported in the entorhinal cortex at E70, similarly to tyrosine-hydroxylase+ catecholaminergic axons [140]. In human cortical anlage, one can suggest that the early afferents of serotoninergic axons as described for the catecholaminergic afferents may penetrate the cortical anlage around GW8 and invade the fetal cortex at midgestation in a mature-like pattern [102, 141]. In parallel, SERT expression in developing TCAs have been detected at GW10 in human cortical anlage [142]. Comparable expression has been described for the visual sensory system in the marmoset [143].

### 3.3. Other sources of serotonin

The first demonstrations showing that 5-HT was influencing very early embryonic development were provided by pioneer groups showing that *ex vivo* application of 5-HT or alteration of 5-HT levels altered normal development of various embryonic structures before serotoninergic neurons have innervated these structures [144–149]. Several studies suggest that 5-HT derives from maternal or placental sources (see **Figure 5** that recapitulates those studies).

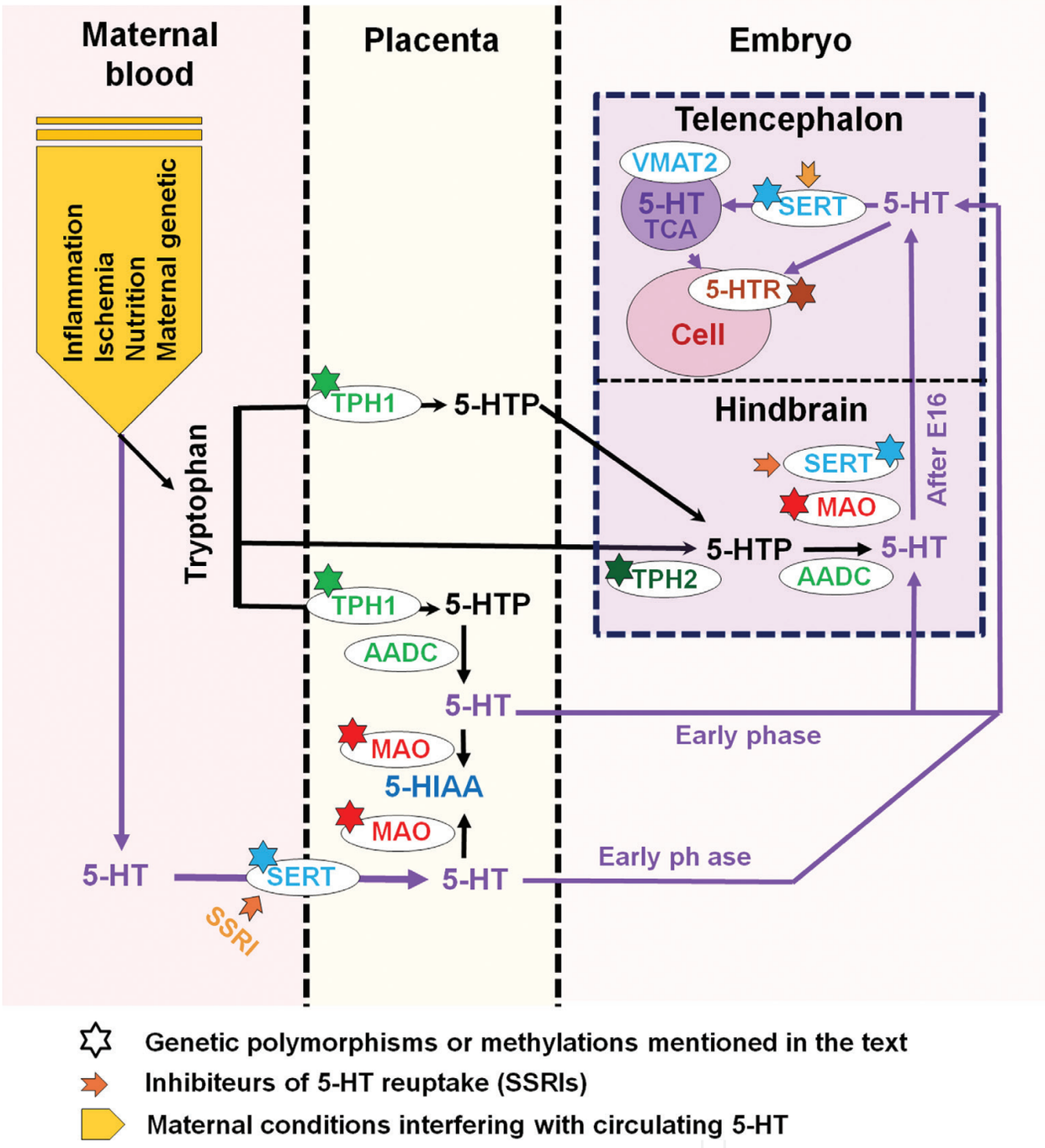


**Figure 5.** Maternal, placental, genetic and pharmacological conditions determining the amount of serotonin supply to the developing telencephalon. Tryptophan is provided to the embryo but could also be converted into 5-HTP (5-hydroxytryptamine) or further into serotonin (5-HT) in the placenta via the expression of various metabolic enzymes expressed in the placenta. In addition, 5-HT from maternal sources could be taken up by the placenta that also expressed serotonin transporter (SERT). During early embryonic stages 5-HT could be delivered directly to the developing embryo. After E15-E16, when 5-HT axons of the hindbrain reach the cortex, 5-HT could act on various target cells (Cell) expressing selected arrays of 5-HT receptors. At this stage 5-HT could also be taken up and stored by thalamocortical afferents (TC) and released after specific stimulation. In addition 5-HTP is provided to the (tryptophan hydroxylase type 2) Tph2 and the (aromatic amino acid decarboxylase) AADC containing neurons that synthetize 5-HT. In this drawing adapted from [19], we have pointed in the large left arrow the maternal conditions that are best known to interfere with 5-HT availability to the embryo. We have also indicated that inhibitors of 5-HT uptake (SSRIs) that cross all barriers affect SERT function at all levels. Genetic polymorphisms or methylations mentioned in the text are indicated by a star. The major catabolic enzymes of 5-HT, monoamine oxidases are indicated (MAO). Tryptophan hydroxylase type 1; Tph1.

Several groups have suggested that, at early stages, 5-HT arises from maternal sources. Indeed, this was suggested when analysing the phenotype of embryos generated from $Tph1^{+/-}$ or $Tph1^{+/+}$ mothers. $Tph1^{-/-}$ and $Tph1^{+/-}$ embryos obtained from crosses between heterozygous parents were indistinguishable from their wild-type littermates (the crown-rump length (CRL) was of 7.4–7.5 mm). By contrast, 80–88.9% of $Tph1^{-/-}$ and $Tph1^{+/-}$ embryos born $Tph1^{-/-}$ mothers displayed low CRL values (5.8–7.4 mm). This suggests that the partial lack of maternal 5-HT provided to the embryo may be sufficient to explain some of the littermates phenotypes [18, 123].

Recently, the placenta (that is of embryonic origin) has been identified as an important source of 5-HT for the developing embryo. The placenta (syncytiothrophoblastic cells and sinusoidal throphoblastic giant cells) of the placenta contain Tph1, AADC and MAO [124, 125, 127], and convert tryptophan of maternal origin into 5-HT as soon as E10-E11 [150]. Homozygote knock-out embryos in which 5-HT neurons fail to fully differentiate or to produce normal amounts of 5-HT levels do not display severe cortical defects when gestating in heterozygous dams. Examples include mice lacking the transcription factors Lmx1b [151] or Pet-1 [152], in which all or 70–80% of 5-HT raphe neurons fail to develop, and mice lacking Tph2 [153, 154]. Further analysis revealed that Pet-1 knock-out embryos developing in heterozygous dams have normal 5-HT levels before the closure of the BBB (before E15 [68]). These studies suggest that 5-HT produced by the placenta may buffer maternal deficiency. However, the compensatory mechanisms remain to be clarified.

Outside the CNS, 5-HT is also produced in the periphery of the developing embryo: from the myenteric plexus (from E15-E16), from enterochromaffin cells of the lining lumen of the digestive tract (from E18), from neuroepithelial cells of the respiratory tracts, from the parafollicular cells of the thyroid and from pinealocytes (belonging to the CNS; from E12). 5-HT could also be taken up by SERT expressing cells and further delivered to a distant region. SERT is expressed in platelets and mast cells [155, 156] that become numerous around E12 in mice. These cells could cross the BBB, transit across blood vessels that start to invade the developing cortex by E10-E11 in mice [68]. Whether these structures and mechanisms provide substantial amount of 5-HT to the developing telencephalon remains to be clarified.

Transiently, sensory thalamic neurons express SERT (E15-P15 in mice) and the vesicular monoamine transporter type 2 (VMAT2) that are respectively responsible for the uptake and packaging of 5-HT into synaptic vesicles [37, 157, 158]. Sensory thalamic neurons do not contain MAOs [159] but are equipped to release 5-HT, possibly with other transmitters (e.g. glutamate), after specific stimulation (review in Ref. [15]). Interestingly, it has been suggested that thalamocortical axons (TCAs) could be implicated in the proliferation and migration of glutamatergic neurons [160, 161] in addition to their well-known role on axonal refinement (see below).

Tryptophan is provided to the embryo but could also be converted into 5-hydroxytryptamine (5-HTP) or further into serotonin (5-HT; violet) in the placenta via the expression of various metabolic enzymes expressed in the placenta. In addition, 5-HT from maternal sources could be taken up by the placenta that also expressed serotonin transporter (SERT). During early embryonic stages, 5-HT could be delivered directly to the developing embryo. After E15-E16, when 5-HT axons of the hindbrain reach the cortex, 5-HT could act on various target cells (Cell; maroon) expressing selected arrays of 5-HT receptors. At this stage, 5-HT could also be

taken up and stored by thalamocortical afferents (TC) and released after specific stimulation. In addition, 5-HTP is provided to the tryptophan hydroxylase type 2 (Tph2) and the aromatic L-amino acid decarboxylase (AADC) containing neurons that synthesize 5-HT. In this drawing adapted from Ref. [19], we have pointed in orange the maternal conditions that are best known to interfere with 5-HT availability to the embryo. We have also indicated that inhibitors of 5-HT uptake (SSRIs) that cross all barriers affect SERT function at all levels. Genetic polymorphisms or methylations mentioned in the text are indicated by a star. The major catabolic enzymes of 5-HT, monoamine oxidases (MAO) are indicated.

*Serotonin receptor signalling has been shown to regulate various cellular events. However, the large spectrum of serotonin receptors still need to be investigated in cortical development in rodent and even more in primate.*

## 4. Serotonin receptors with specific attention to the 5-HT$_{3A}$

### 4.1. Transducing pathways

At least fourteen genes encoding for 5-HT receptors have been identified and cloned in the mammalian brain [162–165]. In addition, isoform diversity, alternative splicing of some subtypes and RNA editing add to the diversity of serotoninergic receptors. With the exception of the 5-HT$_3$ receptors, all 5-HT receptors are coupled to G-proteins. According to their second messenger coupling pathways, 5-HT receptors have been categorized into four groups. The 5-HT$_1$ and 5-HT$_5$ receptors are coupled to Gi/Go proteins and exert their inhibitory effects on adenylate cyclase, inhibiting cAMP formation. The 5-HT$_2$ receptors are coupled to Gq proteins and stimulate phospholipase C to increase the hydrolysis of inositol phosphates and elevate intracellular $Ca^{2+}$. The 5-HT$_{4,6,7}$ receptors are coupled to Gs proteins and are positively linked to adenylate cyclase and increase cAMP formation. 5-HT$_3$ receptors belong to a family of ligand-gated ion channel receptors that include nicotinic acetylcholine receptors, GABA$_A$ receptors and glycine receptors and are modulated by intracellular cyclic AMP [162]. The 5-HT$_3$ receptors respond to neurotransmitter release via direct (through the 5-HT$_3$ receptor itself) or indirect activation of the voltage-gated $Ca^{2+}$ channels and lead to $Ca^{2+}$ entry into the cell [166]. 5-HT$_3$ receptors are composed of five subunits, with the majority being homomers of 5-HT$_{3A}$ receptors. Heteromeric 5-HT$_{3AB}$ receptors have been observed in specific brain regions and display lower $Ca^{2+}$ permeability than the homomeric 5-HT$_{3A}$ receptors [167–169].

### 4.2. Expression patterns

Despite the efforts of many laboratories and open databases, a complete description of the developmental expression pattern of 5-HT receptors in the cerebral cortex is still lacking in rodent and very few studies have been performed in primate. However, pictures are emerging in the rodent brain. For example, 5-HT$_{1A,F}$ are expressed in neocortical proliferative zones in E14.5 rodent brain [17] and the 5-HT$_{2B}$ are expressed in the proliferative zones of the human occipital cortex [129] and in all microglial cells [170, 171]. The 5-HT$_{1A,B,D}$, 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_{3A}$, are expressed in specific subpopulations of post-mitotic neurons [17, 88, 91, 167, 168, 172, 173], whereas the 5-HT$_6$ is expressed in both migrating interneurons and pyramidal neurons [174, 175].

The dynamic expression pattern of the 5-$HT_{3A}$ receptor has been described in details recently in mice. In the developing cortex, 5-$HT_{3A}$ is expressed as early as E11-E12 in neurons expressing reelin (Cajal-Retzius cells) and/or GABA cells located in the PP [88, 173]. The 5-$HT_{3A}$ is expressed by newly post-mitotic GABAergic neurons located in the CGE and AEP/PO, where about 30% of cortical GABAergic neurons are generated ([52, 88]; see **Figure 3A** and **B**). Using homochronic in utero grafting in combination with a transgenic mouse line expressing GFP under the control of the 5-$HT_{3A}$ promoter (5-$HT_{3A}$:GFP animals), we have shown that this expression was protracted in two large subpopulations of cortical GABAergic neurons: the multipolar interneurons expressing NPY displaying late spiking and accommodating properties and in VIP+ interneurons displaying adapting and bursting properties [52, 88, 176]. In addition, subpopulations of NO+ and reelin+ interneurons also express 5-$HT_{3A}$ ([52, 55]; **Figure 1A**). By post-natal stages and decreasing with age, 5-$HT_{3A}$ is also expressed by young neurons expressing doublecortin and/or calretinin generated in the SVZ and migrating towards the olfactory bulb (rostral medial stream) and various cortical and subcortical regions [90, 91]. In addition, we have reported that transient-amplifying precursors located in the white matter ventrally to the anterior cingulate cortex produced neurons destined to populate the anterior cingulate cortex and its vicinity [91].

*Serotonin homeostasis and signalling act as a sculptor of cortical circuitry. In this section, we will review the different steps of cortical assembly that have been shown to be modulated by serotonin.*

## 5. Impact of serotonin imbalance on cortical circuit assembly

### 5.1. Serotonin and cell proliferation

It has been postulated for some time that 5-HT regulates the proliferation of a wide variety of cell types including cortical neurons. Pharmacological studies inducing depletion of several monoamines triggered drawbacks due to the non-selectivity of the drugs used and they will not be discussed here.

Recently, transgenic models selectively targeting specific serotonin-related genes in different neuronal populations have started to provide more insights. For instance, mice deficient in Tph1 or Tph2 showed body weight reduction and delayed maturation of cortical layers [18, 153, 177]. Heterozygous embryos growing in null mutant $Tph1^{-/-}$ mice showed an average of 30% reduction in proliferating cells (BrdU+) in the VZ after a 2 h pulse of BrdU administration, an analog of thymidine that is incorporated during the S phase of the cell cycle [18]. Although these studies suggest that 5-HT from Tph1+ sources may regulate the proliferation of neuronal precursors, additional studies are needed to refine these observations.

Hyposerotonin-induced microcephaly could also be due to increased death of post-mitotic neurons or neuronal progenitors. Indeed, 5-$HT_2$ stimulation promotes the survival of glutamatergic neurons *in vitro* with a maximal effect observed for stages E16 and E18 in rats [178], and 5-$HT_{1A}$ stimulation increases neuroprotection in models of ischaemia and protects neuronal cultures against serum withdrawal [179, 180]. Furthermore, activation of 5-$HT_2$ reverts

increased apoptosis observed in VMAT2:KO mice, in which dopamine, norepinephrine and 5-HT are depleted [181].

The analysis of mice lacking MAOA and B, which displays high 5-HT levels but normal dopamine and norepinephrine levels during development, revealed a specific reduction of symmetric divisions of intermediate precursors cells [76] in SVZ during late corticogenesis (E17.5) [182]. This unexpected alteration was reverted after pharmacological inhibition of 5-HT synthesis (with p-chlorophenylalanine; PCPA) between E14.5-E19.5. In addition, neurosphere formation was modulated by 5-HT in a dose-dependent manner *in vitro*, with proliferative effects observed for concentration ranging from 10 to 100 ng/ml and inhibitory effects observed for higher concentration (1000 ng/ml). In this study, these inhibitory effects were associated with decreased 5-$HT_{1A}$ labelling of neuronal precursors [182] previously known to trigger neurogenesis in adult dentate gyrus. Hence, 5-HT might modulate cortical density through its proliferation-inducing action on progenitors.

During early development, 5-HT could also promote gap junction coupling through 5-$HT_2$ stimulation [183] that coordinates cell-cell assembly during cell cycle [184].

### 5.2. Serotonin and neuronal migration

In most phyla, 5-HT triggers motility of various cell types including vertebrate lymphocytes (chick, fish, rodent [185, 186]) and microglia towards the CNS [170]. In the mammalian cortex, a role for 5-HT in regulating the migration of cortical neurons has recently emerged. In this context, 5-HT produces opposite consequences depending on its concentration.

One of the first experiments to address this question was made *ex vivo* on cortical explants maintained in a serum-free medium and supplemented with low 5-HT concentration. The migration of glutamatergic neurons was examined and was found be faster in explants supplied with 5-HT suggesting that low 5-HT dosage may enhance the radial migration. Furthermore, decreasing 5-HT levels during development delayed or disrupted cortical migration, suggesting that 5-HT produces a positive drive on cortical migration [181]. In rats depleted in 5-HT by PCPA during the peak of migration (E12/E13 to E17 in rats), abnormal accumulation of GABAergic neurons below the subplate at E17 and a marked reduction of calretinin+ and CCK/VIP+ GABAergic neurons at adult stage were reported [187]. Interestingly, mice lacking Tph2 also display reductions of selective GABAergic populations in limbic structures [188]. 5-$HT_{3A}$ is protractedly expressed by 30% of GABAergic neurons leading to calcium entry into the cell (see above). Using electrophysiological recording and calcium imaging, it was recently shown that CGE-derived interneurons that expressed 5-$HT_{3A}$ increase their response to 5-$HT_3$ activation while they migrate radially and integrate the cortical plate (late phase of migration; see **Figure 6A**). This activation leads to an increased growth cone activity and to a decrease resting-state of 5-$HT_{3A}$+ interneurons. Further, using *in vivo* graft of 5-$HT_{3A}$ deficient interneurons into wild-type host, it was shown that this role was cell-autonomous. Interestingly, long-lasting alteration in the positioning of reelin+ cortical interneurons was reported. This suggests that 5-$HT_{3A}$ activation acts as a migratory signal for CGE-derived interneurons and alters definitively the positioning of their subpopulation [189]. A similar conclusion was suggested using SERT:KO animals that showed a specific increase in the migratory speed and positioning of VIP+ interneurons [92].

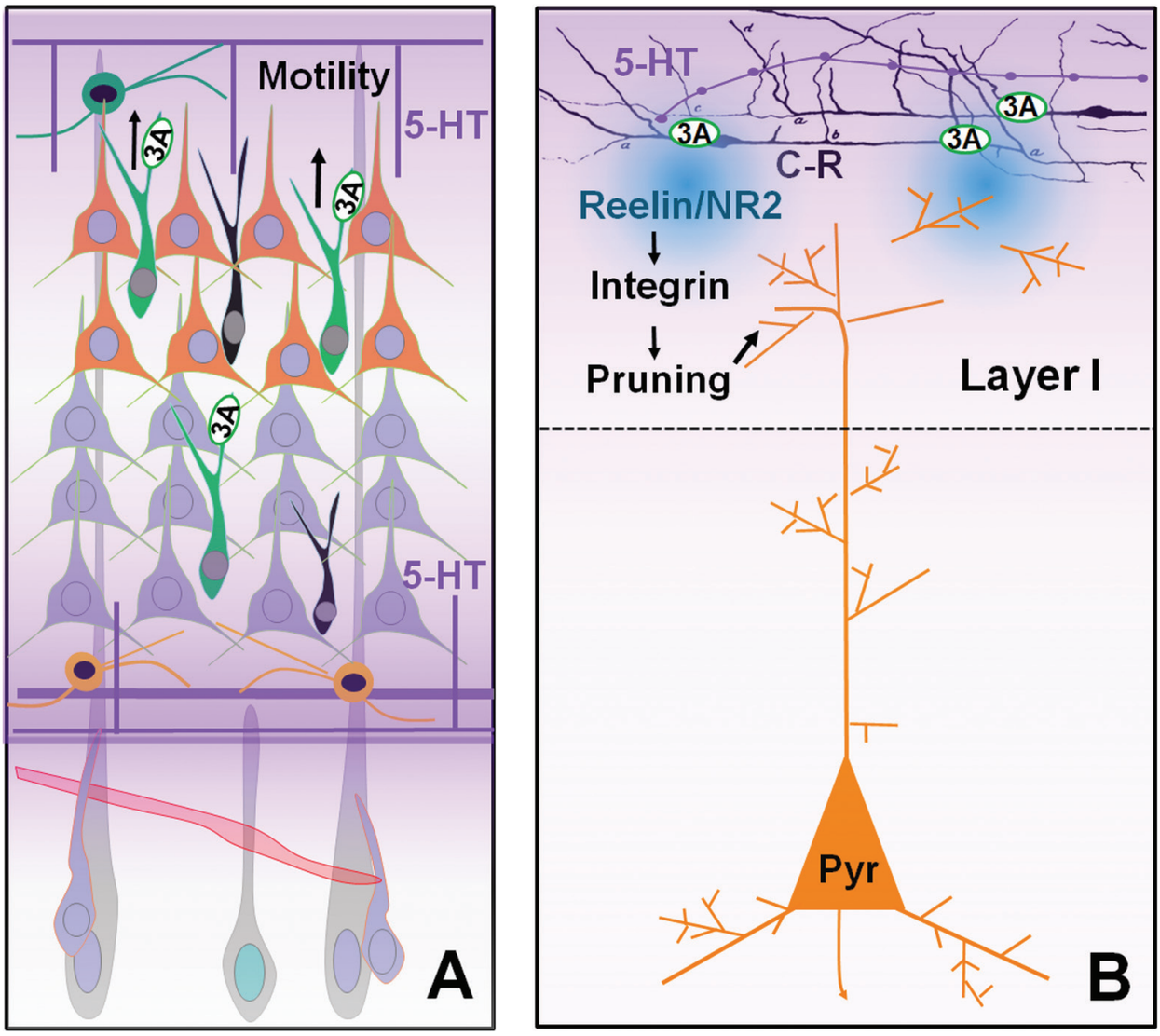


**Figure 6.** Modulation of cerebral circuit formation by 5-HT$_{3A}$ A, 5-HT$_{3A}$ (3A) is expressed by migrating interneurons generated in the caudal ganglionic eminence (CGE). Physiological concentration of serotonin (5-HT), induce an acceleration of the radial migration of 5-HT3A+ interneurons at E17. B, At early postnatal stage, Cajal-Retzius cells (C-R) that express 5-HT$_{3A}$, respond to 5-HT application by releasing reelin that through the activation of the integrin signaling pathway induce pruning of apical dendrites of pyramidal neurons (Pyr). This figure is adapted from [200].

Although dynamic expression pattern of 5-HT receptors is lacking in developing primate and human cortex, a very recent study by the group of Alvarez-Bulla showed that in human, late-born interneurons continue to migrate in the cingulate cortex even after birth. These interneurons expressed a combination of transcription factors and a substantial fraction of them expressed COUP-TFII or SP8 (22 or 28% respectively) that are mainly specific of 5-HT$_{3A}$+ interneurons suggesting that 5-HT could also modulate the migration and positioning of these neurons in human [97]. Interestingly, in the primate cortex, it was shown that 5-HT$_{3A}$ is expressed by a subset of small GABA+, substance P+ or calbindin+ neurons and by medium-size CR+ neurons [190].

By contrast, 5-HT excess appears to have opposite role on migrating neurons. Using high dosage of 5-HT *ex vivo* on cortical slices, it has been shown that 5-HT induces a decrease in the

migratory speed of non-GABAergic and GABAergic neurons [174]. High 5-HT levels induced a retraction of the leading processes of GABAergic neurons migrating into the intermediate zone and cortical plate. This effect was shown to be mediated, at least in part, by the 5-$HT_6$ receptor activating the cAMP-signalling pathway [191]. Such role was also reported for glutamatergic neurons (for review, see Ref. [175]).

### 5.3. Serotonin and differentiation of cortical neurons and afferents

Lauder and Krebs were the first to report that depletion of 5-HT delayed the cessation of cell division, a marker of cell differentiation [144, 192]. After these pioneering studies, numerous groups have shown that 5-HT can influence dendritic and axonal morphogenesis during cortical development.

#### *5.3.1. Serotonin and dendritic maturation of cortical neurons*

5-HT was shown to regulate the physiology of C-R cells known to be key regulators of various aspects of cortical development including dendritic arborization. This role is largely mediated by the secretion of the glycoprotein, reelin [72, 74]. C-R cells receive serotoninergic projections with which they make transient synaptic contacts [134] and reelin secretion was shown to be regulated in part by the amount of brain 5-HT. Pharmacological perturbation of the serotoninergic system by 5-methoxytryptamine (a non-selective 5-HT receptor agonist) reduces reelin levels circulating in the blood flow at P0 [134], leading to the formation of abnormal microcolumns in the mice P7 presubicular cortex, a feature that is observed in autistic syndromes (ASDs). The activation of C-R cells was proposed to be modulated by 5-$HT_{1A}$ or by the 5-$HT_{3A}$ receptors, as they were both suspected to be expressed in the marginal zone during development [167, 193]. Interestingly, the 5-$HT_{3A}$ has been shown to be expressed by C-R cells (averaging 80% at P0) and the synaptic activation of 5-$HT_{3A}$ was shown to be sufficient to induce action-potential firing on C-R cells suggesting that 5-$HT_{3A}$ could play a role in dendritic development [173]. The contribution of the 5-$HT_{3A}$ was further analysed. The deletion or blockade of 5-$HT_{3A}$ receptors was shown to induce excessive arborization of layers II-III apical dendrites of pyramidal neurons. Application of the N-terminal region of reelin, that induces the activation of a signalling pathway that is independent from the classic ApoER2/VLDL-pathway, rescued the dendritic phenotype of cortical pyramidal neurons in 5-$HT_{3A}$:KO cortical slices, whereas reelin blockade leads to an increased growth of apical dendrites ([173]; see **Figure 6B**). This study suggested that increased reelin secretion due to over-activation of the 5-$HT_{3A}$ receptor could induce a decreased growth of apical dendrites. Interestingly, fluoxetine (an inhibitor of 5-HT uptake, SSRI) administration from E8 to E18 decreased the dendritic basal and apical arbor complexity of layer II/III pyramidal neurons in the somatosensory cortex. Such a role is specific to a selective developmental period and SSRIs have opposite functions at mature stages [194]. Furthermore, the effects of SSRIs on developing dendrites were abolished when administered in the 5-$HT_{3A}$:KO mice or after pharmacological blockade of the 5-$HT_{3A}$ receptor [173, 195]. Moreover, the fine tuning of 5-$HT_{3A}$ signalling has been shown to be responsible for the anxiety-like behaviours that are induced by prenatal fluoxetine treatment in wild type mice [196]. These results suggest that developmental excess of serotonin increases reelin secretion by over-

activating 5-$HT_{3A}$ receptors expressed on C-R cells, consequently inhibiting dendritic growth of pyramidal neurons. Whether 5-$HT_{3A}$+ interneurons participate in this process remains unclear.

Animals fed with low tryptophan diet [197, 198] display cortical pyramidal neurons with decreased dendritic complexity and spine density. Thus, 5-HT may regulate dendritic maturation and spine density through different types of 5-HT receptors that remain to be identified. In this respect, the 5-$HT_{1A}$ is strongly expressed in the developing cortical plate [17] and is known to be necessary for the dendritic maturation of CA1 pyramidal neurons [199]. The 5-$HT_6$ receptor also appears as a good candidate for controlling neuritic and dendritic development due to its ability to engage signalling pathways (e.g. Fyn, mTOR and Cdk5) playing roles in these processes. *In vitro* studies strongly suggest a role of 5-$HT_6$ on neuritic extension (for review see [175]). However, a clear view on the implication of the variety of 5-HT receptors expressed in the developing cortex remains to be elucidated.

*5.3.2. Serotonin and axonal development within the cerebral cortex*

The first clear demonstration that serotonin acts on cellular processes involved in the formation of cortical circuits comes from works performed on the rodent barrel field in the somatosensory cortex (S1). The serendipitous generation of a mouse displaying deficiency in the gene encoding for MAOA was at the starting point of these discoveries. These studies showed that excessive 5-HT amounts (ninefold increase at P0) in the developing cortex induced an abnormal organization of thalamocortical afferents (TCAs) growing in the layer IV of the primary somatosensory cortex [36, 37]. These alterations were later interpreted as an abnormal refining of TC axons due to a specific rise of 5-HT occurring during a sensitive period (P0-P4: [201]). In addition, pharmacological normalization of 5-HT levels in MAOA:KO mice by P0-P4 PCPA-treatment was sufficient to revert to normal the organization of S1 in MAOA:KO mice [37]. Later, it was shown that genetic SERT deficiency affected S1 organization similarly in rodent. These alterations are not only structural but also impair whisker-mediated perception [10]. Hyper-activation of the 5-$HT_{1B}$ receptor, transiently expressed on TCAs during development, plays a key role in this process. Indeed, SERT:KO and MAOA:KO mice that are deficient in 5-$HT_{1B}$ receptors are rescued [202–205]. Interestingly, serotonin excess does not only impairs S1 organization, but also such a role could probably be generalized in other regions displaying transient 5-HT uptake [158] as this was shown for the visual system [202, 205, 206]. Moreover, such a role could also occur in primate cortex since SERT is transiently expressed in the visual sensory thalamic neurons, at least in the marmoset [143]. So far due to the difficulty to obtain human embryonic samples of late stages, clear sets of data are still lacking but numerous non-serotoninergic fibres, presumably TCAs, labelled by SERT have been detected at GW10 [142].

Surprisingly, perinatal 5-HT deficiency only induces a reduction of barrel field organization without altering its general organization [177, 207, 208]. Nevertheless, further studies need to be carried since early reduction of 5-HT during embryonic development induces the emergence of altered behaviour [153].

Other studies suggest a prenatal role for 5-HT in regulating initial TCAs pathfinding. TCAs express SERT, 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors at a time when TCAs are navigating towards the pallium. Embryonic down-regulation of 5-$HT_{1B/C}$ receptors in TCAs using *in utero* electropora-

tion leads to abnormal TCAs pathfinding [209]. Furthermore, it has been shown that 5-HT modifies the attractive versus repulsive responsiveness of TCAs to netrin-1 [209], an important guidance molecule for TCAs. Given these findings, it is thus likely that abnormal 5-HT levels could also affect these earlier stages of TCAs pathfinding and lead to abnormal long range of TCAs wiring [19, 150].

### 5.4. Serotonin and the regulation of astrocytes and microglial cell functions

Astrocytes and microglial cells have been shown to be implicated in key processes—from neurogenesis to synaptogenesis—involved in cortical development (for review, see Ref. [61]). These cells bear several 5-HT receptors depending on their stage and state (resting or activated) making 5-HT an indirect actor of cortical development via the modulation of their functions [170]. Pioneer studies have shown that 5-$HT_{1A}$ and 5-$HT_{2}$ are expressed by both immature and mature astrocytes in human and rodent cortex, and that 5-HT stimulates the release of several trophic factor produced by glial cells that promote neuritic extension and synaptogenesis of cortical and serotoninergic neurons such as S100β or BDNF. Conversely, lesions of the serotoninergic system were shown to increase GFAP and to decrease the release of several trophic factors [210, 211].

More recently, several groups have focused their attention on the implications of microglial cells that colonize the embryonic telencephalon at the very beginning of its formation in rodent and human (see above; [63, 212]). Through local phagocytic activities and the release of various molecules (such as interleukin-1beta or tumor necrosis factor-alpha), microglial cells have been shown to regulate neurogenesis, to participate in axonal and dendritic organizations and pruning [212–216]. From early stage of colonization, microglial cells have been shown to express, at least, the 5-$HT_{2B}$ receptor and at later stages or upon stimulation (such as inflammation), several other 5-HT receptors have been detected in rodent (5-$HT_{1F,2A,2B,3B,5A}$ and 5-$HT_{7;}$ [170]). The activation of these receptors has been shown to regulate their motility, their phagocytic properties and selective reshaping of axonal and dendritic arborizations. For instance, 5-$HT_{2B}$ has recently been shown to induce synaptic refinement of retinal projections to the thalamus since this process is impaired in mice lacking 5-$HT_{2B}$ selectively in microglial cells [171].

*During early development, the serotoninergic system is challenged by various genetic and epigenetic factors such as medications altering 5-HT transporter function, by nutrition and stress including ischaemia/hypoxia. In this section, we review how these factors may induce the emergence of various pathological disorders in primate and human.*

## 6. Serotonin imbalance and consequences in human pathology

### 6.1. Serotonin imbalance and 5-$HT_3$ receptor modulation in human pathology

Developmental imbalance of 5-HT homeostasis or serotonin receptor signalling impacts various processes involved in the formation of cortical circuits and has consequences on the emergence of abnormal behaviour in rodent. Some similarities have been detected in primate and

human but many aspects remain to be tested, in particular, the cellular processes implicated (conditioned by SERT or 5-HT receptors expressions) and the time windows of vulnerability.

In human, three major causes of 5-HT imbalance leading to psychiatric diseases have been clearly identified: abnormal metabolism of 5-HT, exposure of fetuses to SSRIs and genetic inheritance of SERT variants (these points of vulnerability have been indicated in **Figure 5**). Following the discovery of the lack of MAOA in Norrie disease [217], abnormal regulation of the enzymes implicated in 5-HT metabolism has been known for long to be associated with neuro-psychiatric diseases (recently reviewed by Naoi et al. [218]). However, it is not known whether the alteration in prenatal or post-natal human life induces such illness. Pharmacological SSRIs treatment gave clearer answers. Indeed, SSRIs during pregnancy are still largely used among women ((2–13%) [219]); despite the high incidence of mood disorders in pregnant women (around 20% of pregnant women are affected) and the deleterious effect of maternal stress on fetal development. However, SSRIs crossing the placenta, are detectable in breast milk, reach the developing brain. Both, short-term (e.g. fetal cardiovascular malformations) and long-term drawbacks of the treatments have been revealed (see below). During gestation, SSRIs induced a reduction of blood flow in the middle cerebral artery at GW36 [220] and reduced fetal head growth [221]. SSRIs induce reduced motor movements and altered speech perception at 6–10 months of age, increased irritability, and persistent blunted pain reactivity [222, 223]. Children exposed to SSRIs during pregnancy have poor scores on psychomotor developmental scales [224] and higher risks to develop autism spectrum disorders [225]. The risk appeared higher when exposure to SSRIs occurred during the second trimester and with higher dosage of SSRIs, suggesting deleterious effects on early neural circuit formation. The third well-known cause of excessive 5-HT-signalling in human is of genetic origin. There are two variants of SERT alleles leading to different levels of SERT expression: the short form that induces decreased levels of SERT expression and SERT hypofunction [41] and the long form. Hypofunctional s-allele has been shown to increase the risk for a wide range of psychopathological traits. When combined with maternal anxiety during pregnancy, infants and children carrying the s-allele showed higher levels of negative emotionality compared to l-allele carriers [42] and increased scores of anxiety and depression [43, 226]. Interestingly, platelets that bear SERT (generally accepted to be identical to neuronal SERT), VMAT2 and 5-$HT_2$ receptors have been suspected to play a role in the emergence of autistic disease in human. Dysregulation in platelets function has been largely used as a marker of autism, however clarifications need to emerge from further studies (for review, see Refs. [227, 228].

Although the consequences are subtle, they reveal that both genetic and environmental SERT deficiency impact human development and increase the risks of future psychiatric diseases [229, 230]. Overall, these findings point to the general conclusion that various clinical pathological traits, including autism, depression and anxiety-related phenotypes are associated to conditions of SERT deficiency during development. One should also consider that alteration of other genes may have synergistic effect on the emergence of those diseases or by contrast that bearing allelic variants of other genes could dampen the negative effects of SSRIs [231].

Rodent studies have revealed that the 5-$HT_{3A}$ regulates cellular events involved in cortical circuit formation (see above). Human genetic studies have recently explored more deeply the involvement of 5-$HT_{3A}$ polymorphisms and methylation in the emergence of various pathological

traits and they now provide compelling evidence for such a role. In human genetic studies, it has been shown that a single-nucleotide polymorphism in 5-$HT_{3A}$ (SNP; rs1062613) was associated with bipolar disease [232]. Moreover, allelic variants or specific levels of methylation of the 5-$HT_{3A}$ have been shown to be tightly linked with alcohol-dependence, modulation of emotional networks and increase of depressive-related symptoms [233]. The emergence of depressive-like diseases was associated at the structural level with a decreased grey matter in the fronto-limbic region. Interestingly, 5-$HT_{3A}$ has been shown to interact with the brain-derived neurotrophic factor, a key factor for circuit formation and consolidation [234, 235]. Thus, genetic polymorphism or methylation of 5-$HT_{3A}$ appears as a marker of susceptibility to develop a large panel of diseases.

Together, this further confirms complex connections between early-life stress and the serotoninergic systems.

### 6.2. Linking serotoninergic system and neonatal inflammation/ischaemia with the emergence of neuropsychiatric diseases in children and adults

Early-life inflammation modulates adulthood-inflammatory response [236]. In early brain injuries, activation of the immune system during fetal and neonatal life affects critical phases of brain development, with long-lasting consequences for neurological and mental health [237]. Neonatal stroke, systemic infection, or excitotoxicity/hypoxia-ischaemia (see **Figure 5**) induce perinatal insults activating the immune system and trigger peripheral and central responses that involve immune mediators (cytokines and chemokines), reactive oxygen species (ROS), reactive nitrosative species, excitotoxicity, mitochondrial impairment, and vascular integrity. In general, neonatal encephalopathy is of complex aetiology, encompassing several causal events, with strong evidence of fetal exposure to infection. The complex and multifactorial process of perinatal brain injury involves sensitization, whereby factors not severe enough by themselves to induce significant brain damage make the developing brain more susceptible to a second insult [238]. Substantial numbers of preclinical studies have demonstrated the sensitizing effects of gestational or neonatal systemic inflammation, gestational chronic mild maternal stress, and gestational hypoxia on perinatal excitotoxic or hypoxic-ischaemic lesions. Genetic factors have also been shown to influence the developing brain's response to sensitizing factors. Efforts to design therapies aimed to reduce the sensitizing effects of inflammation have been undertaken as neuroprotective agents, such as therapeutic hypothermia which have been performed mainly in models of pure hypoxia-ischaemia [238]. One of the main alterations following perinatal infection/inflammation is a persistent low-grade inflammation characterized by higher expression of inflammatory mediators and also microglial reactivity during adulthood [236]. Adult rodent exposed during early-life to LPS-enhanced expression of CD11b, IL-1β and IL-6 and also more activated microglia in the hippocampus, the striatum and substantia nigra/ventral tegmental area [239, 240]. This persistent low-grade inflammation sensitizes the brain to secondary injuries, which can lead to neurological disorders such as cerebral palsy, mood disorder, schizophrenia, or Parkinson disease [241].

Serotoninergic central system is vulnerable following a neonatal hypoxic-ischemic insult induced in a rat model [242] with a significant reduction in 5-HT levels, 5-HT transporter expression and 5-HT+ neurons is the dorsal raphe, 6 weeks after insult compared to control

animals. Inhibition of neuroinflammation by Minocycline within the first week after injury is sufficient to prevent long-term neuroinflammation as well as serotonergic system damage still. The loss of dorsal raphe 5-HT+ neurons has been suspected to be induced by an alteration of one of their major target tissues: the prefrontal cortex [243].

## 7. Conclusion and perspectives

Both genetic and environmental factors that influence serotonin signalling during specific sensitive periods of development impact specific cellular events involved in the development of cortical circuits. Such alterations depending on the cellular target and the time of occurrence could result in a predisposition to a large spectrum of cognitive or psychiatric illnesses including autism and depression.

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