Serotonin and 5-HT_{2B} receptors in microglia control of behavior

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Abstract

Serotonin has been widely involved in neuropsychiatric diseases, including major depressive disorders, or schizophrenia, and in neurodegenerative diseases, including Alzheimer's disease or amyotrophic lateral sclerosis. Independent evidence also suggests the contribution of inflammatory mediators in these pathologies. Indeed, peripheral infections as well as chronic diseases of the central nervous system cause activation of microglia, the resident macrophages of the brain. Emerging evidence indicate that serotonin might regulate brain inflammation, either developmentally, acutely, or during neurodegenerative diseases, and in turn influence the course of these diseases. In this review, we summarize evidence for serotonin involvement via 5-HT_{2B} receptors in inflammatory responses in central nervous system. We show how microglial activation may participate in behavioral changes associated to pathologies including depression, psychosis and neurodegenerative diseases.

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Introduction

The immune system has long been suspected to participate in neuropsychiatric diseases. The connections between immune system and psychiatric pathologies could result from an imbalance between inflammatory/anti-inflammatory circulating factors like cytokines, as was illustrated by the "macrophage theory of depression", for example (Smith, 1991). Such connection is supported by abnormal cytokine levels in patients suffering of depression, schizophrenia, or in persons committing suicide. Furthermore, maternal inflammation during pregnancy has been linked to neurodevelopmental defects of the progeny. This is based on epidemiological studies in humans linking influenza with the frequency of schizophrenia diagnosed 20 years later, and on animal studies identifying defects in behavior, neurogenesis, or synapse maturation, in mice that are exposed to a prenatal immune challenge (Bitanirwe *et al*, 2010). In addition, evidence indicates that the brain resident macrophages or microglial cells (unique immune cells of the brain), are essential for the proper wiring of neuronal networks, through the elimination of supernumerary synapses (Paolicelli *et al*, 2011; Squarzoni *et al*, 2014).

Serotonin (5-Hydroxytryptamine, 5-HT) defects have also been associated to many psychiatric diseases including depression, addiction, impulsivity or psychosis (Meltzer, 2013; Yohn *et al*, 2017). However, the identification of the cellular mechanisms underlying the actions of serotonin in psychiatric diseases remain unclear. Apart for this role in adult neurophysiology, serotonin has been convincingly identified as a key factor in neurodevelopment, for example in mediating the synaptic refinement that allows the formation of retinal and whisker maps in thalamus and cortex, respectively (Kepser and Homberg, 2015; Teissier *et al*, 2017).

One of the puzzling question is how brain can be informed by stimuli from the periphery. Several mechanisms are proposed as involved in central nervous system (CNS) communication with periphery. In fact, most of serotonin is located outside the CNS, being synthesized in gastrointestinal tract and stored in high abundance in platelets. In the gut, the immune system protects the organism from ingested environmental pathogens. In response to microbial stimuli, gut neuroendocrine cells can release serotonin, whereas activated immune cells including mucosal macrophages and mast cells can release various cytokines, see for review (Margolis *et al*, 2016). The resulting production of immune factors (e.g. cytokines) and/or serotonin may stimulate the vagus nerve, or activate the hypothalamic–pituitary–adrenal axis (Margolis *et al*, 2016) and therefore instruct brain responses to peripheral information.

Independently, interactions between the immune and serotonin systems have been linked to psychiatric pathologies by acting at the level of the CNS. This review will concentrate on the contribution of 5-HT_{2B} receptors in brain inflammation.

1. Microglia

1.1. Origin, renewal, and regionalization of microglia

Microglia, the brain resident macrophages, are derived from yolk sac mesodermal hematopoietic stem cell precursors, primitive myeloid progenitors common to all myeloid cells that diverged from the other hematopoietic lineages in the developing embryos before embryonic day 8 (Ginhoux *et al*, 2010). Serotonin has independently been shown to be involved in early steps of differentiation of various hematopoietic lineages; mice deficient in the peripheral serotonin synthesizing enzyme tryptophan 1 (TPH1), *Tph1*^{-/-} mice, display reduced differentiation of embryonic erythroid precursors (Amireault *et al*, 2011). Furthermore, serotonin can significantly enhance hematopoietic early stem and multilineage committed progenitors (Yang *et al*, 2007) via 5-HT₂ receptors. Blocking the 5-HT_{2B} receptor

was shown to decrease colony-forming capacity by inhibition of myeloid lineage formation, endothelial precursor cells differentiation, and monocyte/macrophage release (Launay *et al*, 2012). It is therefore, likely, that serotonin contributes to the differentiation of early hematopoietic myeloid lineage at the origin of microglial cells. Work using adult parabiotic mice (mice with a shared circulatory system, a model for studying the contribution of circulating cells to tissue-resident populations) provided clear evidence that adult microglial population is maintained by local self-renewal and independently of peripheral monocyte precursors under physiological conditions, see for review (Ginhoux and Garel, 2018). Microglial self-renewal under physiological conditions has been shown to constitute a slow random process (Askew *et al*, 2017; Réu *et al*, 2017). In pathological inflammatory conditions, this process shifts to fast clonal microglial expansion. In the resolution phase, excess of microglia is normalized by additional cell migration and apoptosis to restore the original microglial network (Tay *et al*, 2017). Similarly, following depletion, the microglial population recovers at very high speed starting from the few cells that survived (Rossi and Lewis, 2018).

Interestingly, it was recently recognized that microglia establish a dense brain network with regional, significant differences in morphology, membrane properties, and mRNA transcriptomes across brain regions. Region-specific phenotypes of microglia have been shown to emerge during the second postnatal week and to be re-established following microglial ablation in the adult, indicating that local cues play an ongoing role in modeling microglial diversity (De Biase *et al*, 2017). Local cues do not exclude the existence of other mechanisms, such as epigenetic programming. Indeed, peripheral inflammatory stimuli induce acute immune activation and instruction to the brain and lead to epigenetic reprogramming of microglia that persists for months (Wendeln *et al*, 2018). Furthermore, a recent study identified an epigenetic mechanism that underlies normal microglial heterogeneity in different brain regions: the polycomb repressive complex 2 epigenetically restricts the expression of genes that support clearance activity of microglia (Ayata *et al*, 2018). This finding also recalls immune training during the progression of some neurodegenerative conditions in humans over the course of decades (Bilbo, 2018). Strikingly, the regional microglial density has been shown to be regulated differentially in the raphe (the origin of serotonergic neurons in CNS) of suicidal vs. non-suicidal patients (Brisch *et al*, 2017). The mechanisms underlying the heterogeneous identities and functions of microglia in different brain regions, including the emergence and stability of such heterogeneity, remain intensely studied but largely unknown. Nevertheless, this heterogeneity may explain the fairly low number of microglia that responds to a particular neurotransmitter (Pannell *et al*, 2014).

1.2. Microglia are dynamic cells responding to many stimuli including serotonin.

As resident immune cells of the brain, microglia are mostly studied in the context of immune and inflammatory responses (Prinz and Priller, 2014). Pioneering *in vivo* imaging studies indicated that, in resting physiological conditions, microglia demonstrate highly mobile and ramified processes, continuously monitoring the brain microenvironment (Davalos *et al*, 2005; Nimmerjahn *et al*, 2005). Transient intracellular calcium (Ca^{2+}) signals have been observed in cultured newborn and adult microglia upon addition of ATP, endothelin-1, substance P, histamine or serotonin (Pannell *et al*, 2014). If most microglial cells responded to ATP, only a fraction of them also responded to serotonin (Seifert *et al*, 2011). One of the major functions of microglia is to respond to disruptions in homeostatic states following inflammatory stimuli through the release of proinflammatory cytokines. *In vitro* studies in a murine microglia cell line showed that microglia release cytokines, including IL-1 β , in exosomes following inflammatory stimuli (Prada *et al*, 2013). Furthermore, serotonin through 5-HT₂-receptor activation plays a role in the release of exosomes in cultured primary

microglia (Glebov *et al*, 2015). The release of cytokines by microglia may in turn modulate serotonin neurotransmission, such as SERT-mediated clearance of released serotonin (Robson *et al*, 2017). In this manner, serotonin may ultimately be able to regulate cytokine release and immune processes within the CNS similarly to immune function in the periphery.

1.3. Microglia regulate neuronal activity and plasticity

Evidence also indicates that microglia are essential partners for synapse development and function. Microglia interact structurally and functionally with synaptic elements, in particular in axon terminals and dendritic spines (Tremblay *et al*, 2010; Wake *et al*, 2012). Microglia indeed release a large array of signaling molecules, including cytokines, growth factors and transmitters, which can modulate synaptic functions in normal and pathological conditions. Microglia have been shown to play a critical role in pruning synapses during development (Paolicelli *et al*, 2011) by engulfing presynaptic inputs (Schafer *et al*, 2012). Furthermore, reduced synaptic pruning during development observed in mice lacking the chemokine fractalkine receptor CX3CR1 expressed in microglia lead to an impairment in social skills associated with persistent deficits in synaptic connectivity between brain regions implicated in the control of social behavior (Zhan *et al*, 2014). Microglia may also favor learning by promoting formation of glutamatergic synapses through the release of brain-derived neurotrophic factor (Parkhurst *et al*, 2013). Several studies indicated that microglia could regulate excitatory postsynaptic current frequency in the adult mice and zebrafish (Li *et al*, 2012; Parkhurst *et al*, 2013; Pascual *et al*, 2012). On these issues, it has been shown that alterations of microglial marker signaling, including the TREM2 adaptor DAP12, the CX3CR1 receptor, the CD200R receptor of the neuronal protein CD200, or the P2Y12 receptor of ATP, induce dysfunction in long term potentiation, a key cellular expression of learning and memory (Chung *et al*, 2015; Costello *et al*, 2011; Maggi *et al*, 2011; Rogers *et al*, 2011; Roumier *et al*, 2004). These observations imply that microglia could be involved in the fine-tuning of neural circuits under physiological conditions. Interestingly, developmental effects of serotonin also occur during critical periods of postnatal life, at the same period as microglia-dependent mechanisms remodel neural circuits. This was found during the maturation of sensory brain maps in the barrel cortex and the visual system, but also in serotonergic raphe nuclei and in limbic circuits, see for review (Teissier *et al*, 2017). These findings may be of particular relevance in understanding the impact of transient serotonin dysfunction during postnatal life on later development of psychiatric illnesses and emotional disorders in adulthood.

Microglia are thus involved in the fine-tuning of neural circuits during postnatal development, which overlaps with developmental functions of serotonin.

2. Microglia, serotonin and 5-HT_{2B} receptors.

The mediators controlling the activity of microglia are important in their functional consequences, but remain largely unknown, although microglia express a whole range of neurotransmitter-responsive receptors, including those for serotonin.

2.1. Functions of serotonin on microglia via the 5-HT_{2B} receptors

Expression of 5-HT_{2B} receptors was shown by qPCR analysis of RNA isolated from primary cultured and from acutely isolated mouse microglia (Krabbe *et al*, 2012). Importantly, the 5-HT_{2B} receptor was found to be expressed in microglia as early as postnatal day 3 in mice (Kolodziejczak *et al*, 2015). Using two-photon microscopy and focal stimulation of microglia on acute postnatal brain slices, we showed that serotonin induced directional growth of

microglial processes. Using slices from *Htr_{2B}^{-/-}* animals and pharmacological blockers, we confirmed that this effect was mediated by 5-HT_{2B} receptors (Kolodziejczak *et al*, 2015). Transient Ca²⁺ signals have also been observed in newborn and adult microglia cultures upon addition of serotonin (Pannell *et al*, 2014) and about half of microglial cells that respond to ATP also responded to serotonin (Seifert *et al*, 2011). In acute brain slices taken at adult stage and in the presence of serotonin, microglial processes have been shown to move more rapidly towards a laser induced lesion, which is considered a chemotactic response. The chemotactic response to ATP in cultured microglia has also been reported to be enhanced by serotonin (Krabbe *et al*, 2012). The uptake of fluorescent microspheres (as a marker of microglial phagocytic activity) revealed that serotonin application decreased phagocytic activity of amoeboid microglia. Finally, the presence of functional serotonin receptors was confirmed by patch clamp experiments in cultured and amoeboid neonatal microglia (Krabbe *et al*, 2012).

2.2. Developmental functions of serotonin on microglia via the 5-HT_{2B} receptors

Serotonin, in addition to its role as a neurotransmitter, actively participates in postnatal establishment and refinement of brain wiring in mammals. Brain resident macrophages, i.e. microglia, also play an important role in developmentally regulated neuronal death as well as in synaptic maturation and elimination. Microglial cells have been shown to modulate synapse formation, and thus to shape neuronal circuits (Miyamoto *et al*, 2016). The mouse retinogeniculate system is a classic model for studying developmental synapse elimination. During the first postnatal weeks, a massive synaptic pruning eliminates redundant synapses and evokes a segregated phenotype of the axonal projections from each retina (Upton *et al*, 1999). A role for microglia in activity-dependent synaptic pruning has been demonstrated in the post-natal retinogeniculate system (Schafer *et al*, 2012). Eye-specific segregation of retinal projections in the thalamus also depends on an appropriate serotonin level (Gaspar *et al*, 2003; Upton *et al*, 1999). Thus, both microglia and serotonin participate in segregation of retinal projections. Using this model as an *in vivo* model of synaptic refinement during early brain development, we investigated the maturation of the retinal projections to the thalamus. We observed that *Ht2b^{-/-}* mice present anatomical alterations of the ipsilateral projecting area of retinal axons into the thalamus (Kolodziejczak *et al*, 2015). In addition, in primary microglia cultures from neonates, inflammatory markers were upregulated in *Ht2b^{-/-}* microglia compared to *Ht2b^{+/+}* control (Kolodziejczak *et al*, 2015), supporting the possibility that microglia and serotonin may participate in these developmental events.

2.3. Microglial activation and peripheral inflammation

Immune cells can detect and recognize microbial-associated molecular patterns through pattern recognition receptors such as toll-like receptors (TLRs) and nucleotide oligomerization domain (NOD)-like receptors. Serotonin has been shown to inhibit the effects of lipopolysaccharide (LPS) that mimics bacterial infection via stimulation of TLR4 on immune cells (Hennessy *et al*, 2010). Independently, serotonin was reported to inhibit LPS-induced release of proinflammatory cytokines by macrophages, to reduce IFN type I-mediated signaling, and to promote an anti-inflammatory signature via stimulation of 5-HT_{2B} receptors (de Las Casas-Engel *et al*, 2013). Other work showed that NOD1, TLR2 and TLR4 activation inhibits activity of the serotonin transporter (SERT) and its expression in intestinal epithelial cells (Layunta *et al*, 2018).

Pioneering work by Clarke *et al.*, (2013) showed that germ-free animals exhibit a significant increase in serotonin concentration in the hippocampus that cannot be reversed in post-weaning animals by microbiota colonization. Remarkably, the absence of microbiome in germ-free mice impacts both prenatal and postnatal microglia. Antibiotic treatment of adult mice can also trigger modified microglial responses revealing both acute and long-term

effects of microbiota depletion (Thion *et al*, 2018). Therefore, there are relations between intestinal serotonergic and innate immune system, and brain inflammation.

In conclusion, several studies support the hypothesis that serotonin interacts with microglial cells and these interactions may participate in brain maturation mainly through 5-HT_{2B} receptors.

3. Microglia, serotonin and psychiatric disorders

Accumulating evidence suggests that neuroinflammation is closely associated with the pathogenesis of various psychiatric disorders including major depressive disorders (MDD) and schizophrenia. The role of microglia activation is increasingly recognized in these disorders (Sandiego *et al*, 2015). Furthermore, antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), are some of the most commonly prescribed drugs worldwide (Yohn *et al*, 2017). The serotonergic system has also been implicated in the pathogenesis of schizophrenia (Meltzer, 2013; Meltzer and Massey, 2011) that includes a neurodevelopmental inflammatory component (Patterson, 2007).

3.1. Depression

Mounting evidence indicates that inflammatory signaling in the brain affects mood, cognition, and behavior, and is linked to the etiology of anxiety and MDD. Several microglia activation markers have been found associated with depressive disorders, including the mitochondrial translocator protein TSPO, the major histocompatibility complex class II cell surface receptor HLA-DR, or the receptor of the chemokine CX3CL1, CX3CR1.

3.1.1. Depression and inflammation

As marker of microglial activation, TSPO measured by PET scan using distribution volume (TSPO-VT) was found increased in a subset of patients with MDD (Setiawan *et al*, 2015). Moreover, higher expression of the HLA-DR in microglia was found in the dorsal raphe of the non-suicidal depressed subgroup, compared to depressed suicidal cases (Brisch *et al*, 2017; Heinisch and Kirby, 2009). Stress is a major inducer of MDD, and the therapeutic effects induced by SSRIs are initially triggered by blocking SERT that increases the extracellular concentration of serotonin and rely on long-term adaptations of serotonergic autoreceptors. Preclinical rodent models of stress-induced depression have provided insight into how peripheral and central immune components can initiate and propagate inflammatory signals in the brain (Hodes *et al*, 2015; Wohleb *et al*, 2016). When exposed to stress, resident microglia show morphological and functional alterations that are associated in the development of anxiety and depressive-like behaviors. Behavioral alterations related to microglial activity are usually transient, but may be long-lasting if the microglia changes persist, for examples, in association with chronic infections, trauma or chronic psychological stress (Powell *et al*, 2013). Several reports indicate that stress-induced neuroinflammation is mediated by activation of resident microglia that are then involved in stress-induced psychopathology (Wohleb *et al*, 2012). Indeed, evidence from murine models shows that chronic exposure to unpredictable stress induces depressive-like behavior associated to several changes in microglia activity (Kreisel *et al*, 2014). Furthermore, mice lacking CX3CR1, a marker of microglia and receptor of CX3CL1, have impaired microglial and neuronal responsiveness to chronic stress (Milić *et al*, 2016).

3.1.2. Depression and 5-HT_{2B} receptors

The 5-HT_{2B} receptor is the main receptor for serotonin expressed on microglia (Kolodziejczak *et al*, 2015). In humans, the lack of a function polymorphism in *HTR2B* gene segregates with

impulsive and suicidal behavior. In mice, the lack of the 5-HT_{2B} receptor is associated with impulsivity (Bevilacqua *et al*, 2010; Delprato *et al*, 2018), memory and social deficits (Pitychoutis *et al*, 2015), and resistance to SSRI treatments (Diaz *et al*, 2016). Mice which developmentally lack the 5-HT_{2B} receptor exhibit, thus, behavioral alterations suggestive of psychiatric disorders that may include an inflammatory contribution, *e.g.* depression and schizophrenia.

Stress response promotes adaptive plasticity, but may become maladaptive and harmful when the response is overused or dysregulated. Chronic exposure to an adverse social environment is associated in both humans and animal models to increased expression of genes involved in inflammation (Powell *et al*, 2013), leading to greater incidence of psychiatric disorders, including depression (Hodes *et al*, 2015). *Htr_{2B}^{-/-}* mice can develop depressive-like behaviors when socially isolated during four weeks. Furthermore, the effects of the SSRI fluoxetine, including behavioral or plastic effects were neither detected in non-stressed *Htr_{2B}^{-/-}* mice nor in chronically stressed *Htr_{2B}^{-/-}* mice (Diaz *et al*, 2016). Our preliminary observations indicate that *Htr_{2B}^{-/-}* mice developed depressive-like phenotype in response to chronic social defeat stress earlier compared to control mice. These behavioral alterations have face validity with psychiatric symptoms of major depression, see the abstract (D'Andrea *et al*, 2017). However, the contribution of inflammatory processes in these behavioral alterations remains unknown.

A peripheral inflammation is sufficient to induce a sickness response, characterized by physiological and behavioral changes reminiscent of a depressive state, and strong peripheral inflammation is sometimes used in mice as a model of depression (Dantzer *et al*, 2008). In order to test whether sensing of serotonin by microglia could play a role in the sickness response, we compared the effect of peripheral LPS injections on wildtype and constitutive knockout *Htr_{2B}^{-/-}* mice and found that sickness behavior (body temperature variation, weight loss, decreased grooming) worsened and recovery was delayed in *Htr_{2B}^{-/-}* mice (Béchade *et al*, 2018). Using a recently developed conditional *Htr2b* knock-out mouse, *Htr2b^{microgliaKO}*, in which 5-HT_{2B} receptors are inactivated only in microglia, our preliminary data confirmed these findings supporting the contribution of microglial 5-HT_{2B} receptors in these behavioral changes (Béchade *et al*, 2018). Furthermore, following stroke, the expression of *Htr2b* mRNA is markedly increased in the perilesional area. The increased 5-HT_{2B} receptor expression close to degenerating neurons was found associated with activated microglia. Treatment with fluoxetine attenuated the expression of *Htr2b* mRNA, stimulated post-stroke neurogenesis in the subventricular zone, and was associated with an improved anhedonic behavior and an increased activity in the forced swim test (Buga *et al*, 2016). These findings support that serotonin via 5-HT_{2B} receptors limits the brain inflammatory response.

3.1.3. Depression, serotonin, and tryptophan metabolism

The enzyme indoleamine 2,3-dioxygenase, a key enzyme of the tryptophan/kynurenine metabolism, is driven by pro- and anti-inflammatory cytokines, reduces serotonin synthesis, and generates neuroactive kynurenine (Dantzer *et al*, 2008). In the brain, kynurenine can be further metabolized into kynurenic acid and quinolinic acid, which act on the glutamatergic neurotransmission as N-methyl-D-aspartate antagonist and agonist, respectively (Müller, 2014). Microglia preferentially produce quinolinic acid, whereas astrocytes produce kynurenic acid. In light of recent evidence suggesting a role of heightened glutamate receptor activity in major depression, an imbalance of kynurenine pathway metabolites might underlie some inflammation-associated depressive disorders. The tryptophan/kynurenine metabolism is thus one indirect mechanisms regulating serotonin levels.

These findings indicate that inflammation can be implicated in depressive disorders and support the possibility that serotonin via 5-HT_{2B} receptors expressed by microglia may participate in inflammatory processes associated to depressive state.

3.2. Schizophrenia

Schizophrenia is a devastating, complex and costly neuropsychiatric disorder that affects about 1% of the world's population (Olesen *et al*, 2012). Schizophrenia is typically characterized by three symptom clusters: positive (e.g. hallucinations, delusions, disordered thoughts), negative (e.g. flattened affects, social withdrawal) and cognitive (e.g. attention and working memory deficits) (Simpson *et al*, 2010). It has long been known that the serotonergic system is implicated in the pathogenesis of schizophrenia (Meltzer, 2013; Meltzer *et al*, 2011). Indeed, atypical antipsychotics, of which clozapine is the prototype, consist of agents that are more potent antagonists of 5-HT₂ receptors over dopamine D₂ receptors (Gonzalez-Maeso *et al*, 2008). Furthermore, the lack of 5-HT_{2B} receptor induced psychotic-like behaviors in mice (Pitychoutis *et al*, 2015).

3.2.1. Schizophrenia and inflammation

Several lines of evidence suggest that schizophrenia is a neurodevelopmental disorder favored by mobilization of the immune system. Fetal microglia activation has been proposed as a link between *in utero* viral exposure or maternal infection and adult psychotic behavior (Blank and Prinz, 2013). Consistent with this hypothesis, maternal injection of poly(I:C) (mimicking viral double stranded RNA) in rodents during pregnancy yields offspring with adulthood deficits in social interaction, in latent inhibition, and in prepulse inhibition (sensorimotor gating) that are similarly impaired in schizophrenia (Patterson, 2007). In parallel to these observations, it was shown that prenatal inflammation or functional knock-out of DAP12, an immune (microglial) gene, induces defects in glutamatergic synapses and in synaptic plasticity similar to those observed in psychosis (Roumier *et al*, 2004; Roumier *et al*, 2008). A number of other studies have unraveled alterations in neurogenesis, in neuronal migration and in behavior upon prenatal immune challenge (Meyer *et al*, 2006).

Clozapine, the atypical antipsychotic agent used in the treatment of schizophrenia and severe mood disorders (Meltzer, 2013) exerts an anti-inflammatory activity by unknown mechanisms. It was recently observed that clozapine suppressed LPS-induced phosphorylation of IκBα, the inhibitor of the nuclear factor-κB (NF-κB), which results in the release of NF-κB and its nuclear translocation, increasing the NF-κB-dependent transcriptional activity in microglial cells (Jeon *et al*, 2018). The Akt activation appears critical in LPS-induced NF-κB activation in microglia. Therefore, clozapine may exhibit anti-inflammatory activity of microglia through the inhibition of Akt-mediated NF-κB activation (Jeon *et al*, 2018). Clozapine and all atypical antipsychotics have been reported to have higher affinity for 5-HT₂ receptors than for dopamine receptors (Newman-Tancredi and Kleven, 2011; Wainscott *et al*, 1996). Furthermore, many atypical antipsychotics show similar affinity for 5-HT_{2B} and 5-HT_{2A} receptors (e.g. clozapine, amisulpride, asenapine, aripiprazole or cariprazine) (Abbas *et al*, 2009; Kiss *et al*, 2010; Shahid *et al*, 2009; Shapiro *et al*, 2003). The 5-HT_{2B} receptor being the only 5-HT₂ receptor expressed by microglia (Kolodziejczak *et al*, 2015), it is tempting to speculate that the 5-HT_{2B} receptor participates in the anti-inflammatory activity of atypical antipsychotics.

3.2.2. Schizophrenia and 5-HT_{2B} receptors

Domains related to the positive, negative and cognitive symptom-clusters of schizophrenia are present in *Htr_{2B}^{-/-}* mice, as revealed by deficits in sensorimotor gating, in selective attention, in social interactions, and in learning and memory processes (Pitychoutis *et al*, 2015). The 5-

HT_{2B} receptor deficiency confers, thus, a wide spectrum of schizophrenic-like behavioral phenotypes in mice and provide evidence for a role of 5-HT_{2B} in the neurobiology of psychotic disorders. Many pathological conditions including schizophrenia are associated with a general impairment in behavioral or cognitive flexibility. Indeed, the ability to modify the behavior in response to environmental changes is critical to develop adaptive strategy against aversive stimuli. We have shown that *Htr2b*^{-/-} mice present memory deficits in contextual fear conditioning (Pitychoutis *et al*, 2015) similar to mice lacking the microglial marker CX3CR1 (*Cx3cr1*^{-/-} mice) that show contextual fear conditioning and Morris water maze deficits (Rogers *et al*, 2011), linking an increase in inflammation with deficits in synaptic plasticity and cognition. We performed preliminary experiments testing the ability of mice lacking 5-HT_{2B} receptors exclusively in microglia, *Htr2b*^{microgliaKO}, to acquire and reverse simple association (reversal learning). Mice were trained to learn a simple association with one arm of the Y-maze containing a reward of a palatable sucrose pellet. In the reversal phase, the reward is relocated in the opposite arm. Although the control and transgenic mice acquired the initial task, the *Htr2b*^{microgliaKO} mice failed to learn the reversal task. These preliminary unpublished results indicate that *Htr2b*^{microgliaKO} mice are impaired in behavioral flexibility, characterized by reduced ability for reversal learning, see the abstract (D'Andrea *et al*, 2017).

Overall, these findings point to a role for microglia and serotonin via 5-HT_{2B} receptors to drive psychotic behaviors in response to the environmental challenges.

4. Neurodegeneration, serotonin, and microglia

Accumulating evidence suggests that neuroinflammation, microglial activation, and serotonin are also closely associated with the pathogenesis of various neurodegenerative diseases.

4.1. Alzheimer's disease

Soluble A β oligomers that accumulate in the brain are thought to initiate Alzheimer's disease and to trigger synapse failure and memory loss (Benilova *et al*, 2012). Recent data showed that exposure to A β oligomers triggers depressive-like behavior in mice and are associated with microglial activation, aberrant TNF- α signaling, and decreased brain serotonin levels. Furthermore, serotonin blocked A β oligomers-induced microglial activation and elevated TNF- α production and release. Inactivation or ablation of microglia also blocked the increase in brain TNF- α and abolished depressive-like behavior induced by A β oligomers (Ledo *et al*, 2016). These findings support that serotonin acts as a negative regulator of microglial activation. In addition, A β oligomers failed to induce depressive-like behavior in TLR4-deficient mice and in mice harboring a nonfunctional TLR4 variant in myeloid cells. These results establish that A β oligomers trigger depressive-like behavior via a double impact on brain serotonin levels and on microglial activation, revealing a cross-talk between brain innate immunity and serotonergic signaling as a key player in mood alterations in Alzheimer's disease (Ledo *et al*, 2016). It was independently reported that treatment with A β promoted neuronal-like differentiation of bone marrow-derived mesenchymal stem cells without toxic effects. The effect of A β was mediated by the Gq-protein coupled receptors, 5-HT_{2B}, via phosphatidylinositol-3-OH kinase-dependent activation of the MAPK/ERK1/2 (Jin *et al*, 2009).

Together, these findings support the hypothesis that serotonin participate via 5-HT_{2B} receptors in preventing microglia-dependent change in mood during Alzheimer's disease progression.

4.2. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS, motoneuron disease, Lou Gehrig's, or Charcot's disease) is a progressive neurodegenerative disorder characterized by motor neuron dysfunction. Microglia are strongly activated in both ALS patients and animal models of ALS expressing mutant forms of SOD1 (mSOD1 mice). Central serotonergic neurons degenerate during ALS in both patients and in mSOD1 mice (Dentel *et al*, 2013). Although serotonergic neurons degenerate during ALS, the 5-HT_{2B}-receptor expression was found strongly increased at disease onset *i.e.* the time point of maximal microglial activation (El Oussini *et al*, 2016). This upregulation was robustly observed in both spinal cord and brainstem of various models of ALS, SOD1(G86R) and SOD1 (G37R) mice, expressing ALS-linked SOD1 mutations, and p150(G59S) mice, expressing a mutant form of dynactin linked to ALS cases and this upregulation was restricted to cells positive for CD11b, a marker of microglia.

To understand the putative role of 5-HT_{2B} receptors in ALS, SOD1 (G86R) mice were crossed with *Htr_{2B}^{-/-}* mice. Interestingly, the complete loss of 5-HT_{2B} receptors shortened survival of SOD1 (G86R) mice by 30%, while the loss of only one copy of 5-HT_{2B} yielded an intermediate effect. Disease onset, as assessed either by peak of body weight (Boillée *et al*, 2006) or by peak of rotarod performance, was not changed. Therefore, ablation of 5-HT_{2B} receptors potentially accelerated disease progression after onset (El Oussini *et al*, 2016). Consistent with its late overexpression in ALS, 5-HT_{2B} receptor upregulation potentially moderate disease progression of SOD1(G86R) mice after onset.

Ablation of 5-HT_{2B} receptor in transgenic ALS SOD1 (G86R) mice resulted in increased degeneration of microglia, as evidenced by fragmentation of cellular processes that are positive for the microglial marker, Iba1 (El Oussini *et al*, 2016). This was accompanied at end-point by decreased expression of key neuroinflammatory genes but also loss of expression of homeostatic microglial genes. Furthermore, the C allele of the SNP rs10199752 in *HTR2B* gene was associated with longer survival in a large cohort of ALS patients, and patients carrying this allele showed increased 5-HT_{2B} receptor mRNA expression in spinal cord and displayed less pronounced degeneration of Iba1⁺ microglial cells than patients carrying two copies of the more common A allele. Thus, the 5-HT_{2B} receptor is able to limit degeneration of spinal cord microglia, and slows disease progression in ALS (El Oussini *et al*, 2016).

In summary, the lack of 5-HT_{2B} receptor exacerbates ALS symptoms and progression of the disease. Considering the expression and upregulation of 5-HT_{2B} receptor in microglia, its protective effects likely result from the regulation of microglial functions.

Conclusion

In this review, we have summarized data showing that serotonin interacts with microglial cells and these interactions may participate in brain maturation and inflammation through 5-HT_{2B} receptors. Serotonin via 5-HT_{2B} receptors expressed by microglia may prevent inflammatory processes associated to depressive state and psychotic behaviors in response to the environmental challenges. The 5-HT_{2B} receptor also participates in microglia-dependent change in mood during Alzheimer's disease progression, and the lack of 5-HT_{2B} receptor exacerbates ALS symptoms, is associated with microglial alterations, and with the progression of the disease. This set of data revealed that serotonin via 5-HT_{2B} receptors expressed in microglia limit neuroinflammatory events and may have protective effect in neurodegenerative diseases. Whether this is a developmental/differentiation process or an acute effect of serotonin remains unknown.

Figure: Environmental factors and/or lack of 5-HT_{2B} receptors in microglia contribute to depressive disorders, schizophrenia, and/or neurodegeneration

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