Regulation of microglia by neuromodulators: Modulations in major and minor modes

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ABSTRACT

Microglial cells, the brain resident macrophages, participate to brain development and function and help maintaining its homeostasis. To play these roles, they need to detect and adapt to modifications of their environment, including changes in the activity of neurons. The neuromodulators serotonin, dopamine, norepinephrine, acetylcholine and histamine are synthesized and released by specialized neurons to coordinate the activity of other neurons in different regions.

In this review, we summarize the current evidence obtained in vitro or in vivo that neuromodulators act on microglia. On the short term, they can modify their motility, morphology and phagocytic activity; on the mid-long term they can modulate their transition between different immune activation states. Lastly, we review some recent data suggesting that these regulations of microglia by neuromodulators are involved in vivo in some aspects of central nervous system development, function and homeostasis.

KEYWORDS

Microglia; neuromodulators; inflammation; development; norepinephrine; serotonin; acetylcholine; histamine; dopamine; motility; chemotaxis; phagocytosis

ABBREVIATIONS:

5-HT: serotonin
Aß: amyloid ß peptide
ACh: acetylcholine
ATP: adenosine triphosphate
Ca^{2+}: calcium
cAMP: cyclic adenosine monophosphate
CNS: central nervous system
EGFP: enhanced green fluorescent protein
GPCR: G protein-coupled receptor

IFN: interferon
IL: interleukin
IPSc: induced pluripotent stem cells
LPS: lipopolysaccharide
MAPK: mitogen-activated protein kinase
mAChRs: muscarinic acetylcholine receptors
nAChRs: nicotinic acetylcholine receptor
PGE2: prostaglandin E2
PI3K: phosphoinositide 3 kinase
PKA: protein kinase A
POA: preoptic area
RT: reverse-transcription
SERT: serotonin transporter
TNF: tumor necrosis factor

Introduction

Microglia are the resident tissue macrophages of the central nervous system (CNS). Besides their involvement in the immune response, they have been proposed as mediators of CNS development, contributors of CNS homeostasis and functions, and key players during neurodegenerative and psychiatric disorders. To engage in these roles, they need to detect and adapt to modifications of their environment, like the presence of pathogens or cell death, but also to global changes in neuronal activity. Neuronal activity in specific brain areas can be coordinated by specialized neurons which synthesize and release the so-called neuromodulators serotonin (5-HT), dopamine, norepinephrine, acetylcholine (ACh) and histamine. One intriguing hypothesis is that these typical neuromodulators[1] might not only act on neuronal targets, but also directly tune microglia in harmony. Indeed, neuromodulators, which are mainly released through asynaptic volume transmission, can reach targets located a few micrometers away [1–5], and early ultrastructural studies identified glial elements close to sites of neuromodulators release [1,6,7].

This review aims to provide the major keys to appreciate how neuromodulators can strengthen or inhibit microglial functions and properties. We will show, based on studies on primary cultures of microglia, brain slices or *in vivo*, that neuromodulators can act on the short term, by modulating microglia motility, morphology, phagocytic activity, and on the mid and long terms, by driving or modulating their transition between different immune activation states. Lastly, we will discuss some recent data suggesting that these regulations participate to CNS development, function and homeostasis.

1. The regulation of microglia by neuromodulators is allowed by spatial vicinity between microglial processes and axons, and the expression of neuromodulator receptors.

The ability of microglia to respond to a subset of neuromodulators, neurotransmitters and neuropeptides has been examined in systematic studies on microglia in primary culture or freshly purified from whole brain. Using intracellular calcium (Ca^{2+}) rise as a readout, these studies showed that a fraction (2 - 20%) of microglial cells is responsive to 5-HT, dopamine, nicotine and histamine. These percentages are generally higher after a 24h-treatment with lipopolysaccharide (LPS), interferon (IFN)-γ or interleukin (IL)-4 [8,9]. Although the intensity of the elicited Ca^{2+} rise and the percentage of responsive cells are lower than for adenosine triphosphate (ATP), the gold standard for Ca^{2+} response in microglia, these studies demonstrate that microglia can respond to each of these neuromodulators. In addition, interestingly, different subpopulations respond either to one or to subsets of neuromodulators [9]. This functional diversity is consistent with recent global analyses documenting the heterogeneity of microglia among brain regions [10–12] and during development [13,14]. Thus, the percentage of microglia able to respond to a given neuromodulator may vary drastically depending on place, age and condition.

A first requirement for being modulated by a neuromodulator *in vivo* is to be located close to its site of release. Pioneer electron microscopy studies provided ultrastructural evidence of contacts between non synaptic varicosities containing norepinephrine or 5-HT and glial processes [1,6,7].

[1] This definition of neuromodulators excludes adenosine triphosphate (ATP) and endocannabinoids, which have modulatory effects on neurons and microglia, but are not synthetized by specific neurons. Their effects on microglia have been addressed in, for example, [81,82].

Recently, we quantified by confocal imaging and 3D reconstruction that, in the thalamus at P6, one microglia is surrounded by 43±7 serotonergic varicosities closer than 1 micrometer [15], a distance compatible with volume transmission. We observed similar features in different regions and at several developmental stages. Figure 1 illustrates an example of the proximity of microglia and serotonergic axons in the hippocampus of adult mice (Fig. 1A), with even some extremely close contacts (Fig. 1B). Close proximity of microglial processes and noradrenergic axons have been observed in the somatosensory cortex [16]. To our knowledge, similar studies have not yet been performed for the other neuromodulators. However, considering the density of dopaminergic, cholinergic and histaminergic fibers in sub-regions of the CNS and the mode of transmission of these neuromodulators, it is likely that microglial cells can detect them if they express the appropriate receptors. Thus, the second requirement for sensing neuromodulators is the expression of adequate receptors. In the next paragraphs and in Table 1, we will summarize what is currently known about this expression in microglia *in vivo* and in primary cultures.

Serotonin (5-HT)

In the brain, 5-HT is synthetized by serotonergic neurons of the raphe nuclei located in the brain stem, and released in close and distant regions of the whole CNS. 5-HT can also be released locally by mast cells, which are immune cells lining the outside of brain blood vessels [17].

Fifteen subtypes of 5-HT receptors exist, divided in seven classes. While the 5-HT3 receptors are ion channels, all the others are G protein-coupled receptors (GPCRs). It has been shown by real time (RT-) and quantitative PCR that 5-HT2B receptors are the main serotonergic receptors expressed in primary microglial cultures [15,18]. Consistently, an agonist of 5-HT2B receptor elicits Ca^{2+} responses in cultured microglia [18]. Expression of this receptor in microglia has also been confirmed at the neonatal [15] and adult stages [18] *in vivo*. In adulthood, 5-HT2B is consistently expressed in microglia throughout the brain, but additional serotonergic receptor subtypes may be expressed in specific regions [18].

Dopamine

Most of the dopaminergic neurons are clustered in the substantia nigra and the ventral tegmental area. Their axons project to many brain regions including the basal ganglia, the prefrontal cortex, the nucleus accumbens and the amygdala. Moreover, some dopaminergic neurons are located in the arcuate nucleus of the hypothalamus and project to the pituitary gland.

Dopamine receptors are GPCRs classified into two major families, activating either $G_{s/olf}$ (D1-like, *i.e*. D1 and D5 receptors) or $G_{i/o}$ proteins (D2-like, *i.e*. D2, D3 and D4 receptors). The first direct evidence of the presence of dopamine receptors (D1, D2, D4, D5R) in rodent microglia in primary cultures and acute brain slices dates back to 2005 [19]. Human elderly microglial cultures have a similar but not identical (D1 to D4, not D5R) expression pattern [20]. Functionally, rat primary microglia cultures respond to a selective D2 receptor agonist, but not to D1R nor D3R agonists, by increasing tumor necrosis factor (TNF)-α mRNA [21]. The expression pattern of dopamine receptors might be region dependent Indeed, in rats, D1 and D2 receptors were observed in CD11b+ microglia isolated from the nucleus accumbens but not from the hippocampus [22]. It is also likely to be context dependent: according to immunohistochemistry and to a detailed examination of transgenic mice which express enhanced green fluorescent protein (EGFP) under the control of the *Drd2a* (D2R-encoding gene)

promoter, D2 receptor is found in microglia activated by cerebral ischemia [23] but not in the healthy mouse cortex [24].

Norepinephrine

Norepinephrine is released by noradrenergic neurons, whose soma are located in the locus coeruleus in the brain stem. Their complex projections allow norepinephrine to be released in the whole CNS. Two classes of receptors for norepinephrine exist: the alpha (α1-A, B, C and α2-A, B, C) and beta (ß1, ß2 and ß3) adrenergic receptors, all GPCRs.

In microglial primary culture, there is a consensus on the expression of the ß2 receptor subtype, based on mRNA expression analysis [25–29] and/or pharmacological approaches [30–33]. Expression of ß2 adrenergic receptor in microglia *in vivo* was confirmed by transcriptome analysis on microglia acutely purified from the postnatal whole brain or from cortex and hippocampus [14,34]. The expression of ß1 is more controversial but has been reported in primary cultures [25,26] and *in vivo* [14,35]. Interestingly, the expression pattern of adrenergic receptors may depend on the physio-pathological state of the brain. Indeed, in microglial primary culture, treatment with LPS decreases the expression of ß2 and upregulates α2A adrenergic receptor [28,29], which has a higher affinity for norepinephrine.

Acetylcholine (ACh)

Besides being a fast-acting neurotransmitter at the neuromuscular junction, ACh acts as neuromodulator in the CNS [36]. The main source of ACh in the CNS are projecting neurons of discrete nuclei (pedunculopontine tegmental and lateral dorsal tegmental nuclei, medial habenula, etc.), which diffusely innervate the CNS. ACh is also released locally by cholinergic interneurons in the striatum and the nucleus accumbens.

ACh receptors are classified into two main classes: nicotinic receptors (nAChRs), which are ionotropic cation channels permeable to Na^+ and K^+, and muscarinic GPCRs (mAChRs). There are five subtypes of muscarinic receptors: M1, M3 and M5 coupled to G_q and M2 and M4 coupled to $G_{i/o}$ proteins. The $\alpha7$ nAChRs are the major components of the cholinergic anti-inflammatory pathway, a mechanism that down-regulates peripheral inflammation through ACh released by the vagus nerve that acts on macrophages through their $\alpha7$ nAChRs [37,38]. Interestingly, $\alpha7$ nAChRs are also present on microglia, in primary cultures [39–42]. *In vivo*, the expression of nAChRs in microglia seems to increase upon inflammatory conditions. For instance, in an animal model of Alzheimer's disease, microglial $\alpha7$ nAChR level, assessed by immunohistochemistry, strongly increases when activated microglia appear at sites of Aβ deposition [43]. In human, in the brain of Alzheimer patients, $\alpha7$ nAChRs are detected by immunohistochemistry in microglia surrounding the amyloid plaques [44]. Moreover, immunohistochemistry demonstrated overexpression of the $\alpha4\beta2$ nAChRs in microglia after cerebral ischemia, consistent with *in vivo* increased positron emission tomography signal for a specific ligand [45]. The presence and function of metabotropic ACh receptors on microglia is poorly documented. Using mAChRs agonists in cultured microglia, two groups reported a transient increase in intracellular Ca^{2+}[46,47]. Additionally, a flow cytometry analysis of mouse adult brain identified a M3-positive subpopulation representing around 15% of the microglial cells [8].

Histamine

In the brain, histamine is released by neurons from the tuberomammillary nucleus of the hypothalamus, or can be degranulated, like 5-HT, by activated mast cells [48] (a role for this source of histamine will be illustrated in section 4). Moreover, microglia in primary cultures express histidine decarboxylase (*Hdc*), the enzyme responsible for histamine production, and *Hdc* level increases upon LPS treatment [49]. The previously mentioned investigation of microglial response to neuromodulators indicates that only 5 to 10% of cultured or freshly purified microglia respond to histamine by a Ca^{2+} increase. This percentage reaches 60% in cells treated for 24h with LPS [9]. Four receptors exist for histamine, H1 to H4, all GPCRs. Bulk transcriptome analyses on acutely purified microglia report the expression mainly of *Hrh2* [14,34], especially in cortical microglia [11]. In primary cultures, mRNA expression analysis as well as pharmacological and functional approaches support the expression of *Hrh3* [49], *Hrh1* and *Hrh4* genes [50].

2. Acute modulation of microglia by neuromodulators: effects on morphology, motility and phagocytic activity

Dynamic interactions between microglia and other cells rely on the motility of their fine processes, as well as their migration and phagocytic activity. We will hereby summarize how these functions can be rapidly regulated by neuromodulators (Fig. 2).

Serotonin (5-HT)

We demonstrated that a local application of 5-HT on acute brain slices induces a directional motility of microglial processes, mediated by the microglial 5-HT2B receptor [15,51]. This is consistent with a previous study reporting that 5-HT promotes the increase of microglia processes motility in response to a laser lesion in acute brain slices, and enhances the chemotactic effect of ATP in microglia primary cultures [18]. The same study described an inhibitory effect of high concentrations of 5-HT or 5-HT2 agonists on the phagocytosis of artificial microspheres by microglia in primary cultures and in acute brain slices [18].

Dopamine

In primary microglial cultures, dopamine reduces the number of cellular processes and increases microglial cell area in basal conditions [52]. Regarding microglial migration, it is enhanced by dopamine and D1- and D2-receptor agonists in rodent cultures [19] as well as in human elderly microglial cultures [20].

Norepinephrine

The effect of norepinephrine on microglial morphology has been first studied on acute brain slices and on primary microglia cultivated in a tridimensional matrix. In these experimental models, norepinephrine, through activation of ß2 adrenergic receptors, induces a reduction of microglial arborization within minutes, and inhibits the effect of ATP on microglial processes outgrowth [28]. Interestingly, retraction of microglial processes upon norepinephrine application is also seen in microglia stimulated by LPS for 24h, but this effect is mediated by α2A adrenergic receptors. As ß2 and α2A are likely to have different coupling (G_s and G_i, respectively, in non-microglial cells), and norepinephrine does not induce Ca^{2+} rise [28], the signaling pathways mediating the retraction of microglial processes remain to be elucidated. Recently, the control of microglia morphology by norepinephrine has been confirmed *in vivo* by two independent studies. Both showed that in awake

mice, norepinephrine tonically limits, though activation of ß2 receptors, the arborization and the motility of microglia, thus suppressing their surveillance of the parenchyma. This suppressive effect is abolished, and thus surveillance increases, in the presence of anesthetic agents which inhibit noradrenergic neurons activity in the locus coeruleus [16,53] . Further experiments will be required to determine whether a physiological reduction of noradrenergic neurons activity, e.g. during sleep, also significantly increases microglial surveillance. To note is that under anesthesia, when microglia are more ramified and motile than during wakefulness, activation of ß2 receptors prevents the recruitment of microglial processes toward a focal lesion injury [53]. This effect is reminiscent of the inhibitory effect of ß2 receptors agonists on microglial processes attraction toward ATP, observed in acute slices and mentioned above [28]. Overall, these results suggest that, *in vivo*, variations in norepinephrine tone upon stress or sleep-wake cycle may alter microglial interaction with synapses, neurons, vasculature and responsiveness to damaged tissues.

In primary cultures, norepinephrine does not seem to promote nor inhibit migration of microglial cells when applied alone [19], but modulatory effects on chemotaxis induced by other compounds have been reported. On one hand, high doses (30 or 100 µM) of norepinephrine inhibit chemotaxis toward ATP [28] and C5a complement [33]. The latter effect is likely to occur through ß2 adrenergic receptors and activation of adenylate cyclase, protein kinase A (PKA) and phosphoinositide 3 kinase (PI3K). On the other hand, low doses of norepinephrine or ß2 adrenergic agonist potentiate chemotaxis toward TNF-α or amyloid (A)ß peptide [54]. This discrepancy might come from the different attractants used, the dose of adrenergic agonists, or both. *In vivo*, in a mouse model of Alzheimer's disease, transplanted microglia fail to migrate toward amyloid plaques if the terminals from the locus coeruleus neurons have been depleted of norepinephrine [54]. This supports a positive role of norepinephrine on microglial migration *in vivo*, at least in this pathological context.

In primary microglial cultures, norepinephrine potentiates the phagocytosis and degradation of Aß peptide, through ß2 adrenergic receptors [27,54]. This is consistent with *in vivo* observations in models of Alzheimer's disease. Indeed, toxin-based depletion of norepinephrine reduces the number of microglia around amyloid plaques and their content in Aß peptide, and injury of the locus coeruleus increases amyloid plaques deposit and glial inflammation [54,55]. Altogether, this suggests a defect in Aß elimination *in vivo* when norepinephrine level is reduced.

Acetylcholine (ACh)

To our knowledge, the potential effect of ACh on microglial migration has not been investigated *per se*; however, it has been shown that agonists of mACh receptors such as carbachol induce chemotaxis in primary cultures of microglia [8].

Regarding phagocytosis, metabotropic and ionotropic receptors of ACh may have different effects, or their effect might depend on the target to be phagocytosed. Indeed, on one hand, carbachol —an agonist of mAChR— diminishes the phagocytosis of latex beads in primary cultures of microglia [8]. On the other hand, galantamine —an allosteric activator of α7 nAChR— significantly enhances the phagocytosis of Aβ by rat primary cultures of microglia in presence of an orthosteric ligand of α7 nAChRs [44]. Thus, mAChRs and nAChRs seem to have opposite effects on phagocytosis in primary cultures, and it would be interesting to see if the expression of these receptors is coordinately regulated *in vivo* to promote one effect or the other.

Several efforts were made to characterize the ACh- and nicotine-induced regulation of intracellular signaling pathways. Both ACh and nicotine lead to a transient increase in intracellular Ca^{2+} [9,41]. However, surprisingly —considering the ionotropic nature of nAChR—, nicotine-induced intracellular Ca^{2+} increase is independent of extracellular Ca^{2+}, and blocked by inhibitors of phospholipase C or inositol phosphate 3 receptor. This supports the hypothesis that α7 nAChRs in microglia may not function only as conventional ion channels, but rather drive a signaling cascade triggering Ca^{2+} release from intracellular stores [41].

Histamine

Histamine triggers motility of microglia out of cortical explants, in a H4R-dependent manner [56], but it inhibits, through H3R, ATP-induced chemotaxis in primary cultures [49]. These apparently opposite effects could be imputable to the different experimental models (tissue explants *vs* primary cultures) or the different read-outs used (spontaneous migration *vs* chemotaxis).

Regarding phagocytosis, incubation for one hour with histamine or a H3R agonist inhibits phagocytosis of artificial microspheres by primary cultures of microglia [49].

3. Involvement of neuromodulators in the regulation of microglia immune polarization

Microglia show a continuum of activation states (reviewed in [57]). Depending on age and context, microglia can indeed release a vast array of factors with pro- or anti-inflammatory activity, neurotoxic or neuroprotective effects. They can also express a considerable variety or receptors, and differ by their phagocytic capacity. Transition from one state to another can be triggered by bacterial or viral compounds, or by endogenous factors like cytokines, aggregated proteins, etc. In this section and in Figure 2, we will show how neuromodulators also endorse a role of “immune” modulators by regulating the polarization of microglia toward different activation states.

Serotonin (5-HT)

A role of 5-HT in microglia functional polarization is supported by the observation that lack of 5-HT2B receptor induces the overexpression of some cytokines and cytokine receptors by microglia in primary cultures [15]. In addition, in a model of amyotrophic lateral sclerosis, microglia adopt an abnormal phenotype if mice are also knocked-out for this receptor gene [58], but the implication of the *microglial* 5-HT2B receptor remains to be demonstrated. This would be consistent with a tonic role of 5-HT in preventing microglia activation, as shown for human peripheral macrophages [59]. 5-HT might also regulate the dialog between microglia and other cells by stimulating the release of exosomes from microglial primary cultures [60].

Dopamine

In primary microglial cultures, dopamine generally exerts a protective effect against LPS-induced inflammation, attenuating the release of a prominent indicator of microglial activation, nitric oxide [19]. This anti-inflammatory action of dopamine might be imputable to inhibition of the Angiotensin-1/NADPH-oxidase/superoxide axis [61].

In vivo, an increase of dopamine extracellular level in the striatum, in response to cocaine, stimulates microglia. Indeed, these elevated dopamine levels not only activate D1 receptors on striatal neurons, but also target D2 receptors expressed by microglia. This increases TNF-α mRNA level in these cells [21]. TNF-α, besides its widely known pro-inflammatory effects, is a potent

inducer of synaptic changes [62,63], which here alleviate drug-induced behavioral alterations such as cocaine sensitization [21]. These results suggest that modulation of microglia might be of therapeutical interest in drug addiction.

Norepinephrine

In primary cultures of microglia, norepinephrine counteracts the effect of LPS or Aß peptide on the expression or release of TNF-α, IL-1ß, IL-6, IL-12p40, nitric oxide and reactive oxygen species [19,26,29,31,32,54,64,65]. In addition, it limits LPS-induced toxicity of microglia toward cortical [64] and dopaminergic neurons [31,32].

The signaling pathway linking norepinephrine to inhibition of microglial activation in culture is unclear, as well as the signal transduction for its effects on microglial processes (section 2). Pharmacological approaches generally point to a role of ß2 adrenergic receptors, whose canonical coupling is G_s. However, several studies deny a role of cyclic adenosine monophosphate (cAMP)- and PKA-dependent, as well as Ca^{2+}-mediated, pathways, in these effects of norepinephrine [28,29,31,32,66]. Non-G protein coupling would be in accordance with the observation that ß2 and α2A adrenergic receptors have the same morphological effect on microglia [28], although they are supposed to have opposite effects on adenylate cyclase. An alternative pathway could be mediated by β arrestin-2 [31]. To note is that a report, furthermore, questions the involvement of adrenergic receptors themselves in some effects of norepinephrine on microglia [32].

In vivo, decreasing norepinephrine levels potentiates brain inflammation in rodent models of Parkinson's [65] and Alzheimer's diseases [55], and in response to systemic inflammation [67]. Although non-microglial noradrenergic-sensitive cells, like astrocytes, could participate to this neuroprotective and anti-inflammatory effect of norepinephrine [64], these experiments are consistent with the results obtained on primary cultures of microglia. This anti-inflammatory role of norepinephrine may also explain in part why, in Parkinson's and Alzheimer's patients, neuronal loss in the locus coeruleus —where noradrenergic neurons are located— occurs prior to neurodegeneration in other brain regions [68,69].

Acetylcholine (ACh)

In the periphery, macrophages are controlled by ACh released by the vagus nerve and acting onto their α7 nAChRs (a mechanism called "cholinergic anti-inflammatory pathway")[37,38]. Similarly, the role of nAChRs in modulating microglia activation state has been broadly investigated. A short pretreatment of rodent microglial primary cultures with ACh or nicotine drastically reduces their response to LPS in terms of mitogen-activated protein kinase (MAPK) activation and expression or release of pro-inflammatory factors, such as TNF-α [39–41]. Such pretreatment also increases the production of anti-inflammatory factors such as prostaglandin (PG) E2 [40]. Transcriptomic analyses on primary microglial cultures from sheep fetal brain are in line with these results. In this animal model, α7nAChR agonists limit the acquisition of a pro-inflammatory phenotype in response to LPS, while antagonizing α7nAChRs does the opposite [70]. Eventually, stimulation of microglial α7 nAChR was shown to limit the neurotoxicity of LPS-activated microglia on neurons cultured from hippocampus and cortex [71]. A neuroprotective effect of α7 nAChR stimulation, microglia-dependent, was also uncovered in models of stroke in organotypic slices and *in vivo* [72].

Beside its immunomodulatory action, stimulation of α7 nAChR might be neuroprotective by increasing the expression of the glutamate transporter GLAST in microglia, thereby maintaining glutamate extracellular concentration below its excitotoxic threshold [42].

Histamine

In primary microglia, histamine can promote the release of PGE2 by microglia [50], which is synaptogenic on neurons of the preoptic area but not in the hippocampus [73]. This will be further discussed in section 4. *In vivo*, several studies report a protective and anti-inflammatory effect of antagonists of histamine receptors in various pathologies such as Alzheimer's and Parkinson's disease and multiple sclerosis (reviewed in [17,74]). Noteworthy, histamine receptors are expressed in various cell types of the brain and of the peripheral immune system. Studies on mutants conditionally invalidated for histamine receptors in microglia only will therefore be needed to definitely implicate these cells in the effects of histamine receptor ligands.

4. Microglia as effectors of neuromodulators

Many events can affect the extracellular level of neuromodulators in the CNS and thus trigger changes in microglia. During development, this level will regionally vary according to the ontogeny of each of the neuromodulatory systems. It will particularly depend on the growth of the axons releasing the neuromodulators, and on the maturation of their release and reuptake machineries. For 5-HT, transient and ectopic expression of 5-HT transporter (SERT) in non-serotonergic neurons may impact on local availability of 5-HT [75]. On a different time-scale, the levels of histamine, 5-HT and norepinephrine undergo circadian variations, with lower levels during sleep than wake phases. External cues or pathologies superimpose their effects onto these physiological variations. To provide a few examples, peripheral inflammation increases the level of 5-HT and dopamine in the brain for a few hours [76]; norepinephrine is released upon stress; antidepressant treatments with selective 5-HT or norepinephrine reuptake inhibitors, or with monoamine oxidase inhibitors, increase the level of 5-HT, norepinephrine or other monoamines, by decreasing their reuptake or degradation, respectively. Based on the evidences presented in the previous sections, these variations can induce transient or long-lasting changes in microglia. Whether these microglial changes are required to mediate some of the neuronal and behavioral effects induced by these variations remains to be fully understood.

We hereby discuss few recent works which support the hypothesis that regulation of microglia by neuromodulators is needed for proper CNS development and the control of higher brain functions in physiological or pathological conditions.

Histamine, microglia, and masculinization of the brain

Sexual dimorphism exists in the brain as in other organs and results from developmental programs regulated by sexual hormones. In adult rodents, one difference between male and female brains is a larger number of dendritic spines in the preoptic area (POA) of males, a hypothalamic region essential for expression of copulatory behavior. It results from an increased synaptogenesis during neonatal development, which is triggered by PGE2 locally produced under the control of estradiol [73,77]. It is known that when sexual dimorphism appears in the POA of neonates, microglial cells are more ameboid, and probably more "active" in males than in females [78]. Recently, it was proposed that microglia are the source of PGE2, but under the intermediate control of histamine.

Indeed, manipulating histamine release by mast cells during the neonatal period impacts on the levels of PGE2 in the POA and on the establishment of male sexual behavior. Moreover, using combinations of primary cultures from different cell types (microglia, mast cells, neurons), Lenz and colleagues demonstrated that estradiol induces the degranulation of histamine by mast cells, which, in turn, activates H1R and H4R on microglia and potentiates their release of PGE2 [50]. PGE2, lastly, promotes dendritic spines formation. Thus, microglia could be effectors of histamine for the release of PGE2 and the masculinization of brain and behavior, but additional experiments are required to demonstrate that this regulation occurs identically *in vivo*.

Norepinephrine, microglia, and the neuroprotective effect of an enriched environment

Enriching the environment of rodents is a paradigm of cognitive reserve in humans. Indeed, an enriched environment promotes rodent's resistance to neuronal and cognitive deficits and limits microglial activation in models of Alzheimer's disease [79]. ß2 adrenergic receptor was known to be required for some of the neuroprotective effects of an enriched environment [80].A recent study unraveled its additional role in limiting the activation of microglia in a model of Alzheimer's disease induced by human Aß oligomers injection [66]. Indeed, in this model, the anti-inflammatory effect of an enriched environment is suppressed by treatment with ß-adrenergic antagonists. It is also absent in knock-outs of ß1/ß2 adrenergic receptors. Conversely, the inhibition of microglia activation can be mimicked by administration of ß-adrenergic agonists. In addition, the authors show that administration of Aß oligomers decreases the expression of ß-adrenergic receptors in microglia as well as the extracellular content in norepinephrine, and that both alterations are absent in mice raised in an enriched environment [66]. In summary, these data are consistent with a model where activation of microglial ß2 adrenergic receptors prevents Alzheimer's disease progression. In this model, the beneficial effect of an enriched environment would be to sustain the expression of microglial ß2 adrenergic receptors, which tends to decrease in the presence of Aß oligomers. Although an analysis of mice specifically invalidated for microglial ß2 adrenergic receptors would be required to validate this model, these data support the idea that regulation of microglia by norepinephrine is relevant for the maintenance of brain homeostasis in a pathological context.

Conclusion and perspectives

Here, we first summarized the evidence that microglial cells are equipped to respond to neuromodulators. We then illustrated how neuromodulators can impact rapidly on microglial processes motility and phagocytosis, and more durably on their immune polarization. These regulations of microglial cells can, in turn, impact on neurons by modulating synapse formation, neuronal survival, etc. Altogether, these experiments support the hypothesis that some of the effects of the neuromodulators on CNS development and homeostasis may be performed through the modulation of microglia. There are now indications that this could be the case for some developmental functions, like masculinization of the brain, and for the neuroprotective effect of enriched environment. Nonetheless, the involvement of microglia in many other potential roles of neuromodulators, for example on mood, sleep-wake cycle or response to inflammation, remain to be addressed.

To note is that several of the regulations described in this review have been demonstrated on cultivated or freshly purified microglia and need to be confirmed *in vivo*, by inhibiting or activating

receptors selectively in microglia. To do this, crossing transgenic mouse lines is to date the most straightforward but time-consuming approach. Hopefully, the development of better experimental systems, such as microglial targeting with dendrimers and induced pluripotent stem cells (IPSc)-derived microglia that can be more easily genetically engineered, will allow to further explore this exciting research area in the near future.

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None.

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Table 1: Expression of receptors for neuromodulators in microglia.

Biological material: C: primary Culture of microglia, FP: Freshly Purified microglia, S: brain Slices, IV: In Vivo. When not specified, experiments have been performed on rodents.

Receptor subtypes	Evidence for the expression of the receptors in microglia	References, biological material
Histamine		
?	Histamine application induces a calcium increase in 5-10% of freshly purified or cultured microglia (60% if cells were pretreated with LPS for 24h)	[9] [C, FP]
H1, H4	Antagonists for H1R and H4R limit the effects of mast cells supernatant on the production of PGE2 by microglia	[50] [C]
H2	RNA-Seq	[11,14,34] [FP]
H3	H3R agonist has similar effects than histamine on phagocytosis, chemotaxis by ATP and response to LPS	[49] [C]
Norepinephrine		
ß	ß adrenergic receptors antagonists have the same effect than norepinephrine on cytokine release, phagocytosis, migration, in response to amyloid peptide	[54] [C]
ß	An agonist of ß adrenergic receptors has the same inhibitory effect than norepinephrine on migration induced by C5a	[33] [C]
α1 and 2, ß1 and 2	RT-PCR	[26] [C]
α, ß1 and 2	The anti-inflammatory effect of norepinephrine on LPS-treated microglia is blocked by ß1 and ß2 adrenergic receptors antagonists; membrane currents are modulated by norepinephrine and by α- and β-adrenergic agonists	[19] [C]
ß 1 and 2	RT-PCR; norepinephrine and agonists of ß1 or ß2 induce cAMP, and antagonists of ß1 or ß2 block the induction of cAMP by norepinephrine	[25] [C]
ß 1 and 2	RNA-Seq (found in microglia purified from adult, but not newborn, animals)	[14] [FP]
ß 1 and 2	A ß-adrenergic agonist prevents the induction of cytokines by amyloid peptide; *Adrb1* and *Adrb2* genes are detected by QPCR and ß2 is detected by Western-Blot	[66] [C, FP]
ß2	QPCR	[27] [C]

ß2	Some anti-inflammatory effects of norepinephrine are blocked by antagonists of ß2 adrenergic receptors	[30] [C]
ß2 (high), ß1 and α2A (low)	RNA-Seq	[34] [FP]
ß2 (and low ß1) in basal conditions; α2A (and low ß2) after LPS	QPCR; norepinephrine and agonists of ß1 and ß2 have the same inhibitory effect on the LPS-induced release of inflammatory factors	[29] [C]
Serotonin (5-HT)		
?	5-HT application induces a calcium increase in 5-20% of freshly purified or cultured microglia	[9] [C, FP]
5-HT2, 5A, 7, 1A, 1F, depending on age and preparation	QPCR; membrane currents are modulated by 5-HT and 5-HT2 agonist DOI	[18] [C, FP]
5-HT2B	RT-PCR; oriented growth of microglial processes toward 5-HT, abolished in 5-HT2B KO mice or in presence of a 5-HT2B-specific antagonist	[15] [C, FP, S]
Dopamine		
?	Dopamine induces an increase in intracellular Ca^{2+} in 5-15% of microglia in primary cultures or freshly purified from adult brain	[9] [C, FP]
?	Dopamine induces morphological remodeling in primary cultures of microglia	[52] [C]
D1- and D2-like receptors	Membrane currents are modulated by dopamine and D1- and D2-like agonists, dopamine increases motility and inhibits NO production	[19] [C, S]
D1 to D4	RT-PCR and Immunofluorescence in human elderly cultures of microglia. Dopamine induces chemoattraction on these microglia, which is partly prevented by a D2-like antagonist	[20] [C, human]
D1, D2	mRNA for D1 and D2R are detected by QPCR in microglia from the Nucleus Accumbens. In addition, 7-8% of microglia (CD11b -positive cells) are found D1R-positive by flow cytometry, in this brain region	[22] [FP]
D1, D2	Dopamine induces the expression of angiotensin-1 and 2, and this is prevented by D2R antagonists. Agonists of D1 or D2R prevent the induction of angiotensin-1 and 2 and the increase in NADPH oxidase activity upon LPS treatment	[61] [C]
D2	D2R is detected by immunofluorescence and western-blot in primary cultures of microglia. *In vivo*, three days after an experimentally induced ischemia, D2R is detected in microglia in ischemic areas, likely due to neo-expression and not to phagocytosis of D2R-containing	[23] [C, IV after ischemia]

	element. **NB: no detection in normal conditions,** consistently with [24]	
D2	Application of a selective D2R agonist upregulates TNF-α mRNA in rat microglia primary culture	[21] [C]
Acetylcholine (ACh)		
nAChR	Nicotine induces an increase in intracellular Ca^{2+} in 5-15% of freshly purified or cultured microglia	[9] [C, FP]
α7	RT-PCR; ACh and nicotine inhibit the release of TNF-α by LPS-treated microglia and this effect is inhibited by antagonists of α7 nAChR	[39] [C]
α7	RT-PCR	[40] [C]
α7	RT-PCR, Western-Blot; nicotine inhibits the induction of signaling pathways and TNF-α release by LPS, and this effect of nicotine is blocked by a specific antagonist of α7 nAChR	[41] [C]
α7	RT-PCR, Western-Blot, Immunofluorescence (in cultures, and post-mortem in the brain of an Alzheimer's disease patient); nicotine increases phagocytosis of Amyloid ß peptides, this is prevented by a specific antagonist of α7 nAChR	[44] [C, IV human]
α7	RT-PCR and Western-Blot; nicotine induces GLAST expression in cultured microglia and this is prevented by a specific antagonist of α7 nAChR	[42] [C]
α7	By immunohistochemistry, in a model of Alzheimer's disease, anti-α7 labeling increases and decreases in parallel with microglial activation	[43] [IV]
α7	Several effects of LPS are inhibited by nicotine and this inhibitory effect is blocked in presence of a specific antagonist of α7 nAChR	[71] [C]
α7	An agonist and an antagonist of α7 nAChR have opposite effects on the inflammatory profile induced by LPS in microglial cultures	[70] [C]
α4ß2	Seven days after ischemia, increased signal with a α4ß2 ligand in positron emission tomography; colocalization of anti-CD11b and anti-α4ß2 labelings demonstrated by immunohistochemistry	[45] [IV]
mAChR?	Carbachol, a mAChR agonist, induces an increase of intracellular Ca^{2+} in rat or human cultured microglia	[47] [C]; [46] [C, human]
M3	A fraction of freshly isolated microglia (11% in basal conditions, 25-60% in models of Alzheimer's disease or stroke) respond to the muscarinic receptors' agonist carbachol by a Ca^{2+} increase. In a flow cytometry analysis, 16% of microglia are M3R-positive. In culture, carbachol is chemoattractant and inhibits phagocytosis	[8] [FP, C]

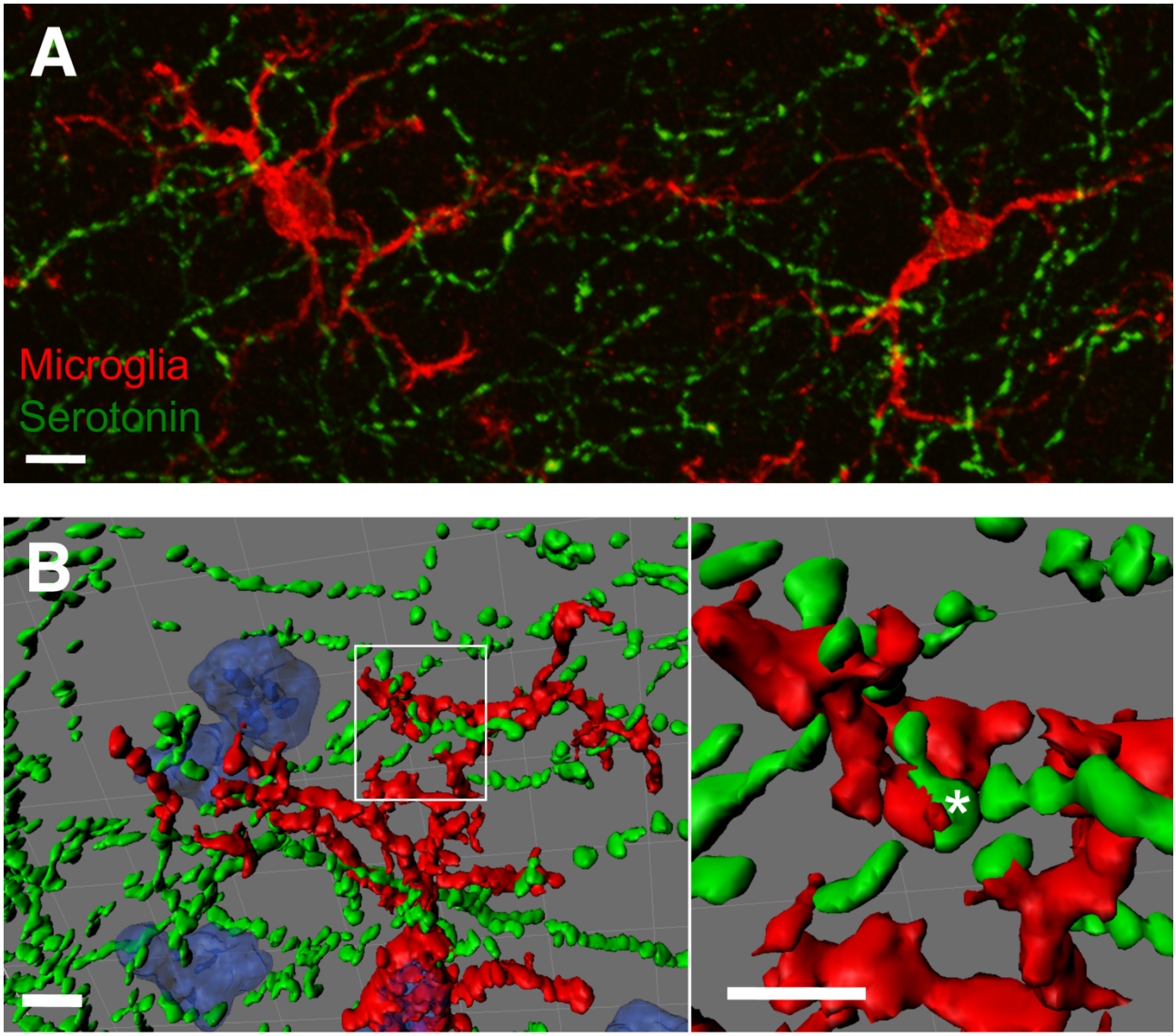

Figure 1: Close contacts between microglial processes and serotonergic varicosities in the adult brain. (A) Projection of a 20 µm-thick stack of confocal images from the hippocampus of an adult mouse, illustrating proximity of microglia (red, stained with anti-Iba1 antibody) and serotonergic axons (green, stained with anti-serotonin antibody; note the typical varicosities along the axons). Noteworthy, to estimate distances and visualize appositions, a projection image is not sufficient, and 3D reconstruction is required. Scale bar: 10 µm. (B) Imaris 3D reconstruction from a stack of confocal images allows to show some close appositions (square) of microglial processes and serotonergic axons, in addition to the general presence of varicosities at a micrometer-range distance of microglia. The discontinuous appearance of the serotonergic axons in the reconstruction is due to the contrast between the strongly stained varicosities, where serotonin-containing vesicles accumulate, and the rest of the axons. Nuclei, stained with Bis-Benzimide, are in blue. Left: overview, right: detail showing a microglia process surrounding a serotonin varicosity (asterisk). Scale bars: 5 µm.

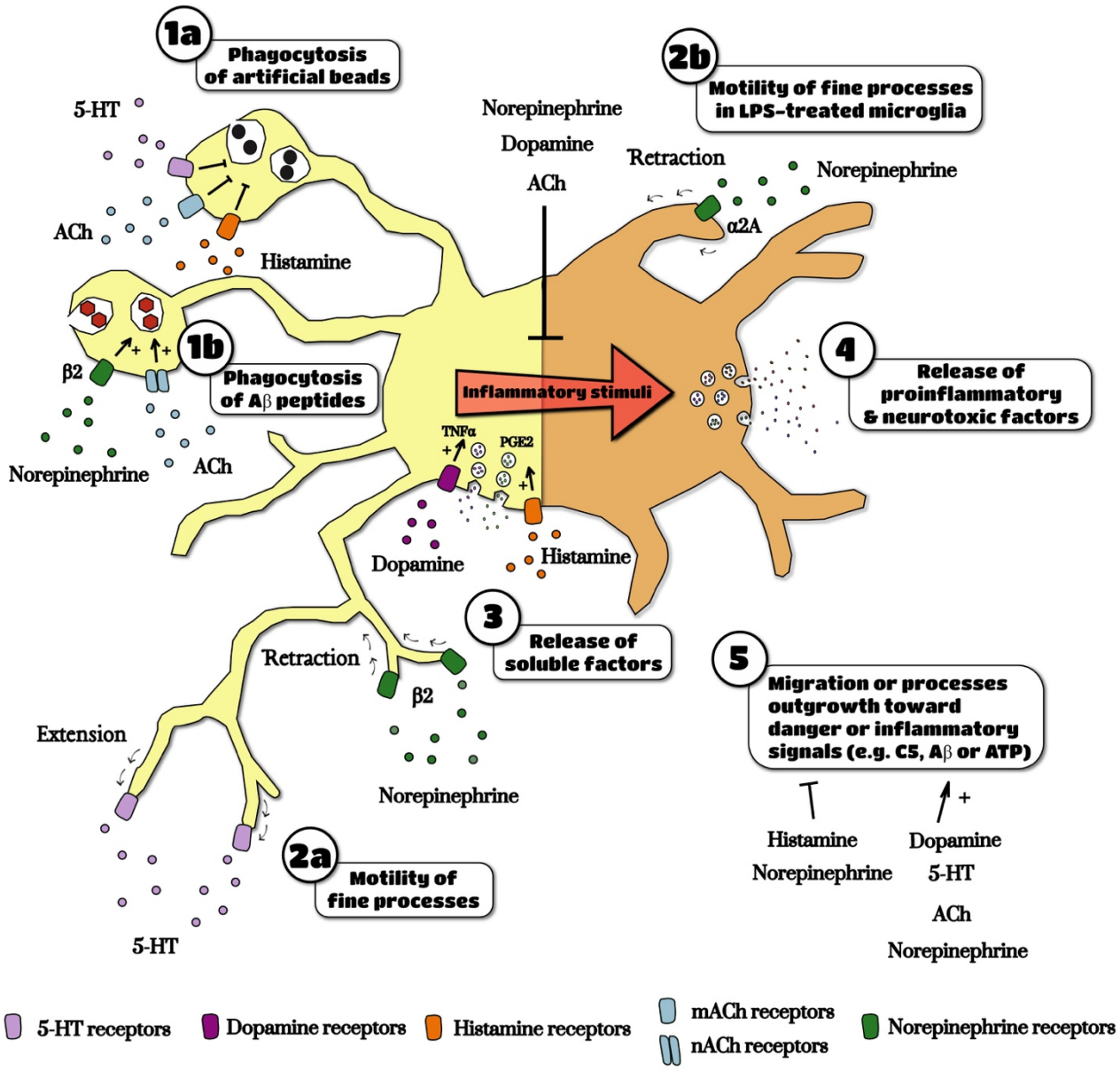

Figure 2. Regulation of microglial functions by neuromodulators.

Microglia can act on neurons and other cell types through physical interactions and by releasing a vast array of soluble factors, and neuromodulators were shown to regulate many of these processes. **1**: Phagocytosis of dying neurons, excessive synapses or unwanted compounds is pivotal during CNS development and to maintain CNS homeostasis. Several neuromodulators can regulate phagocytosis, although the final effect seems to be dependent on the target to be phagocytosed. **(1a)** On the one hand, clearance of artificial beads is reduced after application of 5-HT [18], histamine [49], and carbachol, an agonist of mACh receptors [8]; **(1b)** on the other hand, phagocytosis and degradation of Aβ are increased by norepinephrine acting on β2 receptors [27,54] and ACh acting on α7 nAChR [44]. **2**: To scan the environment and make physical interactions, microglia have to be highly motile, extending and retracting their fine processes. **(2a)** In normal conditions, besides potentiating ATP-induced increase in microglia process motility (not illustrated

here [18]), local applications of 5-HT on acute brain slices induces a directional extension of microglial processes, an effect dependent on 5-HT2B receptors [15]. Norepinephrine instead promotes retraction of microglial processes through β2 receptors [28][16,53] and prevents ATP- or lesion-induced motility (not illustrated here [28][53]). **(2b)** In microglia treated with LPS, norepinephrine induces a reduction of microglial arborization, this time mediated by α2A receptors [28]. **3 and 4**: Several experiments demonstrate that neuromodulators actively regulate microglial immune polarization and influence their release of soluble factors. **3:** Some of these factors are released by microglia in basal conditions, such as PGE2, whose release is promoted by histamine [50], or TNF-α, whose release is promoted by dopamine in the striatum [62]. **4:** However, in a number of pathological conditions, microglia can become neurotoxic and release pro-inflammatory cytokines, factors and reactive oxygen species. Most neuromodulators, including dopamine, norepinephrine and ACh, exert a protective action by inhibiting this polarization of microglia toward a toxic phenotype after exposure to inflammatory stimuli (numerous references, cited in section 3). **5:** Eventually, "danger" or inflammatory signals promote microglial migration and process outgrowth, which are potentiated by 5-HT [18] and agonists of mAChR [8], and inhibited by histamine [49]. Norepinephrine modulates microglial chemotaxis toward danger signals in a dual way: high doses inhibit process outgrowth toward ATP and C5 complement [28,33], but low doses increase chemotaxis toward Aβ and TNF-α [54]. 5-HT: serotonin; α2A: α2A adrenergic receptors; ß2: ß2 adrenergic receptors; Aβ: amyloid β; ACh: acetylcholine; ATP: adenosine triphosphate; C5: C5 complement protein; LPS: lipopolysaccharide; mAChR: muscarinic acetylcholine receptors; nAChR: nicotinic acetylcholine receptors; PGE2: prostaglandin E2; TNF- α: tumor necrosis factor α.