



Neurotransmitter signaling in the pathophysiology of microglia

María Domercq^{1,2,3}, Nuria Vázquez-Villoldo^{1,2,3} and Carlos Matute^{1,2,3*}

¹ Departamento de Neurociencias, Universidad del País Vasco-UPV/EHU, Leioa, Spain

² Achucarro Basque Center for Neuroscience-UPV/EHU, Zamudio, Spain

³ Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Leioa, Spain

Edited by:

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Hiroaki Wake, National Institute for Basic Biology, Japan

*Correspondence:

Carlos Matute, Laboratorio de Neurobiología, Departamento de Neurociencias, Facultad de Medicina y Odontología, Universidad del País Vasco (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Vizcaya, Spain.
e-mail: carlos.matute@ehu.es

Microglial cells are the resident immune cells of the central nervous system. In the resting state, microglia are highly dynamic and control the environment by rapidly extending and retracting motile processes. Microglia are closely associated with astrocytes and neurons, particularly at the synapses, and more recent data indicate that neurotransmission plays a role in regulating the morphology and function of surveying/resting microglia, as they are endowed with receptors for most known neurotransmitters. In particular, microglia express receptors for ATP and glutamate, which regulate microglial motility. After local damage, the release of ATP induces microgliosis and activated microglial cells migrate to the site of injury, proliferate, and phagocytose cells, and cellular compartments. However, excessive activation of microglia could contribute to the progression of chronic neurodegenerative diseases, though the underlying mechanisms are still unclear. Microglia have the capacity to release a large number of substances that can be detrimental to the surrounding neurons, including glutamate, ATP, and reactive oxygen species. However, how altered neurotransmission following acute insults or chronic neurodegenerative conditions modulates microglial functions is still poorly understood. This review summarizes the relevant data regarding the role of neurotransmitter receptors in microglial physiology and pathology.

Keywords: microglia, ATP, glutamate, purinergic and glutamatergic receptors

INTRODUCTION

Microglial cells constitute the resident immune cell population of the mammalian central nervous system (CNS). Postnatally, microglia are present in all regions of the CNS in a non-overlapping territorial manner and comprise a large proportion of the total cellular makeup of the CNS, which is estimated to be as high as 12% (Lawson et al., 1990). Similar to macrophages, their peripheral counterparts, microglia display remarkable ranges of morphology and activity depending, in part, on the state of the surrounding tissue (Lynch, 2009; Ransohoff and Perry, 2009). “Resting” microglia are not functionally silent cells, but extremely dynamic *in vivo*, perpetually changing their morphology by extending and retracting highly motile processes on a time scale of minutes (Davalos et al., 2005; Nimmerjahn et al., 2005). In response to local damage (few micron lesions), microglial processes rapidly and automatically converge on the site of injury without cell body movement. The microglial branching response mediated by ATP release aims to shield and/or scavenge the affected side. In addition, part of the dynamic motility of surveying microglial processes *in vivo* is directed toward synapses, suggesting that microglia vigilantly monitor and respond to the functional status of synapses (Wake et al., 2009). In addition, microglia have been reported to be capable of sensing defunct synapses and phagocytose them in normal brain (Wake et al., 2009; Tremblay et al., 2010). Synaptic pruning by microglia is essential during development for the remodeling of synaptic circuits [Paolicelli et al., 2011; see also the reviews by Tremblay

(2011) and Wake et al. (2013)]. Microglia also efficiently phagocytose apoptotic neurons in the neurogenic niche (Sierra et al., 2010).

In addition to its functions in normal brain, microglia are involved in most, if not all, known CNS pathologies. More than a decade ago, Georg Kreutzberg coined the term “microglial sensor of pathology” (Kreutzberg, 1996), which captures the essence of microglial cell function. Microglia are the brain’s intrinsic immune cells and serve as damage sensors within the brain. Any type of injury or pathological process leads to the activation of these cells from their surveillant/resting state. In response to injury, microglia change their highly branched and ramified morphology by retracting their processes and taking on an ameboid appearance. Activated microglial cells can then migrate to the site of injury, proliferate, and release substances that affect pathological processes. These substances include pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α , and interleukin (IL)-6 or IL-12, which signal the invading T lymphocytes.

Multiple signals converge on microglial cells to actively maintain or alter their functional state and orchestrate the specific repertoire of microglial functions. Transitions between surveillance and activated states are triggered when microglia perceive a sudden appearance, abnormal concentration, or unusual molecular format of certain factors (Hanisch and Kettenmann, 2007). This review focuses on the role of neurotransmitter receptors, particular ATP and glutamate receptors, in the control of microglial physiology and pathology. For the role of other

receptors or channels, see these other excellent reviews (Pocock and Kettenmann, 2007; Kettenmann et al., 2011).

ATP RECEPTORS

EXPRESSION OF ATP RECEPTORS IN MICROGLIA

Purines and pyrimidines act as widespread extracellular signaling molecules. The physiological effects of purines and pyrimidines are mediated through an extended family of purinoceptors activated by adenosine, classified as P1 receptors, or by ATP, classified as P2 receptors (Ralevic and Burnstock, 1998; North, 2002). Purinergic receptors are expressed in the majority of living cells and are particularly abundant in glia (Pocock and Kettenmann, 2007; Kettenmann et al., 2011). ATP activates a family of metabotropic P2Y, P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃, P2Y₁₄, and ionotropic P2X1-7 receptors. Extracellular ATP is degraded to adenosine by ectonucleotidases, such as CD39 and CD73, which are known to be present in microglial cells (Braun et al., 2000) and adenosine activates G protein-coupled adenosine receptors A₁, A_{2A}, A_{2B}, and A₃. The A₁ and A₃ receptors can inhibit adenylyl cyclase or activate phospholipase C, whereas A_{2A} and A_{2B} receptors activate cyclic AMP production (Fredholm et al., 2001). Collectively, the actions of ATP and its degradation products produce responses that last from milliseconds to minutes, and even longer time scales through changes in gene regulation via second messengers (Khakh and North, 2012). P2X receptors are non-selective cation channels with high Ca²⁺ permeability that carry a depolarizing current under standard physiological conditions. In some cells, P2X channels are also significantly permeable to anions, such as the full-length P2X5 receptor (P2X5R), which is permeable to Cl⁻ (North, 2002). Functional homomeric P2X1R and P2X3Rs have fast desensitization properties. The other receptor types have slow desensitization properties, except P2X7R, which does not desensitize (Khakh and North, 2012). After prolonged activation, P2X7Rs open a large pore, causing cytolytic cell death (Surprenant et al., 1996). Signaling diversity is increased by the broad range of ATP sensitivities exhibited by ATP receptors, ranging from nanomoles in the case of P2Y receptors to hundreds of micromoles for P2X7Rs (North, 2002). Thus, ATP receptors respond over remarkably broad spatiotemporal scales, making ATP signaling highly dynamic.

P2X-mediated currents were identified in microglial cultures prepared from human and rodent brains more than two decades ago (Walz et al., 1993). Patch-clamp recordings have shown that cultured microglial cells respond to extracellular ATP (100 μM) with the activation of a transient inward non-selective cationic current, which is followed, in some cases, by an outward K⁺ current (Walz et al., 1993; Nörenberg et al., 1994; McLarnon et al., 1999). These results were recently corroborated and extended in acute slices. Thus, ATP triggers a non-selective cationic inward current in association with the activation of P2X7Rs, and an outward K⁺ current associated with the activation of P2Y6 and P2Y12 metabotropic receptors (Boucsein et al., 2003; Avignone et al., 2008). Importantly, these studies describe diverse electrophysiological properties for microglial cell types, one subtype with a lower resting membrane potential (between -50 mV and -60 mV) and another subtype with

a higher membrane potential (-20 to -30 mV), and on the basis of different responses to ATP. These differences may be associated with the different functional roles of microglia.

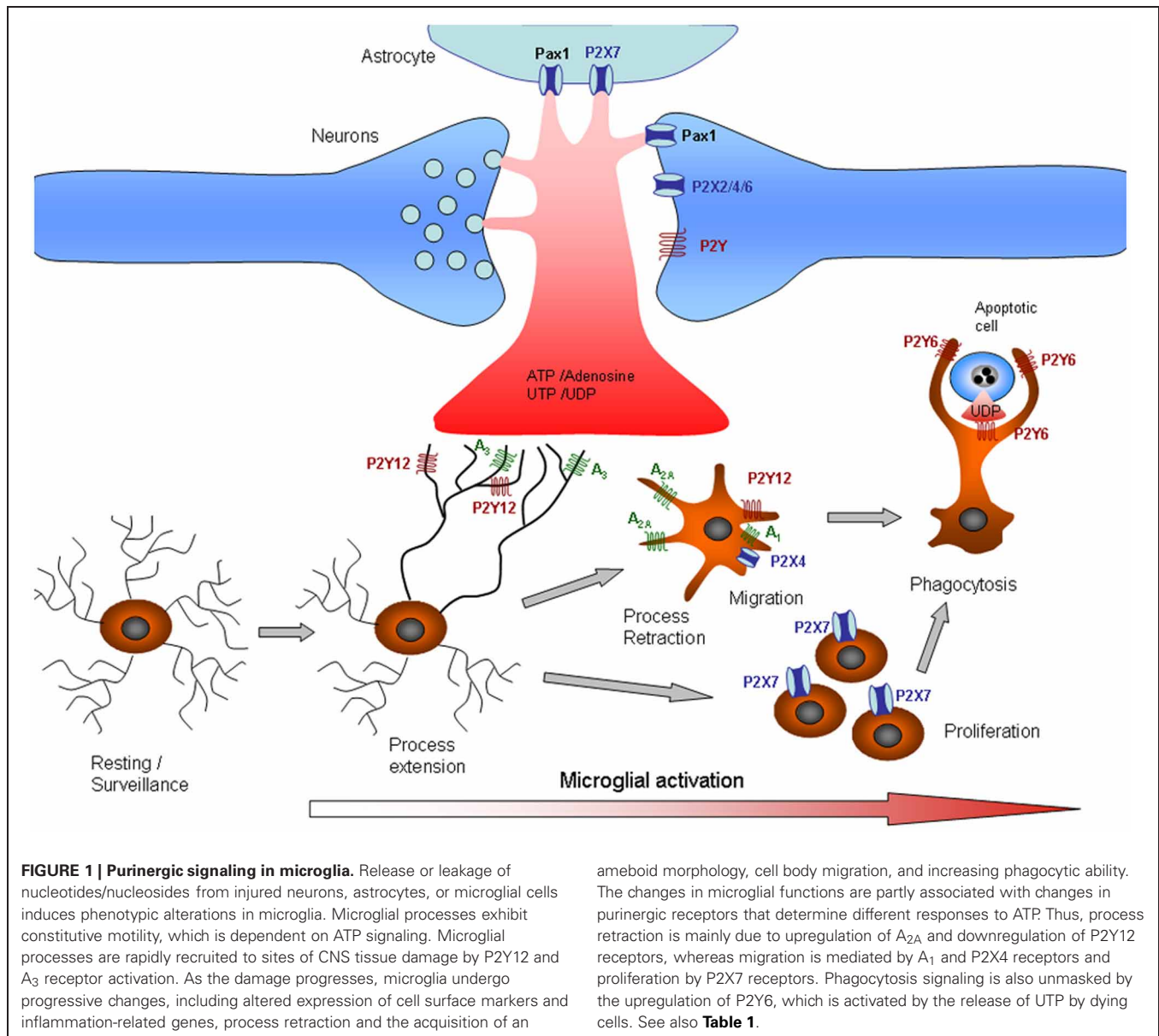
Immunohistochemical studies have shown the expression of low levels of P2X4 (Ulmann et al., 2008) and P2X7 (Matute et al., 2007) and high levels of P2Y12 (Haynes et al., 2006) in microglia in the adult normal brain. The expression of purinergic receptors changes during development (Xiang and Burnstock, 2005). Thus, at embryonic day 16, the majority of microglial cells express P2X1 and P2X4 subunits, whereas only 30% of these cells express P2X7. From postnatal day 7, P2X4-positive microglia locate preferentially around blood vessels. At postnatal P30, the cells expressing P2X1 virtually disappear, the P2X7-positive cells are distributed widely through the forebrain, whereas cells bearing P2X4Rs are mainly localized around blood vessels and lining the subarachnoid space (Xiang and Burnstock, 2005). Constitutive expression of P2X4 in pervascular cells was also described in the adult spinal cord (Guo and Schluesener, 2005). Pervascular P2X4⁺ cells are ED1⁺/OX42⁺, indicating that correspond to infiltrating monocytes/microglia, but not to lymphocytes.

From postnatal day 7, many microglial cells with P2X4 receptor-immunoreactivity were seen around the blood vessels. At postnatal day 30, microglial cells with P2X1 receptor-immunoreactivity disappeared and the cells with P2X4 receptor-immunoreactivity were mainly localized around blood vessels and lining the subarachnoid space. From postnatal day 30, the microglial cells with P2X7 receptor-immunoreactivity were found to be distributed widely in the forebrain. Cells with P2X7 receptor-immunoreactivity from P30 were not labeled by ED1, but some were labeled by isolectin B4. The expression of P2X1, P2X4, and P2X7 receptor mRNA and protein on primary cultures of rat microglial cells and on the N9 microglial cell line was demonstrated with immunocytochemistry and RT-PCR. This is the first report that the P2X1 receptor is expressed on microglial cells, at least in early development, before postnatal day 30.

Regarding adenosine receptors, *in vitro* functional studies have identified the expression of all P1 adenosine receptors, A₁, A_{2A}, A_{2B}, and A₃ receptors in microglia (Haskó et al., 2005; Abbracchio and Ceruti, 2007). *In vivo*, the A_{2A} receptor appears to be expressed only in activated microglia after systemic lipopolysaccharide (LPS) injection (Orr et al., 2009). In contrast, adult healthy brains express relatively higher levels of A₁ and A₃ receptors (Koizumi et al., 2013).

FUNCTIONS OF ATP RECEPTORS IN MICROGLIA

The initial microglial responses that occur after brain injury and in various neurological diseases are characterized by microglial accumulation in the affected sites of the brain as a result of the migration and proliferation of these cells. The early-phase signal responsible for this accumulation is likely to be transduced by rapidly diffusible factors, such as ATP. Purinergic receptors control several microglial functions, including the motility of their fine processes, migration, cytokine release, and phagocytosis (Figure 1). Low ATP concentrations almost exclusively activate chemotaxis in order to recruit cells at the site of injury or inflammation. When the ATP concentration increases, additional



effector functions, such as phagocytosis and cytokine secretion, are also triggered (**Table 1**).

Movement of the fine microglial processes is controlled primarily through the activation of P2Y₁₂ receptors, which are expressed at high levels in microglia in normal brain. However, P2Y₁₂ receptors are downregulated in microglia after stroke or activation by LPS (Haynes et al., 2006). Microglial chemotaxis is characterized by cell body movement, as well as process movement, and is mediated through the activation of P2X₄ and P2Y₁₂ receptors (Honda et al., 2001; Ohsawa et al., 2007). Haynes et al. (2006) showed that, in P2Y₁₂ receptor-null mice undergoing focal laser cortical ablation, the chemotactic response of microglia was markedly impaired in the first 40 min of the observation period compared to wild-type animals. However, when microglia from mutant mice were examined 2 h after injury, the degree of chemotaxis approached that observed in wild-type animals. Moreover,

P2Y₁₂ receptor expression on microglia was barely observable 24 h after injury. The loss of P2Y₁₂ expression was accompanied by microglial transformation from highly ramified to an amoeboid state. These observations indicate that P2Y₁₂ receptors are involved in the early, rather than late, responses of microglia to injury (Haynes et al., 2006). In contrast, P2X₄ has been shown to be markedly upregulated after microglial activation; thus, chemotaxis after injury could be mediated by this receptor (Ohsawa et al., 2007).

Phagocytosis is the terminal removal of cellular debris by phagocytes. In vertebrates, phagocytosis is performed mostly by macrophages and other specialized innate immune cells engulfing the cellular debris in phagosomes, membrane protrusions that fuse with lysosomes for terminal degradation. Although phagocytosis is activated primarily by the expression of “eat-me” signals on the surface of damaged or dead cells, injured neurons

Table 1 | Expression and function of purinergic receptors on microglia.

Receptor type	Presence	Function	References
P1			
A ₁	+	Migration/Neuropathic pain/Anti-inflammatory properties	Färber et al., 2008; Haselkorn et al., 2010; Luongo et al., 2012
A _{2A}	+	Process retraction/Microglial activation	Orr et al., 2009; Yao et al., 2012
A _{2B}	+	Anti-inflammatory properties; release of IL-10	Koscsó et al., 2012
A ₃	+	Process extension and migration	Ohsawa et al., 2012
P2			
Ionotropic			
P2X ₁	–	–	Cavaliere et al., 2005; Xiang and Burnstock, 2005
P2X ₂	–	–	Unpublished observation
P2X ₃	+	–	Unpublished observation
P2X ₄	+	Migration/Neuropathic pain	Ohsawa et al., 2007; Beggs et al., 2012
P2X ₅	?	–	–
P2X ₆	–	–	Cavaliere et al., 2005
P2X ₇	+	Microglial proliferation/Inflammasome signaling	Rigato et al., 2012; Volonte et al., 2012
Metabotropic			
P2Y ₁	?	Purine release/Activation of a K ⁺ current	Boucsein et al., 2003; Ballerini et al., 2005
P2Y ₂	+	A β (1–42) degradation and uptake	Kim et al., 2012
P2Y ₄	+	Not determined	–
P2Y ₆	+	Phagocytosis	Koizumi et al., 2007
P2Y ₁₁	?	Microglial activation	Brandenburg et al., 2010
P2Y ₁₂	+	Process extension/Migration	Haynes et al., 2006
P2Y ₁₃	+	Neuropathic pain	Kobayashi et al., 2012
P2Y ₁₄	+	Neuropathic pain	Kobayashi et al., 2012

?, Not determined

leak diffusible UDP signals that activate P2Y₆-dependent phagocytic signaling in microglia (Koizumi et al., 2007). UDP, which also acts on G_q-coupled P2Y₆ receptors, induces the expression of chemokines (Kim et al., 2011). In contrast to the positive effect of P2Y₆, the stimulation of P2X₇ or P1 receptors attenuates microglial phagocytosis (Fang et al., 2009; Bulavina et al., 2012). Phagocytosis by microglia is also regulated by the ratio of P2/P1 activation. The interplay between P2 and P1 receptor activation is controlled by a cascade of extracellular enzymes that dephosphorylate purines, resulting in the formation of adenosine. In microglia, the capacity to degrade ATP and ADP depends on the expression of ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1, also called CD39). Deletion of CD39 practically abolishes ATP degradation and increases microglial phagocytic activity (Bulavina et al., 2012). Interestingly, the P2X₇ receptor has also been detected in phagosomes. Lipids stimulate both actin assembly and the transport of ADP across the phagosomal membrane into the lumen. In the lumen, ADP is converted to ATP by adenylate kinase activity and activates P2X₇ receptors in phagosomes, triggering actin assembly on the cytoplasmic membrane surface (Kuehnel et al., 2009a,b). These data indicate a distinct role of intracellular P2X₇ receptors in phagocytosis.

In addition to its role in normal CNS function, ATP signaling is involved in neuroinflammation in a broad range of CNS pathologies (Di Virgilio et al., 2009). The extracellular concentration of ATP increases dramatically during inflammation (Idzko et al., 2007; Pellegatti et al., 2008), and P2X₇ receptors are overexpressed in microglial cells in the neuroinflammatory

foci of numerous neurodegenerative conditions (Weisman et al., 2012). The overexpression of P2X₇ receptors in microglia, in the absence of a pathological insult, is sufficient to drive the activation and proliferation of microglia (**Figure 1**), which depends on the pore-forming capacity of this receptor (Monif et al., 2009). The activation of P2X₇ receptors is coupled to the maturation and secretion of the key pro-inflammatory cytokine IL-1 β [Ferrari et al., 1996; reviewed in Di Virgilio et al. (2009)], a signaling pathway that depends on P2X₇ coupling with pannexin-1 and subsequent caspase-1 activation (Pelegri and Surprenant, 2006). Activation of the large-pore P2X₇ receptor also leads to the release of TNF α (Suzuki et al., 2004), the endocannabinoid 2-arachidonylglycerol (Witting et al., 2004), and superoxide (Parvathani et al., 2003). However, whether the pore capacity of P2X₇ depends on its coupling with pannexin-1 is a matter of debate. In neurons and astrocytes, pannexin1 appears to be the molecular substrate for the permeabilization pore (or death receptor channel) recruited into the P2X₇ receptor signaling complex (Locovei et al., 2007; Silverman et al., 2009). In innate immune cells, including microglia and macrophages, P2X₇ signaling appears to be independent of pannexin-1 (Hanley et al., 2012; Rigato et al., 2012). Thus, microglia proliferate and phagocytose dying motor neurons during early embryonic spinal cord invasion (Rigato et al., 2011). Notably, microglial invasion and proliferation are controlled by P2X₇ receptor signaling in a pannexin-1-independent manner (Rigato et al., 2012). The pore dilation capacity of P2X₇ receptors in microglia does not depend on the expression of pannexin-1 at this embryonic stage (Rigato et al., 2012).

The main function of adenosine receptors is the control of innate immune function. Adenosine receptors A_1 and A_3 block TNF α release by microglia, whereas A_{2B} stimulates the production and secretion of anti-inflammatory cytokine IL-10 (Koscsó et al., 2012), indicating an anti-inflammatory role of P1 receptor activation in the brain. Signaling through adenosine receptor A_{2A} drives the proliferation of spinal microglia after nerve injury (Bura et al., 2008), and intrathecal blockade of this receptor has been shown to abolish neuropathic pain in the same model (Loram et al., 2009). Finally, adenosine can suppress inflammation and aid in tissue restitution, in part, by promoting alternative macrophage activation. Alternative activation occurs in a Th2 cytokine environment and promotes the immunomodulatory and anti-inflammatory, rather than pro-inflammatory, properties of macrophages/microglia. Adenosine treatment of IL-4- or IL-13-activated macrophages augments the expression of alternative macrophage markers, primarily through the activation of A_{2B} receptors, though A_{2A} receptors also contribute to the effect (Csóka et al., 2012). Acting through A_3 receptors, adenosine is also involved in the extension and migration of microglial processes (Ohsawa et al., 2012). Interestingly, simultaneous stimulation of P2Y₁₂ and A_3 receptors is required for microglial process extension, suggesting that intimate crosstalk occurs between P2Y₁₂ and A_3 receptors.

CHANGES IN ATP RECEPTOR EXPRESSION IN MICROGLIA

Microglia adopt an appropriate stimulus modality-dependent phenotype in response to injury or disease. The phenotypic catalog of microglia includes proliferative, migrational, and phagocytic responses, though how distinct the discrete molecular fingerprints of the phenotypes are is not clear (Hanisch and Kettenmann, 2007). Microglia undergo dramatic changes in shape and gene expression patterns within hours of *in vivo* activation, including modulation of the expression and function of purinergic receptors (Figure 1). Remodeling of purinoceptor expression has been observed *in situ* in various pathological models. Thus, epileptic seizures induced by kainate injections trigger an activation of microglia in hippocampal slices (Avignone et al., 2008), accompanied by an upregulation of the expression of mRNA specific for P2X₁, P2X₄, P2X₇, P2Y₆, P2Y₁₂, and P2Y₁₃ receptors. Functionally, this upregulation manifests as an increase in ATP-induced membrane currents and ATP-induced microglial motility (Avignone et al., 2008). Depending on the microglial stage, changes in purinergic expression determine the responses, sometimes with opposite effects, to extracellular purines. For example, the microglial chemotactic response to ATP is reversed following microglial activation. The switch from process attraction to repulsion is driven by upregulation of the G_s -coupled A_{2A} receptor (Orr et al., 2009) concomitant with downregulation of the G_i -coupled P2Y₁₂ receptor (Haynes et al., 2006).

The extended and divergent time course of microglial activation suggests that the activation process is regulated by complex mechanisms, which may differ significantly depending on the initiating stimulus. *In vivo*, P2Y₁₂ receptor expression decreases as microglia become activated after LPS injection in the striatum (Haynes et al., 2006). In contrast, facial-nerve axotomy, a classical model of microglial activation, induces an upregulation of

microglial P2Y₁₂ mRNA (Sasaki et al., 2003). Other stimuli, such as epileptic seizures or trauma, also lead to rapid upregulation of P2Y₁₂ mRNA and protein in microglia (Franke et al., 2007; Avignone et al., 2008; Tozaki-Saitoh et al., 2008).

The P2X₄R has been reported to be associated with the activation of microglia/macrophages after CNS injury and may play roles in inflammatory cascades involved in secondary brain damage. The development of mechanical allodynia temporally correlates with an increase in spinal P2X₄R expression in microglia (Ulmann et al., 2008). Microglial P2X₄R upregulation, the P2X₄R⁺ state of microglia, seems to be common in most acute and chronic neurodegenerative diseases associated with inflammation. Microglial activation after traumatic brain injury also parallels a significant increase in P2X₄R expression in microglia, which is suppressed by systemic treatment with dexamethasone (Zhang et al., 2007). The upregulation of microglial P2X₄R has also been observed in animals expressing superoxide dismutase 1 mutant, an animal model of amyotrophic lateral sclerosis (D'Ambrosi et al., 2009), in the acute experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) (Guo and Schluesener, 2005), after spinal cord injury (Schwab et al., 2005), and in cerebral ischemia (Cavaliere et al., 2003). Different regulators of P2X₄R expression in microglia have been described, such as the chemokine CCL2 (also known as monocyte chemoattractant protein, MCP-1; Biber et al., 2011; Toyomitsu et al., 2012), interferon- γ (Tsuda et al., 2009), and the extracellular matrix molecule fibronectin acting through Lyn kinase (Tsuda et al., 2008, 2009).

The role of P2X₄R in microglial activation and how its expression affects microglial functions is unclear. In neurons, P2X₄R influences inflammasome activation after spinal cord injury (de Rivero Vaccari et al., 2012). The inflammasome is a multiprotein complex that promotes the activation of caspase-1 and release of mature inflammatory cytokines, such as IL-1 β and IL-18. This complex likely controls many aspects of neuroinflammatory processes. P2X₄ knock-out mice exhibit impaired inflammasome signaling in the spinal cord, resulting in decreased IL-1 β levels and reduced infiltration of neutrophils and monocyte-derived M1 macrophages, resulting in significant tissue sparing and improved functional outcomes (de Rivero Vaccari et al., 2012).

The metabotropic P2Y₆R that controls microglial phagocytosis is highly expressed in surveying microglia, but it is also slightly upregulated in microglia following neuronal damage induced by kainic acid injection in the hippocampus (Koizumi et al., 2007), by trauma (Franke et al., 2007), or in animals expressing dismutase 1 mutant (D'Ambrosi et al., 2009). These data point to a role of this receptor in controlling the phagocytosis of necrotic cells after damage (Koizumi et al., 2007). In contrast, the phagocytosis of apoptotic cells prevents the spillover of cellular contents. Thus, whether UDP levels are sufficient to activate the phagocytosis of apoptotic cells or debris by microglia under normal physiological conditions remains to be determined.

Finally, the expression of adenosine receptors varies depending on microglial activation. Thus, surveying microglia express high levels of A_1 and A_3 receptors, and activation leads to their downregulation. In contrast, the expression level of the adenosine A_{2A} receptor in surveying/resting microglia is relatively low, but LPS

dramatically increases its expression (Orr et al., 2009). The upregulation of A_{2A} receptor expression is also observed in pathological states, such as Parkinson disease and ischemia (Pedata et al., 2001; Schwarzschild et al., 2006).

GLUTAMATE RECEPTORS

EXPRESSION OF GLUTAMATE RECEPTORS IN MICROGLIA

Glutamate is the major excitatory neurotransmitter of the CNS and perturbations in this transmitter's homeostasis have been reported in most neurodegenerative diseases. Glutamate activates both ionotropic and metabotropic receptors. Ionotropic receptors are classified into α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) subtypes according to their preferred agonist. Molecular cloning has revealed that each receptor subtype is composed of several subunits with high homology within each receptor class. Thus, AMPA receptors are formed by GluR1-4, kainate receptors by GluR5-7 and KA1-2, and NMDA receptors by NMDAR1, NMDAR2A-D, and NMDAR3A-B subunits (Cull-Candy and Leszkiewicz, 2004). AMPA receptors are activated by AMPA and kainate, whereas kainate receptors are activated by kainate and are best functionally isolated in the presence of GYKI53655, a selective AMPA receptor antagonist (Lerma, 2003). Similarly, metabotropic GluRs (mGluRs) can be classified as group I (mGluR1, mGluR5), group II (mGluR2, mGluR3), and group III (mGluR4, mGluR6-8) seven transmembrane receptors (Swanson et al., 2005). The individual mGluR groups are coupled to various G-proteins that activating phospholipase C (PLC, group I) or inhibit adenylate cyclase (groups II and III).

Few studies have characterized the functional expression of ionotropic glutamate receptors in microglial cells. An early study showed inward currents corresponding to the activation of low-Ca²⁺ permeability AMPA-type glutamate receptors (expressing the GluR2 subunit) in cultured microglia, the activation of which leads to TNF- α release [Noda et al., 2000; reviewed by Pocock and Kettenmann (2007)]. AMPA-type glutamate receptor activation also leads to *c-fos* expression (Eun et al., 2004). In contrast, no direct evidence has been found for the functional expression of kainate receptors in microglial cells. In contrast to *in vitro* conditions, electrophysiological recordings of microglial cells in retina or hippocampus slices clamped at -50 mV failed to detect any inward or outward current in response to glutamate or AMPA (Wu and Zhuo, 2008; Fontainhas et al., 2011). The expression of functional NMDA receptors in microglia in normal brain has not been reported, but the activation of microglia after the induction of transient forebrain ischemia leads to NMDAR1 subunit upregulation (Gottlieb and Matute, 1997). The functional significance of NMDA receptor upregulation in microglia is still unknown. However, NMDA injection into the somatosensory cortex of newborn rats triggers transient microglial activation (Acarin et al., 1996), whereas systemic administration of MK-801 prevents rapid microglial activation in the hippocampus, secondary to ischemic insults (Streit et al., 1992) or LPS treatment (Thomas and Kuhn, 2005). Whether NMDA receptor activation controls microglial activation directly or indirectly remains to be determined. Therefore, additional studies are necessary to characterize the existence of functional ionotropic glutamate receptors

in the resident and activated microglia of slices, which could respond to glutamate release during synaptic activity or damage.

Regarding metabotropic glutamate receptors, different subunits of metabotropic groups I (mGluR5), II (mGluR2 and 3), and III (mGluR4, 6, and 8, but not mGluR7) are expressed by microglia and regulate microglial transformation into neuroprotective (via group III mGluRs) or neurotoxic (via group II mGluRs) phenotypes (Biber et al., 1999; Taylor et al., 2002, 2003, 2005; Pocock and Kettenmann, 2007). Microglial activation of group II mGluRs, particularly mGluR2, induces TNF- α and Fas ligand release, which trigger neuronal caspase-3 activation via TNFR1 (also known as p55) and Fas receptor, leading to neuronal death (Taylor et al., 2005). However, an agonist of mGluR3, a member of group II, has been shown to inhibit the toxicity of microglia toward oligodendrocytes (Pinteaux-Jones et al., 2008). Activation of groups II and III metabotropic glutamate receptors also modulates LPS-induced glutamate release by the xCT antiporter in microglia (McMullan et al., 2012), suggesting a neuroprotective role of its activation. Groups I and III metabotropic glutamate receptors also modulate the activity of NADPH oxidases, the main source of superoxide anions (Mead et al., 2012).

FUNCTIONS OF GLUTAMATE RECEPTORS IN MICROGLIA

Similar to ATP, glutamate is a chemotactic neurotransmitter for microglia. Microglia stimulated by kainate, via either AMPA or kainate receptors, undergo dramatic morphological and cytoskeletal changes characterized by the condensation of cytoplasmic actin filaments, rapid depolymerization and repolymerization, and cytoplasmic redistribution of condensed actin bundles (Christensen et al., 2006). Actin filament rearrangement is thought to be involved in locomotion and phagocytosis and to indicate an increased level of activation. Microglia cells exposed to glutamate exhibit increased cell membrane ruffling and migrate to a source of glutamate in cell culture and spinal cord slices. This chemotaxis is mediated by AMPA and metabotropic glutamate receptors on the microglia, and is dependent on the redistribution of actin filaments and tubulin following receptor activation (Liu et al., 2009).

However, the role of glutamate in regulating baseline motility remains controversial. Initial studies showed that neuronal neurotransmission and activity-dependent synaptic plasticity does not affect surveying microglial motility in the hippocampus (Wu and Zhuo, 2008). However, other studies have suggested that endogenous glutamatergic neurotransmission positively regulates the dendritic morphology and process motility of surveying microglia (Fontainhas et al., 2011). The processes of surveying microglia have been demonstrated to make brief (~ 5 min) and direct contacts with neuronal synapses at a frequency of approximately once per hour. These contacts are activity-dependent and reduce in frequency with reduced neuronal activity (Wake et al., 2009; Li et al., 2012). Neuronal activity affects the direction but not the basal level of microglial process motility (Li et al., 2012), which could explain the previous discrepancy with the article by Wu and Zhuo. Thus, neuronal activity steers surveying/resting microglia and facilitates their contacts with highly active neurons. This effect is not direct, as microglia do not express glutamate

receptors in processes and lack direct responses to glutamatergic agonists *in situ*. Instead, these influences are mediated indirectly via extracellular ATP, which is released in response to glutamatergic neurotransmission through probenecid-sensitive pannexin hemichannels (Fontainhas et al., 2011; Li et al., 2012). The consequences of these microglia-synapse contacts depend probably on the nature and intensity of the stimulus. After transient cerebral ischemia, the duration of these microglia-synapse contacts is markedly prolonged (~1 h) and is frequently followed by the disappearance of the presynaptic bouton (Wake et al., 2009). However, an increase in spontaneous neuronal activity (i.e., by glutamate uncaging or kir channels overexpression in neurons) leads to the formation of microglial bulbous endings contacts with neurons that, surprisingly, reduce the activity of contacted neurons. This study suggests a role of surveying microglial in homeostatic regulation of neuronal activity (Li et al., 2012). These results suggest that microglia vigilantly monitor and respond to the functional status of synapses either to eliminate dysfunctional synapses or to silence them. Additional studies are needed to explore the mechanism and neurotransmitter involved in microglial detection of the functional status of synapses.

Glutamate is involved in the transmission of the death signal to microglia. Using the optically transparent larval zebrafish brain, rapidly propagating Ca^{2+} waves have been shown to determine the range of microglial responses to neuronal cell death. Though Ca^{2+} -mediated microglial responses require ATP, the spreading of intercellular Ca^{2+} waves is ATP independent, and glutamate has been identified as a potent inducer of Ca^{2+} -transmitted microglial attraction. Thus, the real-time analysis revealed the existence of a mechanism controlling microglial-targeted migration to neuronal injuries initiated by glutamate and proceeding across the brain in the form of a Ca^{2+} wave (Sieger et al., 2012).

ROLE OF MICROGLIAL NEUROTRANSMITTER RECEPTORS IN PATHOLOGY

Early during a pathological process, microglia may be stimulated by either non-self pathogens (stranger signals) or injured-self components (danger signals). Both stimuli can activate pattern-recognition receptors, such as the Toll-like receptors, scavenger receptors, and NOD system. The effector outputs of these stimuli focus on the clearance of tissue debris, generation of cues for tissue restoration, and resistance to pathogens. Together, these reactions comprise innate immune responses. Subsequent events may require the establishment of responses, including lymphocyte effector functions, antibodies, and immunological memory—collectively termed adaptive immunity. Microglial cells contribute to this process with antigen presentation, including the instruction of T cells to adopt varied effector programs (Th1, Th2, Th17) and, in some cases, directing them to the tissue from which the pathogenic material originated.

Although the innate immune response is beneficial in principle, an excessive and sustained activation of microglial cells is detrimental to neurons and oligodendrocytes (Merrill et al., 1993; Bezzi et al., 2001). Microglia activation has been described extensively in most pathological conditions in the CNS (Block and Hong, 2005), though its role is still debated (Schwartz et al.,

2006). The outcome of microglial activation is complex and likely dependent on context. Microglia and macrophages can be activated by the cytokines interferon- γ (IFN- γ), IL-17, or LPS to a pro-inflammatory phenotype (M1), whereas IL-4 or IL-13 induce a state of alternative activation (M2) that is associated with neuroprotective functions that promote repair (Butovsky et al., 2006; Ponomarev et al., 2007; Kawanokuchi et al., 2008). Understanding the different processes and regulators of microglial activation will be important to unraveling their many complex functions. The thinking behind this dichotomization is that understanding these two microglial responses may minimize the harmful effects and capitalize on the beneficial effects (Popovich et al., 2011). In addition, a given facet of microglial activation that is beneficial in principle, such as phagocytosis, could turn detrimental under other circumstances. For example, phagocytosis under inflammatory conditions actively induces neuronal death (Neher et al., 2011) because inflammation causes viable neurons to express the “eat-me” signal, phosphatidylserine, on their surface, leading to their death through phagocytosis.

ROLE OF PURINERGIC RECEPTORS IN CNS INJURY

Purinergic signaling regulates both innate and adaptive immune responses and is involved in numerous acute insults and chronic neurodegenerative diseases of the CNS (Burnstock, 2008) because purine homeostasis is compromised in most diseases. However, relatively few studies have described the specific contribution of purinoceptor signaling pathways in microglia to neuropathology. In this section we summarize the data demonstrating the beneficial role of blocking P2 and P1 receptors, particularly P2X4R and P2X7R, in different CNS pathologies.

P2 RECEPTORS IN NEUROPATHIC PAIN: P2X4

Spinal microglia react and undergo a series of changes that directly influence the establishment of neuropathic pain states. Purinergic signaling via P2X4R is at the center of this reactivity (Beggs et al., 2012). Microglial P2X4 upregulation determines the behavioral manifestations of neuropathic pain arising from peripheral nerve injury and is sufficient to convert the response to normal non-painful peripheral inputs, from innocuous to nociceptive (review in Beggs et al., 2012). These findings are the basis for the concept that the microglial phenotype characterized by dramatic upregulation of P2X4Rs. The P2X4R⁺ state, is critical in the etiology of neuropathic pain (Beggs et al., 2012). However, other microglial purinergic receptors, such as P2X7R, P2Y12R, and P1 receptors, are also involved in neuropathic pain. The blockage of P2X7Rs has been shown to alleviate chronic pain in three different models of neuropathic pain (Honore et al., 2009). Microglial P2X7Rs might participate in the neuronal hyperexcitability of dorsal horn neurons and development of neuropathic pain through the production of pro-inflammatory cytokines and chemokines (Tsuda et al., 2013). P2X7R also participates in microglial P2X4R trafficking and assembly (Boumechache et al., 2009), which could indirectly modulate P2X4R-regulated neuropathic pain. In macrophages and microglia, P2X4 and P2X7 form homotrimers that interact. P2X7Rs are found predominantly at the cell surface, whereas P2X4Rs are primarily intracellular. However, microglial activation induces a rapid translocation of

P2X4R to the surface, which is an efficient means of enhancing the function of P2X4R (Boumechache et al., 2009).

P2Y12R expression levels are also dramatically increased in microglial cells in the spinal cord after peripheral nerve injury, and the blockage of P2Y12Rs alleviates neuropathic pain (Kobayashi et al., 2008; Tozaki-Saitoh et al., 2008). After nerve injury, microglia are more abundant in layers II-III of the dorsal horn than in other areas, and some of them adhere to and engulf both injured and uninjured myelinated axons. This microglial engulfment is controlled by P2Y12R activation and directly involved in tactile allodynia (Maeda et al., 2010). The upregulation of other metabotropic P2 receptors, such as P2Y6R, P2Y13R, and P2Y14R, after nerve injury was reported recently (Kobayashi et al., 2012), and the concomitant block of the three receptors results in a longer suppressive effect on pain behavior (Kobayashi et al., 2012). In view of the crucial role played by different purinergic receptors in neuropathic pain, investigating the mechanisms of ATP release and how to modulate it as a means of attenuating neuropathic pain will be important.

Interestingly, a recent paper describes the involvement of microglial P2X4Rs in hyperalgesia produced by the gold-standard opiate analgesics. Morphine and other opiates are indispensable in the treatment of moderate-to-severe postoperative and chronic pain, but the use of these drugs is plagued by the development of two major problems: hyperalgesia and tolerance. Hyperalgesia is a sensitization process in which opioids, paradoxically, cause pain hypersensitivity. The spinal dorsal horn lamina I neurons are central targets for the analgesic effects of morphine and other opiates, and mediate morphine-induced hyperalgesia and tolerance. In particular, morphine induces analgesia via inhibition in lamina I neurons. In contrast, morphine induces hyperalgesia via the P2X4R-BDNF-KCC2 disinhibition cascade between microglia and lamina I neurons. Thus, BDNF release by activation of P2X4Rs in microglia impairs Cl^- homeostasis by downregulating $\text{K}^+ - \text{Cl}^-$ co-transporter KCC2 in those neurons (Ferrini et al., 2013).

P2X4Rs are also involved in other acute insults. Thus, the activation of microglia after hypoxia in the neonatal rat brain, a model of periventricular white matter damage, is mediated by P2X4R signaling (Li et al., 2011). P2X4 is also upregulated in microglial cells in the CA1 and transition zone to CA2 regions of the hippocampus after ischemia (Cavaliere et al., 2003), and its blockade confers neuronal protection (Cavaliere et al., 2005). Conversely, activation of P2X4 purinergic signaling in glia after traumatic injury stimulates the synthesis and release of thrombospondin-1, an extracellular matrix molecule that induces synapse formation during development, and it may play a role in CNS repair and remodeling after injury (Tran and Neary, 2006).

P2X7 RECEPTORS: A PROMISING TARGET FOR NEUROPROTECTION

In immune cells, P2X7R activation promotes assembly of the inflammasome, and caspase-1-dependent cleavage and release of biologically active IL-1 β and IL-18 *in vitro* and *in vivo*, ultimately leading to a rapid form of cell death called pyroptosis (Miao et al., 2011). P2X7R antagonists improve neuronal viability by inhibiting P2X7R-activated NLRP3 inflammasome formation and the subsequent IL-1 β release from glia (Murphy et al., 2012).

P2X7R stimulation in neurons also induces inflammasome activation in these cells (Silverman et al., 2009). In addition, P2X7R activation in neurons and oligodendrocytes leads to a massive calcium influx that induces mitochondrial damage and initiates the apoptotic cascade (Matute et al., 2007; Díaz-Hernández et al., 2009; Arbeloa et al., 2012). P2X7Rs have unique properties that could be relevant to pathological conditions. First, P2X7Rs have high Ca^{2+} permeability, similar to that of NMDA receptors. Second, in contrast to NMDA receptors, P2X7Rs can be activated at resting membrane potentials and do not require membrane depolarization. Finally, P2X7Rs do not desensitize and open a large pore that causes cytolytic cell death after prolonged activation (Surprenant et al., 1996). Prolonged activation of P2X7Rs kills all CNS cells, including neurons (Jun et al., 2007; Díaz-Hernández et al., 2009; Arbeloa et al., 2012), oligodendrocytes (Matute et al., 2007), astrocytes (Kim et al., 2011), and microglia (Harada et al., 2011).

Acute insults, such as trauma and ischemia, lead to a massive release of nucleotides from disrupted cells at a level sufficient to activate low-affinity P2X7Rs in neighboring neurons and oligodendrocytes, leading to their death by excitotoxicity (Wang et al., 2004; Domecq et al., 2010; Arbeloa et al., 2012). Both insults induced the activation of microglia and dramatic remodeling of purinoceptors in microglia, which could influence microglial functions with beneficial or detrimental consequences. P2X7R antagonists modulate microglial activation and the inflammatory response after ischemia, which could contribute to the therapeutic value of these antagonists (Melani et al., 2006; Chu et al., 2012). Cerebral microvascular occlusion elicits microvascular injury, mimicking the different degrees of stroke severity observed in patients. Recently, a role of microglial P2X7R in this type of injury has been proposed. After inducing focal microsphere embolism to microvessels, microglia are recruited to the lesion site through a P2X7R-dependent mechanism and release FasL contributing to neuroinflammation (Lu et al., 2012). The microglial response to P2X7R activation appears to be region specific. Thus, in the status epilepticus (SE), microglia appear amoeboid or phagocytic in the dentate gyrus (DG) and piriform cortex due to P2X7R activation, but elongated in the CA1 hippocampal regions and frontoparietal cortex (Choi et al., 2012).

P2X7R antagonists have also been protective in animal models of MS, amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, and Alzheimer's disease (Table 2). Whether protection is mediated by blocking neuronal or oligodendroglial excitotoxicity, inflammation, or both remains to be determined in most neurodegenerative diseases. In Alzheimer's disease, different mechanisms determine the beneficial effects of P2X7R antagonists. *In vivo* administration of P2X7R antagonists reduce amyloid plaque formation through a signaling cascade involving the activation of GSK-3 kinase and increased non-amyloidogenic amyloid precursor protein processing by α -secretase (Diaz-Hernandez et al., 2012). However, P2X7Rs are necessary for β -amyloid-induced microglial activation (Sanz et al., 2009). The treatment of chronic EAE, the animal model of MS, with P2X7R antagonists reduces demyelination and ameliorates associated neurological symptoms (Matute et al., 2007). Because ATP signaling can trigger oligodendrocyte excitotoxicity (Matute et al.,

2007), the beneficial effect of P2X7R antagonists in this pathology may be due its protective role in oligodendrocytes and axons, more than the possible interference of the immune system. Importantly, sustained activation of P2X7Rs *in vivo* causes lesions that are reminiscent of the major features of MS plaques, and P2X7 RNA and protein levels are elevated in normal-appearing axon tracts in MS patients, suggesting that oligodendroglial signaling through P2X7Rs is enhanced in MS, which may render this cell type more vulnerable to ATP dysregulation (Matute et al., 2007).

P2X7Rs have a low affinity for ATP (100 μ M–10 mM), and ATP levels in the extracellular space are in the low nanomolar range due to its rapid inactivation by powerful ubiquitous ecto-ATPases, whether this receptor is activated under physiological conditions is unclear. However, ATP is available at high concentrations within the cytoplasm (1–3 mM) and quickly released in sufficient quantities to activate P2X7Rs following cell damage in acute insults and chronic neurodegenerative diseases. These characteristics indicate an almost exclusive activation of P2X7Rs in pathological states and a low or negligible interference with

Table 2 | Neuroprotective properties of P2X7 receptor antagonists.

Disease	P2X7 receptor involvement	References
CHRONIC NEURODEGENERATION		
Alzheimer's disease	P2X7 mediates microglial neuroinflammatory reaction in different models of Alzheimer's disease	Parvathenani et al., 2003; Rampe et al., 2004; McLarnon et al., 2006
	P2X7 receptor blocks α -secretase activity/P2X7 triggers α -secretase activity	Delarasse et al., 2011; León-Otegui et al., 2011
	<i>In vivo</i> P2X7 inhibition reduces amyloid plaques in Alzheimer's disease	Díaz-Hernandez et al., 2012
	Upregulation of P2X7 in microglia in the cerebral cortex of the APP ^{swe} /PS1 ^{dE9} mice, a mouse model of AD	Lee et al., 2011
Amyotrophic lateral sclerosis	P2X7 receptor activation in spinal cord SOD1(G93A) astrocytes leads to motor neuron death	Gandelman et al., 2010
	P2X7 is overexpressed in activated microglial in ALS	Yiangou et al., 2006
Parkinson's disease	ATP mediates necrotic cell death in SN4741 dopaminergic neurons through P2X7 receptors	Jun et al., 2007
	P2X7 increases in astrocytes in the rotenone Parkinson's disease model	Gao et al., 2011
Huntington's disease	P2X7 antagonists prevented neuronal apoptosis in HD mice	Díaz-Hernández et al., 2009
Multiple sclerosis	P2X7 ^{-/-} mice are more susceptible to EAE, the MS model	Chen and Brosnan, 2006; Witting et al., 2006
	P2X7 mediates ATP excitotoxicity to oligodendrocytes and P2X7 blockage improves neurological damage in EAE	Matute et al., 2007
	Association of gain of function P2X7 variants with MS	Oyanguren-Desez et al., 2011
ACUTE INSULTS		
Epilepsy	Enhanced purinergic signaling in microglia in status epilepticus	Avignone et al., 2008
	P2X7 ^{-/-} mice and Panx1 gene silencing showed greater susceptibility to pilocarpine-induced seizures	Kim and Kang, 2011
	P2X7 antagonists as well Panx1 gene silencing blocked status epilepticus induced by kainic acid	Santiago et al., 2011; Engel et al., 2012
	P2X7 antagonists prevented astroglial apoptosis in status epilepticus	Kim et al., 2009
Ischemia	P2X7 receptors is overexpressed in activated microglia and in neurons in different models of <i>in vitro</i> and <i>in vivo</i> ischemia	Cavaliere et al., 2004, 2005; Franke et al., 2004
	P2X7 antagonists reduces neuronal damage and infarct size after transient focal ischemia	Le Feuvre et al., 2003; Melani et al., 2006; Arbeloa et al., 2012
	P2X7 blockage ameliorates oligodendroglial and axonal damage after white matter ischemia	Domecq et al., 2010
Trauma	P2X7 receptor inhibition improves recovery after spinal cord injury	Wang et al., 2004; Peng et al., 2009

normal brain functionality. Therefore, P2X7R could be an ideal therapeutic target for neurodegenerative diseases.

ADENOSINE RECEPTORS

Adenosine plays a relevant role as a neuromodulator and, thus, contributes to MS, a chronic disease with an autoimmune and inflammatory basis. A₁ receptor-null mice have been shown to develop more severe demyelination and motor symptoms in chronic EAE compared to their wild-type counterparts (Tsutsui et al., 2004). The aggravation of EAE is mostly mediated by cells in the microglial lineage and involves the release of toxic factors by macrophages/microglia lacking A₁ receptors (Tsutsui et al., 2004). More recently, a protective role of adenosine A_{2A} receptors was reported in this model. Genetic inactivation of the A_{2A} receptor exacerbates EAE pathology in mice. In addition, A_{2A} receptor knockout mice display increased inflammatory cell infiltration and enhanced microglial cell activation in the cortex, brainstem, and spinal cord (Mills et al., 2012; Yao et al., 2012).

Inflammation also contributes to post-ischemic delayed cerebral damage. A₃ adenosine receptor expression is modulated by the activation state of inflammatory cells and, in turn, its activation regulates the inflammatory activity of immune cells (Bar-Yehuda et al., 2007; Ochaion et al., 2009). The administration of A₃ agonists before or immediately after ischemic insults has been shown to significantly protect the brain in rodent ischemia models (Von Lubitz et al., 1994, 2001; Chen et al., 2006). Importantly, A₃ agonists protect against ischemic brain injury when applied 7 h after the ischemic insult (5.5 h after starting reperfusion) (Choi et al., 2011). The effect could be due to an inhibitory effect of the A₃ agonist on microglial/monocyte migration through the regulation of Rho GTPases (Choi et al., 2011).

ROLE OF GLUTAMATE RECEPTORS IN CNS INJURY

The possible contribution of microglia glutamate signaling to pathology has not been analyzed thoroughly. Few studies have demonstrated that the activation of ionotropic glutamate receptors in microglia has deleterious consequences to neurons and oligodendrocytes. NMDA receptor expression is upregulated in activated microglia following ischemia (Gottlieb and Matute, 1997; Kaur et al., 2006), which contributes to oligodendrocyte damage in hypoxic postnatal rats, a model of periventricular white matter damage. The activation of NMDA receptors in microglia leads to NO release in response to NF- κ B signaling, which is known to induce oligodendrocyte cell death (Li et al., 2005). Pharmacological inhibition of NMDA receptors (MK801), NF- κ B (BAY), and iNOS (1400w) prevents oligodendrocyte cell death (Murugan et al., 2011). Thus, NMDA receptor blockade protects oligodendrocytes by reducing the release of NO from microglia (Tahraoui et al., 2001; Murugan et al., 2011), in addition to the effect of direct blockage of NMDA receptors in these cells (Manning et al., 2008). In contrast, kainate-activated microglia induce IL-1 β and TNF- α release, which mediate increased excitability of hippocampal CA3 neurons (Zheng et al., 2010; Zhu et al., 2010), an effect that could be relevant in acute insults.

The activation of microglial metabotropic receptors has been reported to regulate superoxide production by modulating

NADPH oxidase (Nox) activity. Nox enzymes are major generators of ROS, which contribute to the progression of CNS disorders as diverse as amyotrophic lateral sclerosis, schizophrenia, Alzheimer's disease, Parkinson's disease, and stroke. Microglia are the predominant cells expressing Nox enzymes (Harrigan et al., 2008). Nox activation is elicited by agonists of metabotropic mGlu3 receptors, promotes neurotoxicity, and is inhibited by antagonists of mGluR5 receptors (Mead et al., 2012). For example, the regulation of Nox activity by mGluRs could contribute to limiting microglial activation after traumatic brain injury, improving motor and cognitive recovery (Byrnes et al., 2012).

ROLE OF MICROGLIA IN GLUTAMATE AND ATP HOMEOSTASIS AND ITS CONTRIBUTION TO NEURODEGENERATIVE DISEASES

ATP HOMEOSTASIS

ATP homeostasis is compromised in most CNS pathologies. Immediately after acute CNS injury, astrocytes and damaged cells release ATP, resulting in rapid activation of microglia. ATP and UTP are released by apoptotic cells as a "find-me" signal in the earliest stages of death to recruit phagocytes to the plasma membrane channel pannexin 1 (PANX1). Pharmacological inhibition and siRNA-mediated knockdown of PANX1 leads to decreased nucleotide release and monocyte recruitment by apoptotic cells (Chekeni et al., 2010). Pannexins also open following ischemic insult (Thompson et al., 2006; Domecq et al., 2010; MacVicar and Thompson, 2010), in response to high extracellular K⁺ (Silverman et al., 2009), after NMDA receptor stimulation (Thompson et al., 2008), and, surprisingly, in response to caspase cleavage (Chekeni et al., 2010), which suggests that pannexins may open in most pathological contexts. However, the expression of pannexins in microglia and their possible influence on ATP release under normal conditions and after microglial activation has not been characterized. Finally, microglia are able to release ATP after activation with LPS, leading to an increase in excitatory neurotransmission (Pascual et al., 2012). Different mechanisms have been proposed, including microglial release of ATP via zinc uptake by zinc transporters (Higashi et al., 2011). In addition, lysosomes in microglia contain abundant ATP and exhibit Ca²⁺-dependent exocytosis in response to various stimuli (Dou et al., 2012).

GLUTAMATE HOMEOSTASIS

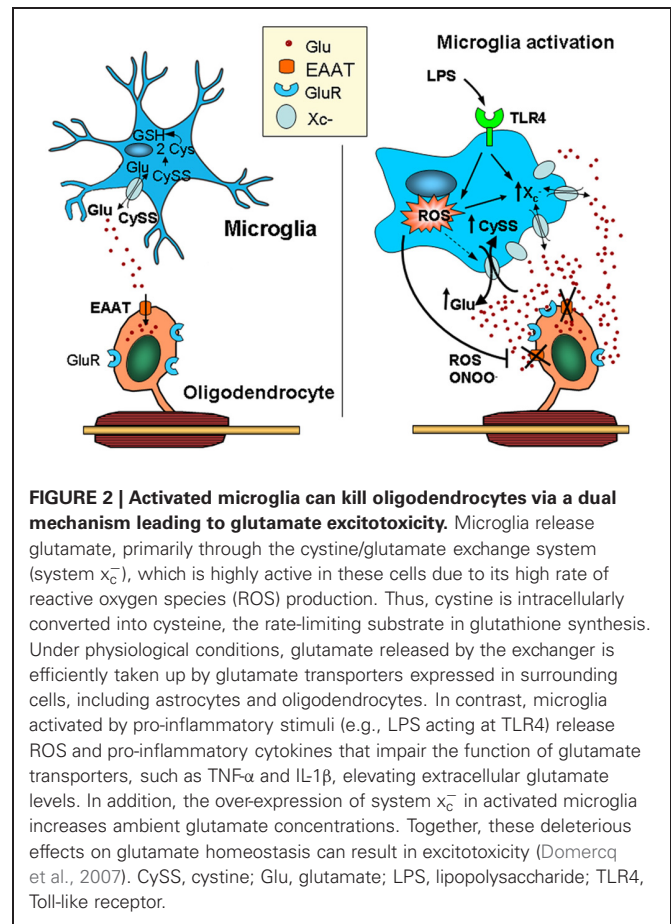
Since the discovery of excitotoxicity and its contribution to neuronal cell death in neuropathology, numerous studies have aimed to understand the origin of alterations in glutamate homeostasis, which determines lethal overactivation of glutamate receptors. *In vitro* studies have demonstrated that activated microglia play a deleterious role by releasing glutamate or altering its homeostasis. Surprisingly, a recent study demonstrated a neuroprotective role of surveying microglia in excitotoxicity-induced neurodegeneration in the hippocampus (Vinet et al., 2012). This region exhibits differential sensitivity to excitotoxicity, with the CA1 region more vulnerable than CA3 or DG neurons. However, ablation of ramified microglia in the latter two areas exacerbates neuronal cell death, suggesting a protective role of surveying microglia in these

areas (Vinet et al., 2012). The mechanism by which surveying microglia confer protection remains to be determined.

Microglia, mainly in the activated state, contribute to alterations in neurotransmitter homeostasis via three main mechanisms: (1) release of excitotoxins, including glutamate (Piani et al., 1992), quinolinate (Heyes et al., 1996), D-serine (Wu et al., 2004), and ATP; (2) interfering with glutamate uptake, which is mainly carried out by astrocytes, leading to extracellular glutamate accumulation; or (3) altering astrocyte gliotransmitter release (including glutamate) or synaptic transmission.

A key determinant of microglial neurotoxicity is the release of excitotoxins, such as glutamate. The vast majority of glutamate exported from activated microglia can be attributed to the x_c^- exchange mechanism (a.k.a., SLC7A11 or CCBRI). This antiporter is a membrane-bound, Cl^- -dependent, Na^+ -independent antiporter that mediates the cellular uptake of cystine in exchange for glutamate at a 1:1 ratio, primarily following the relative concentration gradients of each of these amino acids (Bridges et al., 2012). This mechanism becomes extremely active in microglia because it is the primary route for internalizing cystine, which is converted to cysteine intracellularly, the rate-limiting substrate in glutathione synthesis (Bridges et al., 2012). As activated microglia produce ROS, they place themselves under severe oxidative stress. Although the bulk of superoxide produced by Nox is released from microglia, some remains intracellular. Thus, the microglial oxidative burst creates a GSH shortage that is alleviated by cystine influx through the x_c^- antiporter, extruding glutamate in the balance (Barger et al., 2007). The obligate exchange of glutamate could be deleterious to neuronal cells and other tissues that are susceptible to excitotoxic damage. Accordingly, the cystine/glutamate exchanger has been implicated in glutamate-associated disorders, such as glioma-derived epileptic seizures (Buckingham et al., 2011), oxidative glutamate toxicity (Oka et al., 1993; Albrecht et al., 2010), Alzheimer's disease (Barger and Basile, 2001; Qin et al., 2006), bacterial infection/LPS (Taguchi et al., 2007), MS (Domerqcq et al., 2007; Pampliega et al., 2011), Parkinson's disease, AIDS (Zeng et al., 2010), virally-induced encephalopathy (Espey et al., 1998; Qin et al., 2010), tumor proliferation (Ogunrinu and Sontheimer, 2010), antigen presentation (D'Angelo et al., 2010), and hypoxia (Fogal et al., 2007; Jackman et al., 2010).

Both surveying and activated microglia release glutamate through the cystine/glutamate antiporter (Domerqcq et al., 2007) (Figure 2). However, glutamate released through surveying microglia is rapidly and efficiently removed by glutamate transporters in other glial cells, mainly astrocytes, though also oligodendrocytes (Domerqcq et al., 1999). In contrast, the activation of microglia induces the release of factors, such as ROS, $TNF-\alpha$, and $IL-1\beta$, that impair the function of EAATs, resulting in an increase in the extracellular levels of glutamate (Domerqcq et al., 2007). In addition, autoantigen-activated myelin basic protein-specific T cells also inhibit EAATs (Korn et al., 2005), suggesting that these mechanisms could contribute to alterations in glutamate homeostasis in the plasma and cerebrospinal fluid of MS patients. Gliomas also achieve excitotoxic levels of glutamate through high levels of system x_c^- activity coupled with a relative absence of sodium-dependent transport (Ye and Sontheimer,



1999; Ye et al., 1999; Kim et al., 2001; Takano et al., 2001; Rothstein, 2002; Chung et al., 2005). Glutamate released by system x_c^- in gliomas triggers excitotoxic cell death in the regions surrounding the tumor, allowing the tumor cells to migrate and invade (Lyons et al., 2007). Interestingly, glutamate export by the microglial cystine/glutamate antiporter is inhibited by mGluRII and III metabotropic glutamate receptor activation (McMullan et al., 2012).

Finally, microglia may regulate astrocyte-dependent synaptic modulation, called gliotransmission (Perea et al., 2009; Pascual et al., 2012). The first evidence of gliotransmission came from the seminal observation that glutamate release from cultured astrocytes (in this case stimulated via CXCR4 chemokine receptors) is dramatically amplified by the presence of activated microglia in the astrocytic microenvironment (Bezzi et al., 2001). Constitutive $TNF-\alpha$ was reported to control astrocyte gliotransmission under physiological conditions (Stellwagen and Malenka, 2006; Santello et al., 2011). However, microglia activated by LPS release massive concentrations of $TNF-\alpha$ (approximately 10-fold); at these high concentrations, $TNF-\alpha$ changes its mode of action, not only gating, but also directly causing glutamate release from astrocytes. This alternative $TNF-\alpha$ action is mediated by prostaglandin E_2 (PGE_2) and was found to induce slow excitotoxic neuronal damage, both in cell culture and *in vivo* (Bezzi et al., 2001).

CONCLUSIONS

Neurotransmitter signaling is relevant to microglial functions. Microglia express receptors for neurotransmitters and contribute to neurotransmitter homeostasis. In particular, microglia are endowed with virtually all types of purinergic receptors that are differentially expressed at different stages and mediate process extension and retraction, cytokine release, migration, proliferation, and phagocytosis. In turn, activated microglia can release ATP and modulate synaptic activity. In addition, microglia also have an ample variety of glutamate receptors that mediate chemotaxis, ATP release, and surveillance of the functional status of synapses. Notably, microglia are critical

controllers of glutamate homeostasis via the cystine/glutamate Xc⁻ exchanger. Both ATP and glutamate receptors in microglia are relevant to neuroinflammation in various pathological conditions, including neurodegenerative diseases, and can be valuable targets for drug development in neuropathic pain and neuroprotection.

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