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Keywords: Astrocyte; microglia, oligodendrocyte; cognition; dementia; neurodegeneration; neuropsychiatric diseases; purinoceptors; purinergic signalling

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Abstract: Neuroglia, represented by astrocytes, oligodendrocytes, NG glia and microglia are homeostatic, myelinating and defensive cells of the brain. neuroglial cells express various combinations of purinoceptors, which contribute to multiple intercellular signalling pathways in the healthy and diseased nervous system. Neurological diseases are invariably associated with profound neuroglial remodelling, which is manifest by reactive gliosis, pathological remodelling and functional atrophy of various types of glial cells. Gliopathology is disease and region specific and produces multiple glial phenotypes that may be neuroprotective or neurotoxic. In this review we summarise recent knowledge on the role of glial purinergic signalling in cognitive related neurological diseases.

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Professor Bruno Frenguelli
Editor in Chief
Neuropharmacology - Special issue

19 July 2015

Re: Revision of NEUROPHARM-D-15-00501 "Purinergic neurone-glia signalling in cognitive-related pathologies"

Dear Bruno,

Thank you very much for your kind letter and referees comments. We have revised the paper according to their suggestions (see point by point reply below) and hope that the revised manuscript meets acceptance criteria

We are very much looking forward to hearing from you soon.

On behalf of the authors,
With best regards, I shall remain,

Yours sincerely

Replies to referees comments:

Reviewer #2:

This is an interesting and comprehensive review that brings together the well recognised aspect of ATP in glial pathophysiology and the less well recognised potential importance of glia in cognitive-related pathologies.

The review is logically laid out and is easy to follow for the non-expert. However, although the reason for focusing on purinergic signalling may be clear to the expert, it may be less clear to the non-expert. A primary function of astrocytes is glutamate clearance and dysregulation of glutamate signalling is a unifying aspect of most neuropathologies. Hence, the review may benefit from a paragraph explaining that multiple neurotransmitter signalling mechanisms are involved in cognitive-related pathologies and these are covered elsewhere (cite some key reviews) and explain why this review focuses on the purinergic system.

Our reply: We are very much grateful for these positive comment and we had modified teh text accordingly.

In addition, there are typographical and grammatical errors and the paper requires careful proof-reading. For example, oligodendrocyte is misspelt in the highlight figure, and on page 3, the sentence at the end of the second paragraph is a little clumsy: "NG2 cells are related; the former are mainly responsible for myelination and axonal support, whereas the latter have multiple function; and are probably associated with adult myelination".

Our reply: All corrected and proof-reading performed.

Reviewer #3: The authors of the present review are recognized expert in the fields of purinergic signaling and glial cell biology. They have made important contributions to the progress of the topics covered in the review.

Present manuscript represents an interesting review regarding our current understanding of purinergic signalling in glial cells and the potential implications in several CNS diseases.

It is a fair and well balanced review, well written, clear and very informative. It will certainly be helpful for general readers as well as experts in the field to appreciate the current knowledge in the topics covered. I really enjoyed very much reading the manuscript.

I do not have comments for the authors to improve such very informative review. I have only the suggestion of including a few recent references that the author may want to consider to help the reader and to improve this highly interesting review:

Our reply: Thank you for teh most flattering and kind words.

1. When discussing the concept of fast gliotransmission, besides the cited references, authors may want to include a reference to the recent review in Neuron 2014 81:728-739 that thoroughly discuss the issue.

2. In the paragraph ATP release from glial cells, authors might want to include a sentence stating that glial ATP has been shown to regulate synaptic transmission, and include some related references, e.g., Serrano et al, J Neurosci 2006; Pascual et al., Science 2005; Zhang et al., Neuron 2003; Newman, J Neurosci 2005.

Our reply: We added all suggested references

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Our reply: We added all suggested references

Highlights

- Neuroglia is fundamental for evolution of all neurological disorders.
- Neurons and glial cells interact with each other using various signalling molecules
- Purinergic signalling system contributes to pathogenesis of multiple cognitive-related neuropathologies.

Purinergic neurone-glia signalling in cognitive-related pathologies

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Running title: Glia and ATP in cognitive pathologies

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Abstract

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1. Introduction: Neuronal-glia unity provides the substrate for cognisant/intelligent brain

The brain is composed of many types of cells that are integrated into interconnected networks with distinct functions. Evolution of the nervous system resulted in remarkable redistribution of cellular specialisations with electrically excitable neurones responsible for information processing and electrically non-excitable glia providing for homeostatic support. These two cell types are the main components of the nervous tissue; their activities are precisely coordinated through elaborated signalling pathways. Impairment of these cellular communications constitutes the substrate for neuropathology, which often result in deficits in cognitive function. The focus of this review lies in the neuroglia; by doing so we shall try to mend the prevalent view that regards neurones the main and often the sole site for morbid changes in the brain. The neuron-centric view on neuropathology has been challenged rather recently, despite prophetic ideas of Rudolf Virchow who regarded neuroglia as the most frequent foci of the disease (Virchow, 1858; Kettenmann and Verkhratsky, 2008).

We shall start this narration from brief overview of the functions of glial cells thus helping the clinically oriented reader to appreciate the fundamental role glial cells assume in the brain function. Neuroglial cells are represented by the macroglia which are cells of ectodermal or neural origin (i.e. like neurones are the direct descendants of neuroepithelial cells) and microglial cells which derive from mesoderm, from foetal macrophages (see (Kettenmann and Ransom, 2013; Verkhratsky and Butt, 2013) for comprehensive referencing). Macroglial cells of the CNS are further divided into astroglia, oligodendroglia and NG2 glia, all these types having distinct structure, physiology and function. The oligodendrocytes and NG2 cells are lineage-related. Oligodendrocytes are mainly responsible for myelination and axonal support, whereas the functions of NG2 cells remain to be elucidated.

The term of astroglia covers many types of cells with highly heterogeneous morphology and functional properties, which nonetheless serve the same basic function of maintaining the homeostasis of the central nervous system (Oberheim et al., 2006; Parpura et al., 2012; Verkhratsky, 2010; Verkhratsky et al., 2015). Astrocytes are responsible for virtually every conceivable homeostatic task that occurs in developing and mature CNS. In particular astroglia provides for adult neurogenesis and structural organization of the nervous tissue (astrocytes for example divide grey matter into neuro-astroglia-vascular units). Astroglia

regulate the formation and regulation of brain-blood and brain-cerebrospinal fluid barriers. Astrocytes are critical elements of synaptic connections, supporting synaptogenesis, maturation, maintenance and extinction of synapses (Verkhratsky and Nedergaard, 2014). Astrocytes are fundamental for regulation and maintenance of synaptic transmission through specific uptake of neurotransmitters such as glutamate, γ -aminobutyric acid (GABA), glycine and adenosine by specific transporters, through catabolising glutamate and adenosine by glutamine synthetase and adenosine kinase expressed almost solely in astroglia. Through glutamine-glutamate and glutamine-GABA shuttles astrocytes supply neurones with glutamine which is a compulsory precursor for glutamate and GABA (Hertz and Zielke, 2004; Rose et al., 2013). In addition astroglial cells express a wide array of membrane transporters that control homeostasis of ions (K^+ , Na^+ , Ca^{2+} , H^+ , Zn^{2+} and Cl^-), and water, contribute to reactive oxygen species removal (by supplying glutathione and ascorbic acid) and transport lactate that may act as an important energy substrate for neurones. There are many more homeostatic functions of astroglia that has been extensively reviewed in recent years (Clarke and Barres, 2013; Iadecola and Nedergaard, 2007; Kettenmann and Ransom, 2013; Kimelberg and Nedergaard, 2010; Kirischuk et al., 2012; Nedergaard and Verkhratsky, 2012; Oberheim et al., 2012; Parpura et al., 2012; Parpura and Verkhratsky, 2012; Verkhratsky et al., 2015; Zhang and Barres, 2010).

The oligodendrocytes are the myelinating cells of the CNS, and localise in the grey and the white matter. The main role of oligodendroglia is myelinating the axons in the brain and in the spinal cord, which allows fast action potential propagation in thin axons (0.1 to 3 μm) that form CNS connectome. This latter represents a highly developed system of axons formed by ~ 10 billions of cortical projections neurones. The complexity of the connectome and its dimensions increased remarkably in evolution so that the white matter comprises more than a half of human brain. Oligodendrocytes not only form myelin but also provide many lines of axonal support, including directed transport of lactate utilised by axons for energy production. Oligodendrocytes express multiple receptors and are capable of secreting signalling molecules that allow bi-directional axonal-oligodendroglial communications (Bradl and Lassmann, 2010; Emery, 2010; Miyata et al., 2015; Nave and Ehrenreich, 2014; Tomassy and Fossati, 2014). Oligodendroglial precursor and oligodendroglia-related NG2 glial cells (Stallcup, 1981) maintain oligodendroglial diversity and provide for adult oligodendrogenesis that is linked to brain plasticity, learning and regeneration; incidentally myelination in human brain lasts for 3 or 4 decades after birth. These oligodendroglial precursors are similarly critical for

remyelination and connectome repair in pathology (Crawford et al., 2014; Ruckh et al., 2012; Walhovd et al., 2014).

The last class of neuroglia is represented by microglial cells that are of mesodermal (i.e. non-neuronal) origin. The microglial precursors (which are primitive c-kit⁺ erythromyeloid precursors (Kierdorf et al., 2013)) derive from the yolk sac and enter the neural tube very early in the development, at about embryonic day 7 in mice, and about embryonic day 19 in humans (Ginhoux et al., 2013). After entering the neural tissue these precursors transform into foetal macrophage, which constitute the pool of true microglial progenitors. Microglial cells disseminate throughout the CNS and acquire idiosyncratic "ramified" morphology and begin to express multiple receptors to neurotransmitters and neurohormones. The processes of ramified microglial cells are constantly moving through their respective territories with a speed of about 1.2–1.5 $\mu\text{m}/\text{min}$. In this way microglial cell can scan its territorial domain every several hours. Lesions to the nervous tissue trigger activation of microglia that represents an important part of brain defence (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011).

2. Neuroglia in higher brain functions

The neuronal doctrine dominates the neuroscientific thoughts; the plastic remodelling of synaptic contacts is generally considered the sole mechanisms for learning, memory and cognition (Markram, 2006). Neuronal networks however are embedded into and hence exist and perform surrounded with neuroglia, the role of which in cognition is a matter of intense debate (Blank and Prinz, 2013; Fields, 2008; Krugel et al., 2013; Pannasch and Rouach, 2013; Pereira and Furlan, 2010; Robertson, 2013; Verkhratsky et al., 2011). The concept of tripartite (and later multipartite) synapse, in which neuronal pre- and postsynaptic compartments are closely interacting and can be modulated by astroglia (and possibly by microglia) proposed direct contribution of neuroglia in synaptic integration and therefore in information processing and cognition (Araque et al., 1999; Araque et al., 2014; De Pitta et al., 2015; Halassa et al., 2009; Perea et al., 2009; Wang and Bordey, 2008; Zorec et al., 2012). Participation of astrocytes in fast synaptic transmission in its characteristic microsecond temporal domain remains, however, dubious (Hamilton and Attwell, 2010; Nedergaard and Verkhratsky, 2012). Astroglia are fully capable of releasing numerous neuromodulators, neurotransmitters and neurohormones and yet this secretion generally is rather slow (due to

distinct mechanisms of exocytosis and Ca^{2+} signalling - see e.g. (Zorec et al., 2015) and is not confined to specified sites; and hence the role of astrocytes in modulating neuronal networks seems to embrace multiple synapses or synaptic fields. At the same time astrocytes are fundamental to brain plasticity through employing multiple mechanisms distinct from fast synaptic modulation; these mechanisms include for example regulation of synaptogenesis and homeostatic support (Verkhratsky and Nedergaard, 2014). Astroglial cells are functionally coupled into anatomically segregated syncytia (Giaume and Liu, 2012); this "glial reticulum" is formed by gap junction contacts that are composed out of many hundreds of intercellular connexon-based channels. These channels in turn are permeable for relatively large signalling molecules such as second messengers or metabolites that may represent a distinct information processing mechanism acting in parallel to synaptic integration in neuronal networks.

The role of oligodendrocytes in cognition is similarly fundamental because of their critical role in maintaining CNS connectome; incidentally these are oligodendrocytes that suffer significantly in neuropsychiatric diseases, in ageing and in neurodegenerative pathology (Fields, 2008; Nave and Ehrenreich, 2014). Likewise microglia is critical for developing and shaping neuronal ensembles through, for example, pruning synaptic contacts in healthy brain (Kettenmann et al., 2013). All in all, neuroglia is a fundamental player in cognitive functions, and pathological impairment of glial cells is an important part of neurological disorders.

All in all, chemical signalling between neuroglia and neurones is widespread and involve multiple neurotransmitters, neuromodulators, neurohormones and other signalling molecules we shall focus this review on purinergic signalling system that utilised ATP and its derivatives as extracellular signalling messengers. Receptors to purines (ATP, ADP, AMP and adenosine) are almost universal for all types of neuroglia and hence purinergic signalling plays multiple roles in glial physiology and pathophysiology.

3. Purinergic signalling in neuronal glial-interactions

The purinergic signalling system, which utilises purines and pyrimidines as extracellular messengers is the most ubiquitous one, being operational in animals and plants and acting in virtually all types of living tissues. The omnipresence of purinergic signalling reflects its ancient and broad evolutionary roots (Burnstock and Verkhratsky, 2009; Verkhratsky and

Burnstock, 2014). Adenosine triphosphate, the ATP, is a principal transmitter of the purinergic signalling; ATP is released from many types of cells by several distinct pathways, which include exocytosis of secretory vesicles or lysosomes or diffusion through plasmalemmal channels (Abbracchio et al., 2009; Burnstock and Verkhratsky, 2012; Pankratov et al., 2006). After being released, ATP is rapidly degraded by numerous ectonucleotidases (Zimmermann et al., 2012) thus generating a trail of ADP, AMP and adenosine, which also act as signalling molecules. At the cellular level effects of ATP are mediated through families of purinoceptors broadly classified as metabotropic adenosine P0 receptors, metabotropic P1 adenosine receptors, metabotropic P2Y purinoceptors and ionotropic P2X purinoceptors (Burnstock and Verkhratsky, 2012; Thimm et al., 2013; Verkhratsky and Burnstock, 2014). The latter 3 types of the receptors are widely expressed in all types of glial cells (Verkhratsky et al., 2009); glial expression of P0 receptors has not yet been studied.

3.1. ATP release from glial cells

As mentioned before, astrocytes may release a wide variety of neurotransmitters including ATP by exocytotic mechanisms involving $[Ca^{2+}]_i$ and SNARE-proteins (Halassa and Haydon, 2010; Lalo et al., 2011c; Perea and Araque, 2010). Furthermore, ATP released from astroglia has been demonstrated to affect synaptic transmission (Newman et al., 2005; Pascual et al., 2005; Serrano et al., 2006; Zhang et al., 2003). It has been postulated, that synapses consist of presynaptic and postsynaptic neuronal specializations as well as astrocytic perisynaptic process thus constituting a “tripartite synapse” (Araque et al., 1999). This hypothesis presumes that transmitters released both from presynaptic terminals and astrocytic process may cause neuronal stimulation. Originally, a convincing argument for gliotransmission was that in transgenic mouse models harbouring supposedly astrocyte-specific dnSNARE the glial release of transmitters was prevented (Lalo et al., 2014), thus affecting LTP in hippocampal slices (Hines et al., 2013), CNS damage after stroke (Hines and Haydon, 2013), and progressive development of temporal lobe epilepsy (Clasadonte et al., 2013). The widespread expression of the dnSNARE transgene, however, was demonstrated in cortical neurones of such mice, without a major preference for astrocytes (Fujita et al., 2014). The concept of fast “gliotransmission” remains debatable, due to the lack of selective experimental tools in glia research (Hamilton and Attwell, 2010; Nedergaard and Verkhratsky, 2012).

An alternative, non-vesicular pathway for ATP release from astrocytes appears to utilize hemichannels formed by the mammalian gap junction proteins connexin-43 or pannexin-1 (Suadicani et al., 2012). These hemichannels have been implicated in ATP release as inferred from the inhibition by certain gap junction channel blockers and connexin-mimetic peptides as well as in astrocytic cultures prepared from pannexin-1 deficient mice. There is evidence that pannexin-1 channels are opened by activation of the P2X₇-type ATP receptor (Bennett et al., 2012); although these data have not been universally confirmed (Browne et al., 2013; Ma et al., 2012). Release of ATP from astrocytes participates in the regulation of respiratory activity. It has been reported that astrocytes of the brainstem chemoreceptor areas (but not of the cerebral cortex) responded to physiological decreases in pH with vigorous elevations in $[Ca^{2+}]_i$ and the subsequent secretion of ATP (Gourine et al., 2010; Kasymov et al., 2013). ATP propagated astrocytic $[Ca^{2+}]_i$ waves, activated chemoreceptor neurones and triggered robust respiratory responses, probably via P2Y₁ receptor activation.

ATP was shown to be released from microglia by vesicular or lysosomal exocytosis (Dou et al., 2012; Imura et al., 2013). Unfortunately, evidence for the exocytotic release mechanism might again be handicapped by the failure of the pharmacological inhibitors used to selectively act at their microglial targets. Nonetheless, these and similar results allowed adding microglia as an active source for modulating neuronal responses (Kettenmann et al., 2013). It was reported that activation of microglia by lipopolysaccharide induced a rapid increase of spontaneous excitatory currents in CA1 hippocampal neurones (Pascual et al., 2012). Apparently, activated microglia secreted small amounts of ATP which stimulated in turn P2Y₁ receptors on astrocytes, leading to the release of glutamate and the activation of metabotropic glutamate receptors on neurones. Similarly, mild neurotoxins released ATP from microglia, which again utilized astrocytic P2Y₁ receptors, releasing the neuroprotective interleukin-6 onto neurones in organotypic hippocampal slices (Shinozaki et al., 2014).

3.2. Purinoceptors at glial cells and their physiological functions

In the following section we shall discuss the presence and functional significance of purinergic receptors at astrocytes, oligodendrocytes and microglia. Although adult neural stem cells giving rise to the production of neurones, astrocytes and oligodendrocytes originate from embryonic radial glia, and are influenced in many ways by purinergic signalling (Ulrich

and Illes, 2014; Zimmermann, 2011), they are not the subject of this review (consult Ulrich et al., this issue).

3.2.1. P2X receptors

Although both mRNA and protein for P2X receptors were found in astrocytes isolated from different areas of the brain and kept in culture (Illes et al., 2012; Koles et al., 2011; Verkhratsky et al., 2009), the informative value of these results is limited because of a number of reasons: (i) RT-PCR or Western blotting does not supply any information about the source of the receptor message, which represents a serious problem, because all glial cell cultures are contaminated with neurones, endothelial cells, and microglia. (ii) Astrocytes are exceptionally plastic and are known to alter their receptor complement when surviving *in vitro*. (3) In immunocytochemical experiments, the presumed astrocytes are not always double-stained for glial markers such, for example, glial fibrillary acidic protein (GFAP) to unequivocally confirm the cellular identity.

Activation of P2X receptors by definition (ligand-gated cation channel) mediate rapid responses to ATP. Electrical stimulation of neuronal afferents in brain slices prepared from the somatosensory cortex of mice evoked in layer II astrocytes synaptic currents (Lalo et al., 2011a). After sequential blockade of excitatory amino acid transporters and ionotropic glutamate receptors, a small P2X receptor-mediated component sensitive to the non-selective P2X/Y receptor antagonist PPADS was dissected (Lalo et al., 2011b). It was assumed that the leading mechanism for neurotransmitter delivery to astroglial membranes was associated with spillover (Lalo et al., 2011b; Lalo et al., 2011c). In conclusion, activation of P2X receptors is responsible for a minor fraction of synaptic currents at astrocytes; however, the receptor-type involved was not characterized with selective pharmacological tools or by means of knockout animals.

In astrocytes, mechanically isolated from the cerebral cortex of mice, ATP induced current responses with complex kinetics (rapid peak followed by a steady-state plateau) (Lalo et al., 2008; Palygin et al., 2010). These responses were similar to those mediated by heteromeric P2X_{1/5} receptors in artificial expression systems, reacting to very low ATP concentrations, and exhibiting pharmacological and biophysical properties distinct from other homomeric or heteromeric P2X receptors. The P2X_{1/5} receptor requires the binding of intracellular

phosphatidylinositol 4,5-bisphosphate to its P2X₁ subunit in order to properly function (Ase et al., 2010).

3.2.2. P2Y receptors

P2Y receptor mRNA and protein has been repeatedly identified in astrocytes cultured from the CNS of different species, including humans (Franke and Illes, 2014b; Hashioka et al., 2014; Koles et al., 2011). P2Y₁ and P2Y₂ receptors have been shown to initiate propagating Ca²⁺ waves following the release of ATP/ADP from neighbouring astrocytes; the intercellular diffusion of inositol 1,3,5-trisphosphate (InsP₃) via connexin-based channels also supported this process (Verkhratsky et al., 2009). Astroglial release of neurotransmitters and neuromodulators, such as glutamate and the N-methyl-D-aspartate (NMDA) co-agonist D-serine was triggered by ATP-induced increase in [Ca²⁺]_i. Thereby, the major physiological function of P2Y receptors was to support the astrocyte-astrocyte and astrocyte-neurone communication in glial and glial-neuronal networks.

Metabotropic P2Y₁ receptors play important roles in purinergic neurone-glia communication in two sensory systems, in the visual and in the olfactory system (Grosche et al., 2013; Lohr et al., 2014; Thyssen et al., 2013). In both retina and olfactory bulb, ATP is released from neurones and evokes [Ca²⁺]_i transients in astrocytes or Müller glial cells of the retina, or in astrocyte-like ensheathing cells of the olfactory bulb. Glial Ca²⁺ signalling in turn affects homeostasis of the nervous tissue such as volume regulation and control of blood flow. In addition, gliotransmitter release upon Ca²⁺ signalling – evoked by purinoceptor stimulation – modulates neuronal activity, thus contributing to the processing of sensory information. In contrast to these short-term effects mediated by P2Y receptors, long-term effects on neuronal development and axonal growth have also been described (Del Puerto et al., 2013; Zimmermann, 2011). Hippocampal neurones express in the distal domains of their axons P2Y₁ and P2Y₁₃ receptors, the former being a positive, while the latter the negative regulators of axonal growth (Del Puerto et al., 2013).

4. Neuroglia in neuropathology: General principles

Neuroglia represents the main intrinsic defensive system of the CNS and hence is fundamentally important for evolution of neuropathology; neuroglial component is present in

all neurological disorders (Fig. 1). This understanding of pathological potential of glia gained popularity in the recent decade, when neuron-centric philosophy of neurology was challenged (see (Burda and Sofroniew, 2014; Molofsky et al., 2012; Pekna and Pekny, 2012; Sofroniew, 2015; Verkhratsky et al., 2014b; Verkhratsky et al., 2013, 2014c; Verkhratsky et al., 2012; Zeidan-Chulia et al., 2014) for detailed overview and relevant references). Neuroglial contribution to pathology are multifaceted and include homeostatic responses. Gliodegeneration resulting in atrophy are asthenia, pathological remodelling and reactivity. Importantly, the gliopathology seems to be disease-specific and different disorders witness distinct changes in neuroglia. Gliodegeneration and glial asthenia are documented in many diseases from neurodegenerative pathologies to neuropsychiatric disorders (Rajkowska and Stockmeier, 2013; Verkhratsky et al., 2014a; Verkhratsky et al., 2014d; Williams et al., 2013); the degenerative changes contribute to overall diminution of homeostatic glial capabilities and hence facilitate neurotoxicity. Neuroglial reactivity represents another, active face of glial contribution to brain defence and pathology. Lesions to the CNS, regardless their aetiology, instigate evolutionary conserved glial defensive response, which, through complex and context-specific biochemical, structural and physiological remodelling produces "reactive" glial phenotypes. Conceptually, gliotic response is classified into reactive astrogliosis, reactive activation of NG2 glia, reactive remodelling of oligodendroglia (which is partly manifested as Wallerian degeneration) and the activation of microglia.

Reactive astrogliosis is often considered as purely pathological and destructive process that facilitates and multiplies pathology; this set of beliefs gradually surrenders to a modern concept that defines astroglial response as an intrinsically defensive and protective programme aimed at the preservation and regeneration of the neural tissue (Burda and Sofroniew, 2014; Pekny et al., 2014; Sofroniew, 2015; Verkhratsky et al., 2014b). Inhibition of astroglial reactivity invariably exacerbates pathological progression and impairs post-pathological regeneration (Kraft et al., 2013; Robel et al., 2011; Sofroniew, 2015). Similarly, activation of microglia that emerges in almost all forms in neuropathology, is a complex process that produces numerous activated phenotypes, many of which are neuroprotective; again depression of microglia often reduces defensive capabilities of the CNS (Heneka et al., 2015; Kettenmann et al., 2011).

5. Neuroglia in cognitive-related pathologies

Evidence accumulated in recent years in clinical and experimental studies implicate widespread neuroglial abnormalities in all disorders associated with cognitive impairments. The neurogliopathology includes atrophy and reactivity of astroglia, activation of microglia, and substantial oligodendroglial abnormalities; all these changes are present, to various degrees, in neuropsychiatric diseases such as schizophrenia, bipolar disease, major depression, in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson disease (PD) Huntington disease (HD), amyotrophic lateral sclerosis (ALS) and in neurodevelopmental diseases represented by numerous forms of autism spectrum disorders (Edgar and Sibille, 2012; Heneka et al., 2015; Miyata et al., 2015; Molofsky et al., 2012; Nave and Ehrenreich, 2014; Rajkowska and Stockmeier, 2013; Verkhratsky et al., 2014d; Zeidan-Chulia et al., 2014).

5.1. Neuropsychiatric disorders

5.1.1. Toxic encephalopathies

Astrocytes are the main targets of CNS lesions caused by various toxic agents, which are often manifested by reactive psychosis. Heavy metals (manganese, lead, aluminium or methylmercury) that are frequently associated with environmental brain damage are accumulated into astrocytes by transporters specifically expressed in astroglia. This in turn causes decrease in expression of glutamate transporters that in turn reduces astroglial glutamate uptake and casus excitotoxic neuronal death (Verkhratsky et al., 2013; Yin et al., 2007).

Astrocytes are also selectively damaged by hyperammonemia, which occurs for example in liver failure and accounts for hepatic encephalopathy that has mental symptomatology such as confusion, forgetfulness, irritability as well as alterations of consciousness represented by lethargy, somnolence and, in the terminal stages, coma (Felipo, 2013; Rama Rao and Norenberg, 2014). Ammonium is detoxified by astroglia-specific glutamine synthetase; when in excess ammonium can occlude this pathway and impair conversion of glutamate into glutamine. In addition however hyperammonemia causes severe failure in astroglia homeostatic pathways including glutamate clearance, K^+ buffering, pH regulation and causes aberrant astrocytic Ca^{2+} signalling and excessive release of glutamate (Haack et al., 2014; Liang et al., 2014; Obara-Michlewska et al., 2014; Rangroo Thrane et al., 2013; Wang et al.,

2015); impairment of all these pathways severely affects CNS homeostasis and is likely to be directly linked with psychotic symptoms.

5.1.2. Korsakoff psychosis

Astrocytes are the primary elements responsible for the massive excitotoxic neuronal death underling the Wernicke's encephalopathy, which is a substrate for the Korsakoff syndrome manifested by ante- and retrograde amnesia and confabulations. The encephalopathy develops as a result of thiamine deficiency and is related to alcoholism. In Wernicke's encephalopathy a profound (60 - 70%) down-regulation of astroglial glutamate transporters EAAT1 and EAAT 2 expression occurs, which causes severe deficiency of glutamate uptake and excitotoxicity. Similar down-regulation of these transporters is responsible for nutritional thiamine deficiency that causes the beriberi disease (Hazell, 2009; Hazell et al., 2009).

5.1.3. Schizophrenia

Pathophysiological mechanisms lying behind schizophrenic development are many and they are far from being fully understood. Modern concepts of schizophrenia regard the overall misbalance of neurotransmission with a primary impairment of glutamatergic transmission and pathological modification of connectome as a fundamental core of the disease (Laruelle, 2014; Nave and Ehrenreich, 2014; Rubinov and Bullmore, 2013). Astrocytes are the principal elements of glutamatergic transmission, being responsible for control of extracellular glutamate in the CNS (through synaptic glutamate uptake by EAAT1/2 transporters and glutamate release and extrasynaptic glutamate secretion through Sxc⁻ cystine-glutamate transporter (Bridges et al., 2012; Zhou and Danbolt, 2013) and for supplying neuronal terminals with glutamine for glutamate synthesis (Hertz and Zielke, 2004). Therefore astroglial pathology is now considered to be relevant for schizophrenia development (Verkhatsky et al., 2014b). Investigations of post-mortem human brains did not find signs of astrogliosis; instead the astroglial degeneration and decrease in astroglial numbers and packing density have been revealed (Rajkowska et al., 2002; Rajkowska et al., 1998; Schnieder and Dwork, 2011; Williams et al., 2013). Functional astroglial asthenia in schizophrenia is mainly manifested by decrease in expression of EAAT1/2 glutamate transporters (Spangaro et al., 2012; Takahashi and Sakurai, 2013), and impaired metabolism of D-serine. This latter is for example altered after the expression of mutant Disrupted-In-Schizophrenia-1 (DISC1) gene in

astrocytes (Ma et al., 2013). Astroglial cells are also linked to schizophrenic development through production and release of kynurenic acid, the endogenous inhibitor of NMDA and acetylcholine receptors. Kynurenic acid is produced in astrocytes, which specifically express kynurenine aminotransferase II. The role for this pathways is particularly important for a specific form of schizophrenia associated with infection by *Toxoplasma gondii*; this protozoan specifically affects astrocytes, which results in an increased production of kynurenic acid, and hence increases risk of schizophrenia (Schwarcz and Hunter, 2007). The role for abnormal differentiation of oligodendrocytes has also been suggested as pathologically relevant step in schizophrenia (Miyata et al., 2015).

5.1.4. Mood disorders

Decrease in the number of neuroglial cells, particularly of oligodendrocytes and astrocytes, is well documented for mood disorders including major depressive disorder and bipolar disorder (Miyata et al., 2015; Rajkowska and Stockmeier, 2013). Substantial loss of oligodendroglial cells affects brain connectome and decreases axonal survival, which can be instrumental in formation of abnormal neuronal connectivity (Nave and Ehrenreich, 2014). Decrease in several astroglial markers (such as GFAP, aquaporin 4, connexins, glutamate transporters and glutamine synthetase), is associated with general decline in astroglial homeostatic capabilities and may also cause aberrant neurotransmission (Barley et al., 2009; Bernard et al., 2011; Rajkowska and Stockmeier, 2013; Sequeira et al., 2009).

5.1.5. Addictive disorders

Both astrodegeneration (evidenced in decreased numbers of astroglial cells and morphological fragmentation of astrocytes (Miguel-Hidalgo, 2009) and astroglial reactivity (Armstrong et al., 2004; Fattore et al., 2002; Weber et al., 2013) have been observed in post-mortem human tissues and in animal models of drugs abuse. Astrocytes may contribute to altered neurotransmission through aberrant glutamate regulation: it is claimed that in addictive brains the overall glutamate concentration is increased which reflects the changes in expression of EAAT1/2 transporters and Sxc⁻ exchanger (Moussawi et al., 2011; Reissner and Kalivas, 2010).

5.2. Neurodegenerative disorders

5.2.1. Amyotrophic lateral sclerosis

The functional asthenia of astroglia emerges as one of the leading pathogenic mechanisms in ALS (Rossi, 2015; Valori et al., 2014). Prominent astrodegeneration associated with compromised expression of glial glutamate transporters rendering deficient glutamate uptake has been found in a transgenic animal model of ALS expressing a human mutated gene for superoxide dismutase 1, hSOD1, linked to a family form of the disease (Rossi et al., 2008). Decrease in glutamate uptake in turn drives excitotoxic neuronal death thus contributing to ALS progression. The role for astroglia was further corroborated in experiments demonstrating that ALS in humans and in animal models are associated with significant loss of expression of astroglial form of glutamate transporter, the excitatory amino acid transporter 2, EAAT2 (Staats and Van Den Bosch, 2009). Inhibition of EAAT2 expression in organotypic slices induced death of motor neurones (Rothstein et al., 1996). Incidentally, silencing of mutated hSOD1 gene in astrocytes has been shown to partially restore glutamate uptake thus delaying ALS symptoms (Rossi et al., 2008; Yamanaka et al., 2008).

5.2.2. Alzheimer's disease

The main pathological feature of the AD is a progressive atrophy of brain tissue resulting in dementia. The AD exists in inherited family form and in the sporadic form, which are different in prevalence (sporadic form accounts for 95 - 99% of all cases) and clinical idiosyncrasies. Histopathology of AD includes senile plaques (which are extracellular deposits of β -amyloid protein surrounded by activated glial cells) and abnormal tau-protein filaments, known as neurofibrillary tangles that emerge inside neurones (Braak et al., 1998).

Profound changes in neuroglia are linked to the AD. Astrocytes demonstrate both atrophy and reactivity. Astroglial reactivity is associated with extracellular deposition of β -amyloid with hypertrophic astrocytes being an essential component of senile plaques (Verkhatsky et al., 2014a; Verkhatsky et al., 2010). In experimental AD models astroglial reactivity can precede plaque formation; the reactive astrocytes were forming agglomerates which possibly marked the foci for subsequent plaques formation (Heneka et al., 2005). Astroglial reactivity, however, differed between brain regions; in AD mouse model for example, reactive astrogliosis was observed in hippocampus but not in the entorhinal and prefrontal cortices, which may explain

higher vulnerability of these two regions to AD pathology (Kulijewicz-Nawrot et al., 2012; Olabarria et al., 2010; Yeh et al., 2011). Deficits in astroglial response to β -amyloid may be associated with impairments of Ca^{2+} signalling which are specific to astrocytes in entorhinal and prefrontal cortices (Grolla et al., 2013; Lim et al., 2014). Morphological atrophy of astroglia was observed in several AD animal models and appeared before the formation of senile plaques in the hippocampus, entorhinal cortex and prefrontal cortex (Beauquis et al., 2013; Beauquis et al., 2014; Kulijewicz-Nawrot et al., 2012; Olabarria et al., 2010; Yeh et al., 2011).

Oligodendroglia is severely damaged in AD, with white matter demonstrating dramatic shrinkage and remodelling which may even be employed for disease diagnostics (Fieremans et al., 2013; Sun et al., 2014). Pathological processes which impair upon oligodendroglia in the AD context remain however unknown. Finally microglia demonstrates widespread activation in AD being one of the leading players in the neuroinflammatory component of the disease (Heneka et al., 2015).

5.2.3. Huntington's disease

Huntington's disease (HD) is an inherited, autosomal dominant and progressive neurodegenerative pathology resulting in cognitive decline and motor symptoms. The HD is caused by a genetic aberration represented by the triplet nucleotide repeat cytosine-adenine-guanine (CAG), which encodes glutamine, in exon 1 of the huntingtin gene (Ross et al., 2014). Expression of this mutant gene translates into a mutant huntingtin protein (mhtt) containing an expanded polyglutamine section in its N-terminal portion; the higher the number of CAG repeats, the more severe is the disease progression (Zoghbi and Orr, 2000).

In the context of HD, astrocytes undergo functional atrophy represented by significant reduction in expression of the EAAT2/GLT1 glutamate transporter with associated failure of astroglial glutamate uptake that causes excitotoxicity and neuronal death (Faideau et al., 2010; Hassel et al., 2008). In addition HD-affected astrocytes demonstrate pathologically increased exocytotic secretion of glutamate, which further exacerbates excitotoxic neuronal damage (Lee et al., 2013). Another aspect of astroglial functional atrophy in HD is manifested by down-regulation of expression of inward rectifying $\text{K}_{\text{ir}}4.1$ channels that causes deficient K^{+}

buffering further adding to neuronal distress and pathological progression of the disease (Tong et al., 2014).

6. Pathological potential of purinergic signalling in neuropathology

6.1. The astroglial/microglial death receptor P2X₇

The distinct members of the family, P2X₇ receptors differ from the other P2X receptors in that their long lasting or repetitive activation by ATP results in the formation of a pore allowing the permeation of large molecular weight organic molecules with molecular weight up to 1000 Da (Sperlagh and Illes, 2014)). The P2X₇ receptor subunit is also characterized by an exceptionally long intracellular C-terminal end with an extra 200 amino acid residues compared with P2X₁₋₆. The C-terminus of P2X₇ has been implicated in regulating receptor function including signalling pathway activation, cellular localization, protein-protein interaction and post-translational modification (Costa-Junior et al., 2011). There are several splice variants of mammalian P2X₇ receptors, all of which are widely expressed in the nervous system (Adinolfi et al., 2010). The gene encoding the human P2X₇ receptor exhibits several non-synonymous single nucleotide polymorphisms, which result in a change in amino acid sequence and the expression of different receptor variants, further increasing the structural diversity of P2X₇ receptors.

The activation of P2X₇ receptors is followed by Ca²⁺ influx and cytokine release. This receptor is a key regulatory element of the inflammasome molecular complex, providing the external stimulus necessary for the post-translational modification and subsequent release of the pro-inflammatory cytokine interleukine-1 β (IL-1 β) (Di Virgilio, 2015). Stimulation of P2X₇ receptor induces membrane blebbing and microvesicle shedding, which is a secretory pathway for IL-1 β (Turola et al., 2012). Lipopolysaccharide (LPS), a ligand for toll-like receptor 4 (TLR 4), triggers microglia activation and neural cells injury and is used as an agent reproducing CNS inflammation (Barbierato et al., 2013). In addition, LPS is a necessary pre-requisite for the processing and release of IL-1 β in microglia and astrocytes in response to P2X₇ receptor activation (Skaper et al., 2010). Of note, the “suicidal” P2X₇ receptor instigates death not only of neurones but also of the microglia and astrocytes themselves.

The cell-type specific localisation of P2X₇ receptors in the CNS has been the subject of long-standing debate, which had never reached general consensus; immunohistochemical findings are heterogeneous and contradict data obtained by physiological and neurochemical methods (Sperlagh and Illes, 2014). In contrast to immunohistochemistry, the available electrophysiological evidence convincingly demonstrates the presence of these receptors on cortical and spinal cord astrocytes in culture and in freshly prepared brain slices of rats and mice (Ficker et al., 2014; Norenberg et al., 2010; Oliveira et al., 2011). Comparable data were reported also on microglia in culture or in acute brain slices (Arnoux et al., 2013).

Although often P2X₇ receptors convey death to glial cells, they have also trophic functions; for example this supposedly suicide receptor promotes cell cycle progression and proliferation in microglia (Monif et al., 2009) and may act as a scavenger for the removal of apoptotic cells in the absence of its ATP ligand (Gu et al., 2011). Schwann cells (Luo et al., 2013) and oligodendrocytes (Matute, 2011) that myelinate, respectively, peripheral and central neurones, also possess P2X₇ receptors. In Schwann cells P2X₇ receptors are involved in control of myelination in development (Faroni et al., 2014). At the same time differentiation of adipose cells into Schwann phenotype is accompanied with expression of P2X₇ receptors that regulate cellular death (Faroni et al., 2013). Similarly, in the presence of high extracellular ATP concentrations, P2X₇ receptors cause the loss of oligodendrocytes and demyelination (Butt, 2011).

6.2. *The proliferative/antiproliferative astroglial/microglial receptors P2Y_{1,2,6,12}*

P2Y_{1,2,6} receptors couple to G_q proteins stimulating membrane-bound phospholipase C (PLC), which then cleaves phosphatidylinositol-bisphosphate (PIP₂) in the membrane into two second messengers, InsP₃ and diacylglycerol (DAG) (Franke and Illes, 2014a, b). The InsP₃ triggers Ca²⁺ release from the endoplasmic reticulum store, whereas DAG activates protein kinase C (PKC) initiating various intracellular responses. The ATP/ADP-sensitive P2Y₁₂ receptors bind preferentially to the G_{i/o} subunits of G proteins. G_i activation is associated with the inhibition of adenylate cyclase and reduces intracellular levels of cyclic adenosine 5'-monophosphate (cAMP). The stimulation of G_q protein-linked P2Y_{1,2} receptors leads to the transactivation of the second-messenger pathways of growth factor receptors such as mitogen-activated protein kinase (MAPK)/extracellular signal-regulated protein kinase (ERK) and also cyclooxygenase-2 (COX-2), which have prominent roles in neuroinflammation (Franke and

Illes, 2014a, b). The ATP/ADP-sensitive P2Y₁ receptors mediate the effects of ATP on IL-1 β -induced transcription factors, as well as NF- κ B and activator protein-1 activation. The ATP/UTP-sensitive P2Y₂ receptors bind via an RGD (Arg-Gly-Asp) motif in their first extracellular loop to integrins, whereupon the integrin signalling pathway regulates cytoskeletal reorganization and cell motility (Weisman et al., 2012). The C-terminus of the P2Y₂ receptor contains two Src-homology-3 binding domains that promote association with Src and transactivation of growth factor receptors.

Astrogliosis is a pathological hallmark of CNS injury, characterized by profound morphological, molecular and functional changes in astrocytes that occur within hours and evolve as time elapses after injury (Burda et al., 2015; Burda and Sofroniew, 2014; Franke et al., 2012; Sofroniew, 2015; Verkhratsky et al., 2012). Conceptually, astrogliosis is a defence mechanism to minimize and repair the initial damage, although it may also have some detrimental effects. Although it is generally accepted that reactive astrocytes contribute to isolating damaged brain areas from surrounding healthy cells and synthesize neurotrophins and pleiotrophins that participate in neuronal recovery, these cells also release several potentially toxic compounds (e.g. nitric oxide and arachidonic acid metabolites) (Verkhratsky et al., 2012). In focal lesions, astrogliosis results in the formation of irreversible glial scarring that acts as regeneration barrier (Burda et al., 2015; Franke et al., 2012).

Astrocytes respond to ATP/UTP with hypertrophy, swelling of the cell body and main processes, and proliferation that ultimately result in full blown astrogliotic phenotype with subsequent formation of the glial scar (Tozaki-Saitoh et al., 2011; Weisman et al., 2012). Microglia are one of the identified sources of extracellular ATP in neuroinflammatory conditions; this ATP may act on neighbouring astrocytes and neurones (Dou et al., 2012; Imura et al., 2013). P2Y₁₂ receptors regulate the migration of microglia to the site of ATP release and P2Y₆ receptor activation by UDP results in a drastic increase in the motility of microglia, leading to engulfment and phagocytosis of targeted molecules (Koizumi et al., 2013). These microglial effects are deeply involved in the regulation of astrogliosis, in that microglia prevent the proliferative effects of adenine nucleotides and favour an inhibitory effect of uracil nucleotides (Quintas et al., 2011). Hence UTP appears to support astrogliosis by direct stimulation of astrocytic P2Y₂ receptors, but at the same time UDP attenuates it by activating microglial P2Y₆ receptors.

6.3. Purinergic receptors and long-lasting consequences of acute CNS damage

6.3.1. Epilepsy

The temporal lobe epilepsy can be modelled in rodents by chemical induction of status epilepticus using injections of kainite or pilocarpine (Engel et al., 2012). Complex partial seizures and status epilepticus can cause hippocampal neuronal death, mossy fibre sprouting, astrogliosis, and molecular reorganization of membrane proteins. After chemically-induced epilepsy in rodents, the P2X₇ receptor-immunoreactivity is up-regulated in neurones and microglia, paralleled by astroglial loss (Jimenez-Pacheco et al., 2013). Treatment with selective P2X₇ receptor antagonists were protective against epileptic seizures and counteracted astroglial loss. It is likely that the damage to astrocytes is mostly a consequence of microglial activation.

6.3.2. CNS trauma, hypoxia and stroke

Immunohistochemical investigations in the nucleus accumbens of rats showed that after stab wound injury, previously absent P2X₇ receptor-immunoreactivity was observed on cells labelled for the astrocytic marker GFAP (Bai and Li, 2013; Burnstock et al., 2011). Mechanical injury to the rat spinal cord resulted in an immediate, reversible loss of tissue at the lesion site as well as a secondary expansion of tissue damage over time (Peng et al., 2009) (Fig. 2). The secondary tissue damage was at least partially due to the release of ATP and a deleterious effect mediated by P2X₇ receptors. Thus, the application of the P2X₇ receptor antagonists oxidized ATP or Brilliant Blue G (BBG) even 15 min after spinal cord injury resulted in a faster recovery of the hind limb motor function than without drug application. Similar findings were reported for the cerebral cortex of spontaneously hypertensive rats, where medial cerebral artery occlusion (MCAO) led to the up-regulation of P2X₇ immunoreactivity in the penumbra surrounding the necrotic region (Franke and Illes, 2014b) (Fig. 2). This immunoreactivity was co-localized with astrocytic and neuronal markers. Further investigations demonstrated that the up-regulation of P2X₇ receptors is accompanied by necrosis/apoptosis of CNS tissue. Treatment with P2X₇ receptor antagonists, such as BBG, oxidized ATP, and A-438079 reduced the extent of cellular damage in cerebral ischemia/reperfusion injury (Arbeloa et al., 2012; Chu et al., 2012). The broad spectrum P2

receptor antagonist PPADS had a similar effect, but did not allow to differentiate between P2X and P2Y receptors (Lammer et al., 2011).

There is a general belief that traumatic injury or hypoxic/ischemic damage of astrocytes and neurones leads to a massive outflow of ATP from the intra- to the extracellular space (Bai and Li, 2013). ATP consequently activates P2X₇ receptors and hence initiates excitotoxic cell injury in ischemia/stroke as well as in respective *in vitro* models. Activated P2X₇ receptors contribute directly to a rapid increase of intracellular Ca²⁺ due to massive Ca²⁺ influx through membrane pores. The high [Ca²⁺]_i activates nucleases and proteases (e.g. calpains) which eventually result in cell death. Meanwhile the mitochondrial matrix also takes up Ca²⁺ from the cytoplasm, the mitochondrial membrane potential collapses, the permeability pore opens and solutes flow into the cytoplasm (Csordas and Hajnoczky, 2009) activating the apoptotic caspase enzyme system (Dubyak, 2012). Ischemic damage to white matter causes oligodendrocyte death, myelin disruption, and axon dysfunction, and it is partially mediated by glutamate and ATP release (Domercq et al., 2010). In cultured oligodendrocytes, chemical ischemia - a blockade of glycolysis and mitochondrial function by iodoacetate and antimycin, respectively in a medium lacking glucose – activated inward currents. These currents were blocked by PPADS and BBG, indicating that they were triggered by P2X₇ receptor activation. In contrast to astrocytes and neurones, hypoxia/ischemia might down-regulate P2X₇ receptor expression (studied by immunofluorescence, RT-PCR, Western blot and Ca²⁺ imaging) in cultured oligodendrocytes (Wang et al., 2009). In perfect agreement with the demonstrated difference between astrocytic and oligodendrocytic reactions, chemical ischemia induced inward currents in CA1 neurones and stratum oriens astrocytes independent of P2X₇ receptor activation (Leichsenring et al., 2013).

Increased microglial activation and enhanced P2X₇ receptor expression are seen in ischemic stroke (Arbeloa et al., 2012; Bai and Li, 2013). In the early stages of hypoxia/ischemia the massive release of ATP drives microglial activation triggering the phagocytosis of cell debris but also a vicious cycle of neuronal cell death. It has been shown that the immediate up-regulation of P2X₇ receptor-immunoreactivity by MCAO is due to a microglial reaction, whereas somewhat later P2X₇ receptor-immunoreactivity is up-regulated in neurones and astrocytes (Franke et al., 2012).

P2Y₁ receptors were also shown to be critically involved in traumatic brain injury and hypoxic/ischemic damage of the brain via astrocytic mechanisms. However, these data are

quite heterogeneous. One group of authors reports that P2Y₁ receptor agonists lead to an increase of infarct volume after transient MCAO as well as an enhanced expression of various neuroinflammatory cytokines such as that of IL-6 and tumour necrosis factor- α (Kuboyama et al., 2011). Immunoreactivity of P2Y₁ receptors has been shown to be co-localized with GFAP-positive astrocytes, and antagonists for this receptor were beneficial in regulating the cytokine/chemokine response to MCAO. In agreement with these data cognitive deficits after both traumatic brain injury and focal cerebral stroke could be improved by P2Y₁ receptor blockade or genetic ablation (Chin et al., 2013; Choo et al., 2013). As a reason for disturbed cognition, the overt purinergic astrocyte signalling from the penumbra to neural circuits distant from the injury epicentre has been assumed.

Another group of authors came to a diametrically opposite conclusion. They report that both traumatic brain injury (Talley Watts et al., 2013) and ischemia induced by phototrombolysis (Zheng et al., 2013; Zheng et al., 2010) cause astrocyte swelling, which is the primary cause of brain damage. P2Y₁ receptor stimulation decreases cytotoxic oedema in astrocytes and lessens the size of brain infarcts by increased InsP₃-mediated intracellular Ca²⁺ release, which in turn activates Ca²⁺ sensitive dehydrogenases and leads to increased ATP production in mitochondria. Higher ATP levels can be used for any energy-dependent function that is needed by astrocytes during brain injury. Hence, P2Y₁ receptor agonists rather than antagonists improve the consequences of traumata or vascular occlusion in the brain.

6.4. Major depression

Linkage studies suggested that variations of the chromosome 12q24.31 containing candidate genes for P2X₇ and P2X₄ receptors as well as calmodulin-dependent protein kinase b (CaMKKb) may be associated with major depressive disorders (Sperlagh and Illes, 2014). In view of the fact that microglia and astroglia are sources of inflammatory cytokines, P2X₇ receptors and mood-related behaviour has been intensively studied in animal models. The genetic deletion of P2X₇ receptors resulted in antidepressive-like behaviour (Basso et al., 2009; Csolle et al., 2013). On a larger scale, several potential mechanisms were identified for the antidepressant phenotype of P2X₇^{-/-} mice, such as absence of P2X₇ receptor-mediated glutamate release, elevated basal brain-derived neurotrophic factor (BDNF) production, enhanced neurogenesis, and increased serotonin bioavailability in the hippocampus (Csolle et al., 2013). Although some of these processes are neuronal, the glial ones are certainly of

utmost significance. Low abundance of ATP was observed in the brains of mice that were susceptible to chronic social defeat and the administration of ATP induced a rapid antidepressant-like effect in these mice (Cao et al., 2013). In addition, transgenic blockade of vesicular gliotransmission induced deficiencies in astrocytic ATP release, causing depressive-like behaviours that could be rescued via the administration of ATP.

6.5. Neurodegenerative illnesses

6.5.1. Alzheimer's disease (AD) and dementia

Astrocytes, microglia and oligodendrocytes all have biological properties that could influence learning and cognition (Fields et al., 2014). Myelination by oligodendrocytes increases conduction velocity, affecting spike timing and oscillations in neuronal activity; astrocytes can modulate synaptic transmission and may couple multiple neurones and synapses into functional assemblies; microglia can remove synapses in an activity-dependent manner altering neural networks. In fact, injection of the astrocyte-specific toxin L- α -aminoadipate in the medial prefrontal cortex (mPFC) of rats showed that astrocytic loss in the mPFC triggers subsequent neuronal damage leading to cognitive impairment in tasks depending on the integrity of this brain region (Lima et al., 2014).

It is generally believed that in AD a key role is played by abnormal metabolism of the amyloid precursor protein (APP) which is cleaved by β - and γ -secretases to generate the β -amyloid peptides, which are present in large amounts in the amyloid plaques of the AD patients' brain; it has to be noted however that β -amyloid hypothesis remains debatable (Castellani and Smith, 2011). There seems to be a tight relationship between microglial P2X₇ receptors and neurodegeneration during AD (Burnstock et al., 2011; Del Puerto et al., 2013). Microglia are specifically up-regulated around senile plaques in an animal model of AD and P2X₇ receptor agonists release copious amounts of superoxide from microglia. In addition, β -amyloid triggers increases in intracellular Ca²⁺ concentrations, ATP release, IL-1 β secretion and plasma membrane permeabilisation in microglia from wild-type but not from P2X₇ receptor-deficient mice (Sanz et al., 2009). Likewise, intra-hippocampal injection of A β caused a large accumulation of IL-1 β in wild-type but not in P2X₇ KO mice. Overall these results added P2X₇ receptors as new players mediating microglial purinergic inflammatory responses in AD brain. More recent data, however, suggested that P2X₇ may also have

beneficial effect via the stimulation of non-amyloidogenic processing of APP by α -secretase, leading to proteolytic cleavage with the A β peptide sequence and shedding of the soluble APP ectodomain (sAPP α), which in turn has been reported to exert neuroprotection (Delarasse et al., 2011).

Stimulation of P2Y₁ receptors in the rat mPFC deteriorated performance accuracy and caused both working memory disturbances and impaired impulse control (Koch et al., 2015; Krugel et al., 2013). P2Y₁ receptor activation induced long-term depression in the hippocampal CA1 region (Chen et al., 2013) and at layer V pyramidal cells in the mPFC (Guzman et al., 2010). To selectively induce Ca²⁺ influx through the plasma membrane of astrocytes, a light-gated Ca²⁺ permeable channel channelrhodopsin-2 was specifically expressed in these cells (Chen et al., 2013). It was found that light stimulation of this astrocytic channel resulted in long-term synaptic depression in neighbouring neurones, which could be antagonized by blocking P2Y₁ receptors or buffering astrocyte intracellular Ca²⁺ at a low level. Astroglial hyperactivity (probably leading to cognitive deterioration in AD) could be normalized by the inhibition of P2Y₁ receptors strongly expressed by reactive astrocytes surrounding amyloid plaques (Delekate et al., 2014).

6.5.2. Parkinson' disease (PD)

The unilateral intrastratial application of the dopaminergic neurotoxin 6-OH-dopamine (6-OHDA) to rats caused rotational behaviour after receiving systemic injection of the dopaminergic agonist apomorphine. In this model of PD P2X₇ receptor-immunoreactivity was mainly found in microglia but also in astroglia (Marcellino et al., 2010). The P2X₇ receptor antagonist A-438079 partially prevented the 6-OHDA-induced depletion of striatal DA stores, although this was not associated with a reduction of DA cell loss. In the same animal model, P2X₇ receptors appeared to contribute to PD pathogenesis through a triple impact on synaptotoxicity, gliosis and neurotoxicity, highlighting the therapeutic potential of P2X₇ receptor antagonists in PD (Carmo et al., 2014).

6.5.3. Amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS)

With respect to purinergic signalling and ALS, up-regulation of P2X₇ receptors has been observed in transgenic mice over-expressing human superoxide dismutase 1 (SOD1), an

animal model of ALS (D'Ambrosi et al., 2009). During the progression of ALS, microglia, astrocytes and motor neurones might enter in crosstalk via ATP release/degradation and P2X₇ receptor activation, generating a feedback loop that drives the sustained pro-inflammatory and detrimental response (Amadio et al., 2010; Volonte et al., 2011), which is prevented by P2X₇ receptor antagonists (Del Puerto et al., 2013; Gandelman et al., 2010). In *post mortem* sections of the cerebral cortex from MS patients, the P2Y₁₂ receptor is present in myelin and interlaminar astrocytes but absent in demyelinated axons (Amadio et al., 2010). Decreased P2Y₁₂ receptor-immunoreactivity in the proximity of the lesions is directly correlated with the extent of demyelination, suggesting that the loss of these receptors might perturb tissue integrity.

7. Conclusions

In this review we summarized general principles of neurogliopathology, and presented pathological potential of glial cells in various neurological diseases. We further outlined the importance of purinergic signalling system in regulation of neuronal-glial signalling in health and disease. All glial cells express purinergic receptors, albeit in widely different combinations. The principal purinergic transmitter ATP is released from synaptic terminals and from glia through several mechanisms; when neuronal tissue is damaged, massive release of ATP acts as a danger signal. In pathological conditions ATP and its derivatives, acting through purinergic receptors, control glial reactivity and hence define the balance between neuroprotection and neurotoxicity.

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Figure legends

Figure 1. Neuroglia is fundamental for evolution of neuropathology; neuroglial component is present in all neurological disorders. There is a close interaction between neurones, astrocytes, oligodendrocytes and microglia during acute or chronic damage inflicted upon the CNS by e.g. neurotrauma or ischemia on the one hand, and major depression or neurodegenerative illnesses on the other. Toxic encephalopathy, Korsakoff psychosis, schizophrenia, mood disorders, and addictive disorders are discussed in the text but not shown in this Fig. Neurons and glial cells interact with each other under pathological conditions by means of neuro- and gliotransmitters (including nucleotides) as well as additional signalling molecules such as cytokines, chemokines, growth factors, etc. (see above).

Figure 2. Mechanical trauma, hypoxia/ischemia and stroke inflict a relatively circumscribed lesion to the CNS. In the core of the injured area there is necrotic tissue which is irreversibly dead. Around the core region there is tissue which is reversibly injured and may favourably react to neuroprotective/neuroregenerative pharmacotherapy. Nucleotides are one of the harmful molecules accumulating in the extracellular space and aggravating the core injury as well as causing secondary damage by diffusing to neighbouring areas. Glial cells are both sources of extracellular nucleotides and targets of nucleotide effects mediated by P2X and P2Y receptors. Stimulation of astrocytic purinoceptors may cause the outflow of excitotoxic, metabolically toxic and neuroinflammatory signalling molecules.

Figure 1

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Figure 2
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