REVIEW

Implication of Neuronal *Versus* Microglial P2X4 Receptors in Central Nervous System Disorders

Alexia Duveau^{1,2} · Eléonore Bertin^{1,2} · Eric Boué-Grabot^{1,2}

Received: 18 February 2020 / Accepted: 6 May 2020 / Published online: 5 September 2020
© Shanghai Institutes for Biological Sciences, CAS 2020

Abstract The P2X4 receptor (P2X4) is an ATP-gated cation channel that is highly permeable to Ca^{2+} and widely expressed in neuronal and glial cell types throughout the central nervous system (CNS). A growing body of evidence indicates that P2X4 plays key roles in numerous central disorders. P2X4 trafficking is highly regulated and consequently in normal situations, P2X4 is present on the plasma membrane at low density and found mostly within intracellular endosomal/lysosomal compartments. An increase in the *de novo* expression and/or surface density of P2X4 has been observed in microglia and/or neurons during pathological states. This review aims to summarize knowledge on P2X4 functions in CNS disorders and provide some insights into the relative contributions of neuronal and glial P2X4 in pathological contexts. However, determination of the cell-specific functions of P2X4 along with its intracellular and cell surface roles remain to be elucidated before its potential as a therapeutic target in multiple disorders can be defined.

Keywords Purinergic signaling · P2X · ATP · Ligand-gated ion channels · Chronic pain · Brain trauma · Ischemia · Neurodegenerative diseases · Alcohol · Neuropsychiatric disorders

Introduction

Initially discovered as one of the main sources of energy inside cells, adenosine 5' triphosphate (ATP) is now recognized as a ubiquitous extracellular cell-to-cell signaling molecule in the peripheral (PNS) and central nervous system (CNS), as well as in peripheral organs [1]. Although the release of ATP by sensory nerve cells was reported in the 1950s [2], Geoffrey Burnstock was the first to propose the concept of purinergic signaling using ATP as a fast neurotransmitter in 1972 [3]. ATP was later shown to mediate fast neurotransmission in the PNS and CNS alike, but is nowadays mostly considered to be a neuromodulator co-released with other classical neurotransmitters such as GABA or glutamate at inhibitory or excitatory synapses in the CNS [4]. ATP is also a gliotransmitter released by glial cell types such as astrocytes and microglia [5–7]. ATP released by neurons and glia exerts multiple actions in the neuromodulation of synaptic activity and plasticity, communication in glial networks, and directly between glia and neurons *via* the activation of metabotropic P2Y and ionotropic P2X receptors, or after the conversion of ATP by ectonucleotidases into ADP and adenosine by the activation of P2Y and adenosine P1 receptors [4, 8].

There is also growing evidence for the involvement of purinergic signaling in major CNS disorders including chronic pain, brain trauma, hypoxia/ischemia, epilepsy, and neurodegenerative diseases associated with neuroinflammation (for a recent review, see [9]). In these noxious conditions, extracellular levels of ATP increase and serve as a danger signal along with activation of several purinergic receptor subtypes that are widely distributed both in neurons at pre- and post-synaptic sites and in glial cells [4, 6, 8, 10, 11]. Besides P2X7, P2X4, P2Y1, and A2_{AR} , P2Y6 (microglial phagocytosis) and

✉ Eric Boué-Grabot
eric.boue-grabot@u-bordeaux.fr

¹ Université de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, 33000 Bordeaux, France

² CNRS, Institut des Maladies Neurodégénératives, UMR 5293, 33000 Bordeaux, France

P2Y₁₂ (microglial chemotaxis) also participate in ATP danger signaling [9], and increasing evidence indicates that microglial or neuronal P2X₄ receptors are upregulated and play key roles in many CNS disorders. In this review, we focus on the function of neuronal and glial P2X₄ function in physiological and pathological conditions.

Properties and Function of P2X₄ Receptors

P2X receptors are trimeric ATP-gated cation channels formed by homomeric or heteromeric associations from 7 different subunits (P2X₁–P2X₇) encoded by seven genes in mammals. Functional P2X receptors are non-selective cation channels permeable to Na⁺, K⁺, and Ca²⁺ and their activation by ATP-binding leads to cell depolarization and Ca²⁺ influx. With the exception of P2X₆, all homomeric P2X receptors are functional and display different ATP sensitivities, current kinetics, and pharmacological properties. Moreover, the variety in expression pattern of the seven P2X subunits and the existence of heteromeric associations between these subunits contribute to the diversity and widespread action of ATP signaling [8]. Among the seven P2X subunits, P2X₄ is the most widely distributed in various cell types throughout the body [1, 12]. All P2X subunits are expressed in the CNS in a heterogeneous manner throughout the different structures, cell types, and subcellular compartments [13], although P2X₄ is one of the main subunits found in both neurons and glial cells such as microglia and more controversially

in astrocytes, oligodendrocytes, and Schwann cells (Table 1 and Fig. 1) [14]. However, the subunit composition of P2X receptors in most CNS cells is not well defined, and the physiological roles of P2X₄ or P2X₄-containing receptors is far from being deciphered due to the paucity of selective pharmacological tools. TNP-ATP, a weak and non-selective P2X₄ antagonist, or 5-BDBD, a selective allosteric P2X₄ antagonist, have been commonly used to show the involvement of P2X₄ in rodent CNS tissue [12] *in vitro* and *in vivo*. New potent antagonists of rodent P2X₄ (BAY-1797 and NP-1815-PX) have recently been identified [15, 16] as well as a highly-selective human P2X₄ antagonist (BX430) that has no effect on rodent P2X₄ [17]. Ivermectin, which is an agonist of invertebrate glutamate-gated Cl[−] channels, is used as an antiparasitic drug in human medicine [18]. This compound is also a very potent positive modulator of mammalian P2X₄. However, although highly selective for the P2X₄ subunit among P2X subtypes, Ivermectin potentiates human P2X₇ currents and modulates other ligand-gated ion channels such as GABA_A and nicotinic receptors [19–22]. Ivermectin is a positive allosteric modulator of P2X₄ that stabilizes this subunit in the open state [20], which consequently may also prevent P2X₄ internalization [21, 22].

Left, P2X₄ in normal states is mostly intracellular with low expression on the surface of neurons and glia. P2X₄ expression in astrocytes and oligodendrocyte precursors remains controversial (indicated by a question mark). Activation of P2X₄ by extracellular ATP released by

Table 1 Expression of P2X₄ in the CNS.

CNS regions	P2X ₄ mRNA ^a	P2X ₄ protein	P2rx4 reporter mice [82, 83]	P2X ₄ mCherryIN mice [84]	Cell type specificity	References
Amygdala	++	NA	NA	NA	NA	
Basal ganglia	++	+	NA	NA	Neurons, Astrocytes	[48, 49, 71]
Cerebellum	++	++	++	+	Neurons	[48, 49, 57–59]
Cerebral cortex	+++	+	++	++	Neurons Astrocytes ^b	[52, 58, 59]
Hindbrain	+	++	++	NA	Neurons	[58]
Hippocampus	++	++	++	++	Neurons, Microglia, Astrocytes ^b	[13, 14, 57–59]
Hypothalamus	+	+	++	NA	Neurons	[51, 58]
Midbrain	+	+	++	NA	Neurons	[58]
Olfactory region	++	+++	+++	++	Neurons, Epithelial cells	[49, 57–59]
Spinal Cord	++	++	++	NA	Motoneurons, Microglia	[58, 89, 109]
Thalamus	+	+	+	NA	Neurons	[58]

Expression levels are indicated on a semi-quantitative scale where (+) indicates the presence and relative abundance of P2X₄ expression. NA, no specific data set.

^aData from the Allen Brain Atlas obtained by *in situ* hybridization and available at <http://mouse.brain-map.org/experiment/show/75551474>.

^bP2X₄ expression detected by electronic microscopy in astrocytes of P2X₄mCherryIN mice using anti-RFP antibodies.

Fig. 1 P2X4 expression in CNS diseases.

neurons or glial cells exerts mostly neuromodulatory actions in the physiological state. Right, in pathological states, higher extracellular ATP concentration and *de novo* expression of P2X4 as well as increased surface trafficking of intracellular pools of P2X4 lead to an increase in the number of surface P2X4s in distinct cell types in numerous central disorders and the exacerbation of purinergic signaling.

P2X4 Trafficking

Indeed, in contrast to other P2X subunits, P2X4 is constitutively and highly internalized, and as a result, is found mainly in endosomal/lysosomal compartments thereby ensuring low surface expression of these receptors in basal states [23]. Recent work has shown that intracellular P2X4 in these acidic compartments is functional and may have important actions in lysosome membrane fusion [24]. In various pathological states, such as trauma, ischemia, chronic pain, neurodegenerative processes, and

several neuropsychiatric disorders, *de novo* expression of P2X4 and/or an increase in cell surface P2X4 density has been reported in microglia and/or neurons, thus suggesting possible key and multiple roles of neuronal and microglial P2X4 receptors in the establishment and/or maintenance of these pathologies [25]. Changes in the intracellular expression of P2X4 may also have important consequences in the pathophysiological context.

Similar to all seven P2X subunits, P2X4 has a topology with 2 transmembrane regions linked by a large extracellular domain and two intracellular amino and carboxyl termini [26]. The first X-ray crystal structure of a P2X receptor was resolved in a truncated form of the zebrafish P2X4 receptor in its closed state [27] and then in complex with ATP [28] from crystallized human P2X3 in different states [29], which confirmed the subunit topology and trimeric organization of P2X receptors. The structure of the head domain of rat P2X4 has also been investigated [30]. These data revealed the importance of a large extracellular disulfide-rich region packed with N-linked glycosylation

moieties and the existence of three inter-subunit binding sites. They also showed that the binding of three molecules of ATP is required to induce conformational changes leading to the opening of the channel pore formed by the three transmembrane regions [28]. P2X subunits share several conserved motifs within the intracellular domains, such as a YXXXK in the C-terminus that regulates surface expression [31] and a TXK/R in the N-terminus which is a putative protein kinase C phosphorylation site regulating current desensitization [32]. However, in terms of sequence and length, the C-terminus domain is the most divergent between P2X subunits [12]. The mammalian P2X4 subunit displays two endocytic tyrosine-based and di-leucine motifs within the C- and N-termini, respectively, which are responsible for its constitutive internalization and main localization in lysosome-related intracellular organelles [33]. This distribution is in contrast to other P2X receptors such as P2X2, which are mainly found at the level of the plasma membrane [34]. Interestingly, the clathrin-dependent constitutive internalization of P2X4 is mediated by an interaction between the non-canonic motif YXXGΦ and the μ2 subunit of adaptive protein 2 (AP2), whereas the canonic YXXΦ motif is not involved [34, 35]. Mutations of the endocytic motif YXXGL or blockade of clathrin-mediated internalization lead to an increase both in the number of P2X4 receptors at the plasma membrane and the associated ATP currents [22, 34–36]. Being highly glycosylated, the extracellular loop of P2X4 allows it to resist the very acidic intra-lysosomal environment and proteolysis. Intracellular P2X4 pools can thus either remain intracellular or recycle back to the cell surface and dynamically regulate the number of surface P2X4 receptors [23, 34, 37–39]. Several studies have shown that in macrophages and microglia, the surface trafficking of P2X4 is upregulated in response to stimuli promoting lysosomal exocytosis or altering endocytosis [33, 40, 41]. In numerous pathophysiological conditions, *de novo* expression of P2X4 and/or increased surface trafficking has been reported [42] suggesting an important role of the dynamic regulation of P2X4 surface trafficking in diseases (Fig. 1).

Intracellular P2X4 Function

Finally, growing evidence suggests that lysosomal P2X4 receptors also have important physiological functions (for review, see [43]). In lysosomes, the extracellular domain of P2X4 containing the agonist binding sites faces the luminal compartment, which contains high concentrations of ATP [44, 45] transported by the vesicular nucleotide transporter VNUT/SLC17A9 present in the lysosomal membrane. Alkalinization of the intra-lysosomal pH may lead to P2X4 activation and participate in endolysosomal fusion and

vacuolation [43]. Indeed, Ca^{2+} flux through P2X4 activates calmodulin, which subsequently associates with the receptor channel and promotes vesicular fusion [46].

Neuronal P2X4

P2X4 is expressed in various tissue cell types of peripheral organs including epithelial cells, kidney, pancreas, lung, liver, the cardiovascular system, and immune cells (for review, see [12]) in which it has important functions. In the CNS (Table 1), P2X4 along with P2X2 and P2X6, is the most widely expressed P2X subunit in both neurons and glial cells [14, 47]. P2X4 was first identified and cloned from brain tissue in 1996 by two independent groups [14, 48]. Their studies also showed the expression of P2X4 mRNA in the dentate gyrus, CA1/CA3 pyramidal cells of the hippocampus, and in cerebellar Purkinje cells [14, 48]. Further immunohistochemical studies with anti-P2X4 antibodies confirmed the presence of P2X4 at the protein level in GABAergic interneurons of the hippocampus, cerebellum and olfactory bulb [13, 49], spiny neurons of the striatum and substantia nigra [49, 50], and in the hypothalamus and hypophysis [51]. Other studies have reported P2X4 expression in somatosensory cortical neurons [52], sensory nerves and ganglia [53–55], and the retina [56]. Electron microscopy has indicated that neuronal P2X4 is located both pre- and post-synaptically, mainly at the edge of the postsynaptic density in the peri- and extra-synaptic space [13]. This is consistent with the idea that activation of synaptic P2X receptors by ATP co-released with other neurotransmitters such as glutamate or GABA does not mediate fast synaptic transmission but modulates excitatory and inhibitory synapses in the CNS [6]. The widespread distribution of P2X4 in CNS neurons has been confirmed in P2X4-knockout mice expressing β-galactosidase instead of P2X4 [57] and recently in a transgenic reporter mouse expressing tdTomato under the control of a P2X4 promoter and subsequently using a novel internalization-defective P2X4mcherryIN knock-in mouse [58, 59].

Neuronal P2X receptors located at pre- and post-synaptic sites act mainly as neuromodulators. However, in most cases the specific contribution of P2X4 has been difficult to ascertain due to the paucity of appropriate pharmacological or genetic tools. In addition, the P2X4 subunit may not only form functional homomeric receptors, but can also assemble in heteromeric association with P2X6 (P2X4/6) and as heterotrimeric P2X2/4/6 [60, 61]. Activation of pre-synaptic P2X4 increases the release of neurotransmitters such as glutamate and GABA in neurons of the arcuate nucleus of the hypothalamus to regulate food intake [58]. At the post-synaptic level, ATP released by neurons or glial cells acting on postsynaptic P2X4

receptors modulates the activity and plasticity of excitatory and inhibitory synapses in several areas of the brain [10, 62]. P2X4 can modulate excitatory synaptic transmission and plasticity by acting on glutamatergic NMDA and AMPA receptors [63]. The influx of Ca^{2+} *via* the opening of either P2X2 or P2X4 channel pores can trigger the internalization of AMPARs by phosphorylation of the GluA1 subunit [10, 11] *in vitro*, although in hippocampal neurons, this action seems to be mediated by P2X2 rather than P2X4, consistent with the low surface expression of hippocampal neuronal P2X4 in basal conditions [10]. P2X4 receptors can also modulate the expression of NMDA-dependent long-term potentiation (LTP) in CA1 neurons of the hippocampus. Indeed, the pharmacological blockade of P2X4 facilitates the induction of NMDAR-dependent LTP [64], suggesting that P2X4 receptors have a negative impact on LTP. However, another study has shown that potentiation of P2X4 by ivermectin, a positive modulator of P2X4, increases LTP in the hippocampal CA1 region [57]. This effect was not found in P2X4-KO mice. The results suggested that the Ca^{2+} influx *via* postsynaptic P2X4, by promoting the incorporation of synaptic NMDARs, would strengthen synaptic activity during LTP [65]. ATP originating from individual excitatory synapses or glial cells can activate P2X4 in neocortical neurons and in turn reduce the currents induced by NMDAR activation. This effect does not occur in P2X4KO mice in which LTP is increased [62]. Altogether, these results point to the multiple neuromodulatory actions of P2X4 at central excitatory synapses.

P2X4 is also capable of modulating inhibitory GABA synapses. The physical interaction between postsynaptic P2X4 and GABA_A receptors in the ventromedial nucleus of the hypothalamus leads to an inhibition of GABA-mediated postsynaptic currents and consequently enhances neuronal excitability [36, 66]. Interestingly, this effect was found solely after blockade of P2X4 internalization using a competitive peptide, leading to an increase in the surface expression of P2X4 [36]. P2X4-mediated attenuation of tonic and phasic GABAergic inhibition has also been reported in cortical neurons. In this case, the effect is apparently mediated by P2X4-induced Ca^{2+} influx and a PKC-dependent downregulation of GABA_A receptors [6, 67].

Glial P2X4

Glial cells are themselves capable of releasing ATP, which can play an important role in neuron-glia and glial network signaling such as propagating glial Ca^{2+} waves [68]. P2X4 is expressed in microglia with P2X7, and both receptors can interact with each other. The existence of P2X4/7 heteromers remains controversial and the interaction may occur directly or indirectly between homomeric P2X4 and

P2X7 [69]. The *de novo* expression of P2X4 and/or increased surface trafficking of P2X4 reported in activated microglia [42] play key roles in many CNS diseases (see below). The upregulation of microglial P2X4 was revealed following lipopolysaccharide-induced inflammation in the hippocampus and selectively involved activated microglia in *dtTomato* reporter mice and internalization-defective P2X4mCherryIN knock-in mice [58, 59].

The P2X4 receptor has been revealed by immunostaining in S100 β -positive astrocytes of the rat hippocampus in the CA1, CA3 regions and the dentate gyrus [70]. P2X4 expression has also been detected in astrocytes in hippocampal cultures from P2X4mCherryIN mice, as well as in astrocyte end-feet contacting endothelial cells in brain slices from these mice solely by electron microscopy [59]. P2X4 receptors have also been detected in GFAP-positive astrocytes of the rat nucleus accumbens [71]. Nevertheless, P2X4 expression in astrocytes remains debated [72], although this discrepancy may merely reflect the heterogeneity of astrocytes across the different regions of the CNS. Indeed, a growing body of evidence suggests that astrocytes as well as microglia and oligodendrocytes display regional and contextual heterogeneity in terms of gene expression patterns, phenotypes, and roles in controlling physiological and pathological brain functions [73–75].

Recent studies have shown the presence of P2X4 in satellite glial cells (SGCs) in dorsal root ganglia stained by astrocytic markers. This expression was observed solely during neuropathic pain [76] and diabetes-induced neuropathy [77], suggesting a pathology-induced upregulation of P2X4 in SGCs. The expression of several types of P2 receptors, including P2X4, has also been demonstrated in oligodendroglial precursor cells [78]. However, its function is not yet fully understood, in contrast to P2X7 receptors which contribute to ATP excitotoxicity in oligodendrocytes and the pathogenesis of experimental autoimmune encephalomyelitis [79].

P2X4 has also been found in Schwann cells, mainly localized intracellularly in lysosomes, under normal physiological conditions. After peripheral nerve injury, overexpression of P2X4 receptors and their increase on the surface of Schwann cells has been shown to promote, through the release of brain-derived neurotrophic factor (BDNF), motor and sensory functional recovery and ameliorate nerve remyelination [80].

P2X4 in CNS Diseases

Neuropathic Pain

Acute pain serves a protective function in promoting reflex withdrawal from painful stimuli and the avoidance of

injury or damage. Acute pain is initiated by the activation of nociceptive neurons in peripheral dorsal root ganglia (DRG), information being processed and integrated within the spinal cord before transmission to the brain [81]. While acute pain is a normal physiological function of the CNS, chronic pain results from pathological and persistent alterations in the PNS and CNS [82]. Chronic pain can be induced by nerve damage, so-called neuropathic pain, or by persistent inflammatory pain associated with the healing of damaged tissue [83]. Chronic pain persists even after the expected period of healing and becomes disabling for the individual [84].

Peripheral nerve injury (PNI) has been shown to activate microglia in the spinal dorsal horn [85, 86] by several pathways involving interferon- γ and platelet-derived growth factor [87, 88]. Activation of spinal microglia causes tactile allodynia, a symptom of neuropathic pain, and several studies following the pioneered work of Tsuda *et al.* [89] showed that a specific microglial response phenotype characterized by the *de novo* expression of P2X4 is critical for the pathogenesis of neuropathic pain [67]. The expression of P2X4 increases exclusively in the reactive microglia after PNI and is confined to the ipsilateral dorsal horn (Fig. 2). Moreover, the genetic ablation or downregulation of P2X4 using either P2X4-KO mice or antisense RNA as well as the blockade of P2X4 by the antagonist TNP-ATP dramatically reduces the tactile allodynia after PNI [89–91]. Interestingly, activation of

microglia has also been reported in P2X4-KO mice after PNI [91], indicating that P2X4 is not required for microglial activation but is necessary and sufficient for the development of allodynia by neuron–glia communication. Indeed, activation of microglial P2X4 by ATP reduces GABA or glycine receptor-mediated inhibitory currents within the spinal cord. This disinhibition results from an increase in the intracellular concentration of Cl^- that becomes sufficient to block GABA- or glycine-mediated inhibitory responses or to eventually convert inhibition into excitation, thereby inducing the hypersensitivity of spinal neurons and tactile allodynia [92, 93]. Changes in Cl^- concentration are due to the downregulation of the K^+/Cl^- co-transporter KCC2, which is mediated by the extracellular release of BDNF and the activation of TrkB receptors in inhibitory neurons. Several studies have established the key role played by BDNF in this signaling cascade and the resulting disinhibition and pain-related behavior [92, 94]. Also established is the requirement for ATP-mediated activation of P2X4 receptors to elicit BDNF release by microglia [91], although how P2X4 actually triggers this release remains to be determined. In the dorsal horn, ATP can be released by the terminals of primary sensory neurons [95], dorsal horn neurons [96], and glial cells [7].

De novo P2X4 expression and/or increased surface trafficking of intracellular pools occur in glia or neurons of the spinal cord (inset) in various chronic pain conditions and amyotrophic lateral sclerosis (ALS). The contribution

Fig. 2 Implication of P2X4 in spinal cord disorders.

of P2X4 in the spinal cord (as well as in sensory ganglia for pain) is indicated. P2X4 potentiation was induced by ivermectin.

A recent study has shown that PNI increases the expression of the vesicular nucleotide transporter (VNUT) and induces a slight increase in the extracellular concentration of ATP in the spinal cord. By cell-specific deletion of VNUT, the authors elegantly showed that ATP released by spinal cord neurons and astrocytes, but not by sensory neurons, contributes to neuropathic pain [97], very likely by activating microglial P2X4-mediated signaling cascades. However, ATP is co-released with GABA in dorsal horn neurons that can activate simultaneously postsynaptic P2X and GABA_A receptors [96]. Cross-talk between neuronal P2X, including P2X4 and GABA_A receptors, may downregulate GABA-mediated currents and thus contribute to disinhibition in neuropathic pain [36, 96, 98].

Several mechanisms are involved in the increase of P2X4 expression in microglia following PNI. Two transcription factors of the interferon regulatory factor (IRF) family, IRF8 and IRF5, that are expressed in immune cells including microglia [99, 100] are upregulated after PNI in dorsal horn microglia and silencing IRF8 has been shown to suppress PNI-induced tactile allodynia [100]. IRF8 promotes several actors involved in chronic pain [100], and IRF5 expression in turn directly controls P2X4 transcription by binding to the promoter region of the *p2rx4* gene [100]. Fibronectin also appears to be a key regulator of P2X4 expression through several pathways. The increase in extracellular fibronectin after PNI could induce *de novo* P2X4 expression through several fibronectin/integrin signaling pathways [90, 101], as well as promoting P2X4 trafficking from the lysosome to the cell surface through the release of the chemokine CCL2 and subsequent activation of microglial CCR2 receptors [102].

Upregulation of P2X4 in spinal microglia is likely to play a key role in the tactile allodynia induced by PNI, although interestingly, recent work has suggested that this mechanism occurs only in males [94, 103]. In contrast to male mice following PNI, no increase in P2X4 expression occurs in the dorsal horn microglia of post-PNI female mice and tactile allodynia is not modified by the pharmacological blockade of P2X4 with TNP-ATP [94]. Results suggest that immune cells contribute to pain hypersensitivity in female mice [94, 104], although other studies have indicated that microglial P2X4 and BDNF/TrkB signaling contribute to bone cancer [105] and herpetic pain [16] models in female rodents, thus demonstrating the need for additional studies to clarify this sexual dimorphism.

Besides microglial P2X4 in the spinal cord, P2X4 expression in sensory neurons was recently linked to neuropathic pain. All P2X subunit mRNAs are expressed in sensory DRG neurons [106], but the functional expression

and potential involvement in pain processing was only very recently found to be limited to homomeric P2X3 or heteromeric P2X2/3 receptors expressed in nociceptive neurons [107]. Recent work has shown that P2X4 is expressed at the mRNA and protein levels in sensory DRG neurons [77, 108–110] and that its expression increases in several neuropathic or inflammatory pain models in rodents [111]. P2X4 receptor expression has also been found in satellite glial cells (SGCs) which envelop the cell bodies of sensory neurons in the ganglia [110, 112]. Although it can be difficult to distinguish P2X4 in the plasma membrane of sensory neurons or SCGs due to the apposition of both elements, several lines of evidence have established that P2X4 expression increases in SCGs under neuropathic pain conditions [77, 111, 113]. After PNI, the coupling between the SCGs themselves and between SCGs and neurons increases *via* the formation of gap junctions [114, 115] and activated SCGs release inflammatory mediators and other neuromodulators that contribute to neuronal hyperexcitability and pain hypersensitivity [115].

Chronic Inflammatory Pain

P2X4 receptors expressed in distinct cell types also contribute to inflammatory pain. For example, the presence of P2X4 in skin-resident macrophages contributes to the sensitization of peripheral sensory nerve termini and induces mechanical hypersensitivity due to the production of the inflammatory factor PGE2 [116]. P2X4 also plays a role in mechanical hypersensitivity following chronic inflammation by promoting the hyperexcitability of dorsal horn neurons in mouse models of chronic inflammation [116] (Fig. 2). On the other hand, such hyperexcitability is downregulated in P2X4-deficient mice [117]. A recent study showed that the expression of P2X4 receptors in small-diameter nociceptive neurons of DRGs doubles during chronic inflammation [109]. In addition, these sensory neurons express BDNF, a key factor in both neuropathic and inflammatory pain. Indeed, in chronic inflammatory conditions, BDNF contributes to mechanical hypersensitivity using a similar pathway. However, unlike neuropathic pain, during chronic inflammation, BDNF is released in a P2X4-dependent manner by sensory neurons termini in the dorsal horn, thereby leading *via* the TrkB/KCC2 cascade to spinal disinhibition [118]. P2X4 expressed in SCGs may also contribute to chronic inflammatory pain [112, 119] by releasing pro-inflammatory cytokines such as IL1 β and TNF α following activation of the NLRP1 inflammasome [116, 119].

Migraine

Chronic migraine is a neurological disorder characterized by repeated attacks that can be caused by an increase in the excitability of central neurons in the trigeminal nociceptive pathway, principally the trigeminal nucleus caudalis (TNC), leading to central sensitization [120]. Similar to neuropathic pain, microglia surrounding TNC neurons have been suggested to play direct and indirect roles in the establishment of central sensitization [121]. Indeed, microglial activation has been found when nitroglycerin (NTG) is applied chronically in a model of chronic migraine, with a resultant effect on central sensitization [121]. As reported during neuropathic pain [89, 91], several studies have suggested a role for BDNF expressed in the trigeminovascular system in the pathophysiology of migraine [122] and an increase in P2X4 and BDNF expression has been recently reported in the TNC after chronic intermittent administration of NTG [121, 123]. Moreover, pharmacological inhibition of P2X4 has very recently been found to prevent hyperalgesia induced by NTG, and associated with an inhibition of p-ERK phosphorylation and calcitonin gene-related peptide (CGRP) release in the TNC [123]. This study also showed that in BV2 microglial cells, ATP triggers BDNF synthesis and release, which is in turn reduced by the P2X4 antagonists 5-BDBD and SB203580 [123]. In addition, potentiation of P2X4 by ivermectin induces sustained hyperalgesia and significantly increases the levels of p-ERK and CGRP release in the TNC [123]. These results therefore clearly indicate that microglial P2X4 contributes to the TNC neuronal hyperexcitability involved in chronic migraine.

Post-ischemic Inflammation

Brain ischemia is associated with post-ischemic inflammation, including glial cell activation along with an increase in extracellular ATP levels. Several studies have reported an upregulation of P2X4 receptors in activated microglial cells after ischemic brain injury [124–127] (Fig. 3). During hypoxic conditions, ATP is released in large amounts from cells damaged by ischemia and the activation of both P2Y₁₂ and P2X4 receptors that are present at a higher density in reactive microglia, mediates the chemotaxis of motile microglia towards the lesion site as well as the release of proinflammatory cytokines via the PI3K signaling pathway [125, 128, 129]. More recently, a neuroprotective role of P2X4 during ischemia has also been reported [130]. Specifically, the activation of P2X4 expressed in vascular endothelial cells by ATP release consequent to pressure shocks mediated by vascular ischemia promotes the upregulation of osteopontin, a neuroprotective molecule [130]. These results therefore highlight a new

mechanism whereby endothelial P2X4 induces ischemic tolerance.

In various brain disorders, *de novo* P2X4 expression and/or an increased surface trafficking of intracellular pools occur in either microglia, neurons, or both cell types. The relative contributions of neuronal and glial P2X4 in brain disorders are indicated (MS, multiple sclerosis; AD, Alzheimer's disease; PD, Parkinson's disease). P2X4 potentiation was induced by ivermectin. Increased P2X4 corresponds to results from P2X4mCherryIN knock-in mice.

Epilepsy

Epilepsy is a chronic neurological condition characterized by recurrent seizures that spread to neighboring cortices and lead to hippocampal neuronal loss and microglial activation (Fig. 3). To date, as for P2X₇, the involvement of P2X₄ in epilepsy remains unclear, and whether the latter is up- or down-regulated in the hippocampus following status epilepticus (SE) is still debated. Some studies have reported a lack of change, or even a decrease in P2X₄ immunoreactivity, 24 h after SE induced either by systemic pilocarpine or by intra-amygdala kainate injection. Kang *et al.* reported similar findings in seizure-sensitive compared with seizure-resistant gerbils [131], although the specificity of the anti-P2X₄ antibody used in these studies has not been established.

Subsequently, in an epileptic mouse model using intraperitoneal injection of kainate, hippocampal P2X₄ transcript and protein levels were found to increase 24 and 48 h after SE, and particularly in activated microglia in the stratum radiatum [132]. In P2X₄-KO mice, a decrease in microglial activation as well as subsequent cell death were found after the induction of SE [132]. Even though epileptic seizures induced by kainate are not strongly inhibited in P2X₄-KO mice, there is still a significant reduction of cell death in the hippocampus as well as a decrease in the amplitude of hyperpolarizing outward current activated by microglia [132]. As for neuropathic pain, the increase in *de novo* P2X₄ expression in activated microglia after the induction of epilepsy could facilitate BDNF release and the induction of hyperexcitability, thereby promoting epileptogenesis.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder and is characterized mainly by the accumulation of insoluble aggregates of beta-amyloid fragments (A β _{1–42}) that in turn leads to synaptic dysfunction and neuronal death causing a progressive loss of memory [133, 134]. Like other neurodegenerative diseases,

Fig. 3 Implication of P2X4 in brain disorders.

the increase of extracellular nucleotides concomitantly with exacerbated neuroinflammatory events play a crucial role in AD pathogenesis [135].

It has been proposed that ATP, acting through P2X receptors, could participate directly in some of the toxic effects of A β peptide in neurons, with a particularly important role of P2X4 receptors [136, 137]. Indeed, an accumulation of P2X4 has been reported in hippocampal neurons exposed to the A β peptide [138]. This expression increase is restricted to the cell body after 6 h of treatment, before also reaching the neurites after 12 h [138]. In parallel, after exposure to an A β fragment, the authors revealed the accumulation of a smaller P2X4 fragment, which was prevented by exposure to a caspase inhibitor, thus suggesting the occurrence of proteolytic cleavage of P2X4 receptors (Fig. 3). They concluded that the cleavage by caspase-3 slows down the closing time of the receptor's channel pore and prevents receptor agonist-dependent internalization. This leads to an increase in membrane surface trafficking of the neurons that can in turn induce neuronal toxicity due to increased Ca²⁺ influx [138]. In this

study, moreover, the overexpression of neuronal P2X4 was found to promote the toxic effect of the A β fragment while inhibition of receptor expression had a significant positive effect on neuronal death after A β exposure. Consequently, these findings led to the conclusion that neuronal P2X4 receptors are directly implicated in the neuronal death induced by A β [138]. Consistent with the involvement of neuronal P2X4 in AD, an increased surface density of neuronal P2X4 was recently shown to induce synaptic deficits and alterations in learning and memory functions in P2X4 internalization-defective knock-in mice. Surprisingly, however, the authors also reported a decrease in P2X4 protein levels in the middle frontal and temporal gyri of AD patients with severe cognitive impairment [138].

P2X7 has been shown to play a key role in the neuroinflammatory processes of AD and other neurodegenerative diseases, including PD, ALS, MS, and Huntington's disease, by promoting proinflammatory cytokine production by microglia and astrocytes [139–141]. Like P2X7, P2X4 could also be involved in the neuroinflammation associated with AD [142] and the resultant

potentiation of A β -mediated neuronal death, since similarities exist between these two receptor subtypes as well as neighboring chromosomal localization [69, 143], and because P2X4 has been correlated with several other inflammatory mechanisms [144, 145] and microglial activation processes [89].

Parkinson's Disease

PD is characterized by the progressive and specific loss of dopaminergic nigrostriatal neurons, causing impaired dopamine (DA) homeostasis and associated behaviors leading to motor troubles. A hallmark of PD is the presence of abnormal intracytoplasmic aggregates of α -synuclein, called Lewy bodies, within neuronal cell bodies. It is well known that P2X4 modulates major neurotransmitter systems including glutamatergic and GABAergic functions [10, 11, 36, 57, 62, 64–67, 146–150]. Several early studies suggested that P2X4 receptors are indirectly involved in DA neurotransmission [151, 152] and behavioral deficits are recovered in P2X4-KO mice that may also express DA dysfunction [153, 154]. A recent study has confirmed the receptor's direct role in regulating DA homeostasis that is impaired in PD [155]. Specifically, using P2X4-KO mice, the authors showed that a *p2rx4* deficit affects DA synthesis and transport, and increases DA receptor levels that could in turn alter DA neuronal function and neurotransmission, with a resulting impact on DA-associated behaviors such as motor control and sensorimotor gating [155]. These findings further indicate that P2X4 could be involved in multiple neurological disorders such as PD and psychiatric diseases that involve the impairment of DA homeostasis.

Neuroinflammation is also a main hallmark of PD [156–159] and microglia seem to be central in this neurodegenerative disease [160, 161]. As previously discussed, microglial P2X4 could be involved in the neuroinflammatory mechanisms that are common to neurodegenerative diseases, and for which, in the case of PD, P2X7 is known to play a role [162, 163]. Nevertheless, the involvement of microglia in the physiopathology of PD is still unclear and it remains unknown whether microglial alterations cause, or are a consequence of, DA neuronal degeneration [164].

Amyotrophic Lateral Sclerosis

ALS is a fatal neurodegenerative disease characterized by the progressive and selective loss of spinal motoneurons (MNs) as well as cortical MNs (Fig. 2). The causes of ALS are multifactorial and the mechanisms underlying the selective degeneration of MNs are still unknown.

A link has been established between ALS and P2X4 [165]. For example, a strong increase in P2X4 immunoreactivity in ventral horn MNs immediately preceding their death has been reported in the SOD1-G93A ALS rodent model, suggesting an important role of neuronal P2X4 receptors in the pathogenesis of the disease [166]. P2X4 immunoreactivity has also been associated with degeneration in other neuronal populations including noradrenergic neurons in the locus coeruleus and cerebellar Purkinje cells, and serotonergic neurons of the raphe nucleus [166]. Surprisingly, the same authors subsequently showed, by using anti-P2X4 antibodies directed against the intracellular C-tail of P2X4 subunits, that SOD1-G93A misfolded proteins are detectable in MNs but not in microglia [167]. This finding led to the suggestion that neuronal conformers of SOD1-G93A proteins that are reactive to anti-P2X4 antibodies could play a pathogenic role and induce neuroinflammation activating microglia and astrocytes when injected intracerebrally in normal animals [167].

An increase in P2X4 expression, both at the mRNA and protein levels, has also been reported in the microglia of SOD1-G93A mice [168]. More recently, Volonté and colleagues demonstrated for the first time an increase in P2X4 and P2X7 expression in the peripheral nervous system of SOD1-G93A mice, particularly in the sciatic nerve [165].

Other work has shown that potentiation of P2X4 function by ivermectin has a dual effect on MN survival depending on ATP concentration, with a beneficial action at low ATP levels, but becoming deleterious at higher levels [146]. The authors deduced that ivermectin potentiates the neuroprotective effect of P2X4 on MNs at low ATP levels, probably by increasing P2X4 function and/or the receptor's cell surface density [20, 22]. Moreover, the oral administration of ivermectin *via* the animal's drinking water from a presymptomatic stage (P50), caused a 10% increase in the lifespan of SOD1-G93A mice [146].

As for other neuroinflammatory diseases, the P2X4 receptor is also likely to be involved in neuroinflammatory processes associated with ALS and is known to be involved in the mechanisms of MN degeneration [169]. Several studies have highlighted a role of P2X7 in this disease [170], notably *via* the modulation of autophagy [171] as well as of microglia-mediated neuroinflammatory processes that may be both beneficial and deleterious according to the stage of the disease, [172, 173]. An interaction between P2X4 and P2X7 receptor signaling pathways could also be involved in microglial inflammatory actions, one pathway activating the signaling of the other. Such cross-talk is already known in macrophages, where P2X4 receptors regulate the inflammatory functions of P2X7 receptors [174]. Although the involvement of P2X4 in the

pathogenesis of ALS remains to be clearly established, this receptor subtype appears to be a potential therapeutic target for confronting this disease.

Multiple Sclerosis

MS is a chronic T-cell-mediated auto-immune disease characterized by massive infiltration of immune cells, demyelination, and axonal loss. This neurodegenerative disease is also characterized by early inflammation that is beneficial and initially delays the onset of neurodegeneration, which then evolves in a complex manner as the disease progresses [175]. The initial regenerative phase is due to the repairing actions of anti-inflammatory infiltrating macrophages and microglia.

Interestingly, P2X4 has been shown to be overexpressed in microglia and macrophages in a rat model of MS and experimental autoimmune encephalomyelitis (EAE) [127] (Fig. 3). But in contrast to neuropathic pain, where P2X4 blockers have been proposed to be potential therapeutic drugs [16], P2X4 antagonists exacerbate the neurological symptoms in animal models of MS, whereas P2X4 potentiation by ivermectin and the upregulation of P2X4 expression are both beneficial in EAE [176]. Moreover, microglial P2X4 activation is known to induce BDNF release [92] and it has been shown that BDNF release from microglia as a result of P2X4 potentiation or overexpression in Schwann cells promotes remyelination [80, 176].

In contrast, P2X4 potentiation by ivermectin increases myelin engulfment and degradation [176] and P2X4 activation in microglia induces endolysosomal membrane fusion and lysosome acidification, both of which are essential to phagocytic pathways [46, 176] that could control myelin phagocytosis. Lysosomes are also involved in secretion and myelin biogenesis [177, 178] and P2X4 has been implicated in lysosome exocytosis contributing to surfactant secretion in lung tissue [179]. Altogether, these findings therefore suggest a potential role for lysosomal P2X4 receptors in secretion and phagocytosis in myelin disorders.

Alcohol-Related Disorders

Several studies have pointed to a role of P2X4 in alcohol-induced behavior [180–183]. P2X4 receptors are expressed in brain regions involved in the reinforcing properties of alcohol and other drugs [184]. Furthermore, a recent study suggested the involvement of P2X4 in regulating striatal dopamine homeostasis [155], supporting a role of P2X4 in the mesolimbic dopamine system involved in the reward system (Fig. 3).

An increase in ethanol intake occurs in P2X4-KO mice, suggesting that P2X4 could also be involved in alcohol

intake and/or preference [182, 185]. Other studies have demonstrated lower P2X4 expression in rats that prefer alcohol, confirming the correlation between P2X4 expression and alcohol preference [183, 184]. Moreover, a direct link between P2X4 and the regulation of dopamine neurotransmission in the mesolimbic system has been established, further indicating that through its presence in dopaminergic neurons of the mesolimbic system, P2X4 plays a role in mediating alcohol drinking behavior [183].

Interestingly, low ethanol concentrations inhibit P2X4 *in vitro*, probably by blocking opening of the channel without affecting its deactivation [186, 187]. Conversely, ivermectin antagonizes the inhibition of P2X4 by ethanol, indicating that this positive modulator interferes with the linkage site of ethanol on the P2X4 receptor [188]. Altogether, these findings suggest that ethanol can reinforce alcohol intake *via* P2X4 inhibition and that pharmacological potentiation of P2X4 may reduce alcohol intake and preference.

Alcohol abuse is also known to promote neuroinflammation [189] and microglial P2X4 has recently been reported to play a role in this mechanism [190, 191]. Gofman and collaborators demonstrated that an alcohol-induced increase of P2X4 expression in microglia affects microglial migration and phagocytosis, and is reversed by a P2X4 selective antagonist [190]. Later, the same group found that, *via* P2X4, alcohol decreases the phosphorylation of key regulatory proteins and increases CREB transcriptional activity [191]. Cerebral ischemic events have also been reported in alcohol abuse, involving an upregulation of P2X4 in microglia following ischemia and hypoxia as described above [128, 192].

Neuropsychiatric Disorders

Depression, bipolar disorder, schizophrenia, attention deficit hyperactivity, and anxiety are neuropsychiatric disorders in which DA homeostasis is impaired. As noted above, P2X4 has been implicated in the regulation of DA homeostasis and sensory-motor gating [155] and there is increasing evidence that P2X4 plays a critical role in psychiatric disorders [59, 126, 155, 193, 194].

Consistent with an involvement of P2X4 receptors, ivermectin has been found to induce anxiolytic-like and depressive-like behavior in mice [193]. Very interestingly and in line with these findings, data from a new conditional P2X4 internalization-defective knock-in mouse, namely P2X4mCherryIN, that displays an increase in the number of P2X4 receptors at the surface of targeted cells, further supports the link between neuronal P2X4 and anxiety-like behavior as well as in memory [59]. The anxiolytic effects of a selective increase in surface P2X4 density in mouse excitatory forebrain neurons further underlines the role of

neuronal P2X4 in anxiety [59]. Another study on the role of P2X4 in ischemic stroke using P2X4-KO mice showed that P2X4 deletion predisposes animals to chronic depression-like behavior after stroke [126]. Significantly in this context, a recent study demonstrated that the potentiation of P2X4 by ivermectin leads to DA hyperactivity and the disruption of information processing because of a potential perturbation of the interaction between P2X4 and DA receptors [194]. Together, these results suggest that P2X4 antagonists could serve as novel anti-psychotic treatments for psychiatric disorders arising from sensorimotor gating impairments linked to the disruption of DA homeostasis [194].

Concluding Remarks

P2X4 is the most widespread P2X subunit expressed in several neuronal and glial cell types throughout the CNS. Homomeric and heteromeric P2X4-containing receptors are ATP-gated channels are highly permeable to Ca^{2+} and in basal conditions, are localized in the plasma membrane at low density and mostly in intracellular endosomal/lysosomal compartments. Growing evidence suggest that endo/lysosomal P2X4 receptors are functional and play important intracellular signaling roles. Intracellular P2X4 can also be rapidly mobilized to the cell surface and contribute, together with receptors expressed *de novo*, to the upregulation in specific cell types in many central disorders such as pain, neurodegenerative disorders, and neuropsychiatric diseases. However, establishing the function of upregulated P2X4 expressed in glial *versus* neuronal cells remain elusive. We have recently developed conditional internalization-defective P2X4mCherryIN knock-in mice with increasing numbers of P2X4 receptors at the surface of targeted cells and have demonstrated the role of surface neuronal P2X4 in synaptic plasticity and memory [59]. The development of such a transgenic knock-in mouse, along with the possibility of generating a conditional P2X4-KO line that targets specific cell populations, represent major tools to gain in the near future a better understanding of the role of surface P2X4 in various physiological and pathological conditions and to evaluate the potential of P2X4 as a therapeutic target in these various disorders. Intracellular P2X4 also plays important roles and extensive studies are now needed to evaluate the respective functions of intracellular *versus* surface P2X4 receptors, which may differ between the various cell types expressing P2X4. It is also important to identify the mechanisms and regulation of P2X4 trafficking leading to the cell surface increase in pathological conditions, as well as determining the functional significance of the equilibrium between intracellular and surface P2X4 receptors.

Acknowledgements This review was supported by the Centre National de la Recherche Scientifique, University of Bordeaux, and grants from Association pour la Recherche sur la Sclérose Latérale Amyotrophique, Initiative d'Excellence de Bordeaux, and Laboratoire d'Excellence BRAIN ANR-10-LABX-43.

Conflict of interest All authors declare that they have no competing interests.

References

1. North RA. P2X receptors. *Philos Trans R Soc Lond B Biol Sci* 2016, 371.
2. Holton P. The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. *J Physiol* 1959, 145: 494–504.
3. Burnstock G. Purinergic nerves. *Pharmacol Rev* 1972, 24: 509–581.
4. Khakh BS, North RA. Neuromodulation by extracellular ATP and P2X receptors in the CNS. *Neuron* 2012, 76: 51–69.
5. Pankratov Y, Lalo U, Verkhratsky A, North RA. Vesicular release of ATP at central synapses. *Pflügers Arch* 2006, 452: 589–597.
6. Boué-Grabot E, Pankratov Y. Modulation of central synapses by astrocyte-released ATP and postsynaptic P2X receptors. *Neural Plast* 2017, 2017: 9454275.
7. Imura Y, Morizawa Y, Komatsu R, Shibata K, Shinozaki Y, Kasai H, *et al.* Microglia release ATP by exocytosis. *Glia* 2013, 61: 1320–1330.
8. Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H. Purinergic signalling in the nervous system: An overview. *Trends Neurosci* 2009, 32: 19–29.
9. Rodrigues RJ, Tome AR, Cunha RA. ATP as a multi-target danger signal in the brain. *Front Neurosci* 2015, 9: 148.
10. Pougnet JT, Toulme E, Martinez A, Choquet D, Hosy E, Boué-Grabot E. ATP P2X receptors downregulate AMPA receptor trafficking and postsynaptic efficacy in hippocampal neurons. *Neuron* 2014, 83: 417–430.
11. Pougnet JT, Compans B, Martinez A, Choquet D, Hosy E, Boué-Grabot E. P2X-mediated AMPA receptor internalization and synaptic depression is controlled by two CaMKII phosphorylation sites on GluA1 in hippocampal neurons. *Sci Rep* 2016, 6: 31836.
12. Suurvali J, Boudinot P, Kanellopoulos J, Ruutel Boudinot S. P2X4: A fast and sensitive purinergic receptor. *Biomed J* 2017, 40: 245–256.
13. Rubio ME, Soto F. Distinct Localization of P2X receptors at excitatory postsynaptic specializations. *J Neurosci* 2001, 21: 641–653.
14. Soto F, Garcia-Guzman M, Karschin C, Stuhmer W. Cloning and tissue distribution of a novel P2X receptor from rat brain. *Biochem Biophys Res Commun* 1996, 223: 456–460.
15. Werner S, Mesch S, Hillig RC, Ter Laak A, Klint J, Neagoe I, *et al.* Discovery and characterization of the potent and selective P2X4 inhibitor N-[4-(3-Chlorophenoxy)-3-sulfamoylphenyl]-2-phenylacetamide (BAY-1797) and structure-guided amelioration of its CYP3A4 induction profile. *J Med Chem* 2019, 62: 11194–11217.
16. Matsumura Y, Yamashita T, Sasaki A, Nakata E, Kohno K, Masuda T, *et al.* A novel P2X4 receptor-selective antagonist produces anti-allodynic effect in a mouse model of herpetic pain. *Sci Rep* 2016, 6: 32461.
17. Ase AR, Honson NS, Zaghdane H, Pfeifer TA, Seguela P. Identification and characterization of a selective allosteric

- antagonist of human P2X4 receptor channels. *Mol Pharmacol* 2015, 87: 606–616.
18. Twum-Danso NA. Serious adverse events following treatment with ivermectin for onchocerciasis control: A review of reported cases. *Filaria J* 2003, 2 Suppl 1: S3.
  19. Khakh BS, Proctor WR, Dunwiddie TV, Labarca C, Lester HA. Allosteric control of gating and kinetics at P2X(4) receptor channels. *J Neurosci* 1999, 19: 7289–7299.
  20. Silberberg SD, Li M, Swartz KJ. Ivermectin Interaction with transmembrane helices reveals widespread rearrangements during opening of P2X receptor channels. *Neuron* 2007, 54: 263–274.
  21. Stokes L. Rab5 regulates internalisation of P2X4 receptors and potentiation by ivermectin. *Purinergic Signal* 2012.
  22. Toulme E, Soto F, Garret M, Boue-Grabot E. Functional properties of internalization-deficient P2X4 receptors reveal a novel mechanism of ligand-gated channel facilitation by ivermectin. *Mol Pharmacol* 2006, 69: 576–587.
  23. Robinson LE, Murrell-Lagnado RD. The trafficking and targeting of P2X receptors. *Front Cell Neurosci* 2013, 7: 233.
  24. Murrell-Lagnado RD. A role for P2X4 receptors in lysosome function. *J Gen Physiol* 2018, 150: 185–187.
  25. Burnstock G. Purinergic signalling and disorders of the central nervous system. *Nat Rev Drug Discov* 2008, 7: 575–590.
  26. Brake AJ, Wagenbach MJ, Julius D. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature* 1994, 371: 519–523.
  27. Kawate T, Michel JC, Birdsong WT, Gouaux E. Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. *Nature* 2009, 460: 592–598.
  28. Hattori M, Gouaux E. Molecular mechanism of ATP binding and ion channel activation in P2X receptors. *Nature* 2012, 485: 207–212.
  29. Mansoor SE, Lu W, Oosterheert W, Shekhar M, Tajkhorshid E, Gouaux E. X-ray structures define human P2X(3) receptor gating cycle and antagonist action. *Nature* 2016, 538: 66–71.
  30. Igawa T, Abe Y, Tsuda M, Inoue K, Ueda T. Solution structure of the rat P2X4 receptor head domain involved in inhibitory metal binding. *FEBS Lett* 2015, 589: 680–686.
  31. Chaumont S, Jiang LH, Penna A, North RA, Rassendren F. Identification of a trafficking motif involved in the stabilization and polarization of P2X receptors. *J Biol Chem* 2004, 279: 29628–29638.
  32. Boue-Grabot E, Archambault V, Seguela P. A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of P2X(2) ATP-gated channels. *J Biol Chem* 2000, 275: 10190–10195.
  33. Qureshi OS, Paramasivam A, Yu JC, Murrell-Lagnado RD. Regulation of P2X4 receptors by lysosomal targeting, glycan protection and exocytosis. *J Cell Sci* 2007, 120: 3838–3849.
  34. Bobanovic LK, Royle SJ, Murrell-Lagnado RD. P2X receptor trafficking in neurons is subunit specific. *J Neurosci* 2002, 22: 4814–4824.
  35. Royle SJ, Bobanovic LK, Murrell-Lagnado RD. Identification of a non-canonical tyrosine-based endocytic motif in an ionotropic receptor. *J Biol Chem* 2002, 277: 35378–35385.
  36. Jo YH, Donier E, Martinez A, Garret M, Toulme E, Boue-Grabot E. Cross-talk between P2X4 and gamma-aminobutyric acid, type A receptors determines synaptic efficacy at a central synapse. *J Biol Chem* 2011, 286: 19993–20004.
  37. Boumechache M, Masin M, Edwardson JM, Gorecki DC, Murrell-Lagnado R. Analysis of assembly and trafficking of native P2X4 and P2X7 receptor complexes in rodent immune cells. *J Biol Chem* 2009, 284: 13446–13454.
  38. Royle SJ, Qureshi OS, Bobanovic LK, Evans PR, Owen DJ, Murrell-Lagnado RD. Non-canonical YXXGPhi endocytic motifs: recognition by AP2 and preferential utilization in P2X4 receptors. *J Cell Sci* 2005, 118: 3073–3080.
  39. Xu J, Chai H, Ehinger K, Egan TM, Srinivasan R, Frick M, *et al.* Imaging P2X4 receptor subcellular distribution, trafficking, and regulation using P2X4-pHluorin. *J Gen Physiol* 2014, 144: 81–104.
  40. Toulme E, Garcia A, Samways D, Egan TM, Carson MJ, Khakh BS. P2X4 receptors in activated C8-B4 cells of cerebellar microglial origin. *J Gen Physiol* 2010, 135: 333–353.
  41. Stokes L, Surprenant A. Dynamic regulation of the P2X4 receptor in alveolar macrophages by phagocytosis and classical activation. *Eur J Immunol* 2009, 39: 986–995.
  42. Trang T, Salter MW. P2X4 purinoceptor signaling in chronic pain. *Purinergic Signal* 2012, 8: 621–628.
  43. Murrell-Lagnado RD, Frick M. P2X4 and lysosome fusion. *Curr Opin Pharmacol* 2019, 47: 126–132.
  44. Zhang Z, Chen G, Zhou W, Song A, Xu T, Luo Q, *et al.* Regulated ATP release from astrocytes through lysosome exocytosis. *Nat Cell Biol* 2007, 9: 945–953.
  45. Besnard A, Gautherot J, Julien B, Tebbi A, Garcin I, Doignon I, *et al.* The P2X4 purinergic receptor impacts liver regeneration after partial hepatectomy in mice through the regulation of biliary homeostasis. *Hepatology* 2016, 64: 941–953.
  46. Cao Q, Zhong XZ, Zou Y, Murrell-Lagnado R, Zhu MX, Dong XP. Calcium release through P2X4 activates calmodulin to promote endolysosomal membrane fusion. *J Cell Biol* 2015, 209: 879–894.
  47. Bo X, Kim M, Nori SL, Schoepfer R, Burnstock G, North RA. Tissue distribution of P2X4 receptors studied with an ectodomain antibody. *Cell Tissue Res* 2003, 313: 159–165.
  48. Seguela P, Haghighi A, Soghomonian JJ, Cooper E. A novel neuronal P2x ATP receptor ion channel with widespread distribution in the brain. *J Neurosci* 1996, 16: 448–455.
  49. Lê KT, Villeneuve P, Ramjaun AR, McPherson PS, Beaudet A, Seguela P. Sensory presynaptic and widespread somatodendritic immunolocalization of central ionotropic P2X ATP receptors. *Neuroscience* 1998, 83: 177–190.
  50. Amadio S, Montilli C, Picconi B, Calabresi P, Volonte C. Mapping P2X and P2Y receptor proteins in striatum and substantia nigra: An immunohistological study. *Purinergic Signal* 2007, 3: 389–398.
  51. Stojilkovic SS. Purinergic regulation of hypothalamopituitary functions. *Trends Endocrinol Metab* 2009, 20: 460–468.
  52. Lalo U, Verkhatsky A, Pankratov Y. Ivermectin potentiates ATP-induced ion currents in cortical neurones: evidence for functional expression of P2X4 receptors? *Neurosci Lett* 2007, 421: 158–162.
  53. Tan Y, Zhao B, Zeng QC, Shi CM, Zhao FB, Li ZW. Characteristics of ATP-activated current in nodose ganglion neurons of rats. *Neurosci Lett* 2009, 459: 25–29.
  54. Luo J, Yin GF, Gu YZ, Liu Y, Dai JP, Li C, *et al.* Characterization of three types of ATP-activated current in relation to P2X subunits in rat trigeminal ganglion neurons. *Brain Res* 2006, 1115: 9–15.
  55. Ito K, Chihara Y, Iwasaki S, Komuta Y, Sugawara M, Sahara Y. Functional ligand-gated purinergic receptors (P2X) in rat vestibular ganglion neurons. *Hear Res* 2010, 267: 89–95.
  56. Wheeler-Schilling TH, Marquardt K, Kohler K, Guenther E, Jabs R. Identification of purinergic receptors in retinal ganglion cells. *Brain Res Mol Brain Res* 2001, 92: 177–180.
  57. Sim JA, Chaumont S, Jo J, Ulmann L, Young MT, Cho K, *et al.* Altered hippocampal synaptic potentiation in P2X4 knock-out mice. *J Neurosci* 2006, 26: 9006–9009.
  58. Xu J, Bernstein AM, Wong A, Lu XH, Khoja S, Yang XW, *et al.* P2X4 receptor reporter mice: Sparse brain expression and

- feeding-related presynaptic facilitation in the arcuate nucleus. *J Neurosci* 2016, 36: 8902–8920.
59. Bertin E, Deluc T, Pilch KS, Martinez A, Pougnet JT, Doudnikoff E, *et al.* Increased surface P2X4 receptor regulates anxiety and memory in P2X4 internalization-defective knock-in mice. *Mol Psychiatry* 2020.
  60. Le KT, Babinski K, Seguela P. Central P2X4 and P2X6 channel subunits coassemble into a novel heteromeric ATP receptor. *J Neurosci* 1998, 18: 7152–7159.
  61. Antonio LS, Stewart AP, Varanda WA, Edwardson JM. Identification of P2X2/P2X4/P2X6 heterotrimeric receptors using atomic force microscopy (AFM) imaging. *FEBS Lett* 2014, 588: 2125–2128.
  62. Lalo U, Palygin O, Verkhratsky A, Grant SG, Pankratov Y. ATP from synaptic terminals and astrocytes regulates NMDA receptors and synaptic plasticity through PSD-95 multi-protein complex. *Sci Rep* 2016, 6: 33609.
  63. Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron* 2009, 61: 340–350.
  64. Pankratov Y, Lalo U, Krishtal OA, Verkhratsky A. P2X receptors and synaptic plasticity. *Neuroscience* 2009, 158: 137–148.
  65. Baxter AW, Choi SJ, Sim JA, North RA. Role of P2X4 receptors in synaptic strengthening in mouse CA1 hippocampal neurons. *Eur J Neurosci* 2011, 34: 213–220.
  66. Jo YH, Boue-Grabot E. Interplay between ionotropic receptors modulates inhibitory synaptic strength. *Commun Integr Biol* 2011, 4: 706–709.
  67. Lalo U, Palygin O, Rasooli-Nejad S, Andrew J, Haydon PG, Pankratov Y. Exocytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex. *PLoS Biol* 2014, 12: e1001747.
  68. Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, Kater SB. ATP released from astrocytes mediates glial calcium waves. *J Neurosci* 1999, 19: 520–528.
  69. Craigie E, Birch RE, Unwin RJ, Wildman SS. The relationship between P2X4 and P2X7: a physiologically important interaction? *Front Physiol* 2013, 4: 216.
  70. Kukley M, Barden JA, Steinhäuser C, Jabs R. Distribution of P2X receptors on astrocytes in juvenile rat hippocampus. *Glia* 2001, 36: 11–21.
  71. Franke H, Grosche J, Schädlich H, Krugel U, Allgaier C, Illes P. P2X receptor expression on astrocytes in the nucleus accumbens of rats. *Neuroscience* 2001, 108: 421–429.
  72. Stokes L, Layhadi JA, Bibic L, Dhuna K, Fountain SJ. P2X4 Receptor function in the nervous system and current breakthroughs in pharmacology. *Front Pharmacol* 2017, 8: 291.
  73. Tan YL, Yuan Y, Tian L. Microglial regional heterogeneity and its roles in the brain. *Mol Psychiatry* 2020, 25: 351–367.
  74. Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcao A, Xiao L, *et al.* Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science* 2016, 352: 1326–1329.
  75. John Lin CC, Yu K, Hatcher A, Huang TW, Lee HK, Carlson J, *et al.* Identification of diverse astrocyte populations and their malignant analogs. *Nat Neurosci* 2017, 20: 396–405.
  76. Deng Z, Li C, Du E, Liu C, Xia B, Chen H, *et al.* Catestatin enhances neuropathic pain mediated by P2X4 receptor of dorsal root ganglia in a rat model of chronic constriction injury. *Cell Physiol Biochem* 2018, 51: 812–826.
  77. Teixeira JM, Dos Santos GG, Neves AF, Athie MCP, Bonet IJM, Nishijima CM, *et al.* Diabetes-induced neuropathic mechanical hyperalgesia depends on P2X4 receptor activation in dorsal root ganglia. *Neuroscience* 2019, 398: 158–170.
  78. Agresti C, Meomartini ME, Amadio S, Ambrosini E, Volonte C, Aloisi F, *et al.* ATP regulates oligodendrocyte progenitor migration, proliferation, and differentiation: involvement of metabotropic P2 receptors. *Brain Res Brain Res Rev* 2005, 48: 157–165.
  79. Matute C, Torre I, Perez-Cerda F, Perez-Samartin A, Alberdi E, Etchebarria E, *et al.* P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. *J Neurosci* 2007, 27: 9525–9533.
  80. Su WF, Wu F, Jin ZH, Gu Y, Chen YT, Fei Y, *et al.* Overexpression of P2X4 receptor in Schwann cells promotes motor and sensory functional recovery and remyelination via BDNF secretion after nerve injury. *Glia* 2019, 67: 78–90.
  81. Todd AJ. Neuronal circuitry for pain processing in the dorsal horn. *Nat Rev Neurosci* 2010, 11: 823–836.
  82. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell* 2009, 139: 267–284.
  83. Ji RR. Recent progress in understanding the mechanisms of pain and itch: the second special issue. *Neurosci Bull* 2018, 34: 1–3.
  84. Inoue K. Role of the P2X4 receptor in neuropathic pain. *Curr Opin Pharmacol* 2019, 47: 33–39.
  85. Gilmore SA. Proliferation of non-neuronal cells in spinal cords of irradiated, immature rats following transection of the sciatic nerve. *Anat Rec* 1975, 181: 799–811.
  86. Gilmore SA, Skinner RD. Intraspinal non-neuronal cellular responses to peripheral nerve injury. *Anat Rec* 1979, 194: 369–387.
  87. Masuda J, Tsuda M, Tozaki-Saitoh H, Inoue K. Intrathecal delivery of PDGF produces tactile allodynia through its receptors in spinal microglia. *Mol Pain* 2009, 5: 23.
  88. Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. *Proc Natl Acad Sci U S A* 2005, 102: 5856–5861.
  89. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, *et al.* P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 2003, 424: 778–783.
  90. Tsuda M, Toyomitsu E, Kometani M, Tozaki-Saitoh H, Inoue K. Mechanisms underlying fibronectin-induced up-regulation of P2X4R expression in microglia: distinct roles of PI3K-Akt and MEK-ERK signalling pathways. *J Cell Mol Med* 2009, 13: 3251–3259.
  91. Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, *et al.* Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J Neurosci* 2008, 28: 11263–11268.
  92. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, *et al.* BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005, 438: 1017–1021.
  93. Trang T, Beggs S, Wan X, Salter MW. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J Neurosci* 2009, 29: 3518–3528.
  94. Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, *et al.* Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat Neurosci* 2015, 18: 1081–1083.
  95. Nakatsuka T, Gu JG. ATP P2X receptor-mediated enhancement of glutamate release and evoked EPSCs in dorsal horn neurons of the rat spinal cord. *J Neurosci* 2001, 21: 6522–6531.
  96. Jo YH, Schlichter R. Synaptic corelease of ATP and GABA in cultured spinal neurons. *Nat Neurosci* 1999, 2: 241–245.
  97. Masuda T, Ozono Y, Mikuriya S, Kohro Y, Tozaki-Saitoh H, Iwatsuki K, *et al.* Dorsal horn neurons release extracellular ATP in a VNUT-dependent manner that underlies neuropathic pain. *Nat Commun* 2016, 7: 12529.

98. Boue-Grabot E, Toulme E, Emerit MB, Garret M. Subunit-specific coupling between gamma-aminobutyric acid type A and P2X2 receptor channels. *J Biol Chem* 2004, 279: 52517–52525.
99. Masuda T, Tsuda M, Yoshinaga R, Tozaki-Saitoh H, Ozato K, Tamura T, *et al.* IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep* 2012, 1: 334–340.
100. Masuda T, Iwamoto S, Yoshinaga R, Tozaki-Saitoh H, Nishiyama A, Mak TW, *et al.* Transcription factor IRF5 drives P2X4R+ reactive microglia gating neuropathic pain. *Nat Commun* 2014, 5: 3771.
101. Tsuda M, Toyomitsu E, Komatsu T, Masuda T, Kunifusa E, Nasu-Tada K, *et al.* Fibronectin/integrin system is involved in P2X(4) receptor upregulation in the spinal cord and neuropathic pain after nerve injury. *Glia* 2008, 56: 579–585.
102. Toyomitsu E, Tsuda M, Yamashita T, Tozaki-Saitoh H, Tanaka Y, Inoue K. CCL2 promotes P2X4 receptor trafficking to the cell surface of microglia. *Purinergic Signal* 2012, 8: 301–310.
103. Mapplebeck JC, Beggs S, Salter MW. Sex differences in pain: a tale of two immune cells. *Pain* 2016, 157 Suppl 1: S2–S6.
104. Tsuda M. Modulation of pain and itch by spinal glia. *Neurosci Bull* 2018, 34: 178–185.
105. Jin XH, Wang LN, Zuo JL, Yang JP, Liu SL. P2X4 receptor in the dorsal horn partially contributes to brain-derived neurotrophic factor oversecretion and toll-like receptor-4 receptor activation associated with bone cancer pain. *J Neurosci Res* 2014, 92: 1690–1702.
106. Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, Surprenant A, *et al.* Cloning OF P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J Neurosci* 1996, 16: 2495–2507.
107. Toulme E, Tsuda M, Khakh BS, Inoue K. On the role of ATP-Gated P2X receptors in acute, inflammatory and neuropathic pain. *Translational Pain Research: From Mouse to Man* 2010.
108. Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, *et al.* Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* 2015, 18: 145–153.
109. Lalisse S, Hua J, Lenoir M, Linck N, Rassendren F, Ulmann L. Sensory neuronal P2RX4 receptors controls BDNF signaling in inflammatory pain. *Sci Rep* 2018, 8: 964.
110. Kobayashi K, Yamanaka H, Noguchi K. Expression of ATP receptors in the rat dorsal root ganglion and spinal cord. *Anat Sci Int* 2013, 88: 10–16.
111. Ying M, Liu H, Zhang T, Jiang C, Gong Y, Wu B, *et al.* Effect of artemisinin on neuropathic pain mediated by P2X4 receptor in dorsal root ganglia. *Neurochem Int* 2017, 108: 27–33.
112. Kushnir R, Cherkas PS, Hanani M. Peripheral inflammation upregulates P2X receptor expression in satellite glial cells of mouse trigeminal ganglia: a calcium imaging study. *Neuropharmacology* 2011, 61: 739–746.
113. Yuan H, Ouyang S, Yang R, Li S, Gong Y, Zou L, *et al.* Osthole alleviated diabetic neuropathic pain mediated by the P2X4 receptor in dorsal root ganglia. *Brain Res Bull* 2018, 142: 289–296.
114. Zhang H, Mei X, Zhang P, Ma C, White FA, Donnelly DF, *et al.* Altered functional properties of satellite glial cells in compressed spinal ganglia. *Glia* 2009, 57: 1588–1599.
115. Hanani M, Blum E, Liu S, Peng L, Liang S. Satellite glial cells in dorsal root ganglia are activated in streptozotocin-treated rodents. *J Cell Mol Med* 2014, 18: 2367–2371.
116. Ulmann L, Hirbec H, Rassendren F. P2X4 receptors mediate PGE2 release by tissue-resident macrophages and initiate inflammatory pain. *EMBO J* 2010, 29: 2290–2300.
117. Aby F, Whitestone S, Landry M, Ulmann L, Fossat P. Inflammatory-induced spinal dorsal horn neurons hyperexcitability is mediated by P2X4 receptors. *Pain Rep* 2018, 3: e660.
118. Zhang W, Liu LY, Xu TL. Reduced potassium-chloride cotransporter expression in spinal cord dorsal horn neurons contributes to inflammatory pain hypersensitivity in rats. *Neuroscience* 2008, 152: 502–510.
119. Zhao S, Zhou Y, Fan Y, Gong Y, Yang J, Yang R, *et al.* Involvement of purinergic 2X4 receptor in glycoprotein 120-induced pyroptosis in dorsal root ganglia. *J Neurochem* 2019, 151: 584–594.
120. Dodick D, Silberstein S. Central sensitization theory of migraine: clinical implications. *Headache* 2006, 46 Suppl 4: S182–191.
121. Long T, He W, Pan Q, Zhang S, Zhang Y, Liu C, *et al.* Microglia P2X4 receptor contributes to central sensitization following recurrent nitroglycerin stimulation. *J Neuroinflammation* 2018, 15: 245.
122. Martins LB, Teixeira AL, Domingues RB. Neurotrophins and Migraine. *Vitam Horm* 2017, 104: 459–473.
123. Long T, He W, Pan Q, Zhang S, Zhang D, Qin G, *et al.* Microglia P2X4R-BDNF signalling contributes to central sensitization in a recurrent nitroglycerin-induced chronic migraine model. *J Headache Pain* 2020, 21: 4.
124. Cavaliere F, Florenzano F, Amadio S, Fusco FR, Viscomi MT, D'Ambrosi N, *et al.* Up-regulation of P2X2, P2X4 receptor and ischemic cell death: prevention by P2 antagonists. *Neuroscience* 2003, 120: 85–98.
125. Cheng RD, Ren JJ, Zhang YY, Ye XM. P2X4 receptors expressed on microglial cells in post-ischemic inflammation of brain ischemic injury. *Neurochem Int* 2014, 67: 9–13.
126. Verma R, Cronin CG, Hudobenko J, Venna VR, McCullough LD, Liang BT. Deletion of the P2X4 receptor is neuroprotective acutely, but induces a depressive phenotype during recovery from ischemic stroke. *Brain Behav Immun* 2017, 66: 302–312.
127. Vazquez-Villoldo N, Domercq M, Martin A, Llop J, Gomez-Vallejo V, Matute C. P2X4 receptors control the fate and survival of activated microglia. *Glia* 2014, 62: 171–184.
128. Li F, Wang L, Li JW, Gong M, He L, Feng R, *et al.* Hypoxia induced amoeboid microglial cell activation in postnatal rat brain is mediated by ATP receptor P2X4. *BMC Neurosci* 2011, 12: 111.
129. Ohsawa K, Irino Y, Nakamura Y, Akazawa C, Inoue K, Kohsaka S. Involvement of P2X4 and P2Y12 receptors in ATP-induced microglial chemotaxis. *Glia* 2007, 55: 604–616.
130. Ozaki T, Muramatsu R, Sasai M, Yamamoto M, Kubota Y, Fujinaka T, *et al.* The P2X4 receptor is required for neuroprotection via ischemic preconditioning. *Sci Rep* 2016, 6: 25893.
131. Kang TC, An SJ, Park SK, Hwang IK, Won MH. P2X2 and P2X4 receptor expression is regulated by a GABA(A) receptor-mediated mechanism in the gerbil hippocampus. *Brain Res Mol Brain Res* 2003, 116: 168–175.
132. Ulmann L, Levavasseur F, Avignone E, Peyrourou R, Hirbec H, Audinat E, *et al.* Involvement of P2X4 receptors in hippocampal microglial activation after status epilepticus. *Glia* 2013, 61: 1306–1319.
133. Murphy MP, LeVine H, 3rd. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis* 2010, 19: 311–323.
134. Sadigh-Eteghad S, Sabermarouf B, Majidi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. *Med Princ Pract* 2015, 24: 1–10.
135. Erb L, Woods LT, Khalafalla MG, Weisman GA. Purinergic signaling in Alzheimer's disease. *Brain Res Bull* 2019, 151: 25–37.
136. Saez-Orellana F, Godoy PA, Bastidas CY, Silva-Grecchi T, Guzman L, Aguayo LG, *et al.* ATP leakage induces P2XR activation and contributes to acute synaptic excitotoxicity

- induced by soluble oligomers of beta-amyloid peptide in hippocampal neurons. *Neuropharmacology* 2016, 100: 116–123.
137. Godoy PA, Ramirez-Molina O, Fuentealba J. Exploring the Role of P2X Receptors in Alzheimer's Disease. *Front Pharmacol* 2019, 10: 1330.
  138. Varma R, Chai Y, Troncoso J, Gu J, Xing H, Stojilkovic SS, *et al.* Amyloid-beta induces a caspase-mediated cleavage of P2X4 to promote purinotoxicity. *Neuromolecular Med* 2009, 11: 63–75.
  139. Martin E, Amar M, Dalle C, Youssef I, Boucher C, Le Duigou C, *et al.* New role of P2X7 receptor in an Alzheimer's disease mouse model. *Mol Psychiatry* 2019, 24: 108–125.
  140. Martinez-Frailes C, Di Lauro C, Bianchi C, de Diego-Garcia L, Sebastian-Serrano A, Bosca L, *et al.* Amyloid peptide induced neuroinflammation increases the P2X7 receptor expression in microglial cells, impacting on its functionality. *Front Cell Neurosci* 2019, 13: 143.
  141. Sperlagh B, Illes P. P2X7 receptor: an emerging target in central nervous system diseases. *Trends Pharmacol Sci* 2014, 35: 537–547.
  142. McGeer PL, Rogers J, McGeer EG. Inflammation, antiinflammatory agents, and Alzheimer's disease: The last 22 years. *J Alzheimers Dis* 2016, 54: 853–857.
  143. Weinhold K, Krause-Buchholz U, Rodel G, Kasper M, Barth K. Interaction and interrelation of P2X7 and P2X4 receptor complexes in mouse lung epithelial cells. *Cell Mol Life Sci* 2010, 67: 2631–2642.
  144. Li F, Guo N, Ma Y, Ning B, Wang Y, Kou L. Inhibition of P2X4 suppresses joint inflammation and damage in collagen-induced arthritis. *Inflammation* 2014, 37: 146–153.
  145. Layhadi JA, Turner J, Crossman D, Fountain SJ. ATP evokes Ca(2+) responses and CXCL5 secretion via P2X4 receptor activation in human monocyte-derived macrophages. *J Immunol* 2018, 200: 1159–1168.
  146. Andries M, Van Damme P, Robberecht W, Van Den Bosch L. Ivermectin inhibits AMPA receptor-mediated excitotoxicity in cultured motor neurons and extends the life span of a transgenic mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 2007, 25: 8–16.
  147. Boue-Grabot E, Emerit MB, Toulme E, Seguela P, Garret M. Cross-talk and co-trafficking between rho1/GABA receptors and ATP-gated channels. *J Biol Chem* 2004, 279: 6967–6975.
  148. Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS. Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat Neurosci* 2005, 8: 1078–1086.
  149. Gu JG, MacDermott AB. Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. *Nature* 1997, 389: 749–753.
  150. Pankratov YV, Lalo UV, Krishtal OA. Role for P2X receptors in long-term potentiation. *J Neurosci* 2002, 22: 8363–8369.
  151. Krugel U, Kittner H, Franke H, Illes P. Purinergic modulation of neuronal activity in the mesolimbic dopaminergic system in vivo. *Synapse* 2003, 47: 134–142.
  152. Xiao C, Zhou C, Li K, Davies DL, Ye JH. Purinergic type 2 receptors at GABAergic synapses on ventral tegmental area dopamine neurons are targets for ethanol action. *J Pharmacol Exp Ther* 2008, 327: 196–205.
  153. Zhou FC, Zhang JK, Lumeng L, Li TK. Mesolimbic dopamine system in alcohol-preferring rats. *Alcohol* 1995, 12: 403–412.
  154. Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA. Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: differential effects of D1 and D2 receptor antagonists. *J Neurosci* 2001, 21: 305–313.
  155. Khoja S, Shah V, Garcia D, Asatryan L, Jakowec MW, Davies DL. Role of purinergic P2X4 receptors in regulating striatal dopamine homeostasis and dependent behaviors. *J Neurochem* 2016, 139: 134–148.
  156. Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. *J Neural Transm Suppl* 2000: 143–151.
  157. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol* 2003, 106: 518–526.
  158. Mount MP, Lira A, Grimes D, Smith PD, Faucher S, Slack R, *et al.* Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci* 2007, 27: 3328–3337.
  159. Doorn KJ, Moors T, Drukarch B, van de Berg W, Lucassen PJ, van Dam AM. Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. *Acta Neuropathol Commun* 2014, 2: 90.
  160. Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. *Nat Rev Immunol* 2011, 11: 775–787.
  161. Joers V, Tansey MG, Mulas G, Carta AR. Microglial phenotypes in Parkinson's disease and animal models of the disease. *Prog Neurobiol* 2017, 155: 57–75.
  162. Wang XH, Xie X, Luo XG, Shang H, He ZY. Inhibiting purinergic P2X7 receptors with the antagonist brilliant blue G is neuroprotective in an intranigral lipopolysaccharide animal model of Parkinson's disease. *Mol Med Rep* 2017, 15: 768–776.
  163. Crabbe M, Van der Perren A, Bollaerts I, Kounelis S, Baekelandt V, Bormans G, *et al.* Increased P2X7 receptor binding is associated with neuroinflammation in acute but not chronic rodent models for Parkinson's disease. *Front Neurosci* 2019, 13: 799.
  164. Le W, Wu J, Tang Y. Protective microglia and their regulation in Parkinson's disease. *Front Mol Neurosci* 2016, 9: 89.
  165. Volonte C, Apolloni S, Parisi C, Amadio S. Purinergic contribution to amyotrophic lateral sclerosis. *Neuropharmacology* 2016, 104: 180–193.
  166. Casanovas A, Hernandez S, Tarabal O, Rossello J, Esquerda JE. Strong P2X4 purinergic receptor-like immunoreactivity is selectively associated with degenerating neurons in transgenic rodent models of amyotrophic lateral sclerosis. *J Comp Neurol* 2008, 506: 75–92.
  167. Hernandez S, Casanovas A, Piedrafita L, Tarabal O, Esquerda JE. Neurotoxic species of misfolded SOD1G93A recognized by antibodies against the P2X4 subunit of the ATP receptor accumulate in damaged neurons of transgenic animal models of amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 2010, 69: 176–187.
  168. D'Ambrosi N, Finocchi P, Apolloni S, Cozzolino M, Ferri A, Padovano V, *et al.* The proinflammatory action of microglial P2 receptors is enhanced in SOD1 models for amyotrophic lateral sclerosis. *J Immunol* 2009, 183: 4648–4656.
  169. Liu J, Wang F. Role of neuroinflammation in amyotrophic lateral sclerosis: Cellular mechanisms and therapeutic implications. *Front Immunol* 2017, 8: 1005.
  170. Cieslak M, Roszek K, Wujak M. Purinergic implication in amyotrophic lateral sclerosis-from pathological mechanisms to therapeutic perspectives. *Purinergic Signal* 2019, 15: 1–15.
  171. Fabbriozzi P, Amadio S, Apolloni S, Volonte C. P2X7 Receptor activation modulates autophagy in SOD1-G93A mouse microglia. *Front Cell Neurosci* 2017, 11: 249.
  172. Apolloni S, Amadio S, Montilli C, Volonte C, D'Ambrosi N. Ablation of P2X7 receptor exacerbates gliosis and motoneuron death in the SOD1-G93A mouse model of amyotrophic lateral sclerosis. *Hum Mol Genet* 2013, 22: 4102–4116.
  173. Sluyter R, Bartlett R, Ly D, Yerbury JJ. P2X7 receptor antagonism in amyotrophic lateral sclerosis. *Neural Regen Res* 2017, 12: 749–750.

174. Kawano A, Tsukimoto M, Mori D, Noguchi T, Harada H, Takenouchi T, *et al.* Regulation of P2X7-dependent inflammatory functions by P2X4 receptor in mouse macrophages. *Biochem Biophys Res Commun* 2012, 420: 102–107.
175. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* 2015, 15: 545–558.
176. Zabala A, Vazquez-Villoldo N, Rissiek B, Gejo J, Martin A, Palomino A, *et al.* P2X4 receptor controls microglia activation and favors remyelination in autoimmune encephalitis. *EMBO Mol Med* 2018, 10.
177. Feldmann A, Amphornrat J, Schonherr M, Winterstein C, Mobius W, Ruhwedel T, *et al.* Transport of the major myelin proteolipid protein is directed by VAMP3 and VAMP7. *J Neurosci* 2011, 31: 5659–5672.
178. Chen G, Zhang Z, Wei Z, Cheng Q, Li X, Li W, *et al.* Lysosomal exocytosis in Schwann cells contributes to axon remyelination. *Glia* 2012, 60: 295–305.
179. Fois G, Winkelmann VE, Bareis L, Staudenmaier L, Hecht E, Ziller C, *et al.* ATP is stored in lamellar bodies to activate vesicular P2X4 in an autocrine fashion upon exocytosis. *J Gen Physiol* 2018, 150: 277–291.
180. Yardley MM, Wyatt L, Khoja S, Asatryan L, Ramaker MJ, Finn DA, *et al.* Ivermectin reduces alcohol intake and preference in mice. *Neuropharmacology* 2012, 63: 190–201.
181. Wyatt LR, Finn DA, Khoja S, Yardley MM, Asatryan L, Alkana RL, *et al.* Contribution of P2X4 receptors to ethanol intake in male C57BL/6 mice. *Neurochem Res* 2014, 39: 1127–1139.
182. Franklin KM, Asatryan L, Jakowec MW, Trudell JR, Bell RL, Davies DL. P2X4 receptors (P2X4Rs) represent a novel target for the development of drugs to prevent and/or treat alcohol use disorders. *Front Neurosci* 2014, 8: 176.
183. Franklin KM, Hauser SR, Lasek AW, Bell RL, McBride WJ. Involvement of Purinergic P2X4 Receptors in Alcohol Intake of High-Alcohol-Drinking (HAD) Rats. *Alcohol Clin Exp Res* 2015, 39: 2022–2031.
184. Kimpel MW, Strother WN, McClintick JN, Carr LG, Liang T, Edenberg HJ, *et al.* Functional gene expression differences between inbred alcohol-preferring and -non-preferring rats in five brain regions. *Alcohol* 2007, 41: 95–132.
185. Asatryan L, Nam HW, Lee MR, Thakkar MM, Saeed Dar M, Davies DL, *et al.* Implication of the purinergic system in alcohol use disorders. *Alcohol Clin Exp Res* 2011, 35: 584–594.
186. Davies DL, Kuo ST, Alkana RL. Differential effects of propofol and ethanol on P2X4 receptors expressed in *Xenopus* oocytes. *Int Congr Ser* 2005, 1283: 285–287.
187. Ostrovskaya O, Asatryan L, Wyatt L, Popova M, Li K, Peoples RW, *et al.* Ethanol is a fast channel inhibitor of P2X4 receptors. *J Pharmacol Exp Ther* 2011, 337: 171–179.
188. Asatryan L, Popova M, Perkins D, Trudell JR, Alkana RL, Davies DL. Ivermectin antagonizes ethanol inhibition in purinergic P2X4 receptors. *J Pharmacol Exp Ther* 2010, 334: 720–728.
189. Potula R, Haorah J, Knipe B, Leibhart J, Chastil J, Heilman D, *et al.* Alcohol abuse enhances neuroinflammation and impairs immune responses in an animal model of human immunodeficiency virus-1 encephalitis. *Am J Pathol* 2006, 168: 1335–1344.
190. Gofman L, Cenna JM, Potula R. P2X4 receptor regulates alcohol-induced responses in microglia. *J Neuroimmune Pharmacol* 2014, 9: 668–678.
191. Gofman L, Fernandes NC, Potula R. Relative role of Akt, ERK and CREB in alcohol-induced microglia P2X4R receptor expression. *Alcohol Alcohol* 2016, 51: 647–654.
192. Wixey JA, Reinebrant HE, Carty ML, Buller KM. Delayed P2X4R expression after hypoxia-ischemia is associated with microglia in the immature rat brain. *J Neuroimmunol* 2009, 212: 35–43.
193. Bortolato M, Yardley MM, Khoja S, Godar SC, Asatryan L, Finn DA, *et al.* Pharmacological insights into the role of P2X4 receptors in behavioural regulation: lessons from ivermectin. *Int J Neuropsychopharmacol* 2013, 16: 1059–1070.
194. Khoja S, Asatryan L, Jakowec MW, Davies DL. Dopamine receptor blockade attenuates purinergic P2X4 receptor-mediated prepulse inhibition deficits and underlying molecular mechanisms. *Front Cell Neurosci* 2019, 13: 331.