



HAL
open science

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

Alexia Duveau, Éléonore Bertin, Eric Boué-Grabot

► **To cite this version:**

Alexia Duveau, Éléonore Bertin, Eric Boué-Grabot. Implication of Neuronal Versus Microglial P2X4 Receptors in Central Nervous System Disorders. *Neuroscience Bulletin*, 2020, 10.1007/s12264-020-00570-y . hal-02997812

HAL Id: hal-02997812

<https://hal.science/hal-02997812>

Submitted on 10 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

This is an accepted pre-copyedit version of an article published in Neurosci. Bull.

The final authenticated version is available online Neurosci Bull. 2020;10.1007/

s12264-020-00570-y. doi:10.1007/s12264-020-00570-y

Implication of neuronal versus microglial P2X4 receptors in central nervous system disorders

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

¹Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France

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

*: Corresponding author: Eric Boué-Grabot (EB-G) Institut des Maladies Neurodégénératives (IMN) CNRS UMR 5293, Université de Bordeaux, 146 rue Léo Saignat, 33076 Bordeaux cedex, France; phone: 33 (0)5 33 51 47 95; email: eric.boue-grabot@u-bordeaux.fr

Key words : purinergic signaling / P2X / ATP / ligand-gated ion channels / chronic pain / brain trauma / ischemia / neurodegenerative diseases / alcohol / neuropsychiatric disorders

Abstract

The P2X4 receptor (P2X4) is an ATP-gated cation channel that is highly permeable to calcium and widely expressed in neuronal and glial cell types throughout the 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 *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 the potential of P2X4 as a therapeutic target in multiple disorders can be defined.

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 observed in the 1950s ^[2], Geoffrey Burnstock was the first to propose in 1972, the concept of purinergic signaling using ATP as a fast neurotransmitter ^[3]. ATP was later shown to mediate fast neurotransmission in the PNS and CNS alike, but is nowadays mostly considered as 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 or microglia ^[5-7]. Neuronal and glial released ATP exerts multiple actions in the neuromodulation of synaptic activity or 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 neuro-inflammation (for 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 A_{2A}R also P2Y6 (microglial

1
2
3 phagocytosis) and P2Y12 (microglial chemotaxis) participate in ATP danger signaling [9],
4
5 increasing evidence indicates that microglial or neuronal P2X4 receptors are upregulated and play
6
7 key roles in many CNS disorders. In this review, we focus on the function of neuronal and glial
8
9 P2X4 function in physiological and pathological conditions.
10
11
12
13
14
15
16
17
18

19 **Properties and function of P2X4 receptors**

20
21
22
23
24 P2X receptors are trimeric ATP-gated cation channels that are formed by homo or heteromeric
25
26 associations from 7 different subunits (P2X1 - P2X7) encoded by seven genes in mammals.
27
28 Functional P2X receptors are non-selective cation channels permeable to sodium (Na^+), potassium
29
30 (K^+) and calcium (Ca^{2+}) and their activation by ATP binding leads to cell depolarization and Ca^{2+}
31
32 influx. With the exception of P2X6, all homomeric P2X receptors are functional and display
33
34 different ATP sensitivities, current kinetics and pharmacological properties. Moreover, the variety
35
36 in expression pattern of the seven P2X subunits and the existence of heteromeric associations
37
38 in expression pattern of the seven P2X subunits and the existence of heteromeric associations
39
40 between these subunits contribute to the diversity and widespread action of ATP signaling [8].
41
42 Among the seven P2X subunits, P2X4 is the most widely distributed in various cell types
43
44 throughout the body [1, 12]. All P2X subunits are expressed in the CNS, in a heterogeneous manner
45
46 throughout the different structures, cell types and subcellular compartments [13], although P2X4 is one
47
48 of the main subunits found in both neurons and glial cells such as microglia and more
49
50 controversially in astrocytes, oligodendrocytes and Schwann cells (Table 1 and Figure 1) [14].
51
52 However, the subunit composition of P2X receptors in most CNS cells is not well defined, and the
53
54 physiological roles of P2X4 or P2X4-containing receptors is far from being deciphered due to the
55
56 paucity of selective pharmacological tools. TNP-ATP a weak and non-selective P2X4 antagonist or
57
58
59
60

1
2
3 5-BDBD a selective allosteric P2X4 antagonist, have been commonly used to show the involvement
4
5 of P2X4 in rodent CNS tissue ^[12] *in vitro* and *in vivo*. New potent antagonists of rodent P2X4
6
7 (BAY-1797 or NP-1815-PX) were recently identified ^[15, 16] as well as a highly selective human
8
9 P2X4 antagonist (BX430) with no effect on rodent P2X4 ^[17]. Ivermectin, which is an agonist of
10
11 invertebrate glutamate-gated chloride channels, is used as an antiparasitic drug in human medicine
12
13 ^[18]. This compound is also a very potent positive modulator of mammalian P2X4. However,
14
15 although highly selective for the P2X4 subunit among P2X subtypes, Ivermectin potentiates
16
17 human P2X7 currents and can modulate other ligand-gated ion channels such as GABA_A or
18
19 nicotinic receptors ^[19-22]. Ivermectin is a positive allosteric modulator of P2X4 that stabilizes this
20
21 subunit in the open state ^[20], which consequently may also prevent P2X4 internalization ^[21, 22].
22
23
24
25
26
27

28 **P2X4 trafficking**

29
30 Indeed, in contrast to other P2X subunits, P2X4 is constitutively and highly internalized, and as a
31
32 result, is found mainly in endosomal/lysosomal compartments thereby ensuring low surface
33
34 expression of these receptors in basal states ^[23]. Recent work showed that intracellular P2X4 in these
35
36 acidic compartments are functional and may have important functions in lysosome membrane fusion
37
38 ^[24]. In various pathological states, such as trauma, ischemia, chronic pain, neurodegenerative
39
40 processes and several neuropsychiatric disorders, *de novo* expression of P2X4 and/or an increase
41
42 in cell surface P2X4 density was observed in microglia and/or neurons, thus suggesting possible
43
44 key and multiple roles of neuronal and microglial P2X4 receptors in the establishment and/or
45
46 maintenance of these pathologies ^[25]. Changes in the intracellular expression of P2X4 may also have
47
48 important consequences in the pathophysiological context.
49
50
51

52
53 Similar to all seven P2X subunits, P2X4 has a topology with 2 transmembrane regions, linked by
54
55 a large extracellular domain and two intracellular amino and carboxyl termini ^[26]. The first X-ray
56
57 crystal structure of a P2X receptor resolved in a truncated form of the zebrafish P2X4 receptor in
58
59
60

1
2
3 its closed state ^[27] and then in complex with ATP ^[28] from crystallized human P2X3 in different
4
5 states ^[29] confirmed the subunit topology and trimeric organization of P2X receptors. The structure
6
7 of head domain of rat P2X4 has also been investigated ^[30]. These data also revealed the importance
8
9 of a large extracellular disulfide-rich region packed with N-linked glycosylation moieties and the
10
11 existence of three inter-subunit binding sites. They also showed that the binding of three molecules
12
13 of ATP is required to induce conformational changes leading to the opening of the channel pore
14
15 formed by the three transmembrane regions ^[28]. P2X subunits share several conserved motifs
16
17 within the intracellular domains, such as a YXXXK in the C-terminus that regulates surface
18
19 expression ^[31] and a TXK/R in the N-terminus which is a putative protein kinase C
20
21 phosphorylation site regulating current desensitization ^[32]. However, in terms of sequence and
22
23 length, the C-terminus domain is the most divergent between P2X subunits ^[12]. The mammalian
24
25 P2X4 subunit displays two endocytic tyrosine-based and di-leucine motifs within the C- and N-
26
27 termini, respectively, which are responsible for its constitutive internalization and main
28
29 localization in lysosome-related intracellular organelles ^[33]. This distribution is in contrast to other
30
31 P2X receptors such as P2X2, which are mainly found at the level of the plasma membrane ^[34].
32
33 Interestingly, the clathrin-dependent constitutive internalization of P2X4 is mediated by an
34
35 interaction between the non-canonic motif YXXGΦ and the μ2 subunit of the adaptive protein 2
36
37 (AP2), whereas the canonic YXXΦ motif is not involved ^[34, 35]. Mutations of the endocytic motif
38
39 YXXGL or blockade of clathrin-mediated internalization lead to an increase both in the number
40
41 of P2X4 at the plasma membrane and associated ATP currents^[22, 34-36]. Being highly glycosylated,
42
43 the extracellular loop of P2X4 allows it to resist the very acidic intralysosomal environment and
44
45 proteolysis. Intracellular P2X4 pools can thus remain intracellular or either recycle back to the cell
46
47 surface and dynamically regulate the number of surface P2X4 receptors ^[23, 34, 37-39]. Several studies
48
49 have shown that in macrophages and microglia, the surface trafficking of P2X4 is upregulated in
50
51 response to stimuli promoting lysosomal exocytosis or altering endocytosis ^[33, 40, 41]. In numerous
52
53
54
55
56
57
58
59
60

1
2
3 pathophysiological conditions, *de novo* expression of P2X4 and/or increased surface trafficking
4
5 had been observed [42] suggesting an important role of the dynamic regulation of P2X4 surface
6
7 trafficking in diseases (Figure 1).
8
9

10 11 12 **intracellular P2X4 function**

13
14 Finally, growing evidence suggests that lysosomal P2X4 may also have important physiological
15
16 functions (for review, see [43]). In lysosomes, the extracellular domain of P2X4 containing the
17
18 agonist binding sites faces the luminal compartment, which contains high concentrations of ATP
19
20 [44, 45] transported by the vesicular nucleotide transporter VNUT/SLC17A9 present in the
21
22 lysosomal membrane. Alkalinization of the intralysosomal pH may lead to P2X4 activation and
23
24 participate in endolysosomal fusion and vacuolation [43]. Indeed, Ca²⁺ flux through P2X4 activates
25
26 calmodulin which subsequently associates with the receptor channel and promotes vesicular fusion
27
28
29
30
31 [46].
32
33
34

35 **Neuronal P2X4**

36
37 P2X4 is expressed in various tissue cell types of peripheral organs including epithelial cells,
38
39 kidney, pancreas, lung, liver, cardiovascular system or immune cells (for review, see [12]) in which
40
41 it has important functions. In the CNS (Table 1), P2X4 along with P2X2 and P2X6, is the most
42
43 widely expressed P2X subunit in both neurons and glial cells [14, 47]. P2X4 was first identified and
44
45 cloned from brain tissues in 1996 by two independent groups [14, 48]. Their studies also showed the
46
47 expression of P2X4 mRNA in the dentate gyrus, CA1/CA3 pyramidal cells of the hippocampus
48
49 and in cerebellum Purkinje cells [14, 48]. Further immunohistochemical studies with anti-P2X4
50
51 antibodies confirmed the presence of P2X4 at the protein level in GABAergic interneurons of the
52
53 hippocampus, cerebellum and olfactory bulb [13, 49], spiny neurons of the striatum and substantia
54
55 nigra [49, 50], and in the hypothalamus and hypophysis [51]. Other studies reported P2X4 expression
56
57
58
59
60

1
2
3 in somatosensory cortical neurons ^[52], sensory nerves or ganglia ^[53-55] and the retina ^[56]. Electron
4
5 microscopy has indicated that neuronal P2X4 is located both pre- and post-synaptically, mainly at
6
7 the edge of the postsynaptic density in the peri- and extra-synaptic space ^[13]. This is consistent
8
9 with the idea that activation of synaptic P2X receptors by ATP coreleased with other
10
11 neurotransmitters such as glutamate or GABA did not mediate fast synaptic transmission but
12
13 modulate excitatory or inhibitory synapses in the CNS ^[6]. The widespread distribution of P2X4 in
14
15 CNS neurons was confirmed with P2X4 knockout mice expressing β -galactosidase instead of
16
17 P2X4 ^[57] and recently with a transgenic reporter mice expressing tdTomato under the control of a
18
19 P2X4 promoter and subsequently using a novel internalization-defective P2X4mcherryIN knockin
20
21 mouse ^[58, 59].

22
23
24
25
26 Neuronal P2X receptors located at pre- and post-synaptic sites act mainly as neuromodulators.
27
28 However, in most cases the specific contribution of P2X4 has been difficult to ascertain due to the
29
30 paucity of appropriate pharmacological or genetic tools. In addition, the P2X4 subunit may not
31
32 only form functional homomeric receptors, but can also assemble in heteromeric association with
33
34 P2X6 (P2X4/6) or as heterotrimeric P2X2/4/6 ^[60, 61]. Activation of pre-synaptic P2X4 increases
35
36 the release of neurotransmitters such as glutamate or GABA in neurons of the arcuate nucleus of
37
38 the hypothalamus regulating food intake ^[58]. At the post-synaptic level, ATP released by neurons
39
40 or glial cells acting on postsynaptic P2X4 receptors modulates activity and plasticity of excitatory
41
42 and inhibitory synapses in several areas of the brain ^[10, 62]. P2X4 can modulate excitatory synaptic
43
44 transmission and plasticity by acting on glutamatergic NMDA and AMPA receptors ^[63]. The influx
45
46 of Ca^{2+} *via* the opening of either P2X2 or P2X4 channel pores can trigger the internalization of
47
48 AMPARs by phosphorylation of the GluA1 subunit ^[10, 11] *in vitro*, although in hippocampal
49
50 neurons, this action seems to be mediated by P2X2 rather than P2X4, consistent with the low
51
52 surface expression of hippocampal neuronal P2X4 in basal conditions ^[10]. P2X4 receptors can also
53
54 modulate the expression of NMDA-dependent long-term potentiation (LTP) in CA1 neurons of
55
56
57
58
59
60

1
2
3 the hippocampus. Indeed, the pharmacological blockade of P2X4 facilitates the induction of
4 NMDAR-dependent LTP ^[64], suggesting that P2X4 have a negative impact on LTP. However,
5
6 another study has shown that potentiation of P2X4 by ivermectin (IVM), a positive modulator of
7
8 P2X4, increases LTP in the hippocampal CA1 region ^[57]. This effect was not observed in P2X4KO
9
10 mice. The results suggested that the Ca²⁺ influx *via* postsynaptic P2X4, by promoting the
11
12 incorporation of synaptic NMDARs, would strengthen synaptic activity during LTP ^[65]. ATP
13
14 originating from individual excitatory synapses or glial cells, can activate P2X4 in neocortical
15
16 neurons and in turn reduce the currents induced by NMDAR activation. This effect is not observed
17
18 in P2X4KO mice in which LTP is increased ^[62]. Altogether, these results point to the multiple
19
20 neuromodulator actions of P2X4 at central excitatory synapses.
21
22

23
24 P2X4 is also capable of modulating GABA inhibitory synapses. The physical interaction between
25
26 post-synaptic P2X4 and GABA_A receptors in the ventromedial nucleus of the hypothalamus leads
27
28 to an inhibition of GABA-mediated postsynaptic currents and consequently enhances neuronal
29
30 excitability ^[36, 66]. Interestingly, this effect was observed solely after blockade of P2X4
31
32 internalization using a competitive peptide, leading to the increase of surface expression of P2X4
33
34 ^[36]. P2X4-mediated attenuation of tonic and phasic GABAergic inhibition was also observed in
35
36 cortical neurons. In this case the effect is apparently mediated by P2X4-induced Ca²⁺ influx and a
37
38 PKC-dependent downregulation of GABA_A receptors ^[6, 67].
39
40
41
42
43
44
45
46

47 **Glial P2X4**

48
49 Glial cells are themselves capable of releasing ATP, which can play an important role in neuron-
50
51 glia and glial network signaling such as propagating glial calcium waves ^[68]. P2X4 is expressed
52
53 in microglia with P2X7 and both receptors can interact with each other. The existence of P2X4/7
54
55 heteromers remains controversial and interaction may also occur by direct or indirect interaction
56
57 between homomeric P2X4 and P2X7 ^[69]. A *de novo* expression of P2X4 and/or an increased
58
59
60

1
2
3 surface trafficking of P2X4 observed in activated microglia ^[42] play key roles in many CNS
4 diseases (see below). The upregulation of microglial P2X4 was revealed following LPS-induced
5 inflammation in the hippocampus and selectively involving activated microglia using *dtTomato*
6 reporter mice and internalization-defective P2X4mCherryIN knockin mice ^[58, 59].

7
8
9
10
11
12 The P2X4 receptor has been revealed by immunostaining in S100 β -positive astrocytes of the rat
13 hippocampus in the CA1, CA3 regions and the dentate gyrus ^[70]. P2X4 expression was
14 additionally detected in astrocytes from hippocampal cultures of P2X4mCherryIN mice, as well
15 as in astrocyte end-feet contacting endothelial cells in brain slices of these mice solely by electron
16 microscopy ^[59]. P2X4 were also detected in GFAP-positive astrocytes of the rat nucleus
17 accumbens ^[71]. Nevertheless, P2X4 expression in astrocytes remains debated ^[72], although this
18 discrepancy may merely reflect the heterogeneity of astrocytes across the different regions of the
19 CNS. Indeed, a growing body of evidence suggests that astrocytes as well as microglia and
20 oligodendrocytes display regional and contextual heterogeneities in terms of gene expression
21 pattern, phenotypes and roles in controlling physiological and pathological brain functions ^[73-75].

22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38 Recent studies have shown the presence of P2X4 in satellite glial cells (SGCs) stained by astrocytic
39 markers of dorsal root ganglia. This expression was observed solely during neuropathic pain ^[76]
40 and diabetes-induced neuropathy ^[77] suggesting a pathology-induced upregulation of P2X4 in
41 SGCs. The expression of several types of P2 receptors, including P2X4, has also been
42 demonstrated in oligodendroglial precursor cells ^[78]. However, its function is not yet fully
43 understood, in contrast to P2X7 receptors which contribute to ATP excitotoxicity in
44 oligodendrocytes and the pathogenesis of experimental autoimmune encephalomyelitis^[79].

45
46
47
48
49
50
51
52
53
54
55
56 P2X4 has also been observed in Schwann cells, mainly localized intracellularly in lysosomes,
57 under normal physiological conditions. After peripheral nerve injury, an overexpression of P2X4
58
59
60

1
2
3 receptors and their increase on the surface of Schwann cells was shown to promote, through the
4
5 release of BDNF, motor and sensory functional recovery and ameliorate nerve remyelination [80].
6
7
8
9

10 11 12 **P2X4 in CNS diseases**

13 14 15 16 17 **Neuropathic Pain**

18
19 Acute pain serves a protective function in promoting reflex withdrawal from painful stimuli and
20
21 the avoidance of injury or damage. Acute pain is initiated by an activation of nociceptive neurons
22
23 in peripheral dorsal root ganglia (DRG), with information being processed and integrated within
24
25 the spinal cord (SC) before transmission to the brain [81]. Whereas acute pain is a normal
26
27 physiological function of the CNS, chronic pain results from pathological and persistent alterations
28
29 in the peripheral and central nervous systems [82]. Chronic pain can be induced by nerve damage,
30
31 so-called neuropathic pain, or by persistent inflammatory pain associated with the healing of
32
33 damaged tissue[83]. Chronic pain persists even after the expected period of healing and becomes
34
35 disabling for the individual [84].
36
37
38
39
40
41

42
43 Peripheral nerve injury (PNI) was shown to induce an activation of microglia in the spinal cord
44
45 dorsal horn [85, 86] by several pathways involving interferon- γ (IFN- γ) or platelet-derived growth
46
47 factor (PDGF) [87, 88]. Activation of spinal microglia causes tactile allodynia, a symptom of
48
49 neuropathic pain, and several studies following the pioneered work of Tsuda *et al.* [89] showed that
50
51 a specific microglial response phenotype characterized by the *de novo* expression of P2X4 is
52
53 critical for the pathogenesis of neuropathic pain [67]. The expression of P2X4 increases exclusively
54
55 in the reactive microglia after PNI and is confined to the ipsilateral spinal dorsal horn (Figure 2).
56
57
58 Moreover, the genetic ablation or downregulation of P2X4 using either P2X4KO mice or RNA
59
60

1
2
3 antisense as well as the blockade of P2X4 by the antagonist TNP-ATP dramatically reduces tactile
4 allodynia after PNI [89-91]. Interestingly, an activation of microglia is also observed in P2X4KO
5 mice after PNI [91] indicating that P2X4 is not required for microglial activation but is necessary
6 and sufficient for the development of allodynia by neuro-glial communication. Indeed, activation
7 by ATP of microglial P2X4 reduces GABA or Glycine receptor-mediated inhibitory currents
8 within the spinal cord. This disinhibition results from an increase in the intracellular concentration
9 of chloride that becomes sufficient to block GABA or Glycine mediated-inhibitory responses or
10 to eventually convert inhibition into excitation, thereby inducing the hypersensitivity of spinal
11 neurons and tactile allodynia [92, 93]. Changes in chloride concentration is due to the downregulation
12 of the potassium-chloride co-transporter KCC2, which is mediated by the extracellular release of
13 BDNF and the activation of Trk-B receptors in inhibitory neurons. Several studies have established
14 the key role played by BDNF in this signaling cascade and the resulting disinhibition and pain-
15 related behavior [92, 94]. Also established is the requirement for ATP-mediated activation of P2X4
16 receptors to elicit BDNF release by microglia [91], although how P2X4 actually triggers the release
17 of BDNF remains to be determined. In the dorsal horn of the spinal cord, ATP can be released by
18 the terminals of primary sensory neurons [95], dorsal horn neurons [96], or glial cells [7].

19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42 A recent study showed that PNI increased the expression of the vesicular nucleotide transporter
43 (VNUT) and induced a slight increase in the extracellular concentration of ATP in the spinal cord.
44 By cell-specific deletion of VNUT, the authors elegantly showed that ATP released by spinal cord
45 neurons and astrocytes, but not by sensory neurons, contributes to neuropathic pain^[97] very likely
46 by activating microglial P2X4-mediated signaling cascades. However, ATP is co-released with
47 GABA in spinal dorsal horn neurons that can activate simultaneously postsynaptic P2X and
48 GABA_A receptors^[96]. A cross-talk between neuronal P2X, including P2X4 and GABA_A receptors,
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 may downregulate GABA-mediated currents and thus contribute to disinhibition in neuropathic
4 pain [36, 96, 98].

5
6
7 Several mechanisms are involved in the increase of P2X4 expression in microglia following PNI.
8
9
10 Two transcription factors of the Interferon Regulatory Factor (IRF) family, IRF8 and IRF5, that
11 are expressed in immune cells including microglia [99, 100] are upregulated after PNI in dorsal horn
12 microglia and silencing IRF8 was shown to suppress PNI-induced tactile allodynia^[100]. IRF8
13 promotes several actors involved in chronic pain ^[100] and IRF5 expression which in turn controls
14 directly P2X4 transcription by binding to the promoter region of p2rx4 gene ^[100]. Fibronectin also
15 appears to be a key regulator of P2X4 expression through several pathways. The observed increase
16 in extracellular fibronectin after PNI could induce *de novo* P2X4 expression through several
17 fibronectin/integrin signaling pathways ^[90, 101], as well as promoting P2X4 trafficking from the
18 lysosome to the cell surface through the release of a chemokine CCL2 and subsequent activation
19 of microglia CCR2 receptors ^[102].

20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35 An upregulation of P2X4 in spinal microglia is likely to play a key role in tactile allodynia induced
36 by PNI, although interestingly, recent work has suggested that this mechanism occurs only in
37 males ^[94, 103]. In contrast to male mice following PNI, no increase in P2X4 expression was
38 observed in dorsal horn microglia of post-PNI female mice and tactile allodynia was not modified
39 by the pharmacological blockade of P2X4 with TNP-ATP ^[94]. Results suggest that immune cells
40 contribute to pain hypersensitivity in female mice ^[94, 104], although other studies have indicated
41 that microglial P2X4 and BDNF/TrkB signaling contribute to bone cancer ^[105] and herpetic pain
42 ^[16] models in female rodents, thus demonstrating the need for additional studies to clarify this
43 sexual dimorphism.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Besides microglial P2X4 in the spinal cord, P2X4 expression in sensory neurons was recently
4 linked to neuropathic pain. All P2X subunit mRNAs are expressed in sensory DRG neurons [106],
5
6 but the functional expression and potential involvement in pain processing was only until very
7
8 recently found to be limited to homomeric P2X3 or heteromeric P2X2/3 receptors expressed in
9
10 nociceptive neurons [107]. Recent work showed that P2X4 is expressed at mRNA and protein levels
11
12 in DRG sensory neurons [77, 108-110] and that its expression increases in several neuropathic or
13
14 inflammatory pain models in rodents [111]. P2X4 receptor expression was also observed in satellite
15
16 glial cells (SGC) which enwrap the cell bodies of sensory neurons within the ganglia [110, 112].
17
18 Although it can be difficult to distinguish P2X4 in the plasma membrane of sensory neurons or
19
20 SCGs due to the apposition of both elements, several lines of evidence have established that P2X4
21
22 expression increases in SCGs under neuropathic pain conditions [77, 111, 113]. After PNI, the coupling
23
24 between the SCGs themselves and between SCGs and neurons increases via the formation of gap
25
26 junctions [114, 115] and activated SCGs release inflammatory mediators and other neuromodulators
27
28 that contribute to neuronal hyperexcitability and pain hypersensitivity [115].
29
30
31
32
33
34
35
36
37
38

39 **Chronic inflammatory pain**

40 P2X4 receptors expressed in distinct cell types also contribute to inflammatory pain. For example,
41
42 the presence of P2X4 in skin-resident macrophages contributes to the sensitization of peripheral
43
44 sensory nerve termini and induces mechanical hypersensitivity due to the production of the
45
46 inflammatory factor PGE2 [116]. P2X4 also plays a role in mechanical hypersensitivity following
47
48 chronic inflammation by promoting the hyperexcitability of spinal dorsal horn neurons in chronic
49
50 inflammation mouse models [116] (Figure 2). On the other hand, such hyperexcitability is
51
52 downregulated in P2X4-deficient mice [117]. A recent study showed that the expression of P2X4
53
54 receptors in small diameter nociceptive neurons of DRGs is increased by twofold during chronic
55
56 inflammation [109]. In addition, these sensory neurons express BDNF, a key factor in both
57
58
59
60

1
2
3 neuropathic and inflammatory pain. Indeed, in chronic inflammatory conditions, BDNF
4 contributes to mechanical hypersensitivity using the similar pathway. However, unlike
5
6 neuropathic pain, during chronic inflammation, BDNF is released in a P2X4-dependent manner
7
8 by sensory neurons termini in the dorsal horn of the spinal cord, thereby leading *via* the
9
10 TrkB/KCC2 cascade to spinal disinhibition [118]. P2X4 expressed in SGCs may also contribute to
11
12 chronic inflammatory pain [112, 119] by releasing pro-inflammatory cytokines such as IL1 β and
13
14 TNF α following activation of the inflammasome NLRP1 [116, 119].
15
16
17
18
19
20
21

22 **Migraine**

23
24 Chronic migraine is a neurological disorder characterized by repeated attacks that can be caused
25
26 by an increase in the excitability of central neurons in the trigeminal nociceptive pathway,
27
28 principally the trigeminal nucleus caudalis (TNC), leading to central sensitization [120]. Similar to
29
30 neuropathic pain, microglia surrounding TNC neurons has been suggested to play direct and
31
32 indirect roles in the establishment of central sensitization [121]. Indeed, microglial activation has
33
34 been observed when nitroglycerin (NTG) is applied chronically in a model of chronic migraine,
35
36 with a resultant effect on central sensitization [121]. As observed during neuropathic pain [89, 91],
37
38 several studies suggested a role for BDNF expressed in the trigeminovascular system in migraine
39
40 pathophysiology [122] and an increase in P2X4 and BDNF expression has been recently reported in
41
42 the TNC after chronic intermittent administration of NTG [121, 123]. Moreover, P2X4
43
44 pharmacological inhibition has been very recently found to prevent hyperalgesia induced by NTG,
45
46 associated with an inhibition of p-ERK phosphorylation and calcitonin gene-related peptide
47
48 (CGRP) release in the TNC [123]. This study also showed that in BV2 microglial cells, ATP triggers
49
50 BDNF synthesis and release, which is in turn reduced by the P2X4 antagonists 5-BDBD or
51
52 SB203580 [123]. In addition, a potentiation of P2X4 by ivermectin induced sustained hyperalgesia
53
54 and significantly increased levels of p-ERK and CGRP release in the TNC [123]. Those results
55
56
57
58
59
60

1
2
3 therefore clearly indicate that microglial P2X4 contributes to TNC neuronal hyperexcitability
4
5 involved in chronic migraine.
6
7
8
9

10 **Post-ischemic inflammation**

11
12 Brain ischemia is associated with post-ischemic inflammation, including glial cell activation along
13
14 with an increase in extracellular ATP levels. Several studies have reported an upregulation of
15
16 P2X4 receptors in activated microglial cells after ischemic brain injury ^[124-127] (Figure 3). During
17
18 hypoxic conditions, ATP is released in large amounts from cells damaged by ischemia and the
19
20 activation of both P2Y12 and P2X4 receptors present in a higher density in reactive microglia
21
22 mediates, *via* the PI3K signaling pathway, chemotaxis of motile microglia towards the lesion site
23
24 as well as the release of proinflammatory cytokines ^[125, 128, 129]. More recently, a neuroprotective
25
26 role of P2X4 during ischemia has also been reported ^[130]. Specifically, the activation of P2X4
27
28 expressed in vascular endothelial cells by ATP release consequently to pressure shocks mediated
29
30 by vascular ischemia promotes the upregulation of osteopontin, a neuroprotective molecule ^[130].
31
32 These results therefore highlight a new mechanism whereby endothelial P2X4 induces an ischemic
33
34 tolerance.
35
36
37
38
39
40
41

42 **Epilepsy**

43
44 Epilepsy is a chronic neurological condition characterized by recurrent seizures that spread to
45
46 neighboring cortices, and lead to hippocampal neuron loss and microglia activation (Figure 3). To
47
48 date, as for P2X7, the implication of P2X4 in epilepsy remains unclear, and whether the latter is
49
50 up- or down-regulated in the hippocampus following status epilepticus (SE) is still debated. Some
51
52 studies have reported a lack of change, or even a decrease in P2X4 immunoreactivity, 24 hrs after
53
54 SE induced either by systemic pilocarpine or by intra-amygdala kainate injection . Kang et al.
55
56
57
58
59
60

1
2
3 reported similar findings in seizure-sensitive compared with seizure-resistant gerbils ^[131], although
4 the specificity of the antibody anti-P2X4 used in these studies has not been established.

5
6
7 Subsequently, in an epileptic mouse model using intraperitoneal injection of kainate, hippocampal
8 P2X4 transcript and protein levels were found to increase 24 and 48 hrs after the SE, and
9 particularly in activated microglia in the stratum radiatum ^[132]. In P2X4KO mice, a decrease in
10 microglial activation as well as subsequent cellular death were observed after the induction of the
11 SE ^[132]. Even though epileptic seizures induced by kainate are not strongly inhibited in P2X4KO
12 mice, there is still a significant reduction of cell death in the hippocampus as well as a decrease in
13 the amplitude of hyperpolarizing outward current activated by microglia ^[132]. As for neuropathic
14 pain, the increase in *de novo* P2X4 expression in activated microglia after the induction of epilepsy
15 could facilitate BDNF release and the induction of hyperexcitability, thereby promoting
16 epileptogenesis.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33 **Alzheimer's disease**

34
35 Alzheimer's disease (AD) is the most common neurodegenerative disorder and is characterized
36 mainly by the accumulation of insoluble aggregates of beta-amyloid fragments ($A\beta_{1-42}$) that in turn
37 lead to synaptic dysfunction and neuronal death causing a progressive loss of memory ^[133, 134].
38 Like other neurodegenerative diseases, the increase of extracellular nucleotides concomitantly
39 with exacerbated neuroinflammatory events play a crucial role in AD pathogenesis ^[135].

40
41
42 It has been proposed that ATP, acting through P2X receptors, could participate directly in some
43 of the toxic effects of $A\beta$ peptide observed in neurons, with a particularly important role of P2X4
44 receptors ^[136, 137]. Indeed, an accumulation of P2X4 has been observed in hippocampal neurons
45 exposed to the $A\beta$ peptide ^[138]. This expression increase was found to be restricted to the cell body
46 after 6 hrs of treatment, before also reaching the neurites after 12 hrs ^[138]. In parallel, after exposure
47 to an $A\beta$ fragment, the authors revealed an accumulation of a smaller P2X4 fragment, which could
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 be prevented by exposure to a caspase inhibitor, thus suggesting occurrence of a proteolytic
4 cleavage of P2X4 receptors (Figure 3). They concluded that the cleavage by caspase-3 slows down
5
6 the closing time of the receptor's channel pore and prevents receptor agonist-dependent
7
8 internalization. This leads to an increase in membranal surface trafficking of the neurons that can
9
10 in turn induce neuron toxicity due to increased calcium influx [138]. In this study, moreover, the
11
12 overexpression of neuronal P2X4 was found to promote the toxic effect of the A β fragment while
13
14 inhibition of receptor expression has a significant positive effect on neuronal death after A β
15
16 exposure. Consequently, these findings led to the conclusion that neuronal P2X4 receptors are
17
18 directly implicated in neuronal death induced by A β [138]. Consistent with the implication of
19
20 neuronal P2X4 in AD, an increased surface density of neuronal P2X4 was recently shown to
21
22 induce synaptic deficits and alterations in learning and memory functions of P2X4 internalization-
23
24 defective knock-in mice. Surprisingly, however, the authors also reported a decrease in P2X4
25
26 protein levels in the middle frontal and temporal gyri of AD patients with severe cognitive
27
28 impairment [138].
29
30
31
32
33
34

35 P2X7 has been shown to play a key role in neuroinflammatory processes of AD and other
36
37 neurodegenerative diseases, including Parkinson's disease (PD), amyotrophic lateral sclerosis
38
39 (ALS), multiple sclerosis (MS) and Huntington disease (HD), in promoting proinflammatory
40
41 cytokine production by microglia and astrocytes [139-141]. Like P2X7, P2X4 could also be
42
43 implicated in neuroinflammation associated with AD [142] and the resultant potentiation of A β -
44
45 mediated neuronal death, since similarities exist between these two receptor subtypes as well as a
46
47 neighboring chromosomal localization [69, 143], and because PX4 has been correlated with several
48
49 other inflammatory mechanisms [144, 145] and microglial activation processes [89].
50
51
52
53
54
55

56 **Parkinson's disease**

57
58
59
60

1
2
3 PD is characterized by the progressive loss of dopaminergic nigrostriatal neurons specifically,
4 causing impaired dopamine (DA) homeostasis and associated behaviors leading to motor troubles.
5
6 A hallmark of PD is the presence of abnormal intracytoplasmic aggregates of α -synuclein, called
7
8 Lewy bodies (LB), within neuronal cell bodies. It is well known that P2X4 modulates major
9
10 neurotransmitter systems including glutamatergic and GABAergic functions [10, 11, 36, 57, 62, 64-67, 146-
11
12 150]. Several early studies suggested that P2X4 receptors are indirectly involved in dopamine (DA)
13
14 neurotransmission [151, 152] and behavioral deficits were recovered in P2X4 KO mice that may also
15
16 express DA dysfunction [153, 154]. A recent study has confirmed the receptor's direct role in
17
18 regulating DA homeostasis that is impaired in PD [155]. Specifically, using P2X4 KO mice, the
19
20 authors showed that a *p2rx4* deficit affects DA synthesis and transport, and increases DA receptor
21
22 levels that could in turn alter DA neuron function and neurotransmission, with a resulting impact
23
24 on DA associated behaviors such as motor control and sensorimotor gating [155]. These findings
25
26 further indicate that P2X4 could be implicated in multiple neurological disorders such as PD and
27
28 psychiatric diseases that involve DA homeostasis impairment.
29
30
31
32
33

34
35 Neuroinflammation is also a main hallmark of PD [156-159] and microglia seems to be central in this
36
37 neurodegenerative disease [160, 161]. As previously discussed, microglial P2X4 could be involved in
38
39 those neuroinflammatory mechanisms that are common to neurodegenerative diseases, and for
40
41 which, in the case of PD, P2X7 is known to play a role [162, 163]. Nevertheless, the implication of
42
43 microglia in the physiopathology of PD is still unclear and it remains unknown whether microglial
44
45 alterations are causal to, or a consequence of, DA neuron degeneration [164].
46
47
48
49
50

51 **Amyotrophic lateral sclerosis (ALS)**

52
53 ALS is a fatal neurodegenerative disease characterized by the progressive and selective loss of
54
55 spinal motoneurons (MN) as well as cortical motor neurons (Figure 2). The causes of ALS are
56
57 multifactorial and the mechanisms underlying the selective degeneration of MN are still unknown.
58
59
60

1
2
3 A link has been established between ALS and P2X4 [165]. For example, a strong increase in P2X4
4 immunoreactivity in ventral horn MN immediately preceding their death has been reported in the
5
6 SOD1-G93A ALS rodent model, suggesting an important role of neuronal P2X4 receptors in the
7
8 pathogenesis of the disease [166]. P2X4 immunoreactivity has also been associated with a
9
10 degeneration of other neuronal populations including noradrenergic neurons in the *locus*
11
12 *coeruleus*, cerebellar Purkinje cells and serotonergic neurons of raphe nucleus [166]. Surprisingly,
13
14 the same authors subsequently showed, by using anti-P2X4 antibodies directed against the
15
16 intracellular C-tail of P2X4 subunits, that SOD1-G93A misfolded proteins were detectable in MN
17
18 but not in microglia [167]. This finding led to the suggestion that neuronal conformers of SOD1-
19
20 G93A proteins that are reactive to anti-P2X4 antibodies could play a pathogenic role and induce
21
22 neuroinflammation activating microglia and astrocytes when injected intracerebrally in normal
23
24 animals [167].
25
26
27
28
29
30
31
32

33 An increase in P2X4 expression, both at mRNA and protein levels, was also reported in microglia
34
35 of SOD1-G93A mice [168]. More recently, Volonté and colleagues demonstrated for the first time
36
37 an increase in P2X4 and P2X7 expression in the peripheral nervous system of SOD1-G93A mice,
38
39 particularly in the sciatic nerves [165].
40
41

42 Other work has shown that a potentiation of P2X4 function by ivermectin has a dual effect on MN
43
44 survival depending on ATP concentrations, with a beneficial action at low ATP levels, but
45
46 becoming deleterious at higher levels [146]. The authors deduced that ivermectin potentiates a
47
48 neuroprotective effect of P2X4 on MN at low ATP levels, probably by increasing P2X4 function
49
50 and/or the receptor's cell surface density [20, 22]. Moreover, the oral administration of ivermectin
51
52 via the animal's drinking water from a presymptomatic stage (P50), caused a 10% increase in the
53
54 lifespan of SOD1-G93A mice [146].
55
56
57
58
59
60

1
2
3 As for other neuroinflammatory diseases, P2X4 receptor is also likely to be involved in
4 neuroinflammation processes associated with ALS and known to be implicated in MN
5 degeneration mechanisms [169]. Several studies have highlighted a role of P2X7 in this disease [170],
6 notably via a modulation of autophagy [171] as well as of microglia-mediated neuroinflammation
7 processes that may be both beneficial or deleterious according to the stage of the disease, [172, 173].
8 An interaction between P2X4 and P2X7 receptors signaling pathways could also be involved in
9 microglial inflammatory actions, with one pathway activating signaling of the other. Such a cross-
10 talk is already known for macrophages, where P2X4 receptors regulate the inflammatory functions
11 of P2X7 receptors [174]. Although the implication of P2X4 in the pathogenesis of ALS remains to
12 be clearly established, this receptor subtype appears to be a potential therapeutic target for
13 confronting this disease.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **Multiple Sclerosis**

32 Multiple sclerosis (MS) is a chronic T-cell mediated auto-immune disease characterized by a
33 massive infiltration of immune cells, demyelination and axonal loss. This neurodegenerative
34 disease is also characterized by an early inflammation that is beneficial and initially delays the
35 onset of neurodegeneration, which then evolves in a complex manner as the disease progresses
36 [175]. The initial regenerative phase is due to the repairing actions of anti-inflammatory infiltrating
37 macrophages and microglia.
38
39
40
41
42
43
44
45

46 Interestingly, P2X4 has been shown to be overexpressed in microglia and macrophages in a rat
47 model of MS and experimental autoimmune encephalomyelitis (EAE) [127] (Figure 3). But in
48 contrast to neuropathic pain, where P2X4 blockers have been proposed to be potential therapeutic
49 drugs [16], P2X4 antagonists exacerbate neurological symptoms in MS animal models, whereas
50 P2X4 potentiation by ivermectin or P2X4 expression upregulation are both beneficial in EAE [176].
51 Moreover, microglial P2X4 activation is known to induce BDNF release [92] and it has been shown
52
53
54
55
56
57
58
59
60

1
2
3 that BDNF release from microglia as a result of P2X4 potentiation or overexpression in Schwann
4
5 cells promotes remyelination [80, 176].
6

7
8 In contrast, P2X4 potentiation by ivermectin increases myelin engulfment and degradation [176]
9
10 and P2X4 activation in microglia induces endolysosomal membrane fusion and lysosome pH
11
12 acidification, both of which are essential to phagocytic pathways [46, 176] that could control myelin
13
14 phagocytosis. Lysosomes are also involved in secretion and myelin biogenesis [177, 178] and P2X4
15
16 has been shown to be implicated in lysosome exocytosis contributing to surfactant secretion in
17
18 lung tissue [179]. Altogether these findings therefore suggest a potential role for lysosomal P2X4
19
20 receptors in secretion and phagocytosis in myelin disorders.
21
22
23
24
25

26 **Alcohol-related disorders**

27
28 Several studies have pointed to a role of P2X4 in alcohol-induced behavior [180-183]. P2X4 receptors
29
30 are expressed in brain regions involved in the reinforcing properties of alcohol and other drugs
31
32 [184]. Furthermore, a recent study suggested the involvement of P2X4 in regulating striatal
33
34 dopamine homeostasis [155], supporting a role of P2X4 in the mesolimbic dopamine system
35
36 implicated in the reward system of the brain (Figure 3).
37
38

39
40 An increase in ethanol intake is observed in P2X4 KO mice, suggesting that P2X4 could also be
41
42 implicated in alcohol intake and/or preference [182, 185]. Other studies have demonstrated a lower
43
44 P2X4 expression in rats that prefer alcohol, confirming the correlation between P2X4 expression
45
46 and alcohol preference [183, 184]. Moreover, a direct link between P2X4 and the regulation of
47
48 dopamine neurotransmission in the mesolimbic system has been established, further indicating
49
50 that through its presence in dopaminergic neurons of the mesolimbic system, P2X4 plays a role in
51
52 mediating alcohol drinking behavior [183].
53
54

55
56 Interestingly, low ethanol concentrations are able to inhibit P2X4 *in vitro*, probably by blocking
57
58 opening of the channel without affecting its deactivation [186, 187]. Conversely, ivermectin is able to
59
60

1
2
3 antagonize the inhibition of P2X4 by ethanol, indicating that this positive modulator interferes
4 with the linkage site of ethanol on the P2X4 receptor [188]. Altogether, these findings suggest that
5 ethanol can reinforce alcohol intake *via* P2X4 inhibition and that pharmacological potentiation of
6 P2X4 may reduce alcohol intake and preference.
7
8
9

10
11 Alcohol abuse is also known to promote neuroinflammation [189] and microglial P2X4 has been
12 recently reported to play a role in this mechanism [190, 191]. Gofman and collaborators demonstrated
13 that an alcohol-induced increase of P2X4 expression in microglia affects microglial migration and
14 phagocytosis, and can be reversed by a P2X4 selective antagonist [190]. Later, the same group found
15 that *via* P2X4, alcohol decreases the phosphorylation of key regulatory proteins and increases
16 CREB transcriptional activity [191]. Cerebral ischemic events have also been reported in alcohol
17 abuse, involving an upregulation of P2X4 in microglia following ischemia and hypoxia as
18 described above [128, 192].
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33 **Neuropsychiatric disorders**

34
35 Depression, bipolar disorder, schizophrenia, attention deficit hyperactivity and anxiety are
36 neuropsychiatric disorders where DA homeostasis is impaired. As mentioned above, P2X4 has
37 been shown to be implicated in the regulation of DA homeostasis and sensory motor gating [155]
38 and there is increasing evidence that P2X4 plays a critical role in psychiatric disorders [59, 126, 155,
39 193, 194].
40
41
42
43
44
45

46
47 Consistent with an involvement of P2X4 receptors, ivermectin has been found to produce
48 anxiolytic-like and depressive-like behavior in mice [193]. Very interestingly and in line with these
49 findings, data from a new conditional P2X4 internalization-defective knock-in mouse, namely
50 P2X4mCherryIN, that display an increase in number of P2X4 receptors at the surface of targeted
51 cells, further supported the link between neuronal P2X4 and anxiety-like behavior as well as in
52 memory [59]. The anxiolytic effects of a selective increase in surface P2X4 density in mouse
53
54
55
56
57
58
59
60

1
2
3 excitatory forebrain neurons further underlined the role of neuronal P2X4 in anxiety ^[59]. Another
4 study using P2X4KO mice on the role of P2X4 in ischemic stroke showed that P2X4 deletion
5 predisposes animals to chronic depression-like behavior after stroke ^[126]. Significantly in this
6 context, a recent work demonstrated that the potentiation of P2X4 by ivermectin can lead to DA
7 hyperactivity and disruption of information processing because of a potential perturbation of the
8 interaction between P2X4 and DA receptors ^[194]. Together these results suggest that P2X4
9 antagonists could serve as novel anti-psychotic treatments for psychiatric disorders arising from
10 sensorimotor gating impairments linked to the disruption of DA homeostasis ^[194].
11
12
13
14
15
16
17
18
19
20
21
22

23 **Concluding remarks**

24
25
26
27
28 P2X4 is the most widespread P2X subunit expressed in several neuronal and glial cell types
29 throughout the CNS. Homomeric or heteromeric P2X4-containing receptors are ATP-gated
30 channels that are highly permeable to calcium and in basal conditions, are localized in the plasma
31 membrane at low density and mostly in intracellular endosomal/lysosomal compartments.
32 Growing evidence suggest that endo/lysosomal P2X4 receptors are functional and play important
33 intracellular signaling roles. Intracellular P2X4 can also be rapidly mobilized to the cell surface
34 and contribute, together with *de novo* expressed receptors, to the upregulation observed in specific
35 cell types in many central disorders such as pain, neurodegenerative disorders and
36 neuropsychiatric diseases. However, establishing the function of upregulated P2X4 expressed in
37 glial *versus* neuronal cells remain elusive. We have recently developed conditional internalization-
38 defective P2X4mCherryIN knockin-mice with increasing numbers of P2X4 at the surface of
39 targeted cells and have demonstrated the role of surface neuronal P2X4 in synaptic plasticity and
40 memory^[59]. The development of such a transgenic knockin mouse, along with the possibility of
41 generating a conditional P2X4KO line that targets specific cell populations, represent major tools
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 to gaining in the near future a better understanding of the role of surface P2X4 in various
4
5 physiological and pathological conditions and to evaluate the potential of P2X4 as a therapeutic
6
7 target in these various disorders. Intracellular P2X4 can also play important roles and extensive
8
9 studies are now needed to evaluate the respective functions of intracellular *versus* surface P2X4
10
11 receptors, which may differ between the various cell types expressing P2X4. It will also be
12
13 important to identify mechanisms and regulation of P2X4 trafficking leading to the cell surface
14
15 increase in pathological conditions, as well as determining the functional significance of the
16
17 equilibrium between intracellular and surface P2X4 receptors.
18
19
20
21
22

23 **Conflict of Interest**

24
25
26
27
28 All authors declare that they have no competing interests
29
30
31
32

33 **Acknowledgments**

34
35
36
37 This work was supported by CNRS, University of Bordeaux, and grants from ARSLA, IdEx of
38
39 Bordeaux and LabEx BRAIN ANR-10-LABX-43.
40
41
42
43
44
45
46

47 **References**

- 48
49
50
51 [1] North RA. P2X receptors. *Philos Trans R Soc Lond B Biol Sci* 2016, 371.
52
53 [2] Holton P. The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves.
54
55 *J Physiol* 1959, 145: 494-504.
56
57
58 [3] Burnstock G. Purinergic nerves. *Pharmacol Rev* 1972, 24: 509-581.
59
60

- 1
2
3 [4] Khakh BS, North RA. Neuromodulation by extracellular ATP and P2X receptors in the CNS.
4
5 Neuron 2012, 76: 51-69.
6
7 [5] Pankratov Y, Lalo U, Verkhratsky A, North RA. Vesicular release of ATP at central synapses.
8
9 Pflugers Arch 2006, 452: 589-597.
10
11 [6] Boué-Grabot E, Pankratov Y. Modulation of Central Synapses by Astrocyte-Released ATP
12
13 and Postsynaptic P2X Receptors. Neural Plast. 2017, 2017: 9454275.
14
15 [7] Imura Y, Morizawa Y, Komatsu R, Shibata K, Shinozaki Y, Kasai H, *et al.* Microglia release
16
17 ATP by exocytosis. Glia 2013, 61: 1320-1330.
18
19 [8] Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H. Purinergic signalling in the
20
21 nervous system: an overview. Trends Neurosci 2009, 32: 19-29.
22
23 [9] Rodrigues RJ, Tome AR, Cunha RA. ATP as a multi-target danger signal in the brain. Front
24
25 Neurosci 2015, 9: 148.
26
27 [10] Pougnet JT, Toulme E, Martinez A, Choquet D, Hosy E, Boue-Grabot E. ATP P2X receptors
28
29 downregulate AMPA receptor trafficking and postsynaptic efficacy in hippocampal neurons.
30
31 Neuron 2014, 83: 417-430.
32
33 [11] Pougnet JT, Compans B, Martinez A, Choquet D, Hosy E, Boue-Grabot E. P2X-mediated
34
35 AMPA receptor internalization and synaptic depression is controlled by two CaMKII
36
37 phosphorylation sites on GluA1 in hippocampal neurons. Sci Rep 2016, 6: 31836.
38
39 [12] Suurvali J, Boudinot P, Kanellopoulos J, Ruutel Boudinot S. P2X4: A fast and sensitive
40
41 purinergic receptor. Biomed J 2017, 40: 245-256.
42
43 [13] Rubio ME, Soto F. Distinct Localization of P2X receptors at excitatory postsynaptic
44
45 specializations. J Neurosci 2001, 21: 641-653.
46
47 [14] Soto F, Garcia-Guzman M, Karschin C, Stuhmer W. Cloning and tissue distribution of a novel
48
49 P2X receptor from rat brain. Biochem Biophys Res Commun 1996, 223: 456-460.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [15] Werner S, Mesch S, Hillig RC, Ter Laak A, Klint J, Neagoe I, *et al.* Discovery and
4
5 Characterization of the Potent and Selective P2X₄ Inhibitor N-[4-(3-Chlorophenoxy)-3-
6
7 sulfamoylphenyl]-2-phenylacetamide (BAY-1797) and Structure-Guided Amelioration of Its
8
9 CYP3A4 Induction Profile. *J Med Chem* 2019, 62: 11194-11217.
- 11
12 [16] Matsumura Y, Yamashita T, Sasaki A, Nakata E, Kohno K, Masuda T, *et al.* A novel P2X₄
13
14 receptor-selective antagonist produces anti-allodynic effect in a mouse model of herpetic pain. *Sci*
15
16 *Rep* 2016, 6: 32461.
- 17
18 [17] Ase AR, Honson NS, Zaghdane H, Pfeifer TA, Seguela P. Identification and characterization
19
20 of a selective allosteric antagonist of human P2X₄ receptor channels. *Mol Pharmacol* 2015, 87:
21
22 606-616.
- 23
24 [18] Twum-Danso NA. Serious adverse events following treatment with ivermectin for
25
26 onchocerciasis control: a review of reported cases. *Filaria J* 2003, 2 Suppl 1: S3.
- 27
28 [19] Khakh BS, Proctor WR, Dunwiddie TV, Labarca C, Lester HA. Allosteric control of gating
29
30 and kinetics at P2X₄ receptor channels. *J Neurosci* 1999, 19: 7289-7299.
- 31
32 [20] Silberberg SD, Li M, Swartz KJ. Ivermectin Interaction with transmembrane helices reveals
33
34 widespread rearrangements during opening of P2X receptor channels. *Neuron* 2007, 54: 263-274.
- 35
36 [21] Stokes L. Rab5 regulates internalisation of P2X₄ receptors and potentiation by ivermectin.
37
38 *Purinergic Signal* 2012.
- 39
40 [22] Toulme E, Soto F, Garret M, Boue-Grabot E. Functional properties of internalization-
41
42 deficient P2X₄ receptors reveal a novel mechanism of ligand-gated channel facilitation by
43
44 ivermectin. *Mol Pharmacol* 2006, 69: 576-587.
- 45
46 [23] Robinson LE, Murrell-Lagnado RD. The trafficking and targeting of P2X receptors. *Front*
47
48 *Cell Neurosci* 2013, 7: 233.
- 49
50 [24] Murrell-Lagnado RD. A role for P2X₄ receptors in lysosome function. *J Gen Physiol* 2018,
51
52 150: 185-187.
- 53
54
55
56
57
58
59
60

- 1
2
3 [25] Burnstock G. Purinergic signalling and disorders of the central nervous system. *Nat Rev Drug*
4 *Discov* 2008, 7: 575-590.
5
6
7 [26] Brake AJ, Wagenbach MJ, Julius D. New structural motif for ligand-gated ion channels
8 defined by an ionotropic ATP receptor. *Nature* 1994, 371: 519-523.
9
10 [27] Kawate T, Michel JC, Birdsong WT, Gouaux E. Crystal structure of the ATP-gated P2X(4)
11 ion channel in the closed state. *Nature* 2009, 460: 592-598.
12
13 [28] Hattori M, Gouaux E. Molecular mechanism of ATP binding and ion channel activation in
14 P2X receptors. *Nature* 2012, 485: 207-212.
15
16 [29] Mansoor SE, Lu W, Oosterheert W, Shekhar M, Tajkhorshid E, Gouaux E. X-ray structures
17 define human P2X(3) receptor gating cycle and antagonist action. *Nature* 2016, 538: 66-71.
18
19 [30] Igawa T, Abe Y, Tsuda M, Inoue K, Ueda T. Solution structure of the rat P2X4 receptor head
20 domain involved in inhibitory metal binding. *FEBS Lett* 2015, 589: 680-686.
21
22 [31] Chaumont S, Jiang LH, Penna A, North RA, Rassendren F. Identification of a trafficking
23 motif involved in the stabilization and polarization of P2X receptors. *J Biol Chem* 2004, 279:
24 29628-29638.
25
26 [32] Boue-Grabot E, Archambault V, Seguela P. A protein kinase C site highly conserved in P2X
27 subunits controls the desensitization kinetics of P2X(2) ATP-gated channels. *J Biol Chem* 2000,
28 275: 10190-10195.
29
30 [33] Qureshi OS, Paramasivam A, Yu JC, Murrell-Lagnado RD. Regulation of P2X4 receptors by
31 lysosomal targeting, glycan protection and exocytosis. *J Cell Sci* 2007, 120: 3838-3849.
32
33 [34] Bobanovic LK, Royle SJ, Murrell-Lagnado RD. P2X receptor trafficking in neurons is
34 subunit specific. *J Neurosci* 2002, 22: 4814-4824.
35
36 [35] Royle SJ, Bobanovic LK, Murrell-Lagnado RD. Identification of a non-canonical tyrosine-
37 based endocytic motif in an ionotropic receptor. *J Biol Chem* 2002, 277: 35378-35385.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [36] Jo YH, Donier E, Martinez A, Garret M, Toulme E, Boue-Grabot E. Cross-talk between P2X4
4 and gamma-aminobutyric acid, type A receptors determines synaptic efficacy at a central synapse.
5 J Biol Chem 2011, 286: 19993-20004.
6
7
8
9
10 [37] Boumechache M, Masin M, Edwardson JM, Gorecki DC, Murrell-Lagnado R. Analysis of
11 assembly and trafficking of native P2X4 and P2X7 receptor complexes in rodent immune cells. J
12 Biol Chem 2009, 284: 13446-13454.
13
14
15
16 [38] Royle SJ, Qureshi OS, Bobanovic LK, Evans PR, Owen DJ, Murrell-Lagnado RD. Non-
17 canonical YXXGPhi endocytic motifs: recognition by AP2 and preferential utilization in P2X4
18 receptors. J Cell Sci 2005, 118: 3073-3080.
19
20
21
22 [39] Xu J, Chai H, Ehinger K, Egan TM, Srinivasan R, Frick M, *et al.* Imaging P2X4 receptor
23 subcellular distribution, trafficking, and regulation using P2X4-pHluorin. J Gen Physiol 2014,
24 144: 81-104.
25
26
27
28 [40] Toulme E, Garcia A, Samways D, Egan TM, Carson MJ, Khakh BS. P2X4 receptors in
29 activated C8-B4 cells of cerebellar microglial origin. J Gen Physiol 2010, 135: 333-353.
30
31
32
33 [41] Stokes L, Surprenant A. Dynamic regulation of the P2X4 receptor in alveolar macrophages
34 by phagocytosis and classical activation. Eur J Immunol 2009, 39: 986-995.
35
36
37
38 [42] Trang T, Salter MW. P2X4 purinoceptor signaling in chronic pain. Purinergic Signal 2012,
39 8: 621-628.
40
41
42
43 [43] Murrell-Lagnado RD, Frick M. P2X4 and lysosome fusion. Curr Opin Pharmacol 2019, 47:
44 126-132.
45
46
47
48 [44] Zhang Z, Chen G, Zhou W, Song A, Xu T, Luo Q, *et al.* Regulated ATP release from
49 astrocytes through lysosome exocytosis. Nat Cell Biol 2007, 9: 945-953.
50
51
52
53 [45] Besnard A, Gautherot J, Julien B, Tebbi A, Garcin I, Doignon I, *et al.* The P2X4 purinergic
54 receptor impacts liver regeneration after partial hepatectomy in mice through the regulation of
55 biliary homeostasis. Hepatology 2016, 64: 941-953.
56
57
58
59
60

- 1
2
3 [46] Cao Q, Zhong XZ, Zou Y, Murrell-Lagnado R, Zhu MX, Dong XP. Calcium release through
4 P2X4 activates calmodulin to promote endolysosomal membrane fusion. *J Cell Biol* 2015, 209:
5 879-894.
6
7
8
9
10 [47] Bo X, Kim M, Nori SL, Schoepfer R, Burnstock G, North RA. Tissue distribution of P2X4
11 receptors studied with an ectodomain antibody. *Cell Tissue Res* 2003, 313: 159-165.
12
13 [48] Seguela P, Haghghi A, Soghomonian JJ, Cooper E. A novel neuronal P2x ATP receptor ion
14 channel with widespread distribution in the brain. *J Neurosci* 1996, 16: 448-455.
15
16 [49] Lê KT, Villeneuve P, Ramjaun AR, McPherson PS, Beaudet A, Seguela P. Sensory
17 presynaptic and widespread somatodendritic immunolocalization of central ionotropic P2X ATP
18 receptors. *Neuroscience* 1998, 83: 177-190.
19
20 [50] Amadio S, Montilli C, Picconi B, Calabresi P, Volonte C. Mapping P2X and P2Y receptor
21 proteins in striatum and substantia nigra: An immunohistological study. *Purinergic Signal* 2007,
22 3: 389-398.
23
24 [51] Stojilkovic SS. Purinergic regulation of hypothalamopituitary functions. *Trends Endocrinol*
25 *Metab* 2009, 20: 460-468.
26
27 [52] Lalo U, Verkhatsky A, Pankratov Y. Ivermectin potentiates ATP-induced ion currents in
28 cortical neurones: evidence for functional expression of P2X4 receptors? *Neurosci Lett* 2007, 421:
29 158-162.
30
31 [53] Tan Y, Zhao B, Zeng QC, Shi CM, Zhao FB, Li ZW. Characteristics of ATP-activated current
32 in nodose ganglion neurons of rats. *Neurosci Lett* 2009, 459: 25-29.
33
34 [54] Luo J, Yin GF, Gu YZ, Liu Y, Dai JP, Li C, *et al.* Characterization of three types of ATP-
35 activated current in relation to P2X subunits in rat trigeminal ganglion neurons. *Brain Res* 2006,
36 1115: 9-15.
37
38 [55] Ito K, Chihara Y, Iwasaki S, Komuta Y, Sugasawa M, Sahara Y. Functional ligand-gated
39 purinergic receptors (P2X) in rat vestibular ganglion neurons. *Hear Res* 2010, 267: 89-95.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [56] Wheeler-Schilling TH, Marquardt K, Kohler K, Guenther E, Jabs R. Identification of
4 purinergic receptors in retinal ganglion cells. *Brain Res Mol Brain Res* 2001, 92: 177-180.
5
6
7 [57] Sim JA, Chaumont S, Jo J, Ulmann L, Young MT, Cho K, *et al.* Altered hippocampal synaptic
8 potentiation in P2X4 knock-out mice. *J Neurosci* 2006, 26: 9006-9009.
9
10
11 [58] Xu J, Bernstein AM, Wong A, Lu XH, Khoja S, Yang XW, *et al.* P2X4 Receptor Reporter
12 Mice: Sparse Brain Expression and Feeding-Related Presynaptic Facilitation in the Arcuate
13 Nucleus. *J Neurosci* 2016, 36: 8902-8920.
14
15
16 [59] Bertin E, Deluc T, Pilch KS, Martinez A, Pougnet JT, Doudnikoff E, *et al.* Increased surface
17 P2X4 receptor regulates anxiety and memory in P2X4 internalization-defective knock-in mice.
18 *Mol Psychiatry* 2020.
19
20
21 [60] Le KT, Babinski K, Seguela P. Central P2X4 and P2X6 channel subunits coassemble into a
22 novel heteromeric ATP receptor. *J Neurosci* 1998, 18: 7152-7159.
23
24
25 [61] Antonio LS, Stewart AP, Varanda WA, Edwardson JM. Identification of P2X2/P2X4/P2X6
26 heterotrimeric receptors using atomic force microscopy (AFM) imaging. *FEBS Lett* 2014, 588:
27 2125-2128.
28
29
30 [62] Lalo U, Palygin O, Verkhratsky A, Grant SG, Pankratov Y. ATP from synaptic terminals and
31 astrocytes regulates NMDA receptors and synaptic plasticity through PSD-95 multi-protein
32 complex. *Sci Rep* 2016, 6: 33609.
33
34
35 [63] Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron* 2009, 61:
36 340-350.
37
38
39 [64] Pankratov Y, Lalo U, Krishtal OA, Verkhratsky A. P2X receptors and synaptic plasticity.
40 *Neuroscience* 2009, 158: 137-148.
41
42
43 [65] Baxter AW, Choi SJ, Sim JA, North RA. Role of P2X4 receptors in synaptic strengthening
44 in mouse CA1 hippocampal neurons. *Eur J Neurosci* 2011, 34: 213-220.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [66] Jo YH, Boue-Grabot E. Interplay between ionotropic receptors modulates inhibitory synaptic
4 strength. *Commun Integr Biol* 2011, 4: 706-709.
5
6
7 [67] Lalo U, Palygin O, Rasooli-Nejad S, Andrew J, Haydon PG, Pankratov Y. Exocytosis of ATP
8 from astrocytes modulates phasic and tonic inhibition in the neocortex. *PLoS Biol* 2014, 12:
9 e1001747.
10
11
12 [68] Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, Kater SB. ATP released
13 from astrocytes mediates glial calcium waves. *J Neurosci* 1999, 19: 520-528.
14
15 [69] Craigie E, Birch RE, Unwin RJ, Wildman SS. The relationship between P2X4 and P2X7: a
16 physiologically important interaction? *Front Physiol* 2013, 4: 216.
17
18 [70] Kukley M, Barden JA, Steinhauser C, Jabs R. Distribution of P2X receptors on astrocytes in
19 juvenile rat hippocampus. *Glia* 2001, 36: 11-21.
20
21 [71] Franke H, Grosche J, Schadlich H, Krugel U, Allgaier C, Illes P. P2X receptor expression on
22 astrocytes in the nucleus accumbens of rats. *Neuroscience* 2001, 108: 421-429.
23
24 [72] Stokes L, Layhadi JA, Bibic L, Dhuna K, Fountain SJ. P2X4 Receptor Function in the
25 Nervous System and Current Breakthroughs in Pharmacology. *Front Pharmacol* 2017, 8: 291.
26
27 [73] Tan YL, Yuan Y, Tian L. Microglial regional heterogeneity and its role in the brain. *Mol*
28 *Psychiatry* 2020, 25: 351-367.
29
30 [74] Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcao A, Xiao L, *et al.*
31 Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science*
32 2016, 352: 1326-1329.
33
34 [75] John Lin CC, Yu K, Hatcher A, Huang TW, Lee HK, Carlson J, *et al.* Identification of diverse
35 astrocyte populations and their malignant analogs. *Nat Neurosci* 2017, 20: 396-405.
36
37 [76] Deng Z, Li C, Du E, Liu C, Xia B, Chen H, *et al.* Catestatin Enhances Neuropathic Pain
38 Mediated by P2X4 Receptor of Dorsal Root Ganglia in a Rat Model of Chronic Constriction
39 Injury. *Cell Physiol Biochem* 2018, 51: 812-826.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [77] Teixeira JM, Dos Santos GG, Neves AF, Athie MCP, Bonet IJM, Nishijima CM, *et al.*
4
5 Diabetes-induced Neuropathic Mechanical Hyperalgesia Depends on P2X4 Receptor Activation
6
7 in Dorsal Root Ganglia. *Neuroscience* 2019, 398: 158-170.
8
9
10 [78] Agresti C, Meomartini ME, Amadio S, Ambrosini E, Volonte C, Aloisi F, *et al.* ATP regulates
11
12 oligodendrocyte progenitor migration, proliferation, and differentiation: involvement of
13
14 metabotropic P2 receptors. *Brain Res Brain Res Rev* 2005, 48: 157-165.
15
16
17 [79] Matute C, Torre I, Perez-Cerda F, Perez-Samartin A, Alberdi E, Etxebarria E, *et al.* P2X(7)
18
19 receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental
20
21 autoimmune encephalomyelitis. *J Neurosci* 2007, 27: 9525-9533.
22
23
24 [80] Su WF, Wu F, Jin ZH, Gu Y, Chen YT, Fei Y, *et al.* Overexpression of P2X4 receptor in
25
26 Schwann cells promotes motor and sensory functional recovery and remyelination via BDNF
27
28 secretion after nerve injury. *Glia* 2019, 67: 78-90.
29
30
31 [81] Todd AJ. Neuronal circuitry for pain processing in the dorsal horn. *Nat Rev Neurosci* 2010,
32
33 11: 823-836.
34
35
36 [82] Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain.
37
38 *Cell* 2009, 139: 267-284.
39
40
41 [83] Ji RR. Recent Progress in Understanding the Mechanisms of Pain and Itch: the Second Special
42
43 Issue. *Neurosci Bull* 2018, 34: 1-3.
44
45
46 [84] Inoue K. Role of the P2X4 receptor in neuropathic pain. *Curr Opin Pharmacol* 2019, 47: 33-
47
48 39.
49
50
51 [85] Gilmore SA. Proliferation of non-neuronal cells in spinal cords of irradiated, immature rats
52
53 following transection of the sciatic nerve. *Anat Rec* 1975, 181: 799-811.
54
55
56 [86] Gilmore SA, Skinner RD. Intraspinous non-neuronal cellular responses to peripheral nerve
57
58 injury. *Anat Rec* 1979, 194: 369-387.
59
60

- 1
2
3 [87] Masuda J, Tsuda M, Tozaki-Saitoh H, Inoue K. Intrathecal delivery of PDGF produces tactile
4 allodynia through its receptors in spinal microglia. *Mol Pain* 2009, 5: 23.
5
6
7 [88] Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like receptor 4 in innate
8 neuroimmunity and painful neuropathy. *Proc Natl Acad Sci U S A* 2005, 102: 5856-5861.
9
10
11 [89] Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, *et al.*
12 P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 2003,
13 424: 778-783.
14
15
16 [90] Tsuda M, Toyomitsu E, Kometani M, Tozaki-Saitoh H, Inoue K. Mechanisms underlying
17 fibronectin-induced up-regulation of P2X4R expression in microglia: distinct roles of PI3K-Akt
18 and MEK-ERK signalling pathways. *J Cell Mol Med* 2009, 13: 3251-3259.
19
20
21 [91] Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, *et al.* Up-regulation
22 of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and
23 neuropathic pain. *J Neurosci* 2008, 28: 11263-11268.
24
25
26 [92] Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, *et al.* BDNF from microglia
27 causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005, 438: 1017-
28 1021.
29
30
31 [93] Trang T, Beggs S, Wan X, Salter MW. P2X4-receptor-mediated synthesis and release of
32 brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated
33 protein kinase activation. *J Neurosci* 2009, 29: 3518-3528.
34
35
36 [94] Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, *et al.* Different immune
37 cells mediate mechanical pain hypersensitivity in male and female mice. *Nat Neurosci* 2015, 18:
38 1081-1083.
39
40
41 [95] Nakatsuka T, Gu JG. ATP P2X receptor-mediated enhancement of glutamate release and
42 evoked EPSCs in dorsal horn neurons of the rat spinal cord. *J Neurosci* 2001, 21: 6522-6531.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [96] Jo YH, Schlichter R. Synaptic corelease of ATP and GABA in cultured spinal neurons. *Nat*
4
5 *Neurosci* 1999, 2: 241-245.
6
7 [97] Masuda T, Ozono Y, Mikuriya S, Kohro Y, Tozaki-Saitoh H, Iwatsuki K, *et al.* Dorsal horn
8
9 neurons release extracellular ATP in a VNUT-dependent manner that underlies neuropathic pain.
10
11 *Nat Commun* 2016, 7: 12529.
12
13 [98] Boue-Grabot E, Toulme E, Emerit MB, Garret M. Subunit-specific coupling between gamma-
14
15 aminobutyric acid type A and P2X2 receptor channels. *J Biol Chem* 2004, 279: 52517-52525.
16
17 [99] Masuda T, Tsuda M, Yoshinaga R, Tozaki-Saitoh H, Ozato K, Tamura T, *et al.* IRF8 is a
18
19 critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep* 2012,
20
21 1: 334-340.
22
23 [100] Masuda T, Iwamoto S, Yoshinaga R, Tozaki-Saitoh H, Nishiyama A, Mak TW, *et al.*
24
25 Transcription factor IRF5 drives P2X4R⁺-reactive microglia gating neuropathic pain. *Nat*
26
27 *Commun* 2014, 5: 3771.
28
29 [101] Tsuda M, Toyomitsu E, Komatsu T, Masuda T, Kunifusa E, Nasu-Tada K, *et al.*
30
31 Fibronectin/integrin system is involved in P2X(4) receptor upregulation in the spinal cord and
32
33 neuropathic pain after nerve injury. *Glia* 2008, 56: 579-585.
34
35 [102] Toyomitsu E, Tsuda M, Yamashita T, Tozaki-Saitoh H, Tanaka Y, Inoue K. CCL2 promotes
36
37 P2X4 receptor trafficking to the cell surface of microglia. *Purinergic Signal* 2012, 8: 301-310.
38
39 [103] Mapplebeck JC, Beggs S, Salter MW. Sex differences in pain: a tale of two immune cells.
40
41 *Pain* 2016, 157 Suppl 1: S2-6.
42
43 [104] Tsuda M. Modulation of Pain and Itch by Spinal Glia. *Neurosci Bull* 2018, 34: 178-185.
44
45 [105] Jin XH, Wang LN, Zuo JL, Yang JP, Liu SL. P2X4 receptor in the dorsal horn partially
46
47 contributes to brain-derived neurotrophic factor oversecretion and toll-like receptor-4 receptor
48
49 activation associated with bone cancer pain. *J Neurosci Res* 2014, 92: 1690-1702.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [106] Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, Surprenant A, *et al.* Cloning
4 OF P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-
5 gated ion channels. *J Neurosci* 1996, 16: 2495-2507.
6
7
8
9
10 [107] Toulme E, Tsuda M, Khakh BS, Inoue K. On the Role of ATP-Gated P2X Receptors in
11 Acute, Inflammatory and Neuropathic Pain. *Translational Pain Research: From Mouse to Man*
12 2010.
13
14
15
16 [108] Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, *et al.* Unbiased classification
17 of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* 2015, 18: 145-
18 153.
19
20
21
22
23 [109] Lalisie S, Hua J, Lenoir M, Linck N, Rassendren F, Ulmann L. Sensory neuronal P2RX4
24 receptors controls BDNF signaling in inflammatory pain. *Sci Rep* 2018, 8: 964.
25
26
27
28 [110] Kobayashi K, Yamanaka H, Noguchi K. Expression of ATP receptors in the rat dorsal root
29 ganglion and spinal cord. *Anat Sci Int* 2013, 88: 10-16.
30
31
32
33 [111] Ying M, Liu H, Zhang T, Jiang C, Gong Y, Wu B, *et al.* Effect of artemisinin on neuropathic
34 pain mediated by P2X4 receptor in dorsal root ganglia. *Neurochem Int* 2017, 108: 27-33.
35
36
37
38 [112] Kushnir R, Cherkas PS, Hanani M. Peripheral inflammation upregulates P2X receptor
39 expression in satellite glial cells of mouse trigeminal ganglia: a calcium imaging study.
40 *Neuropharmacology* 2011, 61: 739-746.
41
42
43
44 [113] Yuan H, Ouyang S, Yang R, Li S, Gong Y, Zou L, *et al.* Osthole alleviated diabetic
45 neuropathic pain mediated by the P2X4 receptor in dorsal root ganglia. *Brain Res Bull* 2018, 142:
46 289-296.
47
48
49
50
51 [114] Zhang H, Mei X, Zhang P, Ma C, White FA, Donnelly DF, *et al.* Altered functional
52 properties of satellite glial cells in compressed spinal ganglia. *Glia* 2009, 57: 1588-1599.
53
54
55
56 [115] Hanani M, Blum E, Liu S, Peng L, Liang S. Satellite glial cells in dorsal root ganglia are
57 activated in streptozotocin-treated rodents. *J Cell Mol Med* 2014, 18: 2367-2371.
58
59
60

- 1
2
3 [116] Ulmann L, Hirbec H, Rassendren F. P2X4 receptors mediate PGE2 release by tissue-resident
4 macrophages and initiate inflammatory pain. *EMBO J* 2010, 29: 2290-2300.
5
6
7 [117] Aby F, Whitestone S, Landry M, Ulmann L, Fossat P. Inflammatory-induced spinal dorsal
8 horn neurons hyperexcitability is mediated by P2X4 receptors. *Pain Rep* 2018, 3: e660.
9
10
11 [118] Zhang W, Liu LY, Xu TL. Reduced potassium-chloride co-transporter expression in spinal
12 cord dorsal horn neurons contributes to inflammatory pain hypersensitivity in rats. *Neuroscience*
13 2008, 152: 502-510.
14
15
16 [119] Zhao S, Zhou Y, Fan Y, Gong Y, Yang J, Yang R, *et al.* Involvement of purinergic 2X4
17 receptor in glycoprotein 120-induced pyroptosis in dorsal root ganglia. *J Neurochem* 2019, 151:
18 584-594.
19
20
21 [120] Dodick D, Silberstein S. Central sensitization theory of migraine: clinical implications.
22 *Headache* 2006, 46 Suppl 4: S182-191.
23
24
25 [121] Long T, He W, Pan Q, Zhang S, Zhang Y, Liu C, *et al.* Microglia P2X4 receptor contributes
26 to central sensitization following recurrent nitroglycerin stimulation. *J Neuroinflammation* 2018,
27 15: 245.
28
29
30 [122] Martins LB, Teixeira AL, Domingues RB. Neurotrophins and Migraine. *Vitam Horm* 2017,
31 104: 459-473.
32
33
34 [123] Long T, He W, Pan Q, Zhang S, Zhang D, Qin G, *et al.* Microglia P2X4R-BDNF signalling
35 contributes to central sensitization in a recurrent nitroglycerin-induced chronic migraine model. *J*
36 *Headache Pain* 2020, 21: 4.
37
38
39 [124] Cavaliere F, Florenzano F, Amadio S, Fusco FR, Viscomi MT, D'Ambrosi N, *et al.* Up-
40 regulation of P2X2, P2X4 receptor and ischemic cell death: prevention by P2 antagonists.
41 *Neuroscience* 2003, 120: 85-98.
42
43
44 [125] Cheng RD, Ren JJ, Zhang YY, Ye XM. P2X4 receptors expressed on microglial cells in
45 post-ischemic inflammation of brain ischemic injury. *Neurochem Int* 2014, 67: 9-13.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [126] Verma R, Cronin CG, Hudobenko J, Venna VR, McCullough LD, Liang BT. Deletion of
4 the P2X4 receptor is neuroprotective acutely, but induces a depressive phenotype during recovery
5 from ischemic stroke. *Brain Behav Immun* 2017, 66: 302-312.
6
7
8
9
10 [127] Vazquez-Villoldo N, Domercq M, Martin A, Llop J, Gomez-Vallejo V, Matute C. P2X4
11 receptors control the fate and survival of activated microglia. *Glia* 2014, 62: 171-184.
12
13 [128] Li F, Wang L, Li JW, Gong M, He L, Feng R, *et al.* Hypoxia induced amoeboid microglial
14 cell activation in postnatal rat brain is mediated by ATP receptor P2X4. *BMC Neurosci* 2011, 12:
15 111.
16
17
18
19
20 [129] Ohsawa K, Irino Y, Nakamura Y, Akazawa C, Inoue K, Kohsaka S. Involvement of P2X4
21 and P2Y12 receptors in ATP-induced microglial chemotaxis. *Glia* 2007, 55: 604-616.
22
23 [130] Ozaki T, Muramatsu R, Sasai M, Yamamoto M, Kubota Y, Fujinaka T, *et al.* The P2X4
24 receptor is required for neuroprotection via ischemic preconditioning. *Sci Rep* 2016, 6: 25893.
25
26 [131] Kang TC, An SJ, Park SK, Hwang IK, Won MH. P2X2 and P2X4 receptor expression is
27 regulated by a GABA(A) receptor-mediated mechanism in the gerbil hippocampus. *Brain Res Mol*
28 *Brain Res* 2003, 116: 168-175.
29
30 [132] Ulmann L, Levavasseur F, Avignone E, Peyroutou R, Hirbec H, Audinat E, *et al.*
31 Involvement of P2X4 receptors in hippocampal microglial activation after status epilepticus. *Glia*
32 2013, 61: 1306-1319.
33
34 [133] Murphy MP, LeVine H, 3rd. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers*
35 *Dis* 2010, 19: 311-323.
36
37 [134] Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-
38 beta: a crucial factor in Alzheimer's disease. *Med Princ Pract* 2015, 24: 1-10.
39
40 [135] Erb L, Woods LT, Khalafalla MG, Weisman GA. Purinergic signaling in Alzheimer's
41 disease. *Brain Res Bull* 2019, 151: 25-37.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [136] Saez-Orellana F, Godoy PA, Bastidas CY, Silva-Grecchi T, Guzman L, Aguayo LG, *et al.*
4
5 ATP leakage induces P2XR activation and contributes to acute synaptic excitotoxicity induced by
6
7 soluble oligomers of beta-amyloid peptide in hippocampal neurons. *Neuropharmacology* 2016,
8
9 100: 116-123.
10
11
12 [137] Godoy PA, Ramirez-Molina O, Fuentealba J. Exploring the Role of P2X Receptors in
13
14 Alzheimer's Disease. *Front Pharmacol* 2019, 10: 1330.
15
16
17 [138] Varma R, Chai Y, Troncoso J, Gu J, Xing H, Stojilkovic SS, *et al.* Amyloid-beta induces a
18
19 caspase-mediated cleavage of P2X4 to promote purinotoxicity. *Neuromolecular Med* 2009, 11:
20
21 63-75.
22
23
24 [139] Martin E, Amar M, Dalle C, Youssef I, Boucher C, Le Duigou C, *et al.* New role of P2X7
25
26 receptor in an Alzheimer's disease mouse model. *Mol Psychiatry* 2019, 24: 108-125.
27
28
29 [140] Martinez-Frailes C, Di Lauro C, Bianchi C, de Diego-Garcia L, Sebastian-Serrano A, Bosca
30
31 L, *et al.* Amyloid Peptide Induced Neuroinflammation Increases the P2X7 Receptor Expression
32
33 in Microglial Cells, Impacting on Its Functionality. *Front Cell Neurosci* 2019, 13: 143.
34
35
36 [141] Sperlagh B, Illes P. P2X7 receptor: an emerging target in central nervous system diseases.
37
38 *Trends Pharmacol Sci* 2014, 35: 537-547.
39
40
41 [142] McGeer PL, Rogers J, McGeer EG. Inflammation, Antiinflammatory Agents, and
42
43 Alzheimer's Disease: The Last 22 Years. *J Alzheimers Dis* 2016, 54: 853-857.
44
45
46 [143] Weinhold K, Krause-Buchholz U, Rodel G, Kasper M, Barth K. Interaction and interrelation
47
48 of P2X7 and P2X4 receptor complexes in mouse lung epithelial cells. *Cell Mol Life Sci* 2010, 67:
49
50 2631-2642.
51
52
53 [144] Li F, Guo N, Ma Y, Ning B, Wang Y, Kou L. Inhibition of P2X4 suppresses joint
54
55 inflammation and damage in collagen-induced arthritis. *Inflammation* 2014, 37: 146-153.
56
57
58
59
60

- 1
2
3 [145] Layhadi JA, Turner J, Crossman D, Fountain SJ. ATP Evokes Ca(2+) Responses and
4 CXCL5 Secretion via P2X4 Receptor Activation in Human Monocyte-Derived Macrophages. *J*
5 *Immunol* 2018, 200: 1159-1168.
6
7
8
9
10 [146] Andries M, Van Damme P, Robberecht W, Van Den Bosch L. Ivermectin inhibits AMPA
11 receptor-mediated excitotoxicity in cultured motor neurons and extends the life span of a
12 transgenic mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 2007, 25: 8-16.
13
14
15 [147] Boue-Grabot E, Emerit MB, Toulme E, Seguela P, Garret M. Cross-talk and co-trafficking
16 between rho1/GABA receptors and ATP-gated channels. *J Biol Chem* 2004, 279: 6967-6975.
17
18
19 [148] Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS.
20 Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat Neurosci* 2005,
21 8: 1078-1086.
22
23
24 [149] Gu JG, MacDermott AB. Activation of ATP P2X receptors elicits glutamate release from
25 sensory neuron synapses. *Nature* 1997, 389: 749-753.
26
27
28 [150] Pankratov YV, Lalo UV, Krishtal OA. Role for P2X receptors in long-term potentiation. *J*
29 *Neurosci* 2002, 22: 8363-8369.
30
31
32 [151] Krugel U, Kittner H, Franke H, Illes P. Purinergic modulation of neuronal activity in the
33 mesolimbic dopaminergic system in vivo. *Synapse* 2003, 47: 134-142.
34
35
36 [152] Xiao C, Zhou C, Li K, Davies DL, Ye JH. Purinergic type 2 receptors at GABAergic
37 synapses on ventral tegmental area dopamine neurons are targets for ethanol action. *J Pharmacol*
38 *Exp Ther* 2008, 327: 196-205.
39
40
41 [153] Zhou FC, Zhang JK, Lumeng L, Li TK. Mesolimbic dopamine system in alcohol-preferring
42 rats. *Alcohol* 1995, 12: 403-412.
43
44
45 [154] Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA. Prepulse inhibition deficits and
46 perseverative motor patterns in dopamine transporter knock-out mice: differential effects of D1
47 and D2 receptor antagonists. *J Neurosci* 2001, 21: 305-313.
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [155] Khoja S, Shah V, Garcia D, Asatryan L, Jakowec MW, Davies DL. Role of purinergic P2X4
4 receptors in regulating striatal dopamine homeostasis and dependent behaviors. *J Neurochem*
5 2016, 139: 134-148.
6
7
8
9
10 [156] Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. *J Neural*
11 *Transm Suppl* 2000: 143-151.
12
13
14 [157] Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution
15 of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's
16 disease brains. *Acta Neuropathol* 2003, 106: 518-526.
17
18
19
20 [158] Mount MP, Lira A, Grimes D, Smith PD, Faucher S, Slack R, *et al.* Involvement of
21 interferon-gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci* 2007, 27:
22 3328-3337.
23
24
25
26
27
28 [159] Doorn KJ, Moors T, Drukarch B, van de Berg W, Lucassen PJ, van Dam AM. Microglial
29 phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy
30 body disease cases and Parkinson's disease patients. *Acta Neuropathol Commun* 2014, 2: 90.
31
32
33
34 [160] Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. *Nat Rev*
35 *Immunol* 2011, 11: 775-787.
36
37
38
39 [161] Joers V, Tansey MG, Mulas G, Carta AR. Microglial phenotypes in Parkinson's disease and
40 animal models of the disease. *Prog Neurobiol* 2017, 155: 57-75.
41
42
43
44 [162] Wang XH, Xie X, Luo XG, Shang H, He ZY. Inhibiting purinergic P2X7 receptors with the
45 antagonist brilliant blue G is neuroprotective in an intranigral lipopolysaccharide animal model of
46 Parkinson's disease. *Mol Med Rep* 2017, 15: 768-776.
47
48
49
50 [163] Crabbe M, Van der Perren A, Bollaerts I, Kounelis S, Baekelandt V, Bormans G, *et al.*
51 Increased P2X7 Receptor Binding Is Associated With Neuroinflammation in Acute but Not
52 Chronic Rodent Models for Parkinson's Disease. *Front Neurosci* 2019, 13: 799.
53
54
55
56
57
58
59
60

- 1
2
3 [164] Le W, Wu J, Tang Y. Protective Microglia and Their Regulation in Parkinson's Disease.
4
5 Front Mol Neurosci 2016, 9: 89.
6
7 [165] Volonte C, Apolloni S, Parisi C, Amadio S. Purinergic contribution to amyotrophic lateral
8
9 sclerosis. Neuropharmacology 2016, 104: 180-193.
10
11 [166] Casanovas A, Hernandez S, Tarabal O, Rossello J, Esquerda JE. Strong P2X4 purinergic
12
13 receptor-like immunoreactivity is selectively associated with degenerating neurons in transgenic
14
15 rodent models of amyotrophic lateral sclerosis. J Comp Neurol 2008, 506: 75-92.
16
17 [167] Hernandez S, Casanovas A, Piedrafita L, Tarabal O, Esquerda JE. Neurotoxic species of
18
19 misfolded SOD1G93A recognized by antibodies against the P2X4 subunit of the ATP receptor
20
21 accumulate in damaged neurons of transgenic animal models of amyotrophic lateral sclerosis. J
22
23 Neuropathol Exp Neurol 2010, 69: 176-187.
24
25 [168] D'Ambrosi N, Finocchi P, Apolloni S, Cozzolino M, Ferri A, Padovano V, *et al.* The
26
27 proinflammatory action of microglial P2 receptors is enhanced in SOD1 models for amyotrophic
28
29 lateral sclerosis. J Immunol 2009, 183: 4648-4656.
30
31 [169] Liu J, Wang F. Role of Neuroinflammation in Amyotrophic Lateral Sclerosis: Cellular
32
33 Mechanisms and Therapeutic Implications. Front Immunol 2017, 8: 1005.
34
35 [170] Cieslak M, Roszek K, Wujak M. Purinergic implication in amyotrophic lateral sclerosis-
36
37 from pathological mechanisms to therapeutic perspectives. Purinergic Signal 2019, 15: 1-15.
38
39 [171] Fabbriozio P, Amadio S, Apolloni S, Volonte C. P2X7 Receptor Activation Modulates
40
41 Autophagy in SOD1-G93A Mouse Microglia. Front Cell Neurosci 2017, 11: 249.
42
43 [172] Apolloni S, Amadio S, Montilli C, Volonte C, D'Ambrosi N. Ablation of P2X7 receptor
44
45 exacerbates gliosis and motoneuron death in the SOD1-G93A mouse model of amyotrophic lateral
46
47 sclerosis. Hum Mol Genet 2013, 22: 4102-4116.
48
49 [173] Sluyter R, Bartlett R, Ly D, Yerbury JJ. P2X7 receptor antagonism in amyotrophic lateral
50
51 sclerosis. Neural Regen Res 2017, 12: 749-750.
52
53
54
55
56
57
58
59
60

- 1
2
3 [174] Kawano A, Tsukimoto M, Mori D, Noguchi T, Harada H, Takenouchi T, *et al.* Regulation
4 of P2X7-dependent inflammatory functions by P2X4 receptor in mouse macrophages. *Biochem*
5
6
7 *Biophys Res Commun* 2012, 420: 102-107.
8
9
10 [175] Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev*
11
12 *Immunol* 2015, 15: 545-558.
13
14 [176] Zabala A, Vazquez-Villoldo N, Rissiek B, Gejo J, Martin A, Palomino A, *et al.* P2X4
15
16
17 receptor controls microglia activation and favors remyelination in autoimmune encephalitis.
18
19 *EMBO Mol Med* 2018, 10.
20
21 [177] Feldmann A, Amphornrat J, Schonherr M, Winterstein C, Mobius W, Ruhwedel T, *et al.*
22
23
24 Transport of the major myelin proteolipid protein is directed by VAMP3 and VAMP7. *J Neurosci*
25
26
27 2011, 31: 5659-5672.
28
29 [178] Chen G, Zhang Z, Wei Z, Cheng Q, Li X, Li W, *et al.* Lysosomal exocytosis in Schwann
30
31
32 cells contributes to axon remyelination. *Glia* 2012, 60: 295-305.
33
34 [179] Fois G, Winkelmann VE, Bareis L, Staudenmaier L, Hecht E, Ziller C, *et al.* ATP is stored
35
36
37 in lamellar bodies to activate vesicular P2X4 in an autocrine fashion upon exocytosis. *J Gen*
38
39 *Physiol* 2018, 150: 277-291.
40
41 [180] Yardley MM, Wyatt L, Khoja S, Asatryan L, Ramaker MJ, Finn DA, *et al.* Ivermectin
42
43
44 reduces alcohol intake and preference in mice. *Neuropharmacology* 2012, 63: 190-201.
45
46 [181] Wyatt LR, Finn DA, Khoja S, Yardley MM, Asatryan L, Alkana RL, *et al.* Contribution of
47
48
49 P2X4 receptors to ethanol intake in male C57BL/6 mice. *Neurochem Res* 2014, 39: 1127-1139.
50
51 [182] Franklin KM, Asatryan L, Jakowec MW, Trudell JR, Bell RL, Davies DL. P2X4 receptors
52
53
54 (P2X4Rs) represent a novel target for the development of drugs to prevent and/or treat alcohol use
55
56
57
58
59
60 disorders. *Front Neurosci* 2014, 8: 176.

- 1
2
3 [183] Franklin KM, Hauser SR, Lasek AW, Bell RL, McBride WJ. Involvement of Purinergic
4 P2X4 Receptors in Alcohol Intake of High-Alcohol-Drinking (HAD) Rats. *Alcohol Clin Exp Res*
5 2015, 39: 2022-2031.
6
7
8
9 [184] Kimpel MW, Strother WN, McClintick JN, Carr LG, Liang T, Edenberg HJ, *et al.* Functional
10 gene expression differences between inbred alcohol-preferring and -non-preferring rats in five
11 brain regions. *Alcohol* 2007, 41: 95-132.
12
13 [185] Asatryan L, Nam HW, Lee MR, Thakkar MM, Saeed Dar M, Davies DL, *et al.* Implication
14 of the purinergic system in alcohol use disorders. *Alcohol Clin Exp Res* 2011, 35: 584-594.
15
16 [186] Davies DL, Kuo ST, Alkana RL. Differential effects of propofol and ethanol on P2X4
17 receptors expressed in *Xenopus* oocytes. *Int Congr Ser* 2005, 1283: 285-287.
18
19 [187] Ostrovskaya O, Asatryan L, Wyatt L, Popova M, Li K, Peoples RW, *et al.* Ethanol is a fast
20 channel inhibitor of P2X4 receptors. *J Pharmacol Exp Ther* 2011, 337: 171-179.
21
22 [188] Asatryan L, Popova M, Perkins D, Trudell JR, Alkana RL, Davies DL. Ivermectin
23 antagonizes ethanol inhibition in purinergic P2X4 receptors. *J Pharmacol Exp Ther* 2010, 334:
24 720-728.
25
26 [189] Potula R, Haorah J, Knipe B, Leibhart J, Chrastil J, Heilman D, *et al.* Alcohol abuse enhances
27 neuroinflammation and impairs immune responses in an animal model of human
28 immunodeficiency virus-1 encephalitis. *Am J Pathol* 2006, 168: 1335-1344.
29
30 [190] Gofman L, Cenna JM, Potula R. P2X4 receptor regulates alcohol-induced responses in
31 microglia. *J Neuroimmune Pharmacol* 2014, 9: 668-678.
32
33 [191] Gofman L, Fernandes NC, Potula R. Relative Role of Akt, ERK and CREB in Alcohol-
34 Induced Microglia P2X4R Receptor Expression. *Alcohol Alcohol* 2016, 51: 647-654.
35
36 [192] Wixey JA, Reinebrant HE, Carty ML, Buller KM. Delayed P2X4R expression after hypoxia-
37 ischemia is associated with microglia in the immature rat brain. *J Neuroimmunol* 2009, 212: 35-
38 43.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 [193] Bortolato M, Yardley MM, Khoja S, Godar SC, Asatryan L, Finn DA, *et al.* Pharmacological
4 insights into the role of P2X4 receptors in behavioural regulation: lessons from ivermectin. *Int J*
5
6 *Neuropsychopharmacol* 2013, 16: 1059-1070.
7

8
9
10 [194] Khoja S, Asatryan L, Jakowec MW, Davies DL. Dopamine Receptor Blockade Attenuates
11
12 Purinergic P2X4 Receptor-Mediated Prepulse Inhibition Deficits and Underlying Molecular
13
14 Mechanisms. *Front Cell Neurosci* 2019, 13: 331.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only

Figure Legends

Figure 1: P2X4 expression in CNS diseases

Left, P2X4 in normal states is mostly intracellular with low surface expression at the surface of neurons and glia. P2X4 expression in astrocytes and oligodendrocytes precursors remain controversial (indicated by a question mark). Activation of P2X4 by extracellular ATP released by neurons or glial cells exerts mostly neuromodulatory actions in 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 the increase of the number of surface P2X4 in distinct cell types in numerous central disorders and exacerbation of purinergic signaling.

Figure 2: Implication of P2X4 in spinal cord disorders

de novo P2X4 expression and/or an 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 of P2X4 in spinal cord (as well as in sensory ganglia for pain) is indicated. P2X4 potentiation was induced by ivermectin.

Figure 3: Implication of P2X4 in brain disorders

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only

Table 1. Expression of P2X4 in the CNS

CNS regions	P2X4 mRNA ^a	P2X4 protein	P2rx4 reporter mice [82, 83]	P2X4 mCherryIN mice [84]	Cell types specificity	References
Amygdala	++	NA	NA	NA	NA	
Basal ganglia	++	+	NA	NA	Neurons, Astrocytes	[48, 49, 71]
Cerebellum	++	++	++	+	Neurons	[48, 49, 57, 58, 59,]
Cerebral cortex	+++	+	++	++	Neurons Astrocytes ^b	52, 58] [59]
Hindbrain	+	++	++	NA	Neurons	[58]
Hippocampus	++	++	++	++	Neurons, Microglia, Astrocytes ^b	[13, 14, 57, 58, 59]
Hypothalamus	+	+	++	NA	Neurons	[51, 58]
Midbrain	+	+	++	NA	Neurons	[58]
Olfactory region	++	+++	+++	++	Neurons, Epithelial cells	[49, 57, 58, 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. (a) data are from the Allen brain atlas obtained by in situ hybridization and available at <http://mouse.brain-map.org/experiment/show/75551474>. (b) P2X4 expression was detected by electronic microscopy in astrocytes of P2X4mCherryIN mice using anti-RFP antibodies.

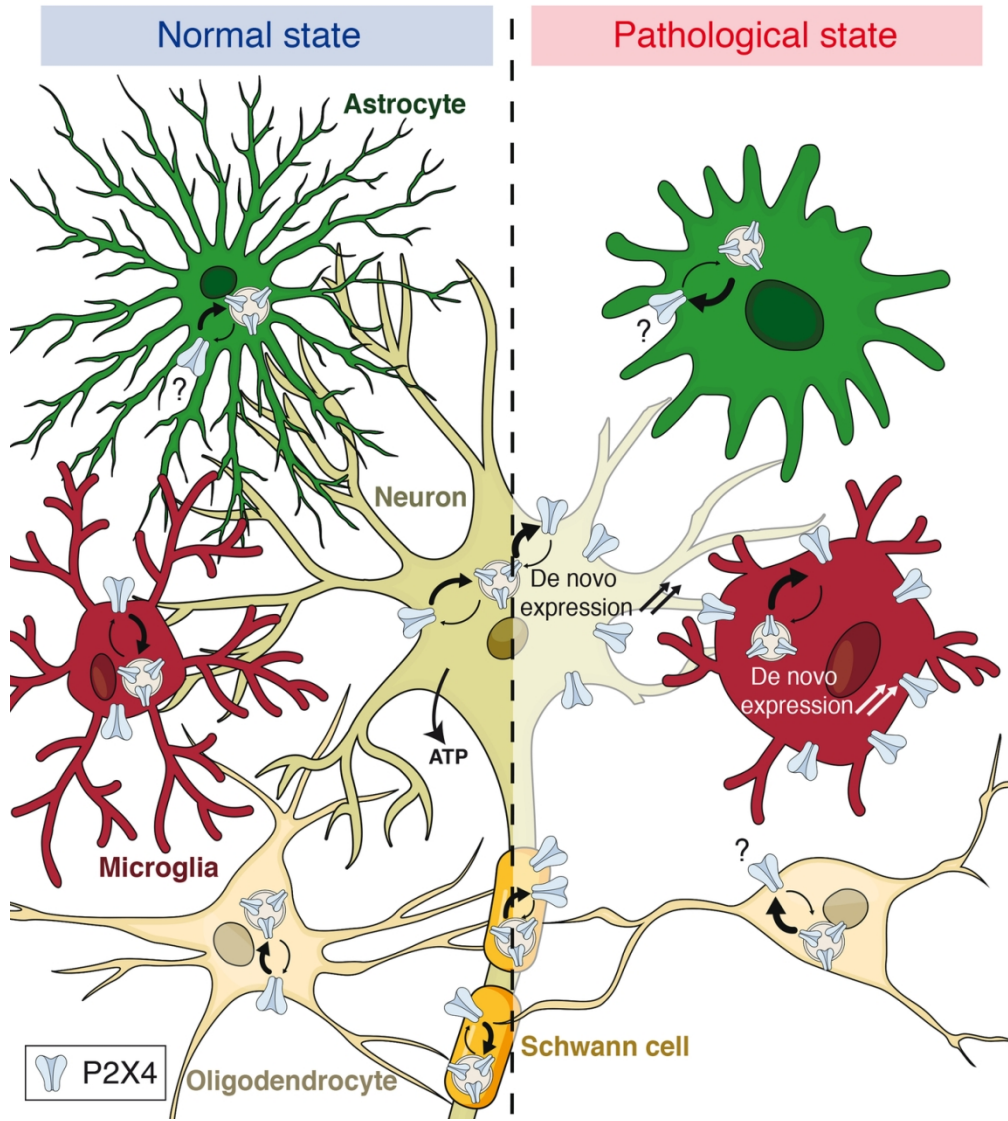


Figure 1: P2X4 expression in CNS diseases

119x134mm (300 x 300 DPI)

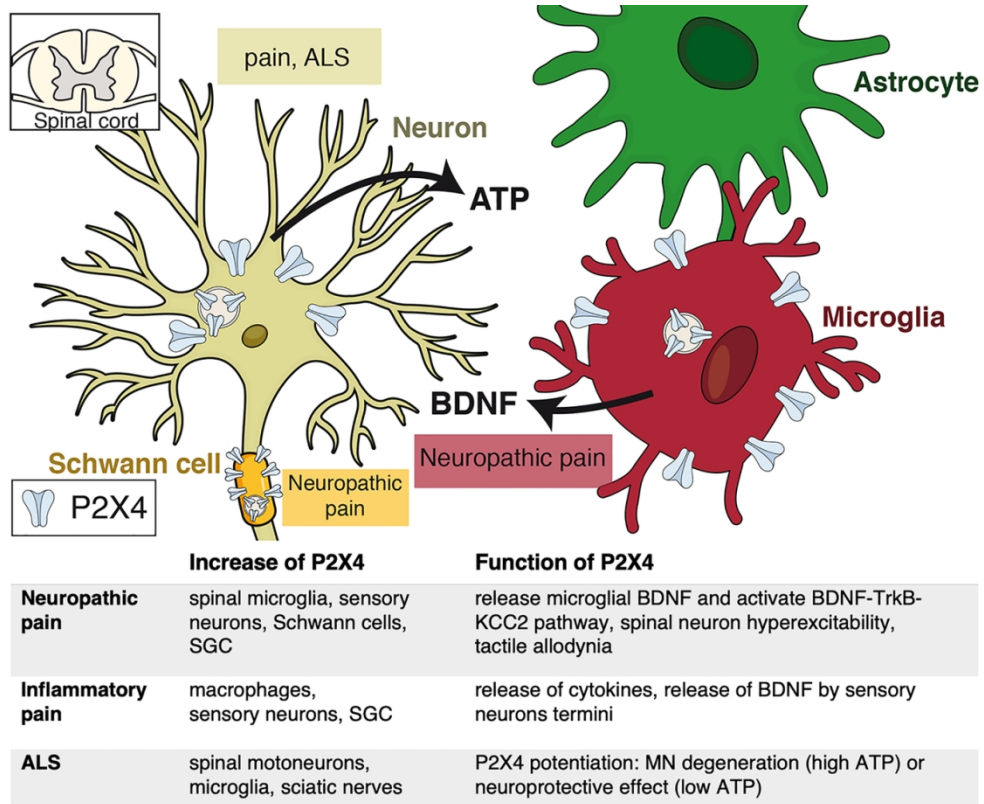
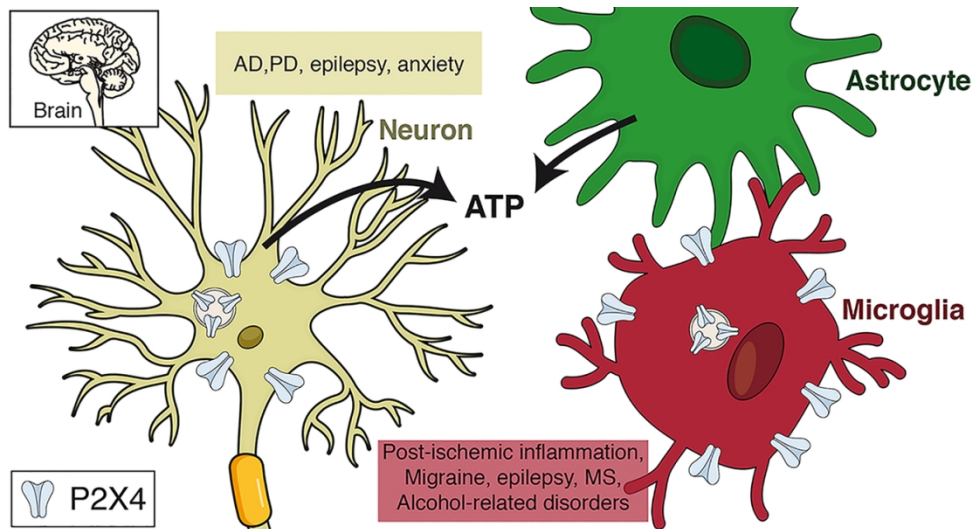


Figure 2: Implication of P2X4 in spinal cord disorders

118x96mm (300 x 300 DPI)



	Increase of P2X4	Function of P2X4
Post-ischemic inflammation	brain microglia, vascular endothelial cells	chemotaxis of microglia, release of cytokines upregulation of osteopontin (neuroprotective)
AD	hippocampal neurons	promote the toxic effect of beta-amyloid fragments increased P2X4: induced alteration of synaptic plasticity and memory deficits
Migraine	trigeminal nucleus caudalis microglia	microglial release of BDNF P2X4 potentiation: MN degeneration (high ATP), Increase p-ERK and CGRP release
Epilepsy	hippocampal neurons, stratum radiatum microglia	release of BDNF, neuronal hyperexcitability, cell death
MS	microglia, macrophages	release of microglial BDNF; Increase of myelin phagocytosis P2X4 potentiation: promotes remyelination
Alcohol-related disorders	dopaminergic neurons, microglia	ethanol induced P2X4 inhibition, reinforce alcohol intake/preference
PD	dopaminergic neurons	regulate DA homeostasis
Anxiety	forebrain neurons	increased P2X4: induced anxiolytic effects

Figure 3: Implication of P2X4 in brain disorders

114x123mm (300 x 300 DPI)