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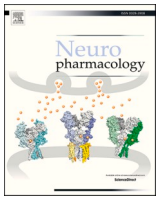

Role of purines in brain development, from neuronal proliferation to synaptic refinement

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ABSTRACT

The purinergic system includes P1 and P2 receptors, which are activated by ATP and its metabolites. They are expressed in adult neuronal and glial cells and are crucial in brain function, including neuromodulation and neuronal signaling. As P1 and P2 receptors are expressed throughout embryogenesis and development, purinergic signaling also has an important role in the development of the peripheral and central nervous system. In this review, we present the expression pattern and activity of purinergic receptors and of their signaling pathways during embryonic and postnatal development of the nervous system. In particular, we review the involvement of the purinergic signaling in all the crucial steps of brain development i.e. in neural stem cell proliferation, neuronal differentiation and migration as well as in astrogliogenesis and oligodendrogenesis. Then, we review data showing a crucial role of the ATP and adenosine signaling pathways in the formation of the peripheral neuromuscular junction and of central GABAergic and glutamatergic synapses. Finally, we examine the consequences of deregulation of the purinergic system during development and discuss the therapeutic potential of targeting it at adult stage in diseases with reactivation of the ATP and adenosine pathway.

1. Introduction

The development of an organism is a complex process of cellular and molecular interactions, highly regulated and time-precise. The formation of the central nervous system (CNS) includes proliferation of neural stem cells (NSCs) that differentiate into neuronal and astroglial lineages, that are required to organize and integrate in an intricate structure (Götz and Huttner, 2005). Moreover, the maturation of newborn neurons includes the formation of dendritic spines and synapses in a process named synaptogenesis (Südhof, 2018). Due to its immense intricacy, the developmental process occurs under tight regulation by a plethora of regulatory mechanisms, including by the purinergic system. Here we will detail the contribution of purines, their receptors and signaling pathways in brain development. We will firstly describe the purinergic receptors expressed, some transiently, during development. Then, we will discuss the contribution of the purinergic system in all steps of neuronal development (i.e. neurogenesis, neuronal migration, axon and dendrite arborization, synaptogenesis), as well as in astrogliogenesis and oligodendrogenesis.

2. Expression of purines and purine receptors during development

2.1. Purines

All brain cells produce and release purines, such as adenosine triphosphate (ATP) and its metabolites adenosine diphosphate (ADP), adenosine monophosphate (AMP) or adenosine, which have essential physiological functions. ATP is known to constitute the cellular source of energy, but it also has a role in cellular interaction and communication. In the CNS, **ATP can be stored alone or with neurotransmitters** in vesicles and can be released by glial cells through exocytosis (Zhang et al., 2003) or by neurons as a co-transmitter together with glutamate or γ-butyric acid (GABA) at excitatory glutamatergic and GABAergic synapses. Purines can be exocytosed in response to electrical stimulation, glutamate receptor agonist or in case of injury (Pankratov et al., 2006, 2007; Wall et al., 2013). In neurons, the amount of ATP in synaptic vesicles is comparable to GABA and glutamate suggesting an important role of this nucleotide in synaptic function (Zisapel and Zurgil, 1979). Astrocytes also largely contribute to extracellular ATP, via other

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mechanisms than exocytosis. Still controversial, several studies propose **ATP release by astrocytic junctions formed by connexins** (Kang et al., 2008) **or pannexins** (Iglesias et al., 2009) but also by **anion channels** such as volume-regulated anion channels (VRAC) (Blum et al., 2010) and Maxi anion channels (Zhao et al., 2017). It has been proposed that adenosine may be exocytosed in an action potential- and calcium-dependent manner by hippocampal neurons (Wall et al., 2013). However, other data showed that reducing extracellular calcium levels in hippocampal slices increases adenosine release from **equilibrative nucleoside transporters** (ENTs) (Diez et al., 2017; Baldwin et al., 2004). Extracellular and intracellular ATP is rapidly hydrolyzed by enzymes localized on glial cells and neuronal membranes (Bjelobaba et al., 2007). In rat hippocampal slices, **extracellular ATP degradation** occurs within milliseconds (Dunwiddie et al., 1997) through a **channeling process** allowing a local increase in adenosine (Cunha et al., 1998). **ATP is metabolized into AMP** by **CD39** ectonucleotidase and then into adenosine by **CD73** ectonucleotidase. The ectonucleotidases CD39 and CD73 are detected at synapses during the period of synaptogenesis, allowing local purinergic receptor activation at the synapse (Grković et al., 2019). Moreover, a transient increase in evoked activity-dependent release of ATP and adenosine has been shown during the period of synaptogenesis (i.e. at postnatal day 7, P7) as compared to adult stage (P60) in the rodent hippocampus (Gomez-Castro et al., 2021), suggesting a role of this signaling pathway in brain development. Once in the extracellular space, purines activate purinergic receptors composed of 2 subfamilies: P1 receptors (P1R) and P2 receptors (P2R) (see below).

2.2. P1 receptors

P1 receptors are G protein coupled receptors (GPCRs) that preferentially increase or decrease cAMP production via the activation or inhibition of adenylyl cyclases (ACs) depending on the nature of the G protein to which they are coupled (Fredholm et al., 1994). Since adenosine is the main agonist of P1 receptors, the latter are known as adenosine receptors. These are divided into four subtypes, the A_1 (A_1R), A_{2A} ($A_{2A}R$), A_{2B} ($A_{2B}R$) and A_3 (A_3R) receptors, which have a high degree of structural homology. **A_1R** are coupled to $G_{i/o}$, **$A_{2A}R$** to $G_{s/olf}$, **$A_{2B}R$** to $G_{s/q}$ **and A_3R** to $G_{i/q}$ proteins (Fredholm et al., 2005). $A_{2A}R$ are positively coupled to AC/Cyclic adenosine monophosphate (cAMP) pathway that in turn activates the protein kinase A (**PKA**) that phosphorylates several targets such as the cAMP-responsive element binding protein (**CREB**) and dopamine- and cAMP-regulated phosphoprotein (**DARPP-32**) (Fredholm et al., 2005). In contrast, A_1R inhibit the activity of the AC/cAMP/PKA pathway. Other P1R signaling pathways have been identified such as the mitogen-activated protein kinase (**MAPK**) pathway (Haq et al., 1998; Merighi et al., 2017). Changes in phospholipase C (**PLC**) activity following P1R activation have also been described depending on the cell type and adenosine concentration (Ribeiro et al., 2002). Interestingly, a common feature for all P1Rs is the coupling to the Extracellular signal-regulated kinases 1/2 (**ERK1/2**) (Schulte et al., 2000). In addition, A_1R activation can lead to **channel regulation** by activating several potassium channels via the β/γ subunits of the G protein (Hosseinzadeh and Stone, 1998; Rotermund et al., 2018), or by inactivating N, P and Q-type calcium channels (Hartwick et al., 2004). Finally, the carboxy-terminal part of $A_{2A}R$ can bind **regulatory proteins** as translin-associated protein X TRAX (Sun et al., 2006) and β-arrestin (Borroto-Escuela et al., 2011; Nagaoka et al., 2023).

P1R expression during development has been shown to be time-dependent. Purinergic receptors are one of the first cell surface receptors expressed during development, with adenosine receptors being present in mid-late embryogenesis onwards (Burnstock and Dale, 2015). Using in situ hybridization and binding assays, it was shown that **A_1R** expression begins at embryonic day 14 (**E14**) and then gradually **increases until E21** to stabilize at a level that **persists into adulthood**. A_1R are ubiquitous in the brain. They are detected in the hippocampus, thalamus, midbrain, cortex, and cerebellum (Rivkees, 1995; Weaver, 1996). In basal activity conditions, the weak extracellular adenosine concentration preferentially activates A_1R because this receptor displays a stronger affinity for adenosine in comparison with $A_{2A}R$ (Lopes et al., 2002). A_1R are expressed by **neurons**, but they are also present in **glial cells** (Fredholm et al., 2005). The Brain RNA-Seq website from the laboratory of B. Barres (https://www.brainrnaseq.org/) allows comparing the expression of A_1R transcripts in the different cell types of the mouse and human nervous system at the early and mature developmental stages. It shows that **A_1R transcripts are highest in OPCs**. They are also detected at a **higher level in astrocytes, newly formed oligodendrocytes or myelinating oligodendrocytes than in neurons**. A_1R is also found in **microglia** and **macrophages** but at lower levels than in neurons.

$A_{2A}R$, they are **detected at E14** in the striatum (Johansson et al., 1997), with expression **increasing markedly after birth** reaching **adult levels at P14**. $A_{2A}R$ are restricted to the striatum, hippocampus, olfactory bulb, cerebellum, and cortex (Doriat et al., 1996; Weaver, 1993). Consultation of the Brain RNA-Seq website (https://www.brainrnaseq.org/) for cell type comparison of $A_{2A}R$ mRNAs reveals that **$A_{2A}R$ transcripts** are detected at **high level** in mouse **astrocytes** and human fetal astrocytes, as well as in **neurons**, and at **low level in OPCs and microglia**. However, its expression is **much higher in endothelial cells** than in other brain cell types.

Recently, the **adenosine signaling pathway** has been investigated during the **developmental period of synaptogenesis** (between P3 and P16) in the rat and mice hippocampus *in vitro* and *in vivo*. First, a transient increase in evoked activity-dependent release of ATP and adenosine has been shown during the period of synaptogenesis as compared to adult stage (P7 vs P60) in the rodent hippocampus (Gomez-Castro et al., 2021), suggesting a role of this signaling pathway in brain development. The ectonucleotidases CD39 and CD73, two enzymes involved in ATP metabolism and adenosine production, are detected at synapses during the period of synaptogenesis (Grković et al., 2019). This transient vesicular release and local production of adenosine at the synapse is associated with an increase in the expression of $A_{2A}R$ but not of A_1R in the hippocampus (Gomez-Castro et al., 2021). This **increase in $A_{2A}R$ expression occurs at synapses**. Using electron microscopy, $A_{2A}R$ was detected in the developing (P7) hippocampus *in vivo* in the postsynaptic neuron, either within the postsynaptic density or at the periphery of the synapse at symmetric GABAergic synapses (Gomez-Castro et al., 2021). In contrast, $A_{2A}R$ was homogeneously detected along dendrites at P60. Using super-resolution imaging in primary rat hippocampal cultures, a perisynaptic accumulation of $A_{2A}R$ coincides with the period of synaptogenesis (Gomez-Castro et al., 2021). The receptor forms clusters at 30–40% of inhibitory synapses at 14 days *in vitro* (DIV14) suggesting that it is associated with a subset of inhibitory synapses (Gomez-Castro et al., 2021), where it regulates the **stabilization of nascent synapses** (see below).

Compared with A_1R and $A_{2A}R$, **$A_{2B}R$ and A_3R** are less expressed in the brain. They respond only to very high concentrations of adenosine (in the μM range). RNA-Seq analysis shows that **$A_{2B}R$** transcripts are detected at a high level in **mature astrocytes**, whereas they are **poorly expressed in fetal astrocytes** (https://www.brainrnaseq.org/). They are also expressed at **high level in OPCs**. The expression of **$A_{2B}R$ transcripts is higher in astrocytes and OPCs than in neurons,** suggesting a specific role in these cells. In contrast**, A_3Rs are mostly expressed by microglia during brain development** (Zhang et al., 2014) although they have also been found at **lower level** in human **olgodendrocytes and in mice OPCs** (https://www.brainrnaseq.org/).

2.3. P2 receptors

P2Rs are subdivided into **ionotropic P2X receptors and metabotropic P2Y receptors**, with ATP and ADP being their main ligands. Each

subtype is expressed in the brain and is implicated in many physiological functions including synaptic transmission, long-term plasticity (LTP) and neuroglial interactions (Jourdain et al., 2007; Khakh and North, 2012; Koizumi et al., 2013). ATP signaling is extremely broad because of the different subtypes of receptors that have different sensitivities to ATP (nM to μM range) and its effectors. For example, the **kinetics and sensitivity of P2XRs** vary among the receptor subtypes. Each **P2XR** has its own functional properties and can be found as homomers or heteromers (Khakh and North, 2012). **P2XRs open Na^+/Cl^- ion channels** leading to the modulation of **intracellular cation homeostasis** and the activation of various intracellular signaling molecules including **MAPK/ERK** and **p38** mitogen-activated protein kinases (Khakh and North, 2012; Weisman et al., 2006).

P2X4, P2X7 receptor mRNA are **expressed** in **subventricular zone-derived neurospheres** from adult mice (Grimm et al., 2009; Stafford et al., 2007), and P2X7 are expressed by subventricular zone and dentate gyrus NSCs (Tsao et al., 2013), suggesting a role for these receptors in **postnatal neurogenesis**. **P2X3** is expressed very early during the development of the nervous system. It is present in neural crest cells in the developing zebrafish embryo (Norton et al., 2000). In the rat nervous system, its **expression starts at E11** (Cheung and Burnstock, 2002), and progressively increases during the course of the development of the autonomic nervous system, the brain and spinal cord, highlighting its participation in the **formation of sensory nerves and craniofacial motoneurons** (Massé et al., 2007; Massé and Dale, 2012). **P2X5** may also contribute to the generation of motoneurons since it is detected in the spinal cord at E9 and in **motoneurons** at E11 (Guo et al., 2013). In the developing brain, **microglial cells** express purine metabolism-related enzymes (Dalmau et al., 2003) as well as **P2X1 and P2X4** (Harry, 2013).

Eight **P2YR subtypes**, all **GPCRs**, with different pharmacological and physiological properties have been described. P2Y1R and P2Y2R are thought to be the most important PYRs in the CNS (Weisman et al., 2006). **P2Y1R and P2Y2R** activation leads to **inositol trisphosphate (IP3) production** and the **mobilization of calcium from intracellular calcium stores**. The concomitant activation of P2Y1R and P2Y2R leads to maximal calcium wave propagation in the cells expressing both receptors such as in **astrocytes** (Weisman et al., 2006).

In the developing rat brain, the expression of the different **P2Y receptors varies in time and space**, suggesting different implications in the development of the nervous system. **P2Y1 and P2Y4 are expressed early in development (between E11 and E18), while P2Y2 and P2Y6 appear later** (Cheung et al., 2003). However, P2Y4 expression decreases after birth suggesting a specific role for this receptor in the development of the prenatal brain. P2Ys, through their presence at different times of development, in the spinal cord (**P2Y1**), the ventral horn of the spinal cord (**P2Y2 and P2Y4**), the spinal motor nerves (P2Y2), and the dorsal root ganglia (P2Y2), must be important players in **spinal cord and motoneuron development**. Furthermore, P2YR mRNAs are detected in **subventricular zone-derived neurospheres** of adult mice (Grimm et al., 2009; Stafford et al., 2007). **P2Y1R** expression during development matches with a role of this receptor **in neurogenesis and neuronal migration while its expression decreases during neuronal cell differentiation** (Resende et al., 2007).

3. Role in brain development

Since purinergic P1 and P2 receptors and the purinergic synthesizing and degrading molecules are expressed during embryogenesis and postnatal development, this raises the question of their contribution in brain development. In fact, purines have been shown to be involved in several steps of brain development that we will now review.

3.1. Neural stem cell proliferation

Neural stem cells (NSCs) are self-renewing multipotent cells with the capacity to give rise to neurons, oligodendrocytes, and astrocytes that are present in the embryonic as well as the adult CNS. In the adult rodent brain, neurogenesis occurs in the subventricular zone (SVZ) of the lateral ventricles and the dentate gyrus (DG) of the hippocampus (Altman and Das, 1965; Eriksson et al., 1998; Alvarez-Buylla and Garcia-Verdugo, 2002). NSCs can proliferate in an asymmetric and symmetric manner, thus maintaining their own pool of cells and allowing the differentiation into neurons, astrocytes, and oligodendrocytes to build a functional brain (Götz and Huttner, 2005). Purines are involved in different steps of neurogenesis with its effects depending on the receptor involved.

Concerning P1Rs, A_1R was described to be the most expressed receptor in adult NSCs of the SVZ and its activation promotes proliferation *in vitro* (Migita et al., 2008). Importantly, A_1R activation has been shown to inhibit SVZ neurogenesis and stimulate astrogliogenesis both *in vitro* and *in vivo* (Benito-Muñoz et al., 2016). Regarding **$A_{2A}R$,** its *in vivo* blockade has no impact on SVZ cell proliferation during embryogenesis (Alçada-Morais et al., 2021). *In vitro* studies have suggested that activation of A_1R or $A_{2A}R$ promotes neural progenitor cell proliferation (Lv et al., 2018). Interestingly, in adult animals under physiological conditions $A_{2A}R$ activation increases the number of newborn neurons in the dentate gyrus without affecting cell proliferation both *in vitro* and *in vivo* (Ribeiro et al., 2021). Moreover, $A_{2A}R$ KO present cognitive impairments associated with a reduction in immature neuroblast proliferation in the hippocampus (Moscoso-Castro et al., 2017). Interestingly, KO mice for the equilibrative nucleoside transporter type 1 express less $A_{2A}R$, have exacerbated impulsivity, and present an impairment in cell proliferation and neuroblast development (Oliveros et al., 2017).

Concerning P2Rs, P2Y1R is one of the main ATP receptor controlling embryonic neurogenesis. In radial glia, **P2Y1R** induces calcium release from calcium stores through the IP3R signaling and thereby the release of ATP, growth factors and neurotransmitters (Elias and Kriegstein, 2008). P2Y1R activation generates calcium waves between radial glial cells across the entire cortex that are involved in the **proliferation of neuronal progenitors and their synchronization in the S-phase of the cell cycle** (Weissman et al., 2004). Consistently, the outgrowth index of striata embryonic neurospheres decreases when P2Y1R is blocked meaning P2Y1R stimulates the proliferation of NSCs (Scemes et al., 2003). Similarly, cell proliferation is inhibited in adult mice SVZ neurospheres treated with a P2Y1R antagonist or in neuropheres from P2Y1R KO mice (Mishra et al., 2006). P2Y1R is not only involved in **the proliferation of embryonic NSCs** but it also acts as a **regulator of their differentiation** (Lin et al., 2007), P2Y1R is downregulated to permit the cell differentiation (Lin et al., 2007). **Proliferation** can also be **promoted by the activation of P2X7R**, and its **downregulation leads to neurogenesis** (Glaser et al., 2014). Under pathological conditions, the blockade of P2X receptors during oxygen and glucose deprivation has been described to upregulate SVZ neurogenesis (Vergni et al., 2009).

3.2. Neuronal migration

Neurons born in the ventricular zone of the neural tube migrate to establish the cortical layers and to reach the right location in the brain according to their cell fate.

Regarding P1Rs, $A_{2A}R$ is involved in **radial and tangential migrations** (Alçada-Morais et al., 2021; Silva et al., 2013). Treating pregnant female mice with an $A_{2A}R$ antagonist decreases somatostatin GABA interneurons number in the hippocampus at P6, whereas the number of somatostatin interneurons is similar to that of control offspring at adult stage indicating a delay in neuronal tangential migration (Silva et al., 2013). $A_{2A}R$ also controls the migration of cortical projection neurons, as using short hairpin (sh)$A_{2A}R$ in E14 mice lead to an accumulation of migratory neurons at the lower intermediate zone (IZ) region (Alçada-Morais et al., 2021). Interestingly $A_{2A}R$ activation stimulates cAMP production and there is evidence of an

implication **of cAMP/PKA pathway in neuronal migration** (Stoufflet et al., 2020). To our knowledge, A_1R, $A_{2B}R$ or A_3R implication in neuronal migration has not been demonstrated so far.

Regarding P2Rs, **P2Y1R controls neuronal migration** (Lin et al., 2007; Liu et al., 2008; Scemes et al., 2003). P2Y1R is expressed in cells of the ventricular zone of the SVZ (Liu et al., 2008). Adding P2Y1R selective antagonist on mice striatal embryonic neurospheres decreases radial migration of neuroblasts (Scemes et al., 2003) and P2Y1R knock down at E14 interferes with the migration of neuronal progenitors to the SVZ (Liu et al., 2008). **P2Y1R acts by propagating calcium waves** in cells of the ventricular and SVZ (Liu et al., 2008).

3.3. Neuronal and glial differentiation

Neural stem cell division during brain development maintains a pool of undifferentiated NSCs and generates progenitors that will differentiate into neurons, astrocytes, or oligodendrocytes (Götz and Huttner, 2005). Purines are involved in all stages of NSC differentiation, whether in the growth of neuronal extensions or in the production of astrocytes and oligodendrocytes. Following differentiation, neural progenitors undergo axonal elongation and dendritic branching.

Purines play a role in **neurite outgrowth at embryonic and early postnatal stages**. **Regarding P1Rs**, **$A_{2A}R$ enhances axonal elongation** through a **mechanism independent of brain-derived neurotrophic** factor (BDNF) (Ribeiro et al., 2016) although it is essential for BDNF-mediated neuronal differentiation (Alçada-Morais et al., 2021) via PKA activity. Indeed, $A_{2A}R$ activation leads to tropomyosin related kinase B (**TrkB) receptor transactivation** (Lee and Chao, 2001), and BDNF can be produced upon $A_{2A}R$-dependent AC/cAMP/PKA stimulation (Jeon et al., 2011). Inhibition of this kinase in neuroblastoma cells inhibits neuritogenesis induced by $A_{2A}R$ agonist (Canals et al., 2005). Although the role of $A_{2A}R$ in this process is clear, findings are contradictory regarding A_1R. A_1R agonist in an *in vitro* model of cancer cells induces neurite outgrowth (Canals et al., 2005). In contrast, **A_1R has an inhibitory action on neurite outgrowth in primary cultures of hippocampal neurons**. Neurons treated with N6-Cyclopentyladenosine (CPA), a selective agonist of A_1R, shows a decrease in axonal length (Thevananther et al., 2001). A_1R exerts its action **through Ras** homology family member A (RhoA also known as Rho-associated, coiled-coil containing protein kinase ROCK) activation since a pharmacological inhibition of Rho kinase prevents CPA-mediated inhibition of neurite growth (Thevananther et al., 2001). RhoA modulates **cytoskeletal actin filaments and axon elongation** (Stern et al., 2021). The activity of the protein RhoA is regulated by Rho kinase (Katoh et al., 1998). This may be related to intracellular cAMP levels as Rho kinase is inhibited in conditions of high cAMP concentration (Akakpo et al., 2017). A_1R is a G_i-coupled GPCR that inhibits the activity of AC and decreases cAMP levels. This may in turn activates Rho kinase leading to cytoskeletal retraction and inhibition of axon elongation. **$A_{2B}Rs$ are also involved in axonal outgrowth** in the CNS. Their activation **promotes intracellular cAMP level and netrin1**, a chemoattractant signal for axon, involved in neuronal differentiation in dorsal spinal cord explants from E11 rat embryo (Corset et al., 2000). A_3R has not been implicated in this brain development step.

Concerning P2Rs, **P2Y1R inhibits differentiation**, as it must be downregulated to permit the differentiation of embryonic neural precursors (Lin et al., 2007). In addition, **P2X7R promotes proliferation and maintenance of undifferentiated states** (Glaser et al., 2014; Yuahasi et al., 2012). Importantly, **P2Y1R positively while P2X7R and P2Y13R negatively regulate axonal outgrowth** (del Puerto et al., 2012a; Díaz-Hernandez et al., 2008a,b). Adding ATP to primary cultures of mouse hippocampal neurons decreases axonal length. This requires an increase in intracellular calcium levels in axons via the activation of ligand-gated cationic channels. Conversely, P2X7R knockdown in neurons using shRNA approach increases the axonal length of neurons at 3 days *in vitro* (3 DIV) (Díaz-Hernandez et al., 2008a,b). On the same line, neurons transfected with a shRNA against P2Y13R develop axons almost twice longer compared to control neurons (del Puerto et al., 2012b). On the contrary, the positive effect of ADP on axonal length is prevented in shP2Y1R transfected neurons (del Puerto et al., 2012b). Moreover, **an increase in local cAMP level induces axonal growth** (Batty et al., 2017; Shelly et al., 2010). This increase has been linked to P2X7, P2Y13 and P2Y1 activation and the capacity of these receptors to inhibit or activate adenylyl cyclase 5 (AC5). Indeed, these receptors regulate intracellular cAMP level through different ways: **P2Y13R is coupled to a G_i protein able to inhibit AC5, and P2X7R activation leads to an intracellular calcium elevation also able to inhibit AC5**. Conversely, **P2Y1R is coupled to G_q that activates AC5** (del Puerto et al., 2012b).

Differentiation of NSCs can also drive astrocyte and oligodendrocyte production, i.e., respectively **astrogliogenesis and oligodendrogenesis**. Evidence for the **involvement of P1Rs** in **astrogliogenesis** comes from the observed decrease in astrocyte density in the striatum and hippocampus of pup daily i.p. injected with caffeine, a non-competitive $A_{2A}R$ and A_1R antagonist, between P3 and P10 (Desfrere et al., 2007). This effect is transient because it is observed at P7, P10 and P15 but not at P20 and P40 i.e., after the period of astrocyte proliferation and maturation (Desfrere et al., 2007). **A_1R is not involved** in astrogliogenesis because a treatment with its agonist CPA or antagonist 8-(*p*-sulfophenyl) 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) does not modify astrocytic density. In contrast, **$A_{2A}R$ is involved** since the treatment with a selective $A_{2A}R$ agonist (CGS21680) inhibits the caffeine-induced loss of astrocytes (Desfrere et al., 2007). This effect of caffeine on astrocyte density could be a **consequence of reduced NSC proliferation**, as A_1R and $A_{2A}R$ promote self-renewal of adult NSC (Migita et al., 2008; Ribeiro et al., 2021). With regard to **A_3R**, it has been shown in primary rat cultures at P7 that the receptor **influences astrocyte viability** as a function of adenosine concentration, via regulation of the level of the **chemokine CCL2**, which is protective or apoptotic (Abbracchio et al., 1998; Wittendorp et al., 2004).

Purines are also involved in **oligodendrogenesis**. **Opposite to ATP and the activation of certain ATP receptors, adenosine treatment inhibits the proliferation and promotes the maturation of OPCs**. Treating rat cultures of OPCs with the potent non-selective adenosine A_1, A_{2A}, A_{2B} and A_3 receptor agonist, 5′-N-ethylcarboxamido adenosine (NECA), in the presence of the mitogen platelet-derived growth factor (PDGF), increases the proportion of OPCs expressing O4 and O1 maturation markers (Stevens et al., 2002). Moreover, **P1R activation promotes myelination in the same model** but the receptors involved and the underlying mechanisms are unclear (Stevens et al., 2002). However, neonatal rats treated with A_1R agonists present a reduction in white and gray matter volume and ventriculomegaly (Turner et al., 2002). Furthermore, **$A_{2A}R$ inhibits the differentiation of oligodendrocytes** in primary cultures prepared from P1 rats, inhibiting K^+ current (I_K) an essential current for OPC differentiation (Coppi et al., 2013).

3.4. Synaptogenesis

After axon elongation, the neuron finds its target to establish synaptic connections. Synaptogenesis corresponds to the steps of synapse formation between neurons in the central nervous system or between neuron and (skeletal/smooth/cardiac) muscle in the **peripheral nervous system** e.g. at the neuromuscular junction (NMJ). Once formed, active synapses releasing neurotransmitters are stabilized while the inactive ones are rapidly destabilized and eliminated. Since NMJs are larger and more accessible than **central synapses**, the mechanisms of synapse formation and elimination have been first studied and understood for the NMJ. However, neuron-neuron and neuron-muscle synaptogenesis share similar mechanisms, which are largely regulated by purines (see below).

3.4.1. Neuromuscular junction

Initially, the immature NMJ is innervated by two or more axons.

Subsequently, the axons compete to leave a mono-innervated NMJ (Balice-Gordon and Lichtman, 1993; Keller-Peck et al., 2001; Redfern, 1970). The process of axon elimination occurs within two weeks of birth in rodents. Thus, at the mature NMJ, a muscle fiber is innervated by only one axon, forming a muscle unit. The neurotransmitter released by the motoneuron ending at the NMJ is acetylcholine (ACh). It has several receptors including nicotinic receptors (nAChR) mostly postsynaptic and presynaptic muscarinic M1, M2, M3 and M4 receptors (mAChR). The initial steps of synapse formation at the NMJ involve the postsynaptic recruitment of nAChRs with the aggregation of existing receptors. Then, the maturation of the synapse occurs through an increase in the density of postsynaptic nAChRs by local synthesis of receptors and a modification in receptor subunit composition (Bruneau and Akaaboune, 2006; Missias et al., 1996). NMJ formation is **regulated by electrical activity**: blocking of voltage-dependent Na^+ channels, responsible for the generation of action potentials, by tetrodotoxin (TTX) in rat sciatic nerves at P9 (Thompson et al., 1979), or inhibition of postsynaptic nAchRs in the soleus muscle by bungarotoxin at P5 (Duxson, 1982) leads to poly-innervated NMJs. **Neuronal mAChRs** allow an auto-regulation of the synapse upon presynaptic release of Ach: they **inhibit or stimulate ACh release** depending on the type of mAChR activated and control axonal competition (Nadal et al., 2016a). **ATP is co-released with the neurotransmitter ACh** at the axon terminal but also **by the muscle at the NMJ** (Dowdall et al., 1974; Silinsky and Hubbard, 1973). ATP present in the synaptic cleft is implicated in **synapse maturation** by **controlling the number and type of postsynaptic nAChRs** (Fu, 1995; Jia et al., 2007). **ATP stabilizes nAChRs** in the membrane of cultured rat myotubes (O'Malley et al., 1997). ATP is also involved in **activity-dependent modifications of the NMJ during development**. Indeed, **ATP controls motoneuron axon elimination** by selecting the axon that will innervate the muscle fiber (Fu, 1995; Jia et al., 2007).

Regarding the implication of P1Rs in the formation of the motor endplate, the terminal nerves express **A_1R and $A_{2A}R$, which are mostly detected presynaptically** (Garcia et al., 2013). The expression and complex interplay of these receptors at the NMJ **regulates ACh transmission** during development **to drive the maturation and axonal competition**. The four P1Rs are expressed in motor terminals in newborn mice even if the **$A_{2A}R$ is predominant at early development** stage (Garcia et al., 2013, 2014). Both **A_1R and $A_{2A}R$ are implicated in synapse elimination** at the NMJ during development. Adenosine receptors have opposite roles depending on the developmental stage of the mouse. Blocking A_1R with DPCPX or $A_{2A}R$ with 2-(2-furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e] [1, 2,4] triazolo[1,5-*c*]pyrimidin-5-amine (SCH58261) during the period of axonal elimination (P5-P15) accelerates the elimination **at P7** meaning that at this age adenosine signaling **delays axonal elimination**. In contrast, **at P9**, these presynaptic adenosine receptors **accelerate axonal loss for the competition and promote postsynaptic nAChR clustering** (Nadal et al., 2016b). This is associated with a **balance between A_1R and $A_{2A}R$ expressed at motor terminals**, depending on the developmental stage and the amount of adenosine present in the synaptic cleft, which would activate either A_1R or $A_{2A}R$ (at higher adenosine concentrations) in order to decrease or increase, respectively, the **synaptic release of ACh** (Correia-de-Sá et al., 1991; Pousinha et al., 2010; Ribeiro et al., 1996). Interestingly, some **presynaptic mAChR can potentiate the effects of adenosine receptors on axonal competition whereas others have antagonistic effect** (Nadal et al., 2016a). For example, **A_1R and mAChR M2** signaling pathways **converge downstream of calcium influx on the modulation of AC activity**, with a negative crosstalk during intense synaptic activity to mitigate the inhibitory effect of ACh release (Oliveira et al., 2009; Shakirzyanova et al., 2006). This underlies the functional interaction between presynaptic receptors in synapse maturation, as a **result of PKA and PKC pathway balance**. At presynaptic site, **PKA and PKC regulate calcium-dependent ACh release** (Besalduch et al., 2010; Lanuza et al., 2014; Santafé et al., 2009). **At postsynaptic site, PKC phosphorylates the delta subunit of the nAChR to destabilise it while, conversely, PKA stabilizes the nAChR by phosphorylating the epsilon subunit** (Lanuza et al., 2010). In contrast to A_1R and $A_{2A}R$, the role of $A_{2B}R$ and A_3R have been less studied during the development of the NMJ (Garcia et al., 2014). **$A_{2B}R$ is involved in promotion of end plate potential at the adult NMJ** (Bernareggi et al., 2018) whereas **A_3R inhibits ACh release** (Cinalli et al., 2013).

Concerning P2Rs, during development, P2X2R expression parallels the formation of the NMJ (Ryten et al., 2001). A direct involvement of this receptor in **the formation of the NMJ** was then demonstrated in KO animals: NMJs of **P2X2R–KO mice are disorganized** with a misalignment of pre- and postsynaptic elements and a reduction in the density of folds, meaning less invagination of the postsynaptic part and smaller area to express nAChR, that persist at adult stage (Ryten et al., 2007). Therefore, P2X2R regulates more probably the **maturation of the NMJ** than the initial step of formation.

During NMJ development, ATP released at the synapse binds to and activates presynaptic P2 receptors, resulting in a decrease in ACh release and thus the stabilization of the most activated synapse (Jia et al., 2007). A role of presynaptic **P2Y13R in adult mice was shown**, where P2Y13R activation, with its selective agonist inosine 5-diphosphate sodium salt (IDP), **decreases evoked release of ACh** of phrenic nerve **to stabilize the synapse** (Guarracino et al., 2016). Some studies tried to understand the pathway underlying P2Y activation leading to an inhibition of neurotransmission on mature systems (Sokolova et al., 2003). They concluded that **presynaptic P2Y13R are coupled with $G_{i/o}$ protein** and that **ATP inhibitory effect depends on PLC and PKC**. Another study reported the **role of presynaptic PKC in activity-dependent synapse modulation of the NMJ** (Li et al., 2004). Perhaps this mechanism could be established from the developmental stage.

The NMJ is a **tripartite synapse** composed of the motor neuron ending, the muscle fiber and the Schwann cells that are in close contact with the neuromuscular synapse during its development (Love and Thompson, 1998; Ogata, 1988). **Schwann cells express AChR and purinergic receptors** (Robitaille, 1995) and are able to **regulate the release of neurotransmitters at the NMJ.** During development, they **sense synaptic activity via the activation of P2Y1R** by ATP released by neurons, which increase their intracellular calcium level that in turn allow the release of ATP, rapidly degraded into adenosine in the synaptic cleft. **Schwann cells therefore reinforce strong synapses by activating presynaptic $A_{2A}R$** (Darabid et al., 2013, 2018). All the steps of synapse formation and maturation of the NMJ in which purines are involved have been schematized in Fig. 1.

3.4.2. *Central synapses*

Although ATP is known to be released in the synaptic cleft after synaptic stimulation in the CNS (Cunha et al., 1996; Jo and Role, 2002), its role at developing central synapses has been less studied in comparison with the NMJ. During brain development, ATP released from astrocytes in prefrontal cortical slices from neonatal rats (P3) increases the frequency and amplitude of spontaneous excitatory postsynaptic currents (Beamer et al., 2017). However, the receptor involved in this regulation was not identified.

Concerning P1 adenosine receptors, recent works reported that it regulates both GABAergic and glutamatergic synapse stabilization. **The adenosine signaling pathway has been involved in the stabilization of nascent GABAergic synapses in the rodent hippocampus.** A pharmacological blockade of $A_{2A}R$ destabilizes a subset of GABAergic synapses in hippocampal cultures, tissue slices and *in vivo* (Gomez-Castro et al., 2021). This treatment affected the functionally active inhibitory synapses *in vitro* and *ex vivo* (Gomez-Castro et al., 2021). Since the drugs had a very rapid effect (within 20 min) and that it takes several hours to form new synapses (Dobie and Craig, 2011), the adenosine signaling **regulates synapse stabilization** rather than synapse

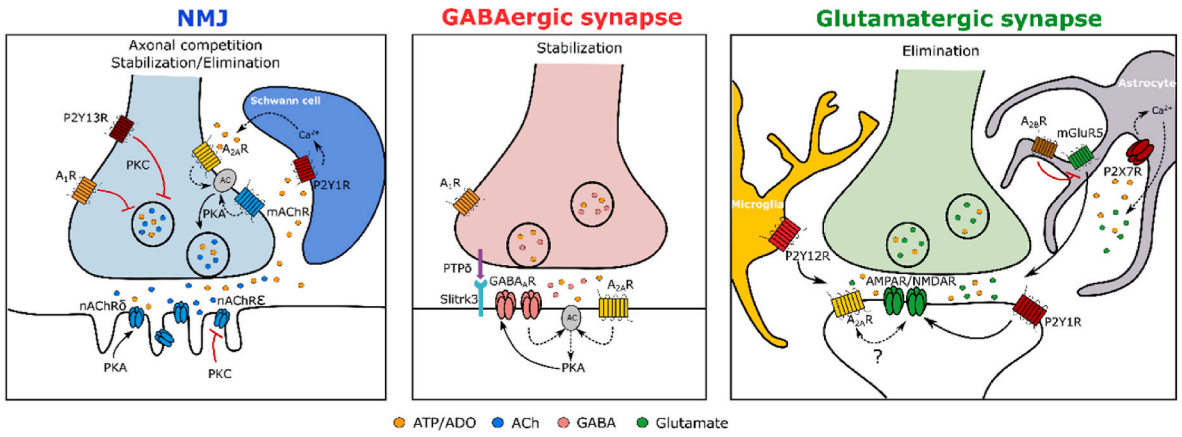

Fig. 1. Involvement of purinergic signaling in the formation, elimination, and maturation of peripheral and central synapses during development.
ATP, which is released together with acetylcholine (Ach) at the NMJ but also with GABA and glutamate at central GABAergic and glutamatergic synapses, is involved in the key steps of synapse formation.
At the developing NMJ, ATP via certain P2YR and adenosine via A_1R and $A_{2A}R$, whose action is regulated by muscarinic ACh receptors (mAChRs), are involved in the regulation of Ach release leading to axonal competition (by eliminating supernumerary axons and selecting the most active axon), and in the postsynaptic maturation of the NMJ (by regulating the type of nAChR subunits). The positive (black arrows) or negative (red lines) effects of ATP and adenosine signaling reflect PKC and PKA activity at the pre- and post-synaptic sites. Schwann cells can release ATP in response to changes in neuronal activity sensed by P2Y1R thus reinforcing strong synapses by activating presynaptic A_{2A}Rs.
At hippocampal GABAergic synapses, postsynaptic $A_{2A}R$ signaling stabilizes newly formed synapses that release ATP and GABA by regulating PKA activity which in turn controls postsynaptic recruitment of $GABA_A$Rs and the synaptogenic *trans*-synaptic proteins Slitrk3-PTPδ. GABA converges on the adenylyl cyclase pathway to boost cAMP production and PKA activation.
At glutamatergic synapses, purinergic receptors mediate synapse elimination (black arrows). $A_{2A}R$ signaling is involved in the formation of the retinogeniculate system by controlling the removal of certain glutamatergic synapses by a mechanism that is still unknown but requires neuronal activity and microglia activation. Microglia is also implicated in the pruning of glutamatergic synapses in the visual cortex via P2Y12R activation. Astrocytic $A_{2B}R$ signaling indirectly plays a role in glutamatergic synapse remodeling in the primary somatosensory cortex by negatively regulating the level of mGluR5 in astrocytes which decrease excitatory synapse number. Astrocytes also tunes neuronal activity through gliotransmitter release of glutamate and ATP after astrocytic P2X7R activation.

formation *per se*. The effect of the adenosine signaling pathway is restricted to the period of synaptogenesis *in vitro* and *ex vivo* (Gomez--Castro et al., 2021), highlighting a specific role during this key period of development. Moreover, the expression *in vitro* and *in vivo* of a shRNA against $A_{2A}R$ in a subset of hippocampal neurons is sufficient to mimic the effect of the bath-applied $A_{2A}R$ antagonists on synapse number, indicating that the effect of the drug is specific, cell-autonomous and that postsynaptic A_{2A}Rs are necessary and sufficient for GABAergic synapse stabilization (Gomez-Castro et al., 2021). It was then proposed that **A_{2A}Rs stabilize nascent GABAergic synapses through the activation of G_s protein and calcium-Calmodulin sensitive AC 1 and/or 8 which in turn lead to the elevation in intracellular cAMP and the activation of PKA**. Then, **PKA phosphorylates gephyrin**, the main scaffolding protein at inhibitory synapses, on a unique PKA phosphosite (Ser305), that in turn allows the **recruitment of ionotropic GABA type A receptors ($GABA_A$R) and of the *trans*-synaptic organizers SlitrK3 and PTP delta adhesion molecules** (Gomez-Castro et al., 2021) (Fig. 1). Like adenosine signaling, GABA stabilizes nascent inhibitory synapses in the developing brain (Huang and Scheiffele, 2008; Oh et al., 2016; Wu et al., 2012). GABA exerts its action by inducing calcium influx at the developing synapse through $GABA_A$R-induced membrane depolarization and the activation of voltage-dependent calcium channels (Leinekugel et al., 1995; Perrot-Sinal et al., 2003). The molecular mechanism downstream calcium stabilizing the nascent synapses remained unclear for a long time. Recently, we demonstrated that **$GABA_A$R signaling converge onto the adenosine signaling by activating calcium-calmodulin, which in turn boost the activity of calcium-sensitive adenylyl cyclase AC 1/8 and the production of cAMP** in the neuron (Gomez-Castro et al., 2021) (Fig. 1). Therefore, the **AC 1/8 may act as coincident detectors of presynaptically** released GABA and adenosine to stabilize nascent GABAergic synapses (Gomez-Castro et al., 2021).

Interestingly, the role of the adenosine signaling during synaptogenesis is not restricted to GABAergic synapses of the hippocampus. $A_{2A}R$ has been shown to be involved in **the pruning of glutamatergic synapses** formed between the retinal ganglion cells (RGC) and the dorsal lateral geniculate nucleus (dLGN) during development (Fig. 1). Treating mice with the **A_{2A}R antagonist** 8-[(1E)-2-(2-(3,4-Dimethoxyphenyl)ethenyl]-1,3-diethyl-3,7-dihydro-7-methyl-1H-purine-2,6-dione (KW6002) in the initial steps of synaptogenesis (between P2 and P4 and not at later stage) **enhances the segregation of the retinogeniculate system** (Miao et al., 2021), indicating a role of this receptor in the refinement of synapses during development. However, in **A_{2A}R KO mice** in which $A_{2A}R$ is absent from the initial steps of brain development, an opposite effect was observed with a **delay in dLGN segregation**. The opposite effects observed on synaptogenesis in KO mice compared to KW6002-treated animals may be explained by an impact of $A_{2A}R$ deletion on neurogenesis, interneuron migration, neurite elongation and/or non-neuronal cell differentiation and function (as seen above) whereas treatment of neonates between P2 and P4 with KW6002 would have a selective effect on synaptogenesis.

Miao et al. (2021) also reported that the effect of $A_{2A}R$ antagonist on the segregation of the retinogeniculate system requires **microglia activation**, indicating the **contribution of neuroglial interactions in the refinement of dLGN synapses** (Fig. 1). This differs from GABAergic synapses in the hippocampus where the neuron plays a central role at least *in vitro* since removal of glial cells from hippocampal mixed neuron-astrocyte-microglia cultures with ARAC did not prevent $A_{2A}R$ blockade from decreasing the number of GABAergic synapses during synaptogenesis (Gomez-Castro et al., 2021). This suggests that the $A_{2A}R$ is involved in a more complex mechanism at glutamatergic synapses than at GABAergic synapses during synaptogenesis. The molecular mechanism by which $A_{2A}R$ controls glutamatergic synaptogenesis is not fully understood. Miao et al. (2021) showed that a transient KW6002 treatment during synaptogenesis reduces the density of postsynaptic scaffolding molecule Homer1 and mGluR5 without altering the density of presynaptic VGluT2 synaptic boutons, suggesting a postsynaptic regulation of the synapse. Future studies will determine whether

adenylyl cyclase also functions as an activity sensor at glutamatergic synapses to activate PKA and stabilize nascent synapses as has been shown at hippocampal GABAergic synapses, which would allow the mechanism to be generalised to central synapses. On the other hand, the study of the mechanisms at play at the glutamatergic synapse should provide further insight into the key role of $A_{2A}R$ in relation to microglia activation and control of synapse pruning.

Astrocytic $A_{2B}R$ indirectly plays a role in synapse remodeling in the primary somatosensory cortex by **negatively regulating the level of mGluR5 in astrocytes** (Tanaka et al., 2021). There is a downregulation of mGluR5 expression during development that can be prevented by $A_{2B}R$ knock down in astrocytes, thus leading to an increase in excitatory synapse number. Therefore, **astrocytic activation of $A_{2B}R$ is involved in the pruning of excitatory synapses** (Fig. 1).

A_1R is known to inhibit the probability of neurotransmitter release at adult stage (Oliet and Poulain, 1999; Shen and Johnson, 1997) and seems to have similar function at immature synapses. A_1R agonist decreases the frequency of miniature inhibitory postsynaptic currents in P12 rat hippocampal slices (Jeong et al., 2003). Consistent with this finding, Gomez-Castro et al. did not report a transient increase in A_1R expression during the period of synaptogenesis, as observed for $A_{2A}R$, and treating hippocampal neurons with the A_1R antagonist DPCPX did not alter GABAergic synapse number. This suggests a **minor contribution of the A_1R compared to $A_{2A}R$ in synaptogenesis**. Indeed, other studies showed that the o**ntogenesis of A_1R largely sprouts after synapogenesis** and regulates synaptic transmission in young adult rat hippocampus (Dumas and Foster, 1998).

Concerning P2 receptors, they have been implicated in synapse elimination and regulation. ATP released from presynaptic neurons can elicit excitatory postsynaptic current in postnatal rat (P20) hippocampus (Pankratov et al., 1998) and cortex (Pankratov et al., 2002, 2003). At the same developmental stage, **astrocytic ATP regulates the activity of glutamatergic synapses** by activating presynaptic **P2Y receptors** in hippocampal cultures and in slices (Zhang et al., 2003). Calcium-dependent **astrocytic ATP release stimulates P2Y1R** (Yang et al., 2016). Electrophysiology and immunostaining demonstrated that **glutamatergic synapse elimination** in ventral posteromedial nucleus (VPm) of the thalamus is prevented at P16 in P2Y1R–KO mice and is not rescued by ATP. In the VPm, only neurons express P2Y1R (on the contrary to the hippocampus) (Yang et al., 2016; Zhu and Kimelberg, 2004), meaning that **synapse elimination occurs through pre- or post-synaptic P2Y1R activation**. In the adult brain, an interaction between P2Y1R and the postsynaptic scaffold protein postsynaptic density protein 95 (PSD95) recruits glutamatergic receptors at excitatory synapses (Siow et al., 2010), in favor of a **postsynaptic mechanism**. Zhang et al. (2003) also shown that **adenosine from astrocytic ATP** degradation decreases the amplitude of excitatory postsynaptic potentials in CA1 neurons and participate to heterosynaptic modulation. On the contrary, during brain development, **ATP released from astrocytes** in prefrontal cortex slices from neonatal (P3) rats **increases the frequency and amplitude of spontaneous excitatory postsynaptic currents** (Beamer et al., 2017). However, the receptor involved in this regulation was not identified.

Astrocytes express ATP receptors (Fumagalli et al., 2003) and these receptors are involved in **synapse regulation**. ATP receptors allow astrocytes to **sense synaptic activity**. **Astrocytic P2X7R** binds ATP released in the synaptic cleft and receptor activation increases intracellular calcium level (Suadicani et al., 2006) that tunes neuronal activity through **gliotransmitter release of glutamate and ATP** (Araque et al., 1998; Parri et al., 2001; Pasti et al., 1997).

Microglia expresses P2X4, P2X7, P2Y2, P2Y4, P2Y6, P2Y12, P2Y14 receptors (Verkhrasky et al., 2009) and all adenosine receptors. **Microglia can detect synaptic release of ATP** from neurons and astrocytes in particular through **P2Y12R** (Haynes et al., 2006). Interestingly, the pharmacological inhibition of P2Y12R, which controls ATP/ADP-dependent microglia chemotaxis and motility, prevents neuronal activity (Badimon et al., 2020). During development, the **microglial P2Y12R** is implicated in **synaptic pruning** of the visual cortex in the critical post-natal window (Sipe et al., 2016). Activation of microglia by lipopolysaccharide (LPS) induces the release of brain-derived neurotrophic factor (BDNF) which is responsible for microglial cell proliferation in an $A_{2A}R$-dependent manner (Gomes et al., 2013). However, the involvement of adenosine receptors in microglia-mediated pruning of synapses has not yet been shown.

All the developmental stages in which purines are involved are listed in Table 1.

4. Purine system deregulation during development

Given the role of purinergic signaling in brain development, it has been shown that deregulation in particular of **A_1R** and **$A_{2A}R$** affect the integrity of the brain.

A_1R have been associated with **brain injury**. The **periventricular leukomalacia** (PVL) is characterized by focal necroses in the periventricular white matter leading to a ventricular volume increase, a feature that can be induced in mice by **hypoxia**. **Impairment in oligodendrocyte development and axonal disruption** may explain the white matter damage (Dammann et al., 2001). Interestingly, the ventricles of A_1R KO mice under hypoxic conditions are the same size as those of WT mice reared under normoxic conditions, indicating that the **A_1R plays an important role in this brain lesion** (Turner et al., 2003). Rats treated with A_1R agonist CPA display reduction in total axonal volume, a lower expression of myelin protein and a ventriculomegaly (Turner et al., 2002). However, it is not known whether this is due to a loss of oligodendrocytes or a functional deficit.

$A_{2A}R$ have been implicated in pathological conditions associated with a defect in **neurogenesis**. Oxygen and glucose deprivation decreases hippocampal precursor cell proliferation that can be prevented by pharmacological blockade of $A_{2A}R$ (Maraula et al., 2013). However, $A_{2A}R$ blockade does not significantly alter the density of proliferating cells in the granule cell layer in a rat model of status epilepticus (Xu et al., 2022). Similarly, in a mouse strain prone to depression, caffeine that non-selectively blocks A_1R and $A_{2A}R$ also does not alter neuron proliferation in the dentate gyrus (Machado et al., 2017).

Impairment in $A_{2A}R$ signaling affects the **establishment of functional networks**. The mammalian visual system is a developmental model of synaptogenesis and neuronal plasticity. Upon monocular enucleation, the retinotectal pathway undergoes plastic rearrangements that are restricted to the first three postnatal weeks corresponding to the critical period. Interestingly, **retinotectal plasticity** is associated with upregulation of **A_1R and $A_{2A}R$** expression and requires $A_{2A}R$-dependent astrocytic reactivity. Indeed, **blocking $A_{2A}R$ prevents astrocytic reactivity and reconnection of the superior colliculus following enucleation** (Tavares-Gomes et al., 2023). Exposing pregnant mice to **caffeine** or to the $A_{2A}R$ selective antagonist KW6002 during the **entire developmental period** i.e., the gestation and lactation period had deleterious consequences in the offspring (Silva et al., 2013). This led to an **interneuron migration deficit** and **a hyperexcitable hippocampus**, with long lasting consequences such as an **increased susceptibility to seizures and a spatial memory deficit** (Silva et al., 2013). **Caffeine exposure during the pregnancy until P15** also affects the **primary visual cortex** (V1) neurons leading to neuronal hyperactivity *in vivo* in this brain region (Fazeli et al., 2017). A **blockade of $A_{2A}R$ activity**, restricted to the **period of synaptogenesis** (between P3 and P16) i.e., the lactation period, by daily intraperitoneal injection of caffeine or a selective antagonist SCH58261, has similar effects on GABAergic synapses and behavior as compared to animals that were treated throughout development. This results in a **40% loss of GABAergic synapses in the hippocampus, increased susceptibility to epilepsy and impairment in some memory tasks** (novel object location but not novel object recognition tasks) (Gomez-Castro et al., 2021). This indicates that the effect of $A_{2A}R$ blockade throughout

Table 1
Roles of purinergic receptors in brain development.

	P2Y	P2X	A_1	A_{2A}	A_{2B}	A_3
Neural stem cell proliferation	P2Y1 ↗ (*Lin et al., 2007; Scemes et al., 2003; Weissman et al., 2004*)	P2X7 ↗ (*Glaser* et al., *2014*)	↗ (postnatal) (*Migita* et al., *2008*)	↗ (only postnatal)(*Alçada-Morais et al., 2021; Ribeiro* et al., *2021*)	–	–
Neuronal migration	P2Y1 ↗ (radial migration) (*Scemes* et al., *2003*; *Liu* et al., *2008*)	–	–	↗ (interneurons) (*Alçada-Morais et al., 2021; Silva* et al., *2013*)	–	–
Neuronal differentiation	P2Y1 ↘ (*Lin* et al., *2007*)	P2X7 ↘ (*Glaser* et al., *2014*; *Yuahasi* et al., *2012*)	–	↗ neuritogenesis (Alçada-Morais et al., 2021; Jeon et al., 2011); Lee and Chao, 2001)	–	–
Axonal outgrowth	P2Y1 ↗ (*del Puerto* et al., *2012a,b*) P2Y13 ↘ (*del Puerto* et al., *2012b*)	P2X7 ↘ (*Díaz-Hernandez et al., 2008*; *del Puerto* et al., *2012a*)	↗ and ↘ (not clear) (*Canals et al., 2005*; *Thevananther* et al., *2001*)	↗ (*Ribeiro* et al., *2016*)	↗ (*Corset* et al., *2000*)	–
Astrogenesis	–	–	–	↗ (*Desfrere et al., 2007*)	–	–
Oligodendrogenesis	–	–	–	↘ differentiation (*Coppi* et al., *2013*)	–	–
			↗ maturation (*Stevens* et al., *2002*)			
NMJ nAChR stabilization	↗ (*O'Malley* et al., *1997*)		↗ (*Nadal* et al., *2016b*)	↗ (*Nadal* et al., *2016b*)	–	–
NMJ mono-innervation	Stabilize active synapses (*Jia* et al.., *2007; Fu* et al., *199;*)		↘ axon elimination at P7 vs ↗ at P9 (*Nadal* et al., *2016b*)	↘ axon elimination at P7 vs ↗ at P9 (*Nadal* et al., *2016b*)	–	–
GABAergic synapse stabilization	–	–	–	↗ (*Gomez-Castro* et al., *2021*)	–	–
Glutamatergic synapse stabilization	P2Y1 ↘ (*Yang* et al., *2016*)	–	–	KO ↗ vs antagonist ↘ (*Miao* et al., *2021*)	↘ (astrocytic) (*Tanaka* et al., *2021*)	–

development including during the gestation and lactation period (Silva et al., 2013) is primarily due to **alterations in synaptogenesis.** Post-synaptic expression of $A_{2A}R$ peaks during the period of synaptogenesis *in vitro* and *in vivo* in the mouse and rat cortex and hippocampus (Gomez-Castro et al., 2021). This transient expression of the receptor coincides with its role in stabilizing GABAergic synapses restricted to the period of synaptogenesis. Indeed, $A_{2A}R$ blockade after the period of synaptogenesis does not affect the number of inhibitory synapses (Gomez-Castro et al., 2021).

It is tempting to propose that **$A_{2A}R$-dependent mechanism of synapse elimination could be abnormally reactivated in pathologies** where extracellular adenosine increases to levels well above physiological conditions such as **in epilepsy** (Dale and Frenguelli, 2009), **aging** (Cunha et al., 1995) and **Alzheimer's disease** in which $A_{2A}R$ expression is also upregulated (Albasanz et al., 2008; Espinosa et al., 2013). In this context, the massive and continuous overflow of extracellular adenosine will then overcome the restricted activation of $A_{2A}Rs$, leading to a predominant role of $A_{2A}Rs$ in the development of neurodegeneration (Carvalho et al., 2019). In line with this, $A_{2A}Rs$ have been implicated in **Parkinson's disease** and clinical trials with an **$A_{2A}R$ antagonist** have already shown their **efficacy** (Armentero et al., 2011; Mizuno et al., 2010). $A_{2A}Rs$ have also been involved in **neurodevelopmental disorders**. An increase in $A_{2A}R$ expression has been reported in spontaneously hypertensive rats (SHR), a model of attention deficit hyperactivity disorder (**ADHD**), and chronic treatment with **caffeine improves attention deficit in SHR** (Pandolfo et al., 2013). In **Fragile X syndrome**, excessive glutamatergic signaling linked to overactivation of the metabotropic glutamatergic receptor mGluR5 is attenuated by a chronic treatment with istradefylline, an **$A_{2A}R$ antagonist**, which also **improves the associated learning deficit** (Ferrante et al., 2021). **Concerning epilepsy, ATP released and its turnover into adenosine during seizures control seizures and the** development of the **disease** (Doná et al., 2016; Lietsche et al., 2016). Seizure-induced ATP release from hippocampal terminals and its metabolism into adenosine may in turn activate $A_{2A}R$ on mossy fibers resulting in mossy fiber sprouting in the molecular layer of the hippocampus in animal models of temporal lobe epilepsy as well as in patients (Xu et al., 2022). Moreover, a local **upregulation of $A_{2A}R$ in glutamatergic synapses is observed in rodent epileptic models** associated with an increase of glutamatergic activity and neurotoxicity (Canas et al., 2018).

5. Conclusions

The brain development is a highly regulated process involving a multitude of mechanism and signaling. In this review, we illustrated the role of the purinergic system in various stages of brain development. The neonatal expression of P1 and P2 receptors have been linked to roles of adenosine and ATP in neurogenesis, neuronal migration and differentiation as well as in synaptogenesis. The involvement of ATP in brain development has been more extensively studied than that of adenosine, but there is growing interest in the role of A_1R and $A_{2A}R$ in brain development. $A_{2A}R$ have a dual role in synaptogenesis: while inhibition of $A_{2A}R$ signaling at neonatal stage alters the development of the neuronal network, the use of $A_{2A}R$ antagonist such as caffeine appears to have beneficial effects on neurodevelopmental and neurodegenerative diseases. In addition, A_1R and $A_{2A}R$ have been respectively described as neuroprotective and neurodegenerative in the adult brain (Cunha, 2005). Therefore, understanding the mechanism of synapse formation involving purines during development should ultimately lead to the development of new therapeutic targets for Alzheimer's disease (Launay et al., 2023) and other diseases in which the ATP and adenosine pathways are reactivated.

Declaration of competing interest

The authors declare no competing interests.

Data availability

The authors do not have permission to share data.

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