# Ion channels and receptors as determinants of microglial function

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## Highlights

- Essential microglial functions depend on membrane potential and ion channels.
- Surveillance is regulated by THIK-1 $K^+$ channels, while directed process motility is controlled by purinergic signalling and $Cl^-$ channels.
- Enhanced $K^+$ efflux via THIK-1 and $K_V$ channels is needed for NLRP3 inflammasome assembly.
- Microglial ion channel and receptor expression *in situ* often differ from those in culture, which result in different morphological and functional states.

**Abstract**

**Microglia provide immune surveillance of the central nervous system (CNS). They display diverse behaviors, including non-directional and directed motility of their processes, phagocytosis of targets such as dying neurons or superfluous synapses, and generation of reactive oxygen species (ROS) and cytokines. Many of these functions are mediated by ion channels and cell surface receptors, the expression of which varies with the many morphological and functional states that microglial cells can adopt. Recent progress in understanding microglial function has resulted from applying classical cell physiological techniques *in situ* such as patch-clamping and live imaging, and cell-specific transcriptomic analyses. Here, we review the contribution of microglial ion channels and receptors to microglial and brain function.**

**Keywords**

Microglia; ion channels; membrane voltage; receptors.

## Microglia: Function shaped by ion channels

Microglia are the resident immune cells of the central nervous system (CNS) and account for 5-12% of brain cells [1]. They exhibit constant movement of their processes to survey the brain for invading organisms, dying neurons and also redundant synapses that need removing during development. They extend processes in a directed manner to regions of damage. When processes arrive at such targets, they engulf and phagocytose cellular debris, apoptotic neurons or synapses, and generate immune-modulators including reactive oxygen species (ROS, which damage invading organisms) and cytokines (which can alter neuronal and immune cell function). Ion channels and cell surface receptors shape many of these functions. Regulation of ion channel activity instantaneously changes the cells' membrane voltage, thus allowing microglial cells to rapidly adapt their behavior in response to acute stimuli. This occurs in addition to slower mechanisms, such as changes of gene expression (including that of ion channels) which typically requires a longer time course (hours). Moreover, given the very high membrane resistance of microglia, modulating the function of just a few ion channels is sufficient to profoundly alter microglial membrane potential and associated functions. However, earlier work has mainly relied on studies *in vitro*, where microglia drastically alter their gene expression profiles and

rarely show complex process ramification or surveillance [2]. Major advances have been provided recently by the application of high-throughput transcriptomic profiling, and cell physiological techniques such as patch-clamping and imaging *in situ*. These are providing a mechanistic basis for understanding the behavior of these cells in the intact brain. Here, we discuss how key microglial functions are shaped by microglial ion channels and the cell surface receptors that regulate them.

**Setting the resting potential of microglia**

Ion channels set the cell resting potential, which powers many other cellular processes. Microglia *in situ* exhibit a depolarized membrane potential ($V_m$) typically around -45 mV [3–6], but ranging from -20 mV [7,8] to -60 mV [9]. This variation could partly reflect shunting by the patch pipette seal resistance (see Box 1, Technical Challenges), but also differences across brain regions, sex, species (see Box 2, Microglial Diversity) and especially age, with microglia from older animals exhibiting a more negative $V_m$ [6,9,10].

For microglia *in situ*, under physiological conditions, THIK-1 (two-pore domain halothane-inhibited $K^+$ channel type 1) $K^+$ channels maintain a negative $V_m$ [5]. These channels are tonically active but extracellular ATP and ADP can act via $P2Y_{12}$ receptors to potentiate THIK-1 activity and hyperpolarize microglia, with functional consequences described below. Nevertheless, the resting potential is independent of tonic purinergic signaling [11], because extracellular nucleotide levels are normally kept too low to activate $P2Y_{12}$ receptors [5,11,12]. THIK-1 is not expressed in cultured microglia [13], for which only $Cl^-$ channels have been shown to contribute to $V_m$ [14]. This reflects the fact that the ion channel and receptor profile of cultured microglia differs profoundly from their counterparts embedded in brain tissue [5,7,15–17]. Indeed, microglia change their gene expression profile within minutes of being in culture [2,13,18,19], downregulating homeostatic genes (e.g. *Tmem119*, *P2ry12*).

Despite the presence of the THIK-1 $K^+$ conductance, the $V_m$ of microglial cells is more positive than the $K^+$ reversal potential ($E_K$ ~ -90 mV), implying that other conductances with a more positive reversal potential are also present. Candidates include volume-regulated $Cl^-$ channels or transient receptor potential (TRP) cation channels. The latter include TRPM7 and TRPV1 [14,20,21], although TRPV1 is primarily located on intracellular membranes [22], as well as TRPM2/4 and TRPV2/4 which are also expressed *in situ* [23] and could contribute. Microglial $Cl^-$ and TRPV1

channels have been documented *in situ* [23–27], but without investigating their role in controlling $V_m$.

Although voltage-gated, $Ca^{2+}$-gated and inwardly-rectifying $K^+$ channels are only weakly or transiently [28] expressed by microglia *in situ* under healthy conditions [3,5,7,10,16], both $K_{ir}2.1$ and $K_v1.3$ are up-regulated with age and when microglial cells become activated in pathology [4,10,15,16,29]. In these situations, it is less clear whether voltage-gated $K^+$ channels contribute to the cells' $V_m$, though a correlation between increased expression of $K_{ir}$ $K^+$ channels and a more negative $V_m$ has been suggested [4,9,10]. The ion channels regulating microglial resting potential are shown in Fig. 1.

**Regulation of microglial surveillance**

In the healthy brain, the ramified processes of non-activated microglia are highly motile, continuously extending and retracting at ~2.5 µm/min [30,31], to perform surveillance of the brain. Microglia have non-overlapping territories for their processes, suggesting an energetically efficient strategy for scanning the brain [32].

Microglial ramification and surveillance depend on the resting potential, and hence on the tonic activity of THIK-1 $K^+$ channels [5]. Particularly highly ramified microglial cells in the *substantia nigra pars reticulata* exhibit a more hyperpolarized resting potential than less ramified cells elsewhere in the basal ganglia [9]. Conversely, depolarization of microglia, either by pharmacological blockade or genetic deletion of THIK-1, or by raising extracellular $[K^+]$, decreases microglial ramification and surveillance [5]. Consequently, pathological rises of $[K^+]_o$ e.g. in epilepsy, or downregulation of THIK-1 with ageing [33] or when microglia become activated in disease [34,35], are expected to decrease microglial surveillance and events that depend on it. Indeed, reduced ramification and motility are characteristic of microglia in old age and disease [36,37]. THIK-1-induced hyperpolarization of microglia due to $P2Y_{12}$ receptor activation by elevated extracellular [ATP] or [ADP] is thus expected to increase microglial ramification and surveillance. However, maintenance of surveillance occurs independently of tonic purinergic signaling [11], and the dominant effect of a local release of ATP in the brain is to attract microglial processes towards the ATP source (see below). The ion channels and receptors regulating surveillance by resting microglia are shown in Fig. 1.

Microglia experience dramatic morphological and functional changes during development. For instance, postnatal microglia display a more amoeboid morphology (i.e. with increased soma size and decreased process ramification) while adult microglia are more ramified [28]. Interestingly, mature microglia become more amoeboid again following activation [38]. Although not much is known about the precise role of microglial ion channels throughout development, these morphological and functional changes may reflect changes in ion channel expression and function (e.g. transient activation of $K_v1.3$ as microglia invade the cortex [38] or more prominent outward rectifier $K^+$ currents in aged microglia [10]), which alter membrane potential.

Microglial ramification and surveillance may also be modulated by cyclic AMP. Under healthy conditions favouring microglial ramification and surveillance, [cAMP] is kept low via $G_i$-coupled receptor (e.g. CX3CR1) signaling, whereas microglial activation tends to be characterized by an upregulation of $G_s$-coupled adenosine $A_{2A}$ receptors and a downregulation of $G_i$-coupled $P2Y_{12}$ receptors [12,39] (although microglial activation is not an all-or-none process, and there can be exceptions to this rule, see below). Direct stimulation of the adenylate cyclase or genetic deletion of the microglial-specific $G_i$-coupled fractalkine receptor CX3CR1, which both lead to a raised [cAMP], reduced ramification and process velocity and thus microglial surveillance [40–42]. A rise of intracellular [cAMP] has also been implicated in the global retraction of processes that occurs when microglia become activated by exposure to lipopolysaccharide (LPS) [39]. ß-adrenoceptor activation, which modulates [cAMP] via coupling to both $G_i$ and $G_s$ proteins, induced a mild process retraction of resting microglia *in situ* [43], but in Aß-treated microglia maintained ramification [44].

Furthermore, microglial surveillance is promoted by tyrosine kinase receptors, which regulate microglial motility *in vitro* [45], while microglial-specific deletion of the TAM tyrosine kinase subtypes Mer and Axl decreased microglial surveillance *in vivo* [46]. Although the underlying mechanism remains elusive, tyrosine kinase receptor signaling may affect two-pore domain $K^+$ channels including THIK-1, which maintains the resting potential (see above), and which is more active when tyrosine phosphorylated [47].

**Targeting processes to damaged areas (chemotaxis)**

Microglia are the first line of defense in brain injury and pathologies. They rapidly send out processes to ensheath injured areas, thereby confining the damage [30]. Chemotaxis is regulated differently from surveillance, and purinergic signaling plays an important role [5,12,30,38,41]. A rise of extracellular [ATP], caused by brain damage or neuronal hyperactivity, is detected by receptors activated by ATP itself ($P2X_4$, $P2X_7$) or its by-products ADP ($P2Y_{12}$) and adenosine ($A_1$, $A_{2A}$ $A_3$) generated by ectonucleotidase-mediated ATP hydrolysis (see [48]). Targeted motility (chemotaxis) of microglial processes mainly depends on ADP-evoked $G_i$-coupled $P2Y_{12}$ receptor signaling [12], which promotes interactions with the extracellular matrix by activating $\beta$-integrins, particularly at the bulbous endings of chemotactic processes [49,50]. A rapid chemotactic response is also triggered by stimulation of microglial $\alpha_M\beta_2$-integrin receptors by fibrinogen, which enters the brain from damaged blood vessels and acts as a strong chemoattractant like ATP [51]. Integrins can also regulate ion channel function, e.g. $K^+$ channels involved in cell migration [52].

The activation of adenosine receptors by adenosine generated via the ecto-nucleotidase NTPDase-1 (CD39) contributes to the chemotactic response of microglia *in situ* [53], although adenosine alone cannot trigger chemotaxis [54]. *In vitro* work suggested an involvement of $A_1$ or $A_3$ adenosine receptors [54,55], but in brain microglia only mRNA for the $A_3$ receptor is found [23].

$G_i$-coupled CX3CR1 fractalkine receptors also promote chemotaxis, since their genetic deletion greatly slowed process extension to focal laser injury or ATP source *in situ* [41,42]. Fractalkine, expressed by neurons, exists in a soluble and a membrane-tethered form. In the undisturbed brain, the latter attenuates microglial activation, antagonizing inflammation and promoting a ramified morphology. However inflammation, or pathologically increased neuronal activity in epilepsy, increases enzymatic shedding of soluble fractalkine, which induces microglial migration and targeted process movements [56,57].

Furthermore, amine transmitters regulate chemotaxis. Serotonin, acting via $G_q$-coupled 5-$HT_{2B}$ receptors, can attract processes of microglial cells in the visual system [58]. The serotonin-evoked chemotactic response resembles that evoked by ATP. Globally deleting 5-$HT_{2B}$ receptors did not attenuate ATP-induced chemotaxis [58], indicating that the purinergic and serotonergic systems may operate independently (although serotonin could act indirectly by triggering ATP release from neurons [59] or astrocytes [60]). Conversely, noradrenaline inhibits injury- and ATP-triggered motility,

acting via $G_s$-coupled $\beta_2$ adrenoceptors [43], suggesting a complex interaction between purinergic and noradrenergic signaling in microglia. Thus, overall, $G_i$- and $G_q$-coupled GPCR signaling promote injury-triggered process elongation while $G_s$-signaling tends to inhibit targeted process elongation to injury and mediates process retraction in surveilling microglia.

Chemotaxis is also controlled by tyrosine kinase signaling, since knockout of the TAM receptor tyrosine kinases Mer and Axl slowed process extension by ~40% *in vivo* [46]. Perhaps relatedly, Triggering Receptor Expressed on Myeloid cells 2 (TREM2), which acts in part via tyrosine phosphorylation, promotes cell migration and outgrowth of microglial processes towards focal damage *in vivo*, by driving expression of genes involved in microglial chemotaxis and migration [61].

Microglial chemotaxis to an ATP source depends on volume-sensitive $Cl^-$ channels [24], which are activated during osmotic stress and membrane stretch. *In vitro*, a similar role has been proposed for $P2X_4$, TRPV1 and TRPM7 cation channels [22,62,63]. As microglial processes grow through narrow extracellular spaces to reach their target, water that is initially outside the cell may have to move inside as the growing process advances through a volume. This water movement may be driven by changes of osmotic pressure produced by ion movements across the cell membrane. In brain slices, activation of microglial $Cl^-$ channels requires ATP release and an intracellular $[Ca^{2+}]$ rise [27]. Both conditions occur during chemotaxis induced by damage-evoked ATP release, possibly amplified by ATP-induced ATP release from astrocytes [30], which evokes intracellular $Ca^{2+}$ transients in microglia via P2Y receptors [64,65]. Interestingly, $Cl^-$ channels can also mediate ATP release [66], suggesting a self-perpetuating mechanism that may sustain local purinergic signaling and promote $P2Y_{12}$ receptor and $Cl^-$ channel activity. Blockade of $Cl^-$ channels abolished chemotaxis but left surveillance unaffected [24], further indicating different mechanisms controlling these two motility modes.

Given the dependence of microglial surveillance and ramification on the resting potential, which is set by tonic THIK-1 $K^+$ channel activity (see above), it is astonishing that targeted motility is unaffected by changes of membrane potential and occurs robustly even when microglia are strongly depolarized [5]. Nevertheless, the microglial cell hyperpolarization that occurs during chemotaxis, via the coupling of $P2Y_{12}$ to THIK-1, suggests a voltage or $K^+$-efflux mediated activation of downstream signaling events such as microglial phagocytosis or inflammasome assembly (see below). The

ion channels and receptors regulating targeted process movement by microglia are shown in Fig. 2.

**Regulation of phagocytosis**

Phagocytosis follows 3 conceptual stages: “find me”, “eat me” and “digest me”. “Find me” involves microglial receptor recognition of target ligands, such as Aβ from amyloid plaques (by TREM2; [67]), LPS on bacteria (by toll-like receptors, [68]), phosphatidylserine on apoptotic cells (by TAM kinases; [69]) or ATP release (by $P2Y_{12}$ to attract processes to a damaged area as described above, and by $P2Y_6$ to trigger phagocytosis, see below). For “eat me”, a phagocytic cup is formed which upon internalization will transform into a phagolysosome that degrades its contents (“digest me”). This depends on cytoskeletal rearrangements controlled by $Ca^{2+}$. While “resting” microglia rarely exhibit spontaneous $Ca^{2+}$ transients *in vivo* [65], damage to nearby neurons rapidly raises microglial [$Ca^{2+}$] via UDP-mediated $P2Y_6$ receptor signaling [64,65] and possibly via ADP-activated $P2Y_{12}$ receptors.

$P2Y_6$ receptor activation triggers phagocytosis via PKC and $IP_3$-dependent $Ca^{2+}$ release from internal stores [70,71] which may trigger subsequent capacitative $Ca^{2+}$ entry through store-operated $Ca^{2+}$ entry (SOCE) channels. SOCE is mediated by the opening of Orai $Ca^{2+}$ channels on the plasma membrane upon interaction with STIM, a $Ca^{2+}$-depletion sensor on the endoplasmic reticulum [72]. Both proteins are present in cultured microglia [73], and microglia have the highest mRNA expression of *Orai1* in the brain [23]. The latter is particularly enriched in podosomes, highly dynamic actin-rich subcellular microglial structures that interact with the extracellular matrix [74]. Knockdown of *Stim1* or *Orai1* prevents microglia from exhibiting UDP-evoked $Ca^{2+}$ elevations, and inhibits their phagocytic activity *in vitro* [73,75]. Thus, SOCE supports [$Ca^{2+}$] rises mediating $P2Y_6$-dependent phagocytosis, possibly by creating $Ca^{2+}$ hotspots near phagosomes (as in neutrophils: [76]).

Phagocytosis in immune cells is accompanied by a sustained hyperpolarisation [77]. This could serve to facilitate SOCE by maintaining sufficient driving force for $Ca^{2+}$ entry and may be mediated by the upregulation of $K_{ir}2.1$ or $K_v3.1$ $K^+$ channels [78]. Interestingly, TREM2 deficiency leads to downregulation of various $K^+$ channels and impaired microglial phagocytosis [67,79], suggesting that TREM2 controls phagocytosis, at least in part, through regulation of the membrane potential. The ion channels that regulate phagocytosis by microglia are shown in Fig. 3.

**Regulation of reactive oxygen species release**

Production of superoxide by the NADPH oxidase (NOX) complex is required for phagocytes to efficiently destroy pathogens, in what is known as a “respiratory burst” of reactive oxygen species (ROS) generation [80]. Once the complex is assembled, the enzyme (mostly NOX2 in microglia [23]) oxidises NADPH molecules to $NADP^+$ and $H^+$, and extrudes an electron across the cell membrane. This electron then reacts with oxygen to generate highly reactive superoxide radicals which contribute to bacterial lysis.

Electron pumping into the phagosome and $H^+$ release into the cytoplasm by NADPH oxidase cause depolarisation and intracellular acidification, respectively. These would readily inhibit NOX activity [81], so a charge compensation and $H^+$ removal mechanism may be required for sustained bacterial killing. $H_v1$ $H^+$ channels, which generate a hyperpolarizing $H^+$ efflux when activated by the depolarization accompanying electron extrusion, were shown to provide such a mechanism in blood granulocytes, where $H^+$ currents are essential for the respiratory burst [82]. $H_v1$-deficient mice lacked voltage-gated $H^+$ currents in leukocytes, and showed an impaired respiratory burst and decreased bacterial clearance [83].

In the brain, $H_v1$ channels are only expressed by microglia (at the mRNA [23] and protein level [84]), and not by neurons [83] or astrocytes [85], but the significance of these currents is uncertain [15] due to inter-species differences. DeSimoni [17] reported no detectable $H^+$ currents in microglia *in situ* in rats, while Wu [85] found that mouse microglia express $H_v1$ currents *in situ* but confirmed that the current was ~12-fold smaller in rats. Microglia from $H_v1$ knockout mice show a larger pH drop following NOX activation by phorbol ester [85], suggesting $H_v1$ mediates $H^+$ extrusion and maintains a negative membrane potential to sustain NOX activity in mice.

In the absence of $H_v1$, in rat microglia other ion channels may contribute to charge compensation maintaining the oxidative burst, such as SK2/SK4, $K_v1.3$ $K^+$ or TRPV1 channels [86,87], which are involved in ROS production *in vitro*. The ion channels regulating the generation of ROS are shown in Fig. 3.

**Regulation of activation state**

It is now clear that the old binary view of microglial activation (i.e. M1/M2) was overly simplified. Instead, microglia can exist in a spectrum of activation states, with different stimuli shifting microglia towards a particular expression profile [88]. For instance, microglial activation is controlled by receptors inducing a transcriptional switch, like one of the Aβ-binding receptors, TREM2 [35]. Microglia activated by Aβ upregulate immune response genes [89] and show altered purinergic signaling and $K^+$ currents [4]. Notably, increased $K_{ir}$- and $K_v$-evoked currents occur in microglia near Aβ plaques [4,29], and may contribute to activation, since the voltage-gated $K^+$ channel blocker 4-aminopyridine suppressed microglial activation following Aβ exposure *in vitro* and *in vivo* [78] (although the concentration of 4-aminopyridine used may also inhibit THIK-1 $K^+$ channels). $K_v$1.3 channels are upregulated when microglia become activated, e.g. by amyloidosis. Inhibiting $K_v$1.3 prevented Aβ-evoked neuronal damage [90], however, the inhibitor applied will block neuronal as well as microglial $K_v$1.3, complicating interpretation of the results. Damaging effects of LPS-induced microglial activation also depend on $K_v$1.3 channels [17], since $K_v$1.3 deletion reduced LPS-evoked expression of inflammatory mediators, impairment of long-term potentiation and antigen presentation [91,92], but again no distinction was possible between microglial and neuronal $K_v$1.3.

**Regulation of cytokine release**

$K^+$ efflux via THIK-1, and via voltage-gated $K^+$ channels upregulated on activation, is needed for NLRP3 inflammasome-mediated release of pro-inflammatory cytokines. NLRP3 function develops in two stages: First, a priming signal (e.g. LPS or cytokines) is required for NFκB-mediated transcriptional upregulation of NLRP3 and IL-1β. Second, an activating signal triggers inflammasome assembly and activation, whereby caspase-1 cleaves interleukin from precursors to allow it to be released from the cell [93]. In this process, $K^+$ efflux, intracellular $Ca^{2+}$ mobilization and decrease of [cAMP] have been identified as crucial checkpoints for NLRP3 assembly and activation in peripheral macrophages [94,95], which share several key functions with microglia such as phagocytosis, chemotactic responses and the production of immune modulators and reactive oxygen species [13,96]. In LPS-primed microglia stimulated with ATP or the $P2Y_{12}$ specific agonist 2-MeSADP, activation of the $P2Y_{12}$-THIK-1

signaling complex is required for IL-1β release *in situ* [5], consistent with a mainly $K^+$ efflux-triggered, $Ca^{2+}$ and cAMP-dependent mechanism.

Despite being close relatives to microglia, macrophages are developmentally distinct cell types originating from bone marrow (unlike microglia which are yolk-sac derived) and have a different gene transcript and protein expression profile [96-98]. Peripheral macrophages are normally excluded from the brain parenchyma by the blood-brain barrier, but during injury and under some chronic disease conditions (e.g. Alzheimer's disease, multiple sclerosis, traumatic brain injury) they can enter the brain where they, instructed by the local brain environment, rapidly adopt a genetic signature similar to microglial cells [98]. Macrophages also express various ionotropic and metabotropic purinergic receptors (predominantly $P2Y_6$, $P2X_4$ and $P2X_7$), inward- and delayed-rectifying voltage-dependent $K^+$ channels and two-pore domain $K^+$ channels of the THIK and TWIK subfamilies, albeit at expression levels different from microglia [96,98,99]. Importantly, in macrophages, activation of the NLRP3 inflammasome and release of IL-1β is, as in microglial cells, also controlled by a purinergic signaling activated two-pore domain $K^+$ channel, in this case tonically active TWIK2 channels that are suggested to be gated by $P2X_7$ [99]. Although IL-1β release was reduced upon genetic deletion of $P2X_7$ or TWIK2, with $P2X_7$ knocked out, ATP still evoked outward TWIK2 $K^+$ currents, suggesting that TWIK2 can also be gated by a P2Y-type G protein coupled purinergic receptor as has been shown for microglia [5].

Depending on the cells' activation state, $K_v$ channel activation may also provide the $K^+$ efflux needed for inflammasome activation [78,90], although the use of pharmacological $K^+$ channel blockers makes it difficult to distinguish effects on microglia from effects on neurons. $K_v1.3$ blockade or knockout also abolishes LPS-induced release of IL-1β and TNFα from microglia [91,92], and reduces inflammation in ischaemic stroke [100]. Interestingly, while $K_v1.3$ is involved in regulating cytokine release *in situ*, chemokine release seems to be regulated by $P2Y_{12}$ [101]. By contrast, other $K^+$ channels, like the inward rectifier $K_{ir}2.1$, are not induced by LPS, and do not regulate cytokine or chemokine release [101]. These data suggest that THIK-1 and $K_v1.3$ mediate the $K^+$ loss required for inflammasome assembly in microglia. Given that THIK-1 $K^+$ channels are tonically active [5], any depolarization of microglia (for example by ATP release activating $P2X_7$ receptors) could act as an initial trigger for increased $K^+$ loss and inflammasome assembly, with upregulation of $K_v1.3$ (and possibly $K_{Ca}3.1$ [102]) following later.

Production of inflammatory mediators may require long-lasting elevations of basal [$Ca^{2+}$], which occur *in vitro* in microglia and macrophages upon LPS treatment [103]. LPS induces microglial upregulation of $P2X_7$ channels [104], which mediate both $K^+$ efflux and $Ca^{2+}$ entry when activated by high extracellular [ATP] and are involved in the production of cytokines *in vitro* [104–107]; however, this may primarily be mediated by $K^+$ efflux, given that in macrophages $K^+$ efflux alone is sufficient to activate the NLRP3 inflammasome [108]. Finally, ATP release via gap junctional hemichannels may contribute to inflammasome activation and $P2X_7$ activation [109] and thus exacerbate inflammation. The ion channels regulating microglial cytokine release are shown in Fig. 4.

## Regulation of complex functions and disease relevance

Ion channel- and cell surface receptor-controlled mechanisms regulate immune surveillance by microglia, and thus interactions with neurons and other CNS cells which are essential to establish complex brain functions. These interactions help to shape synaptic properties, control neuronal survival and confine brain damage. Some of the ion channels and receptors involved are only induced in pathology, e.g. inward- and outward rectifying $K^+$ channels after stroke [110] or $Ca^{2+}$-activated $K^+$ channels in epileptic patients [111]. Due to their profound role in many neural diseases [112], microglial ion channels and receptors are attracting increasing attention as drug targets.

### *Synaptic refinement and plasticity*

Microglia contribute to synapse formation and remodeling during development [113–115], and regulate synaptic strengthening and plasticity [116,113,117,118] via IL-1β release [119] and $P2Y_{12}$ receptor signaling [116]. In turn, synaptic activity can increase both the ramification of microglial processes and the duration of their contacts with synapses [120]. As ramification and surveillance are regulated by THIK-1 activity [5], modulation of THIK-1 may alter synapse numbers and strength.

### *Neuronal damage and loss*

Ion channels regulating microglial phagocytosis [121], NOX activity [85] or neuroinflammation [122] may be targeted therapeutically in stroke, traumatic injury or neurodegenerative disorders. Knockout of $H_v1$ [85,123] or block of $K_v1.3$ [100], which are upregulated by ischaemia, leads to decreased infarct volume after stroke. Inhibition of $P2Y_{12}$ reduces damage by preventing inflammasome induction [124], the blockade

of which improves neuronal function following brain injury [122]. Reducing the enhanced activity of TRPM2 and TRPV1 channels in neurodegenerative conditions may attenuate inflammation and cognitive impairment [26,125]. Furthermore, blockade of $P2X_7$ channels, which are upregulated in Alzheimer's disease [126], may prevent inflammation-driven neuronal loss [104].

***Pain***

Purinergic signaling to microglia is a crucial mediator of pain. $P2X_4$ is induced in spinal [127] microglia following nerve injury, and drives neuropathic pain by inducing microglial release of brain-derived neurotrophic factor [128,129]. Block of $P2X_4$ reversed pain sensations in rats [127]. $P2X_7$ receptors are also implicated in pain sensitivity in humans [130], presumably by mediating cytokine release, since impairment of $P2X_7$ function conferred antinociception and reduced inflammation [131,132]. Similarly, blockade of microglial $P2Y_{12}$ receptors and their downstream THIK-1 $K^+$ channels alleviates neuropathic pain and inflammation [133–135], presumably by blocking the $K^+$ efflux-triggered NLRP3 activation [5].

***Epilepsy***

Neuronal hyperexcitability enhances neuron-microglia interactions, microglial activation and IL-1β release [136,137]. Microglial processes are attracted to highly active neurons, via NMDA receptor activation and neuronal ATP release-mediated activation of microglial $P2Y_{12}$ receptors [138]. Epilepsy enhances purinergic signaling via up-regulation of ionotropic ($P2X_4$, $P2X_7$) and G-protein-coupled ($P2Y_6$, $P2Y_{12}$) receptors, doubling the speed at which microglial processes approach an injury-mimicking ATP source and increasing expression of inflammatory modulators [16]. Global deletion of $P2X_4$ reduces microglial activation and decreased seizure-induced neuronal death [139], although this could reflect $P2X_4$ function in either neurons or microglia.

***Mood disorders***

Brain inflammation is associated with a number of psychiatric disorders including depression [140]. Related to this, microglial activation may reduce serotonin levels [141] and minocycline, which prevents microglial activation, evokes antidepressant effects [142]. Thus, reducing microglial activation and inflammasome formation may provide a promising therapeutic strategy. Pharmacological block and genetic deletion of $P2X_7$ receptors, which are predominantly but not exclusively expressed by microglia [143], confers antidepressive effects [144], while $P2X_7$ agonists

induce depressive-like behavior in rodents [145]. Depressive behavior can also be associated with microglial hyper-ramification, which is dependent on CX3CR1 receptor signaling and can be reversed by antidepressant treatment [146]. Although it is unclear whether the hyper-ramification is a cause or an effect of the disease, interfering with mechanisms that restore a normal microglial morphology may support the action of antidepressants.

## Concluding remarks

The work discussed in this review has revealed how microglial ion channels and cell surface receptors shape microglial function, and thus affect the overall functioning of the nervous system. The last decade has seen tremendous progress in our understanding of ion channels in microglia *in situ* and their roles, although several key aspects remain unresolved (see Outstanding questions). While *in vitro* studies are still widely used, recent research shows that they should be interpreted with caution, and any result described in culture needs validating in an *in situ* context (in brain slices or *in vivo*) in order to assess how interactions with other CNS cells [147] and differential regulation by sex, age or brain region [6,148,149] affect the results. This improved understanding of microglial ion channel function is likely to underpin future therapeutic developments targeting the brain's immune cells.

**BOX 1: Technical challenges in electrophysiology**

Investigations of the electrical properties of cells are routinely performed by patch-clamping, which enables recordings of ionic currents from individual cells at high resolution. However, accurate measurements of $V_m$ using this method require the seal resistance between the glass of the patch pipette and the cell membrane to be much higher than the cell membrane resistance - a condition which is not always fulfilled for microglial cells due to their very high membrane resistance in the gigaohm range. Thus, even subtle (and technically unavoidable) leak currents across the seal resistance can profoundly influence the measured $V_m$ values, which could result in artificially depolarized membrane voltages. Conversely, the cells may also become more hyperpolarized if $Ca^{2+}$ entry, via the leak conductance, activates $Ca^{2+}$-activated $K^+$ channels. Accordingly, the real membrane resistance of microglia will in general be higher than that obtained from patch-clamp experiments, due to the similar magnitudes of the seal and membrane resistances. Indeed, when using voltage-sensitive dyes to overcome these experimental constraints, a much higher membrane resistance has been measured in cultured microglia [14]. The susceptibility of microglia to even subtle changes in their environment requires ongoing effort to develop experimental paradigms that are as little invasive as possible, facilitated by e.g. technical advances in multiphoton imaging or the availability of high-sensitivity genetically encoded voltage and $Ca^{2+}$ sensors.

**BOX 2: Microglial diversity**

Brain region-dependent differences of microglial distribution, ramification and motility affect surveillance. Microglial densities vary significantly (> 4-fold) across different brain regions and are highest in the cortex, the basal ganglia and limbic areas, slightly lower in the diencephalon and brainstem and particularly low in the cerebellum [1,150,151]. Apart from the *substantia nigra* of the basal ganglia, which exhibits the highest density of all brain areas, microglia are fairly equally distributed within each gross brain region [9,151]. There are also marked variations of morphological parameters depending on brain region, with less ramified microglial cells in the cerebellum that cover a smaller territory and thus survey less of the parenchyma, compared to highly branched cells with longer processes in the cortex, hippocampus and *substantia nigra* [9,150]. Furthermore, some cerebellar microglia, in addition to movements of their processes, exhibit rapid and frequent displacements of their cell

bodies [150], a phenomenon that is not seen in their cortical counterparts [30,31]. These brain region-dependent variations of microglial surveillance that occur in healthy conditions suggest distinct functional states and different physical interactions of microglia with other CNS cells - interactions which have been shown to play crucial roles in brain development and homeostasis such as synaptic remodeling, regulation of neuronal activity and removal of apoptotic neurons (see overview in [48]). Apart from this, recent work also highlights significant sex-dependent changes of microglial morphology, distribution and functional aspects [6]. This implies that, apart from the growing awareness of region-specific properties of microglia and the existence of different microglial subtypes, future studies must carefully consider effects in both sexes.

## Outstanding questions

- What is the mechanism coupling microglial membrane voltage to process ramification and motility?
- What is the nature of the tonically active depolarizing ion channels, which keep the microglial membrane potential more positive than the $K^+$ reversal potential ($E_K$)?
- Do microglial ion channels contribute to neurotransmitter (e.g. glutamate, ATP) release from microglia?
- The high motility of microglial processes must cause a high turnover of their cell-surface receptors and ion channels. How is this regulated?
- What is the sensor of the decrease of intracellular [$K^+$] which triggers inflammasome assembly, and does the resulting change in membrane voltage contribute?
- Ion channels in microglia are often studied using pharmacological blockers or global genetic deletion. To what extent do these arise from changes in non-microglial cells or developmental aberrations?

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## Figure legends

**Figure 1. Ion channels and receptors regulating microglial resting potential and surveillance.** A negative membrane potential ($V_m$) in "resting" microglia is maintained by tonically active THIK-1 $K^+$ channels, the activity of which is promoted by tyrosine kinase-mediated phosphorylation. Voltage-gated $K^+$ channels ($K_v$ or $K_{ir}$) may contribute to a negative $V_m$ in activated microglia. $Cl^-$ channels and depolarizing cation channels, such as the non-selective cation channels TRPV1 and TRPM7, may further regulate $V_m$ and counteract a $K^+$ channel-evoked hyperpolarization. A negative $V_m$ (minus signs), $G_i$-protein signaling-induced low cAMP levels (e.g. via CX3CR1) and tyrosine kinase activity promote microglial surveillance and ramification.

**Figure 2. Ion channels and receptors regulating targeted process movements.** Left: G protein coupled receptor signaling mediated by $P2Y_{12}$, adenosine $A_3$, CX3CR1 and $5\text{-}HT_{2B}$ receptors, as well as TAM receptor tyrosine kinases, positively regulate targeted movements of microglial processes towards focal brain injury. Process outgrowth along an [ATP] gradient mediated by $P2Y_{12}$ receptors is shown as an example. Ion channel activity via volume- and membrane stretch-activated $Cl^-$ channels is further required to allow chemotaxis, perhaps to mediate ion movements driving water movements that occur when microglial processes grow through the narrow extracellular space. Right: Close-up of a microglial growth cone as it extends to reach its target. Gi/o-mediated signaling cascades, involving not only a decrease of cAMP concentration, but also phosphorylation of Akt (protein kinase B) and a rise of intracellular $Ca^{2+}$ level, trigger the activation of integrin receptors, which promote interactions of microglial processes with the extracellular matrix. Chloride channels may, in addition to driving water movements, also release ATP to sustain local $P2Y_{12}$-receptor activation as a key driver of targeted motility. $P2Y_{12}$-mediated activation of THIK-1 is not needed for targeted process movements.

**Figure 3. Ion channels and receptors regulating microglial phagocytosis and production of reactive oxygen species (ROS).** Phagocytosis requires actin polymerization and cytoskeletal rearrangements (e.g. for the formation of a phagocytic cup). These require a rise in cytosolic $Ca^{2+}$ concentration, which is achieved by $P2Y_6$ receptors evoking release of $Ca^{2+}$ from the endoplasmic reticulum via $IP_3$ receptor

($IP_3R$) channels. *In vitro* data suggest that capacitative $Ca^{2+}$entry might then also occur, whereby STIM on the endoplasmic reticulum senses store $Ca^{2+}$ depletion and interacts with Orai channels triggering their opening and further entry of extracellular $Ca^{2+}$. $K_v1.3$ and $K_{ir}2.1$ may contribute by providing a driving force for $Ca^{2+}$ influx. A "respiratory burst" occurs during phagocytosis, when NADPH oxidase (NOX) extrudes electrons across the cell membrane. These react with oxygen to form superoxide ($O_2^{\cdot -}$), a form of ROS. The sustained activation of NOX requires $H^+$ buffering or release, and a movement of net positive charge out across the membrane (charge compensation). $H_v1$ $H^+$ channels contribute to this in mice (but far less in rats), with other candidates suggested by *in vitro* data including $K_v1.3$, SK2/4 and TRPV1 channels.

**Figure 4. Ion channels and receptors regulating cytokine release.** Priming of microglia (e.g. by lipopolysaccharide (LPS) on bacteria interacting with TLR4 receptors) triggers transcriptional upregulation of NLRP3. Assembly and activation of the NLRP3 inflammasome complex results in caspase 1 activation, which in turn can cleave interleukin precursors (e.g. pro-IL-1β, pro-IL-18) so that interleukins are released from the cell as active mediators of inflammation. For inflammasome assembly to occur, $K^+$ loss is a crucial checkpoint. It can be achieved by activation of THIK-1 channels via ATP/ADP acting on $P2Y_{12}$ receptors, and via $K_v1.3$ channels which are upregulated in inflammation. $P2X_7$ cation channels have also been implicated, the gating of which may be promoted by ATP released via pannexin (panx) channels.

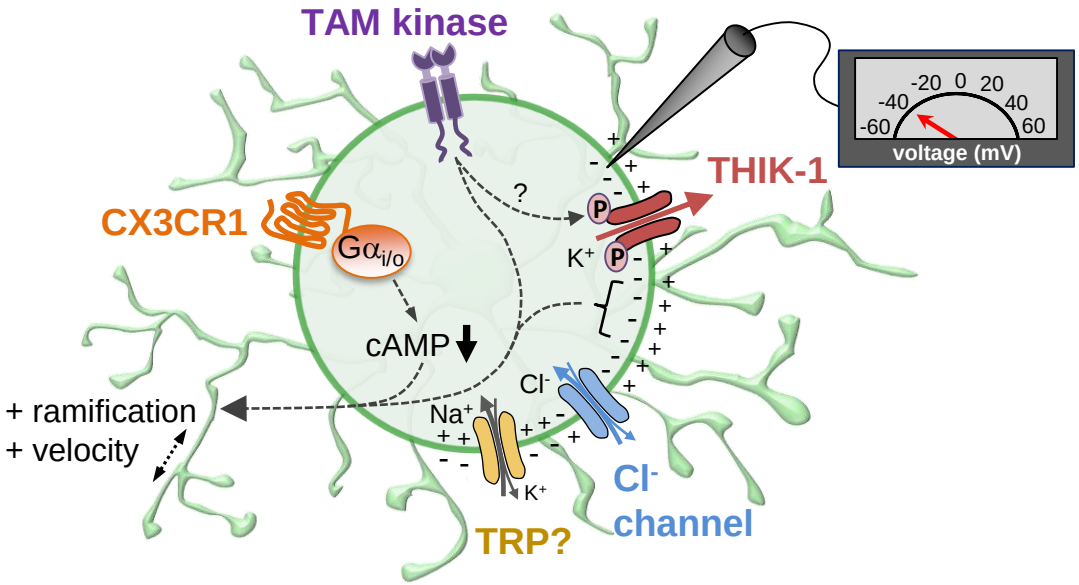
TAM kinase
THIK-1
CX3CR1
Gαi/o
cAMP
?
K+
P
Cl-
Na+
K+
+ ramification
+ velocity
TRP?
Cl- channel
-20 0 20
-40 40
-60 60
voltage (mV)

**Figure 1**

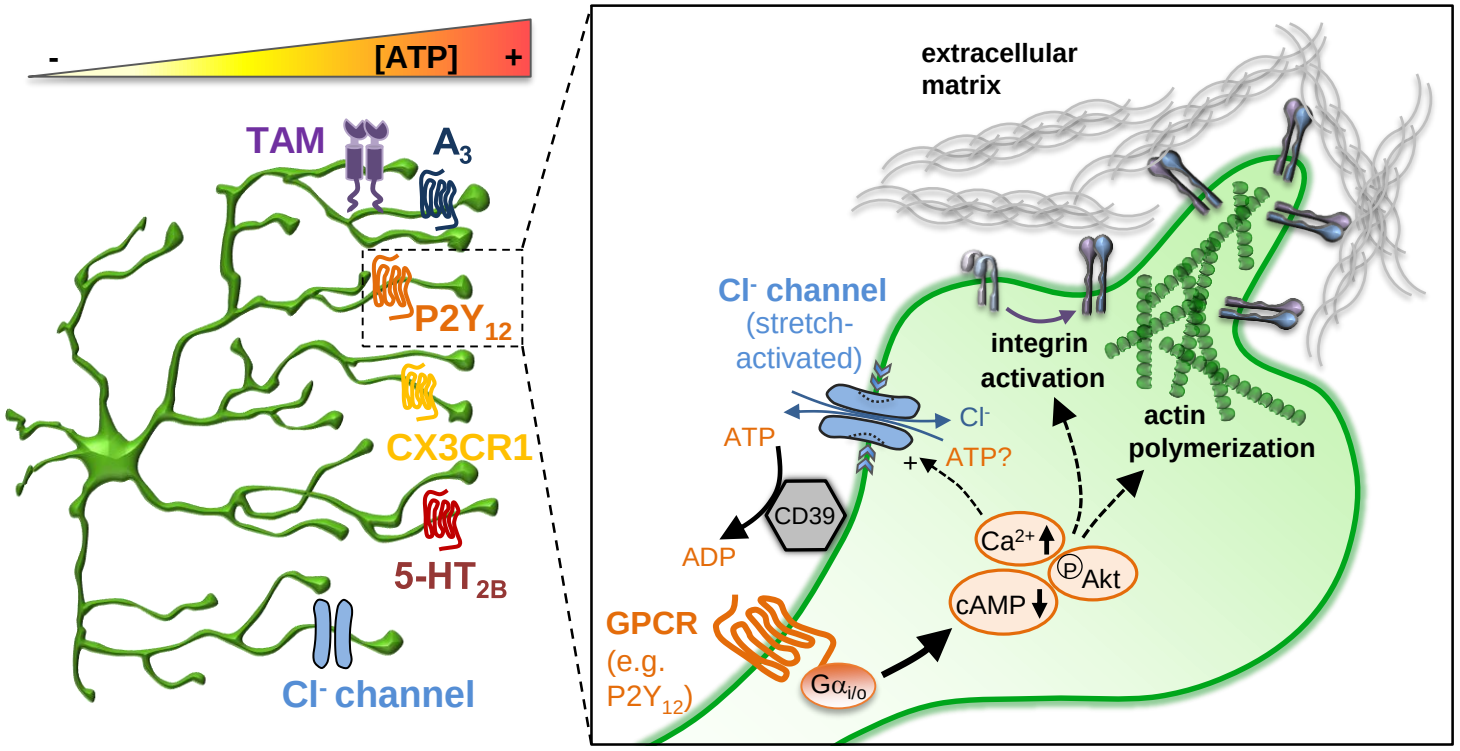
-
[ATP]
+
TAM
A3
P2Y12
CX3CR1
5-HT2B
Cl- channel
extracellular matrix
Cl- channel (stretch-activated)
integrin activation
actin polymerization
ATP
Cl-
ATP?
+
CD39
ADP
Ca2+
PAkt
cAMP
GPCR (e.g. P2Y12)
Gαi/o

**Figure 2**

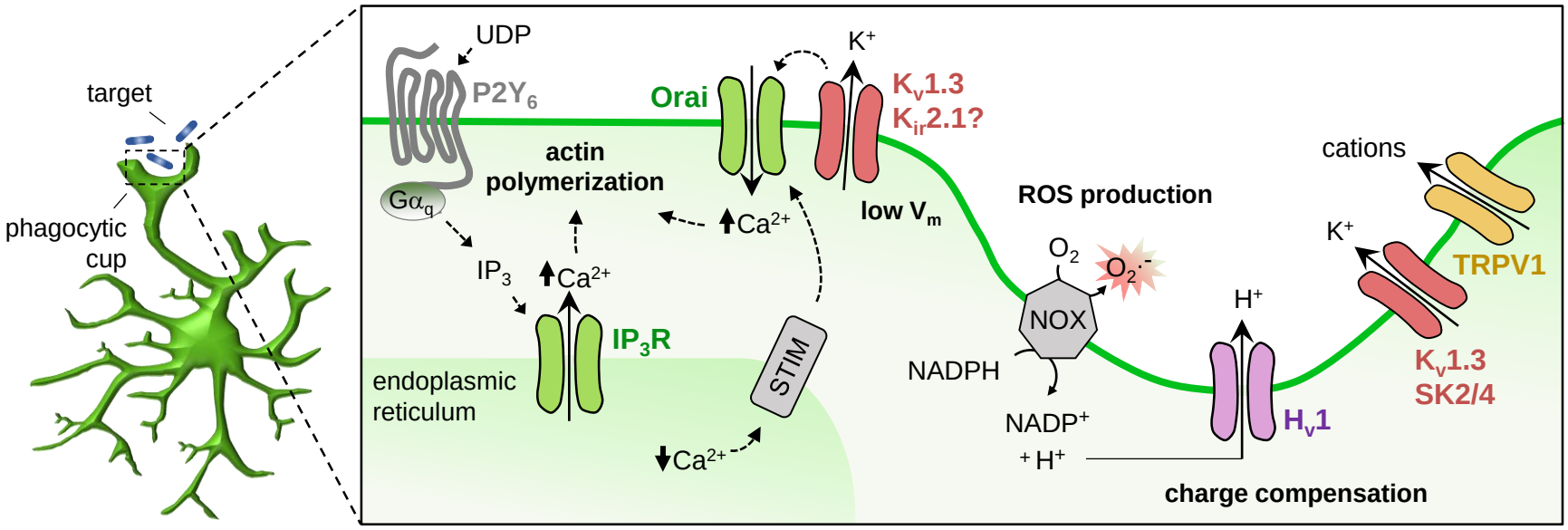
target
phagocytic cup
UDP
P2Y6
Gαq
IP3
Ca2+
IP3R
endoplasmic reticulum
actin polymerization
Orai
K+
Kv1.3
Kir2.1?
Ca2+
low Vm
STIM
Ca2+
ROS production
O2
O2.-
NOX
NADPH
NADP+
+ H+
H+
Hv1
charge compensation
cations
K+
TRPV1
Kv1.3
SK2/4

**Figure 3**

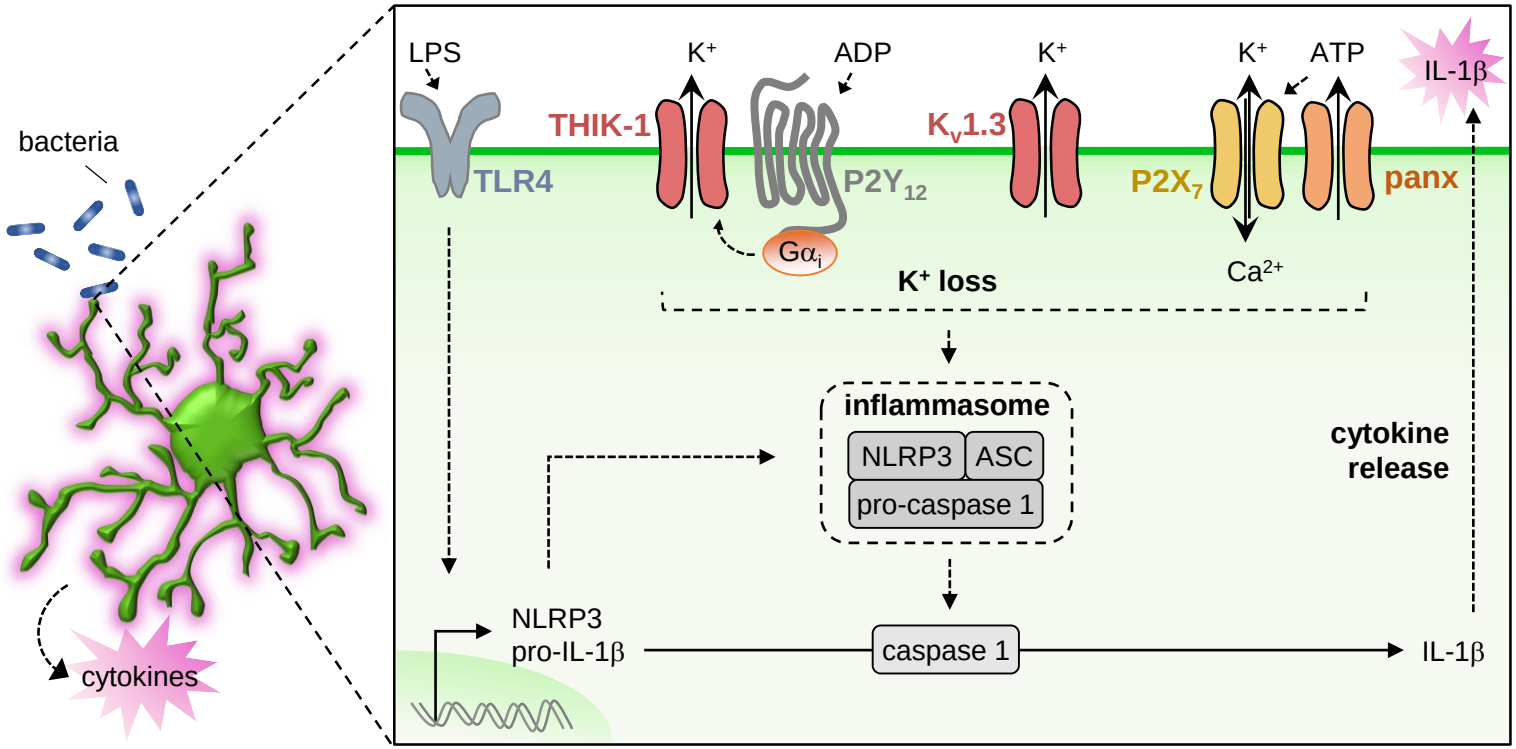
bacteria
cytokines
LPS
TLR4
K+
THIK-1
ADP
P2Y12
Gαi
K+
Kv1.3
K+
ATP
P2X7
panx
Ca2+
IL-1β
K+ loss
inflammasome
NLRP3
ASC
pro-caspase 1
cytokine release
NLRP3
pro-IL-1β
caspase 1
IL-1β

**Figure 4**