

16. Förster C, Burek M, Romero IA, Weksler B, Couraud PO, et al. (2008) Differential effects of hydrocortisone and TNF α on tight junction proteins in an in vitro model of the human blood-brain barrier. *J Physiol* 586: 1937–1949.
17. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, et al. (2007) Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 13: 1173–1175.
18. Minagar A, Alexander JS (2003) Blood-brain barrier disruption in multiple sclerosis. *Mult Scler* 9: 540–549.
19. Minagar A, Long A, Ma T, Jackson T, Kelley R, et al. (2003) Interferon (IFN)- β 1a and IFN- β 1b Block IFN- γ -Induced Disintegration of Endothelial Junction Integrity and Barrier. *Endothelium* 10: 299–307.
20. Mizuno T, Zhang G, Takeuchi H, Kawanokuchi J, Wang J, et al. (2008) Interferon-gamma directly induces neurotoxicity through a neuron specific, calcium-permeable complex of IFN-gamma receptor and AMPA GluR1 receptor. *FASEB J* 22: 1797–1806.
21. Tatsuta T, Naito M, Oh-hara T, Sugawara I, Tsuruo T (1992) Functional involvement of P-glycoprotein in blood-brain barrier. *J Biol Chem* 267: 20383–20391.
22. Sonobe Y, Takeuchi H, Kataoka K, Li H, Jin S, et al. (2009) Interleukin-25 expressed by brain capillary endothelial cells maintains blood-brain barrier function in a protein kinase C ϵ -dependent manner. *J Biol Chem* 284: 31834–31842.
23. Endong L, Shijie J, Sonobe Y, Di M, Hua L, et al. (2011) The gap-junction inhibitor carbenoxolone suppresses the differentiation of Th17 cells through inhibition of IL-23 expression in antigen presenting cells. *J Neuroimmunol* 240–241: 58–64.
24. Conejo-Garcia JR, Buckanovich RJ, Benencia F, Courreges MC, Rubin SC, et al. (2005) Vascular leukocytes contribute to tumor vascularization. *Blood* 105: 679–681.
25. Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, et al. (2000) Interleukin-1 β -induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: a magnetic resonance study. *J Neurosci* 20: 8153–8159.
26. Ransohoff RM, Brown MA (2012) Innate immunity in the central nervous system. *J Clin Invest* 122: 1164–1171.
27. Boström EA, Lundberg P (2013) The Newly Discovered Cytokine IL-34 Is Expressed in Gingival Fibroblasts, Shows Enhanced Expression by Pro-Inflammatory Cytokines, and Stimulates Osteoclast Differentiation. *PLoS one* 8: e81665.
28. Wang Y, Colonna M (2014) Interleukin-34, a cytokine crucial for the differentiation and maintenance of tissue resident macrophages and Langerhans cells. *European journal of immunology*.
29. Ma D, Doi Y, Jin S, Li E, Sonobe Y, et al. (2012) TGF- β induced by interleukin-34-stimulated microglia regulates microglial proliferation and attenuates oligomeric amyloid β neurotoxicity. *Neuroscience letters* 529: 86–91.
30. Nandi S, Gokhan S, Dai X-M, Wei S, Enikolopov G, et al. (2012) The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. *Developmental biology* 367: 100–113.
31. Mitrasinovic OM, Grattan A, Robinson CC, Lapustea NB, Poon C, et al. (2005) Microglia overexpressing the macrophage colony-stimulating factor receptor are neuroprotective in a microglial-hippocampal organotypic coculture system. *The Journal of neuroscience* 25: 4442–4451.
32. Hao C, Guilbert L, Fedoroff S (1990) Production of colony-stimulating factor-1 (CSF-1) by mouse astroglia in vitro. *Journal of neuroscience research* 27: 314–323.
33. Kook S-Y, Hong HS, Moon M, Mook-Jung I (2013) Disruption of blood-brain barrier in Alzheimer disease pathogenesis. *Tissue Barriers* 1: e23993.
34. Zhong Y, Zhang B, Eum SY, Toborek M (2012) HIV-1 Tat triggers nuclear localization of ZO-1 via Rho signaling and cAMP response element-binding protein activation. *J Neurosci* 32: 143–150.
35. Tureci O, Koslowski M, Helftenbein G, Castle J, Rohde C, et al. (2011) Claudin-18 gene structure, regulation, and expression is evolutionary conserved in mammals. *Gene* 481: 83–92.



Interleukin-1 β Induces Blood–Brain Barrier Disruption by Downregulating Sonic Hedgehog in Astrocytes

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Abstract

The blood–brain barrier (BBB) is composed of capillary endothelial cells, pericytes, and perivascular astrocytes, which regulate central nervous system homeostasis. Sonic hedgehog (SHH) released from astrocytes plays an important role in the maintenance of BBB integrity. BBB disruption and microglial activation are common pathological features of various neurologic diseases such as multiple sclerosis, Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease. Interleukin-1 β (IL-1 β), a major pro-inflammatory cytokine released from activated microglia, increases BBB permeability. Here we show that IL-1 β abolishes the protective effect of astrocytes on BBB integrity by suppressing astrocytic SHH production. Astrocyte conditioned media, SHH, or SHH signal agonist strengthened BBB integrity by upregulating tight junction proteins, whereas SHH signal inhibitor abrogated these effects. Moreover, IL-1 β increased astrocytic production of pro-inflammatory chemokines such as CCL2, CCL20, and CXCL2, which induce immune cell migration and exacerbate BBB disruption and neuroinflammation. Our findings suggest that astrocytic SHH is a potential therapeutic target that could be used to restore disrupted BBB in patients with neurologic diseases.

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Introduction

The blood–brain barrier (BBB) is a tight seal composed of capillary endothelial cells, pericytes, and perivascular astrocytes [1]. The BBB contributes to homeostasis in the central nervous system (CNS) by limiting the entry of plasma components, erythrocytes, and immune cells from the circulating blood [2]. Astrocytes play a pivotal role in maintenance of BBB integrity via contact-dependent mechanisms and release of trophic factors [3–5]. In addition, a recent study revealed that Sonic hedgehog (SHH) released from astrocytes promotes BBB formation and integrity by upregulating tight junction (TJ) proteins in capillary endothelial cells [6]. Without SHH, its receptor Patched-1 (Ptch-1) suppresses a G-coupled-protein receptor Smoothed (Smo) which is critical for the activation of a transcription factor Gli-1 [7]. Gli-1 is an important regulator of TJ protein expression and BBB formation. SHH binds and inactivates Ptch-1, which allows Smo to activate Gli-1, which upregulates TJ proteins and enhances BBB integrity. Disruption of BBB integrity is frequently observed in neurologic diseases such as multiple sclerosis (MS), Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease, suggesting that infiltrating molecules and immune cells from the blood perturb CNS homeostasis and exacerbate these disorders [8–13]. Microglial activation is another characteristic

pathologic feature in these diseases [14]. Activated microglia release various cytotoxic factors such as nucleic acids, glutamate, reactive oxygen species (ROS), proteases, and pro-inflammatory cytokines/chemokines [15]. Interleukin-1 β (IL-1 β) is a major microglial pro-inflammatory cytokine that acts on both endothelial cells and astrocytes to increase BBB permeability [16–18]. However, the mechanisms of BBB disruption by IL-1 β have not been fully elucidated. In this study, we demonstrated that IL-1 β suppressed SHH expression in astrocytes and increased BBB permeability by downregulating TJ proteins in endothelial cells. Moreover, IL-1 β stimulated astrocytes to secrete pro-inflammatory chemokines such as CCL2, CCL20, and CXCL2, which induce the migration of immune cells such as neutrophils, monocytes, macrophage, dendritic cells, and pathogenic T cells. Our findings reveal novel mechanisms of BBB disruption by IL-1 β , and suggest that SHH could be used therapeutically against various neurologic diseases.

Methods

Cell cultures

Protocols for animal experiments were approved by the Animal Experiment Committee of Nagoya University (The approval number: 13122).

Mouse primary astrocyte-rich cultures were prepared from primary mixed glial-cell cultures of newborn C57BL/6J mice (SLC, Hamamatsu, Japan), as described previously [19,20]. The purity of astrocytes was >95%, as determined by immunostaining with antibody against glial fibrillary acidic protein. Cells were cultured in maintenance medium (Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 5 μ g/ml bovine insulin, and 0.6% glucose). Astrocytes were plated at a density of 1×10^4 cells/well in 96-well multidishes, 1×10^5 cells/well in 24-well multidishes, or 5×10^5 cells/well in 6-cm culture dishes. For IL-1 β treatment, the cells were incubated with or without 2 ng/ml mouse recombinant IL-1 β (R&D Systems, Minneapolis, MN, USA) for 24 h, and then astrocyte conditioned media (ACM) were collected and used for subsequent experiments.

The mouse brain capillary endothelial cell line, MBEC4 (a kind gift from Dr. T. Tsuruo) [21], was maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and used as an established BBB model.

BBB permeability assay

We used MBEC4 monolayers as an *in vitro* BBB model, as described previously [22]. The permeability of MBEC4 monolayers was evaluated using fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) as a marker. Confluent monolayers of MBEC4 cells on Transwell inserts (3 μ m pore size; BD Falcon, Franklin Lakes, NJ, USA) were incubated for 24 h with 2 ng/ml IL-1 β , ACM, IL-1 β -treated ACM, 1–100 ng/ml recombinant mouse SHH (R&D systems), 0.01–1 μ M purmorphamine (a Smo agonist) (Merck Millipore, Billerica, MA, USA), or 0.3–30 μ M

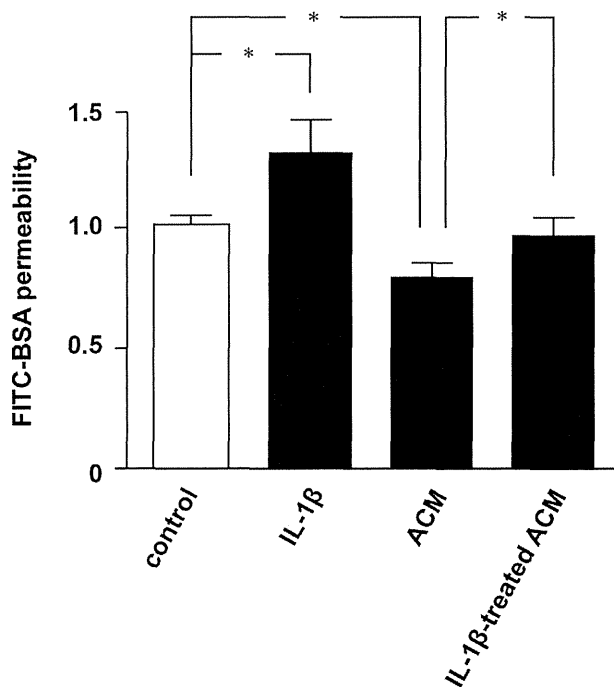


Figure 1. IL-1 β abolishes the protective effect of astrocytes on BBB integrity. MBEC4 cells were treated for 24 h with 2 ng/ml IL-1 β , ACM, or IL-1 β -treated ACM for 24 h. FITC-BSA was loaded onto the luminal side of the insert for 1 h, and then the FITC-BSA levels on the abluminal side were analyzed. All quantitative data are expressed as means \pm SEM (n=5), normalized to the corresponding values from untreated cells. *, $p < 0.001$.

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cyclopamine (a Smo inhibitor) (Merck Millipore). Next, the monolayers were washed with assay buffer (118 mM NaCl, 4.7 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgCl₂, 1.0 mM NaH₂PO₄, 25 mM NaHCO₃, and 11 mM D-glucose, pH 7.4). This buffer (1 ml) was added to the outside of the insert (the abluminal side). Assay buffer containing 4% FITC-BSA (Sigma-Aldrich, St. Louis, MO, USA) was loaded on the luminal side of the insert and incubated for 1 h. The concentration of FITC-BSA in the abluminal chamber was determined by measuring the fluorescence (excitation, 480 nm; emission, 530 nm) using a Wallac 1420 ARVO_{MX} (PerkinElmer Japan, Yokohama, Japan). Assays were carried out in five independent trials.

Quantitative reverse transcription-PCR

Astrocytes were collected after a 6-h incubation with 0.02–2 ng/ml IL-1 β . Total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) and reverse transcribed with SuperScript III (Life Technologies, Carlsbad, CA, USA) as described previously [23]. Expression levels of mRNAs encoding SHH, CXCL2, CCL2, and CCL20 were evaluated by quantitative PCR (qPCR) using TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA, USA) on a Rotor-Gene Q real-time PCR cycler (Qiagen). The following mouse gene-specific primers and probes were obtained from Applied Biosystems: *Shh*, Mm00436528_m1; *Cxcl2*, Mm00436450_m1; *Ccl2*, Mm00411241_m1; *Ccl20*, Mm01268754_m1; *Actb*, Mm00607939_s1; and *Gapdh*, Mm9999915_g1. Gene expression values were determined by the $\Delta\Delta C_T$ method. Levels of the mRNAs of interest were normalized to the geometric mean of *Actb* and *Gapdh* levels. Assays were carried out in five independent trials.

Enzyme-linked immunosorbent assay (ELISA)

Astrocyte conditioned media were collected after a 24-h incubation with 0.02–2 ng/ml IL-1 β , and then assessed for protein levels using ELISA kits specific for mouse SHH, CXCL2, CCL2, and CCL20 (R&D systems). Assays were carried out in five independent trials.

Western blotting

MBEC4 cells were incubated with ACM, 2 ng/ml IL-1 β , 100 ng/ml SHH, 1 μ M purmorphamine, or 30 μ M cyclopamine for 24 h. To assess the protein expression levels of occludin and zonula occludens-1 (ZO-1), cells were lysed in TNES buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, and 0.1% SDS) with protease inhibitor mixture (Complete Mini EDTA-free; Roche Diagnostics, Basel, Switzerland) and a phosphatase inhibitor mixture (Sigma-Aldrich) as described previously [24,25]. Cell lysate proteins dissolved in Laemmli sample buffer (30 μ g/well) were separated on 4–20% SDS-polyacrylamide gels (Mini-Protean TGX; Bio-Rad, Hercules, CA, USA) and transferred to Hybond-P polyvinylidene difluoride membranes (GE Healthcare, Piscataway, NJ, USA). The membranes were blocked for 1 h at room temperature with 5% skim milk in Tris-buffered saline containing 0.05% Tween-20, and then incubated overnight at 4°C with rabbit anti-claudin-5 monoclonal antibody (Zymed Laboratories, South San Francisco, CA, USA), rabbit anti-occludin polyclonal antibody (Zymed Laboratories), rabbit anti-ZO-1 polyclonal antibody (Zymed Laboratories), or mouse anti- β -actin monoclonal antibody (clone AC-15; Sigma-Aldrich), followed by incubation with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare) for 1 h at room temperature. The signals were visualized using SuperSignal West Dura chemiluminescent substrate (Thermo Fisher Scientific,

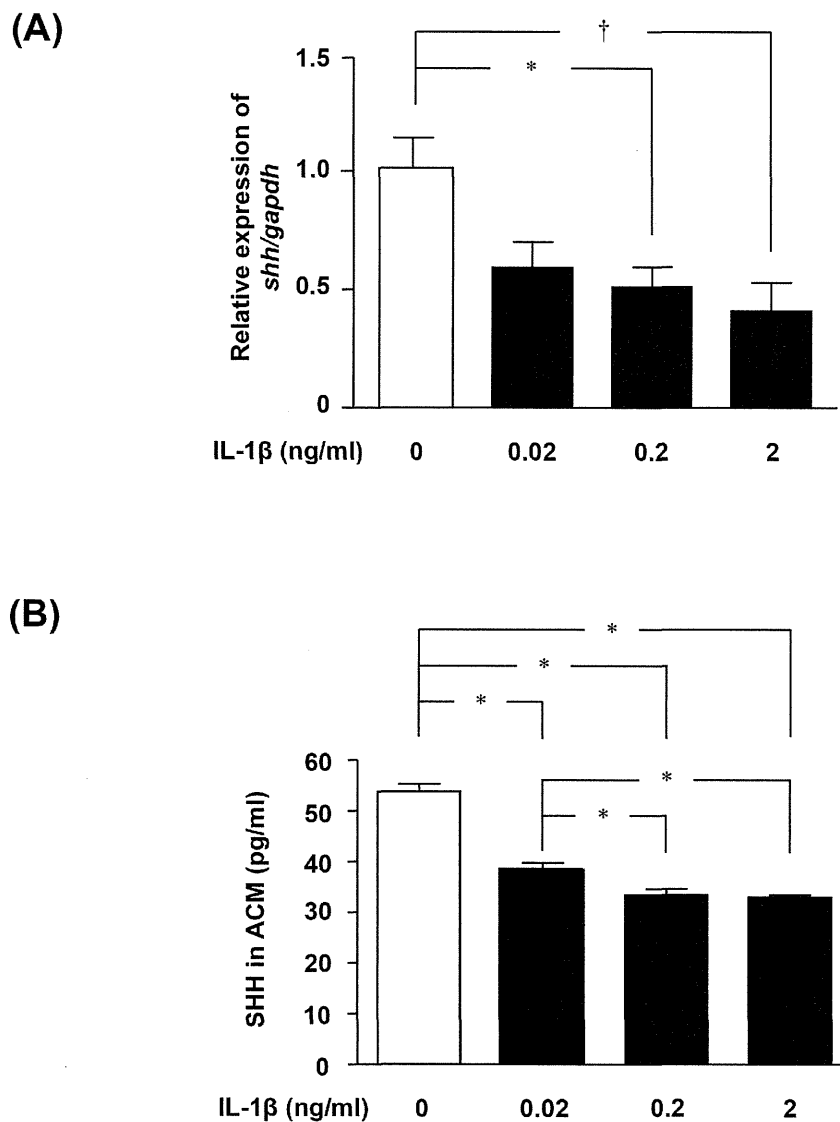


Figure 2. IL-1 β downregulates SHH production in astrocytes. (A) *Shh* mRNA levels in astrocytes, determined by qPCR. Astrocytes were treated with IL-1 β for 6 h. Values are means \pm SEM (n=5). *, $p < 0.05$; †, $p < 0.01$. (B) Protein levels of SHH in ACM, determined using ELISA. Astrocytes were treated with IL-1 β for 24 h. Values are means \pm SEM (n=5). *, $p < 0.001$. doi:10.1371/journal.pone.01110024.g002

Waltham, MA, USA), and quantitated using a CS Analyzer 3.0 system (Atto, Tokyo, Japan). Assays were carried out in five independent trials.

Statistical analysis

Statistical significance was analyzed with one-way analysis of variance followed by post-hoc Tukey's test, using GraphPad Prism6 (GraphPad Software, La Jolla, CA, USA).

Results

IL-1 β suppressed the protective effect of astrocytes on BBB integrity

First, we confirmed the effects of IL-1 β and astrocytes on BBB integrity using MBEC4 monolayers as an *in vitro* BBB model. Astrocyte conditioned media (ACM) significantly decreased the permeability of BBB (Fig. 1). Treatment with IL-1 β alone

significantly increased the permeability of BBB, and conditioned media from IL-1 β -stimulated astrocytes lost the ability to increase BBB integrity (Fig. 1). These findings suggested that IL-1 β disrupts BBB integrity not only directly, but also indirectly via astrocyte dysfunction.

IL-1 β decreased astrocytic production of SHH

Next, we focused on SHH, a soluble factor released from astrocytes that plays an important role in BBB maintenance. Specifically, we investigated whether IL-1 β affects astrocytic SHH expression. Treatment with IL-1 β significantly decreased *Shh* mRNA levels in astrocytes in a dose-dependent manner (Fig. 2A). Similar results were obtained for SHH protein levels in ACM using specific ELISA (Fig. 2B).

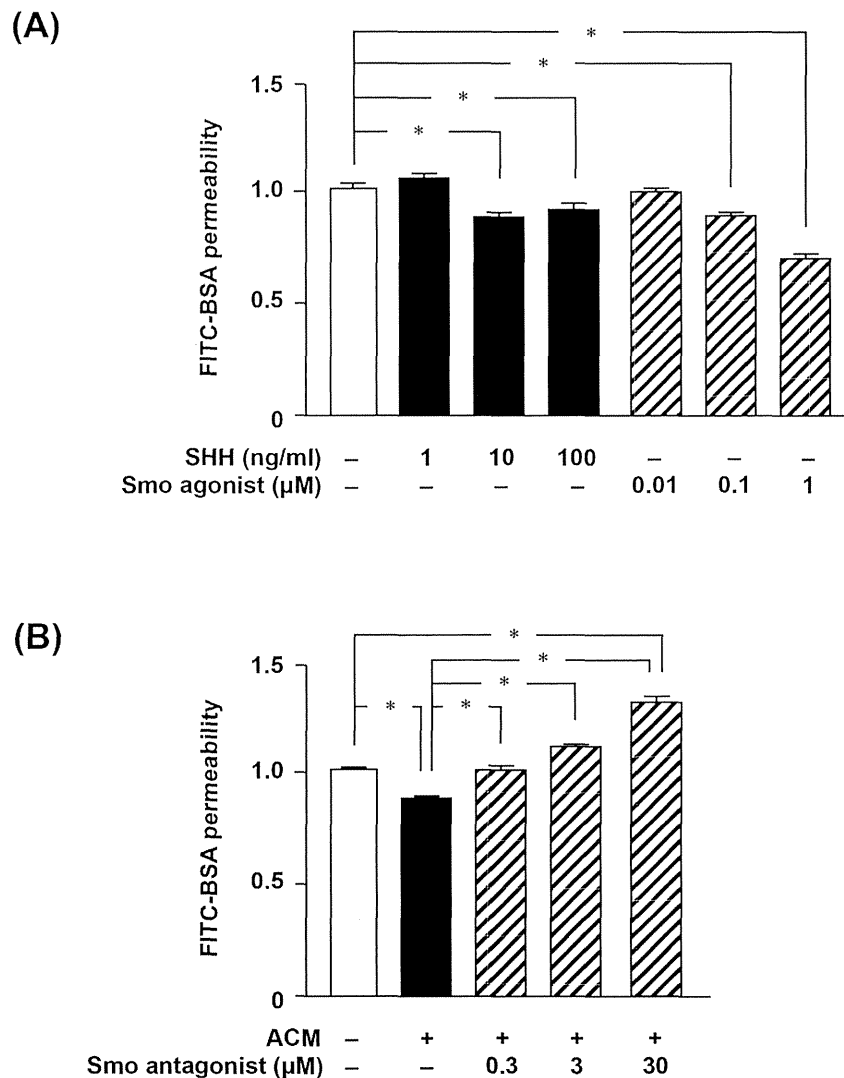


Figure 3. SHH signaling is critical for maintenance of BBB integrity. (A) MBEC4 cells were treated with SHH or the Smo agonist purmorphamine for 24 h. (B) MBEC4 cells were treated with ACM or the Smo antagonist cyclopamine for 24 h. FITC-BSA was loaded onto the luminal side of the insert for 1 h, and then the FITC-BSA levels on the abluminal side were analyzed. All quantitative data are expressed as means \pm SEM ($n=5$), normalized to the corresponding values from untreated cells. *, $p<0.001$. doi:10.1371/journal.pone.0110024.g003

SHH produced by astrocytes is critical for maintenance of BBB integrity by upregulating tight junction proteins

Next, we examined the effect of astrocytic SHH signaling on BBB function. SHH or the Smo agonist (i.e. a SHH signaling enhancer) purmorphamine significantly decreased BBB permeability (Fig. 3A). By contrast, the Smo antagonist (i.e. a SHH signaling inhibitor) cyclopamine abolished the astrocytic effect on the maintenance of BBB function (Fig. 3B). The expression levels of such TJ proteins as claudin-5, occludin, and ZO-1 were closely correlated with BBB integrity (Fig. 4A–C): levels of these proteins were highest when permeability was lowest. Activation of SHH signaling by ACM, SHH, or purmorphamine resulted in significant upregulation of these proteins, whereas the Smo antagonist cyclopamine ablated the astrocytic effect on their expression (Fig. 4A–C). These observations suggested that SHH produced by astrocytes plays a critical role in BBB integrity by upregulating expression of TJ proteins.

IL-1 β stimulated pro-inflammatory chemokine production in astrocytes

Finally, we assessed the effects of IL-1 β on the production of pro-inflammatory chemokines in astrocytes. Treatment with IL-1 β significantly increased the mRNA and protein expression levels of CXCL2, CCL2, and CCL20 in astrocytes (Fig. 5A and 5B). These data imply that IL-1 β also activates astrocytes to release these pro-inflammatory chemokines; induces migration of immune cells such as neutrophils, monocytes, macrophage, dendritic cells, and pathogenic T cells; and leads to further BBB disruption and neuroinflammation.

Discussion

IL-1 β is considered to be a critical factor for astrocyte activation in various neurologic disorders [26]. IL-1 β binds to its receptor, IL-1R, whose downstream signaling activates nuclear factor- κ B (NF- κ B), a key player in the immune and inflammatory response

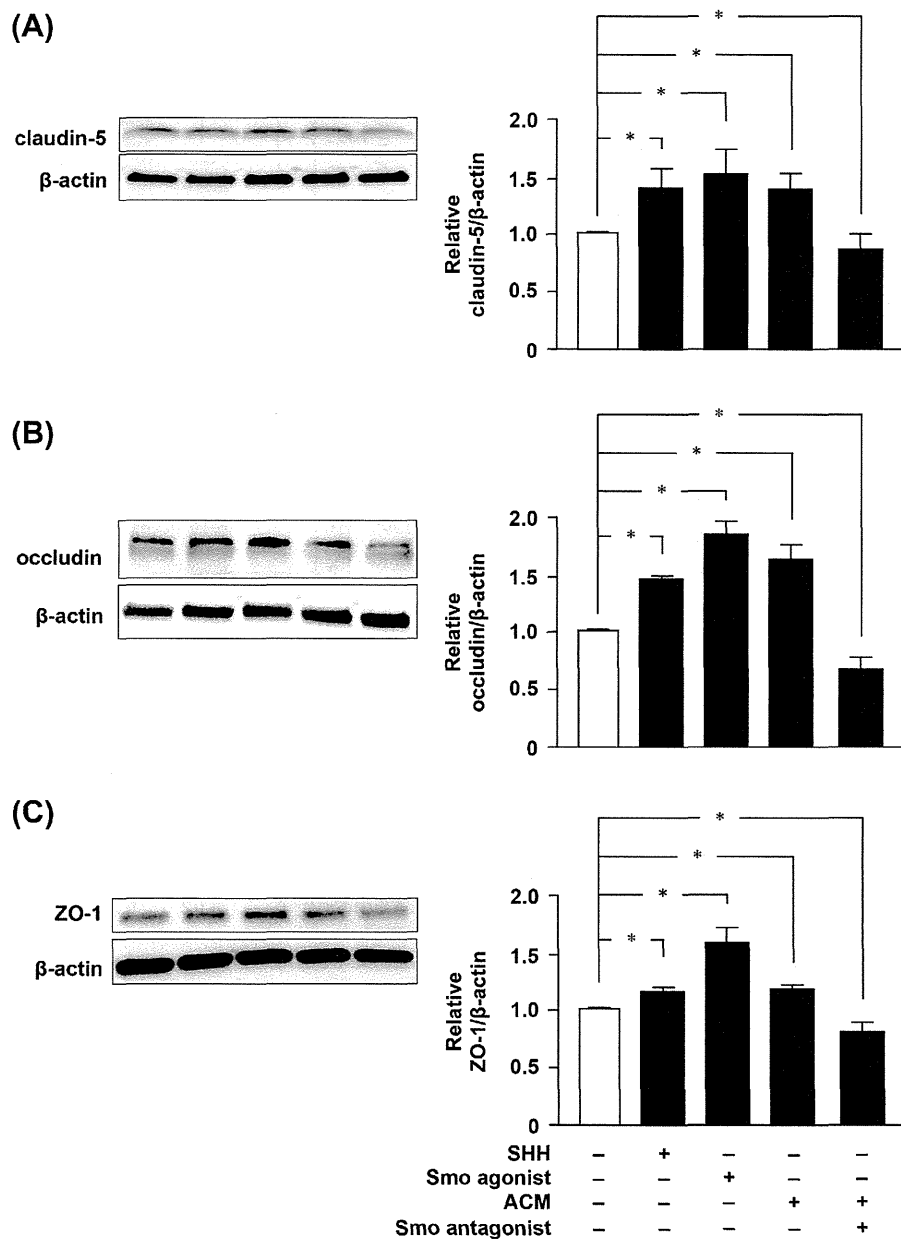


Figure 4. Astrocytic SHH signaling regulates expression of tight junction proteins in BBB. Western blotting of claudin-5 (A), occludin (B) and ZO-1 (C) in MBEC4 cells. Cells were treated for 24 h with ACM, SHH (100 ng/ml), the Smo agonist pumorphamine (1 μ M), or the Smo antagonist cyclopamine (30 μ M). All quantitative data are expressed as means \pm SEM (n = 5), normalized to the corresponding values from untreated cells. *, $p < 0.05$.

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in astrocytes [27,28]. NF- κ B promotes transcription of mediators of inflammation, such as pro-inflammatory cytokines/chemokines [29]. In addition, NF- κ B also increases neurotrophic factor production in astrocytes [30,31]. Therefore, IL-1 β plays two opposing roles in astrocytes. Microglia are the main source of IL-1 β in the CNS. A variety of stimuli, such as damage-associated molecular pattern molecules (DAMPs), amyloid β , and pro-inflammatory cytokines, trigger microglial IL-1 β production via an inflammasome-dependent mechanism [32,33]. Upregulation of IL-1 β is observed in a broad spectrum of neurological diseases, including infections, trauma, stroke, and epilepsy, as well as chronic neurologic diseases such as MS, Parkinson's disease,

amyotrophic lateral sclerosis, and Alzheimer's disease [32]; BBB disruption is associated with progression of these diseases [34]. IL-1 β increases BBB permeability by downregulating TJ proteins [17,18]. In addition, IL-1 β also induces astrocytes to release vascular endothelial growth factor, which increases BBB permeability [16]. Thus, IL-1 β induces BBB breakdown via both direct and indirect pathways.

Here, we propose another novel mechanism for IL-1 β -mediated BBB disruption. SHH is a critical activator of Smo-Gli-1 signaling which upregulates TJ proteins and enhances BBB integrity (Fig. 6A). A decrease in SHH allows Ptc-1 to suppress Smo-Gli-1 signaling. In the healthy state, astrocytes release SHH,

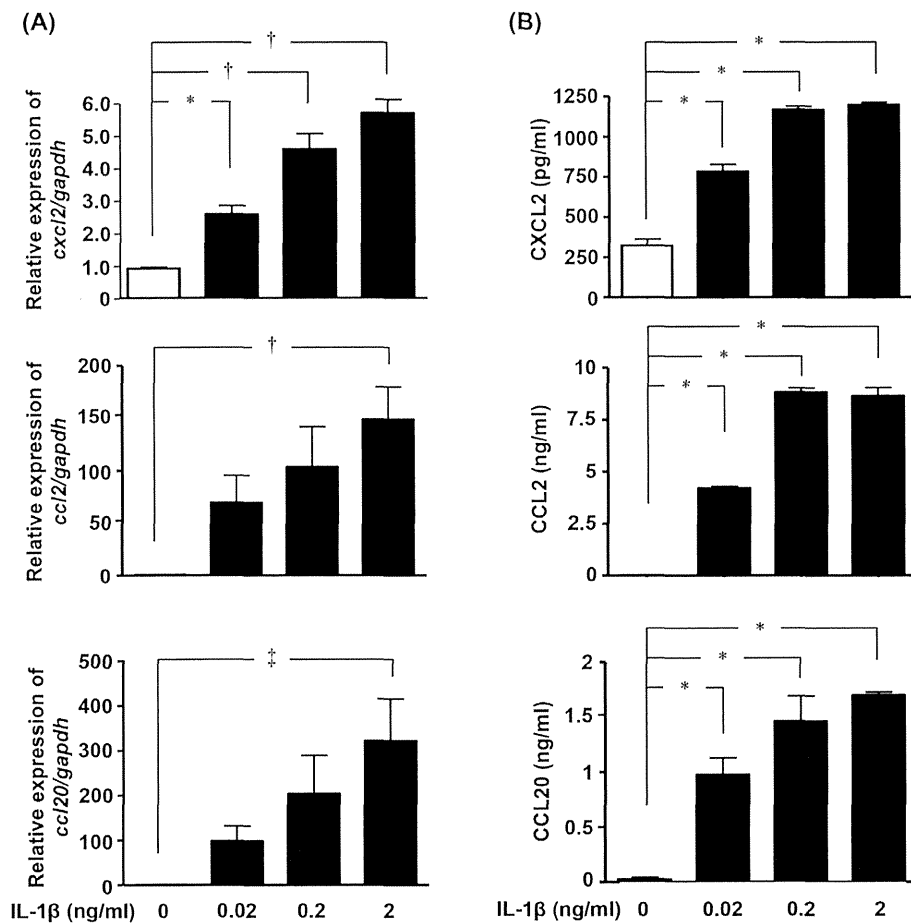


Figure 5. IL-1 β upregulates production of pro-inflammatory chemokines in astrocytes. (A) *Cxcl2*, *Ccl2*, and *Ccl20* mRNA levels in astrocytes, determined by qPCR. Astrocytes were treated with IL-1 β for 6 h. Values are means \pm SEM (n = 5). *, $p < 0.05$; †, $p < 0.01$. (B) Protein levels of CXCL2, CCL2, and CCL20 in astrocytes, determined by ELISA. Astrocytes were treated with IL-1 β for 24 h. Values are means \pm SEM (n = 5). *, $p < 0.001$. doi:10.1371/journal.pone.0110024.g005

which upregulates TJ proteins in endothelial cells and maintains BBB integrity (Fig. 6B, left). Once pathogenic stimuli activate microglia to release IL-1 β (Fig. 6B, right), it suppresses SHH production in astrocytes, downregulates TJ proteins in endothelial cells, and disrupts BBB integrity. Moreover, IL-1 β -stimulated astrocytes secrete the pro-inflammatory chemokines CXCL2, CCL2, and CCL20, which induce migration of immune cells such as neutrophils, monocytes, macrophage, dendritic cells, and pathogenic T cells. Infiltration of these cells exacerbates BBB disintegration and subsequent neuroinflammation.

In this study, the Smo antagonist cyclopamine decreased TJ protein expression levels and BBB integrity exceeding the physiological levels (Figs. 3 and 4). Previous reports suggested that unidentified endogenous ligands of Smo seem to activate this signaling although SHH is the main regulator of Smo-Gli signaling [35,36]. Our data also imply the presence of endogenous ligand(s) of Smo.

BBB disruption is a common pathologic feature of neurologic disorders such as stroke, MS, Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease. Therefore, restoration of BBB integrity has been recognized as a therapeutic target for treatment of these diseases [8,37]. In fact, both glucocorticoids and interferon β , both of which have been widely used for MS treatment, decrease BBB permeability [38,39]. Moreover, the

efficacy of the $\alpha 4$ -integrin antagonist natalizumab has also demonstrated the utility of BBB-targeting drugs in treating MS [40]. By contrast, excessive immunosuppression resulting from conventional therapies for MS sometimes causes progressive multifocal leukoencephalopathy [41]. Thus, from the perspective of adverse effects, restoration of TJ proteins represents a superior therapeutic approach. Inhibition of IL-1 β is a promising potential method for restoring BBB integrity [42]; however, a previous study indicated that simple blockade of IL-1 β runs the risk of increasing BBB disruption, because this cytokine also enhances the protective effects of astrocytes on the BBB [26]. Treatment with SHH may circumvent this dilemma, allowing reinforcement of BBB integrity without loss of the beneficial effects of IL-1 β .

During development, SHH signaling is primarily involved in CNS morphogenic events [43], whereas in adulthood, SHH participates in vascular proliferation, neurogenesis, and tissue repair in the CNS [44]. Dysregulation of SHH occurs in a variety of neurologic disorders; therefore, activation of the SHH signaling pathway, which would enhance neurogenesis and gliogenesis, has been proposed as a potential therapeutic approach for treatment of these diseases [45]. Downregulation of SHH has been observed in MS brains [46], and interferon- β treatment improves symptoms in a MS rodent model, concomitant with reduced BBB breakdown and elevated SHH expression [47]. Taken together, these

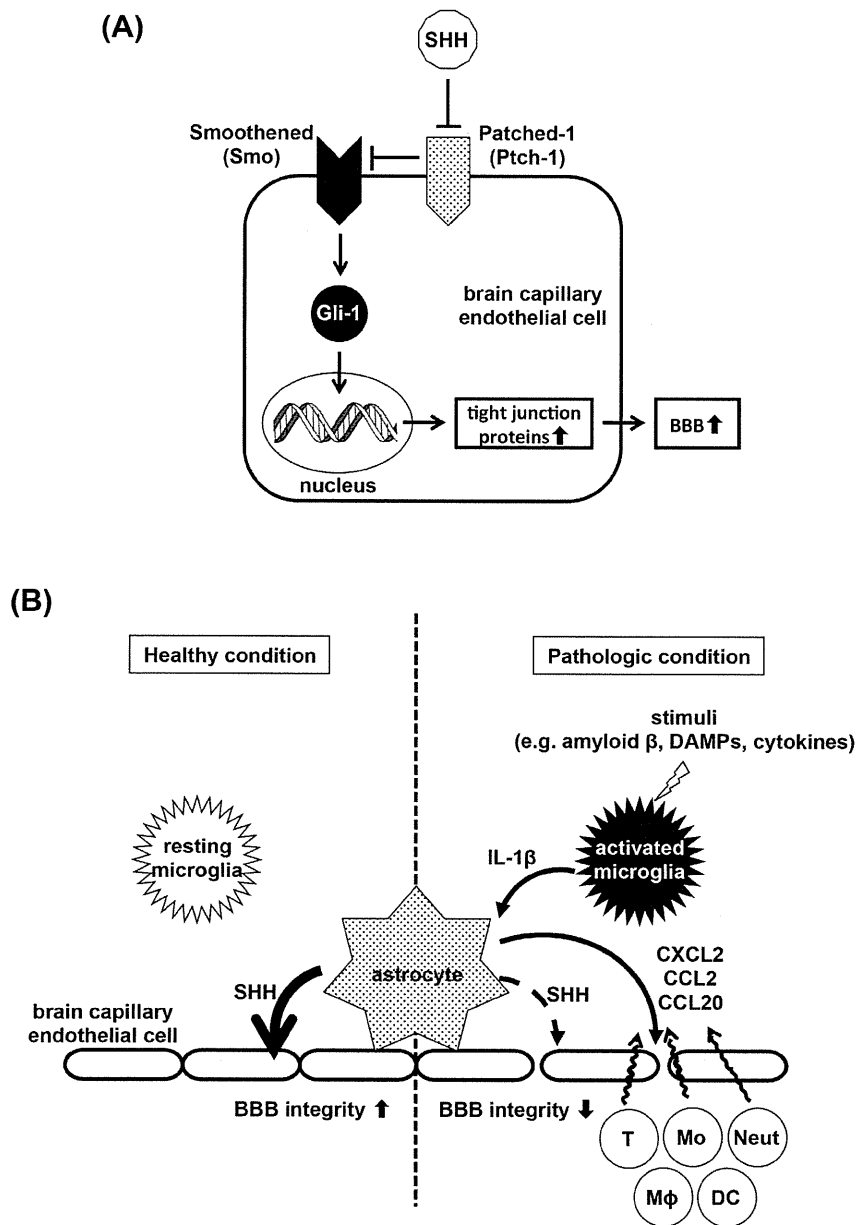


Figure 6. Model of the roles of the SHH and IL-1 β pathways in the BBB. (A) Model of SHH signaling pathway in brain capillary endothelial cells. Secreted SHH binds and inactivates its receptor Patched-1, which allowed Smoothed to activate the transcription factor Gli-1. Gli-1 upregulates tight junction proteins and enhances BBB integrity. (B) Model of BBB breakdown by IL-1 β . Under healthy conditions (left), astrocytes secrete SHH to upregulate tight junction proteins in endothelial cells and maintain BBB integrity. Under pathologic conditions (right), pathogenic stimuli such as amyloid β , DAMPs, or cytokines induce microglia to release IL-1 β . IL-1 β suppresses astrocytic SHH production, leading to downregulation of tight junction proteins in endothelial cells and disintegration of the BBB. IL-1 β also activates astrocytes to release pro-inflammatory chemokines such as CXCL2, CCL2, and CCL20. These chemokines induce migration of immune cells, thereby worsening BBB disruption and neuroinflammation. Neut, neutrophils; Mo, monocytes; M Φ , macrophage; DC, dendritic cells; T, T cells.
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observations suggest that SHH exerts a synergistic therapeutic effect by promoting CNS tissue repair while reinforcing the BBB.

This study reveals a novel mechanism for IL-1 β -mediated BBB disruption: downregulation of SHH expression in astrocytes. Our findings suggest that stimulation of astrocytic SHH production could promote restoration of BBB integrity, and may therefore be useful in treating a variety of neurologic disorders.

Author Contributions

Conceived and designed the experiments: HT YS AS. Performed the experiments: YW SJ YS. Analyzed the data: YW HT SY TM. Contributed reagents/materials/analysis tools: YC HH BP JK. Contributed to the writing of the manuscript: YW HT AS.

References

- Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. *Neurobiol Dis* 37: 13–25.
- Luissint AC, Artus C, Glacial F, Ganeshamoorthy K, Couraud PO (2012) Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS* 9: 23.
- Hayashi Y, Nomura M, Yamagishi S, Harada S, Yamashita J, et al. (1997) Induction of various blood-brain barrier properties in non-neural endothelial cells by close apposition to co-cultured astrocytes. *Glia* 19: 13–26.
- Janzer RC, Raff MC (1987) Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* 325: 253–257.
- Alvarez JL, Katayama T, Prat A (2013) Glial influence on the blood brain barrier. *Glia* 61: 1939–1958.
- Alvarez JL, Dodelet-Devillers A, Kebir H, Ifergan I, Fabre PJ, et al. (2011) The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Science* 334: 1727–1731.
- Osterlund T, Kogerman P (2006) Hedgehog signalling: how to get from Smo to Ci and Gli. *Trends Cell Biol* 16: 176–180.
- Carvey PM, Hendey B, Monahan AJ (2009) The blood-brain barrier in neurodegenerative disease: a rhetorical perspective. *J Neurochem* 111: 291–314.
- Erickson MA, Banks WA (2013) Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. *J Cereb Blood Flow Metab* 33: 1500–1513.
- Grammas P, Martinez J, Miller B (2011) Cerebral microvascular endothelium and the pathogenesis of neurodegenerative diseases. *Expert Rev Mol Med* 13: e19.
- Minagar A, Alexander JS (2003) Blood-brain barrier disruption in multiple sclerosis. *Mult Scler* 9: 540–549.
- Weiss N, Miller F, Cazaubon S, Couraud PO (2009) The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta* 1788: 842–857.
- Zlokovic BV (2008) The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57: 178–201.
- Kempermann G, Neumann H (2003) Neuroscience. Microglia: the enemy within? *Science* 302: 1689–1690.
- Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 8: 57–69.
- Argaw AT, Zhang Y, Snyder BJ, Zhao ML, Kopp N, et al. (2006) IL-1 β regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. *J Immunol* 177: 5574–5584.
- Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, et al. (2000) Interleukin-1 β -induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: a magnetic resonance study. *J Neurosci* 20: 8153–8159.
- Laflamme N, Lacroix S, Rivest S (1999) An essential role of interleukin-1 β in mediating NF- κ B activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. *J Neurosci* 19: 10923–10930.
- Liang J, Takeuchi H, Doi Y, Kawanokuchi J, Sonobe Y, et al. (2008) Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity. *Brain Res* 1210: 11–19.
- Suzumura A, Lavi E, Weiss SR, Silberberg DH (1986) Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. *Science* 232: 991–993.
- Tatsuta T, Naito M, Oh-hara T, Sugawara I, Tsuruo T (1992) Functional involvement of P-glycoprotein in blood-brain barrier. *J Biol Chem* 267: 20383–20391.
- Sonobe Y, Takeuchi H, Kataoka K, Li H, Jin S, et al. (2009) Interleukin-25 expressed by brain capillary endothelial cells maintains blood-brain barrier function in a protein kinase C ϵ -dependent manner. *J Biol Chem* 284: 31834–31842.
- Cheng Y, Takeuchi H, Sonobe Y, Jin S, Wang Y, et al. (2014) Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. *J Neuroimmunol* 269: 38–43.
- Wang J, Takeuchi H, Sonobe Y, Jin S, Mizuno T, et al. (2008) Inhibition of midkine alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population. *Proc Natl Acad Sci U S A* 105: 3915–3920.
- Doi Y, Takeuchi H, Horiuchi H, Hanyu T, Kawanokuchi J, et al. (2013) Fingolimod phosphate attenuates oligomeric amyloid β -induced neurotoxicity via increased brain-derived neurotrophic factor expression in neurons. *PLoS One* 8: e61988.
- Herx LM, Yong VW (2001) Interleukin-1 β is required for the early evolution of reactive astrogliosis following CNS lesion. *J Neuropathol Exp Neurol* 60: 961–971.
- Chakraborty S, Kaushik DK, Gupta M, Basu A (2010) Inflammation signaling at the heart of central nervous system pathology. *J Neurosci Res* 88: 1615–1631.
- Aronica E, Ravizza T, Zurolo E, Vezzani A (2012) Astrocyte immune responses in epilepsy. *Glia* 60: 1258–1268.
- Martinon F, Tschopp J (2005) NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* 26: 447–454.
- Friedman WJ, Thakur S, Seidman L, Rabson AB (1996) Regulation of nerve growth factor mRNA by interleukin-1 in rat hippocampal astrocytes is mediated by NF κ B. *J Biol Chem* 271: 31115–31120.
- Glazner GW, Camandola S, Mattson MP (2000) Nuclear factor- κ B mediates the cell survival-promoting action of activity-dependent neurotrophic factor peptide-9. *J Neurochem* 75: 101–108.
- Walsh JG, Muruve DA, Power C (2014) Inflammation in the CNS. *Nat Rev Neurosci* 15: 84–97.
- Parajuli B, Sonobe Y, Horiuchi H, Takeuchi H, Mizuno T, et al. (2013) Oligomeric amyloid β induces IL-1 β processing via production of ROS: implication in Alzheimer's disease. *Cell Death Dis* 4: e975.
- Muldoon LL, Alvarez JL, Begley DJ, Boado RJ, Del Zoppo GJ, et al. (2013) Immunologic privilege in the central nervous system and the blood-brain barrier. *J Cereb Blood Flow Metab* 33: 13–21.
- Nachtergaele S, Mydock LK, Krishnan K, Rammohan J, Schlesinger PH, et al. (2012) Oxysterols are allosteric activators of the oncoprotein Smoothened. *Nat Chem Biol* 8: 211–220.
- Kristiansen K (2004) Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol Ther* 103: 21–80.
- Rosenberg GA (2012) Neurological diseases in relation to the blood-brain barrier. *J Cereb Blood Flow Metab* 32: 1139–1151.
- Frequin ST, Barkhof F, Lamers KJ, Hommes OR (1992) The effects of high-dose methylprednisolone on gadolinium-enhanced magnetic resonance imaging and cerebrospinal fluid measurements in multiple sclerosis. *J Neuroimmunol* 40: 265–272.
- Kraus J, Oschmann P (2006) The impact of interferon- β treatment on the blood-brain barrier. *Drug Discov Today* 11: 755–762.
- Miller DH, Khan OA, Sheremata WA, Blumhardt LD, Rice GP, et al. (2003) A controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 348: 15–23.
- Yousry TA, Major EO, Ryschkewitsch C, Fahle G, Fischer S, et al. (2006) Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. *N Engl J Med* 354: 924–933.
- Martinez JA, Litwak M, Berman JW, Arezzo JC, Brosnan CF (1990) Pathophysiologic effect of interleukin-1 β in the rabbit retina. *Am J Pathol* 137: 1411–1423.
- Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, et al. (1996) Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* 383: 407–413.
- Araujo GL, Araujo JA, Schroeder T, Tort AB, Costa MR (2014) Sonic hedgehog signaling regulates mode of cell division of early cerebral cortex progenitors and increases astrogliogenesis. *Front Cell Neurosci* 8: 77.
- Traiffort E, Angot E, Ruat M (2010) Sonic Hedgehog signaling in the mammalian brain. *J Neurochem* 113: 576–590.
- Mastroradi FG, daCruz LA, Wang H, Boggs J, Moscarello MA (2003) The amount of sonic hedgehog in multiple sclerosis white matter is decreased and cleavage to the signaling peptide is deficient. *Mult Scler* 9: 362–371.
- Mastroradi FG, Min W, Wang H, Winer S, Dosch M, et al. (2004) Attenuation of experimental autoimmune encephalomyelitis and nonimmune demyelination by IFN- β plus vitamin B12: treatment to modify notch-1/sonic hedgehog balance. *J Immunol* 172: 6418–6426.



Gap junctions and hemichannels composed of connexins: potential therapeutic targets for neurodegenerative diseases

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Microglia are macrophage-like resident immune cells that contribute to the maintenance of homeostasis in the central nervous system (CNS). Abnormal activation of microglia can cause damage in the CNS, and accumulation of activated microglia is a characteristic pathological observation in neurologic conditions such as trauma, stroke, inflammation, epilepsy, and neurodegenerative diseases. Activated microglia secrete high levels of glutamate, which damages CNS cells and has been implicated as a major cause of neurodegeneration in these conditions. Glutamate-receptor blockers and microglia inhibitors (e.g., minocycline) have been examined as therapeutic candidates for several neurodegenerative diseases; however, these compounds exerted little therapeutic benefit because they either perturbed physiological glutamate signals or suppressed the actions of protective microglia. The ideal therapeutic approach would hamper the deleterious roles of activated microglia without diminishing their protective effects. We recently found that abnormally activated microglia secrete glutamate via gap-junction hemichannels on the cell surface. Moreover, administration of gap-junction inhibitors significantly suppressed excessive microglial glutamate release and improved disease symptoms in animal models of neurologic conditions such as stroke, multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease. Recent evidence also suggests that neuronal and glial communication via gap junctions amplifies neuroinflammation and neurodegeneration. Elucidation of the precise pathologic roles of gap junctions and hemichannels may lead to a novel therapeutic strategies that can slow and halt the progression of neurodegenerative diseases.

Keywords: glutamate, microglia, neuroinflammation, neurodegeneration, gap junction, hemichannel, connexin

INTRODUCTION

Microglia are macrophage-like immune cells that reside in the central nervous system (CNS), where they play multiple roles: presenting antigen to initiate immunological reactions, directing attack against non-self antigens, debris clearance, support of neuronal circuit development (Kreutzberg, 1996; Kempermann and Neumann, 2003; Block et al., 2007; Takeuchi, 2010; Boche et al., 2013), and so on. Microglia contribute to maintenance of CNS homeostasis, but abnormal activation of these cells often causes damage to surrounding cells and tissues. Microgliosis, the accumulation of activated microglia, is a characteristic pathological feature in many neurologic conditions such as trauma, stroke, inflammation, epilepsy, and neurodegenerative diseases (Cagnin et al., 2001; Eikelenboom et al., 2002; McGeer and McGeer, 2002; Nelson et al., 2002; Orr et al., 2002; Bruijn et al., 2004; Pavese et al., 2006). Activated microglia release massive amounts of glutamate, at much higher levels than astrocytes and neurons (mM vs. μ M), and destroy neural cells; these processes have been implicated as a major cause of neuronal damage in neurologic diseases (Piani et al., 1992; Barger and Basile, 2001; Schwartz et al., 2003; Ye et al., 2003; Kipnis

et al., 2004; Takeuchi et al., 2005, 2008b; Herman and Jahr, 2007; Liang et al., 2008; Yawata et al., 2008). Therefore, blockade of glutamate signaling and inhibition of microglial activation have been explored as therapeutic candidates for several neurodegenerative diseases. However, glutamate receptor blockers also perturb physiological glutamate signals and cause severe adverse side effects (Parsons et al., 2007). Tetracycline and two of its derivatives (doxycycline and minocycline) have been used as inhibitors of microglial activation, but these compounds exerted little therapeutic benefit, because activated microglia also exert neuroprotective effects such as production of neurotrophic factors and sequestration of neurotoxic substances (Zietlow et al., 1999; Kempermann and Neumann, 2003; Kipnis et al., 2004; Koenigsknecht and Landreth, 2004; Schwab and Schluesener, 2004). Thus, the optimal therapeutic strategy would inhibit the deleterious effects of activated microglia without diminishing their protective roles (Takeuchi, 2010). We recently found that neurotoxic activated microglia secrete glutamate through gap-junction hemichannels. Recent evidence also suggests that neuronal and glial communication by gap junctions amplifies neuroinflammation and neurodegeneration.

Therefore, elucidation of the pathologic roles of gap junctions and hemichannels may provide us with new therapeutic strategies against many neurologic diseases.

MICROGLIA AS THE “ENEMY WITHIN”

Microglia, which originate from bone marrow-derived myeloid cells, account for approximately 10% of cells in the human CNS (Del Rio-Hortega, 1932). Microglia are predominantly observed in gray matter, especially in the olfactory bulb, hippocampus, basal ganglia, and substantia nigra (Lawson et al., 1990). Under healthy physiological conditions, microglia persist in a quiescent state with ramified morphology (resting microglia) and survey the environment of the CNS (Davalos et al., 2005; Nimmerjahn et al., 2005). Under pathological conditions, microglia dramatically change their morphology and adopt an amoeboid appearance in the activated state. Activated microglia express surface molecules such as Fc receptor, CD11b, CD11c, CD14, major histocompatibility complex (MHC) molecules, Toll-like receptors (TLRs), scavenger receptors, and cytokine/chemokine receptors, and they can act as both antigen-presenting cells and immunological effector cells (Suzumura et al., 1987; Rock et al., 2004). In addition to innate immunity, activated microglia also play other beneficial roles, such as neuroprotection mediated by release of neurotrophic factors (Zietlow et al., 1999; Bessis et al., 2007; Liang et al., 2010), maintenance of CNS homeostasis by clearance of cellular debris and toxic substances (Upende and Naegle, 1999; Marin-Teva et al., 2004; Iribarren et al., 2005; Simard et al., 2006; Richard et al., 2008), and guidance of stem-cell migration in neuronal repair and neurogenesis (Aarum et al., 2003; Ziv et al., 2006a,b).

TLRs and scavenger receptors may contribute to diminishing neurotoxicity by sequestering neurotoxic substances such as amyloid β (El Khoury et al., 1996; Coraci et al., 2002; Bamberger et al., 2003; Liu et al., 2005); however, signals downstream of these receptors also enhance microglial neurotoxic effects by producing neurotoxic factors such as cytokines/chemokines, nucleic acids, excitatory amino acids, reactive oxygen species (ROS), and proteases (Kempermann and Neumann, 2003; Takeuchi et al., 2005, 2006; Kawanokuchi et al., 2006; Block et al., 2007). In fact, expression levels of TLRs and scavenger receptors are upregulated in a variety of neurologic diseases (Akiyama and McGeer, 1990; Grewal et al., 1997; El Khoury et al., 1998; Bsibsi et al., 2002; Cho et al., 2005; Carpentier et al., 2008). Therefore, whether activated microglia exert a neurotoxic or a neuroprotective effect may depend on their environment, the spatiotemporal distribution of the microglia themselves, and the type and magnitude of stimuli (Jimenez et al., 2008; Wu et al., 2008; Nakanishi and Wu, 2009; Sawada, 2009). A recently proposed hypothesis suggests, by analogy to macrophage activation, that activated microglia comprise two subpopulations, the neurotoxic (M1) and neuroprotective (M2) species (Mantovani et al., 2002; Henkel et al., 2009; Boche et al., 2013); however, this hypothesis is still open to debate. At least in pathological conditions, deleterious microglial activation is probably involved in the progression of various neurological disorders (Yrjanheikki et al., 1998; Wu et al., 2002; Zhu et al., 2002; Stirling et al., 2004; Boillee et al., 2006; Seabrook et al., 2006). Thus, elucidating the precise mechanism of microglial

neurotoxicity is a necessary step toward development of effective therapeutic strategies against neurologic diseases.

GLUTAMATE AS A MAJOR NEUROTOXIC FACTOR FROM MICROGLIA

Glutamate is the most potently neurotoxic factor released from activated microglia. Excessive glutamate induces severe neuronal damage via excitotoxicity (Piani et al., 1992; Barger and Basile, 2001; Takeuchi et al., 2005, 2006). A common misconception is that inflammatory cytokines produced by activated microglia directly induce neuronal damage. In fact, these cytokines have little direct neurotoxic effect (Takeuchi et al., 2006; Takeuchi, 2010). Although tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) are considered to be the most deleterious inflammatory cytokines produced by activated microglia, these cytokines have only weak direct neurotoxic effects because they also enhance neuroprotective cascades involving mitogen-activated protein kinase (MAPK) and expression of nuclear factor κ B (NF- κ B) (Ghezzi and Mennini, 2001; Kamata et al., 2005). In general, inflammatory cytokines induce neurotoxicity indirectly by stimulating microglia in an autocrine/paracrine manner. These stimuli induce microglia to release high levels of glutamate, resulting in neuronal damage via excitotoxicity. Moreover, a recent paper showed that activated microglial glutamate suppresses astrocytic glutamate transporters, which play a pivotal role in maintenance of the physiological extracellular glutamate level (Takaki et al., 2012); this suppression probably worsens excitotoxic neuronal damage. Although microglia also express glutamate transporters, they seem much less effective at maintaining extracellular glutamate homeostasis than astrocytic glutamate transporters (Liang et al., 2008).

One of the earliest pathologic features of excitotoxicity is formation of neuritic beading, i.e., focal bead-like swelling in dendrites and axons (Takeuchi et al., 2005; Mizuno et al., 2008). Neuritic beading is a common neuropathological hallmark of many neurologic conditions such as ischemia, epilepsy, mechanical pressure, brain tumor, aging, neuroinflammatory diseases, and neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) (Delisle and Carpenter, 1984; Hori and Carpenter, 1994; Takahashi et al., 1997; Trapp et al., 1998; Dickson et al., 1999; Mattila et al., 1999; Swann et al., 2000; Goel et al., 2003; Pavlidis et al., 2003; Saito et al., 2003; Dutta and Trapp, 2007). Recent studies elucidated the detailed role of microglial glutamate in formation of neuritic beading and subsequent neuronal death. Glutamate produced by activated microglia activates neuronal N-methyl-D-aspartate (NMDA) receptor signaling, which promotes Ca^{2+} influx and activates Ca^{2+} /calmodulin-dependent protein kinase (CaMK). CaMK activates neuronal nitric oxide synthase (nNOS) and increases the intracellular concentration of nitric oxide (NO). NO in turn inhibits mitochondrial respiratory chain complex IV, resulting in a rapid reduction in intracellular ATP levels. Ultimately, the loss of intracellular energy pools suppresses dendritic and axonal transport, leading to bead-like accumulation of cytoskeletal and motor proteins along neurites and the formation of neuritic beading. Thus, a low-energy state results in neuronal

dysfunction. Persistence of this neuronal dysfunction eventually causes neuronal death [i.e., excitotoxic neuronal death or non-cell-autonomous neuronal death (Lobsiger and Cleveland, 2007)].

Recent studies have revealed the precise mechanism of glutamate production by activated microglia (Takeuchi et al., 2005, 2006) (**Figure 1**). Two pathways are involved in cellular glutamate synthesis (Newsholme and Newsholme, 1989; Newsholme and Calder, 1997; Yudkoff, 1997; Nissim, 1999). One of these pathways is mediated by glutamate dehydrogenase, which converts α -ketoglutarate to glutamate. Most cells use this pathway to maintain cellular homeostasis of glutamate levels. The other pathway is mediated by glutaminase, which produces glutamate from extracellular glutamine brought into the cell via glutamine transporters. Resting microglia maintain their physiological glutamate level via the glutamate dehydrogenase pathway, as in other cell types, and secrete very little glutamate into the extracellular space (**Figure 1**). By contrast, activated microglia produce excessive amounts of glutamate as a result of upregulation of glutaminase, but not glutamate dehydrogenase. Subsequently, activated microglia release massive amounts of glutamate via gap-junction hemichannels. Inflammatory cytokines such as TNF- α and IFN- γ enhance not only glutaminase expression level but also cell-surface localization of hemichannels in microglia (Eugenin et al., 2001; Takeuchi et al., 2006). These two phenomena may act synergistically to release excess glutamate, leading to excitotoxic neuronal damage (**Figure 1**). Moreover, the extracellular glutamine level is critical for microglial glutamate production (Takeuchi et al., 2006). In the CNS, glutamine from astrocytes is

essential for glutamate production in neurons (Tsacopoulos and Magistretti, 1996), suggesting that it also plays an important role in microglial glutamate production.

GAP JUNCTIONS IN CNS CELLS

Gap junctions contribute to formation of intercellular channels that directly connect the cytoplasmic compartments of neighboring cells (Yeager and Harris, 2007). These channels pass various small molecules (~ 1000 Da) and ions, although the charges and shapes of these molecules may affect the rate of transfer through gap junctions (Goldberg et al., 2004). Each gap junction is composed of a pair of hemichannels docked in a head-to-head configuration. Hemichannels are organized as hexagonal cylinders with central pores, and each hemichannel consists of a hexameric cluster of protein subunits called connexins (in vertebrates) or innexins (in invertebrates). Connexins are encoded by a conserved family of genes with at least 21 members in mammals. There are 21 connexin genes in the human genome and 20 connexin genes in the mouse genome; 19 of these proteins have orthologs in both humans and mice (Willecke et al., 2002; Laird, 2006). The connexin isoforms structurally interact in multiple ways. Homomeric hemichannels consist of a single connexin isoform, whereas heteromeric hemichannels contain two or more different connexin isoforms. Likewise, a homotypic gap junction channel is composed of two identical hemichannels, whereas a heterotypic gap junction channel contains two different hemichannels. Thus, the compositions of gap junctions can be classified into four types: homomeric and homotypic; heteromeric and homotypic; homomeric and heterotypic; and heteromeric and heterotypic

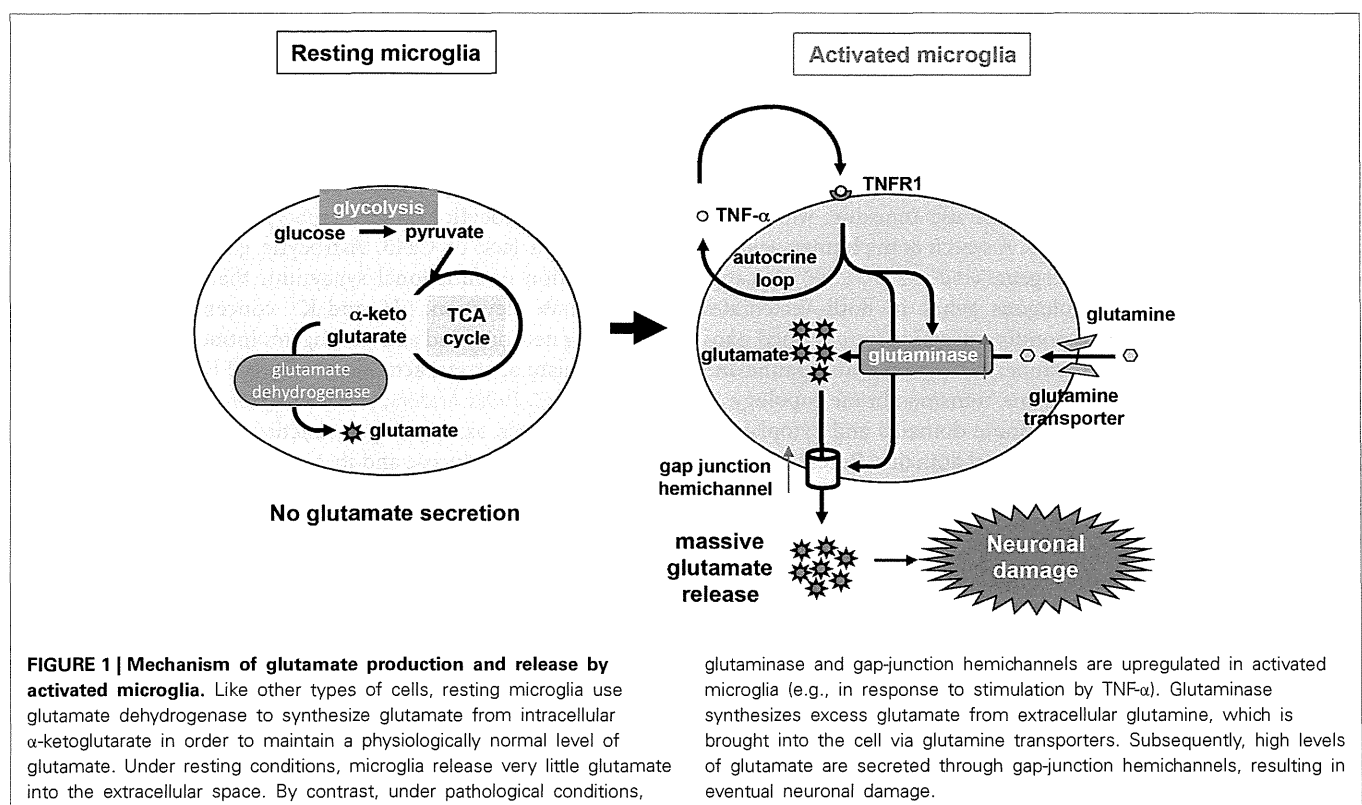
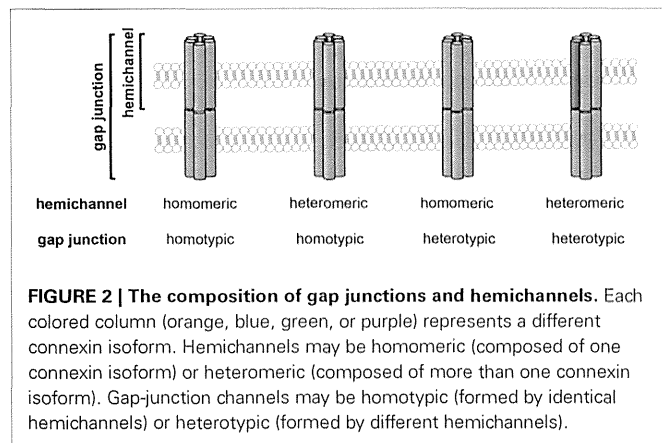


FIGURE 1 | Mechanism of glutamate production and release by activated microglia. Like other types of cells, resting microglia use glutamate dehydrogenase to synthesize glutamate from intracellular α -ketoglutarate in order to maintain a physiologically normal level of glutamate. Under resting conditions, microglia release very little glutamate into the extracellular space. By contrast, under pathological conditions,

glutaminase and gap-junction hemichannels are upregulated in activated microglia (e.g., in response to stimulation by TNF- α). Glutaminase synthesizes excess glutamate from extracellular glutamine, which is brought into the cell via glutamine transporters. Subsequently, high levels of glutamate are secreted through gap-junction hemichannels, resulting in eventual neuronal damage.



(Figure 2). This heterogeneity of connexin configurations confers complexity to the gap junction/hemichannel system.

Gap junctions allow direct intracellular propagation of second messengers (e.g., Ca^{2+} , IP_3 , cAMP, and cGMP), metabolites (e.g., glutamate, glucose, and glutathione), and nucleotides (e.g., ATP, ADP, and RNA) between adjacent cells (Goldberg et al., 1999, 2002; Harris, 2001, 2007; Saez et al., 2003; Valiunas et al., 2005; Laird, 2006). Moreover, recent studies revealed that uncoupled “free” hemichannels facilitate two-way transfer of molecules between the cytosol and extracellular milieu (De Vuyst et al., 2007; Retamal et al., 2007; Laird, 2010). Intracellular communication via gap junctions and hemichannels is regulated by such mechanisms as channel gating via chemicals, pH, and voltage, as well as by changes in connexin transcription, translation, post-translational phosphorylation and ubiquitination, membrane insertion, and hemichannel internalization and degradation (Laird, 2006; Leithe and Rivedal, 2007; Solan and Lampe, 2009). The time courses of these changes range from milliseconds to hours and are influenced by the environmental conditions in cells and tissues.

Whereas vertebrate cells use connexins to form gap junctions and hemichannels, invertebrate cells use innexins, which lack sequence homology to connexins. A search of the human genome identified three innexin-related genes (Barbe et al., 2006). Because of the occurrence of homologous genes in both vertebrates and invertebrates, the corresponding proteins were termed pannexins: pannexin1 (Pannx1), pannexin2 (Pannx2), and pannexin3 (Pannx3). Pannexins have the same transmembrane topology as connexins, with four transmembrane domains and cytoplasmic amino-terminal and carboxyl-terminal domains. Recent evidence indicates that pannexins also form uncoupled hemichannels in mammalian cells; however, it is not clear whether they can form functional gap junctions (Dahl and Locovei, 2006).

Tissues have characteristic connexin expression profiles, and neural cells in the CNS express multiple connexins (Dermietzel et al., 1989, 2000; Bittman and Loturco, 1999; Chang et al., 1999; Nagy and Rash, 2000; Eugenin et al., 2001; Rash et al., 2001; Teubner et al., 2001; Altevogt et al., 2002; Parenti et al., 2002; Rouach et al., 2002; Odermatt et al., 2003; Takeuchi et al., 2006). All neurons express Cx36 and Cx45, whereas other neural connexins are expressed with more specific

spatiotemporal profiles (Sohl et al., 2005). Electrical coupling between neurons has been implicated in neuronal synchronization in the CNS (Christie et al., 1989; Bouskila and Dudek, 1993; Wong et al., 1995). Neuronal gap junctions composed of Cx36 and Cx45 are thought to be homomeric and homotypic (Al-Ubaidi et al., 2000; Teubner et al., 2001), and these junctions are important for formation of electrical synapses (Deans et al., 2001; Hormuzdi et al., 2001). Rodent knockout models have shown that other connexins can compensate for the functions of Cx36 and Cx45, despite differences in conformation or permeability (Frank et al., 2010; Zlomuzica et al., 2010). Furthermore, there is accumulating evidence that gap-junction coupling plays a pivotal role in neuronal differentiation. Mice lacking Cx43 exhibit neonatal death and abnormal migration in the neural crest and neocortex (Lo et al., 1999; Xu et al., 2001; Fushiki et al., 2003). Blockade of gap junctions also hampers retinoic acid-induced neuronal differentiation of NT2 and P19 cells (Bani-Yaghoob et al., 1999a,b). Moreover, Cx36-containing gap junctions are important in neuronal remodeling and short-term spatial memory in some mature organisms (Allen et al., 2011; Hartfield et al., 2011). In contrast to neuron–neuron coupling, for which the evidence is convincing, the existence of functional neuron–glia coupling in the CNS is still a matter of debate (Nadarajah et al., 1996; Alvarez-Maubecin et al., 2000; Rash et al., 2001, 2007).

Astrocytes, the main CNS cells coupled via gap junctions, primarily express Cx43 and Cx30 (Dermietzel et al., 1991; Nagy and Rash, 2000). Consistent with this, Cx43/Cx30 double-knockout mice exhibit minimal gap-junction communication between astrocytes (Wallraff et al., 2006; Rouach et al., 2008), suggesting that functional astrocytic gap junctions are composed predominantly of these two connexins. Cx43-deficient astrocytes exhibit reduced gap-junction coupling, although they express other connexin subtypes including Cx30, Cx26, Cx40, Cx45, and Cx46 (Naus et al., 1997; Scemes et al., 1998; Dermietzel et al., 2000). Although Cx30 has been detected exclusively in astrocytes, Cx30 knockout mice develop only mild abnormalities, including hearing loss due to cochlear degeneration (Teubner et al., 2003). Thus, other astrocytic connexin subtypes do not seem to compensate for a lack of Cx43. Astrocytic gap junctions facilitate the formation of functional syncytium that buffers extracellular glutamate elevation, pH, and K^+ concentrations associated with firing neurons, and also propagates intracellular Ca^{2+} waves that modulate neuronal activities (Walz and Hertz, 1983; Jefferys, 1995; Charles, 1998; Anderson and Swanson, 2000; Ransom et al., 2003). Moreover, astrocytic gap-junction communication facilitates trafficking of glucose and its metabolites, thereby mediating interactions between cerebral vascular endothelium and neurons (Giaume et al., 1997; Goldberg et al., 1999; Tabernero et al., 2006). Thus, astrocytic gap junctions play pivotal roles in modulating neuronal activities and maintaining CNS homeostasis. Astrocyte–astrocyte coupling can be achieved by any of the allowed combinations of homomeric or heteromeric hemichannels in homotypic or heterotypic configurations. Cx30 and Cx26 form both heteromeric and heterotypic channels (Nagy et al., 2003; Altevogt and Paul, 2004), whereas Cx43 forms homomeric and homotypic channels (Orthmann-Murphy et al., 2007). A previous report demonstrated that gap-junction coupling in astrocytes

results in two distinct subpopulations of cells. Astrocytes expressing glutamate transporters are extensively coupled to each other, whereas astrocytes expressing glutamate receptors are not coupled to other astrocytes (Wallraff et al., 2004), suggesting that these cells play a role in buffering extracellular glutamate (Anderson and Swanson, 2000).

Oligodendrocytes primarily express Cx29, Cx32, and Cx47 (Dermietzel et al., 1989; Altevogt et al., 2002; Odermatt et al., 2003). Oligodendrocytic gap junctions facilitate the trafficking of ions and nutrients from somas to myelin layers (Paul, 1995). Mice lacking Cx32 exhibit reduced myelin volume, enhanced excitability in the CNS, and progressive peripheral neuropathies (Anzini et al., 1997; Sutor et al., 2000). Cx32/Cx47 double-knockout mice develop abnormal movements and seizures associated with vacuolated myelin and axonal degeneration in the CNS, whereas Cx47-deficient mice exhibit only minimal CNS abnormalities (Menichella et al., 2003). Cx32 and Cx47 in oligodendrocytes are essential for spatial buffering of K⁺ in response to neuronal activity; failure of this function leads to myelin swelling and subsequent axonal degeneration (Menichella et al., 2006). Oligodendrocyte–oligodendrocyte coupling is mediated by gap junctions in homotypic configurations with homomeric or heteromeric hemichannels containing Cx32 or Cx47 (Orthmann-Murphy et al., 2007). Furthermore, oligodendrocytes also couple with astrocytes. Astrocyte–oligodendrocyte coupling may include heterotypic configurations of Cx43–Cx47, Cx30–Cx32, or Cx26–Cx32 (Nagy et al., 2003; Altevogt and Paul, 2004; Orthmann-Murphy et al., 2007). In addition to astrocyte–astrocyte coupling, astrocyte–oligodendrocyte coupling is important in the glial syncytium to facilitate the propagation of Ca²⁺ waves and the buffering of extracellular K⁺ and neurotransmitters such as glutamate (Walz and Hertz, 1983; Jefferys, 1995; Charles, 1998; Anderson and Swanson, 2000; Ransom et al., 2003).

Microglia express Cx32, Cx36, and Cx43 (Eugenin et al., 2001; Parenti et al., 2002; Garg et al., 2005; Takeuchi et al., 2006; Kielian, 2008; Talaveron et al., 2014), but form few functional gap junctions under resting conditions. The expression of connexins rises in activated microglia, although it remains unclear whether upregulated expression of connexins leads to enhanced formation of functional gap junctions with microglia and other CNS cells (Eugenin et al., 2001; Garg et al., 2005; Kielian, 2008; Takeuchi, 2010; Wasseff and Scherer, 2014). Recent evidence demonstrates that uncoupled microglial hemichannels play important roles in bidirectional trafficking of small molecules between the cytoplasm and extracellular space (Takeuchi et al., 2011; Eugenin et al., 2012).

The evidence described above might give the false impression that CNS cells express only a narrow range of combinations of homomeric hemichannels and gap junctions. However, the precise configuration of these hemichannels [i.e., homomeric and homotypic; heteromeric and homotypic; homomeric and heterotypic; and heteromeric and heterotypic (Figure 2)] has yet to be elucidated. In addition, our recent reverse transcription–PCR analysis using mouse primary cultures indicated that gap junctions/hemichannels in neurons and glial cells may consist of a wider range of combinations of connexins than expected (Table 1) (Takeuchi et al., 2014): neurons predominantly express

Table 1 | mRNA expression levels of mouse connexin in CNS cells.

Mouse		mRNA expression level			
Protein	Gene	Neuron	Microglia	Astrocyte	Oligodendrocyte
Cx43	<i>Gja1</i>	++	±	+++	+
Cx46	<i>Gja3</i>	+	+++	+	++
Cx37	<i>Gja4</i>	+	+++	++	+++
Cx40	<i>Gja5</i>	+	+++	+	+++
Cx33	<i>Gja6</i>	+	++	+	++
Cx50	<i>Gja8</i>	+++	+	±	+
Cx57	<i>Gja10</i>	±	+++	+++	+++
Cx32	<i>Gjb1</i>	+	+++	+	+++
Cx26	<i>Gjb2</i>	+	+	+++	+++
Cx31	<i>Gjb3</i>	++	++	++	++
Cx30.3	<i>Gjb4</i>	++	++	++	++
Cx31.1	<i>Gjb5</i>	+	+	+	+++
Cx30	<i>Gjb6</i>	±	+	+	+++
Cx45	<i>Gjc1</i>	–	+	+++	±
Cx47	<i>Gjc2</i>	±	+++	+	+
Cx29	<i>Gjc3</i>	++	+	±	++
Cx36	<i>Gjd2</i>	+++	++	–	++
Cx30.2	<i>Gjd3</i>	+	++	++	+++
Cx39	<i>Gjd4</i>	±	++	++	+++
Cx23	<i>Gje1</i>	±	++	++	+++

–, none; ±, slight; +, low; ++, moderate; +++, high.

Cx43, Cx50, Cx31, Cx30.3, Cx29, and Cx36; microglia predominantly express Cx46, Cx37, Cx40, Cx33, Cx57, Cx32, Cx31, Cx30.3, Cx47, Cx36, Cx30.2, Cx39, and Cx23; astrocytes predominantly express Cx43, Cx37, Cx57, Cx26, Cx31, Cx30.3, Cx45, Cx30.2, Cx39, and Cx23; and oligodendrocytes predominantly express Cx46, Cx37, Cx40, Cx33, Cx57, Cx32, Cx26, Cx31, Cx30.3, Cx31.1, Cx30, Cx29, Cx36, Cx30.2, Cx39, and Cx23. These findings imply that connexin expression profiles in the CNS are dynamic, both in healthy and pathological states.

GAP JUNCTIONS COMPOSED OF CONNEXINS AS A NOVEL THERAPEUTIC TARGET FOR NEUROLOGIC DISEASES

As mentioned above, glial gap junctions play an important role in maintenance of homeostasis in the CNS under the physiological conditions. These structures, however, also contribute to the initiation and propagation of pathologic conditions (Orellana et al., 2009). Stroke and trauma provide examples that illustrate this mechanism. Ischemia or contusion leads to a rapid decrease in intracellular oxygen levels and subsequent reduction in ATP synthesis, resulting in eventual cell death (Kalogeris et al., 2012). Injured cells contain toxic ions and molecules at high concentrations (e.g., Ca²⁺, K⁺, ROS, and NO). These toxic molecules are propagated from injured cells to healthier cells through gap junctions. Ischemic conditions also induce uncoupled hemichannels to open, leading to paracrine transfer of toxic molecules (Thompson et al., 2006; De Vuyst et al., 2007). These waves of death signals activate astrocytes and microglia, inducing the release of toxic molecules including glutamate, ROS, NO, and pro-inflammatory cytokines and chemokines. This vicious

amplification spiral of signaling could worsen neuroinflammation by recruiting leukocytes and increasing the lesion area (Orellana et al., 2009) (Figure 3). Moreover, gap junction and hemichannel blockers have exerted therapeutic effects in experimental models of stroke and spinal cord injury (Rawanduzy et al., 1997; Frantseva et al., 2002; De Pina-Benabou et al., 2005; Takeuchi et al., 2008a; Tamura et al., 2011; Huang et al., 2012; Umebayashi et al., 2014).

Abnormal expression of glial connexins has been observed in the inflamed lesions in multiple sclerosis (MS) and an animal model of this disease, experimental autoimmune encephalomyelitis (EAE). In particular, downregulation of oligodendrocytic Cx32 and Cx47 and astrocytic Cx43 have been observed in the active lesions of MS patients and EAE mice (Brand-Schieber et al., 2005; Eugenin et al., 2012; Markoullis et al., 2012). Expression levels of Cx47 and Cx32 were upregulated during remyelination, but downregulated in the relapsing phase, and Cx32 deletion resulted in exacerbates symptoms in EAE, specifically increased demyelination and axonal loss (Markoullis et al., 2012). Whereas mice lacking astrocytic expression of Cx43/Cx30

exhibited white-matter vacuolation and hypomyelination, the severity of EAE in these animals was similar to that in wild-type mice (Lutz et al., 2012). Therefore, oligodendrocytic expression levels of Cx32 and Cx47 appear to be associated with the degree of damage and remyelination, whereas astrocytic expression levels of Cx43 do not. However, recent studies showed that a loss of Cx43 in astrocytes precedes demyelination in the MS-related disorders neuromyelitis optica and Balo’s disease (Matsushita et al., 2011; Masaki et al., 2012), suggesting that the temporal expressional pattern of astrocytic Cx43 plays a significant role in the disease process.

Accumulating evidence has also implicated neuroinflammation, including gliosis by activated astrocytes and microglia, in the pathogenesis of such neurodegenerative diseases as HIV encephalopathy, AD, PD, and ALS (Glass et al., 2010; Valcour et al., 2011). Microglial activation followed by astrocytic activation is the earliest pathologic feature in the pre-symptomatic phases of these diseases. Our recent studies have shown that activated microglia release excess glutamate through Cx32 hemichannels, resulting in excitotoxic neuronal death (Takeuchi et al., 2006,

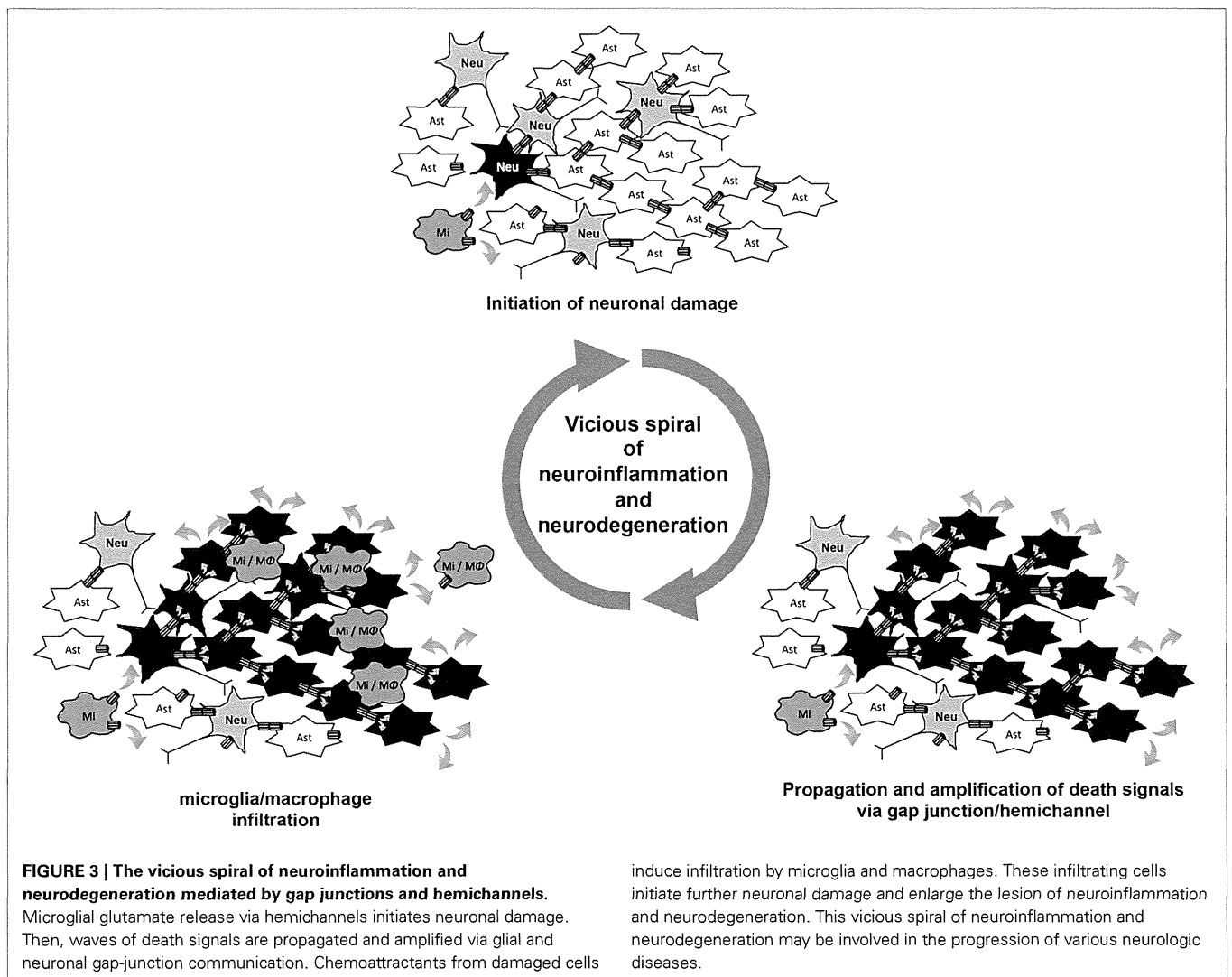


FIGURE 3 | The vicious spiral of neuroinflammation and neurodegeneration mediated by gap junctions and hemichannels. Microglial glutamate release via hemichannels initiates neuronal damage. Then, waves of death signals are propagated and amplified via glial and neuronal gap-junction communication. Chemoattractants from damaged cells

induce infiltration by microglia and macrophages. These infiltrating cells initiate further neuronal damage and enlarge the lesion of neuroinflammation and neurodegeneration. This vicious spiral of neuroinflammation and neurodegeneration may be involved in the progression of various neurologic diseases.

2008a; Yawata et al., 2008). Furthermore, microglia-derived glutamate and pro-inflammatory cytokines induce dysfunction of gap junctions and hemichannels in astrocytes (Kielian, 2008), thereby potentially disrupting CNS homeostasis. On the other hand, reactive astrocytes neighboring amyloid β (A β) plaques in the brains of AD patients expressed elevated levels of Cx43 and Cx30 (Koulakoff et al., 2012). A β peptide induces the release of glutamate and ATP via uncoupled hemichannels in microglia and astrocytes, leading to neuronal death (Orellana et al., 2011). Corroborating this observation, blockade of gap junctions/hemichannels improved memory impairment in a mouse model of AD (Takeuchi et al., 2011). Recent studies also revealed that astrocytic gap junctions/hemichannels are involved in the disease progression of HIV encephalopathy (Eugenin and Berman, 2013; Orellana et al., 2014). PD animal models (MTPT-treated mice and rotenone-treated rats) exhibited upregulation of astrocytic Cx43 expression in affected areas (Rufer et al., 1996; Kawasaki et al., 2009), and a gap junction/hemichannel blocker ameliorated the disease symptoms of a PD mouse model (Suzuki et al., 2014). A recent report revealed that α -synuclein directly binds Cx32, and that overexpression of α -synuclein suppresses the activity of Cx32 in the SH-SY5Y dopaminergic neuroblastoma cell line (Sung et al., 2007). Other studies have shown that microglia and astrocytes are determinants of disease progression in ALS (the non-autonomous neuronal death hypothesis) (Boillee et al., 2006; Yamanaka et al., 2008). Activation of microglia and astrocytes is associated with elevated expression of gap junctions and hemichannels (Cui et al., 2014). In fact, treatment with a gap junction/hemichannel blocker ameliorated disease progression in a mouse model of ALS (Takeuchi et al., 2011). Juvenile neuronal ceroid lipofuscinosis (JNCL) also shows the activation of microglia and astrocytes preceding neuronal loss (Pontikis et al., 2005; Xiong and Kielian, 2013), and treatment with a gap junction/hemichannel blocker attenuated the disease symptoms of a JNCL mouse model (Burkovetskaya et al., 2014). Few reports, however, have focused on the expression profiles and functions of connexins in these diseases. Further studies are needed to elucidate the precise role of glial connexins in the pathogenesis of these diseases.

CONCLUSIONS

A growing body of evidence has demonstrated the pathologic roles of gap junctions and hemichannels in various neurologic diseases. For example, dysfunction and dysregulation of gap junctions and hemichannels in glial cells contribute to neuroinflammation in the CNS, which results in neuronal damage (a situation in which glial cells are “bad neighbors” of neurons) (Block et al., 2007). Despite recent progress in elucidating the pathological roles of gap junctions and hemichannels, many challenges remain, due in part to technical limitations. For instance, few high-quality antibodies against each connexin are available for immunostaining and immunoblotting. Moreover, reagents that are commonly used to block connexin channels are not specific for those channels. In fact, connexin channel blockers such as glycyrrhetic acid, its derivative carbenoxolone, niflumic acid, and octanol also block pannexin channels. Although the most specific gap junction and hemichannel blockers currently

available are mimetic peptides with sequences very similar to that of the extracellular loop of connexins, recent studies showed that mimetic peptides specific for Cx32 (³²gap 24 and ³²gap 27), Cx43 (⁴³gap 27), or Panx1 (¹⁰panx1) non-specifically block both connexins and pannexins (Wang et al., 2007). Although aptamers and siRNA may be used as blockers for specific connexins (Knieps et al., 2007; Xu et al., 2014), they still have a problem of the blood–brain barrier penetration. The heterogeneity of gap-junction and hemichannel configurations (Figure 2) and the ability of various connexins to compensate for the loss of other isoforms (e.g., in connexin-knockout studies) also complicate analysis of this system. Although EGFP-tagged connexins have facilitated live-cell imaging, tagging and/or overexpression of connexins in cultured cells often produce abnormally large gap-junction plaques (Lopez et al., 2001; Gaietta et al., 2002; Hunter et al., 2003). Moreover, tagging the amino-termini of connexins results in non-functional channels, whereas tagging the carboxyl-termini alters the properties of the channels (Bukauskas et al., 2000; Contreras et al., 2003). Therefore, future investigations should attempt to elucidate the spatiotemporal expression profiles of connexin isoforms under pathological conditions in the CNS; this work will require development of specific blockers and tracers for each connexin isoform, hemichannel, and gap junction. Understanding the precise pathologic roles of gap junctions and hemichannels may lead to new therapeutic strategies against multiple chronic neurodegenerative diseases.

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REFERENCES

- Aarum, J., Sandberg, K., Haeberlein, S. L., and Persson, M. A. (2003). Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15983–15988. doi: 10.1073/pnas.2237050100
- Akiyama, H., and McGeer, P. L. (1990). Brain microglia constitutively express beta-2 integrins. *J. Neuroimmunol.* 30, 81–93. doi: 10.1016/0165-5728(90)90055-R
- Allen, K., Fuchs, E. C., Jaschonek, H., Bannerman, D. M., and Monyer, H. (2011). Gap junctions between interneurons are required for normal spatial coding in the hippocampus and short-term spatial memory. *J. Neurosci.* 31, 6542–6552. doi: 10.1523/JNEUROSCI.6512-10.2011
- Al-Ubaidi, M. R., White, T. W., Ripps, H., Poras, I., Avner, P., Gomes, D., et al. (2000). Functional properties, developmental regulation, and chromosomal localization of murine connexin36, a gap-junctional protein expressed preferentially in retina and brain. *J. Neurosci. Res.* 59, 813–826. doi: 10.1002/(SICI)1097-4547(20000315)59:6<813::AID-JNR14>3.0.CO;2-#
- Altevogt, B. M., Kleopa, K. A., Postma, F. R., Scherer, S. S., and Paul, D. L. (2002). Connexin29 is uniquely distributed within myelinating glial cells of the central and peripheral nervous systems. *J. Neurosci.* 22, 6458–6470.
- Altevogt, B. M., and Paul, D. L. (2004). Four classes of intercellular channels between glial cells in the CNS. *J. Neurosci.* 24, 4313–4323. doi: 10.1523/JNEUROSCI.3303-03.2004
- Alvarez-Maubecin, V., Garcia-Hernandez, F., Williams, J. T., and Van Bockstaele, E. J. (2000). Functional coupling between neurons and glia. *J. Neurosci.* 20, 4091–4098.

- Anderson, C. M., and Swanson, R. A. (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* 32, 1–14. doi: 10.1002/1098-1136(200010)32:1<1::AID-GLIA10>3.0.CO;2-W
- Anzini, P., Neuberger, D. H., Schachner, M., Nelles, E., Willecke, K., Zielasek, J., et al. (1997). Structural abnormalities and deficient maintenance of peripheral nerve myelin in mice lacking the gap junction protein connexin 32. *J. Neurosci.* 17, 4545–4551.
- Bamberger, M. E., Harris, M. E., McDonald, D. R., Husemann, J., and Landreth, G. E. (2003). A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. *J. Neurosci.* 23, 2665–2674.
- Bani-Yaghoob, M., Bechberger, J. F., Underhill, T. M., and Naus, C. C. (1999a). The effects of gap junction blockage on neuronal differentiation of human NTERa2/clone D1 cells. *Exp. Neurol.* 156, 16–32. doi: 10.1006/exnr.1998.6950
- Bani-Yaghoob, M., Underhill, T. M., and Naus, C. C. (1999b). Gap junction blockage interferes with neuronal and astroglial differentiation of mouse P19 embryonal carcinoma cells. *Dev. Genet.* 24, 69–81. doi: 10.1002/(SICI)1520-6408(1999)24:1/2<69::AID-DVG8>3.0.CO;2-M
- Barbe, M. T., Monyer, H., and Bruzzone, R. (2006). Cell-cell communication beyond connexins: the pannexin channels. *Physiology (Bethesda)* 21, 103–114. doi: 10.1152/physiol.00048.2005
- Barger, S. W., and Basile, A. S. (2001). Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function. *J. Neurochem.* 76, 846–854. doi: 10.1046/j.1471-4159.2001.00075.x
- Bessis, A., Bechade, C., Bernard, D., and Roumier, A. (2007). Microglial control of neuronal death and synaptic properties. *Glia* 55, 233–238. doi: 10.1002/glia.20459
- Bittman, K. S., and Loturco, J. J. (1999). Differential regulation of connexin 26 and 43 in murine neocortical precursors. *Cereb. Cortex* 9, 188–195. doi: 10.1093/cercor/9.2.188
- Block, M. L., Zecca, L., and Hong, J. S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69. doi: 10.1038/nrn2038
- Boche, D., Perry, V. H., and Nicoll, J. A. (2013). Review: activation patterns of microglia and their identification in the human brain. *Neuropathol. Appl. Neurobiol.* 39, 3–18. doi: 10.1111/nan.12011
- Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., et al. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312, 1389–1392. doi: 10.1126/science.1123511
- Bouskila, Y., and Dudek, F. E. (1993). Neuronal synchronization without calcium-dependent synaptic transmission in the hypothalamus. *Proc. Natl. Acad. Sci. U.S.A.* 90, 3207–3210. doi: 10.1073/pnas.90.8.3207
- Brand-Schieber, E., Werner, P., Jacobas, D. A., Jacobas, S., Beelitz, M., Lowery, S. L., et al. (2005). Connexin43, the major gap junction protein of astrocytes, is down-regulated in inflamed white matter in an animal model of multiple sclerosis. *J. Neurosci. Res.* 80, 798–808. doi: 10.1002/jnr.20474
- Brujijn, L. I., Miller, T. M., and Cleveland, D. W. (2004). Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu. Rev. Neurosci.* 27, 723–749. doi: 10.1146/annurev.neuro.27.070203.144244
- Bsibsi, M., Ravid, R., Gveric, D., and Van Noort, J. M. (2002). Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol.* 61, 1013–1021.
- Bukauskas, F. F., Jordan, K., Bukauskiene, A., Bennett, M. V., Lampe, P. D., Laird, D. W., et al. (2000). Clustering of connexin 43-enhanced green fluorescent protein gap junction channels and functional coupling in living cells. *Proc. Natl. Acad. Sci. U.S.A.* 97, 2556–2561. doi: 10.1073/pnas.050588497
- Burkovetskaya, M., Karpuk, N., Xiong, J., Bosch, M., Boska, M. D., Takeuchi, H., et al. (2014). Evidence for Aberrant Astrocyte Hemichannel Activity in Juvenile Neuronal Ceroid Lipofuscinosis (JNCL). *PLoS ONE* 9:e95023. doi: 10.1371/journal.pone.0095023
- Cagnin, A., Brooks, D. J., Kennedy, A. M., Gunn, R. N., Myers, R., Turkheimer, F. E., et al. (2001). *In-vivo* measurement of activated microglia in dementia. *Lancet* 358, 461–467. doi: 10.1016/S0140-6736(01)05625-2
- Carpentier, P. A., Duncan, D. S., and Miller, S. D. (2008). Glial toll-like receptor signaling in central nervous system infection and autoimmunity. *Brain Behav. Immun.* 22, 140–147. doi: 10.1016/j.bbi.2007.08.011
- Chang, Q., Gonzalez, M., Pinter, M. J., and Balice-Gordon, R. J. (1999). Gap junctional coupling and patterns of connexin expression among neonatal rat lumbar spinal motor neurons. *J. Neurosci.* 19, 10813–10828.
- Charles, A. (1998). Intercellular calcium waves in glia. *Glia* 24, 39–49. doi: 10.1002/(SICI)1098-1136(199809)24:1<39::AID-GLIA5>3.0.CO;2-W
- Cho, S., Park, E. M., Febbraio, M., Anrather, J., Park, L., Racchumi, G., et al. (2005). The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. *J. Neurosci.* 25, 2504–2512. doi: 10.1523/JNEUROSCI.0035-05.2005
- Christie, M. J., Williams, J. T., and North, R. A. (1989). Electrical coupling synchronizes subthreshold activity in locus coeruleus neurons *in vitro* from neonatal rats. *J. Neurosci.* 9, 3584–3589.
- Contreras, J. E., Saez, J. C., Bukauskas, F. F., and Bennett, M. V. (2003). Gating and regulation of connexin 43 (Cx43) hemichannels. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11388–11393. doi: 10.1073/pnas.1434298100
- Coraci, I. S., Husemann, J., Berman, J. W., Hulette, C., Dufour, J. H., Campanella, G. K., et al. (2002). CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. *Am. J. Pathol.* 160, 101–112. doi: 10.1016/S0002-9440(10)64354-4
- Cui, Y., Masaki, K., Yamasaki, R., Imamura, S., Suzuki, S. O., Hayashi, S., et al. (2014). Extensive dysregulations of oligodendrocytic and astrocytic connexins are associated with disease progression in an amyotrophic lateral sclerosis mouse model. *J. Neuroinflammation* 11:42. doi: 10.1186/1742-2094-11-42
- Dahl, G., and Locovei, S. (2006). Pannexin: to gap or not to gap, is that a question? *IUBMB Life* 58, 409–419. doi: 10.1080/15216540600794526
- Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758. doi: 10.1038/nn1472
- Deans, M. R., Gibson, J. R., Sellitto, C., Connors, B. W., and Paul, D. L. (2001). Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36. *Neuron* 31, 477–485. doi: 10.1016/S0896-6273(01)00373-7
- Delisle, M. B., and Carpenter, S. (1984). Neurofibrillary axonal swellings and amyotrophic lateral sclerosis. *J. Neurol. Sci.* 63, 241–250. doi: 10.1016/0022-510X(84)90199-0
- Del Rio-Hortega, P. (1932). *Cytology and Cellular Pathology of the Nervous System*. New York, NY: Penfield Wed.
- De Pina-Benabou, M. H., Szostak, V., Kyrozis, A., Rempe, D., Uziel, D., Urban-Maldonado, M., et al. (2005). Blockade of gap junctions *in vivo* provides neuroprotection after perinatal global ischemia. *Stroke* 36, 2232–2237. doi: 10.1161/01.STR.0000182239.75969.d8
- Dermietzel, R., Gao, Y., Scemes, E., Vieira, D., Urban, M., Kremer, M., et al. (2000). Connexin43 null mice reveal that astrocytes express multiple connexins. *Brain Res. Brain Res. Rev.* 32, 45–56. doi: 10.1016/S0165-0173(99)00067-3
- Dermietzel, R., Hertberg, E. L., Kessler, J. A., and Spray, D. C. (1991). Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis. *J. Neurosci.* 11, 1421–1432.
- Dermietzel, R., Traub, O., Hwang, T. K., Beyer, E., Bennett, M. V., Spray, D. C., et al. (1989). Differential expression of three gap junction proteins in developing and mature brain tissues. *Proc. Natl. Acad. Sci. U.S.A.* 86, 10148–10152. doi: 10.1073/pnas.86.24.10148
- De Vuyst, E., Decrock, E., De Bock, M., Yamasaki, H., Naus, C. C., Evans, W. H., et al. (2007). Connexin hemichannels and gap junction channels are differentially influenced by lipopolysaccharide and basic fibroblast growth factor. *Mol. Biol. Cell* 18, 34–46. doi: 10.1091/mbc.E06-03-0182
- Dickson, T. C., King, C. E., McCormack, G. H., and Vickers, J. C. (1999). Neurochemical diversity of dystrophic neurites in the early and late stages of Alzheimer's disease. *Exp. Neurol.* 156, 100–110. doi: 10.1006/exnr.1998.7010
- Dutta, R., and Trapp, B. D. (2007). Pathogenesis of axonal and neuronal damage in multiple sclerosis. *Neurology* 68, S22–S31; discussion S43–S54.
- Eikelenboom, P., Bate, C., Van Gool, W. A., Hoozemans, J. J., Rozemuller, J. M., Veerhuis, R., et al. (2002). Neuroinflammation in Alzheimer's disease and prion disease. *Glia* 40, 232–239. doi: 10.1002/glia.10146
- El Khoury, J., Hickman, S. E., Thomas, C. A., Cao, L., Silverstein, S. C., and Loike, J. D. (1996). Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. *Nature* 382, 716–719. doi: 10.1038/382716a0
- El Khoury, J., Hickman, S. E., Thomas, C. A., Loike, J. D., and Silverstein, S. C. (1998). Microglia, scavenger receptors, and the pathogenesis of Alzheimer's disease. *Neurobiol. Aging* 19, S81–S84. doi: 10.1016/S0197-4580(98)00036-0
- Eugenin, E. A., Basilio, D., Saez, J. C., Orellana, J. A., Raine, C. S., Bukauskas, F. F., et al. (2012). The role of gap junction channels during physiologic and

- pathological conditions of the human central nervous system. *J. Neuroimmune Pharmacol.* 7, 499–518. doi: 10.1007/s11481-012-9352-5
- Eugenin, E. A., and Berman, J. W. (2013). Cytochrome c dysregulation induced by HIV infection of astrocytes results in bystander apoptosis of uninfected astrocytes by an IP3 and calcium-dependent mechanism. *J. Neurochem.* 127, 644–651. doi: 10.1111/jnc.12443
- Eugenin, E. A., Eckardt, D., Theis, M., Willecke, K., Bennett, M. V., and Saez, J. C. (2001). Microglia at brain stab wounds express connexin 43 and *in vitro* form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4190–4195. doi: 10.1073/pnas.051634298
- Frank, M., Eiberger, B., Janssen-Bienhold, U., De Sevilla Muller, L. P., Tjarks, A., Kim, J. S., et al. (2010). Neuronal connexin-36 can functionally replace connexin-45 in mouse retina but not in the developing heart. *J. Cell Sci.* 123, 3605–3615. doi: 10.1242/jcs.068668
- Frantseva, M. V., Kokorovtseva, L., and Perez Velazquez, J. L. (2002). Ischemia-induced brain damage depends on specific gap-junctional coupling. *J. Cereb. Blood Flow Metab.* 22, 453–462. doi: 10.1097/00004647-200204000-00009
- Fushiki, S., Perez Velazquez, J. L., Zhang, L., Bechberger, J. F., Carlen, P. L., and Naus, C. C. (2003). Changes in neuronal migration in neocortex of connexin43 null mutant mice. *J. Neuropathol. Exp. Neurol.* 62, 304–314.
- Gaietta, G., Deerinck, T. J., Adams, S. R., Bouwer, J., Tour, O., Laird, D. W., et al. (2002). Multicolor and electron microscopic imaging of connexin trafficking. *Science* 296, 503–507. doi: 10.1126/science.1068793
- Garg, S., Md Syed, M., and Kielian, T. (2005). Staphylococcus aureus-derived peptidoglycan induces Cx43 expression and functional gap junction intercellular communication in microglia. *J. Neurochem.* 95, 475–483. doi: 10.1111/j.1471-4159.2005.03384.x
- Ghezzi, P., and Mennini, T. (2001). Tumor necrosis factor and motoneuronal degeneration: an open problem. *Neuroimmunomodulation* 9, 178–182. doi: 10.1159/000049024
- Giaume, C., Taberero, A., and Medina, J. M. (1997). Metabolic trafficking through astrocytic gap junctions. *Glia* 21, 114–123. doi: 10.1002/(SICI)1098-1136(199709)21:1<114::AID-GLIA13>3.0.CO;2-V
- Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., and Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell* 140, 918–934. doi: 10.1016/j.cell.2010.02.016
- Goel, S., Wharton, S. B., Brett, L. P., and Whittle, I. R. (2003). Morphological changes and stress responses in neurons in cerebral cortex infiltrated by diffuse astrocytoma. *Neuropathology* 23, 262–270. doi: 10.1046/j.1440-1789.2003.00510.x
- Goldberg, G. S., Lampe, P. D., and Nicholson, B. J. (1999). Selective transfer of endogenous metabolites through gap junctions composed of different connexins. *Nat. Cell Biol.* 1, 457–459. doi: 10.1038/15693
- Goldberg, G. S., Moreno, A. P., and Lampe, P. D. (2002). Gap junctions between cells expressing connexin 43 or 32 show inverse permselectivity to adenosine and ATP. *J. Biol. Chem.* 277, 36725–36730. doi: 10.1074/jbc.M109797200
- Goldberg, G. S., Valiunas, V., and Brink, P. R. (2004). Selective permeability of gap junction channels. *Biochim. Biophys. Acta* 1662, 96–101. doi: 10.1016/j.bbame.2003.11.022
- Grewal, R. P., Yoshida, T., Finch, C. E., and Morgan, T. E. (1997). Scavenger receptor mRNAs in rat brain microglia are induced by kainic acid lesioning and by cytokines. *Neuroreport* 8, 1077–1081. doi: 10.1097/00001756-199703240-00003
- Harris, A. L. (2001). Emerging issues of connexin channels: biophysics fills the gap. *Q. Rev. Biophys.* 34, 325–472. doi: 10.1017/S0033583501003705
- Harris, A. L. (2007). Connexin channel permeability to cytoplasmic molecules. *Prog. Biophys. Mol. Biol.* 94, 120–143. doi: 10.1016/j.pbiomolbio.2007.03.011
- Hartfield, E. M., Rinaldi, F., Glover, C. P., Wong, L. F., Caldwell, M. A., and Uney, J. B. (2011). Connexin 36 expression regulates neuronal differentiation from neural progenitor cells. *PLoS ONE* 6:e14746. doi: 10.1371/journal.pone.0014746
- Henkel, J. S., Beers, D. R., Zhao, W., and Appel, S. H. (2009). Microglia in ALS: the good, the bad, and the resting. *J. Neuroimmune Pharmacol.* 4, 389–398. doi: 10.1007/s11481-009-9171-5
- Herman, M. A., and Jahr, C. E. (2007). Extracellular glutamate concentration in hippocampal slice. *J. Neurosci.* 27, 9736–9741. doi: 10.1523/JNEUROSCI.3009-07.2007
- Hori, N., and Carpenter, D. O. (1994). Functional and morphological changes induced by transient *in vivo* ischemia. *Exp. Neurol.* 129, 279–289. doi: 10.1006/exnr.1994.1170
- Hormuzdi, S. G., Pais, I., Lebeau, F. E., Towers, S. K., Rozov, A., Buhl, E. H., et al. (2001). Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. *Neuron* 31, 487–495. doi: 10.1016/S0896-6273(01)00387-7
- Huang, C., Han, X., Li, X., Lam, E., Peng, W., Lou, N., et al. (2012). Critical role of connexin 43 in secondary expansion of traumatic spinal cord injury. *J. Neurosci.* 32, 3333–3338. doi: 10.1523/JNEUROSCI.1216-11.2012
- Hunter, A. W., Jourdan, J., and Gourdie, R. G. (2003). Fusion of GFP to the carboxyl terminus of connexin43 increases gap junction size in HeLa cells. *Cell Commun. Adhes.* 10, 211–214. doi: 10.1080/714040429
- Iribarren, P., Chen, K., Hu, J., Gong, W., Cho, E. H., Lockett, S., et al. (2005). CpG-containing oligodeoxynucleotide promotes microglial cell uptake of amyloid beta 1-42 peptide by up-regulating the expression of the G-protein-coupled receptor mFPR2. *FASEB J.* 19, 2032–2034.
- Jefferys, J. G. (1995). Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol. Rev.* 75, 689–723.
- Jimenez, S., Baglietto-Vargas, D., Caballero, C., Moreno-Gonzalez, I., Torres, M., Sanchez-Varo, R., et al. (2008). Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic. *J. Neurosci.* 28, 11650–11661. doi: 10.1523/JNEUROSCI.3024-08.2008
- Kalogeris, T., Baines, C. P., Krenz, M., and Korthuis, R. J. (2012). Cell biology of ischemia/reperfusion injury. *Int. Rev. Cell Mol. Biol.* 298, 229–317. doi: 10.1016/B978-0-12-394309-5.00006-7
- Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H., and Karin, M. (2005). Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120, 649–661. doi: 10.1016/j.cell.2004.12.041
- Kawanokuchi, J., Mizuno, T., Takeuchi, H., Kato, H., Wang, J., Mitsuma, N., et al. (2006). Production of interferon-gamma by microglia. *Mult. Scler.* 12, 558–564. doi: 10.1177/1352458506070763
- Kawasaki, A., Hayashi, T., Nakachi, K., Trosko, J. E., Sugihara, K., Kotake, Y., et al. (2009). Modulation of connexin 43 in rotenone-induced model of Parkinson's disease. *Neuroscience* 160, 61–68. doi: 10.1016/j.neuroscience.2009.01.080
- Kempermann, G., and Neumann, H. (2003). Neuroscience. Microglia: the enemy within? *Science* 302, 1689–1690. doi: 10.1126/science.1092864
- Kielian, T. (2008). Glial connexins and gap junctions in CNS inflammation and disease. *J. Neurochem.* 106, 1000–1016. doi: 10.1111/j.1471-4159.2008.05405.x
- Kipnis, J., Avidan, H., Caspi, R. R., and Schwartz, M. (2004). Dual effect of CD4+CD25+ regulatory T cells in neurodegeneration: a dialogue with microglia. *Proc. Natl. Acad. Sci. U.S.A.* 101(Suppl. 2), 14663–14669. doi: 10.1073/pnas.0404842101
- Knieps, M., Herrmann, S., Lehmann, C., Loer, B., Hoch, M., and Famulok, M. (2007). Anti-innexin 2 aptamers specifically inhibit the heterologous interaction of the innexin 2 and innexin 3 carboxyl-termini *in vitro*. *Biol. Chem.* 388, 561–568. doi: 10.1515/BC.2007.074
- Koenigsnecht, J., and Landreth, G. (2004). Microglial phagocytosis of fibrillar beta-amyloid through a beta1 integrin-dependent mechanism. *J. Neurosci.* 24, 9838–9846. doi: 10.1523/JNEUROSCI.2557-04.2004
- Koulakoff, A., Mei, X., Orellana, J. A., Saez, J. C., and Giaume, C. (2012). Glial connexin expression and function in the context of Alzheimer's disease. *Biochim. Biophys. Acta* 1818, 2048–2057. doi: 10.1016/j.bbame.2011.10.001
- Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312–318. doi: 10.1016/0166-2236(96)10049-7
- Laird, D. W. (2006). Life cycle of connexins in health and disease. *Biochem. J.* 394, 527–543. doi: 10.1042/BJ20051922
- Laird, D. W. (2010). The gap junction proteome and its relationship to disease. *Trends Cell Biol.* 20, 92–101. doi: 10.1016/j.tcb.2009.11.001
- Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170. doi: 10.1016/0306-4522(90)90229-W
- Leithe, E., and Rivedal, E. (2007). Ubiquitination of gap junction proteins. *J. Membr. Biol.* 217, 43–51. doi: 10.1007/s00232-007-9050-z
- Liang, J., Takeuchi, H., Doi, Y., Kawanokuchi, J., Sonobe, Y., Jin, S., et al. (2008). Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity. *Brain Res.* 1210, 11–19. doi: 10.1016/j.brainres.2008.03.012
- Liang, J., Takeuchi, H., Jin, S., Noda, M., Li, H., Doi, Y., et al. (2010). Glutamate induces neurotrophic factor production from microglia via protein

- kinase C pathway. *Brain Res.* 1322, 8–23. doi: 10.1016/j.brainres.2010.01.083
- Liu, Y., Walter, S., Stagi, M., Cherny, D., Letiembre, M., Schulz-Schaeffer, W., et al. (2005). LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. *Brain* 128, 1778–1789. doi: 10.1093/brain/awh531
- Lo, C. W., Waldo, K. L., and Kirby, M. L. (1999). Gap junction communication and the modulation of cardiac neural crest cells. *Trends Cardiovasc. Med.* 9, 63–69. doi: 10.1016/S1050-1738(99)00015-8
- Lobsiger, C. S., and Cleveland, D. W. (2007). Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nat. Neurosci.* 10, 1355–1360. doi: 10.1038/nn1988
- Lopez, P., Balicki, D., Buehler, L. K., Falk, M. M., and Chen, S. C. (2001). Distribution and dynamics of gap junction channels revealed in living cells. *Cell Commun. Adhes.* 8, 237–242. doi: 10.3109/15419060109080730
- Lutz, S. E., Raine, C. S., and Brosnan, C. F. (2012). Loss of astrocyte connexins 43 and 30 does not significantly alter susceptibility or severity of acute experimental autoimmune encephalomyelitis in mice. *J. Neuroimmunol.* 245, 8–14. doi: 10.1016/j.jneuroim.2012.01.007
- Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23, 549–555. doi: 10.1016/S1471-4906(02)02302-5
- Marin-Teva, J. L., Dusart, I., Colin, C., Gervais, A., Van Rooijen, N., and Mallat, M. (2004). Microglia promote the death of developing Purkinje cells. *Neuron* 41, 535–547. doi: 10.1016/S0896-6273(04)00069-8
- Markoullis, K., Sargiannidou, I., Gardner, C., Hadjisavvas, A., Reynolds, R., and Kleopa, K. A. (2012). Disruption of oligodendrocyte gap junctions in experimental autoimmune encephalomyelitis. *Glia* 60, 1053–1066. doi: 10.1002/glia.22334
- Masaki, K., Suzuki, S. O., Matsushita, T., Yonekawa, T., Matsuoka, T., Isobe, N., et al. (2012). Extensive loss of connexins in Balo's disease: evidence for an auto-antibody-independent astrocytopathy via impaired astrocyte-oligodendrocyte/myelin interaction. *Acta Neuropathol.* 123, 887–900. doi: 10.1007/s00401-012-0972-x
- Matsushita, T., Masaki, K., Suzuki, S., Matsuoka, T., Yonekawa, T., Wu, X. M., et al. (2011). Astrocytopathy in neuromyelitis optica, multiple sclerosis and Balo's disease. *Rinsho Shinkeigaku* 51, 898–900. doi: 10.5692/clinicalneuro.51.898
- Mattila, P. M., Rinne, J. O., Helenius, H., and Roytta, M. (1999). Neuritic degeneration in the hippocampus and amygdala in Parkinson's disease in relation to Alzheimer pathology. *Acta Neuropathol.* 98, 157–164. doi: 10.1007/s004010051064
- McGeer, P. L., and McGeer, E. G. (2002). Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve* 26, 459–470. doi: 10.1002/mus.10191
- Menichella, D. M., Goodenough, D. A., Sirkowski, E., Scherer, S. S., and Paul, D. L. (2003). Connexins are critical for normal myelination in the CNS. *J. Neurosci.* 23, 5963–5973.
- Menichella, D. M., Majdan, M., Awatramani, R., Goodenough, D. A., Sirkowski, E., Scherer, S. S., et al. (2006). Genetic and physiological evidence that oligodendrocyte gap junctions contribute to spatial buffering of potassium released during neuronal activity. *J. Neurosci.* 26, 10984–10991. doi: 10.1523/JNEUROSCI.0304-06.2006
- Mizuno, T., Zhang, G., Takeuchi, H., Kawanokuchi, J., Wang, J., Sonobe, Y., et al. (2008). Interferon-gamma directly induces neurotoxicity through a neuron specific, calcium-permeable complex of IFN-gamma receptor and AMPA GluR1 receptor. *FASEB J.* 22, 1797–1806. doi: 10.1096/fj.07-099499
- Nadarajah, B., Thomaidou, D., Evans, W. H., and Parnavelas, J. G. (1996). Gap junctions in the adult cerebral cortex: regional differences in their distribution and cellular expression of connexins. *J. Comp. Neurol.* 376, 326–342. doi: 10.1002/(SICI)1096-9861(19961209)376:2<326::AID-CNE13>3.0.CO;2-J
- Nagy, J. I., Ionescu, A. V., Lynn, B. D., and Rash, J. E. (2003). Coupling of astrocyte connexins Cx26, Cx30, Cx43 to oligodendrocyte Cx29, Cx32, Cx47: implications from normal and connexin32 knockout mice. *Glia* 44, 205–218. doi: 10.1002/glia.10278
- Nagy, J. I., and Rash, J. E. (2000). Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. *Brain Res. Brain Res. Rev.* 32, 29–44. doi: 10.1016/S0165-0173(99)00066-1
- Nakanishi, H., and Wu, Z. (2009). Microglia-aging: roles of microglial lysosome- and mitochondria-derived reactive oxygen species in brain aging. *Behav. Brain Res.* 201, 1–7. doi: 10.1016/j.bbr.2009.02.001
- Naus, C. C., Bechberger, J. F., Zhang, Y., Venance, L., Yamasaki, H., Juneja, S. C., et al. (1997). Altered gap junctional communication, intercellular signaling, and growth in cultured astrocytes deficient in connexin43. *J. Neurosci. Res.* 49, 528–540. doi: 10.1002/(SICI)1097-4547(19970901)49:5<528::AID-JNR3>3.0.CO;2-D
- Nelson, P. T., Soma, L. A., and Lavi, E. (2002). Microglia in diseases of the central nervous system. *Ann. Med.* 34, 491–500. doi: 10.1080/078538902321117698
- Newsholme, E. A., and Calder, P. C. (1997). The proposed role of glutamine in some cells of the immune system and speculative consequences for the whole animal. *Nutrition* 13, 728–730. doi: 10.1016/S0899-9007(97)83034-1
- Newsholme, P., and Newsholme, E. A. (1989). Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. *Biochem. J.* 261, 211–218.
- Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318. doi: 10.1126/science.1110647
- Nissim, I. (1999). Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am. J. Physiol.* 277, F493–F497.
- Odermatt, B., Wellershaus, K., Wallraff, A., Seifert, G., Degen, J., Euwens, C., et al. (2003). Connexin 47 (Cx47)-deficient mice with enhanced green fluorescent protein reporter gene reveal predominant oligodendrocytic expression of Cx47 and display vacuolized myelin in the CNS. *J. Neurosci.* 23, 4549–4559.
- Orellana, J. A., Saez, J. C., Bennett, M. V., Berman, J. W., Morgello, S., and Eugenin, E. A. (2014). HIV increases the release of dickkopf-1 protein from human astrocytes by a Cx43 hemichannel-dependent mechanism. *J. Neurochem.* 128, 752–763. doi: 10.1111/jnc.12492
- Orellana, J. A., Saez, P. J., Shoji, K. F., Schalper, K. A., Palacios-Prado, N., Velarde, V., et al. (2009). Modulation of brain hemichannels and gap junction channels by pro-inflammatory agents and their possible role in neurodegeneration. *Antioxid. Redox Signal.* 11, 369–399. doi: 10.1089/ars.2008.2130
- Orellana, J. A., Shoji, K. F., Abudara, V., Ezan, P., Amigou, E., Saez, P. J., et al. (2011). Amyloid beta-induced death in neurons involves glial and neuronal hemichannels. *J. Neurosci.* 31, 4962–4977. doi: 10.1523/JNEUROSCI.6417-10.2011
- Orr, C. F., Rowe, D. B., and Halliday, G. M. (2002). An inflammatory review of Parkinson's disease. *Prog. Neurobiol.* 68, 325–340. doi: 10.1016/S0301-0082(02)00127-2
- Orthmann-Murphy, J. L., Freidin, M., Fischer, E., Scherer, S. S., and Abrams, C. K. (2007). Two distinct heterotypic channels mediate gap junction coupling between astrocyte and oligodendrocyte connexins. *J. Neurosci.* 27, 13949–13957. doi: 10.1523/JNEUROSCI.3395-07.2007
- Parenti, R., Campisi, A., Vanella, A., and Cicirata, F. (2002). Immunocytochemical and RT-PCR analysis of connexin36 in cultures of mammalian glial cells. *Arch. Ital. Biol.* 140, 101–108.
- Parsons, C. G., Stoffer, A., and Danysz, W. (2007). Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system—too little activation is bad, too much is even worse. *Neuropharmacology* 53, 699–723. doi: 10.1016/j.neuropharm.2007.07.013
- Paul, D. L. (1995). New functions for gap junctions. *Curr. Opin. Cell Biol.* 7, 665–672. doi: 10.1016/0955-0674(95)80108-1
- Pavese, N., Gerhard, A., Tai, Y. F., Ho, A. K., Turkheimer, F., Barker, R. A., et al. (2006). Microglial activation correlates with severity in Huntington disease: a clinical and PET study. *Neurology* 66, 1638–1643. doi: 10.1212/01.wnl.000022734.56412.17
- Pavlidis, M., Stupp, T., Naskar, R., Cengiz, C., and Thanos, S. (2003). Retinal ganglion cells resistant to advanced glaucoma: a postmortem study of human retinas with the carbocyanine dye DiI. *Invest. Ophthalmol. Vis. Sci.* 44, 5196–5205. doi: 10.1167/iovs.03-0614
- Piani, D., Spranger, M., Frei, K., Schaffner, A., and Fontana, A. (1992). Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines. *Eur. J. Immunol.* 22, 2429–2436. doi: 10.1002/eji.1830220936
- Pontikis, C. C., Cotman, S. L., Macdonald, M. E., and Cooper, J. D. (2005). Thalamocortical neuron loss and localized astrocytosis in the Cln3Deltaex7/8 knock-in mouse model of Batten disease. *Neurobiol. Dis.* 20, 823–836. doi: 10.1016/j.nbd.2005.05.018
- Ransom, B., Behar, T., and Nedergaard, M. (2003). New roles for astrocytes (stars at last). *Trends Neurosci.* 26, 520–522. doi: 10.1016/j.tins.2003.08.006
- Rash, J. E., Olson, C. O., Davidson, K. G., Yasumura, T., Kamasawa, N., and Nagy, J. I. (2007). Identification of connexin36 in gap junctions

- between neurons in rodent locus coeruleus. *Neuroscience* 147, 938–956. doi: 10.1016/j.neuroscience.2007.04.061
- Rash, J. E., Yasumura, T., Dudek, F. E., and Nagy, J. I. (2001). Cell-specific expression of connexins and evidence of restricted gap junctional coupling between glial cells and between neurons. *J. Neurosci.* 21, 1983–2000.
- Rawanduzy, A., Hansen, A., Hansen, T. W., and Nedergaard, M. (1997). Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke. *J. Neurosurg.* 87, 916–920. doi: 10.3171/jns.1997.87.6.0916
- Retamal, M. A., Schalper, K. A., Shoji, K. F., Bennett, M. V., and Saez, J. C. (2007). Opening of connexin 43 hemichannels is increased by lowering intracellular redox potential. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8322–8327. doi: 10.1073/pnas.0702456104
- Richard, K. L., Filali, M., Prefontaine, P., and Rivest, S. (2008). Toll-like receptor 2 acts as a natural innate immune receptor to clear amyloid beta 1–42 and delay the cognitive decline in a mouse model of Alzheimer's disease. *J. Neurosci.* 28, 5784–5793. doi: 10.1523/JNEUROSCI.1146-08.2008
- Rock, R. B., Gekker, G., Hu, S., Sheng, W. S., Cheeran, M., Lokensgard, J. R., et al. (2004). Role of microglia in central nervous system infections. *Clin. Microbiol. Rev.* 17, 942–964. doi: 10.1128/CMR.17.4.942-964.2004
- Rouach, N., Avignone, E., Meme, W., Koulakoff, A., Venance, L., Blomstrand, F., et al. (2002). Gap junctions and connexin expression in the normal and pathological central nervous system. *Biol. Cell* 94, 457–475. doi: 10.1016/S0248-4900(02)00016-3
- Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., and Giaume, C. (2008). Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322, 1551–1555. doi: 10.1126/science.1164022
- Rufer, M., Wirth, S. B., Hofer, A., Dermietzel, R., Pastor, A., Kettenmann, H., et al. (1996). Regulation of connexin-43, GFAP, and FGF-2 is not accompanied by changes in astroglial coupling in MPTP-lesioned, FGF-2-treated parkinsonian mice. *J. Neurosci. Res.* 46, 606–617. doi: 10.1002/(SICI)1097-4547(19961201)46:5<606::AID-JNR9>3.0.CO;2-N
- Saez, J. C., Berthoud, V. M., Branes, M. C., Martinez, A. D., and Beyer, E. C. (2003). Plasma membrane channels formed by connexins: their regulation and functions. *Physiol. Rev.* 83, 1359–1400.
- Saito, Y., Kawashima, A., Ruberu, N. N., Fujiwara, H., Koyama, S., Sawabe, M., et al. (2003). Accumulation of phosphorylated alpha-synuclein in aging human brain. *J. Neuropathol. Exp. Neurol.* 62, 644–654.
- Sawada, M. (2009). Neuroprotective and toxic changes in microglia in neurodegenerative disease. *Parkinsonism. Relat. Disord.* 15(Suppl. 1), S39–S41. doi: 10.1016/S1353-8020(09)70011-2
- Scemes, E., Dermietzel, R., and Spray, D. C. (1998). Calcium waves between astrocytes from Cx43 knockout mice. *Glia* 24, 65–73. doi: 10.1002/(SICI)1098-1136(199809)24:1<65::AID-GLIA7>3.0.CO;2-#
- Schwab, J. M., and Schluesener, H. J. (2004). Microglia rules: insights into microglial-neuronal signaling. *Cell Death Differ.* 11, 1245–1246. doi: 10.1038/sj.cdd.4401487
- Schwartz, M., Shaked, I., Fisher, J., Mizrahi, T., and Schori, H. (2003). Protective autoimmunity against the enemy within: fighting glutamate toxicity. *Trends Neurosci.* 26, 297–302. doi: 10.1016/S0166-2236(03)00126-7
- Seabrook, T. J., Jiang, L., Maier, M., and Lemere, C. A. (2006). Minocycline affects microglia activation, Abeta deposition, and behavior in APP-tg mice. *Glia* 53, 776–782. doi: 10.1002/glia.20338
- Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., and Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 49, 489–502. doi: 10.1016/j.neuron.2006.01.022
- Sohl, G., Maxeiner, S., and Willecke, K. (2005). Expression and functions of neuronal gap junctions. *Nat. Rev. Neurosci.* 6, 191–200. doi: 10.1038/nrn1627
- Solan, J. L., and Lampe, P. D. (2009). Connexin43 phosphorylation: structural changes and biological effects. *Biochem. J.* 419, 261–272. doi: 10.1042/BJ20082319
- Stirling, D. P., Khodarahmi, K., Liu, J., McPhail, L. T., McBride, C. B., Steeves, J. D., et al. (2004). Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J. Neurosci.* 24, 2182–2190. doi: 10.1523/JNEUROSCI.5275-03.2004
- Sung, J. Y., Lee, H. J., Jeong, E. I., Oh, Y., Park, J., Kang, K. S., et al. (2007). Alpha-synuclein overexpression reduces gap junctional intercellular communication in dopaminergic neuroblastoma cells. *Neurosci. Lett.* 416, 289–293. doi: 10.1016/j.neulet.2007.02.025
- Sutor, B., Schmolke, C., Teubner, B., Schirmer, C., and Willecke, K. (2000). Myelination defects and neuronal hyperexcitability in the neocortex of connexin 32-deficient mice. *Cereb. Cortex* 10, 684–697. doi: 10.1093/cercor/10.7.684
- Suzuki, H., Ono, K., and Sawada, M. (2014). Protective effect of INI-0602, a gap junction inhibitor, on dopaminergic neurodegeneration of mice with unilateral 6-hydroxydopamine injection. *J. Neural Transm.* doi: 10.1007/s00702-014-1209-z. [Epub ahead of print].
- Suzumura, A., Mezitis, S. G., Gonatas, N. K., and Silberberg, D. H. (1987). MHC antigen expression on bulk isolated macrophage-microglia from newborn mouse brain: induction of Ia antigen expression by gamma-interferon. *J. Neuroimmunol.* 15, 263–278. doi: 10.1016/0165-5728(87)90121-4
- Swann, J. W., Al-Noori, S., Jiang, M., and Lee, C. L. (2000). Spine loss and other dendritic abnormalities in epilepsy. *Hippocampus* 10, 617–625. doi: 10.1002/1098-1063(2000)10:5<617::AID-HIPO13>3.0.CO;2-R
- Taberner, A., Medina, J. M., and Giaume, C. (2006). Glucose metabolism and proliferation in glia: role of astrocytic gap junctions. *J. Neurochem.* 99, 1049–1061. doi: 10.1111/j.1471-4159.2006.04088.x
- Takahashi, T., Yagishita, S., Amano, N., Yamaoka, K., and Kamei, T. (1997). Amyotrophic lateral sclerosis with numerous axonal spheroids in the corticospinal tract and massive degeneration of the cortex. *Acta Neuropathol.* 94, 294–299. doi: 10.1007/s004010050707
- Takaki, J., Fujimori, K., Miura, M., Suzuki, T., Sekino, Y., and Sato, K. (2012). L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the 'collusion' hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. *J. Neuroinflammation* 9:275. doi: 10.1186/1742-2094-9-275
- Takeuchi, H. (2010). Neurotoxicity by microglia: mechanisms and potential therapeutic strategy. *Clin. Exp. Neuroimmunol.* 1, 12–21. doi: 10.1111/j.1759-1961.2009.00001.x
- Takeuchi, H., Jin, S., Suzuki, H., Doi, Y., Liang, J., Kawanokuchi, J., et al. (2008a). Blockade of microglial glutamate release protects against ischemic brain injury. *Exp. Neurol.* 214, 144–146. doi: 10.1016/j.expneurol.2008.08.001
- Takeuchi, H., Jin, S., Suzuki, H., Doi, Y., Liang, J., Kawanokuchi, J., et al. (2008b). Blockade of microglial glutamate release protects against ischemic brain injury. *Exp. Neurol.* 214, 144–146. doi: 10.1016/j.expneurol.2008.08.001
- Takeuchi, H., Jin, S., Wang, J., Zhang, G., Kawanokuchi, J., Kuno, R., et al. (2006). Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J. Biol. Chem.* 281, 21362–21368. doi: 10.1074/jbc.M600504200
- Takeuchi, H., Kawanokuchi, J., Mizuno, T., and Suzumura, A. (2014). Expression profile of connexins in the central nervous system. *Clin. Exp. Neuroimmunol.* doi: 10.1111/cen3.12106. [Epub ahead of print].
- Takeuchi, H., Mizoguchi, H., Doi, Y., Jin, S., Noda, M., Liang, J., et al. (2011). Blockade of gap junction hemichannel suppresses disease progression in mouse models of amyotrophic lateral sclerosis and Alzheimer's disease. *PLoS ONE* 6:e21108. doi: 10.1371/journal.pone.0021108
- Takeuchi, H., Mizuno, T., Zhang, G., Wang, J., Kawanokuchi, J., Kuno, R., et al. (2005). Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport. *J. Biol. Chem.* 280, 10444–10454. doi: 10.1074/jbc.M413863200
- Talaveron, R., Matarredona, E. R., De La Cruz, R. R., Macias, D., Galvez, V., and Pastor, A. M. (2014). Implanted neural progenitor cells regulate glial reaction to brain injury and establish gap junctions with host glial cells. *Glia* 62, 623–638. doi: 10.1002/glia.22630
- Tamura, K., Alessandri, B., Heimann, A., and Kempfski, O. (2011). The effect of a gap-junction blocker, carbenoxolone, on ischemic brain injury and cortical spreading depression. *Neuroscience* 194, 262–271. doi: 10.1016/j.neuroscience.2011.07.043
- Teubner, B., Michel, V., Pesch, J., Lautermann, J., Cohen-Salmon, M., Sohl, G., et al. (2003). Connexin30 (Gjb6)-deficiency causes severe hearing impairment and lack of endocochlear potential. *Hum. Mol. Genet.* 12, 13–21. doi: 10.1093/hmg/ddg001
- Teubner, B., Odermatt, B., Guldenagel, M., Sohl, G., Degen, J., Bukauskas, F., et al. (2001). Functional expression of the new gap junction gene connexin47 transcribed in mouse brain and spinal cord neurons. *J. Neurosci.* 21, 1117–1126.
- Thompson, R. J., Zhou, N., and Macvicar, B. A. (2006). Ischemia opens neuronal gap junction hemichannels. *Science* 312, 924–927. doi: 10.1126/science.1126241