Forum Review Article

Modulation of Brain Hemichannels and Gap Junction Channels by Pro-Inflammatory Agents and Their Possible Role in Neurodegeneration

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Abstract

In normal brain, neurons, astrocytes, and oligodendrocytes, the most abundant and active cells express pannexins and connexins, protein subunits of two families forming membrane channels. Most available evidence indicates that in mammals endogenously expressed pannexins form only hemichannels and connexins form both gap junction channels and hemichannels. Whereas gap junction channels connect the cytoplasm of contacting cells and coordinate electric and metabolic activity, hemichannels communicate the intra- and extracellular compartments and serve as a diffusional pathway for ions and small molecules. A subthreshold stimulation by acute pathological threatening conditions (*e.g.*, global ischemia subthreshold for cell death) enhances neuronal Cx36 and glial Cx43 hemichannel activity, favoring ATP release and generation of preconditioning. If the stimulus is sufficiently deleterious, microglia become overactivated and release bioactive molecules that increase the activity of hemichannels and reduce gap junctional communication in astroglial networks, depriving neurons of astrocytic protective functions, and further reducing neuronal viability. Continuous glial activation triggered by low levels of anomalous proteins expressed in several neurodegenerative diseases induce glial hemichannel and gap junction channel disorders similar to those of acute inflammatory responses triggered by ischemia or infectious diseases. These changes are likely to occur in diverse cell types of the CNS and contribute to neurodegeneration during inflammatory process. *Antiox. Redox Signal.* 11, 369–399.

Introduction

NEURODEGENERATION IS CHARACTERIZED by progressive loss of structure or function of neurons that leads to neuronal death. Neuronal damage can occur either in acute or chronic conditions. In acute injury, such as cerebrovascular events (*e.g.*, ischemic and hemorrhagic stroke) and CNS infections (*e.g.*, bacterial and viral meningitis/encephalitis), rapid neuronal necrosis occurs at the central core of the affected zones. Delayed (*e.g.*, in hours or days) neuronal death also occurs at the penumbral zone located in the periphery of the central core. Several studies support the involvement of pro-inflammatory cytokines released after the primary injury, causing delayed neurodegeneration (13). Chronic conditions characterized by neuronal death include classical neurodegenerative disorders, such as Alzheimer's (AD),

Parkinson's (PD), and Huntington's disease (HD). Because several neurodegenerative diseases are associated with inflammation (335), pro-inflammatory cytokines such as TNF-α and IL-1β are likely to contribute to neuronal cell death.

The most sensitive CNS detectors of threatening conditions are microglia. Upon stimulation, microglial cells release high levels of pro-inflammatory cytokines, leading to dysfunctional astroglial communication (105, 150, 235, 314, 321). Direct communication between astroglial cells occurs mainly through gap junction channels (GJCs) (Fig. 1). Intercellular GJCs are believed to protect neurons through the spatial buffering of neurotoxic substrates. Moreover, reduced gap junctional communication between astroglial cells is associated with neurotoxicity (31, 271). In addition to intercellular channels, glial cells can also communicate through the release of small signaling molecules via hemichannels that

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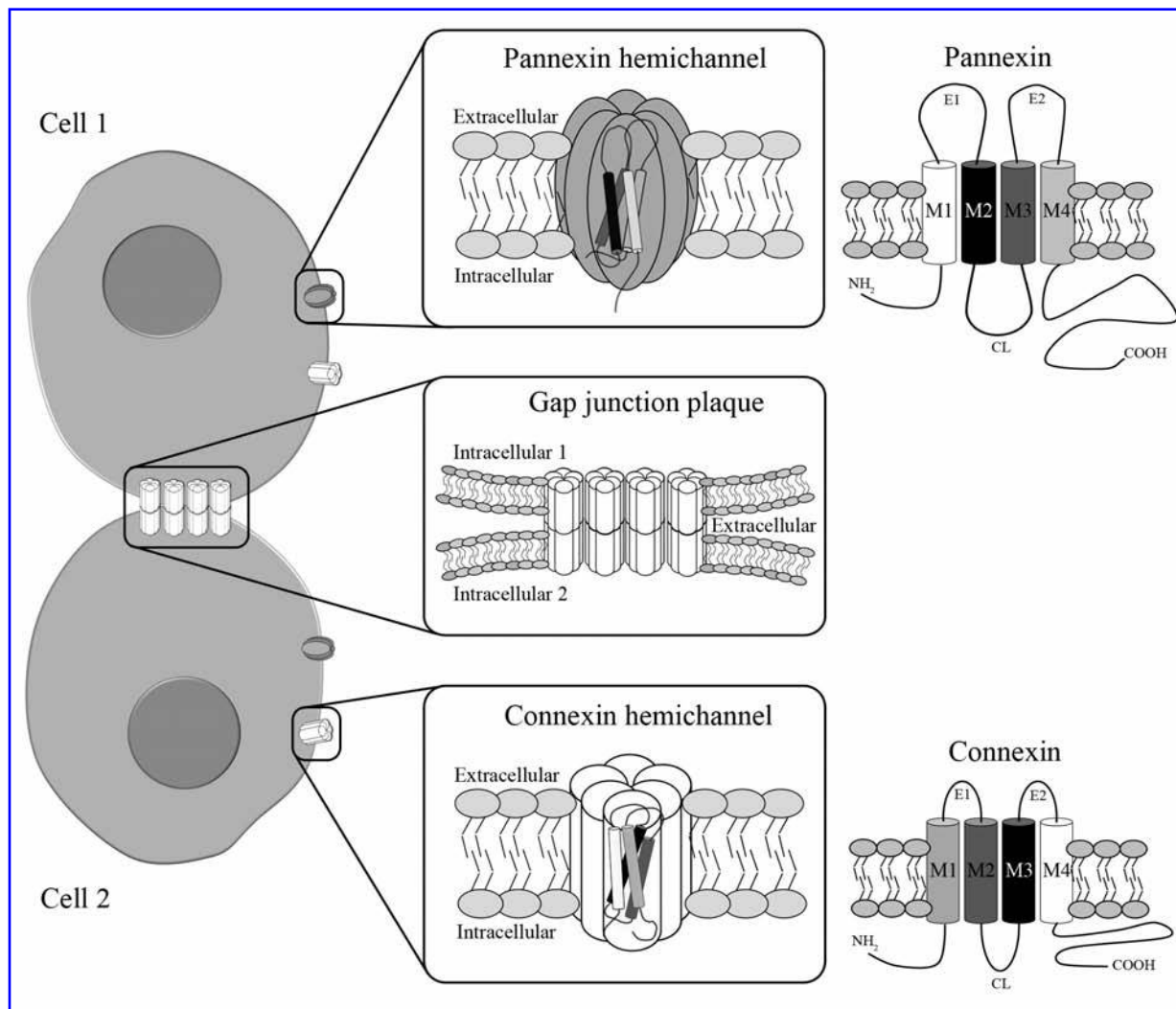[5]Núcleo Milenio Inmunología e Inmunoterapia.

FIG. 1.   Scheme showing the membrane topology of pannexin, connexin, hemichannels, and gap junction channels. *Top and bottom right* correspond to pannexin and connexin in a plasma membrane, respectively. Both protein types have four transmembrane domains (M1–4) with amino (—NH2) and carboxy (—COOH) termini on the cytoplasmic side, two extracellular loops (E1 and E2), and one cytoplasmic loop (CL). *Top and bottom center* show hemichannels formed of six pannexin or connexin subunits each. The *middle center* shows an aggregate of connexin gap junction channels, a section through a gap junction "plaque", at a close contact between cells 1 and 2, as shown in the *left*. Each gap junction channel is formed by two hemichannels docked together (and rotated 30° with respect to one another). Each cell contributes one of hemichannels.

open and close infrequently, but possibly enough to mediate paracrine cell interactions (Fig. 1). However, GJCs and hemichannels may exert opposite effects on neuronal viability, since overactivated glial cells show enhanced hemichannel activity (314) that very likely worsens their neurotoxic effects (352).

Gap junctions are plasma membrane specializations formed by the aggregation of tens to thousands intercellular channels that allow direct but selective cytoplasmic continuity between communicating cells. Each GJC spans the appositional plasma membranes of contacting cells and is formed by the serial docking of two hemichannels or connexons (Fig. 1). Each hemichannel is composed of six protein subunits termed connexins (Cxs), a family of highly conserved proteins encoded by at least 21 different genes in humans (388). Cxs are abundantly expressed in cells of the CNS, and they are named after their predicted molecular mass expressed in kDa, so that Cx43 has a molecular mass of ~43 kDa.

Gap junctions allow the intercellular exchange of metabolites, such as ATP, ADP, glucose, glutamate, and glutathione, and second messengers including cAMP and inositol 1,4,5-triphosphate (128, 129, 170, 199, 265, 296, 328) and other molecules including antigens (236, 263). In addition, GJCs permit the intercellular spread of electrotonic potentials in excitable and nonexcitable tissues (60, 200, 319). All these functions depend on the protein subunit composition of GJCs which defines their conductance and permeability properties. While some GJCs are more permeable to anions, others show preference for cations or exhibit little charge selectivity (57, 95). Consequently, the potential physiological roles of GJCs are limited by the importance of signals and metabolites that can pass through them. Moreover, gap junctions can have an adhesive role (96, 208), and also may act through protein–protein interactions in the cytoplasmic face (125).

Recently, the presence of "free" hemichannels at the unapposed plasma membrane (Fig. 1) has been demonstrated

using several experimental approaches (329). Exogenous expression systems have been used to study the electrophysiological and permeability properties of hemichannels, and identify mechanisms that control their activity. So far, all studied Cxs (Cxs 23, 26, 30, 30.2, 31.9, 35, 37, 38, 43, 44, 45, 45.6, 46, 48.5, 50, 52.6 or 56) expressed in exogenous systems, generate nonselective currents attributed to hemichannel openings (316). Most systems show little hemichannel activity in the presence of physiological $[Ca^{2+}]_o$ (1–2 mM), but this activity can be markedly increased after removal of extracellular divalent cations. Although this regulation has been proposed to control opening of hemichannels under normal conditions (298) it might be more prominent under pathological conditions. In agreement with this possibility, an ~10-fold reduction in extracellular concentration of divalent cations (Ca^{2+} and Mg^{2+}) occurs in ischemic brain (187, 188, 210, 264). Moreover, in low $[Ca^{2+}]_o$, biologically relevant signaling molecules (*e.g.*, ATP, glutamate, glutathione, NAD^+, and PGE_2) can be released through hemichannels (49, 72, 350, 396).

Opening of hemichannels in the presence of physiological concentrations of extracellular divalent cations (Mg^{2+}/Ca^{2+}) occurs in cortical astrocytes under metabolic inhibition (62), after oxygen and glucose deprivation (Orellana JA, Velarde V, Giaume C, Bennett MVL, and Sáez JC, unpublished data) or after exposure to pro-inflammatory cytokines (314). In addition, the activity of hemichannels composed of Cxs 43 or 45, but not Cx26, is enhanced by fibroblast growth factor-1 (FGF-1), mainly due to an increase in surface levels of hemichannels mediated by a rise in the $[Ca^{2+}]_i$ (331). A parallel reduction in cell–cell communication through GJCs occurs under the latter conditions (62, 63, 65, 91, 105, 164, 204, 227, 235, 243, 253, 266, 313–316, 331).

More recently, another gene family encoding a set of membrane proteins, named pannexins (Pxs), has been identified. Pxs have similar functional properties to Cxs and they have been found in various CNS cell types, mostly in neurons (see below). So far, three Px isoforms were discovered in mouse and human. Cxs and Pxs share similar topology, with four α-helical transmembrane domains connected by two extracellular loops and both intracellular N- and C-termini (Fig. 1). However, there is only 16% overall identity when their full-length amino acid sequences are compared.

Here we summarize the current knowledge on the functional modulation of hemichannels and GJCs in brain cells during inflammation, and we discuss its possible role in neurodegeneration.

Where are Connexins and Pannexins in the Mammalian CNS?

Pattern of connexin localization in the CNS

The expression of different Cxs varies according to the developmental stage, cell type and brain region (24, 30, 58, 84, 202, 230, 249, 251, 269, 333, 347, 369). Cx gap junctions form the most common type of electrical synapse between neurons in mammals and other vertebrates (Fig. 2A). Although early on the history dye transfer was found in crayfish septate axon, electrical synapses were first characterized by electrophysiological recordings and were later identified by electron microscopy of thin sections and freeze-fracture replicas in vertebrates (27). Despite the detection of Cxs 26, 30.2, 31.1,

32, 37, 40, 43, 47, and 57 in different neuronal types at different developmental stages (30, 58, 186, 202, 230, 249, 251, 347, 369), only Cxs 36 and 45 have been reproducibly identified at the ultrastructural level in neuronal plaques of the adult rat brain (116, 303, 305–308). In fact, using immunogold labeling of freeze-fracture replicas, Cx36-based neuronal gap junctions were demonstrated in inferior olive, spinal cord, retina, olfactory bulb, visual cortex, suprachiasmatic nucleus, and locus coeruleus, whereas Cx45-based neuronal gap junctions have been found in the retina and olfactory bulb (116, 171, 303, 305–308). Also, functional Cx57 GJCs are expressed between horizontal cells, and horizontal cell receptive fields are reduced in Cx57-deficient mice (152, 338). These findings are in agreement with demonstration of electrical coupling and detection of the Cx type in the corresponding brain regions (27). Cx36 knockout mice show a selective impairment of the hippocampal gamma oscillations (50). In addition, deficit in electrotonic neuronal coupling, behavioral impairments, and disrupted circadian rhythms in Cx36 knockout mice have demonstrated the functional relevance of Cx36 in adult brain (113, 154, 216).

So far, only one report has provided functional evidence supporting the existence of functional Cx36 hemichannels in neurons. In this study, cultured cerebellar granule cells expressing Cx36 show prominent ATP release after depolarization, which is blocked with Cx (but not Px) hemichannels blockers or down regulation of Cx36 with siRNA (333).

Cortical astrocytes express Cxs 26, 30, 40, 43, and 45 (81, 255, 256, 308), but only Cxs 26, 30, and 43 show colocalization at ultrastructurally-defined astroglial gap junctions (254, 308) (Fig. 2B) Cxs 30 and 43 have been detected in astrocytes of the visual cortex (318) and in astrocytic gap junctions of the CA1 and CA2 areas of the hippocampus (34). Nevertheless, single channel conductance studies of GJCs in cultured astrocytes reveal only currents consistent with those found in Cx43 transfectants (51, 82, 120, 191, 241). Cx26 is expressed by cultured astrocytes but is not detected at their cell–cell contacts areas (226). Moreover, cultured astrocytes from Cx43-deficient mice do not form functional gap junctions (261). Thus, Cx43 is thought to be the main functional Cx in cultured astrocytes. However, astrocytes co-cultured with neurons have been shown to express Cx30 (322). Gap junctional communication between astrocytes and neurons in adult mammals is still controversial (Fig. 2H). Although dye transfer and electrical coupling between astrocytes and neurons occurs in the locus coeruleus (11, 369) and in astrocyte–neuron co-cultures (114, 115, 323), there is still no evidence of ultrastructurally-defined neuroastroglial gap junctions *in vivo* (305). The discrepancy between the functional and the morphological observations might be related to the presence of small or less compact junctions that are difficult to identify with ultrastructural techniques.

Oligodendrocyte precursor cells become dye coupled after acquiring the commitment to the oligodendrocyte linage (381). Although there is no ultrastructural evidence of gap junctions between oligodendrocytes in the adult brain (304, 309), gap junctional communication between oligodendrocytes of the gray matter, but not in the white matter of the encephalon and spinal cord, has been consistently reported (80, 176–178, 245, 277, 375). Oligodendrocytes of the gray matter express Cxs 29, 32, 36, 45, and 47 (9, 80, 189, 251, 274, 277, 309, 346, 347), and are exten-
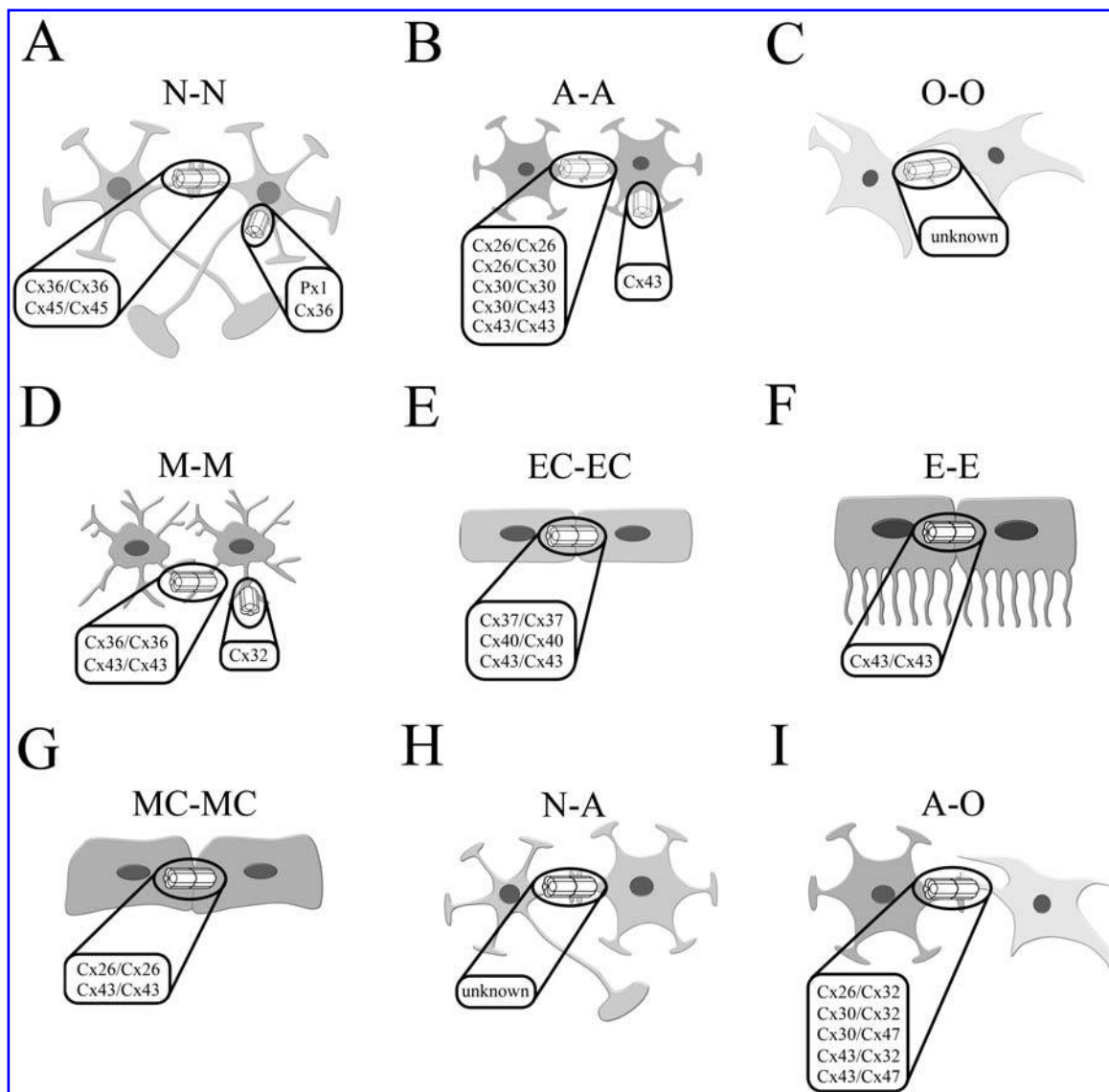
FIG. 2. Cellular distribution of pannexin and connexin hemichannels and connexin gap junctions in brain cells. This figure includes only those cases in which the available information has been obtained *in vivo* and/or *in vitro* with more than one experimental approach. Homocellular (**A–G**) and heterocellular (**H** and **I**) connexin gap junction channels and connexin and pannexin hemichannels (**A**, **B**, and **D**) are indicated within the *encircled regions*. Heterotypic gap junction channels (*i.e.*, with different connexins in the two hemichannels) are indicated in **B** and **I**. Neuron (N), astrocyte (A), oligodendrocyte (O), microglia (M), endothelial cell (EC), ependimocyte (E), and meningeal cell (MC) are denoted in panels **A–I**.

sively coupled to astrocytes via gap junctions (269). By double immunolabeling Cx32 is co-localized at heterocellular contacts with astrocytic Cxs 26, 30, 43, and Cx47 (10, 205) (Fig. 2). Recently, Orthmann–Murphy *et al.* (269), using electrophysiological recordings and immunolabeling, demonstrated heterotypic GJCs formed by Cx47/Cx43 and Cx32/Cx30.

In the adult rat brain, a few microglial cells express low levels of Cx43 (102), and primary cultures of resting microglia (which may be partially activated), express low levels of Cx36, Cx43 and Cx45 (88, 102, 274) (Fig. 2). After microglial activation with cytokines or peptidoglycan (PGN), Cx32 and Cx43 levels increase (102, 119, 361), which is accompanied by augmented functional gap junctions that are absent in microglia of Cx43 knockout mice, suggesting that under those conditions Cx43 is the main microglial gap junction protein (102, 119). In resting heterocellular cultures, approximately one-third of microglial cells show low-strength electrical coupling with neurons through GJCs formed by Cx36 (88). Cx36 has been proposed to form heterocellular gap junctions with neurons and might provide a new mechanism for transfer of death signals from microglia to neurons (88). Cx32 forms hemichannels through which neurotoxic glutamate concentrations can be released (361). Since Cx hemichannels are permeable to ATP (78, 172), it is possible that in microglia they provide a pathway for ATP release which stimulates microglial migration to the sites of injury (76).

The blood-brain barrier (BBB) is another potentially important player in neurodegeneration because it contains the first cellular component affected by blood-borne pro-inflammatory agents and can also be influenced by pro-inflammatory agents generated in the brain parenchyma. *In vivo*, endothelial cells of the BBB express Cx43 and Cx40 (98, 211) (Fig. 2). Cell lines derived from the BBB are well coupled and intracellular photoliberation of inositol-1,4,5-trisphosphate induces Ca^{2+} waves that depend on extracellular ATP released through Cx hemichannels (44). Another functional interaction of Cx40 and Cx43 was reported in primary cultures of porcine BBB endothelial cells, in which these Cxs interact with tight junction proteins occludin, claudin-5, and ZO-1 (250). In this system, oleamide and β-GA, two gap junction blockers, permeabilize the barrier established by tight junctions as determined by measurements of transendothelial electrical resistance and mannitol and inulin paracellular flux (250). Cx37 and Cx43 also form myoendothelial gap junctions (137) that enable electrical coupling between muscle and endothelial layers and facilitate the synchronization of calcium waves and vasomotor activity along arterioles (137). Although, there is no *in vivo* evidence of direct intercellular coupling between astrocytes and endothelial cells at the capillary level, which might be prevented by the basal lamina between the two cell types, *in vitro* studies demonstrate the presence of weak electrical coupling between astrocytes and BBB endothelial cells, which is associated with propagation of both gap junction dependent and independent Ca^{2+} waves (43).

Several other brain cell types including ependymocytes, tanycytes, and leptomeningeal cells can also form gap junctions, but their possible role in brain diseases remain largely unexplored. Ependymocytes are glial cells that line the brain ventricles and the central canal of the spinal cord. They are highly coupled through gap junctions identified at the ultrastructural (304) and functional level (41, 130). Both Cx26 and Cx43 have been consistently detected in ependymocytes (84, 130, 393, 394), whereas presence of Cx30 in these cells has been controversial (190, 256). Interestingly, ependymocytes form Cx43 gap junctions with astrocytes *in vivo* as demonstrated in ultrastructural studies (304). Gap junctions between tanycytes, which are specialized ependymocyte, have been identified at the ultrastructural level (299) and primary cultures of these cells are dye and electrically coupled (162). To our knowledge, the Cx type expressed by tanycytes and the functional role of these junctions remain unknown. Gap junctional communication is wide spread in the developing and adult meningeal membranes, and cultured leptomeningeal cells are strongly dye coupled (83, 349). In cultured cells, dye transfer probably occurs through GJCs formed by Cx26, Cx30, and Cx43 that are expressed at high levels in these cells (237, 256). *In vitro* studies have also identified intercellular communication mediated by gap junctions at heterocellular meningeal cell–astrocyte contacts (132), but their functional significance remains speculative.

Pattern of pannexin localization in the CNS

Pxs are found throughout in the animal kingdom in Cnidaria as well as Bilateria. Three genes have been identified in mammals, termed Pxs 1, 2, and 3, (21, 48). They are homologous to innexins, a term used for gap junction proteins in invertebrates. Primary sequence analyses indicate a membrane topology of Pxs like that of Cxs (Fig. 1) (273). Information on the biosynthesis and assembly of Px channels is still lacking.

Px1 is widely expressed in mammalian tissues (21). In the brain, several regions including cortex, striatum, olfactory bulb, hippocampus, thalamus, inferior olive, inferior colliculus, amygdala, spinal cord, retina, and cerebellum show Px1 immunoreactivity (48, 311, 399). At the cellular level, Px1 has been localized in different neuronal types, including olfactory bulb mitral cells, Purkinje cells, and dopaminergic, cholinergic, and glutamatergic neurons (48, 311, 399). In the cerebral cortex and hippocampus, Px1 is localized in the postsynaptic cell membranes (406). Only one report showed Px1 presence in white matter (48), but others have failed to detect *in vivo* glial expression of Px1 mRNA (311, 379), except in the Bergmann glial cells of the cerebellum that are Px1 immunopositive (399). Px1 was also detected in cultured astrocytes, immature oligodendrocytes, and neurons under resting conditions using immunofluorescence and Western blot analysis, but it has not been found to locate in the cell surface of neurons and astrocytes (157).

Px2 is expressed exclusively in the brain (273), including the olfactory bulb, hippocampus, amygdala, superior colliculus, substantia nigra, cerebellum, hypothalamus, and spinal cord (48, 400). Under resting conditions, hippocampal astrocytes do not express Px2. However, Px2 is expressed in hippocampal astrocytes several hours after ischemia/reperfusion (400). It remains unknown whether it forms functional GJCs and/or hemichannels.

The extent to which Pxs can form functional GJCs remains unclear. Overexpression of Px1 in paired Xenopus oocytes, LNCaP, or C6 glioma cells increases intercellular coupling (48, 196, 373). However, other groups report that expression of Px1 in Xenopus oocytes, or N2A, Neuro2a, or HeLa cells does not induce intercellular communication (35, 157, 284). In addition, Px2 or Px3 do not induce intercellular currents in the oocyte expression system (48), and Px3 expression does not mediate the formation of detectable gap junctional communication in N2A cells (284). Up to now, there is no evidence of gap junction formation by endogenous Px1 in cultured neurons and astrocytes (157). However, functional Px1, Px1/Px2, and Px3 hemichannels have been demonstrated in several exogenous expression systems (20, 22, 47, 48, 215, 282, 284). Moreover, endogenous Px1 hemichannels have been reported in hippocampal neurons (364), but not in cortical astrocytes (157).

Activation of Px1 hemichannels occurs in hippocampal neurons submitted to glucose/oxygen deprivation (364). Moreover, ATP can increase Px1 hemichannels function through $P2X_7$ or P2Y receptors transactivation in erythrocytes and macrophages (215, 282). In addition, the ATP-driven cryopyrin-mediated caspase 1 activation in response to bacterial stimuli requires Px1 activation (173). Activated Px1 hemichannels are permeable to Ca^{2+} and small molecules such as ATP and ethidium (281, 282), calcein (364), sulforhodamine (284, 364), and carboxyfluorescein (215). Px1 hemichannel activity is inhibited by intracellular acidification and classic Cx hemichannel blockers (such as carbenoxolone (CBX), β-GA and flufenamic acid, but they are insensitive to 1-heptanol, 1-octanol, La^{3+} and Gd^{3+} (282).

Glial Cells and Their Implication in Neurodegenerative Diseases

Microglial function and activation

Microglia, the CNS resident macrophages, represent ~12% of total number of brain cells. Under normal conditions, they are sparse within the nervous tissue and present a resting phenotype characterized by a ramified morphology and weak expression of molecules associated with macrophagic function (32). During brain ontogenesis, microglia promote apoptosis in neuronal populations of the cerebellum (223) and cerebral cortex (368). However, they enhance neuronal survival in *in vitro* models (242), possibly by releasing trophic factors, for example, transforming growth factor-β (TGF-β), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Thus, microglia are far from inactive in their resting state. Since in the adult rat brain only a few microglial cells express Cx43 at low levels (102) and primary cultures of resting microglia express low levels of Cx36, Cx43, and Cx45 (88, 102, 274), it is likely that basal microglial function is independent of Cx-based channels. Nonetheless, transient Cx expression and a role in specific cell functions cannot be conclusively ruled out. Moreover, the possible role of Px-based channels in microglial cells (339) remains unexamined.

Although microglial activation, also called microgliosis, plays a relevant role in the innate immune response that should be beneficial, their overactivation may be neurotoxic (32, 291, 351). Microglial activation occurs rapidly in response to acute brain insults, and cells acquire an amoeboid phenotype (397). *In vitro*, microglial activation can be induced by several stimuli, including hypoxia/reoxygenation (195), glutamate (175), lipopolysaccharide (LPS) (220, 324; Froger N, Rétamal MA, Sáez JC, and Giaume C, unpublished data), extracellular proteoglycans (52), prion peptides (23, 46), cell debris (29), amyloid-β peptide (Aβ) (59, 295), α-synuclein (402), and cytokines (244). Nevertheless, molecular mechanisms that determine whether the microglial activation is deleterious or favorable to neuronal survival are not completely understood.

Except for activation via a dectin-1-dependent pathway (337), activation of microglia is most frequently characterized by their synthesis and secretion of pro-inflammatory cytokines such as TNF-α, IL-1α/β, IL-3, IL-6, IL-8, IL-10, IL-12, IL-15, and IL-18 (142), several of which are neurotoxic (229, 363, 376). In this regard, microglia may act on neurons directly through the release of neurotoxic compounds (*e.g.*, cytokines, oxygen- or nitrogen-derived free radicals) or indirectly by promoting astroglial overactivation and dysfunction (see below). In support of the latter, LPS-treated astrocytes are neurotoxic only in the presence of microglia (117, 124, 220, 324). A similar mechanism may operate after microglial activation by other cell insults (*e.g.*, hypoxia/reoxygenation, ischemia/reperfusion, cytokines, Aβ, and α-synuclein). Overactivated microglia may lead to neuronal damage or even death in the normal tissue surrounding a primary affected focus, resulting in propagation of neuronal death. Local vasogenic and cytotoxic edema and damage to microvessels could generate a wave of hypoperfusion contributing to inflammatory response and cell death (see Fig. 8C). Activated microglia release glutamate through hemichannels (361) and their neurotoxic effect is blocked with glutaminase or the gap junction blocker CBX (395). Moreover, glutamate induces astrocyte edema (141, 185) and therefore contributes to the ischemic condition that propagates as a wave to the periphery of the ischemic core (see Fig. 8C). Microglia mediated neurotoxicity is known to be progressive over time (118, 158, 231) and may contribute to the progressive nature of various neurodegenerative diseases. In agreement with this notion, immunosuppression reduces delayed cell death (140).

Which cell type acts as the first threat sensor? Generally, low concentrations of pro-inflammatory molecules activate microglia but do not affect astrocytes or neurons on the same time scale. Concentrations of ~1 μM of Aβ_{25-35} peptide are toxic to microglia *in vitro* (182), but concentrations above 20 μM Aβ_{25-35} are required to induce neuronal death (111, 217, 391). In primary cultures, gap junction-mediated coupling between activated microglia occurs in 30–50% of the cells, is limited to few cells (three–four), and is transient (119). A remarkably similar response has been described for Kupffer cells, the resident macrophages of the liver (103), and other cells of innate immune system such as dendritic cells (64), monocyte/macrophages (101), and granulocytes (45). The transient coupling might be a property of migratory cells and the low number of coupled microglia might be explained by their heterogeneity and various states of activation. Alternatively, the limited gap junctional communication might serve for direct transfer of signals between microglia during a brief period of time, but the functional implications could become evident long after the cells had ceased to be coupled. In other cell types, the formation of GJCs has been shown to require the expression of cell adhesion proteins (166, 248). Activated microglial are known to transiently express surface molecules, such as CD45, CD14, and intercellular adhesion molecule-1 (351), but their possible role in the formation of gap junctions between microglia remains unknown.

The role of gap junctions between activated microglia remains unclear, but studies in other macrophages and/or migratory cells suggest that they might coordinate several cellular functions, including synthesis and release of metalloproteinases (101), diapedesis (293), and antigen cross-presentation (236, 263). In addition, gap junctional communication between activated microglia might enhance the release of cytokines which in turn reduce gap junctions in parenchymal and/or mesenchymal cells. In agreement, LPS does not affect liver parenchymal cells but acts through Kupffer cells to induce downregulation of gap junctional communication between hepatocytes (131). Similarly, in microglia-free astrocyte cultures, LPS does not affect gap junctional communication, but it induces cytokine release from microglia in heterocellular cultures which reduces the astrocytic dye coupling (235, 314). Hence, block of microglial gap junctions might diminish the inflammatory response and consequently limit neurodegeneration. A major current limitation is that all available blockers would also block astroglial gap junctions which might induced adverse neuronal effects (see below). Alternatively, specific blockers of hemichannels that did not block GJCs might reduce the neurotoxic consequences of microglial activation.

Astroglial function and dysfunction

Over the last decade, the perspective on astrocytes, the most abundant brain cells, has changed from supporting cells to a cell type with multiple homeostatic properties. They do not only organize the structural architecture of the brain but also organize its communication pathways and plasticity (97, 262). An important feature of astrocytes is that they are highly coupled to each other, forming extensive intercellular networks (121) (Fig. 3 and Fig. 4). In specific brain area each tri-dimensional astroglial network is poorly coupled via gap junctions with other neighboring networks (155). Domains of resulting intercellular networks take up local extracellular K^+ accumulated during episodes of high neuronal activity, and the excess of intracellular K^+ is dissipated by simple diffusion towards network areas with lower $[K^+]_o$ (Fig. 3). Depolarization spreads electrotonically in the coupled network; K^+ ions are the major charge carrier in the cytoplasm and move in where extracellular K^+ is high and out where it is low. Consequently, action potential depression is prevented in the area with high neuronal activity

(201). The hippocampus of mice with coupling-deficient astrocytes retains a large capacity for K+ clearance, indicating that gap junction-dependent processes only partially account for the K^+ buffering (383). The hippocampal CA1 is the most sensitive CNS region to global ischemia, and dysfunction of Cx-base channels different of gap junctions or hemichannels may play a more prominent role in this brain area.

Astrocyte networks also contribute to maintaining H^+ homeostasis (73) and preventing glutamate excitotoxicity in the brain (74, 334, 386) (Fig. 3). Glutamate accumulated in astrocytes is transformed to glutamine by glutamine synthase, and glutamine can be transferred to neurons to be recycled as a precursor of glutamate and/or GABA (148) (Fig. 3). In addition, glutamate and glutamine have been reported to permeate GJCs in cultured astrocytes (122). In the hippocampus, astroglial cells expressing AMPA-type glutamate receptors are not coupled via gap junctions, while those expressing glutamate transporters are very well coupled (383). A direct relationship between the expression of astroglial Cx43 based channels and expression of glutamate transporters has been recently demonstrated (107), suggesting
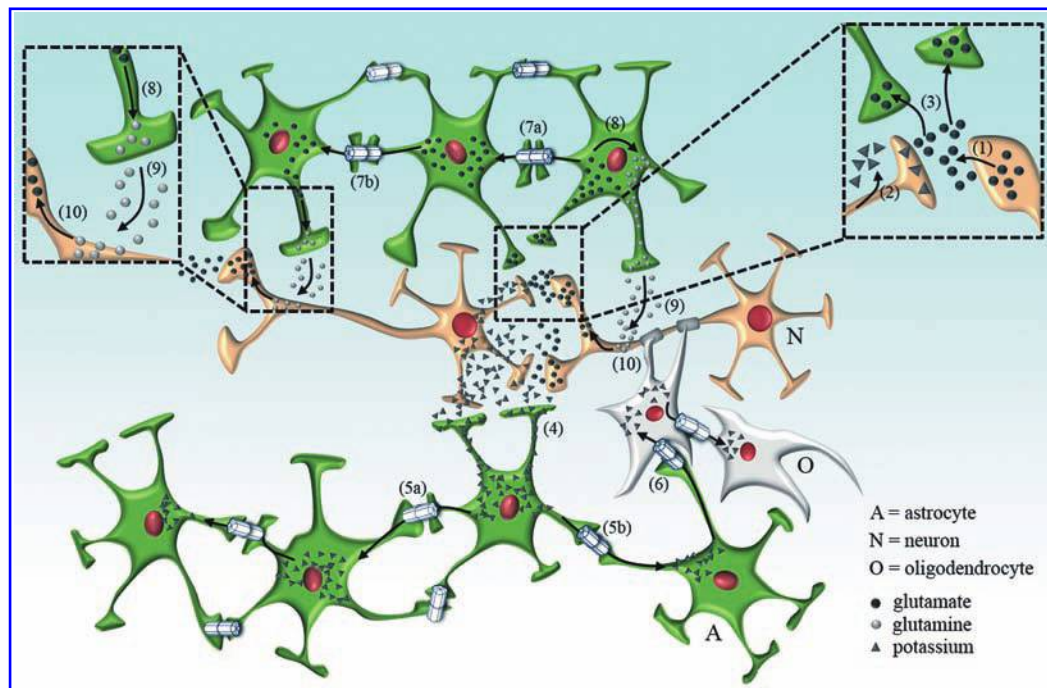

FIG. 3. Role of astroglial gap junction communication in K^+ spatial buffering and the tripartite chemical synapse. Glutamate released from presynaptic neurons (**1**) binds to ionotropic glutamate receptors, triggering a postsynaptic potential in the postsynaptic neuron and promoting the K^+ release (**2**) during repolarization that is more prominent if action potentials are elicited (*Box, enlarged in the right inset*). Astrocytes surrounding the synapses take up glutamate (**3**) through EAAT1 and EAAT2 transporters. During high rates of neuronal activity, K^+ accumulates in the extracellular space, and then is taken up by astrocytes (**4**) through at least inwardly rectifying potassium channels and Na^+/K^+-pumps. K^+ that accumulates inside astrocytes diffuses to neighboring astrocytes (**5a** to **5b**, follow *arrows* in astrocytes *at the bottom*) and oligodendrocytes (**6**) via gap junction channels, a process termed "spatial buffering". Spatial buffering is contributed to by depolarization in regions of K^+ accumulation; the increased positivity causes a current to flow out through membrane that is less depolarized. This outward flow is carried by K^+, which is also the major charge carrier in the cytoplasm. Similarly, glutamate taken up by astrocytes diffuses (**7a** to **7b**, follow *arrows* in astrocytes *at the top*) to neighboring astrocytes through gap junction channels. The glutamate is metabolized to glutamine (**8**) by glutamine synthetase and released to the extracellular milieu from which it is taken up by neurons (**9**) (*Box, enlarged in the left inset*). In neurons, glutamine is transformed to the neurotransmitter, glutamate (or GABA) (**10**). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).
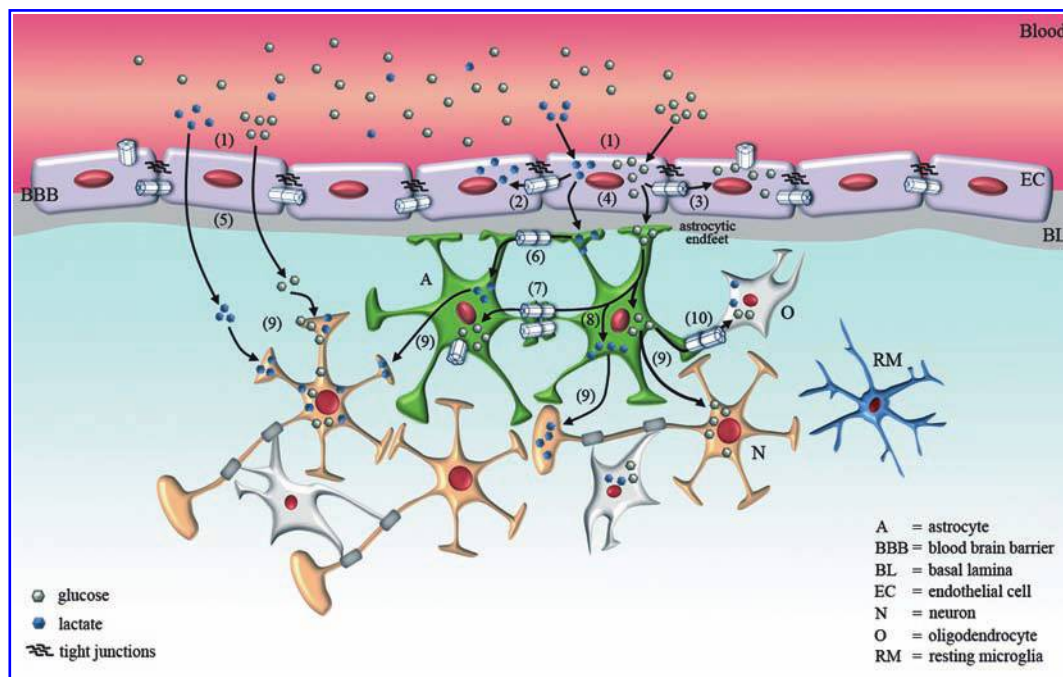
FIG. 4. Role of connexins in neuron/glial metabolic coupling. (1) Under normal conditions, endothelial cells of the blood brain barrier (BBB) take up blood-borne glucose and lactate through GLUT-1 and monocarboxylate transporters, respectively. Both lactate (**2**) or glucose (**3**) may diffuse through gap junction channels between adjacent endothelial cells. Both metabolites are eventually taken up by astrocytic endfeet (**4**) or released to the interstitial space (**5**). Glucose (**6**) and lactate (**7**) can diffuse through astrocytes and their gap junctions with neighboring astrocytes to reach relatively distant areas. Glucose can be metabolized to lactate by astrocytes (**8**), and both can be released into the extracellular space and taken up by neurons (**9**). Astrocytes may transfer lactate and/or glucose to oligodendrocytes through heterocellular gap junction channels between them (**10**). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

that both glutamate transport pathways co-exist and might be under the control of the same transcription factors. Moreover, intercellular astrocyte communication is enhanced in a concentration-dependent manner after elevating extracellular K^+ concentrations (77), suggesting that astrocyte depolarization might enhance gap junction-dependent neuroprotective functions (*e.g.*, spatial buffering capacity and intercellular signaling).

Astrocytes release various neuromodulators and transmitters or so-called "gliotransmitters", which include glutamate (276), GABA (7), ATP (297), taurine (219), adenosine (8), D-serine (240, 272, 332), and eicosanoids (247). The mechanisms underlying the gliotransmitter release are not clear, although a number of studies demonstrate release via hemichannels (350, 396) and vesicle-mediated exocytosis (42, 56, 276). Moreover, the activity of these secretory mechanisms is enhanced by elevation of $[Ca^{2+}]_i$ (276, 294). For example, neural stimulation can increase $[Ca^{2+}]_i$ in astrocytes via ionotropic and/or metabotropic ATP, glutamate, $GABA_B$, and acetylcholine receptors (380). Increased $[Ca^{2+}]_i$ might induce release of gliotransmitters from astrocytes, which increase $[Ca^{2+}]_i$ of neighboring neurons, for example through an NMDA receptor-dependent pathway (276). Moreover, the increase in astroglial $[Ca^{2+}]_i$ does not just occur locally but also can propagate as a Ca^{2+} wave through the astroglial network and affect the neural activity in distant locations (330).

A recently discovered astrocyte function is their involvement as signaling pathway in the neurovascular coupling (Fig. 4). This function is drastically reduced by inhibition of Cx-based channels, which mediate the propagation of Ca^{2+} waves and release of vasoactive arteriolar factors. Thus, astroglial cells have been proposed as sites for production of vasoactive factors in response to neuronal activity causing rapid and localized changes in cerebral blood flow upon changes in energy demand (239, 392, 407) (Fig. 4). Thus, astrocytes may nourish neurons by controlling the glucose availability through the regulation of blood flow as well as by generation of lactic acid in anaerobic metabolism for more efficient ATP generation in the tricarboxilic acid cycle (283) (Fig. 4). Physiopathological conditions that reduce the expression of Cx based channels in astrocytes should also have a negative impact in glucose tissue supply (Fig. 4), increasing the neuronal susceptibility to injuries.

Two mechanisms underlying Ca^{2+} wave propagation have received strong experimental support. One is mediation by the intercellular transfer of IP_3, which spreads through GJCs and causes release of Ca^{2+} from intracellular stores (38, 123). The other is mediation by extracellular ATP acting as a paracrine messenger (66, 67, 123, 135, 143). Thus, ATP released from an astrocyte can activate purinergic receptors (P2X and P2Y) in surrounding cells, leading to increased $[Ca^{2+}]_i$ (67).. Release of ATP from these neighboring cells and then their neighbors allows the spread of a calcium

waves to increase $[Ca^{2+}]_i$ of distant cells. The mechanisms of ATP release from astrocytes are not well defined, but may include vesicle-mediated exocytosis (42, 56) and release through Cx43 hemichannels (62, 350). In addition, cultured astrocytes express Px1, which under resting *in vitro* conditions is not present in the surface membrane (157), but in particular conditions Px1 hemichannels may be inserted and provide a conduit for ATP release (20, 350). Moreover, a recent report describes the activation of an astrocytic macropore termed a maxi-anion channel in response to oxygen–glucose deprivation, which is resistant to several Px/Cx hemichannel blockers, but sensitive to Gd^{3+} and arachidonic acid (213). Further experimental confirmation of these findings and development of experimental paradigms to identify each channel type (formed of Cxs, Pxs, or unrelated proteins) will help to determine the contributions of each to Ca^{2+} wave propagation.

Cultured astrocytes express CD38, an integral membrane ectoenzyme also facing the lumen of the endoplasmic reticulum, and under resting conditions release NAD^+ through hemichannels, possibly formed of Cx43 (377). CD38 may then generate cyclic ADP ribose for autocrine and paracrine signaling. It is possible that enhanced activity of hemichannels also enhances this intercellular signaling pathway.

Astrocytes are also involved in maintaining the redox potential. They produce glutathione, an important free radical scavenger, and transfer it to neurons (90, 374). This process is essential for protecting neurons from free radicals under adverse conditions such as ischemia and acute or chronic inflammation (374). Recently, hemichannels were demonstrated to act as an astrocytic pathway for release of glutathione to the extracellular milieu (302, 352). In addition, Cx43 GJCs are permeable to glutathione; thus, glutathione levels may be similar in a functional network. Nevertheless, during acute inflammation, astrocytes located within the affected area are likely to show enhanced hemichannel activity that would favor the release of glutathione to the interstitial space, and the reduced gap junctional communication will impair the replenishment of glutathione by diffusion from astrocytes located in less regions of the network. Because astrocytes perform numerous homeostatic functions (221) diverse insults that affect them can critically affect neuronal susceptibility to the same or different insults (14). Indeed, in ischemic episodes the metabolism of both neurons and astroglia is affected (138), and astrocytic functions such as glutamate uptake, dissipation of K^+, and/or free radical scavenger activity are impaired contributing to the development of neuronal damage (276, 359).

In several CNS disorders, astrocytes can be activated by various inflammatory mediators, including cytokines (110, 365), lipoteichoic acid (181), LPS (233, 405), and Aβ (384). Some suggested mechanisms that underlie this effect include release of high levels of NO, peroxynitrite, glutamate, TNF-α, and IL-1β (18, 146). Astrocyte activation involves changes in cellular phenotype and gene expression which can be deleterious for neurons (18, 39, 149), Accordingly, activated astrocytes increase neuronal death *in vitro* (71, 156, 180).

A recent study revealed novel astrocytic gap junction functions. Uninfected astrocytes coupled through gap junctions with HIV-infected astrocytes become apoptotic (100). Gap junction blockers abolished apoptosis in uninfected astrocytes, supporting the role of these channels in amplifying cell death. Therefore, this is a novel mechanism of toxicity within the brain, triggered by low numbers of HIV-infected astrocytes and amplified by gap junctions or hemichannels that might contribute to neurodegeneration in AIDS (100). It would be of interest to study the role of Cx-based channels in HIV-infected astrocytes co-cultured with microglia or treated with inflammatory mediators that would mimic the AIDS inflammatory condition. A general cellular reaction observed after brain injury is that astrocytes adjacent to the damaged tissue undergo a process of hypertrophy and proliferation, called reactive astrogliosis (280). These astrocytes might be well coupled via gap junctions since Cx43 mRNA and protein levels are increased in the glial scar of photothrombotic ischemic lesions (145). Moreover, during the astrogliosis response observed 24 h after reperfusion, *de novo* synthesis of Px2 occurs in hippocampal rat astrocytes (400). The functional role of Cx43 and Px2 expression during astrogliosis remains unresolved.

Effect of Free Radicals on Gap Junction Channels and Hemichannels, and Possible Implications for Neurological Disorders

CNS cells are highly susceptible to oxidative insults, due to their high oxygen consumption, high levels of peroxidizable fatty acids, and poor antioxidant defenses (54). Oxidative stress occurs during brain invasion by pathogens, as well during partial ischemic episodes and after ischemia/reperfusion (87, 94, 133, 238). Elevated reactive nitrogen species are also neurotoxic and their generation is particularly exacerbated in cells under metabolic stress (127, 167, 403). Moreover, a large body of evidence suggests an important role of increased ROS production leading to oxidative stress as well as of increased reactive nitrogen species (RNS) in aging and neurodegenerative disorders (55, 292). The increased oxidative alterations of Aβ in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD), and SOD1 in an animal model of amyotrophic lateral sclerosis (ALS) might result in altered protein folding and impaired protein degradation. One consequence of intracellular protein aggregation is ER dysfunction (ER stress), which leads to mitochondrial uncoupling and contributes to excessive production of ROS, thus causing or worsening oxidative stress (54). In fact, postmortem brain tissues from patients with neurodegenerative disorders, including PD, AD, and ALS, display increased indices of ROS in affected brain regions (85, 147, 279).

Membrane damage secondary to ROS-mediated lipid peroxidation can affect the activity of functional membrane proteins (5, 53, 153, 228). Moreover, NO or NO-derived compounds enhance astrocytic Cx hemichannel activity (62, 63, 313, 315) and reduce gap junctional communication (40).

Currently, all the available information on the effect of oxidant agents on gap junctions is only at the functional level. The application of H_2O_2 rapidly increases gap junctional communication, an effect associated with increased death of astrocytes evaluated 24 h later (320). The toxicity is not prevented by gap junction blockers. The effect on gap junctional communication is mimicked by the oxidizing agent diethylmaleate and prevented with reducing agents dithiothreitol (DTT) and *N*-acetyl-cysteine (320). The presence of microglia in astrocyte cultures prevents the effects of H_2O_2 on astrocyte gap junctions and toxicity (320), suggesting that mi-

croglial cells are better equipped with antioxidant mechanisms and might be protective under particular conditions. In contrast, treatment with the NO donor *S*-nitroso-*N*-acetylpenicillamine, the O_2^- (-)-generating system xanthine/xanthine oxidase, and/or peroxynitrite anion inhibits gap junction permeability in rat astrocytes (40). It remains unknown whether Cx-forming gap junctions are modified differentially by different oxidant agents or if hydrogen peroxide and NO oxide-derived compounds cause a differential covalent modification in Cx43 that might explain their opposite functional effect. Moreover, GJCs of normal and injured cells might be oppositely affected by oxidant agents as described for Cx43 hemichannels (315). The opposite effects might be related to the phosphorylation state of Cx43 since in normal cells the protein is phosphorylated, but in ischemic cells it is mainly dephosphorylated (62, 63, 204, 313). For further information on oxidant stress on gap junctions, readers are referred to the reviews published in this issue by Johnson and Koval, Upham and Trosko, and Berthoud and Beyer, respectively.

Gap junctions between astrocytes have been proposed to be neuroprotective during oxidative stress. In agreement with this notion, a transient and pronounced reduction in astroglial dye coupling occurs during metabolic inhibition (227), a condition that induce generation of free radicals; the reduction in intercellular communication is prevented by reducing agents (227). In addition, blockade of gap junctions with 18-α-glycyrrhetinic acid (α-GA) in co-cultures of neurons and coupled astrocytes or in organotypic hippocampal slice cultures after oxidative stress induced by $FeSO_4$ and 4-hydroxynonenal results in a markedly enhanced generation of intracellular peroxides, impairment of mitochondrial function, and neuronal death (31). In contrast, gap junctions could also be deleterious for neurons because they allow the intercellular diffusion of death signals and amplify cell injury and death in astrocytes, a phenomenon known as "*bystander death*" or "glial fratricide" (209). In support of this concept, pretreatment with the gap junction blockers CBX, α-GA, or endothelin, decreases the number of apoptotic neurons in the hippocampus, as compared to the contralateral hippocampus treated with saline in a model of global transient ischemia in rats. A significant reduction in lipid peroxides of the hippocampus treated with CBX suggests that the actions of gap junctional coupling during injuries may be causally related to oxidative stress (285). An alternative interpretation of these findings is that CBX reduces cell death by blocking hemichannels formed by either Cxs or Pxs. Clearly, the molecular mechanisms and biological implications of gap junction modulation in injury by free radicals are still poorly understood and may be related to differences in experimental conditions (*e.g.*, differences in insult intensity and/or duration and differences in cell or tissue type and conditions). In addition, the studies described above did not consider the possible involvement of Cx hemichannels and the differences may be explained by the relative involvement of cell-cell channels and hemichannel.

The number of open Cx43 hemichannels is enhanced in astrocytes under metabolic inhibition (62, 63, 313), a condition that reduces the intracellular redox potential due, in part, to generation of free radicals including nitrogen- and oxygen-derived species (127, 167, 403). The increase of hemichannel activity is abolished by intracellular but not extracellular cystine reducing agents (313), suggesting the involvement of the carboxy terminal cystine residues as sensors of the redox changes. The enhanced hemichannel activity elicited by metabolic inhibition is explained in by an increase in total surface levels of hemichannels, but it is sensitive to cystine reducing agents, suggesting the involvement of a gating mechanism activated by oxidation (62, 313). Application of NO donors enhances astroglial hemichannel activity and induces nitrosylation of intracellular Cx43 cystine residues. S-nitrosylation of Cx43 hemichannels also occurs in astrocytes under metabolic inhibition (313), and thus is likely to occur during ischemia. The enhanced hemichannel activity elicited by NO is not associated with changes in levels of surface hemichannels or changes in the state of phosphorylation of surface Cx43 evaluated by Western blot analysis (313), supporting the involvement of an NO-sensitive gating mechanism in cells under metabolic inhibition. The increased hemichannel activity may compromise the capacity for handling redox changes because Cx43 hemichannels are permeable to free radical scavengers such as reduced glutathione and ascorbic acid (4, 302). Trolox and melatonin, two free radical scavengers, can prevent the increase in hemichannel activity induced by metabolic inhibition in rat astrocytes and blockade of hemichannels delays cell death (62). Activation of hemichannels has also been demonstrated in hippocampal neurons under oxygen–glucose deprivation (401). The effect is mediated by NO and is reversed with DTT (401). Because hippocampal neurons do not express Cx43 but might express Cx36 and/or Px1, it is possible that hemichannels formed by one of these protein subunits are also sensitive to redox changes

How Might Hemichannels and Gap Junction Channels Induce Neuronal Damage in Neurological Disorders?

Cell and tissue responses to injuries depend on properties if the cells (*e.g.*, age, hormonal experience, and stage of cell cycle) and insult (*e.g.*, duration, intensity, and quality). Moreover, tissue responses depend on interactions between their constituent cells, including chemical and electrical transmission as well as paracrine and autocrine signaling (*e.g.*, by cytokines and ROS), possibly mediated by GJCs and hemichannels. Despite the difficulty of assigning contributions to these channels in the pathogenesis of neurodegenerative diseases, recent studies using homo and/or heterocellular cultures have provided clues. In most chronic diseases, additional mechanisms are progressively added to the primary cause and thus, complicating assignment of the contributions of each factor to the final condition. For sake of clarity, in the following sections we first describe acute pathological conditions in which the primary cause or insult is identifiable, and the cell/tissue responses can be attributed to them (*e.g.*, stroke and infection). Then, we described four chronic and progressive neurodegenerative diseases with specific attention to the possible involvement of Cx-based channels and inflammatory responses.

Stroke

The application of a sublethal insult in many cases induces resistance to a subsequent more severe insult, a phenomenon called preconditioning response. Mechanisms of this induced resistance include the release of neuroprotective

agents, but pathways for this release remain unknown. Recently, *in vitro* studies reveal that preconditioning reduces degradation of Cx43, and leads to a marked increase in the number of Cx43 hemichannels in the surface membrane (207). Consequently, more ATP is released which is hydrolyzed to adenosine, a potent neuroprotective molecule (207). In agreement with the possible involvement of hemichannels in preconditioning responses, Cx43 null mice were shown to be insensitive to hypoxic preconditioning but, wild-type littermate mice exhibit a prominent reduction in infarct volume after induction of preconditioning through occlusion of the middle cerebral artery (207). The same year, the involvement of Cx36 hemichannels in preconditioning response of cultured cerebellar granule neurons was demonstrated (333). Most likely these observations will be replicated in numerous cell types that manifest preconditioning and express Cxs and/or Pxs that can form hemichannels at their surface. Cx43 translocation to the mitochondria has been observed in heart during preconditioning (36), but the functional role of mitochondrial Cx43 remains speculative. To our knowledge, similar studies in brain and cultured brain cells have not been reported.

More intense or prolonged ischemia, due to partial or complete blockade of a brain artery, causes detrimental cellular consequences. Among the early cellular changes, the electrochemical gradient across the neuronal and astroglial membrane potential is lost, and both neurons and astroglial cells become depolarized (174). Voltage-gated Ca^{2+} channels are activated and neurotransmitters are released into the extracellular milieu (165, 192). At the same time, the energy-dependent processes such as neurotransmitter re-uptake are reduced which increases accumulation of extracellular glutamate. Consequently, the activation of NMDA and metabotropic glutamate receptors contribute to the increase in $[Ca^{2+}]_i$ (6). It is believed that the increase in $[Ca^{2+}]_i$ activates nuclear and cytoplasmic events that influence the development of cell damage. These changes include the activation of phospholipase A_2, generation of arachidonic acid and metabolism via cyclooxygenase/lipooxygenases leading to increase in formation of free radicals, lipid peroxidation, and plasma membrane damage. All these intracellular Ca^{2+}-dependent events occur with a concomitant reduction in $[Ca^{2+}]_o$, which should enhance opening of Cx43 hemichannels (298), but not Pxs hemichannels (282).

The events described above do not take place uniformly throughout the ischemic brain tissue. In the center, or ischemic core, where there is deficient blood flow, occurs a permanent anoxic depolarization since the beginning of ischemia and cells die quickly. Between the core zone and normal brain tissue is found an area with restricted blood flow and with partial energy supply termed penumbra. Afterwards, this area may undergo excitotoxicity or deleterious secondary phenomena such as propagated depolarization, postischemic inflammation, and apoptosis. After a mild ischemia or in the penumbra zone, specific neurons may die with considerable delay, while other neurons and glia survive. The involvement of hemichannels and GJCs in these events has been discussed previously (63).

In models of ischemia/reperfusion, neurons are the main cell type that dies, in part, because their metabolism is primarily aerobic. However, a prolonged ischemic episode also causes astroglial death (356), and their relative insensitivity

seems to be due to their ability to switch from aerobic to anaerobic metabolism when exposed to hypoxia (288). Under this condition, ischemia is the causal condition but the response can be worsening by conditioning factors. For example, patients or experimental animals with hyperglycemia (a difference in the ischemic episode) and stroke suffer massive death in neuronal and glial cell populations (168, 246). In primary astrocyte cultures, high glucose levels also worsen the reduction in gap junctional communication and increase the enhancement in hemichannel activity (Orellana JA, Velarde V, Bennett MVL, Giaume C, and Sáez JC, unpublished data), suggesting a direct link between astroglial Cx-based channel dysfunction and reduced neuronal and glial viability. Other conditions that might aggravate stroke damage are neurodegenerative diseases such as AD. In an animal model, focal ischemia causes larger infarct volumes in the presence of Aβ (387). A possible mechanism involves Cx-based channels, since Aβ enhances the cytokine-induced reduction in gap junctional communication and increases hemichannel activity in cortical astrocytes (235) (Orellana JA, Sáez JC, and Giaume C, unpublished data).

The enhanced hemichannel activity of astroglia under metabolic inhibition accelerates cell death (62, 63). A similar scenario may occur in other *in vitro* models of ischemia where an increase in Cx or Px hemichannel activity has been observed (203, 316, 341, 364, 401). Demonstration of the relative importance of enhanced hemichannel activity versus reduced gap junctional communication in astroglial as well as neuronal cell viability will require new experimental approaches including drugs that specifically block Cx or Px based cell-cell channels and hemichannels. Currently available reagents are quite unsatisfactory.

In vitro gap junctional communication is only partially inhibited by ischemia or ischemia-like conditions (62, 65, 204, 227), and the remaining gap junctional communication may allow amplification of cellular damage. Ca^{2+} waves are a possible cell death propagation mechanism (209). The first *in vivo* evidence for the involvement of Cx-based channels in the spread of death signals in the CNS was that octanol, a nonselective gap junction and hemichannel blocker, reduces the infarct size after a focal ischemia (310). In addition, damage induced by global ischemia is more pronounced in the CA1 than in the CA3 area, although gap junctions are more common in CA1, and that CA1 damage is reduced by octanol (301).These observations are contradicted by findings obtained in Cx43 knockout heterozygous mouse or mouse astrocytes with the floxed Cx43 gene inactivated by Cre expression, in which focal ischemia causes larger lesions (257, 258, 344). This apparent contradiction might be explained by a differential distribution of Px1 in these neurons (406) where hemichannels could open under ischemia-like insults (364). In addition, ischemia-reperfusion induces more macrophage and microglia recruitment to the injured tissue of Cx43 knockout animals (258), suggesting that more neuronal death leads to more activation of macrophages.

In a rat model of transient global ischemia, pretreatment with compounds that block both hemichannels and GJCs (*i.e.*, CBX, α-GA, and endothelin) reduces the number of TUNEL-positive neurons, as compared to the contralateral hippocampus treated with a saline injection (285). In agreement, the CA1 of mice deficient in Cx32 shows increased sen-

sitivity to global ischemia (268). Cx32 forms hemichannels in activated microglia (361), and in Cx32 knockout animals microglial release of neurotoxins might be reduced. An additional complication to understanding the role of Cx-based channels in stroke is possible change in Cx expression. During ischemia expression of Cx32 and Cx26, but not Cx43, increases selectively in the hippocampal area CA1 before the onset of neuronal death (268). Moreover, the infarct penumbra in $Cx43^{+/-}$ mice shows an increase in Cx30 levels as compared to wild-type $Cx43^{+/+}$ mice (257). The significance of these changes in Cx expression remains speculative that each Cx may play different roles at different stages of the insult and its sequelae. So far, most available *in vivo* information has been obtained using different models and conditions which complicate the interpretation. Future studies using

more homogeneous systems and conditions should further understanding to the role of Cx based channels in stroke.

In vitro studies have also contributed to knowledge of the possible regulatory mechanisms and roles of Cx -and Px-based channels in stroke. Ischemia-like conditions induce dephosphorylation of astroglial Cx43 which is associated with a reduction in gap junctional communication in hippocampal slices and astroglial cultures (62, 65, 204) as well as with activation of Cx43 hemichannels. In primary astrocyte cultures, oxygen–glucose deprivation enhances hemichannel activity to an extent dependent on insult duration (316). In hippocampal slices, the reduction in gap junction coupling is associated with a rise in $[Ca^{2+}]_i$ in astrocytes (65) that might activate Ca^{2+}-dependent protein phosphatases. In agreement with the latter interpretation, inhibition of cal-
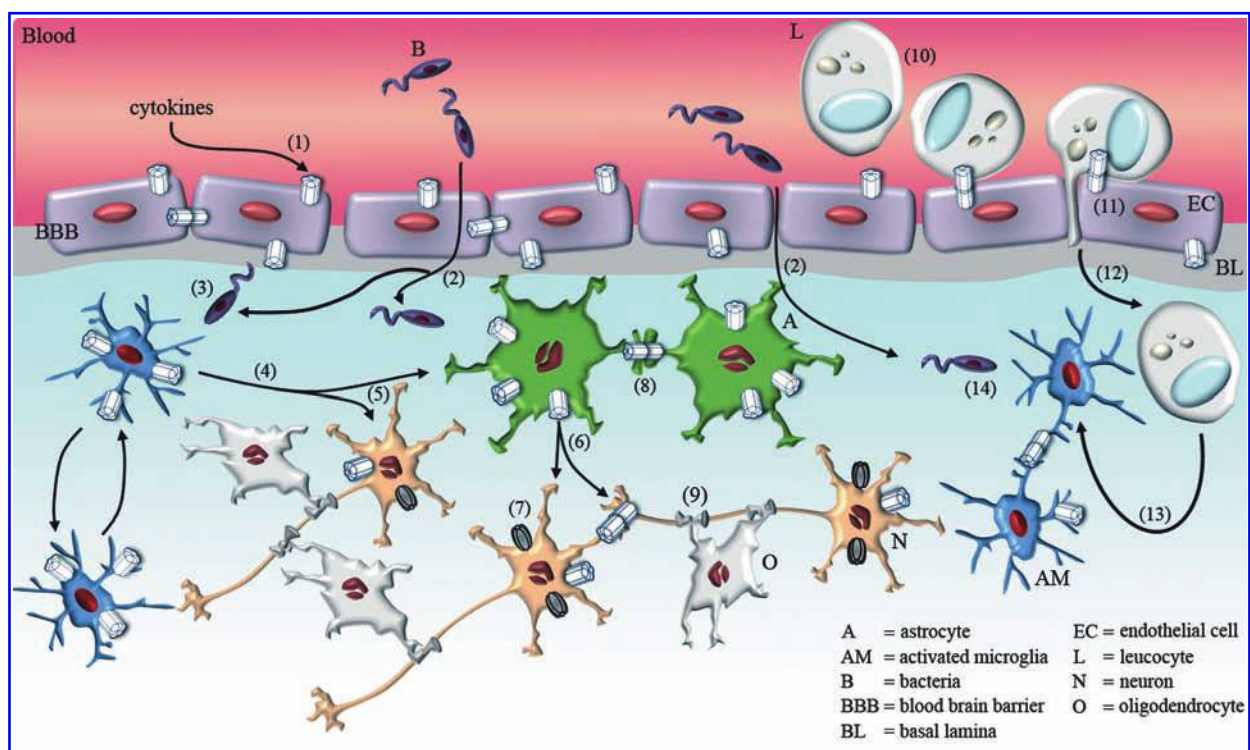

FIG. 5. Connexin based channels in brain cells during bacterial meningitis. During bacterial infection blood levels of cytokine (e.g., TNF-α and IL-β) are elevated. Both, TNF-α and IL-1β enhance hemichannel activity (**1**) of brain endothelial cells. Furthermore, these cytokines induce BBB discontinuity favoring bacterial extravasation (**2**). Once in the interstitium, bacteria and their extracellular wall components such as LPS and PGN are recognized by microglia (**3**) which are activated and release cytokines that further activate them (*reciprocal arrows*). ATP released via hemichannels from microglia (**4**) promotes microglial migration from less affected regions. Activated microglia can also release glutamate through hemichannels and oxygen- and nitrogen-derived free radicals that are neurotoxic (**5**). The enhanced hemichannel activity of activated astrocytes induces neuronal damage through the release of neurotoxic and/or inflammatory compounds such as glutamate and PGE$_2$ (**6**). These compounds may also increase the activity of neuronal pannexin hemichannels (**7**), causing electrochemical imbalance and Ca^{2+} overload in neurons. In contrast to increased opening of hemichannels, astroglial gap junction communication is reduced (**8**) (depicted in the figure as reduction in channel number, but could also be the consequence of a reduction in permeability or open probability), impairing glutamate and K^+ spatial buffering, which enhances neuronal susceptibility to insults. Bacterial meningitis can also induce demyelination (**9**), possibly via microglial cytokine release. Severe inflammation induces recruitment of leucocytes (**10**) to the infected loci. Gap junction communication between leucocytes and endothelial cells (**11**) may contribute to stronger heterocellular adhesion and allow transfer of signals that regulate leucocyte diapedesis across the endothelium (**12**). Activated microglia may perform antigen cross-presentation interaction with infiltrating leucocytes in which gap junctions between them may play an important role (**13**). Direct microglial interaction with LPS or PGN induces gap junction communication between microglia which can coordinate microglial function (**14**). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

cineurin, a Ca^{2+}-dependent protein phosphatase, prevents the reduction in gap junctional communication (63, 204) but not the increase in hemichannel activity (63). Thus, junctional channels and hemichannels are under the control of different mechanisms possibly related to the different molecular compositions of the subcellular compartment where each one is located. It has been proposed that inhibition of astroglial gap junctions reduces neuronal vulnerability to glutamate or oxidant stress or disruption of Ca^{2+} homeostasis (31, 209, 271). However, in these studies the effect of gap junction blockers on hemichannel activity was not evaluated and now it is known that these conditions induce oxidant stress which enhances the activity of Cx43 and Px1 hemichannels (315, 401). In primary cultures of resting astrocytes and neurons Px1, but not Px2, is expressed (157, 400). However, Px1 is not detected at the surface of either neurons or astrocytes (157), suggesting that formation of Px1 hemichannels is hindered. So far, conditions that affect the expression and/or induce insertion of Px1 into the plasma membrane remain unknown.

Bacterial meningitis/encephalitis

Bacterial meningitis promotes inflammation of the pia, arachnoid, and subarachnoid space. Inflammation may also affect the brain parenchyma leading to encephalitis. Bacteria present in the bloodstream induce an innate immune response which produces systemic release of cytokines, mainly TNF-α (86, 317). Then, bacteria colonize and cross the inflamed BBB and components of their wall, such as LPS (19), peptidoglycan (PGN) (19), or streptococcal hemolysin/cytolysin (89), induce BBB activation and permeabilization (112) (Fig. 5). BBB activation is characterized by numerous changes including cytokine production, overexpression of cell adhesion molecules and NO synthesis (112). Changes in the BBB endothelium due to activation facilitate bacterial interaction with endothelial PAF (platelet activating factor) receptor which allows bacterial transmigration (385) into the brain parenchyma where they proliferate (Fig. 5).

The BBB may be a target for inflammatory responses initiated in the brain parenchyma (*e.g.*, inflammation triggered by Aβ) or in the periphery (*e.g.*, sepsis or extensive burn of the body surface) (Fig. 5). Since the BBB restricts movement of substances into or out of the CNS, and many of its functions are likely to be coordinated, it would be of interest to learn how their hemichannels and GJCs are modified by proinflammatory agents or conditions. TNF-α treatment for 2 h reduces gap junction communication and increases basal ATP release in RBE4 cells (370), a cell line derived from rat brain cortical endothelium. In agreement, we found that conditioned medium of microglia activated with LPS for 24 h increases hemichannel activity of RB4 cells in a time-dependent manner (Fig. 6). In addition, 2 h of TNF-α treatment blocks ATP release induced by photoliberation of InsP$_3$ and zero $[Ca^{2+}]_o$, but increases basal ATP release and hemichannel activity in a way not prevented by the gap26 mimetic peptide (370), a purported blocker of Cx43 hemichannels (104). However, gap26 does not block Cx40, which is highly expressed in brain endothelial cells (250), thus the rise in basal activity induced by TNF-α could be related to Cx40 and/or Px hemichannels. Hemichannels and GJCs have not been well studied in BBB cells, but these channels have been
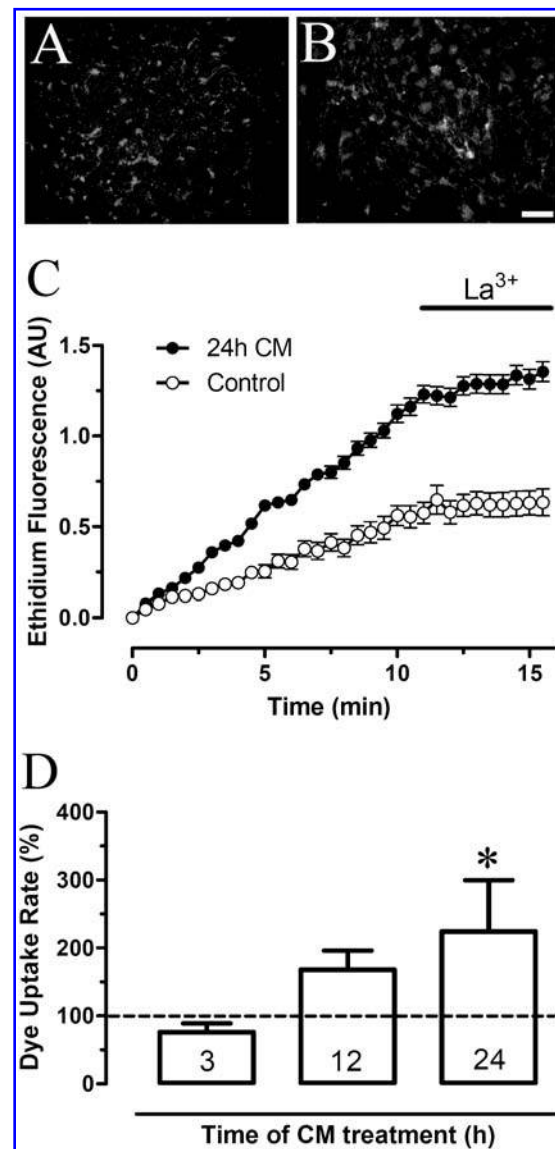

FIG. 6. **Microglial conditioned medium enhances dye uptake in RBE4 cells, a cell line derived from brain endothelial cells.** In RBE4 cells, ethidium (Et) (5 μM) uptake was measured every 30 s as fluorescence emission of Et bound to DNA (518 nm, intensity in arbitrary units, AU). (**A**) Fluorescence micrograph of Et uptake taken after 10 min under control conditions shows little uptake. (**B**) After 24 h of incubation with medium conditioned by microglia treated for 6 h with LPS, uptake is increased after the same exposure to Et (10 min). (**C**) Time lapse measurements of Et uptake in RBE4 cells under control conditions (white circles) and after 24 h of exposure to the conditioned medium of LPS treated microglia (black circles). Under both conditions, Et uptake was blocked by La^{3+} (200 μM) applied after ~11 min. (**D**) Averaged data normalized to control (*dashed line*) of Et uptake rate of RBE4 cells treated for 3, 12, or 24 h with medium conditioned by microglia treated with LPS. Bar = 10 μm.

extensively studied in endothelium of other territories of the circulatory system (69, 108). Readers interested on Cx-based channels in endothelial cells in general are referred to reviews in this issue by Figueroa and Duling (45a) and by Brisset, Isakson, and Kwak (107a) in this issue.

Enhanced endothelial hemichannel activity could enhance release of ATP, which would recruit microglia to the injury site (76). In agreement with a role of hemichannels in ATP release during inflammatory conditions triggered by bacterial infections, *Shigella* infection of epithelial cells promote ATP release through Cx26 hemichannels, resulting in the activation of purinergic receptors on neighboring cells and bacterial dissemination (367). On the other hand, a reduction in gap junction communication between brain endothelial cells may impair astrocyte-endothelial cell calcium signaling (43) and increase BBB permeability by reducing tight junction formation. The latter is in agreement with the observation that block of GJCs increases transendothelial electrical resistance and enhanced paracellular flux of permeability tracers (250).

In the brain parenchyma, bacteria may undergo lysis and release pro-inflammatory and toxic factors such as PGN and LPS (353, 354), while microglia interact directly with intact bacteria (179). Bacterial-derived pro-inflammatory factors such as LPS induce neurodegeneration (295). PGN and LPS stimulate microglial Toll-like receptors (TLRs), induce translocation of nuclear factor (NF) κB (336), and activation of MAPK signaling and transcription of genes encoding inflammatory cytokines (194, 259). Moreover, PGN increases microglial Cx43 mRNA and protein expression which correlates with development of gap junction communication *in vitro* (119). Similarly, treatment with LPS plus IFN-γ increases Cx43 expression in rat microglia and induces gap junction communication (102). In addition, brain stab wounds induce recruitment of Cx43 immunoreactive microglia, suggesting that Cx43 is important for coordinating microglial responses (102). Expression of Cxs and formation of gap junctions, at least at homocellular contacts, might be a common feature of inflammatory macrophagic cells. Cx43 expression has been demonstrated in activated dendritic cells (64, 326), peritoneal macrophages (12, 161), foam cells of atherosclerotic lesions (290), J774 cells (12, 28), Kupffer cells (100), and polymorphonuclear cells in a tissue subjected to ischemia-reperfusion or treated with pro-inflammatory cytokines (45, 161). LPS or TNF-α also promote microglial release of neurotoxic glutamate concentrations via Cx32 hemichannels (361).

While resting microglia have a weak but significant density-dependent inhibitory effect on astroglial Cx43 expression and gap junctional coupling (105, 321), microglia activation by LPS amplifies these effects (235, 314), suggesting that they can modify signaling among astrocytes. In addition, in astrocyte cultures not devoid of microglia, LPS or pro-inflammatory cytokines reduce astroglial coupling via nitric oxide synthase (NOS) induction and a p38 kinase-dependent pathway (40, 91, 139, 164, 235, 408). Both the IL-1β and TNF-α effects on gap junctions occur as early as 2 h after application (139, 408). Because this latency is close to the half-life of Cx43 (197), the action may be due to block of synthesis or increase in degradation of Cx. In general, the reduction in astroglial coupling is associated with reduced Cx43 levels (91, 164, 235). Similar to LPS, PGN reduces astroglial coupling associated with decrease in Cx43 and Cx30 protein levels; whereas under the same conditions expression of Cx26 increases (99). In co-cultures, inhibition of astrocyte dye coupling is induced by LPS-activated microglia which is associated to a reduction in Cx43 expression (235). The effect is mimicked when microglia-free astrocyte cultures are exposed to TNF-α plus IL-1β and is potentiated by Aβ (235), suggesting that reduction of gap junctional communication can result from activation of at least two different pathways. In agreement, activation of the innate immune response by dsRNA viruses and viral replication intermediates occurs via Toll-like receptor 3 (TLR3) and activation of astrocytes with the dsRNA mimetic polyinosinic-cytidylic acid (pI:C) inhibits expression of Cx43 (404). This effect is independent of p38 MAP kinase, ERK, or JNK signaling pathways, whereas it is completely inhibited by a blocker of the PI3 kinase/Akt pathway (404). Thus, activation of innate immune response can reduce astroglial coupling through two independent pathways. It remains unknown whether their simultaneous activation elicits a greater response of hemichannels and gap junctions channels than activation of either alone.

Changes in levels of Cxs 30 and 43 have been observed in astroglial cells of mice infected with Borna disease virus (183, 184). Evaluation of the time course of the inflammatory response in those mice would help to determine whether the Cx level changes are generated by the mechanisms discussed in the present review. Similarly, studies in mixed glial cultures and astroglial cultures devoid of microglia would help to dissect changes in astroglial cells directly caused by the virus from those due to activation of microglia induced by the virus.

Beside downregulation of astrocytic coupling, the presence of a few microglia induces depolarization of the resting membrane potential (139, 150), suggesting the involvement of membrane channels located in the cell surface. Astrocytes treated with conditioned medium from microglia activated with LPS or treated with TNF-α or IL-1β show increased Cx43 hemichannel activity through a p38 MAP kinase-dependent pathway (314). Pro-inflammatory cytokines increase dye uptake mediated by hemichannels as early as 30 min after their addition (314). The astroglial hemichannel activity is sensitive to La^{3+} and L-NAME, and does not occur in astrocytes from Cx43 knockout mice (314). The possible consequences of increase hemichannel opening include enhanced glucose uptake, which might explain changes in the metabolic status of astrocytes under inflammatory conditions (314). Moreover, it could promote neuronal damage through astrocytic release of neurotoxic and/or inflammatory compounds such as glutamate and PGE$_2$, respectively.

Bacterial meningitis also causes axonal damage and demyelination (260). These effects may be related to microglial cytokine release, which could promote opening of oligodendrocyte hemichannels, possibly composed of Cx32 or of Cx29, which does not form gap junctions and faces the periaxonal space (206) and, thus, promoting ion gradient imbalance and calcium overload.

Cerebrospinal fluid (CSF) normally contains leucocytes, complement factors, and antibodies at low abundance. However, severe inflammatory episodes are accompanied by high CSF cytokine and blood-derived leukocyte levels. The latter migrate to the infection zone attracted by soluble factors released by endothelial and microglial cells (362, 409). The invasion of infiltrating leucocytes often induces vasculitis which can produce thrombotic obstruction of vessels, and decreased cerebral perfusion can lead to focal ischemic lesions (224). Close to 2 decades ago, gap junction-like structures between lymphocytes and brain endothelial cells were

discovered (300), suggesting that heterocellular gap junctions may be required for an inflammatory response and extravasation (see below).

Leukocytes adhere weakly to the endothelium and roll along the vessel wall at a rate ~1% of the flow rate of non-adherent red blood cells (198). Rolling is ascribed to weak adhesive interactions between the leukocytes and endothelium surface; firmer adhesion of leukocytes to the endothelium causes cessation of rolling. The increased adhesion may result from formation of leuko-endothelial gap junctions (327). Neutrophils (161, 398) and T lymphocytes (93, 136) form gap junctions with endothelial cells that facilitate leukocyte transmigration across an endothelial cell monolayer (101, 270, 390, 398). Treatment with α-GA reduces monocyte/macrophage transmigration across a BBB model (101). Gap junctions between lymphoma cells and endothelial cells have also been demonstrated and α-GA attenuates the transmigration of lymphoma cells across an endothelial barrier (136). Interpretation of experimental results obtained using gap junction blockers or constitutive Cx knock out animals should also take in account the possible role of homocellular and heterocellular gap junctions express by inflammatory cells. In infected tissues, monocytes/macrophages play a relevant role, whereas neutrophils and lymphocytes are important protagonists in ischemia-reperfusion and autoimmune disease, respectively. This issue is particularly relevant because activated inflammatory cells generate copious free radicals which, as discussed above, can cause hemichannel and gap junction dysfunction.

It was recently shown that microglial cells cross-present antigens to naive and memory CD8$^+$ T-cells *in vitro* (26). Moreover, it is conceivable that leukocytes infiltrated in the brain parenchyma might undergo antigen cross-presentation with activated microglia, and antigenic peptide domains derived from viral or cellular proteins may be transferred between inflammatory cells through gap junctions as has been demonstrated for other antigenic molecules (236, 263). Cross-presentation via GJCs might coordinate and amplify inflammatory responses. Another route of antigen presentation could arise, when one cell engulfs an area of gap junction and takes with it a bit of cytoplasm from the "donor cell". If this vesicle contains antigens and is degraded by lysosomes, some antigenic fragments may be passed to the ER and eventually presented on the surface of the engulfing cell. Thus, skepticism on the role of gap junction in antigen cross-presentation might be reduced if the latter is ruled out.

Alzheimer's disease

Alzheimer's disease (AD) is characterized by the accumulation of the Aβ into amyloid plaques in the extracellular brain parenchyma, formation of tangles inside neurons as a result of abnormal phosphorylation of the microtubule-associated protein tau, dendritic atrophy, and changes in neurotransmission in specific brain regions (275). Aβ is generated by proteolytic cleavage of the amyloid precursor protein (APP), which plays a role in neuronal adhesion, synaptogenesis, and axonal growth (275). High concentrations of Aβ are toxic to several neuronal types (217, 275, 289). The mechanisms underlying Aβ-neurotoxicity are complex but involve activation of NMDA receptors, sustained elevations of $[Ca^{2+}]_i$, and oxidative stress (92, 111) which are effects

common to those induced by ischemia-reperfusion but on a different time scale.

In addition to the above, the cerebral cortex of individuals with AD present activated microglia and astroglia closely associated with amyloid plaques (169, 389). Cx43 immunoreactivity is increased at amyloid plaques where gliosis occurs (252). Imbalance in brain homeostasis may explain the increase in Cx43 expression in amyloid plaques as an (ultimately failed) compensatory mechanism to ensure normal brain function (252). Alternatively, increased astroglial gap junctions may serve as a pathway for the propagation of neuronal damage, transferring death signals generated in the microenvironment of amyloid plaques to distant neurons, as death signals can propagate from C6 glioma cells injured with calcium ionophore (209, 252). In agreement with this interpretation, inhibition of gap junctions with octanol abolishes the ability of Aβ to enhance the velocity and extent of propagation of astroglial calcium waves (144). However, octanol also blocks P2X$_7$ receptors expressed by spinal astrocytes that also show calcium waves (355). In support of P2 receptor mediation of the Aβ-induced increase of calcium wave velocity in cortical astrocytes is the fact that suramin, a P2Y and P2X receptors blocker, reduces this response (144). Nevertheless, P2X receptors as ATP release pathway in astrocytes treated with Aβ remains to be demonstrated. At least in macrophages and lymphocytes cell lines, where both P2X$_7$ receptor and Px1 coexist, the ATP induced dye uptake occurs through Px1 hemichannels resulting from P2X$_7$ activation (282). Although cultured astrocytes express Px1, it is not detected at the cell surface, excluding that ATP release through Px1 hemichannels mediates propagation of calcium waves (157). To be sure, the *in vivo* and *in vitro* preparations discussed may lack factors required for Px1 insertion into the plasma membrane. Studies in AD models will help to confirm or reject the above interpretations.

Overexpression of activated calcineurin induces astroglial activation, and calcineurin is upregulated in AD models (267). Activation of a calcineurin-dependent dephosphorylation pathway is associated with reduced gap junctional communication in astrocytes subjected to metabolic inhibition or glucose/oxygen deprivation followed by reoxygenation (62, 204). Hence, the Aβ-induced reduction in astroglial coupling may be in part due to dephosphorylation of Cx43 GJCs via calneurin.

Inflammatory cells inhibit astroglial coupling through gap junctions in co-cultures of resting microglia and astrocytes (321). Because microglia in primary cultures are partially activated (225) and release low levels of cytokines, the reduction in astroglial coupling in these cultures could be mediated by soluble factors instead of direct cell–cell contacts. TNF-α and IL-1β secreted by activated microglia more strongly inhibit coupling of astroglia (105, 235). This effect is potentiated by Aβ_{25-35}, which is the neurotoxic Aβ domain (289). In addition, TNF-α and IL-1β secreted by activated microglia increase astroglial Cx43-hemichannel activity (314) (Fig. 7), which accelerates death induced by ischemia (62). Thus, neuronal death induced by Aβ-treated microglia could be mediated by cytokines released from astrocytes as well as from microglia (234, 384) that reduce gap junctional coupling and enhance Cx43 hemichannel activity (Fig. 8A). This condition would generate a positive feedback cycle because dead cells activate more microglia which release more cyto-
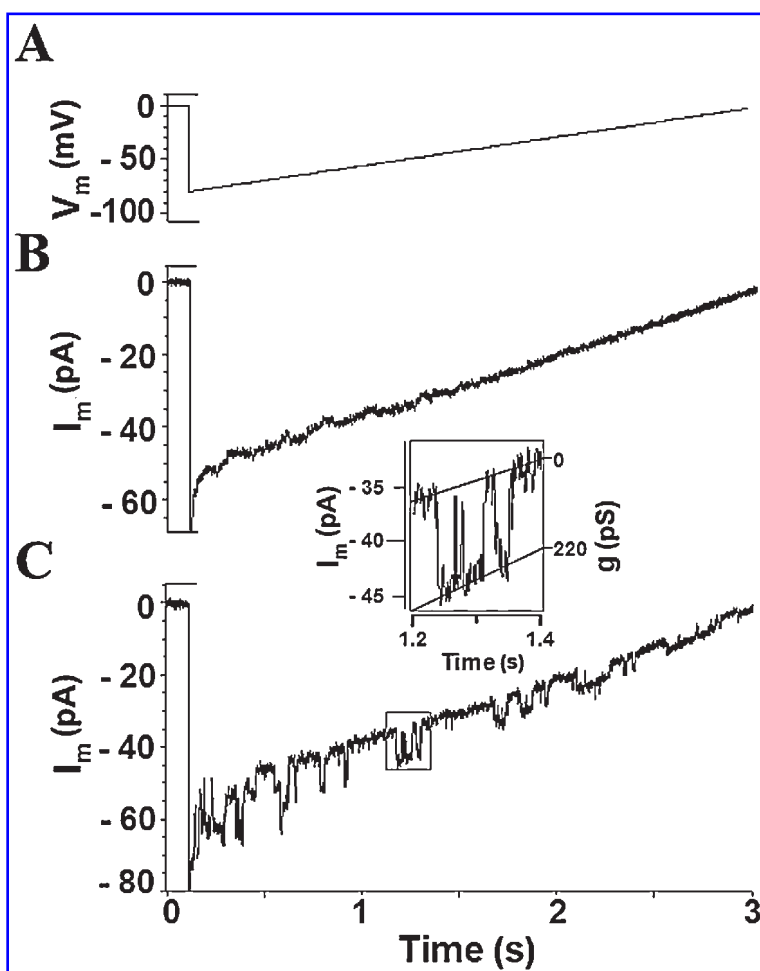
FIG. 7. **Conditioned medium from microglia treated with LPS induces Cx43 hemichannel activity in cortical astrocytes.** (**A**) Voltage ramps from -80 to 0 mV, 3 s in duration, were applied during whole cell recording. The ramp was initiated by a transition from 0 to -80 mV. (**B**) Under control conditions, no hemichannel openings were observed, although there was some low level activity at the more negative potentials. (**C**) In astrocytes treated for 24 h with medium conditioned by microglia treated with LPS, an obvious increase in channel openings was observed. The *boxed region* in **C** is enlarged in the *inset* and shows ~220 pS transitions between closed and open states; this conductance is characteristic of Cx43 hemichannels.

kine, and the inflammatory response causes edema that reduces tissue perfusion causing partial or total ischemia (Fig. 8B and C).

The lack of neuronal protection by astroglial cells could result from astroglial death as well as dysregulation of astroglial gap junctions and hemichannels. In support of the first possibility, 0.1–10 μM Aβ_{25-35} induces astroglial death *in vitro* (15). As previously discussed, a reduction in gap junctional communication impairs the spatial buffering capacity of astroglial networks, and an increase in hemichannel activity could in turn affect the viability of other cells through a paracrine mechanism. Indeed, excessive release or deficient uptake of signaling molecules such as glutamate, arachidonic acid byproducts, PGE$_2$ could enhance neuronal excitotoxicity (3, 238, 361, 396) (Fig. 8B). These mechanisms are likely to be enhanced by Aβ and may potentiate astroglial mediated neuronal death. The Aβ-induced rise in [Ca^{2+}]$_i$ (384) may activate astroglial Cx43 hemichannels and promote as-
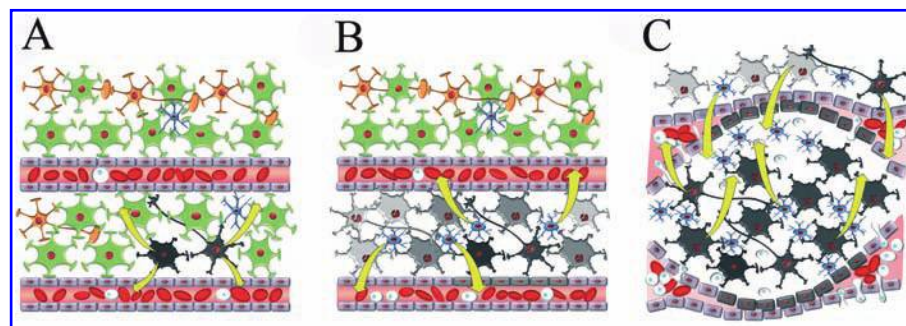

FIG. 8. **Three mechanisms of death amplification.** (**A**) Initially, a brain injury produced by ischemia, infection, or necrosis affecting astrocytes (*green*), neurons (*orange*), or resting microglia (*blue*), could start a wave of death propagated (*yellow arrows*) and amplified through diffusible toxins and molecules (*e.g.*, Ca^{2+}, NO, superoxide ion, peroxinitrite, glutamate, and NAD+) present in high concentration in injured cells (depicted in the figure as dark-colored cells). These molecules could be transferred through connexin gap junctions and connexin and pannexin hemichannels from injured cells (less and more affected cells in *gray* and *black*, respectively) to healthier cells. (**B**) Later, a second wave of death (*yellow arrows*) may be mediated by microglial cells overactivated by ATP and cytokines released by injured cells. (**C**) Still later inflammation-induced edema that reduces tissue perfusion could worsen the inflammatory response, recruiting leucocytes and increasing the extent of the lesion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

troglial death through at least three mechanisms: a) intracellular Ca^{2+} overload caused by excessive Ca^{2+} entry through Cx43 hemichannels leading to activation of lipases, phosphatases, and proteases, b) passive water flow generated by massive Na^+ and Cl^- influx through Cx43 hemichannels causing astroglial swelling, and c) Cx43 hemichannel-mediated release to the extracellular milieu of molecules required in normal metabolism (*e.g.*, glucose and derivates, NADH, ATP, ascorbic acid, and reduced glutathione: GSH).

Aβ plaques are also commonly found in the brain of Down's syndrome patients in whom neurodegeneration is prominent and premature (218). Therefore, mechanisms involving GJCs and hemichannels as those described for AD are likely to occur in Down's syndrome.

Parkinson's disease

The neuropathological hallmarks of Parkinson's disease (PD) patients are characterized by progressive and profound loss of neuromelanin containing dopaminergic neurons in the substantia nigra pars compacta with presence of eosinophillic, intracytoplamic, proteinaceous inclusions termed as Lewy bodies, which contain α-synuclein (348).

The molecular mechanisms of PD remain elusive but important factors in the disease pathogenesis are oxidative stress (163, 212) and α-synuclein accumulation (343). Microglial activation in PD is indicated by extensive proliferation of reactive amoeboid macrophages and microglia in the substantia nigra of PD patients in postmortem studies (232). Moreover, the substantia nigra in PD presents activated glial cells expressing pro-inflammatory cytokines, such as TNF-α, IL-1β, and IFN-γ, as well as iNOS (151, 159).

Overexpression and mutation of α-synuclein are associated with early onset PD (343), suggesting that α-synuclein dysregulation plays an important role in modulating the microglial activation state. Accordingly, fibrillary α-synuclein activates microglia *in vitro* (402) and microglia activation *in vivo* correlates with fibrillar α-synuclein deposition (68). Moreover, microglia from knockout α-synuclein mice (Scna$^{-/-}$) display an increased reactive phenotype under basal conditions and an exacerbated reactive phenotype after stimulation (16), suggesting that deficient α-synuclein contribute to microglia activation. In agreement with the possible role of hemichannel and gap junction dysfunction discussed in the present review, Scna$^{-/-}$ microglia, after stimulation, secrete elevated levels of pro-inflammatory cytokines, including TNF-α (16).

In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned striatum, an animal model of PD, total Cx43 mRNA and astroglial Cx43-immunoreactive punctata are significantly increased, whereas astroglial coupling evaluated with dye coupling of patched cells remained unaffected (325). Moreover, FGF-2, a potent trophic factor for mesencephalic dopaminergic neurons (106) further increases astroglial density and Cx43 immunoreactivity in the vicinity of the FGF-2 source without change astroglial coupling (325). In this system, the hemichannel activity has not been evaluated and is likely to be increased due to the imminent inflammatory response described above

How can the trophic effect of FGF-2 be explained? The role of hemichannels in injury may vary with the course and severity of noxious stimuli, passing from being beneficial af-

ter sublethal insults to accelerating cell death in severely injured tissues (316). Thus, it is likely that FGF-2 promotes hemichannel uptake of energy substrates and/or release of toxic metabolites to the extracellular milieu, which may be beneficial for injured cells. In support of this view, one signaling pathway of FGF-2 depends on activation of the p38 MAP kinase (222), which is associated with an increase in astroglial Cx43 hemichannel activity induced by cytokines (314).

FGF-2 upregulates expression of Cx43 by cultured dopaminergic neurons of the midbrain floor and increases coupling (345). More importantly, block of gap junctions with oleamide abolishes the survival effect of FGF-2 on dopaminergic neurons (345). In the latter study, oleamide would block both astroglial and neuronal coupling, thus, dissection of the possible role of hemichannels and GJCs remains to be elucidated. FGF-2 may permit diffusion of ions and substances from healthy to injured neurons through gap junctions, thereby promoting neuronal survival. FGF-2 also could be beneficial by decreasing opening of neuronal hemichannels composed of Pxs and/or Cxs. Indeed, short-lasting treatment with FGF-2 or FGF-1 decreases Cx43 hemichannel activity (79). The beneficial effect of FGF-2 in PD models is likely to be related to inhibition of astroglial and/or neuronal hemichannel, which could promote both astroglial and neuronal survival.

As in the case of AD, it is probable that increased microglial, astroglial, and neuronal hemichannel activity in PD is accompanied by a reduction in gap junction coupling. Overexpression of α-synuclein inhibits gap junction communication in dopaminergic neuroblastoma cells, and this effect may increase the sensitivity of α-synuclein overexpressing cells to various toxins (357). Moreover, α-synuclein binds to Cx32 (357), a Cx expressed in substantia nigra pars compacta (345, 371) where neurons are electrically coupled (372).

Huntington's disease

Huntington's disease (HD) is a progressive and fatal neurological disorder caused by an expanded CAG repeat in the gene coding for the protein huntingtin (1). This protein accumulates and causes apoptosis of striatal neurons (278). In HD, cortico-striatal circuits are particularly affected. Degeneration of medium spiny neurons and dysfunction of the supplementary motor area and the dopamine system is particularly relevant (382). As in other neurodegenerative diseases, HD causes both microglial (358) and astroglial activation (2). Astroglia expressing mutant huntingtin enhance excitotoxic neuronal damage (340). Moreover, a mutant huntingtin fragment perturbs the microglia transcription program (126). In this view, expression of mutant proteins (*e.g.*, Aβ, α-synuclein, and huntingtin) can perturb the normal glial response, amplifying the initial injury affecting susceptible neurons (214). Alternatively, glia expressing these mutant proteins become themselves a primary source of neurotoxicity, potentially independent of the mutation's effects in neurons at risk (214).

The distribution of Cxs 26, 32, 40, and 43 in the caudate nucleus and globus pallidus of the basal ganglia in normal and HD human brains has been studied using immunohistochemical techniques (378). Cx40, localized in vascular en-

dothelium, is increased in HD brains, apparently due to more numerous and smaller blood vessels. Both Cx26 and Cx32 have a similar distribution in the striatum and globus pallidus of normal and HD brains, with little in the caudate nucleus but clearly detectable amounts in the globus pallidus. In the caudate nucleus the Cx43 density is increased, which correlates with enhanced GFAP expression in patches (378), indicating a reactive astrogliosis around foci of neurodegeneration.

Changes in expression of Cx36, Cx43, and Cx45 also occur in the retina of adult transgenic R6/2 mice, a model of HD (286) (The basal ganglia were not investigated in this study). In the retina, Cx36 is slightly reduced in the outer plexiform layer, whereas Cx45 is strongly reduced in the inner plexiform layer. No changes in Cx43 expression were evident in mutant HD mice. It should be determined if HD is associated with increased astroglial hemichannel activity and/or reduced gap junctional communication that could explain in part the retinal neurodegeneration as well caudate nucleus neurodegeneration (287).

Concluding Remarks

The inflammatory response is the main cause of numerous acute neuropathological processes and can progress in chronic diseases, leading to tissue dysfunction. In the brain, key cells of the inflammatory response are microglia and astrocytes that under resting conditions contribute to normal functioning of neurons. Deleterious conditions can trigger changes in both types of glial cells as well in other cells of the brain, including neurons and endothelial cells. The latter may be targets of inflammatory responses initiated within the nervous tissue as well as of inflammatory responses initiated in the periphery, such as infections, traumas, and burns. If intensity, duration, and quality of the insult are below the threshold to elicit an overactive response, release of ATP via hemichannels mediates a preconditioning response that confers tissue resistance to successive deleterious insults. If the adverse condition is excessive, microglia and astrocytes manifest rapid phenotypic changes to neutralize the threat or to adapt to the new microenvironment. In contrast, neurons do not adapt as well, and excitotoxicity and neuronal death can cause persistent glial activation and a damaging positive feedback loop. Free radicals generated during reoxygenation after an ischemic episode or cytokines and/or free radicals generated during an inflammatory response enhance hemichannel activity and reduce gap junction coupling of astrocytes. These changes tend to deprive neurons of numerous glial protective functions, and neurons become more susceptible and may undergo necrosis or apoptosis. Under acute pathological conditions, these mechanisms can be triggered by debris of necrotic cells or directly by pathogens or foreign molecules detected by microglia. In chronic diseases inflammation can be activated by anomalous molecules. The inflammation induced edema also reduces tissue perfusion worsening the inflammatory response.

The role of microglia and astrocytes in mediating inflammation of nervous tissue has been recognized previously as leading to death of neuron; these cell types can be bad neighbors for neurons (37, 360). Dysfunction of astroglial and microglial hemichannels and GJCs likely are mechanisms commonly elicited in all brain diseases associated with in-

flammatory responses and thus, contribute to acute and chronic neurodegeneration. Therefore, normalization of channel dysfunctions should confer tissue protection, improve quality of life, and extend survival of patients suffering acute or chronic brain inflammatory responses.

Possible changes in Cx-based channels in the brain of neonates remain unknown, and future studies might reveal interesting cues to further understanding of the role of these channels in neurodegeneration. A discussion of disease states in relation to developmental and age-dependent regulation of Cxs such as neonatal *versus* adult hypoxia and hypoxia-ischemia is one example. Other examples include the possible nonchannel function of Cxs and their expression at sites other than the plasma membrane.

Inflammation and neurodegeneration are closely related in other chronic and acquired neurodegenerative diseases not described in the present review, including Niemann–Pick type C disease (25), Down's syndrome (160), ALS (TBP-43) (193), experimental ALS models (75), diabetes (312), Epstein–Barr virus induced multiple sclerosis (366), Creutzfeldt–Jakob disease (17) and HIV-associated dementia (33). Elucidation of the role of hemichannel and GJCs dysfunction in these processes should be a topic of high research interest in the immediate future. How can current treatments be related to the possible role of Cx based channels in neurodegeneration? So far the most specific hemichannel blockers are mimetic peptides, but they also block GJCs (presumably by preventing formation) and most likely their chronic use will be limited by their antigenicity and susceptibility to degradation by extracellular proteases. Classical anti-inflammatory agents such as indomethacin prevent the reduction in astroglial gap junctional communication induced by arachidonic acid (226, 227). Moreover n-3 polyunsaturated fatty acids (DHA) are potent anti-inflammatory components that enhance gap junctional communication between astrocytes (70), but their effects on hemichannels remains unknown. Steroidal anti-inflammatory agents may also restore astroglial functions depressed by inflammatory responses. Corticoids do not affect gap junctions and Cx43 levels in astrocytes under basal conditions, but increase resting cytosolic free $[Ca^{2+}]$ levels and the extent and amplitude of Ca^{2+} wave propagation (342). But interestingly, corticoids prevent TNF-α and IL-1β-induced inhibition of gap junctional communication and Cx43 downregulation (Froger N. and Giaume C., unpublished data). Alternatively, anti-inflammatory cytokines (*e.g.*, TGF-β1 and IFN-β) might be useful, since they prevent the astrocyte uncoupling response elicited by pro-inflammatory cytokines (150). A group of compounds with anti-inflammatory activity that also block hemichannels and GJCs are derived from licorice (61, 109, 134), which suggests a reduction in gap junctional communication is not so detrimental to cells as is excess hemichannel activity.

All above-mentioned compounds that are nonspecific blockers of both hemichannels and GJCs also affect other molecular targets. At this time, block of hemichannels and GJCs can be considered a side effect. From a different perspective, two common conditions known to elicit an inflammatory response, ischemia and infectious agents, reduce gap junctional communication and enhance hemichannel activity in astrocytes, suggesting that these channels could be common targets of different pathways (*e.g.*, p38 kinase-dependent and

–independent pathways) that mediate cellular responses elicited by the innate immune system. Therefore, the development or discovery of compounds with specific inhibitory action either on hemichannels or GJCs and with selectivity for Cx isoforms may offer a new generation of anti-inflammatory compounds with applicability in a much broader spectrum of disease than the compounds currently used.

Acknowledgment

Abbreviations

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ASICs, acid-sensing ion channels; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; $[Ca^{2+}]_o$, extracellular calcium concentration; $[Ca^{2+}]_I$, intracellular calcium concentration CFTR, cystic fibrosis transmembrane conductance regulator; CNS, central nervous system; CSF, cerebral spinal fluid; Cx, connexin; DTT, dithiothreitol; ER, endoplasmatic reticulum; FGF-1, fibroblast growth factor 1; α-GA, 18-α-glycyrrhetinic acid β-GA, 18β-glycyrrhetinic acid; GJC, gap junction channel; HD, Huntington's disease; IFN-γ, interferon-γ; IL-1β, interleukin-1β; LPS, lipopolysaccharide; NGF, nerve growth factor; NO, nitric oxide; NT-3, neurotrophin-3; NT-4, neurotrophin-4; PAF, platelet activating factor; PD, Parkinson's disease; PGE$_2$, prostaglandin E$_2$; PGN, peptidoglycan; PMCA, plasma membrane Ca^{2+}-ATPase; Px, pannexin; RNS, reactive nitrogen species; ROS, reactive oxygen species; siRNA, small interfering ribonucleic acid; SOD1, superoxide dismutase 1; TGF-β, transforming growth factor-β; TLRs, toll-like receptors; TNF-α, tumor necrosis factor-α; TRP, transient receptor potential; ZO-1, tonula occludens protein 1.

References

1. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 72: 971–983, 1993.

2. Aguado F, Ballabriga J, Pozas E, and Ferrer I. TrkA immunoreactivity in reactive astrocytes in human neurodegenerative diseases and colchicine-treated rats. *Acta Neuropathol* 96: 495–501, 1998.

3. Ahmad AS, Zhuang H, Echeverria V, and Dore S. Stimulation of prostaglandin EP2 receptors prevents NMDA-induced excitotoxicity. *J Neurotrauma* 23: 1895–1903, 2006.

4. Ahmad S and Evans WH. Post-translational integration and oligomerization of connexin 26 in plasma membranes and evidence of formation of membrane pores: implications for the assembly of gap junctions. *Biochem J* 365: 693–699, 2002.

5. Aizenman E, Lipton SA, and Loring RH. Selective modulation of NMDA responses by reduction and oxidation. *Neuron* 2: 1257–1263, 1989.

6. Albensi BC. The NMDA receptor/ion channel complex: a drug target for modulating synaptic plasticity and excitotoxicity. *Curr Pharm Des* 13: 3185–3194, 2007.

7. Albrecht J and Rafalowska U. Enhanced potassium-stimulated gamma-aminobutyric acid release by astrocytes derived from rats with early hepatogenic encephalopathy. *J Neurochem* 49: 9–11, 1987.

8. Albrecht J, Simmons M, Dutton GR, and Norenberg MD. Aluminum chloride stimulates the release of endogenous glutamate, taurine and adenosine from cultured rat cortical astrocytes. *Neurosci Lett* 127: 105–107, 1991.

9. Altevogt BM, Kleopa KA, Postma FR, Scherer SS, and Paul DL. Connexin29 is uniquely distributed within myelinating glial cells of the central and peripheral nervous systems. *J Neurosci* 22: 6458–6470, 2002.

10. Altevogt BM and Paul DL. Four classes of intercellular channels between glial cells in the CNS. *J Neurosci* 24: 4313–4323, 2004.

11. Alvarez–Maubecin V, Garcia–Hernandez F, Williams JT, and Van Bockstaele EJ. Functional coupling between neurons and glia. *J Neurosci* 20: 4091–4098, 2000.

12. Alves LA, Coutinho–Silva R, Persechini PM, Spray DC, Savino W, and Campos de Carvalho AC. Are there functional gap junctions or junctional hemichannels in macrophages? *Blood* 88: 328–334, 1996.

13. Allan SM and Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2: 734–744, 2001.

14. Anderson MF, Blomstrand F, Blomstrand C, Eriksson PS, and Nilsson M. Astrocytes and stroke: networking for survival? *Neurochem Res* 28: 293–305, 2003.

15. Assis–Nascimento P, Jarvis KM, Montague JR, and Mudd LM. Beta-amyloid toxicity in embryonic rat astrocytes. *Neurochem Res* 32: 1476–1482, 2007.

16. Austin SA, Floden AM, Murphy EJ, and Combs CK. Alpha-synuclein expression modulates microglial activation phenotype. *J Neurosci* 26: 10558–10563, 2006.

17. Baker CA, Martin D, and Manuelidis L. Microglia from Creutzfeldt-Jakob disease-infected brains are infectious and show specific mRNA activation profiles. *J Virol* 76: 10905–10913, 2002.

18. Bal–Price A and Brown GC. Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J Neurosci* 21: 6480–6491, 2001.

19. Bannerman DD, Fitzpatrick MJ, Anderson DY, Bhattacharjee AK, Novitsky TJ, Hasday JD, Cross AS, and Goldblum SE. Endotoxin-neutralizing protein protects against endotoxin-induced endothelial barrier dysfunction. *Infect Immun* 66: 1400–1407, 1998.

20. Bao L, Locovei S, and Dahl G. Pannexin membrane channels are mechanosensitive conduits for ATP. *FEBS Lett* 572: 65–68, 2004.

21. Baranova A, Ivanov D, Petrash N, Pestova A, Skoblov M, Kelmanson I, Shagin D, Nazarenko S, Geraymovych E, Litvin O, Tiunova A, Born TL, Usman N, Staroverov D, Lukyanov S, and Panchin Y. The mammalian pannexin family is homologous to the invertebrate innexin gap junction proteins. *Genomics* 83: 706–716, 2004.

22. Barbe MT, Monyer H, and Bruzzone R. Cell-cell communication beyond connexins: the pannexin channels. *Physiology (Bethesda)* 21: 103–114, 2006.

23. Bate C, Boshuizen RS, Langeveld JP, and Williams A. Temporal and spatial relationship between the death of PrP-damaged neurones and microglial activation. *Neuroreport* 13: 1695–1700, 2002.

24. Batter DK, Corpina RA, Roy C, Spray DC, Hertzberg EL, and Kessler JA. Heterogeneity in gap junction expression in astrocytes cultured from different brain regions. *Glia* 6: 213–221, 1992.

25. Baudry M, Yao Y, Simmons D, Liu J, and Bi X. Postnatal development of inflammation in a murine model of Niemann–Pick type C disease: immunohistochemical observations of microglia and astroglia. *Exp Neurol* 184: 887–903, 2003.

26. Beauvillain C, Donnou S, Jarry U, Scotet M, Gascan H, Delneste Y, Guermonprez P, Jeannin P, and Couez D. Neonatal and adult microglia cross-present exogenous antigens. *Glia* 56: 69–77, 2008.

27. Bennett MV and Zukin RS. Electrical coupling and neuronal synchronization in the mammalian brain. *Neuron* 41: 495–511, 2004.

28. Beyer EC and Steinberg TH. Evidence that the gap junction protein connexin-43 is the ATP-induced pore of mouse macrophages. *J Biol Chem* 266: 7971–7974, 1991.

29. Beyer M, Gimsa U, Eyupoglu IY, Hailer NP, and Nitsch R. Phagocytosis of neuronal or glial debris by microglial cells: upregulation of MHC class II expression and multinuclear giant cell formation *in vitro*. *Glia* 31: 262–266, 2000.

30. Bittman K, Becker DL, Cicirata F, and Parnavelas JG. Connexin expression in homotypic and heterotypic cell coupling in the developing cerebral cortex. *J Comp Neurol* 443: 201–212, 2002.

31. Blanc EM, Bruce–Keller AJ, and Mattson MP. Astrocytic gap junctional communication decreases neuronal vulnerability to oxidative stress-induced disruption of Ca2+ homeostasis and cell death. *J Neurochem* 70: 958–970, 1998.

32. Block ML and Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog Neurobiol* 76: 77–98, 2005.

33. Block ML, Zecca L, and Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 8: 57–69, 2007.

34. Blomstrand F, Venance L, Siren AL, Ezan P, Hanse E, Glowinski J, Ehrenreich H, and Giaume C. Endothelins regulate astrocyte gap junctions in rat hippocampal slices. *Eur J Neurosci* 19: 1005–1015, 2004.

35. Boassa D, Ambrosi C, Qiu F, Dahl G, Gaietta G, and Sosinsky G. Pannexin1 channels contain a glycosylation site that targets the hexamer to the plasma membrane. *J Biol Chem* 282: 31733–31743, 2007.

36. Boengler K, Dodoni G, Rodriguez–Sinovas A, Cabestrero A, Ruiz–Meana M, Gres P, Konietzka I, Lopez–Iglesias C, Garcia–Dorado D, Di Lisa F, Heusch G, and Schulz R. Connexin 43 in cardiomyocyte mitochondria and its increase by ischemic preconditioning. *Cardiovasc Res* 67: 234–244, 2005.

37. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, and Cleveland DW. Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312: 1389–1392, 2006.

38. Boitano S, Dirksen ER, and Sanderson MJ. Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science* 258: 292–295, 1992.

39. Bolanos JP, Almeida A, Fernandez E, Medina JM, Land JM, Clark JB, and Heales SJ. Potential mechanisms for nitric oxide-mediated impairment of brain mitochondrial energy metabolism. *Biochem Soc Trans* 25: 944–949, 1997.

40. Bolanos JP and Medina JM. Induction of nitric oxide synthase inhibits gap junction permeability in cultured rat astrocytes. *J Neurochem* 66: 2091–2099, 1996.

41. Bouille C, Mesnil M, Barriere H, and Gabrion J. Gap junctional intercellular communication between cultured ependymal cells, revealed by lucifer yellow CH transfer and freeze-fracture. *Glia* 4: 25–36, 1991.

42. Bowser DN and Khakh BS. Vesicular ATP is the predominant cause of intercellular calcium waves in astrocytes. *J Gen Physiol* 129: 485–491, 2007.

43. Braet K, Paemeleire K, D'Herde K, Sanderson MJ, and Leybaert L. Astrocyte-endothelial cell calcium signals conveyed by two signalling pathways. *Eur J Neurosci* 13: 79–91, 2001.

44. Braet K, Vandamme W, Martin PE, Evans WH, and Leybaert L. Photoliberating inositol-1,4,5-trisphosphate triggers ATP release that is blocked by the connexin mimetic peptide gap 26. *Cell Calcium* 33: 37–48, 2003.

45. Branes MC, Contreras JE, and Saez JC. Activation of human polymorphonuclear cells induces formation of functional gap junctions and expression of connexins. *Med Sci Monit* 8: BR313–323, 2002.

45a. Brisset AC, Isakson BE, and Kwak BR. Connexins in vascular physiology and pathology. *Anitoxid Redox Signal* 11:267–282, 2009.

46. Brown DR, Schmidt B, and Kretzschmar HA. Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. *Nature* 380: 345–347, 1996.

47. Bruzzone R, Barbe MT, Jakob NJ, and Monyer H. Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in Xenopus oocytes. *J Neurochem* 92: 1033–1043, 2005.

48. Bruzzone R, Hormuzdi SG, Barbe MT, Herb A, and Monyer H. Pannexins, a family of gap junction proteins expressed in brain. *Proc Natl Acad Sci USA* 100: 13644–13649, 2003.

49. Bruzzone S, Guida L, Zocchi E, Franco L, and De Flora A. Connexin 43 hemi channels mediate Ca2+-regulated transmembrane NAD+ fluxes in intact cells. *FASEB J* 15: 10–12, 2001.

50. Buhl DL, Harris KD, Hormuzdi SG, Monyer H, and Buzsaki G. Selective impairment of hippocampal gamma oscillations in connexin-36 knock-out mouse in vivo. *J Neurosci* 23: 1013–1018, 2003.

51. Bukauskas FF, Bukauskiene A, Bennett MV, and Verselis VK. Gating properties of gap junction channels assembled from connexin43 and connexin43 fused with green fluorescent protein. *Biophys J* 81: 137–152, 2001.

52. Bussini S, Meda L, Scarpini E, Clementi E, Conti G, Tiriticco M, Bresolin N, and Baron P. Heparan sulfate proteoglycan induces the production of NO and TNF-alpha by murine microglia. *Immun Ageing* 2: 11, 2005.

53. Cadiou H, Studer M, Jones NG, Smith ES, Ballard A, McMahon SB, and McNaughton PA. Modulation of acid-sensing ion channel activity by nitric oxide. *J Neurosci* 27: 13251–13260, 2007.

54. Calabrese V, Guagliano E, Sapienza M, Mancuso C, Butterfield DA, and Stella AM. Redox regulation of cellular stress response in neurodegenerative disorders. *Ital J Biochem* 55: 263–282, 2006.

55. Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, and Clark JB. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. *Neurochem Res* 26: 739–764, 2001.

56. Calegari F, Coco S, Taverna E, Bassetti M, Verderio C, Corradi N, Matteoli M, and Rosa P. A regulated secretory pathway in cultured hippocampal astrocytes. *J Biol Chem* 274: 22539–22547, 1999.

57. Cao F, Eckert R, Elfgang C, Nitsche JM, Snyder SA, DF Hu, Willecke K, and Nicholson BJ. A quantitative analysis of connexin-specific permeability differences of gap junctions expressed in HeLa transfectants and Xenopus oocytes. *J Cell Sci* 111 (Pt 1): 31–43, 1998.

58. Cina C, Bechberger JF, Ozog MA, and Naus CC. Expression of connexins in embryonic mouse neocortical development. *J Comp Neurol* 504: 298–313, 2007.

59. Combs CK, Johnson DE, Karlo JC, Cannady SB, and Landreth GE. Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. *J Neurosci* 20: 558–567, 2000.

60. Connors BW and Long MA. Electrical synapses in the mammalian brain. *Annu Rev Neurosci* 27: 393–418, 2004.

61. Contreras JE, Sáez JC, Bukauskas FF, and Bennett ML. Gating and regulation of connexin 43 (Cx43) hemichannels. *Proc Natl Acad Sci USA* 100: 11388–11393, 2003.

62. Contreras JE, Sánchez HA, Eugenin EA, Speidel D, Theis M, Willecke K, Bukauskas FF, Bennett ML, and Sáez JC. Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. *Proc Natl Acad Sci USA* 99: 495–500, 2002.

63. Contreras JE, Sánchez HA, Veliz LP, Bukauskas FF, Bennett ML, and Sáez JC. Role of connexin-based gap junction channels and hemichannels in ischemia-induced cell death in nervous tissue. *Brain Res Brain Res Rev* 47: 290–303, 2004.

64. Corvalán LA, Araya R, Brañes MC, Sáez PJ, Kalergis AM, Tobar JA, Theis M, Willecke K, and Sáez JC. Injury of skeletal muscle and specific cytokines induce the expression of gap junction channels in mouse dendritic cells. *J Cell Physiol* 211: 649–660, 2007.

65. Cotrina ML, Kang J, Lin JH, Bueno E, Hansen TW, He L, Liu Y, and Nedergaard M. Astrocytic gap junctions remain open during ischemic conditions. *J Neurosci* 18: 2520–2537, 1998.

66. Cotrina ML, Lin JH, Alves–Rodrigues A, Liu S, Li J, Azmi–Ghadimi H, Kang J, Naus CC, and Nedergaard M. Connexins regulate calcium signaling by controlling ATP release. *Proc Natl Acad Sci USA* 95: 15735–15740, 1998.

67. Cotrina ML, Lin JH, Lopez–Garcia JC, Naus CC, and Nedergaard M. ATP-mediated glia signaling. *J Neurosci* 20: 2835–2844, 2000.

68. Croisier E, Moran LB, Dexter DT, Pearce RK, and Graeber MB. Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation* 2: 14, 2005.

69. Chadjichristos CE and Kwak BR. Connexins: new genes in atherosclerosis. *Ann Med* 39: 402–411, 2007.

70. Champeil–Potokar G, Chaumontet C, Guesnet P, Lavialle M, and Denis I. Docosahexaenoic acid (22:6n-3) enrichment of membrane phospholipids increases gap junction coupling capacity in cultured astrocytes. *Eur J Neurosci* 24: 3084–3090, 2006.

71. Chao CC, Hu S, Sheng WS, Bu D, Bukrinsky MI, and Peterson PK. Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. *Glia* 16: 276–284, 1996.

72. Cherian PP, Siller-Jackson AJ, Gu S, Wang X, Bonewald LF, Sprague E, and Jiang JX. Mechanical strain opens connexin 43 hemichannels in osteocytes: a novel mechanism for the release of prostaglandin. *Mol Biol Cell* 16: 3100–3106, 2005.

73. Chesler M. Failure and function of intracellular pH regulation in acute hypoxic-ischemic injury of astrocytes. *Glia* 50: 398–406, 2005.

74. Danbolt NC. The high affinity uptake system for excitatory amino acids in the brain. *Prog Neurobiol* 44: 377-396, 1994.

75. Dangond F, Hwang D, Camelo S, Pasinelli P, Frosch MP, Stephanopoulos G, Brown RH, Jr., and Gullans SR. Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter. *Physiol Genomics* 16: 229–239, 2004.

76. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, and Gan WB. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* 8: 752–758, 2005.

77. De Pina–Benabou MH, Srinivas M, Spray DC, and Scemes E. Calmodulin kinase pathway mediates the K+-induced increase in gap junctional communication between mouse spinal cord astrocytes. *J Neurosci* 21: 6635–6643, 2001.

78. De Vuyst E, Decrock E, Cabooter L, Dubyak GR, Naus CC, Evans WH, and Leybaert L. Intracellular calcium changes trigger connexin 32 hemichannel opening. *EMBO J* 25: 34–44, 2006.

79. De Vuyst E, Decrock E, De Bock M, Yamasaki H, Naus CC, Evans WH, and Leybaert L. Connexin hemichannels and gap junction channels are differentially influenced by lipopolysaccharide and basic fibroblast growth factor. *Mol Biol Cell* 18: 34–46, 2007.

80. Dermietzel R, Farooq M, Kessler JA, Althaus H, Hertzberg EL, and Spray DC. Oligodendrocytes express gap junction proteins connexin32 and connexin45. *Glia* 20: 101–114, 1997.

81. Dermietzel R, Gao Y, Scemes E, Vieira D, Urban M, Kremer M, Bennett MV, and Spray DC. Connexin43 null mice reveal that astrocytes express multiple connexins. *Brain Res Brain Res Rev* 32: 45–56, 2000.

82. Dermietzel R, Hertberg EL, Kessler JA, and Spray DC. Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis. *J Neurosci* 11: 1421–1432, 1991.

83. Dermietzel R and Krause D. Molecular anatomy of the blood-brain barrier as defined by immunocytochemistry. *Int Rev Cytol* 127: 57–109, 1991.

84. Dermietzel R, Traub O, Hwang TK, Beyer E, Bennett MV, Spray DC, and Willecke K. Differential expression of three gap junction proteins in developing and mature brain tissues. *Proc Natl Acad Sci USA* 86: 10148–10152, 1989.

85. Dexter DT, Carter CJ, Wells FR, Javoy–Agid F, Agid Y, Lees A, Jenner P, and Marsden CD. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* 52: 381–389, 1989.

86. Dietzman DE, Fischer GW, and Schoenknecht FD. Neonatal *Escherichia coli* septicemia–bacterial counts in blood. *J Pediatr* 85: 128–130, 1974.

87. Dirnagl U, Iadecola C, and Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 22: 391–397, 1999.

88. Dobrenis K, Chang HY, Pina-Benabou MH, Woodroffe A, Lee SC, Rozental R, Spray DC, and Scemes E. Human and mouse microglia express connexin36, and functional gap junctions are formed between rodent microglia and neurons. *J Neurosci Res* 82: 306–315, 2005.

89. Doran KS, Liu GY, and Nizet V. Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. *J Clin Invest* 112: 736–744, 2003.

90. Drukarch B, Schepens E, Jongenelen CA, Stoof JC, and Langeveld CH. Astrocyte-mediated enhancement of neuronal survival is abolished by glutathione deficiency. *Brain Res* 770: 123–130, 1997.

91. Duffy HS, John GR, Lee SC, Brosnan CF, and Spray DC. Reciprocal regulation of the junctional proteins claudin-1 and connexin43 by interleukin-1beta in primary human fetal astrocytes. *J Neurosci* 20: RC114, 2000.

92. Ekinci FJ, Linsley MD, and Shea TB. Beta-amyloid-induced calcium influx induces apoptosis in culture by oxidative stress rather than tau phosphorylation. *Brain Res Mol Brain Res* 76: 389–395, 2000.

93. El–Sabban ME, Merhi RA, Haidar HA, Arnulf B, Khoury H, Basbous J, Nijmeh J, de The H, Hermine O, and Bazarbachi A. Human T-cell lymphotropic virus type 1-transformed cells induce angiogenesis and establish functional gap junctions with endothelial cells. *Blood* 99: 3383–3389, 2002.

94. Elahi MM and Matata BM. Free radicals in blood: evolving concepts in the mechanism of ischemic heart disease. *Arch Biochem Biophys* 450: 78–88, 2006.

95. Elfgang C, Eckert R, Lichtenberg–Frate H, Butterweck A, Traub O, Klein RA, Hulser DF, and Willecke K. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. *J Cell Biol* 129: 805–817, 1995.

96. Elias LA, Wang DD, and Kriegstein AR. Gap junction adhesion is necessary for radial migration in the neocortex. *Nature* 448: 901–907, 2007.

97. Enkvist MO and McCarthy KD. Astroglial gap junction communication is increased by treatment with either glutamate or high K+ concentration. *J Neurochem* 62: 489–495, 1994.

98. Errede M, Benagiano V, Girolamo F, Flace P, Bertossi M, Roncali L, and Virgintino D. Differential expression of connexin43 in foetal, adult and tumour-associated human brain endothelial cells. *Histochem J* 34: 265–271, 2002.

99. Esen N, Shuffield D, Syed MM, and Kielian T. Modulation of connexin expression and gap junction communication in astrocytes by the gram-positive bacterium S. aureus. *Glia* 55: 104–117, 2007.

100. Eugenin EA and Berman JW. Gap junctions mediate human immunodeficiency virus-bystander killing in astrocytes. *J Neurosci* 27: 12844–12850, 2007.

101. Eugenin EA, Brañes MC, Berman JW, and Sáez JC. TNF-alpha plus IFN-gamma induce connexin43 expression and formation of gap junctions between human monocytes/macrophages that enhance physiological responses. *J Immunol* 170: 1320–1328, 2003.

102. Eugenin EA, Eckardt D, Theis M, Willecke K, Bennett ML, and Saez JC. 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 USA* 98: 4190–4195, 2001.

103. Eugenin EA, González HE, Sánchez HA, Brañes MC, and Sáez JC. Inflammatory conditions induce gap junctional communication between rat Kupffer cells both in vivo and in vitro. *Cell Immunol* 247: 103–110, 2007.

104. Evans WH and Leybaert L. Mimetic peptides as blockers of connexin channel-facilitated intercellular communication. *Cell Commun Adhes* 14: 265–273, 2007.

105. Faustmann PM, Haase CG, Romberg S, Hinkerohe D, Szlachta D, Smikalla D, Krause D, and Dermietzel R. Microglia activation influences dye coupling and Cx43 expression of the astrocytic network. *Glia* 42: 101–108, 2003.

106. Ferrari G, Minozzi MC, Toffano G, Leon A, and Skaper SD. Basic fibroblast growth factor promotes the survival and development of mesencephalic neurons in culture. *Dev Biol* 133: 140–147, 1989.

107. Figiel M, Allritz C, Lehmann C, and Engele J. Gap junctional control of glial glutamate transporter expression. *Mol Cell Neurosci* 35: 130–137, 2007.

107a. Figueroa XF and Duling BR. Gap junctions in the control of vascular function. *Antioxid Redox Signal* 11:251–266, 2009.

108. Figueroa XF, Isakson BE, and Duling BR. Vascular gap junctions in hypertension. *Hypertension* 48: 804–811, 2006.

109. Finney RS and Somers GF. The antiinflammatory activity of glycyrrhetinic acid and derivatives. *J Pharm Pharmacol* 10: 613–620, 1958.

110. Fogal B, Li J, Lobner D, McCullough LD, and Hewett SJ. System x(c)-activity and astrocytes are necessary for interleukin-1 beta-mediated hypoxic neuronal injury. *J Neurosci* 27: 10094–10105, 2007.

111. Forloni G, Chiesa R, Smiroldo S, Verga L, Salmona M, Tagliavini F, and Angeretti N. Apoptosis mediated neurotoxicity induced by chronic application of beta amyloid fragment 25-35. *Neuroreport* 4: 523–526, 1993.

112. Freyer D, Manz R, Ziegenhorn A, Weih M, Angstwurm K, Docke WD, Meisel A, Schumann RR, Schonfelder G, Dirnagl U, and Weber JR. Cerebral endothelial cells release TNF-alpha after stimulation with cell walls of Streptococcus pneumoniae and regulate inducible nitric oxide synthase and ICAM-1 expression via autocrine loops. *J Immunol* 163: 4308–4314, 1999.

113. Frisch C, De Souza–Silva MA, Sohl G, Guldenagel M, Willecke K, Huston JP, and Dere E. Stimulus complexity dependent memory impairment and changes in motor performance after deletion of the neuronal gap junction protein connexin36 in mice. *Behav Brain Res* 157: 177–185, 2005.

114. Froes MM, Correia AH, Garcia–Abreu J, Spray DC, Campos de Carvalho AC, and Neto MV. Gap-junctional coupling between neurons and astrocytes in primary central nervous system cultures. *Proc Natl Acad Sci USA* 96: 7541–7546, 1999.

115. Froes MM and de Carvalho AC. Gap junction-mediated loops of neuronal-glial interactions. *Glia* 24: 97–107, 1998.

116. Fukuda T, Kosaka T, Singer W, and Galuske RA. Gap junctions among dendrites of cortical GABAergic neurons establish a dense and widespread intercolumnar network. *J Neurosci* 26: 3434–3443, 2006.

117. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, and Liu B. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J Neurochem* 81: 1285–1297, 2002.

118. Gao HM, Liu B, Zhang W, and Hong JS. Novel anti-inflammatory therapy for Parkinson's disease. *Trends Pharmacol Sci* 24: 395–401, 2003.

119. Garg S, Md Syed M, and Kielian T. *Staphylococcus aureus*-derived peptidoglycan induces Cx43 expression and functional gap junction intercellular communication in microglia. *J Neurochem* 95: 475–483, 2005.

120. Giaume C, Fromaget C, el Aoumari A, Cordier J, Glowinski J, and Gros D. Gap junctions in cultured astrocytes: single-channel currents and characterization of channel-forming protein. *Neuron* 6: 133–143, 1991.

121. Giaume C and McCarthy KD. Control of gap-junctional communication in astrocytic networks. *Trends Neurosci* 19: 319–325, 1996.

122. Giaume C, Tabernero A, and Medina JM. Metabolic trafficking through astrocytic gap junctions. *Glia* 21: 114–123, 1997.

123. Giaume C and Venance L. Intercellular calcium signaling and gap junctional communication in astrocytes. *Glia* 24: 50–64, 1998.

124. Gibbons HM and Dragunow M. Microglia induce neural cell death via a proximity-dependent mechanism involving nitric oxide. *Brain Res* 1084: 1–15, 2006.

125. Giepmans BN. Gap junctions and connexin-interacting proteins. *Cardiovasc Res* 62: 233–245, 2004.

126. Giorgini F, Moller T, Kwan W, Zwilling D, Wacker JL, Hong S, Tsai LC, Cheah CS, Schwarcz R, Guidetti P, and Muchowski PJ. Histone deacetylase inhibition modulates kynurenine pathway activation in yeast, microglia, and mice expressing a mutant huntingtin fragment. *J Biol Chem* 283: 7390–7400, 2008.

127. Globus MY, Prado R, and Busto R. Ischemia-induced changes in extracellular levels of striatal cyclic GMP: role of nitric oxide. *Neuroreport* 6: 1909–1912, 1995.

128. Goldberg GS, Lampe PD, and Nicholson BJ. Selective transfer of endogenous metabolites through gap junctions composed of different connexins. *Nat Cell Biol* 1: 457–459, 1999.

129. Goldberg GS, Moreno AP, and Lampe PD. Gap junctions between cells expressing connexin 43 or 32 show inverse permselectivity to adenosine and ATP. *J Biol Chem* 277: 36725–36730, 2002.

130. González CA, Garcés G, Sáez JC, Schobitz K, and Rodriguez EM. The ependymocytes of the bovine subcommissural organ are functionally coupled through gap junctions. *Neurosci Lett* 262: 175–178, 1999.

131. Gonzalez HE, Eugenin EA, Garces G, Solis N, Pizarro M, Accatino L, and Saez JC. Regulation of hepatic connexins in cholestasis: possible involvement of Kupffer cells and inflammatory mediators. *Am J Physiol Gastrointest Liver Physiol* 282: G991–G1001, 2002.

132. Grafstein B, Liu S, Cotrina ML, Goldman SA, and Nedergaard M. Meningeal cells can communicate with astrocytes by calcium signaling. *Ann Neurol* 47: 18–25, 2000.

133. Granger DN and Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. *Annu Rev Physiol* 57: 311–332, 1995.

134. Guan X, Wilson S, Schlender KK, and Ruch RJ. Gap-junction disassembly and connexin 43 dephosphorylation induced by 18 beta-glycyrrhetinic acid. *Mol Carcinog* 16: 157–164, 1996.

135. Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, and Kater SB. ATP released from astrocytes mediates glial calcium waves. *J Neurosci* 19: 520–528, 1999.

136. Haddad L, El Hajj H, Abou–Merhi R, Kfoury Y, Mahieux R, El–Sabban M, and Bazarbachi A. KSHV-transformed primary effusion lymphoma cells induce a VEGF-dependent angiogenesis and establish functional gap junctions with endothelial cells. *Leukemia* 22: 826–834, 2008.

137. Haddock RE, Grayson TH, Brackenbury TD, Meaney KR, Neylon CB, Sandow SL, and Hill CE. Endothelial coordination of cerebral vasomotion via myoendothelial gap junctions containing connexins 37 and 40. *Am J Physiol Heart Circ Physiol* 291: H2047–2056, 2006.

138. Hagberg H and Mallard C. Effect of inflammation on central nervous system development and vulnerability. *Curr Opin Neurol* 18: 117–123, 2005.

139. Haghikia A, Ladage K, Lafenetre P, Hinkerohe D, Smikalla D, Haase CG, Dermietzel R, and Faustmann PM. Intracellular application of TNF-alpha impairs cell to cell communication via gap junctions in glioma cells. *J Neurooncol* 86: 143–152, 2008.

140. Hailer NP. Immunosuppression after traumatic or ischemic CNS damage: It is neuroprotective and illuminates the role of microglial cells. *Prog Neurobiol* 84: 211–233, 2008.

141. Han BC, Koh SB, Lee EY, and Seong YH. Regional difference of glutamate-induced swelling in cultured rat brain astrocytes. *Life Sci* 76: 573–583, 2004.

142. Hanisch UK. Microglia as a source and target of cytokines. *Glia* 40: 140–155, 2002.

143. Hassinger TD, Guthrie PB, Atkinson PB, Bennett MV, and Kater SB. An extracellular signaling component in propagation of astrocytic calcium waves. *Proc Natl Acad Sci USA* 93: 13268–13273, 1996.

144. Haughey NJ and Mattson MP. Alzheimer's amyloid beta-peptide enhances ATP/gap junction-mediated calcium-wave propagation in astrocytes. *Neuromolecular Med* 3: 173–180, 2003.

145. Haupt C, Witte OW, and Frahm C. Up-regulation of Connexin43 in the glial scar following photothrombotic ischemic injury. *Mol Cell Neurosci* 35: 89–99, 2007.

146. Heales SJ, Bolanos JP, Stewart VC, Brookes PS, Land JM, and Clark JB. Nitric oxide, mitochondria and neurological disease. *Biochim Biophys Acta* 1410: 215–228, 1999.

147. Hensley K, Maidt ML, Yu Z, Sang H, Markesbery WR, and Floyd RA. Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation. *J Neurosci* 18: 8126–8132, 1998.

148. Hertz L and Zielke HR. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci* 27: 735-743, 2004.

149. Hewett SJ, Csernansky CA, and Choi DW. Selective potentiation of NMDA-induced neuronal injury following induction of astrocytic iNOS. *Neuron* 13: 487–494, 1994.

150. Hinkerohe D, Smikalla D, Haghikia A, Heupel K, Haase CG, Dermietzel R, and Faustmann PM. Effects of cytokines on microglial phenotypes and astroglial coupling in an inflammatory coculture model. *Glia* 52: 85–97, 2005.

151. Hirsch EC, Hunot S, Damier P, and Faucheux B. Glial cells and inflammation in Parkinson's disease: a role in neurodegeneration? *Ann Neurol* 44: S115–120, 1998.

152. Hombach S, Janssen–Bienhold U, Sohl G, Schubert T, Bussow H, Ott T, Weiler R, and Willecke K. Functional expression of connexin57 in horizontal cells of the mouse retina. *Eur J Neurosci* 19: 2633–2640, 2004.

153. Hool LC and Corry B. Redox control of calcium channels: from mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 9: 409–435, 2007.

154. Hormuzdi SG, Filippov MA, Mitropoulou G, Monyer H, and Bruzzone R. Electrical synapses: a dynamic signaling system that shapes the activity of neuronal networks. *Biochim Biophys Acta* 1662: 113–137, 2004.

155. Houades V, Rouach, N., Ezan, P., Kirchhoff, F., Koulakoff, A., Giaume, C. Shapes of astrocyte networks in the juvenile brain. *Neuron Glia Biology*, 2006, p. 3–14.

156. Hu J, Ferreira A, and Van Eldik LJ. S100beta induces neuronal cell death through nitric oxide release from astrocytes. *J Neurochem* 69: 2294–2301, 1997.

157. Huang Y, Grinspan JB, Abrams CK, and Scherer SS. Pannexin1 is expressed by neurons and glia but does not form functional gap junctions. *Glia* 55: 46–56, 2007.

158. Huh Y, Jung JW, Park C, Ryu JR, Shin CY, Kim WK, and Ryu JH. Microglial activation and tyrosine hydroxylase immunoreactivity in the substantia nigral region following transient focal ischemia in rats. *Neurosci Lett* 349: 63-67, 2003.

159. Hunot S, Boissiere F, Faucheux B, Brugg B, Mouatt–Prigent A, Agid Y, and Hirsch EC. Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* 72: 355–363, 1996.

160. Hunter CL, Bachman D, and Granholm AC. Minocycline prevents cholinergic loss in a mouse model of Down's syndrome. *Ann Neurol* 56: 675–688, 2004.

161. Jara PI, Boric MP, and Sáez JC. Leukocytes express connexin 43 after activation with lipopolysaccharide and appear to form gap junctions with endothelial cells after isch-

emia-reperfusion. *Proc Natl Acad Sci USA* 92: 7011–7015, 1995.

162. Jarvis CR and Andrew RD. Correlated electrophysiology and morphology of the ependyma in rat hypothalamus. *J Neurosci* 8: 3691–3702, 1988.

163. Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol* 53 Suppl 3: S26-36; discussion S36–28, 2003.

164. John GR, Scemes E, Suadicani SO, Liu JS, Charles PC, Lee SC, Spray DC, and Brosnan CF. IL-1beta differentially regulates calcium wave propagation between primary human fetal astrocytes via pathways involving P2 receptors and gap junction channels. *Proc Natl Acad Sci USA* 96: 11613–11618, 1999.

165. Johnston MV. Excitotoxicity in perinatal brain injury. *Brain Pathol* 15: 234-240, 2005.

166. Jongen WM, Fitzgerald DJ, Asamoto M, Piccoli C, Slaga TJ, Gros D, Takeichi M, and Yamasaki H. Regulation of connexin 43-mediated gap junctional intercellular communication by Ca2+ in mouse epidermal cells is controlled by E-cadherin. *J Cell Biol* 114: 545–555, 1991.

167. Kader A, Frazzini VI, Solomon RA, and Trifiletti RR. Nitric oxide production during focal cerebral ischemia in rats. *Stroke* 24: 1709–1716, 1993.

168. Kagansky N, Levy S, and Knobler H. The role of hyperglycemia in acute stroke. *Arch Neurol* 58: 1209-1212, 2001.

169. Kalaria RN. Microglia and Alzheimer's disease. *Curr Opin Hematol* 6: 15–24, 1999.

170. Kam Y, Kim DY, Koo SK, and Joe CO. Transfer of second messengers through gap junction connexin 43 channels reconstituted in liposomes. *Biochim Biophys Acta* 1372: 384–388, 1998.

171. Kamasawa N, Furman CS, Davidson KG, Sampson JA, Magnie AR, Gebhardt BR, Kamasawa M, Yasumura T, Zumbrunnen JR, Pickard GE, Nagy JI, and Rash JE. Abundance and ultrastructural diversity of neuronal gap junctions in the OFF and ON sublaminae of the inner plexiform layer of rat and mouse retina. *Neuroscience* 142: 1093–1117, 2006.

172. Kang J, Kang N, Lovatt D, Torres A, Zhao Z, Lin J, and Nedergaard M. Connexin 43 hemichannels are permeable to ATP. *J Neurosci* 28: 4702–4711, 2008.

173. Kanneganti TD, Lamkanfi M, Kim YG, Chen G, Park JH, Franchi L, Vandenabeele P, and Nunez G. Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. *Immunity* 26: 433–443, 2007.

174. Katsura K, Kristian T, Smith ML, and Siesjo BK. Acidosis **induced** by hypercapnia exaggerates ischemic brain damage. *J Cereb Blood Flow Metab* 14: 243–250, 1994.

175. Kaushal V and Schlichter LC. Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. *J Neurosci* 28: 2221–2230, 2008.

176. Kettenmann H and Orkand RK. Intracellular SITS injection dye-uncouples mammalian oligodendrocytes in culture. *Neurosci Lett* 39: 21–26, 1983.

177. Kettenmann H, Orkand RK, and Schachner M. Coupling among identified cells in mammalian nervous system cultures. *J Neurosci* 3: 506–516, 1983.

178. Kettenmann H and Ransom BR. Electrical coupling between astrocytes and between oligodendrocytes studied in mammalian cell cultures. *Glia* 1: 64–73, 1988.

179. Kim KS. Pathogenesis of bacterial meningitis: from bacteraemia to neuronal injury. *Nat Rev Neurosci* 4: 376–385, 2003.

180. Kingham PJ, Cuzner ML, and Pocock JM. Apoptotic pathways mobilized in microglia and neurones as a conse-quence of chromogranin A-induced microglial activation. *J Neurochem* 73: 538–547, 1999.

181. Kinsner A, Pilotto V, Deininger S, Brown GC, Coecke S, Hartung T, and Bal–Price A. Inflammatory neurodegeneration induced by lipoteichoic acid from Staphylococcus aureus is mediated by glia activation, nitrosative and oxidative stress, and caspase activation. *J Neurochem* 95: 1132–1143, 2005.

182. Korotzer AR, Pike CJ, and Cotman CW. beta-Amyloid peptides induce degeneration of cultured rat microglia. *Brain Res* 624: 121–125, 1993.

183. Koster–Patzlaff C, Hosseini SM, and Reuss B. Layer specific changes of astroglial gap junctions in the rat cerebellar cortex by persistent Borna Disease Virus infection. *Brain Res* 1219: 143–158, 2008.

184. Koster–Patzlaff C, Hosseini SM, and Reuss B. Persistent Borna Disease Virus infection changes expression and function of astroglial gap junctions in vivo and in vitro. *Brain Res* 1184: 316–332, 2007.

185. Koyama Y, Ishibashi T, Okamoto T, Matsuda T, Hashimoto H, and Baba A. Transient treatments with L-glutamate and threo-beta-hydroxyaspartate induce swelling of rat cultured astrocytes. *Neurochem Int* 36: 167–173, 2000.

186. Kreuzberg MM, Deuchars J, Weiss E, Schober A, Sonntag S, Wellershaus K, Draguhn A, and Willecke K. Expression of connexin30.2 in interneurons of the central nervous system in the mouse. *Mol Cell Neurosci* 37: 119–134, 2008.

187. Kristian T, Gido G, Kuroda S, Schutz A, and Siesjo BK. Calcium metabolism of focal and penumbral tissues in rats subjected to transient middle cerebral artery occlusion. *Exp Brain Res* 120: 503–509, 1998.

188. Kristian T and Siesjo BK. Calcium in ischemic cell death. *Stroke* 29: 705–718, 1998.

189. Kunzelmann P, Blumcke I, Traub O, Dermietzel R, and Willecke K. Coexpression of connexin45 and -32 in oligodendrocytes of rat brain. *J Neurocytol* 26: 17–22, 1997.

190. Kunzelmann P, Schroder W, Traub O, Steinhauser C, Dermietzel R, and Willecke K. Late onset and increasing expression of the gap junction protein connexin30 in adult murine brain and long-term cultured astrocytes. *Glia* 25: 111-119, 1999.

191. Kwak BR, Hermans MM, De Jonge HR, Lohmann SM, Jongsma HJ, and Chanson M. Differential regulation of distinct types of gap junction channels by similar phosphorylating conditions. *Mol Biol Cell* 6: 1707–1719, 1995.

192. Kwak S and Weiss JH. Calcium-permeable AMPA channels in neurodegenerative disease and ischemia. *Curr Opin Neurobiol* 16: 281–287, 2006.

193. Kwong LK, Uryu K, Trojanowski JQ, and Lee VM. TDP-43 proteinopathies: neurodegenerative protein misfolding diseases without amyloidosis. *Neurosignals* 16: 41–51, 2008.

194. Laflamme N and Rivest S. Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. *FASEB J* 15: 155–163, 2001.

195. Lai AY and Todd KG. Hypoxia-activated microglial mediators of neuronal survival are differentially regulated by tetracyclines. *Glia* 53: 809–816, 2006.

196. Lai CP, Bechberger JF, Thompson RJ, MacVicar BA, Bruzzone R, and Naus CC. Tumor-suppressive effects of pannexin 1 in C6 glioma cells. *Cancer Res* 67: 1545–1554, 2007.

197. Laird DW. Life cycle of connexins in health and disease. *Biochem J* 394: 527–543, 2006.

198. Lasky LA. A 'roll' in acute inflammation. *Curr Biol* 3: 680–682, 1993.

199. Lawrence TS, Beers WH, and Gilula NB. Transmission of hormonal stimulation by cell-to-cell communication. *Nature* 272: 501–506, 1978.

200. Lee SM and Clemens MG. Subacinar distribution of hepatocyte membrane potential response to stimulation of gluconeogenesis. *Am J Physiol* 263: G319–326, 1992.

201. Leis JA, Bekar LK, and Walz W. Potassium homeostasis in the ischemic brain. *Glia* 50: 407–416, 2005.

202. Leung DS, Unsicker K, and Reuss B. Expression and developmental regulation of gap junction connexins cx26, cx32, cx43 and cx45 in the rat midbrain-floor. *Int J Dev Neurosci* 20: 63–75, 2002.

203. Li F, Sugishita K, Su Z, Ueda I, and Barry WH. Activation of connexin-43 hemichannels can elevate [Ca(2+)]i and [Na(+)]i in rabbit ventricular myocytes during metabolic inhibition. *J Mol Cell Cardiol* 33: 2145–2155, 2001.

204. Li WE and Nagy JI. Connexin43 phosphorylation state and intercellular communication in cultured astrocytes following hypoxia and protein phosphatase inhibition. *Eur J Neurosci* 12: 2644–2650, 2000.

205. Li X, Ionescu AV, Lynn BD, Lu S, Kamasawa N, Morita M, Davidson KG, Yasumura T, Rash JE, and Nagy JI. Connexin47, connexin29 and connexin32 co-expression in oligodendrocytes and Cx47 association with zonula occludens-1 (ZO-1) in mouse brain. *Neuroscience* 126: 611–630, 2004.

206. Li X, Lynn BD, Olson C, Meier C, Davidson KG, Yasumura T, Rash JE, and Nagy JI. Connexin29 expression, immunocytochemistry and freeze-fracture replica immunogold labelling (FRIL) in sciatic nerve. *Eur J Neurosci* 16: 795–806, 2002.

207. Lin JH, Lou N, Kang N, Takano T, Hu F, Han X, Xu Q, Lovatt D, Torres A, Willecke K, Yang J, Kang J, and Nedergaard M. A central role of connexin 43 in hypoxic preconditioning. *J Neurosci* 28: 681–695, 2008.

208. Lin JH, Takano T, Cotrina ML, Arcuino G, Kang J, Liu S, Gao Q, Jiang L, Li F, Lichtenberg–Frate H, Haubrich S, Willecke K, Goldman SA, and Nedergaard M. Connexin 43 enhances the adhesivity and mediates the invasion of malignant glioma cells. *J Neurosci* 22: 4302–4311, 2002.

209. Lin JH, Weigel H, Cotrina ML, Liu S, Bueno E, Hansen AJ, Hansen TW, Goldman S, and Nedergaard M. Gap-junction-mediated propagation and amplification of cell injury. *Nat Neurosci* 1: 494–500, 1998.

210. Lin MC, Huang YL, Liu HW, Yang DY, Lee CP, Yang LL, and Cheng FC. On-line microdialysis-graphite furnace atomic absorption spectrometry in the determination of brain magnesium levels in gerbils subjected to cerebral ischemia/reperfusion. *J Am Coll Nutr* 23: 561S–565S, 2004.

211. Little TL, Beyer EC, and Duling BR. Connexin 43 and connexin 40 gap junctional proteins are present in arteriolar smooth muscle and endothelium in vivo. *Am J Physiol* 268: H729–739, 1995.

212. Litvan I, Chesselet MF, Gasser T, Di Monte DA, Parker D, Jr., Hagg T, Hardy J, Jenner P, Myers RH, Price D, Hallett M, Langston WJ, Lang AE, Halliday G, Rocca W, Duyckaerts C, Dickson DW, Ben–Shlomo Y, Goetz CG, and Melamed E. The etiopathogenesis of Parkinson disease and suggestions for future research. Part II. *J Neuropathol Exp Neurol* 66: 329–336, 2007.

213. Liu HT, Sabirov RZ, and Okada Y. Oxygen-glucose deprivation induces ATP release via maxi-anion channels in astrocytes. *Purinergic Signal* 4: 147–154, 2008.

214. Lobsiger CS and Cleveland DW. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nat Neurosci* 10: 1355–1360, 2007.

215. Locovei S, Bao L, and Dahl G. Pannexin 1 in erythrocytes: function without a gap. *Proc Natl Acad Sci USA* 103: 7655–7659, 2006.

216. Long MA, Jutras MJ, Connors BW, and Burwell RD. Electrical synapses coordinate activity in the suprachiasmatic nucleus. *Nat Neurosci* 8: 61–66, 2005.

217. Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, and Cotman CW. Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. *Proc Natl Acad Sci USA* 90: 7951–7955, 1993.

218. Lott IT and Head E. Alzheimer disease and Down syndrome: factors in pathogenesis. *Neurobiol Aging* 26: 383–389, 2005.

219. Madelian V, Martin DL, Lepore R, Perrone M, and Shain W. Beta-receptor-stimulated and cyclic adenosine 3′,5′-monophosphate-mediated taurine release from LRM55 glial cells. *J Neurosci* 5: 3154–3160, 1985.

220. Madrigal JL, Feinstein DL, and Dello Russo C. Norepinephrine protects cortical neurons against microglial-induced cell death. *J Neurosci Res* 81: 390–396, 2005.

221. Magistretti PJ. Neuron-glia metabolic coupling and plasticity. *J Exp Biol* 209: 2304–2311, 2006.

222. Maher P. p38 mitogen-activated protein kinase activation is required for fibroblast growth factor-2-stimulated cell proliferation but not differentiation. *J Biol Chem* 274: 17491–17498, 1999.

223. Marin–Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, and Mallat M. Microglia promote the death of developing Purkinje cells. *Neuron* 41: 535–547, 2004.

224. Martin LJ, Al–Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, and Portera–Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: A perspective on the contributions of apoptosis and necrosis. *Brain Res Bull* 46: 281–309, 1998.

225. Martínez AD, Eugenin EA, Brañes MC, Bennett ML, and Sáez JC. Identification of second messengers that induce expression of functional gap junctions in microglia cultured from newborn rats. *Brain Res* 943: 191–201, 2002.

226. Martínez AD and Sáez JC. Arachidonic acid-induced dye uncoupling in rat cortical astrocytes is mediated by arachidonic acid byproducts. *Brain Res* 816: 411–423, 1999.

227. Martínez AD and Sáez JC. Regulation of astrocyte gap junctions by hypoxia-reoxygenation. *Brain Res Brain Res Rev* 32: 250–258, 2000.

228. Matalon S, Hardiman KM, Jain L, Eaton DC, Kotlikoff M, Eu JP, Sun J, Meissner G, and Stamler JS. Regulation of ion channel structure and function by reactive oxygen-nitrogen species. *Am J Physiol Lung Cell Mol Physiol* 285: L1184–1189, 2003.

229. Matsuoka Y, Kitamura Y, Takahashi H, Tooyama I, Kimura H, Gebicke-Haerter PJ, Nomura Y, and Taniguchi T. Interferon-gamma plus lipopolysaccharide induction of delayed neuronal apoptosis in rat hippocampus. *Neurochem Int* 34: 91–99, 1999.

230. Maxeiner S, Kruger O, Schilling K, Traub O, Urschel S, and Willecke K. Spatiotemporal transcription of connexin45 during brain development results in neuronal expression in adult mice. *Neuroscience* 119: 689–700, 2003.

231. McGeer EG and McGeer PL. Inflammatory processes in Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 27: 741–749, 2003.

232. McGeer PL, Itagaki S, Akiyama H, and McGeer EG. Rate of cell death in parkinsonism indicates active neuropathological process. *Ann Neurol* 24: 574–576, 1988.

233. McNaught KS and Jenner P. Altered glial function causes neuronal death and increases neuronal susceptibility to 1-methyl-4-phenylpyridinium- and 6-hydroxydopamine-induced toxicity in astrocytic/ventral mesencephalic co-cultures. *J Neurochem* 73: 2469–2476, 1999.

234. Meda L, Baron P, Prat E, Scarpini E, Scarlato G, Cassatella MA, and Rossi F. Proinflammatory profile of cytokine production by human monocytes and murine microglia stimulated with beta-amyloid[25-35]. *J Neuroimmunol* 93: 45–52, 1999.

235. Meme W, Calvo CF, Froger N, Ezan P, Amigou E, Koulakoff A, and Giaume C. Proinflammatory cytokines released from microglia inhibit gap junctions in astrocytes: potentiation by beta-amyloid. *FASEB J* 20: 494–496, 2006.

236. Mendoza–Naranjo A, Saez PJ, Johansson CC, Ramirez M, Mandakovic D, Pereda C, Lopez MN, Kiessling R, Saez JC, and Salazar–Onfray F. Functional gap junctions facilitate melanoma antigen transfer and cross-presentation between human dendritic cells. *J Immunol* 178: 6949–6957, 2007.

237. Mercier F and Hatton GI. Connexin 26 and basic fibroblast growth factor are expressed primarily in the subpial and subependymal layers in adult brain parenchyma: roles in stem cell proliferation and morphological plasticity? *J Comp Neurol* 431: 88–104, 2001.

238. Mergenthaler P, Dirnagl U, and Meisel A. Pathophysiology of stroke: lessons from animal models. *Metab Brain Dis* 19: 151–167, 2004.

239. Metea MR and Newman EA. Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. *J Neurosci* 26: 2862–2870, 2006.

240. Miller RF. D-Serine as a glial modulator of nerve cells. *Glia* 47: 275–283, 2004.

241. Moreno AP, Rook MB, Fishman GI, and Spray DC. Gap junction channels: distinct voltage-sensitive and -insensitive conductance states. *Biophys J* 67: 113–119, 1994.

242. Morgan SC, Taylor DL, and Pocock JM. Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. *J Neurochem* 90: 89–101, 2004.

243. Morita M, Saruta C, Kozuka N, Okubo Y, Itakura M, Takahashi M, and Kudo Y. Dual regulation of astrocyte gap junction hemichannels by growth factors and a pro-inflammatory cytokine via the mitogen-activated protein kinase cascade. *Glia* 55: 508–515, 2007.

244. Mount MP, Lira A, Grimes D, Smith PD, Faucher S, Slack R, Anisman H, Hayley S, and Park DS. Involvement of interferon-gamma in microglial-mediated loss of dopaminergic neurons. *J Neurosci* 27: 3328–3337, 2007.

245. Mugnaini. Cell junctions of astrocytes, ependyma, and related cells in the mammalian central nervous system, with emphasis on the hypothesis of a generalized functional syncytium of supporting cells. In: *Astrocytes*, edited by Fedoroff S VA. New York: Academic Press, 1986, p. 329–371.

246. Muranyi M, Ding C, He Q, Lin Y, and Li PA. Streptozotocin-induced diabetes causes astrocyte death after ischemia and reperfusion injury. *Diabetes* 55: 349–355, 2006.

247. Murphy S and Pearce B. Eicosanoids in the CNS: sources and effects. *Prostaglandins Leukot Essent Fatty Acids* 31: 165–170, 1988.

248. Musil LS and Goodenough DA. Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques. *J Cell Biol* 115: 1357–1374, 1991.

249. Nadarajah B, Jones AM, Evans WH, and Parnavelas JG. Differential expression of connexins during neocortical development and neuronal circuit formation. *J Neurosci* 17: 3096–3111, 1997.

250. Nagasawa K, Chiba H, Fujita H, Kojima T, Saito T, Endo T, and Sawada N. Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. *J Cell Physiol* 208: 123–132, 2006.

251. Nagy JI, Dudek FE, and Rash JE. Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. *Brain Res Brain Res Rev* 47: 191–215, 2004.

252. Nagy JI, Hossain MZ, Lynn BD, Curpen GE, Yang S, and Turley EA. Increased connexin-43 and gap junctional communication correlate with altered phenotypic characteristics of cells overexpressing the receptor for hyaluronic acid-mediated motility. *Cell Growth Differ* 7: 745–751, 1996.

253. Nagy JI and Li WE. A brain slice model for in vitro analyses of astrocytic gap junction and connexin43 regulation: actions of ischemia, glutamate and elevated potassium. *Eur J Neurosci* 12: 4567–4572, 2000.

254. Nagy JI, Li X, Rempel J, Stelmack G, Patel D, Staines WA, Yasumura T, and Rash JE. Connexin26 in adult rodent central nervous system: demonstration at astrocytic gap junctions and colocalization with connexin30 and connexin43. *J Comp Neurol* 441: 302–323, 2001.

255. Nagy JI, Ochalski PA, Li J, and Hertzberg EL. Evidence for the co-localization of another connexin with connexin-43 at astrocytic gap junctions in rat brain. *Neuroscience* 78: 533–548, 1997.

256. Nagy JI, Patel D, Ochalski PA, and Stelmack GL. Connexin30 in rodent, cat and human brain: selective expression in gray matter astrocytes, co-localization with connexin43 at gap junctions and late developmental appearance. *Neuroscience* 88: 447–468, 1999.

257. Nakase T, Fushiki S, Sohl G, Theis M, Willecke K, and Naus CC. Neuroprotective role of astrocytic gap junctions in ischemic stroke. *Cell Commun Adhes* 10: 413–417, 2003.

258. Nakase T, Sohl G, Theis M, Willecke K, and Naus CC. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin43 in astrocytes. *Am J Pathol* 164: 2067–2075, 2004.

259. Nau R and Bruck W. Neuronal injury in bacterial meningitis: mechanisms and implications for therapy. *Trends Neurosci* 25: 38–45, 2002.

260. Nau R, Gerber J, Bunkowski S, and Bruck W. Axonal injury, a neglected cause of CNS damage in bacterial meningitis. *Neurology* 62: 509–511, 2004.

261. Naus CC, Bechberger JF, Zhang Y, Venance L, Yamasaki H, Juneja SC, Kidder GM, and Giaume C. Altered gap junctional communication, intercellular signaling, and growth in cultured astrocytes deficient in connexin43. *J Neurosci Res* 49: 528–540, 1997.

262. Nedergaard M, Ransom B, and Goldman SA. New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci* 26: 523–530, 2003.

263. Neijssen J, Herberts C, Drijfhout JW, Reits E, Janssen L, and Neefjes J. Cross-presentation by intercellular peptide transfer through gap junctions. *Nature* 434: 83–88, 2005.

264. Nicholson C, Bruggencate GT, Steinberg R, and Stockle H. Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. *Proc Natl Acad Sci USA* 74: 1287–1290, 1977.

265. Niessen H, Harz H, Bedner P, Kramer K, and Willecke K. Selective permeability of different connexin channels to the

second messenger inositol 1,4,5-trisphosphate. *J Cell Sci* 113: 1365–1372, 2000.

266. Nishida M, Futami S, Morita I, Maekawa K, and Murota SI. Hypoxia-reoxygenation inhibits gap junctional communication in cultured human umbilical vein endothelial cells. *Endothelium* 7: 279–286, 2000.

267. Norris CM, Kadish I, Blalock EM, Chen KC, Thibault V, Porter NM, Landfield PW, and Kraner SD. Calcineurin triggers reactive/inflammatory processes in astrocytes and is upregulated in aging and Alzheimer's models. *J Neurosci* 25: 4649–4658, 2005.

268. Oguro K, Jover T, Tanaka H, Lin Y, Kojima T, Oguro N, Grooms SY, Bennett MV, and Zukin RS. Global ischemia-induced increases in the gap junctional proteins connexin 32 (Cx32) and Cx36 in hippocampus and enhanced vulnerability of Cx32 knock-out mice. *J Neurosci* 21: 7534–7542, 2001.

269. Orthmann–Murphy JL, Freidin M, Fischer E, Scherer SS, and Abrams CK. Two distinct heterotypic channels mediate gap junction coupling between astrocyte and oligodendrocyte connexins. *J Neurosci* 27: 13949–13957, 2007.

270. Oviedo–Orta E, Errington RJ, and Evans WH. Gap junction intercellular communication during lymphocyte transendothelial migration. *Cell Biol Int* 26: 253–263, 2002.

271. Ozog MA, Siushansian R, and Naus CC. Blocked gap junctional coupling increases glutamate-induced neurotoxicity in neuron-astrocyte co-cultures. *J Neuropathol Exp Neurol* 61: 132–141, 2002.

272. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, and Oliet SH. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 125: 775–784, 2006.

273. Panchin YV. Evolution of gap junction proteins—the pannexin alternative. *J Exp Biol* 208: 1415–1419, 2005.

274. Parenti R, Campisi A, Vanella A, and Cicirata F. Immunocytochemical and RT-PCR analysis of connexin36 in cultures of mammalian glial cells. *Arch Ital Biol* 140: 101–108, 2002.

275. Parihar MS and Hemnani T. Alzheimer's disease pathogenesis and therapeutic interventions. *J Clin Neurosci* 11: 456–467, 2004.

276. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, and Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature* 369: 744–747, 1994.

277. Pastor A, Kremer M, Moller T, Kettenmann H, and Dermietzel R. Dye coupling between spinal cord oligodendrocytes: Differences in coupling efficiency between gray and white matter. *Glia* 24: 108–120, 1998.

278. Paulsen JS, Ready RE, Hamilton JM, Mega MS, and Cummings JL. Neuropsychiatric aspects of Huntington's disease. *J Neurol Neurosurg Psychiatry* 71: 310–314, 2001.

279. Pedersen WA, Fu W, Keller JN, Markesbery WR, Appel S, Smith RG, Kasarskis E, and Mattson MP. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. *Ann Neurol* 44: 819–824, 1998.

280. Pekny M and Nilsson M. Astrocyte activation and reactive gliosis. *Glia* 50: 427–434, 2005.

281. Pelegrin P and Surprenant A. Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. *J Biol Chem* 282: 2386–2394, 2007.

282. Pelegrin P and Surprenant A. Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. *EMBO J* 25: 5071–5082, 2006.

283. Pellerin L, Bouzier–Sore AK, Aubert A, Serres S, Merle M, Costalat R, and Magistretti PJ. Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* 55: 1251–1262, 2007.

284. Penuela S, Bhalla R, Gong XQ, Cowan KN, Celetti SJ, Cowan BJ, Bai D, Shao Q, and Laird DW. Pannexin 1 and pannexin 3 are glycoproteins that exhibit many distinct characteristics from the connexin family of gap junction proteins. *J Cell Sci* 120: 3772–3783, 2007.

285. Perez-Velazquez JL, Kokarovtseva L, Sarbaziha R, Jeyapalan Z, and Leshchenko Y. Role of gap junctional coupling in astrocytic networks in the determination of global ischaemia-induced oxidative stress and hippocampal damage. *Eur J Neurosci* 23: 1–10, 2006.

286. Petrasch–Parwez E, Habbes HW, Weickert S, Lobbecke–Schumacher M, Striedinger K, Wieczorek S, Dermietzel R, and Epplen JT. Fine-structural analysis and connexin expression in the retina of a transgenic model of Huntington's disease. *J Comp Neurol* 479: 181–197, 2004.

287. Petrasch–Parwez E, Nguyen HP, Lobbecke–Schumacher M, Habbes HW, Wieczorek S, Riess O, Andres KH, Dermietzel R, and Von Horsten S. Cellular and subcellular localization of Huntingtin [corrected] aggregates in the brain of a rat transgenic for Huntington disease. *J Comp Neurol* 501: 716–730, 2007.

288. Peuchen S, Duchen MR, and Clark JB. Energy metabolism of adult astrocytes in vitro. *Neuroscience* 71: 855–870, 1996.

289. Pike CJ, Walencewicz–Wasserman AJ, Kosmoski J, Cribbs DH, Glabe CG, and Cotman CW. Structure-activity analyses of beta-amyloid peptides: contributions of the beta 25-35 region to aggregation and neurotoxicity. *J Neurochem* 64: 253–265, 1995.

290. Polacek D, Lal R, Volin MV, and Davies PF. Gap junctional communication between vascular cells. Induction of connexin43 messenger RNA in macrophage foam cells of atherosclerotic lesions. *Am J Pathol* 142: 593–606, 1993.

291. Polazzi E and Contestabile A. Reciprocal interactions between microglia and neurons: from survival to neuropathology. *Rev Neurosci* 13: 221–242, 2002.

292. Polidori MC, Griffiths HR, Mariani E, and Mecocci P. Hallmarks of protein oxidative damage in neurodegenerative diseases: focus on Alzheimer's disease. *Amino Acids* 32: 553–559, 2007.

293. Pollmann MA, Shao Q, Laird DW, and Sandig M. Connexin 43 mediated gap junctional communication enhances breast tumor cell diapedesis in culture. *Breast Cancer Res* 7: R522–534, 2005.

294. Pryazhnikov E and Khiroug L. Sub-micromolar increase in [Ca(2+)](i) triggers delayed exocytosis of ATP in cultured astrocytes. *Glia* 56: 38–49, 2008.

295. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ, and Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 55: 453–462, 2007.

296. Qu Y and Dahl G. Function of the voltage gate of gap junction channels: selective exclusion of molecules. *Proc Natl Acad Sci USA* 99: 697–702, 2002.

297. Queiroz G, Gebicke–Haerter PJ, Schobert A, Starke K, and von Kugelgen I. Release of ATP from cultured rat astrocytes elicited by glutamate receptor activation. *Neuroscience* 78: 1203–1208, 1997.

298. Quist AP, Rhee SK, Lin H, and Lal R. Physiological role of gap-junctional hemichannels. Extracellular calcium-dependent isosmotic volume regulation. *J Cell Biol* 148: 1063–1074, 2000.

299. Rafols JA and Goshgarian HG. Spinal tanycytes in the adult rat: a correlative Golgi gold-toning study. *Anat Rec* 211: 75–86, 1985.

300. Raine CS, Cannella B, Duijvestijn AM, and Cross AH. Homing to central nervous system vasculature by antigen-specific lymphocytes. II. Lymphocyte/endothelial cell adhesion during the initial stages of autoimmune demyelination. *Lab Invest* 63: 476–489, 1990.

301. Rami A, Volkmann T, and Winckler J. Effective reduction of neuronal death by inhibiting gap junctional intercellular communication in a rodent model of global transient cerebral ischemia. *Exp Neurol* 170: 297–304, 2001.

302. Rana S and Dringen R. Gap junction hemichannel-mediated release of glutathione from cultured rat astrocytes. *Neurosci Lett* 415: 45–48, 2007.

303. Rash JE, Davidson KG, Kamasawa N, Yasumura T, Kamasawa M, Zhang C, Michaels R, Restrepo D, Ottersen OP, Olson CO, and Nagy JI. Ultrastructural localization of connexins (Cx36, Cx43, Cx45), glutamate receptors and aquaporin-4 in rodent olfactory mucosa, olfactory nerve and olfactory bulb. *J Neurocytol* 34: 307–341, 2005.

304. Rash JE, Duffy HS, Dudek FE, Bilhartz BL, Whalen LR, and Yasumura T. Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a "panglial syncytium" that is not coupled to neurons. *J Comp Neurol* 388: 265–292, 1997.

305. Rash JE, Olson CO, Davidson KG, Yasumura T, Kamasawa N, and Nagy JI. Identification of connexin36 in gap junctions between neurons in rodent locus coeruleus. *Neuroscience* 147: 938–956, 2007.

306. Rash JE, Olson CO, Pouliot WA, Davidson KG, Yasumura T, Furman CS, Royer S, Kamasawa N, Nagy JI, and Dudek FE. Connexin36 vs. connexin32, "miniature" neuronal gap junctions, and limited electrotonic coupling in rodent suprachiasmatic nucleus. *Neuroscience* 149: 350–371, 2007.

307. Rash JE, Pereda A, Kamasawa N, Furman CS, Yasumura T, Davidson KG, Dudek FE, Olson C, Li X, and Nagy JI. High-resolution proteomic mapping in the vertebrate central nervous system: close proximity of connexin35 to NMDA glutamate receptor clusters and co-localization of connexin36 with immunoreactivity for zonula occludens protein-1 (ZO-1). *J Neurocytol* 33: 131–151, 2004.

308. Rash JE, Staines WA, Yasumura T, Patel D, Furman CS, Stelmack GL, and Nagy JI. Immunogold evidence that neuronal gap junctions in adult rat brain and spinal cord contain connexin-36 but not connexin-32 or connexin-43. *Proc Natl Acad Sci USA* 97: 7573–7578, 2000.

309. Rash JE, Yasumura T, Davidson KG, Furman CS, Dudek FE, and Nagy JI. Identification of cells expressing Cx43, Cx30, Cx26, Cx32 and Cx36 in gap junctions of rat brain and spinal cord. *Cell Commun Adhes* 8: 315–320, 2001.

310. Rawanduzy A, Hansen A, Hansen TW, and Nedergaard M. Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke. *J Neurosurg* 87: 916–920, 1997.

311. Ray A, Zoidl G, Weickert S, Wahle P, and Dermietzel R. Site-specific and developmental expression of pannexin1 in the mouse nervous system. *Eur J Neurosci* 21: 3277–3290, 2005.

312. Raza H and John A. Glutathione metabolism and oxidative stress in neonatal rat tissues from streptozotocin-induced diabetic mothers. *Diabetes Metab Res Rev* 20: 72–78, 2004.

313. Retamal MA, Cortés CJ, Reuss L, Bennett ML, and Sáez JC. S-nitrosylation and permeation through connexin 43 hemichannels in astrocytes: induction by oxidant stress and reversal by reducing agents. *Proc Natl Acad Sci USA* 103: 4475–4480, 2006.

314. Retamal MA, Froger N, Palacios–Prado N, Ezan P, Sáez PJ, Sáez JC, and Giaume C. Cx43 hemichannels and gap junction channels in astrocytes are regulated oppositely by proinflammatory cytokines released from activated microglia. *J Neurosci* 27: 13781–13792, 2007.

315. Retamal MA, Schalper KA, Shoji KF, Bennett ML, and Sáez JC. Opening of connexin 43 hemichannels is increased by lowering intracellular redox potential. *Proc Natl Acad Sci USA* 104: 8322–8327, 2007.

316. Retamal MA, Schalper KA, Shoji KF, Orellana JA, Bennett ML, and Sáez JC. Possible involvement of different connexin43 domains in plasma membrane permeabilization induced by ischemia-reperfusion. *J Membr Biol* 218: 49–63, 2007.

317. Ring A, Weiser JN, and Tuomanen EI. Pneumococcal trafficking across the blood-brain barrier. Molecular analysis of a novel bidirectional pathway. *J Clin Invest* 102: 347–360, 1998.

318. Rochefort N, Quenech'du N, Ezan P, Giaume C, and Milleret C. Postnatal development of GFAP, connexin43 and connexin30 in cat visual cortex. *Brain Res Dev Brain Res* 160: 252–264, 2005.

319. Rohr S. Role of gap junctions in the propagation of the cardiac action potential. *Cardiovasc Res* 62: 309–322, 2004.

320. Rouach N, Calvo CF, Duquennoy H, Glowinski J, and Giaume C. Hydrogen peroxide increases gap junctional communication and induces astrocyte toxicity: regulation by brain macrophages. *Glia* 45: 28–38, 2004.

321. Rouach N, Calvo CF, Glowinski J, and Giaume C. Brain macrophages inhibit gap junctional communication and downregulate connexin 43 expression in cultured astrocytes. *Eur J Neurosci* 15: 403–407, 2002.

322. Rouach N, Glowinski J, and Giaume C. Activity-dependent neuronal control of gap-junctional communication in astrocytes. *J Cell Biol* 149: 1513–1526, 2000.

323. Rozental R, Andrade–Rozental AF, Zheng X, Urban M, Spray DC, and Chiu FC. Gap junction-mediated bidirectional signaling between human fetal hippocampal neurons and astrocytes. *Dev Neurosci* 23: 420–431, 2001.

324. Ruano D, Revilla E, Gavilan MP, Vizuete ML, Pintado C, Vitorica J, and Castano A. Role of p38 and inducible nitric oxide synthase in the in vivo dopaminergic cells' degeneration induced by inflammatory processes after lipopolysaccharide injection. *Neuroscience* 140: 1157–1168, 2006.

325. Rufer M, Wirth SB, Hofer A, Dermietzel R, Pastor A, Kettenmann H, and Unsicker K. 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, 1996.

326. Sáez J, Araya R, Brañes MC, Concha M, Contreras J, Eugenín EA, Martínez AD, and Palisson FaSM. Gap junctions in inflammatory responses: Connexins, regulation and possible functional roles. In: *Gap Junctions—Molecular Basis of Cell Communication in Health and Diseases. Current Topics in Membranes*, edited by C P. San Diego: Academic Press, 1999.

327. Sáez JC, Berthoud VM, Brañes MC, Martínez AD, and Beyer EC. Plasma membrane channels formed by connexins: their regulation and functions. *Physiol Rev* 83: 1359–1400, 2003.

328. Sáez JC, Gregory WA, Watanabe T, Dermietzel R, Hertzberg EL, Reid L, Bennett ML, and Spray DC. cAMP delays disappearance of gap junctions between pairs of rat hepatocytes in primary culture. *Am J Physiol* 257: C1–11, 1989.

329. Sáez JC, Retamal MA, Basilio D, Bukauskas FF, and Bennett ML. Connexin-based gap junction hemichannels: gating mechanisms. *Biochim Biophys Acta* 1711: 215–224, 2005.

330. Scemes E and Giaume C. Astrocyte calcium waves: what they are and what they do. *Glia* 54: 716–725, 2006.

331. Schalper KA, Palacios–Prado N, Retamal MA, Shoji KF, Martínez AD, and Sáez JC. Connexin hemichannels composition determines the FGF-1-induced membrane permeability and free $[Ca^{2+}]_i$ responses. *Mol Biol Cell* 19: 3501–3513, 2008.

332. Schell MJ, Molliver ME, and Snyder SH. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci USA* 92: 3948–3952, 1995.

333. Schock SC, Leblanc D, Hakim AM, and Thompson CS. ATP release by way of connexin 36 hemichannels mediates ischemic tolerance in vitro. *Biochem Biophys Res Commun* 368: 138–144, 2008.

334. Schousboe A, Westergaard N, Sonnewald U, Petersen SB, Huang R, Peng L, and Hertz L. Glutamate and glutamine metabolism and compartmentation in astrocytes. *Dev Neurosci* 15: 359–366, 1993.

335. Schultzberg M, Lindberg C, Aronsson AF, Hjorth E, Spulber SD, and Oprica M. Inflammation in the nervous system–physiological and pathophysiological aspects. *Physiol Behav* 92: 121–128, 2007.

336. Schwandner R, Dziarski R, Wesche H, Rothe M, and Kirschning CJ. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* 274: 17406–17409, 1999.

337. Shah VB, Huang Y, Keshwara R, Ozment–Skelton T, Williams DL, and Keshvara L. {beta}-Glucan activates microglia without inducing cytokine production in dectin-1-dependent manner. *J Immunol* 180: 2777–2785, 2008.

338. Shelley J, Dedek K, Schubert T, Feigenspan A, Schultz K, Hombach S, Willecke K, and Weiler R. Horizontal cell receptive fields are reduced in connexin57-deficient mice. *Eur J Neurosci* 23: 3176–3186, 2006.

339. Shestopalov VI and Panchin Y. Pannexins and gap junction protein diversity. *Cell Mol Life Sci* 65: 376–394, 2008.

340. Shin JY, Fang ZH, Yu ZX, Wang CE, Li SH, and Li XJ. Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. *J Cell Biol* 171: 1001–1012, 2005.

341. Shintani–Ishida K, Uemura K, and Yoshida K. Hemichannels in cardiomyocytes open transiently during ischemia and contribute to reperfusion injury following brief ischemia. *Am J Physiol Heart Circ Physiol* 293: H1714–1720, 2007.

342. Simard M, Couldwell WT, Zhang W, Song H, Liu S, Cotrina ML, Goldman S, and Nedergaard M. Glucocorticoids-potent modulators of astrocytic calcium signaling. *Glia* 28: 1–12, 1999.

343. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, and Gwinn–Hardy K. alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302: 841, 2003.

344. Siushansian R, Bechberger JF, Cechetto DF, Hachinski VC, and Naus CC. Connexin43 null mutation increases infarct size after stroke. *J Comp Neurol* 440: 387–394, 2001.

345. SiuYi Leung D, Unsicker K, and Reuss B. Gap junctions modulate survival-promoting effects of fibroblast growth factor-2 on cultured midbrain dopaminergic neurons. *Mol Cell Neurosci* 18: 44–55, 2001.

346. Sohl G, Eiberger J, Jung YT, Kozak CA, and Willecke K. The mouse gap junction gene connexin29 is highly expressed in sciatic nerve and regulated during brain development. *Biol Chem* 382: 973–978, 2001.

347. Sohl G and Willecke K. Gap junctions and the connexin protein family. *Cardiovasc Res* 62: 228–232, 2004.

348. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, and Goedert M. Alpha-synuclein in Lewy bodies. *Nature* 388: 839–840, 1997.

349. Spray DC, Moreno AP, Kessler JA, and Dermietzel R. Characterization of gap junctions between cultured leptomeningeal cells. *Brain Res* 568: 1–14, 1991.

350. Stout CE, Costantin JL, Naus CC, and Charles AC. Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. *J Biol Chem* 277: 10482–10488, 2002.

351. Streit WJ. Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia* 40: 133–139, 2002.

352. Stridh MH, Tranberg M, Weber SG, Blomstrand F, and Sandberg M. Stimulated efflux of amino acids and glutathione from cultured hippocampal slices by omission of extracellular calcium: Likely involvement of connexin hemichannels. *J Biol Chem* 283: 10347–10356, 2008.

353. Stuertz K, Schmidt H, Eiffert H, Schwartz P, Mader M, and Nau R. Differential release of lipoteichoic and teichoic acids from Streptococcus pneumoniae as a result of exposure to beta-lactam antibiotics, rifamycins, trovafloxacin, and quin-upristin-dalfopristin. *Antimicrob Agents Chemother* 42: 277–281, 1998.

354. Stuertz K, Schmidt H, Trostdorf F, Eiffert H, Mader M, and Nau R. Lower lipoteichoic and teichoic acid CSF concentrations during treatment of pneumococcal meningitis with non-bacteriolytic antibiotics than with ceftriaxone. *Scand J Infect Dis* 31: 367–370, 1999.

355. Suadicani SO, Brosnan CF, and Scemes E. P2X7 receptors mediate ATP release and amplification of astrocytic intercellular Ca2+ signaling. *J Neurosci* 26: 1378–1385, 2006.

356. Sugawara T, Lewen A, Noshita N, Gasche Y, and Chan PH. Effects of global ischemia duration on neuronal, astroglial, oligodendroglial, and microglial reactions in the vulnerable hippocampal CA1 subregion in rats. *J Neurotrauma* 19: 85-98, 2002.

357. Sung JY, Lee HJ, Jeong EI, Oh Y, Park J, Kang KS, and Chung KC. Alpha-synuclein overexpression reduces gap junctional intercellular communication in dopaminergic neuroblastoma cells. *Neurosci Lett* 416: 289–293, 2007.

358. Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, and Piccini P. Microglial activation in presymptomatic Huntington's disease gene carriers. *Brain* 130: 1759–1766, 2007.

359. Takahashi S, Shibata M, and Fukuuchi Y. Effects of increased extracellular potassium on influx of sodium ions in cultured rat astroglia and neurons. *Brain Res Dev Brain Res* 104: 111–117, 1997.

360. Takano T, Han X, Deane R, Zlokovic B, and Nedergaard M. Two-photon imaging of astrocytic Ca2+ signaling and the microvasculature in experimental mice models of Alzheimer's disease. *Ann NY Acad Sci* 1097: 40–50, 2007.

361. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, Sonobe Y, Mizuno T, and Suzumura A. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J Biol Chem* 281: 21362–21368, 2006.

362. Tauber MG and Moser B. Cytokines and chemokines in meningeal inflammation: biology and clinical implications. *Clin Infect Dis* 28: 1–11; quiz 12, 1999.

363. Thirumangalakudi L, Yin L, Rao HV, and Grammas P. IL-8 induces expression of matrix metalloproteinases, cell cycle and pro-apoptotic proteins, and cell death in cultured neurons. *J Alzheimers Dis* 11: 305–311, 2007.

364. Thompson RJ, Zhou N, and MacVicar BA. Ischemia opens neuronal gap junction hemichannels. *Science* 312: 924–927, 2006.

365. Thornton P, Pinteaux E, Gibson RM, Allan SM, and Rothwell NJ. Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release. *J Neurochem* 98: 258–266, 2006.

366. Torkildsen O, Nyland H, Myrmel H, and Myhr KM. Epstein-Barr virus reactivation and multiple sclerosis. *Eur J Neurol* 15: 106–108, 2008.

367. Tran Van Nhieu G, Clair C, Bruzzone R, Mesnil M, Sansonetti P, and Combettes L. Connexin-dependent inter-cellular communication increases invasion and dissemination of Shigella in epithelial cells. *Nat Cell Biol* 5: 720–726, 2003.

368. Upender MB and Naegele JR. Activation of microglia during developmentally regulated cell death in the cerebral cortex. *Dev Neurosci* 21: 491–505, 1999.

369. Van Bockstaele EJ, Garcia–Hernandez F, Fox K, Alvarez VA, and Williams JT. Expression of connexins during development and following manipulation of afferent input in the rat locus coeruleus. *Neurochem Int* 45: 421–428, 2004.

370. Vandamme W, Braet K, Cabooter L, and Leybaert L. Tumour necrosis factor alpha inhibits purinergic calcium signalling in blood-brain barrier endothelial cells. *J Neurochem* 88: 411–421, 2004.

371. Vandecasteele M, Glowinski J, and Venance L. Connexin mRNA expression in single dopaminergic neurons of substantia nigra pars compacta. *Neurosci Res* 56: 419–426, 2006.

372. Vandecasteele M, Glowinski J, and Venance L. Electrical synapses between dopaminergic neurons of the substantia nigra pars compacta. *J Neurosci* 25: 291–298, 2005.

373. Vanden Abeele F, Bidaux G, Gordienko D, Beck B, Panchin YV, Baranova AV, Ivanov DV, Skryma R, and Prevarskaya N. Functional implications of calcium permeability of the channel formed by pannexin 1. *J Cell Biol* 174: 535–546, 2006.

374. Vargas MR, Pehar M, Cassina P, Beckman JS, and Barbeito L. Increased glutathione biosynthesis by Nrf2 activation in astrocytes prevents p75NTR-dependent motor neuron apoptosis. *J Neurochem* 97: 687–696, 2006.

375. Venance L, Cordier J, Monge M, Zalc B, Glowinski J, and Giaume C. Homotypic and heterotypic coupling mediated by gap junctions during glial cell differentiation in vitro. *Eur J Neurosci* 7: 451–461, 1995.

376. Venters HD, Dantzer R, and Kelley KW. Tumor necrosis factor-alpha induces neuronal death by silencing survival signals generated by the type I insulin-like growth factor receptor. *Ann NY Acad Sci* 917: 210–220, 2000.

377. Verderio C, Bruzzone S, Zocchi E, Fedele E, Schenk U, De Flora A, and Matteoli M. Evidence of a role for cyclic ADP-ribose in calcium signalling and neurotransmitter release in cultured astrocytes. *J Neurochem* 78: 646–657, 2001.

378. Vis JC, Nicholson LF, Faull RL, Evans WH, Severs NJ, and Green CR. Connexin expression in Huntington's diseased human brain. *Cell Biol Int* 22: 837–847, 1998.

379. Vogt A, Hormuzdi SG, and Monyer H. Pannexin1 and Pannexin2 expression in the developing and mature rat brain. *Brain Res Mol Brain Res* 141: 113–120, 2005.

380. Volterra A and Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 6: 626–640, 2005.

381. Von Blankenfeld G, Ransom BR, and Kettenmann H. Development of cell-cell coupling among cells of the oligodendrocyte lineage. *Glia* 7: 322–328, 1993.

382. Walker FO. Huntington's disease. *Lancet* 369: 218–228, 2007.

383. Wallraff A, Odermatt B, Willecke K, and Steinhauser C. Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. *Glia* 48: 36–43, 2004.

384. Wang S, Li J, Xia W, and Geng M. A marine-derived acidic oligosaccharide sugar chain specifically inhibits neuronal cell injury mediated by beta-amyloid-induced astrocyte activation *in vitro*. *Neurol Res* 29: 96–102, 2007.

385. Weber JR and Tuomanen EI. Cellular damage in bacterial meningitis: an interplay of bacterial and host driven toxicity. *J Neuroimmunol* 184: 45–52, 2007.

386. Westergaard N, Sonnewald U, and Schousboe A. Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. *Dev Neurosci* 17: 203–211, 1995.

387. Whitehead SN, Cheng G, Hachinski VC, and Cechetto DF. Progressive increase in infarct size, neuroinflammation, and cognitive deficits in the presence of high levels of amyloid. *Stroke* 38: 3245–3250, 2007.

388. Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Guldenagel M, Deutsch U, and Sohl G. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem* 383: 725–737, 2002.

389. Wisniewski HM and Wegiel J. Spatial relationships between astrocytes and classical plaque components. *Neurobiol Aging* 12: 593–600, 1991.

390. Wong CW, Christen T, and Kwak BR. Connexins in leukocytes: Shuttling messages? *Cardiovasc Res* 62: 357–367, 2004.

391. Xiao XQ, Zhang HY, and Tang XC. Huperzine A attenuates amyloid beta-peptide fragment 25-35-induced apoptosis in rat cortical neurons via inhibiting reactive oxygen species formation and caspase-3 activation. *J Neurosci Res* 67: 30–36, 2002.

392. Xu HL and Pelligrino DA. ATP release and hydrolysis contribute to rat pial arteriolar dilatation elicited by neuronal activation. *Exp Physiol* 92: 647–651, 2007.

393. Yamamoto T, Ochalski A, Hertzberg EL, and Nagy JI. LM and EM immunolocalization of the gap junctional protein connexin 43 in rat brain. *Brain Res* 508: 313–319, 1990.

394. Yamamoto T, Vukelic J, Hertzberg EL, and Nagy JI. Differential anatomical and cellular patterns of connexin43 expression during postnatal development of rat brain. *Brain Res Dev Brain Res* 66: 165–180, 1992.

395. Yawata I, Takeuchi H, Doi Y, Liang J, Mizuno T, and Suzumura A. Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors. *Life Sci* 82: 1111–1116, 2008.

396. Ye ZC, Wyeth MS, Baltan–Tekkok S, and Ransom BR. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J Neurosci* 23: 3588–3596, 2003.

397. Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, and Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci USA* 95: 15769–15774, 1998.

398. Zahler S, Hoffmann A, Gloe T, and Pohl U. Gap-junctional coupling between neutrophils and endothelial cells: a novel modulator of transendothelial migration. *J Leukoc Biol* 73: 118–126, 2003.

399. Zappala A, Cicero D, Serapide MF, Paz C, Catania MV, Falchi M, Parenti R, Panto MR, La Delia F, and Cicirata F. Expression of pannexin1 in the CNS of adult mouse: cellular localization and effect of 4-aminopyridine-induced seizures. *Neuroscience* 141: 167–178, 2006.

400. Zappala A, Li Volti G, Serapide MF, Pellitteri R, Falchi M, La Delia F, Cicirata V, and Cicirata F. Expression of pannexin2 protein in healthy and ischemized brain of adult rats. *Neuroscience* 148: 653–667, 2007.

401. Zhang L, Deng T, Sun Y, Liu K, Yang Y, and Zheng X. Role for nitric oxide in permeability of hippocampal neuronal hemichannels during oxygen glucose deprivation. *J Neurosci Res* 86: 2281–2291, 2008.

402. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhou Y, Hong JS, and Zhang J. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J* 19: 533–542, 2005.

403. Zhang ZG, Chopp M, Bailey F, and Malinski T. Nitric oxide changes in the rat brain after transient middle cerebral artery occlusion. *J Neurol Sci* 128: 22–27, 1995.

404. Zhao J, Brooks DM, and Lurie DI. Lipopolysaccharide-activated SHP-1-deficient motheaten microglia release increased nitric oxide, TNF-alpha, and IL-1beta. *Glia* 53: 304–312, 2006.

405. Zhou F, Yao HH, Wu JY, Yang YJ, Ding JH, Zhang J, and Hu G. Activation of Group II/III metabotropic glutamate receptors attenuates LPS-induced astroglial neurotoxicity via promoting glutamate uptake. *J Neurosci Res* 84: 268–277, 2006.

406. Zoidl G, Petrasch–Parwez E, Ray A, Meier C, Bunse S, Habbes HW, Dahl G, and Dermietzel R. Localization of the pannexin1 protein at postsynaptic sites in the cerebral cortex and hippocampus. *Neuroscience* 146: 9-1-6, 2007.

407. Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, and Carmignoto G. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* 6: 43–50, 2003.

408. Zvalova D, Cordier J, Mesnil M, Junier MP, and Chneiweiss H. p38/SAPK2 controls gap junction closure in astrocytes. *Glia* 46: 323–333, 2004.

409. Zysk G, Bruck W, Huitinga I, Fischer FR, Flachsbarth F, van Rooijen N, and Nau R. Elimination of blood-derived macrophages inhibits the release of interleukin-1 and the entry of leukocytes into the cerebrospinal fluid in experimental pneumococcal meningitis. *J Neuroimmunol* 73: 77–80, 1997.

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4. Claire A. Scott, Daniel Tattersall, Edel A. O'Toole, David P. Kelsell. 2012. Connexins in epidermal homeostasis and skin disease. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1818**:8, 1952-1961. [CrossRef]

5. Annette Koulakoff, Xin Mei, Juan A. Orellana, Juan C. Sáez, Christian Giaume. 2012. Glial connexin expression and function in the context of Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1818**:8, 2048-2057. [CrossRef]

6. Steven Donnelly, Grant English, Eugene A. de Zwart-Storm, Sue Lang, Maurice A. M. Steensel, Patricia E. Martin. 2012. Differential susceptibility of Cx26 mutations associated with epidermal dysplasias to peptidoglycan derived from Staphylococcus aureus and Staphylococcus epidermidis. *Experimental Dermatology* **21**:8, 592-598. [CrossRef]

7. Martin Theis, Christian Giaume. 2012. Connexin-based intercellular communication and astrocyte heterogeneity. *Brain Research* . [CrossRef]

8. Juan Xiong, Maria Burkovetskaya, Nikolay Karpuk, Tammy Kielian. 2012. IL-1RI signaling is essential for host defense and hemichannel activity during acute central nervous system bacterial infection. *ASN NEURO* . [CrossRef]

9. H. V. Danesh-Meyer, N. M. Kerr, J. Zhang, E. K. Eady, S. J. O'Carroll, L. F. B. Nicholson, C. S. Johnson, C. R. Green. 2012. Connexin43 mimetic peptide reduces vascular leak and retinal ganglion cell death following retinal ischaemia. *Brain* **135**:2, 506-520. [CrossRef]

10. Alberto Baroja-Mazo, Maria Barberà-Cremades, Pablo Pelegrín. 2012. The participation of plasma membrane hemichannels to purinergic signalling. *Biochimica et Biophysica Acta (BBA) - Biomembranes* . [CrossRef]

11. Christian Giaume, Xinhe Liu. 2012. From a glial syncytium to a more restricted and specific glial networking. *Journal of Physiology-Paris* **106**:1-2, 34-39. [CrossRef]

12. Christian GiaumeNeuroglial Networks **20112381**, 139-156. [CrossRef]

13. Anandakumar Shunmugavel, Mushfiquddin Khan, Peter C.-te Chou, Inderjit Singh. 2011. Spinal Cord Injury Induced Arrest in Estrous Cycle of Rats Is Ameliorated by S-nitrosoglutathione: Novel Therapeutic Agent to Treat Amenorrhea. *The Journal of Sexual Medicine* no-no. [CrossRef]

14. P. Diaz-Amarilla, S. Olivera-Bravo, E. Trias, A. Cragnolini, L. Martinez-Palma, P. Cassina, J. Beckman, L. Barbeito. 2011. Phenotypically aberrant astrocytes that promote motoneuron damage in a model of inherited amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences* . [CrossRef]

15. Juan A. Orellana, Emilio Díaz, Kurt A. Schalper, Aníbal A. Vargas, Michael V.L. Bennett, Juan C. Sáez. 2011. Cation permeation through connexin 43 hemichannels is cooperative, competitive and saturable with parameters depending on the permeant species. *Biochemical and Biophysical Research Communications* **409**:4, 603-609. [CrossRef]

16. A. K. Singh, M. N. Tiwari, A. Dixit, G. Upadhyay, D. K. Patel, D. Singh, O. Prakash, M. P. Singh. 2011. Nigrostriatal proteomics of cypermethrin-induced dopaminergic neurodegeneration: Microglial activation dependent and independent regulations. *Toxicological Sciences* . [CrossRef]

17. Juan A. Orellana, Nicolas Froger, Pascal Ezan, Jean X. Jiang, Michael V. L. Bennett, Christian C. Naus, Christian Giaume, Juan C. Sáez. 2011. ATP and glutamate released via astroglial connexin 43   hemichannels mediate neuronal death through activation of pannexin 1 hemichannels. *Journal of Neurochemistry* no-no. [CrossRef]

18. Yu. Yu. Sharovskaya. 2011. Intercellular interactions through gap junctions in embryonic stem cells. *Biophysics* **56**:1, 86-89. [CrossRef]

19. Youichirou Higashi, Shohei Segawa, Takaaki Matsuo, Shogo Nakamura, Yukiko Kikkawa, Kentaro Nishida, Kazuki Nagasawa. 2011. Microglial zinc uptake via zinc transporters induces ATP release and the activation of microglia. *Glia* n/a-n/a. [CrossRef]

20. Juan A. Orellana, Pablo J. Sáez, Christian Cortés-campos, Roberto J. Elizondo, Kenji F. Shoji, Susana Contreras-Duarte, Vania Figueroa, Victoria Velarde, Jean X. Jiang, Francisco Nualart, Juan C. Sáez, María A. García. 2011. Glucose increases intracellular free Ca2+ in tanycytes via ATP released through connexin 43 hemichannels. *Glia* n/a-n/a. [CrossRef]

21. Ang Li, Chi Ting Leung, Kim Peterson-Yantorno, W. Daniel Stamer, Claire H. Mitchell, Mortimer M. Civan. 2011. Mechanisms of ATP release by human trabecular meshwork cells, the enabling step in purinergic regulation of aqueous humor outflow. *Journal of Cellular Physiology* n/a-n/a. [CrossRef]

22. J. M. Garre, M. A. Retamal, P. Cassina, L. Barbeito, F. F. Bukauskas, J. C. Saez, M. V. L. Bennett, V. Abudara. 2010. FGF-1 induces ATP release from spinal astrocytes in culture and opens pannexin and connexin hemichannels. *Proceedings of the National Academy of Sciences* **107**:52, 22659-22664. [CrossRef]

23. X. Mei, P. Ezan, C. Giaume, A. Koulakoff. 2010. Astroglial connexin immunoreactivity is specifically altered at #-amyloid plaques in #-amyloid precursor protein/presenilin1 mice. *Neuroscience* **171**:1, 92-105. [CrossRef]

24. Juan C. Sáez, Kurt A. Schalper, Mauricio A. Retamal, Juan A. Orellana, Kenji F. Shoji, Michael V.L Bennett. 2010. Cell membrane permeabilization via connexin hemichannels in living and dying cells. *Experimental Cell Research* **316**:15, 2377-2389. [CrossRef]

25. Nicolas Froger, Juan A. Orellana, Charles-Felix Calvo, Edwige Amigou, Michael G. Kozoriz, Christian C. Naus, Juan C. Sáez, Christian Giaume. 2010. Inhibition of cytokine-induced connexin43 hemichannel activity in astrocytes is neuroprotective. *Molecular and Cellular Neuroscience* **45**:1, 37-46. [CrossRef]

26. Georg Zoidl, Rolf Dermietzel. 2010. Gap junctions in inherited human disease. *Pflügers Archiv - European Journal of Physiology* **460**:2, 451-466. [CrossRef]

27. Christian Giaume, Martin Theis. 2010. Pharmacological and genetic approaches to study connexin-mediated channels in glial cells of the central nervous system. *Brain Research Reviews* **63**:1-2, 160-176. [CrossRef]

28. W. Alan C. Mutch. 2010. New concepts regarding cerebral vasospasm: glial-centric mechanisms. *Canadian Journal of Anesthesia/Journal canadien d'anesthésie* **57**:5, 479-489. [CrossRef]

29. Chen Yu Chiang, Zhaohui Li, Jonathan O. Dostrovsky, Barry J. Sessle. 2010. Central sensitization in medullary dorsal horn involves gap junctions and hemichannels. *NeuroReport* **21**:3, 233-237. [CrossRef]

30. Christian Giaume, Annette Koulakoff, Lisa Roux, David Holcman, Nathalie Rouach. 2010. Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nature Reviews Neuroscience* **11**:2, 87-99. [CrossRef]

31. Johanna Hung, Mathieu Chansard, Shalina S. Ousman, Minh Dang Nguyen, Michael A. Colicos. 2010. Activation of microglia by neuronal activity: Results from a new in vitro paradigm based on neuronal-silicon interfacing technology. *Brain, Behavior, and Immunity* **24**:1, 31-40. [CrossRef]

32. Nicolas Froger, Juan A. Orellana, Martine Cohen-Salmon, Pascal Ezan, Edwige Amigou, Juan C. SÃ¡ez, Christian Giaume. 2009. Cannabinoids prevent the opposite regulation of astroglial connexin43 hemichannels and gap junction channels induced by pro-inflammatory treatments. *Journal of Neurochemistry* **111**:6, 1383-1397. [CrossRef]

33. Martha HerbertAutism 343-387. [CrossRef]

34. M. Freidin, S. Asche, T. A. Bargiello, M. V. L. Bennett, C. K. Abrams. 2009. Connexin 32 increases the proliferative response of Schwann cells to neuregulin-1 (Nrg1). *Proceedings of the National Academy of Sciences* **106**:9, 3567-3572. [CrossRef]

35. Juan A. Orellana, Diego E. Hernández, Pascal Ezan, Victoria Velarde, Michael V. L. Bennett, Christian Giaume, Juan C. Sáez. 2009. Hypoxia in high glucose followed by reoxygenation in normal glucose reduces the viability of cortical astrocytes through increased permeability of connexin 43 hemichannels. *Glia* NA-NA. [CrossRef]