Review Article

Large Pore Ion and Metabolite-Permeable Channel Regulation of Postnatal Ventricular Zone Neural Stem and Progenitor Cells: Interplay between Aquaporins, Connexins, and Pannexins?

Leigh E. Wicki-Stordeur 1 and Leigh Anne Swayne 1, 2, 3, 4

1 Division of Medical Sciences, Island Medical Program, University of Victoria, Victoria, BC, Canada V8W 2Y2
2 Department of Biology, University of Victoria, Victoria, BC, Canada V8W 3N5
3 Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada V8W 3P6
4 Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada V6T 1Z3

Correspondence should be addressed to Leigh Anne Swayne, lswayne@uvic.ca

Received 5 April 2012; Accepted 27 April 2012

Academic Editor: Stefan Liebau

Copyright © 2012 L. E. Wicki-Stordeur and L. A. Swayne. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The birth of new neurons from unspecialized neural stem and progenitor cells surrounding the lateral ventricles occurs throughout postnatal life. This process, termed neurogenesis, is complex and multisteped, encompassing several types of cellular behaviours, such as proliferation, differentiation, and migration. These behaviours are influenced by numerous factors present in the unique, permissive microenvironment. A major cellular mechanism for sensing the plethora of environmental cues directing this process is the presence of different channel forming proteins spanning the plasma membrane. So-called large pore membrane channels, which are selective for the passage of specific types of small molecules and ions, are emerging as an important subgroup of channel proteins. Here, we focus on the roles of three such large pore channels, aquaporin 4, connexin 43, and pannexin 1. We highlight both their independent functions as well as the accumulating evidence for crosstalk between them.

1. Introduction

New neurons are produced in the ventricular zone (VZ) of the lateral ventricles throughout postnatal life [1]. This is a remarkable developmental process, in which unspecialized neural stem and progenitor cells (NSC/NPCs) pass through a complex gauntlet of cell behaviours, such as proliferation, differentiation, and migration. It is now becoming increasingly clear that the highly controlled movement of several ions and small molecules trigger numerous, complex signaling pathways that underscore the regulation of these behaviours (recently reviewed in [2, 3]). As follows, there is a growing body of evidence implicating “large pore” channels in the control of postnatal VZ neurogenesis. In contrast to typical ion channels, which are selective for small ions, large pore channels can additionally (or exclusively) allow passage of small molecules (neutral or charged). Aquaporin 4 (AQP4) connexin 43 (Cx43), and pannexin 1 (Panx1) are three such large pore channels that are expressed in postnatal VZ. Perhaps not surprisingly the roles of these channels appear to be closely linked with one another and also with the functions of other ion channels in the regulation of postnatal VZ NSC/NPC biology.

2. AQP4

There are thirteen known types of AQPs in mammals (AQP0-12; recently reviewed in [4]). These are categorized into two primary subgroups based on function: those selective solely for water (AQP0, AQP1, AQP2, AQP4, AQP5), and those permeable to water as well as small nonpolar solutes such as glycerol and urea (AQP3, AQP7, AQP9, and AQP10). Additional types can conduct ions (AQP6, AQP8), while so-called “unorthodox” members (AQP11, AQP12) are more distantly related to the other aquaporins and are expressed on intracellular membranes [5]. In general, AQP proteins are comprised of about 300 amino acids with six transmembrane...
α-helices arranged in a right-handed bundle with intracellular N- and the C-termini [6, 7]. AQP monomers oligomerize to form tetramers, generating four aqueous pores [8, 9]. Specific motifs within the interhelical loop regions form the water conduit and selectivity filter [10]. Slight variations in peptide sequence between different AQPs have generated variability in the size of the pore. This is part of the basis for water selectivity (small pore) versus simultaneous water and nonpolar solute permeability (larger pore) [8].

AQPs 1, 4, and 9 are present in the central nervous system (CNS), largely in epithelial cells, ependymal cells, and/or astroglia ([11–14], reviewed in [15, 16]), where they facilitate movement of water between blood and brain, and between brain and cerebrospinal fluid compartments. Dysregulation of cell volume in the brain underlies clinical conditions such as edema and hypoxia. Water balance also plays a crucial role in neurogenesis, as NSC/NPCs must move considerable amounts of water into or out of the cell to rapidly change their volume during proliferation, differentiation, and migration.

The major AQP found in brain, AQP4, is highly enriched in the neurogenic regions [11, 14, 17], particularly the VZ, and is the main isoform expressed in adult NSC/NPCs and ependymal cells [17, 18]. As described above, AQP4 is a member of the water-only permeable subgroup. Considerable AQP8 (water plus small nonpolar solutes) and AQP9 (water plus ions) have also been detected in NSC/NPCs in culture [18]. In contrast to AQP4, which is more ubiquitous in the VZ, AQP9 is mainly localized in NSC/NPCs in the dorsolateral corner [17]; however, its exact functional significance in NSC/NPC biology remains to be determined. AQP8 is detected primarily in the mitochondria-enriched fraction, although whether it is present in neurogenic regions in situ has not yet been reported [18].

Most of what is currently known about the role of AQPs in NSC/NPCs comes from recent work on AQP4 [19–21]. Using AQP4 knockout (KO) mice, Kong et al. [19] demonstrated that it controls proliferation, survival, migration, and neuronal differentiation of VZ NSC/NPCs. An observed impairment in neurosphere formation in AQP4 KO mice was attributed to both increased cell apoptosis and decreased cell proliferation due to cell cycle arrest in G2/M phase. Furthermore, upon neurosphere differentiation, the proportion of immature neurons in the AQP4 KO population was significantly lower than in the wildtype population, whereas there was no significant difference in the proportion of astrocytes. To help elucidate the underlying mechanism, the authors investigated the effects of AQP4 loss on Ca2+ oscillations. In NSC/NPCs, L-type Ca2+ channel mediated Ca2+ fluxes [22, 23] and purinergic receptor- (P2R-) dependent Ca2+ oscillations [24–27] play major roles in directing neurogenesis (recently reviewed in [2, 3]), in part through Ca2+-dependent transcriptions [23]. Interestingly, these P2R-mediated Ca2+ oscillations can even occur spontaneously without exogenous stimulation in NSC/NPCs [25, 26]. AQP4 KO increased the frequency but decreased the amplitude of spontaneous Ca2+ oscillations and suppressed high K+-induced Ca2+ influx. Given its demonstrated effects on intracellular Ca2+, it is not surprising that AQP4 KO also affected the expression of other channels: the expression of both Cx43 and the L-type voltage-gated Ca2+ channel Ca,1.2 subtype were reduced.

3. Cx43

Cxs are a family of vertebrate four-pass transmembrane proteins with intracellular N- and C-termini, that oligomerize into hexameric channels known as connexons (hemichannels), which, in turn, can connect neighboring cells across the extracellular space by formation of gap junctions [28]. These junctions provide a physical link between cells through which ions, metabolites, and other messengers of up to 1 kDa in size can diffuse, thereby mediating cell-cell communication through passage of signaling molecules such as ATP [29], IP3, and Ca2+ ([30] reviewed in [31, 32]). Gap junction-independent functions of hemichannels have also recently been identified, in which similar exchanges between the cell and its extracellular environment are facilitated (reviewed in [33]). Furthermore, the variable C-terminal domains of individual Cxs can exert intrinsic functionality independent from channel activity (reviewed in [34]), that appears to be regulated by signaling/adaptor proteins like protein kinases, phosphatases, and structural proteins (reviewed in [35]). Cxs have been shown to widely influence physiological and pathological processes and are key in coordinating metabolic and electrical activities as well as cell growth and proliferation (reviewed in [36]), cytoskeletal dynamics [37], and transcriptional regulation [38–40].

Over twenty mammalian members of the Cx family have been identified, with each respective isoform originally named for its molecular weight (reviewed in [41]). Cx43 (gap-junction protein alpha-1, Gja1) is the most widely and highly expressed Cx in almost every tissue [42], and it is the predominant isoform within the CNS. Within the developing CNS, Cx43 is detected in several cell types including astrocytes, NSC/NPCs, cortical neurons, and dopaminergic neurons of the developing midbrain [43–49]. Cx43 is critical for proper CNS formation and organization, likely through its role in the neurogenic processes of NSC/NPC proliferation [50], differentiation [47, 51], and migration [52–54] during development. Interestingly, studies in human and murine embryonic stem cells have found transcriptional regulatory elements controlled by the NSC transcription factor SOX2 within the Cx43 gene region [55] and have identified Cx43 as necessary for both neuroectodermal specification [56] and stem cell proliferation [57].

In the postnatal and adult brain, Cx43 expression becomes much more highly restricted to astrocytes [58–60]. However, Cx43 remains present in cortical neurons [61], ependymal cells [44], NSC/NPCs, and migratory neuroblasts [62–65]. Within the neurogenic VZ and subsequent rostral migratory stream (RMS), a dramatic increase in Cx43 is noted between neonatal periods and adulthood [66] in the astrocytes, NSC/NPCs, and ependymal cells, all of which exhibit gap-junction-dependent coupling [63, 64, 67]. Within this stem cell environment, Cx43 is further thought to be involved in hemichannel mediated ATP uptake and release.
The postnatal VZ in vivo, this is conserved in the postnatal VZ is unknown. Whether proliferating to maintain cells in proliferative state [50], but whether neurospheres were dependent on Cx43 gap junctional coupling [50]. Moreover, embryonic cortical and astrocytes causes severe inhibition of hippocampal hippocampus, conditional Cx43 knockout in radial glia regulates proliferation. In developing and early postnatal of proliferation, other studies suggest Cx43 is a positive regulator of proliferation. In developing and early postnatal hippocampus, conditional Cx43 knockout in radial glia and astrocytes causes severe inhibition of hippocampal NSC/NPC proliferation [80]. Moreover, embryonic cortical neurospheres were dependent on Cx43 gap functional coupling to maintain cells in proliferative state [50], but whether this is conserved in the postnatal VZ is unknown.

Still, the functional relevance of Cx43 in NSC/NPCs of the postnatal VZ in vivo remains to be discovered. Currently, much is assumed from the previously mentioned cell culture experiments, as well as developmental and postnatal hippocampal studies. Together, it appears a role for Cx43 may be emerging in VZ NSC/NPC self-renewal, differentiation and migration, thereby contributing to the regulation of the postnatal process of neurogenesis.

4. Panx1

Panx1 is part of a three-membered family of proteins with homology to the invertebrate gap junction forming innexins [81]. However, little concrete evidence exists pointing towards gap junction functions for Panxs, which are instead widely considered single-membrane channels (reviewed in [82–84]). Panx1 monomers have a predicted four-pass transmembrane sequence, with a conserved intracellular N-terminus and much longer, variable intracellular C-terminus. These monomers oligomerize into large hexameric pores [85] that may be opened by depolarization [86, 87], increased extracellular K⁺ (independent of depolarization) [88, 89], mechanical stimulation [90], NMDAR activation [91], intracellular Ca²⁺ [92], or low oxygen and glucose conditions [93, 94]. Recently, it has been demonstrated that the C-terminal domain of Panx1 is autoinhibitory, and can be removed by caspase-dependent cleavage, resulting in constitutive activation of this channel [95, 96]. Furthermore, Panx1 activation can be inhibited by dramatically increased extracellular ATP [97] or upon cytoplasmic acidification [92], as well as through mimetic peptides [98] and channel blockers [99, 100]. Once activated, the Panx1 pore may nonselectively pass ions, metabolites, and other signaling molecules up to 1 kDa in size (reviewed in [82–84]); however, recent evidence has pointed towards Panx1 as being selective for anions (e.g., Cl⁻) and anionic small molecules [101]. These channels are involved in several physiological and pathological processes, largely by mediating ATP release in several cell types (reviewed in [82–84]).

Panx1 is found in a wide range of rodent tissues, with an expression profile similar to that of Cx43 [100]. It is abundantly expressed in the brain [102, 103]. Importantly, this relatively newly discovered large pore channel has recently been identified in postnatal VZ NSC/NPCs and their immature neuronal progeny [27]. Using Neuro2a murine neuroblastoma cells and primary postnatal VZ neurosphere cultures, Panx1 overexpression and inhibition dramatically increased and decreased NSC/NPC proliferation, respectively. Furthermore, this regulation was partly due to the ability of Panx1 to release ATP (reviewed in [82, 83, 100, 104]), a potent signalling metabolite, which is released in sporadic bursts from NSC/NPCs [25]. Released ATP triggers intracellular Ca²⁺ mobilization via activation of P2R signaling [24–27]. Ongoing studies will likely uncover additional regulatory roles of Panx1 in neurogenesis, as well as underlying mechanisms.

5. Crosstalk between “Large” Pore Channels and Convergence of Signaling Mechanisms

Figure 1 summarizes the roles of AQP4, Cx43 and Panx1 in postnatal VZ NSC/NPCs. Interestingly, there appears to be multiple levels of crosstalk between each of these large pore channels. Here, we outline three primary interconnected ways in which the regulation and function of these large pore channels converge: solute gradient regulation, cytoskeletal signaling related to cell volume changes, and nucleotide signaling.

5.1. Gradient Regulation. The movement of ions and metabolites is often dependent on the ability to tightly control concentration gradients. These gradients cannot be generated and/or maintained without concomitant control of water volume. The mechanism underlying the effects of
AQP4 loss on Ca\(^{2+}\) oscillations and changes in L-type and Cx43 channel expression have not been fully elucidated; however, it is conceivable that these changes could result, in part, from alterations in ion concentration gradients. Cx43 has also been implicated in volume control (for review see [105]), perhaps through reciprocal relationships with AQP4, as described above. Thus, the ion fluxes through Cx43 and Panx1 are dependent on the capacity of AQP4 to regulate solute concentration gradients.

5.2. Cytoskeletal Signaling. Proliferating, differentiating and migrating NSC/NPCs and neuroblasts must make specific and substantial changes in cell volume and morphology that undoubtedly require the movement of water molecules. For example, cell proliferation required for neurosphere formation is inhibited by a hypertonic medium [106]—in glioma cells this results in sustained cell swelling following transient cell shrinkage [107, 108]. The precise details of volume-sensing signaling mechanisms triggered by AQP4-mediated water movement that are important for neurogenesis remain to be further elucidated. An early study in cultured astrocytes demonstrated that AQP4 knockdown also induced alterations of the actin cytoskeleton [109]. Therefore, AQP4-mediated changes in cell volume could directly regulate Cx43 and Panx1 signaling through stretch activation of the channels and/or the cytoskeletal-associated signaling pathways to which they are linked. Recent work has demonstrated that extracellular matrix stiffness modulates NSC behaviour [110] and that cytoskeletal-regulating Rho GTPases mediate the lineage commitment of hippocampal NSCs [111]. For many years, Cxs have been closely linked to the cytoskeleton in numerous cell types (e.g., see [37, 112–116], for reviews see [105, 117, 118] with actomyosin-mediated contractility actually inhibiting Cx43 hemichannel activity [118]).

As described above, we also now know that Panx1 regulates NSC/NPC proliferation [27] which adds another layer
of complexity. Previous work has shown that these channels can be activated by mechanical stress [90]. Further suggesting the potential for positive crosstalk between Panx1 and the actin cytoskeleton in NSC/NPCs, Panx1 has been demonstrated to interact with the actin cytoskeleton [119] and drive actin remodeling [120]. Moreover, nucleotide-dependent mechanisms (e.g., ATP flux, P2R signaling) are implicated in cytoskeletal remodeling in NSC/NPCs [121]. Interestingly, recent work has demonstrated that, in addition to regulating Cx43 and the actin cytoskeleton, AQP4 knockdown reduces a maxi volume-regulated anion current of unknown molecular identity [122]. Given the discovery of the anion selectivity of Panx1 [123], it is tempting to speculate that Panx1 is the molecular basis of this enigmatic maxi volume regulated anion channel—which, incidentally, also mediates ATP release [124].

5.3. Nucleotide Signaling. Purinergic signaling mechanisms also further link Cx43 and Panx1, albeit somewhat controversially. Prior to the discovery of Panx1, channel-mediated ATP release was mainly attributed to Cx43 hemichannels. Interestingly, Cx43 expression also regulates P2R expression [26] in embryonic VZ NSC/NPCs. Cx43 hemichannel-mediated ATP release was heavily studied in astrocytes (e.g., see [68, 125]), however, this role has recently been challenged in favour of Panx1 [126]. Importantly, while Cx43 did not appear to form hemichannels in Xenopus oocytes [127], numerous studies in mammalian cells have elucidated the intricacies of Cx43 hemichannel activity (e.g., see [73, 74]). Furthermore, the cross-inhibition of Cx hemichannels, Panx3, and volume-activated ion channels by certain pharmacological tools is now well known [98, 104, 128], adding further levels of complexity as several previously identified Cx channel blockers are now known to inhibit Panx1 with equal or greater efficacy. Whether Cx43 has hemichannel activity in postnatal VZ NSC/NPCs may thus be more of an open question than was previously thought and further work is clearly needed to elucidate its role. Given that we now know that Panx1 appears to play an important role in purinergic signaling in NSC/NPCs, likely in part through mediating ATP release [27], it will be important to determine if and how Panx1 and Cx43 functionally interact in the postnatal VZ.

6. Conclusions and Perspectives

Here, we have reviewed literature on the roles of three large pore ion channels, AQP4, Cx43, and Panx1 in the regulation of postnatal VZ neurogenesis. A common thread that has emerged during this process is that the regulation and functions of these channels seem to intimately connected (Figure 1).

Acknowledgments

L. A. Swayne is supported by a Natural Sciences and Engineering Research Council of Canada Discovery grant, a Victoria Foundation Willa and Ella Dawson Fund grant, and a University of Victoria laboratory startup grant.

References

[1] G. L. Ming and H. Song, "Adult neurogenesis in the mammalian brain: significant answers and significant questions," Neuron, vol. 70, no. 4, pp. 687–702, 2011.
[2] L. A. Swayne and L. Wicki-Stordeur, "Ion channels in adult neural stem cells and their progeny," Channels, vol. 6, no. 2, 2012.
[3] T. Yasuda and D. I. Adams, "Physiological roles of ion channels in adult neural stem cells and their progeny," Journal of Neurochemistry, vol. 114, no. 4, pp. 946–959, 2010.
[4] K. Ishibashi, S. Hara, and S. Kondo, "Aquaporin water channels in mammals," Clinical and Experimental Nephrology, vol. 13, no. 2, pp. 107–117, 2009.
[5] A. Rojek, J. Praetorius, J. Frokjaer, S. Nielsen, and R. A. Fenton, "A current view of the mammalian aquaglyceroporins," Annual Review of Physiology, vol. 70, pp. 301–327, 2008.
[6] G. M. Preston and P. Agre, "Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family," Proceedings of the National Academy of Sciences of the United States of America, vol. 88, no. 24, pp. 11110–11114, 1991.
[7] G. M. Preston, J. S. Jung, W. B. Guggino, and P. Agre, "Membrane topology of aquaporin CHIP. Analysis of functional epitope-scanning mutants by vectorial proteolysis," The Journal of Biological Chemistry, vol. 269, no. 3, pp. 1668–1673, 1994.
[8] T. Gonen and T. Walz, "The structure of aquaporins," Quarterly Reviews of Biophysics, vol. 39, no. 4, pp. 361–396, 2006.
[9] B. L. Smith and P. Agre, "Erythrocyte M(r) 28,000 transmembrane protein exists as a multisubunit oligomer similar to channel proteins," The Journal of Biological Chemistry, vol. 266, no. 10, pp. 6407–6415, 1991.
[10] J. S. Jung, G. M. Preston, B. L. Smith, W. B. Guggino, and P. Agre, "Molecular structure of the water channel through aquaporin CHIP. The hourglass model," The Journal of Biological Chemistry, vol. 269, no. 20, pp. 14648–14654, 1994.
[11] H. Hasegawa, T. Ma, W. Skach, M. A. Matthy, and A. S. Verkman, "Molecular cloning of a mercurial-insensitive water channel expressed in selected water-transporting tissues," The Journal of Biological Chemistry, vol. 269, no. 8, pp. 5497–5500, 1994.
[12] S. Nielsen, B. L. Smith, E. I. Christensen, and P. Agre, "Distribution of the aquaporin CHIP in secretary and responsive epithelia and capillary endothelia," Proceedings of the National Academy of Sciences of the United States of America, vol. 90, no. 15, pp. 7275–7279, 1993.
[13] M. N. Mylonakou, P. H. Petersen, E. Rinvik et al., "Analysis of mice with targeted deletion of AQP9 gene provides conclusive evidence for expression of AQP9 in neurons, "
Journal of Neuroscience Research, vol. 87, no. 6, pp. 1310–1322, 2009.

[14] J. S. Jung, R. V. Bhat, G. M. Preston, W. B. Guggino, J. M. Baraban, and P. Agre, “Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance,” Proceedings of the National Academy of Sciences of the United States of America, vol. 91, no. 26, pp. 13052–13056, 1994.

[15] J. L. Venero, M. L. Vizuete, A. Machado, and J. Cano, “Aquaporins in the central nervous system,” Progress in Neurobiology, vol. 63, no. 3, pp. 321–336, 2001.

[16] M. J. Tait, S. Saadoun, B. A. Bell, and M. C. Papadopoulos, “Water movements in the brain: role of aquaporins,” Trends in Neurosciences, vol. 31, no. 1, pp. 37–43, 2008.

[17] C. Cavazzen, D. Ferrari, F. Facchetti et al., “Unique expression and localization of aquaporin-4 and aquaporin-9 in murine and human neural stem cells and in their glial progeny,” Glia, vol. 53, no. 2, pp. 167–181, 2006.

[18] C. A. M. La Porta, P. Gena, A. Gritti, U. Fascio, M. Svelto, and G. Calamita, “Adult murine CNS stem cells express aquaporin channels,” Biology of the Cell, vol. 98, no. 2, pp. 89–94, 2006.

[19] H. Kong, Y. Fan, J. Xie et al., “AQP4 knockout impairs proliferation, migration and neuronal differentiation of adult neural stem cells,” Journal of Cell Science, vol. 121, no. 24, pp. 4029–4036, 2008.

[20] H. Kong, L. L. Sha, Y. Fan et al., “Requirement of AQP4 for antidepressive efficiency of fluoxetine: implication in adult hippocampal neurogenesis,” Neuropsychopharmacology, vol. 34, no. 5, pp. 1263–1276, 2009.

[21] L. L. Xie, X. L. Sun, Y. Fan, H. Kong, J. H. Ding, and G. Hu, “Aquaporin 4 knockout resists negative regulation of neural cell proliferation by cocaine in mouse hippocampus,” International Journal of Neuropsychopharmacology, vol. 12, no. 6, pp. 843–850, 2009.

[22] M. D’Ascenzo, R. Piacentini, P. Casalbore et al., “Role of L-type Ca2+ channels in neural stem/progenitor cell differentiation,” European Journal of Neuroscience, vol. 23, no. 4, pp. 935–944, 2006.

[23] K. Deisseroth, S. Singla, H. Toda, M. Monje, T. D. Palmer, and R. C. Malenka, “Excitation-neurogenesis coupling in adult neural stem/progenitor cells,” Neuron, vol. 42, no. 4, pp. 535–552, 2004.

[24] S. K. Mishra, N. Braun, V. Shukla et al., “Extracellular nucleotide signaling in adult neural stem cells: synergism with growth factor-mediated cellular proliferation,” Development, vol. 133, no. 4, pp. 675–684, 2006.

[25] J. H. C. Lin, T. Takano, G. Arcuino et al., “Purinergic signaling regulates neural progenitor cell expansion and neurogenesis,” Developmental Biology, vol. 302, no. 1, pp. 356–366, 2007.

[26] E. Schemes, N. Duval, and P. Meda, “Reduced expression of P2Y1 receptors in connexin43-null mice alters calcium signaling and migration of neural progenitor cells,” Journal of Neuroscience, vol. 23, no. 36, pp. 11444–11452, 2003.

[27] L. E. Wicki-Stordeur, A. D. Dzugalo, R. M. Swansburg, J. M. Suits, and L. A. Swanye, “Pannexin 1 regulates postnatal neural and progenitor cell proliferation,” Neural Development, vol. 7, no. 1, article 11, 2012.

[28] D. A. Goodenough, “The structure and permeability of isolated hepatocyte gap junctions,” Cold Spring Harbor Symposium on Quantitative Biology, vol. 40, pp. 37–43, 1976.

[29] G. S. Goldberg, A. P. Moreno, and P. D. Lampe, “Gap junctions between cells expressing connexin 43 or 32 show inverse permselectivity to adenosine and ATP,” The Journal of Biological Chemistry, vol. 277, no. 39, pp. 36725–36730, 2002.

[30] J. C. Saez, J. A. Connor, D. C. Spray, and M. V. L. Bennett, “Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions,” Proceedings of the National Academy of Sciences of the United States of America, vol. 86, no. 8, pp. 2708–2712, 1989.

[31] D. B. Alexander and G. S. Goldberg, “Transfer of biologically important molecules between cells through gap junction channels,” Current Medicinal Chemistry, vol. 10, no. 19, pp. 2045–2058, 2003.

[32] H. A. Dbouk, R. M. Mroue, M. E. El-Sabban, and R. S. Talhouk, “Connexins: a myriad of functions extending beyond assembly of gap junction channels,” Cell Communication and Signaling, vol. 7, article 4, 2009.

[33] D. C. Spray, Z. C. Ye, and B. R. Ransom, “Functional connexin ‘hemichannels’: a critical appraisal,” Glia, vol. 54, no. 7, pp. 758–773, 2006.

[34] J. X. Jiang and S. Gu, “Gap junction- and hemichannel-independent actions of connexins,” Biochimica et Biophysica Acta, vol. 1711, no. 2, pp. 208–214, 2005.

[35] B. N. G. Giepmans, “Gap junctions and connexin-interacting proteins,” Cardiovascular Research, vol. 62, no. 2, pp. 233–245, 2004.

[36] E. Kardami, X. Dang, D. A. Iacobas et al., “The role of connexins in controlling cell growth and gene expression,” Progress in Biophysics and Molecular Biology, vol. 94, no. 1-2, pp. 245–264, 2007.

[37] S. Olk, A. Turchinovich, M. Grzendaowski et al., “Proteomic analysis of astroglial connexin43 silencing uncovers a cytoskeletal platform involved in process formation and migration,” Glia, vol. 58, no. 4, pp. 494–505, 2010.

[38] D. A. Iacobas, S. Iacobas, M. Urban-Maldonado, and D. C. Spray, “Sensitivity of the brain transcriptome to connexin ablation,” Biochimica et Biophysica Acta, vol. 1711, no. 2, pp. 183–196, 2005.

[39] J. P. Stains, F. Lecanda, J. Screen, D. A. Towler, and R. Civitelli, “Gap junctional communication modulates gene transcription by altering the recruitment of Sp1 and Sp3 to connexin-response elements in osteoblast promoters,” The Journal of Biological Chemistry, vol. 278, no. 27, pp. 24377–24387, 2003.

[40] J. P. Stains and R. Civitelli, “Gap junctions regulate extra-cellular signal-regulated kinase signaling to affect gene transcription,” Molecular Biology of the Cell, vol. 16, no. 1, pp. 64–72, 2005.

[41] G. Söhl and K. Willecke, “An update on connexin genes and their nomenclature in mouse and man,” Cell Communication and Adhesion, vol. 10, no. 4–6, pp. 173–180, 2003.

[42] E. C. Beyer, D. L. Paul, and D. A. Goodenough, “Connexin43: a protein from rat heart homologous to a gap junction protein from liver,” Journal of Cell Biology, vol. 105, no. 6, pp. 2621–2629, 1987.

[43] D. S. Y. Leung, K. Unsicker, and B. Reuss, “Expression and developmental regulation of gap junction connexins cx26, cx32, cx43 and cx45 in the rat midbrain–floor,” International Journal of Developmental Neuroscience, vol. 20, no. 1, pp. 63–75, 2002.

[44] D. S. Y. Leung, K. Unsicker, and B. Reuss, “Expression and developmental regulation of gap junction connexins cx26, cx32, cx43 and cx45 in the rat midbrain–floor,” International Journal of Developmental Neuroscience, vol. 20, no. 1, pp. 63–75, 2002.

[45] R. Dermietzel, O. Traub, T. K. Hwang et al., “Differential expression of three gap junction proteins in developing and mature brain tissues,” Proceedings of the National Academy of Sciences of the United States of America, vol. 86, no. 24, pp. 10148–10152, 1989.
[45] D. J. Belliveau and C. C. G. Naus, “Cellular localization of gap junction mRNAs in developing rat brain,” *Developmental Neuroscience*, vol. 17, no. 2, pp. 81–96, 1995.

[46] R. Rozental, M. Morales, M. F. Mehler et al., “Changes in the properties of gap junctions during neuronal differentiation of hippocampal progenitor cells,” *Journal of Neuroscience*, vol. 18, no. 5, pp. 1753–1762, 1998.

[47] N. Duval, D. Gomés, V. Calaora, A. Calabrese, P. Meda, and R. Bruzzone, “Cell coupling and Cx43 expression in embryonic mouse neural progenitor cells,” *Journal of Cell Science*, vol. 115, no. 16, pp. 3241–3251, 2002.

[48] K. S. Bittman and J. J. LoTurco, “Differential regulation of connexin 26 and 43 in murine neocortical precursors,” *Cerebral Cortex*, vol. 9, no. 2, pp. 188–195, 1999.

[49] B. Nadarajah, A. M. Jones, W. H. Evans, and J. G. Parnavelas, “Differential expression of connexins during neocortical development and neuronal circuit formation,” *Journal of Neuroscience*, vol. 17, no. 9, pp. 3096–3111, 1997.

[50] A. Cheng, H. Tang, J. Cai et al., “Gap junctional communication is required to maintain mouse cortical neuronal progenitor cells in a proliferative state,” *Developmental Biology*, vol. 272, no. 1, pp. 203–216, 2004.

[51] M. E. Santiago, P. Alcami, K. M. Striedinger, D. C. Spray, and E. Scemes, “The carboxyl-terminal domain of Connexin43 is a negative modulator of neuronal differentiation,” *The Journal of Biological Chemistry*, vol. 285, no. 16, pp. 11836–11845, 2010.

[52] L. A. B. Elias, D. D. Wang, and A. R. Kriegstein, “Gap junction adhesion is necessary for radial migration in the neocortex,” *Nature*, no. 448, pp. 901–907, 2007.

[53] E. Scemes, N. Duval, and P. Meda, “Reduced Expression of P2Y1 Receptors in Connexin43–Null Mice Alters Calcium Signaling and Migration of Neuronal Progenitor Cells,” *Journal of Neuroscience*, vol. 23, no. 36, pp. 11444–11452, 2003.

[54] M. Marins, A. L. R. Xavier, N. B. Viana, F. S. A. Fortes, M. M. Fröes, and J. R. L. Menezes, “Gap junctions are involved in cell migration in the early postnatal subventricular zone,” *Developmental Neurobiology*, vol. 69, no. 11, pp. 715–730, 2009.

[55] L. A. Boyer, T. I. Lee, M. F. Cole et al., “Core transcriptional regulatory circuitry in human embryonic stem cells,” *Cell*, vol. 122, no. 6, pp. 947–956, 2005.

[56] B. Parekkadan, Y. Berdichevsky, D. Irimia et al., “Cell-cell interaction modulates neuroectodermal specification of embryonic stem cells,” *Neuroscience Letters*, vol. 438, no. 2, pp. 190–195, 2008.

[57] M. G. Todorova, B. Soria, and I. Quesada, “Gap junctional intercellular communication is required to maintain embryonic stem cells in a non-differentiated and proliferative state,” *Journal of Cellular Physiology*, vol. 214, no. 2, pp. 354–362, 2008.

[58] J. E. Rash, T. Yasumura, K. G. V. Davidson, C. S. Furman, F. E. Dudek, and J. I. Nagy, “Identification of cells expressing Cx43, Cx30, Cx26, Cx32 and Cx36 in gap junctions of rat brain and spinal cord,” *Cell Communication and Adhesion*, vol. 8, no. 4–6, pp. 315–320, 2001.

[59] M. Theis, G. Söhl, D. Speidel, R. Kühn, and K. Willecke, “Connexin43 is not expressed in principal cells of mouse cortex and hippocampus,” *European Journal of Neuroscience*, vol. 18, no. 2, pp. 267–274, 2003.

[60] P. E. Micevych and L. Abelseth, “Distribution of mRNAs coding for liver and heart gap junction proteins in the rat central nervous system,” *Journal of Comparative Neurology*, vol. 305, no. 1, pp. 96–118, 1991.

[61] B. Nadarajah, D. Thomaidou, W. H. Evans, and J. G. Parnavelas, “Gap junctions in the adult cerebral cortex: regional differences in their distribution and cellular expression of connexins,” *The Journal of Comparative Neurology*, vol. 376, no. 2, pp. 326–342, 1996.

[62] B. Lacar, S. Z. Young, J. C. Platel, and A. Bordey, “Gap junction-mediated calcium waves define communication networks among murine postnatal neural progenitor cells,” *European Journal of Neuroscience*, vol. 34, no. 12, pp. 1895–1905, 2011.

[63] X. Liu, A. J. Bolteus, D. M. Balkin, O. Henschel, and A. Bordey, “GFAP-expressing cells in the postnatal subventricular zone display a unique glial phenotype intermediate between radial glia and astrocytes,” *Glia*, vol. 54, no. 5, pp. 394–410, 2006.

[64] Z. Mirzadeh, F. T. Merkle, M. Soriano-Navarro, J. M. Garcia-Verdugo, and A. Alvarez-Buylla, “Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain,” *Cell Stem Cell*, vol. 3, no. 3, pp. 265–278, 2008.

[65] A. S. Freitas, A. L. Xavier, C. M. Furtado et al., “Dye coupling and connexin expression by cortical radial glia in the early postnatal subventricular zone,” *Developmental Neurobiology*. In press.

[66] F. Miragall, P. Albiez, H. Bartels, U. De Vries, and R. Dermitzeli, “Expression of the gap junction protein connexin43 in the subependymal layer and the rostral migratory stream of the mouse: evidence for an inverse correlation between intensity of connexin43 expression and cell proliferation activity,” *Cell and Tissue Research*, vol. 287, no. 2, pp. 243–253, 1997.

[67] X. Liu, Q. Wang, T. F. Haydar, and A. Bordey, “Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors,” *Nature Neuroscience*, vol. 8, no. 9, pp. 1179–1187, 2005.

[68] C. E. Stout, J. L. Costantin, C. C. G. Naus, and A. C. Charles, “Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels,” *The Journal of Biological Chemistry*, vol. 277, no. 12, pp. 10482–10488, 2002.

[69] K. Braet, S. Aspeslagh, W. Vandamme et al., “Pharmacological sensitivity of ATP release triggered by photoliberation of inositol-1,4,5-trisphosphate and zero extracellular calcium in brain endothelial cells,” *Journal of Cellular Physiology*, vol. 197, no. 2, pp. 205–213, 2003.

[70] N. Dale, “Dynamic ATP signalling and neural development,” *Journal of Physiology*, vol. 586, no. 10, pp. 2429–2436, 2008.

[71] T. A. Weissman, P. A. Riquelme, L. Ivic, A. C. Flint, and A. R. Kriegstein, “Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex,” *Neuron*, vol. 43, no. 5, pp. 647–661, 2004.

[72] D. F. Owens and A. R. Kriegstein, “Patterns of intracellular calcium fluctuation in precursor cells of the neocortical ventricular zone,” *Journal of Neuroscience*, vol. 18, no. 14, pp. 5374–5388, 1998.

[73] M. De Bock, N. Wang, M. Bol et al., “Connexin43 hemichannels contribute to cytoplasmic Ca2+ oscillations by providing a bimodal Ca2+-dependent Ca2+-entry pathway,” *The Journal of Biological Chemistry*, vol. 285, no. 15, pp. 12250–12266, 2012.

[74] E. De Vuyt, N. Wang, E. Decrock et al., “Ca2+ regulation of connexin 43 hemichannels in C6 glioma and glial cells,” *Cell Calcium*, vol. 46, no. 3, pp. 176–187, 2009.
[75] A. G. Reaume, P. A. De Sousa, S. Kulkarni et al., “Cardiac malformation in neonatal mice lacking connexin43,” Science, vol. 267, no. 5205, pp. 1831–1834, 1995.

[76] C. Moorby and M. Patel, “Dual functions for connexins: Cx43 regulates growth independently of gap junction formation,” Experimental Cell Research, vol. 271, no. 2, pp. 238–248, 2001.

[77] A. P. Quist, S. K. Rhee, H. Lin, and R. Lal, “Physiological role of gap-junctional hemichannels: extracellular calcium-dependent isosmotic volume regulation,” Journal of Cell Biology, vol. 148, no. 5, pp. 1063–1074, 2000.

[78] D. J. Belliveau, M. Bani-Yaghoub, B. McGirr, C. C. G. Naus, and W. J. Rushlow, “Enhanced neurite outgrowth in PC12 cells mediated by connexin hemichannels and ATP,” The Journal of Biological Chemistry, vol. 281, no. 30, pp. 20920–20931, 2006.

[79] M. Bani-Yaghoub, T. M. Underhill, and C. C. G. Naus, “Gap junction blockage interferes with neuronal and astroglial differentiation of mouse p19 embryonal carcinoma cells,” Developmental Genetics, vol. 24, no. 1–2, pp. 69–81, 1999.

[80] A. Kunze, M. R. Congreso, C. Hartmann et al., “Connexin expression by radial glia-like cells is required for neurogenesis in the adult dentate gyrus,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 27, pp. 11336–11341, 2009.

[81] Y. Panchina, I. Kelmanson, M. Matz, K. Lukyanov, N. Usman, and S. Lukyanov, “A ubiquitous family of putative gap junction molecules,” Current Biology, vol. 10, no. 13, pp. R473–R474, 2000.

[82] G. E. Sosinsky, D. Boassa, R. Dermietzel et al., “Pannexin channels are not gap junction hemichannels,” Channels, vol. 5, no. 3, pp. 193–197, 2011.

[83] B. A. MacVicar and R. J. Thompson, “Non-junction functions of pannexin1-channels,” Trends in Neurosciences, vol. 33, no. 2, pp. 93–102, 2010.

[84] S. Penuela, R. Gehi, and D. W. Laird, “The biochemistry and function of pannexin channels,” Biochimica et Biophysica Acta. In press.

[85] C. Ambrosi, O. Gassmann, J. N. Pranskevich et al., “Pannexin1 and pannexin2 channels show quaternary similarities to connexons and different oligomerization numbers from each other,” The Journal of Biological Chemistry, vol. 285, no. 32, pp. 24420–24431, 2010.

[86] R. Bruzzone, S. G. Hormuzdi, M. T. Barbe, A. Herb, and H. Monyer, “Pannexins, a family of gap junction proteins expressed in brain,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 23, pp. 13644–13649, 2003.

[87] W. Ma, H. Hui, P. Pelegrin, and A. Surprenant, “Pharmacological characterization of pannexin-1 currents expressed in mammalian cells,” Journal of Pharmacology and Experimental Therapeutics, vol. 328, no. 2, pp. 409–418, 2009.

[88] M. F. Santiago, J. Veliskova, N. K. Patel et al., “Targeting pannexin1 improves seizure outcome,” PLoS ONE, vol. 6, no. 9, article e25178, 2011.

[89] W. R. Silverman, J. P. de Rivero Vaccari, S. Locovei et al., “The pannexin 1 channel activates the inflammasome in neurons and astrocytes,” The Journal of Biological Chemistry, vol. 284, no. 27, pp. 18143–18151, 2009.

[90] L. Bao, S. Locovei, and G. Dahl, “Pannexin membrane channels are mechanosensitive conduits for ATP,” FEBS Letters, vol. 572, no. 1–3, pp. 65–68, 2004.

[91] R. J. Thompson, M. E. Jackson, M. E. Olah et al., “Activation of pannexin-1 hemichannels augments aberrant bursting in the hippocampus,” Science, vol. 322, no. 5907, pp. 1555–1559, 2008.

[92] S. Locovei, J. Wang, and G. Dahl, “Activation of pannexin 1 channels by ATP through P2Y receptors and by cytoplasmic calcium,” FEBS Letters, vol. 580, no. 1, pp. 239–244, 2006.

[93] R. J. Thompson, N. Zhou, and B. A. MacVicar, “Ischemia opens neuronal gap junction hemichannels,” Science, vol. 312, no. 5775, pp. 924–927, 2006.

[94] P. Bargiota, A. Krenz, S. G. Hormuzdi et al., “Pannexins in ischemia-induced neurodegeneration,” Proceedings of the National Academy of Sciences of the United States of America, vol. 108, no. 51, pp. 20772–20777, 2011.

[95] F. B. Chekeni, M. R. Elliott, J. K. Sandilos et al., “Pannexin 1 channels mediate “find-me” signal release and membrane permeability during apoptosis,” Nature, vol. 467, no. 7317, pp. 863–867, 2010.

[96] J. K. Sandilos, Y. H. Chiu, F. B. Chekeni et al., “Pannexin 1, an ATP release channel, is activated by caspase cleavage of its pore-associated C terminal autoinhibitory region,” The Journal of Biological Chemistry. In press.

[97] F. Qiu and G. Dahl, “A permeant regulating its permeation pore: inhibition of pannexin 1 channels by ATP,” American Journal of Physiology, vol. 296, no. 2, pp. C250–C255, 2009.

[98] J. Wang, M. Ma, S. Locovei, R. W. Keane, and G. Dahl, “Modulation of membrane channel currents by gap junction protein mimetic peptides: size matters,” American Journal of Physiology, vol. 293, no. 3, pp. C1112–C1119, 2007.

[99] R. Dando and S. D. Roper, “Cell-to-cell communication in intact taste buds through ATP signalling from pannexin 1 gap junction hemichannels,” Journal of Physiology, vol. 587, no. 24, pp. 5899–5906, 2009.

[100] M. T. Barbe, H. Monyer, and R. Bruzzone, “Cell-cell communication beyond connexins: the pannexin channels,” Physiology, vol. 21, no. 2, pp. 103–114, 2006.

[101] W. Ma, V. Compan, W. Zheng et al., “Pannexin 1 forms an anion-selective channel,” Pfliigers Archiv, vol. 463, no. 4, pp. 585–592, 2012.

[102] A. Vogt, S. G. Hormuzdi, and H. Monyer, “Pannexin1 and Pannexin2 expression in the developing and mature rat brain,” Molecular Brain Research, vol. 141, no. 1, pp. 113–120, 2005.

[103] A. Ray, G. Zoidl, S. Weickert, P. Wahle, and R. Dermietzel, “Site-specific and developmental expression of pannexin1 in the mouse nervous system,” European Journal of Neuroscience, vol. 21, no. 12, pp. 3277–3290, 2005.

[104] R. Bruzzone, M. T. Barbe, N. J. Jakob, and H. Monyer, “Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in Xenopus oocytes,” Journal of Neurochemistry, vol. 92, no. 5, pp. 1033–1043, 2005.

[105] J. C. Sáez, V. M. Berthoud, M. C. Brañes, A. D. Martínez, and E. C. Beyer, “Plasma membrane channels formed by connexins: their regulation and functions,” Physiological Reviews, vol. 83, no. 4, pp. 1359–1400, 2003.

[106] T. Yasuda, P. F. Bartlett, and D. J. Adams, “K_\text{a} and K_\text{c} channels regulate electrical properties and proliferation of adult neural precursor cells,” Molecular and Cellular Neurosciences, vol. 37, no. 2, pp. 284–297, 2008.

[107] J. M. Dubois and B. Rouzaire-Dubois, “The influence of cell volume changes on tumour cell proliferation,” European Biophysics Journal, vol. 33, no. 3, pp. 227–232, 2004.

[108] B. Rouzaire-Dubois, M. Malo, J. B. Mándri, and J. M. Dubois, “Cell size-proliferation relationship in rat glioma cells,” Glia, vol. 45, no. 3, pp. 249–257, 2004.
[109] G. P. Nicchia, M. Srinivas, W. Li, C. F. Brosnan, A. Frigeri, and D. C. Spray, “New possible roles for aquaporin-4 in astrocytes: cell cytoskeleton and functional relationship with connexin43,” FASEB Journal, vol. 19, no. 12, pp. 1674–1676, 2005.

[110] K. Saha, A. J. Keung, E. F. Irwin et al., "Substrate modulus directs neural stem cell behavior," Biophysical Journal, vol. 95, no. 9, pp. 4426–4438, 2008.

[111] A. J. Keung, E. M. de Juan-Pardo, D. V. Schafer, and S. Kumar, “Rho GTPases mediate the mechanosensitive lineage commitment of neural stem cells,” Stem Cells, vol. 29, no. 11, pp. 1886–1897, 2011.

[112] S. Imbeault, L. G. Gauvin, H. D. Toeg et al., “The extracellular matrix controls gap junction protein expression and function in postnatal hippocampal neural progenitor cells,” BMC Neuroscience, vol. 10, article 13, 2009.

[113] J. W. Smyth, J. M. Vogan, P. J. Buch et al., “Actin cytoskeleton rest stops regulate anterograde traffic of connexin 43 vesicles to the plasma membrane,” Circulation Research, vol. 110, no. 7, pp. 978–989, 2012.

[114] R. Francis, X. Xu, H. Park et al., "Connexin43 modulates cell polarity and directional cell migration by regulating microtubule dynamics," PLoS ONE, vol. 6, no. 10, article e26379, 2011.

[115] S. Crespin, J. Bechberger, M. Mesnil, C. C. Naus, and W. C. Sin, “The carboxy-terminal tail of connexin43 gap junction protein is sufficient to mediate cytoskeleton changes in human glioma cells,” Journal of Cellular Biochemistry, vol. 110, no. 3, pp. 589–597, 2010.

[116] T. Toyofuku, M. Yabuki, K. Otsu, T. Kuzuya, M. Hori, and M. Tada, “Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes,” The Journal of Biological Chemistry, vol. 273, no. 21, pp. 12725–12731, 1998.

[117] S. Olk, G. Zoidl, and R. Dermietzel, “Connexins, cell motility, and the cytoskeleton,” Cell Motility and the Cytoskeleton, vol. 66, no. 11, pp. 1000–1016, 2009.

[118] R. Ponsaerts, N. Wang, B. Himpens, L. Leybaert, and G. Bultynck, “The contractile system as a negative regulator of the connexin 43 hemichannel,” Biology of the Cell. In press.

[119] R. Bhalla-Gehi, S. Penuela, J. M. Churko, Q. Shao, and D. W. Laird, “Pannexin1 and pannexin3 delivery, cell surface dynamics, and cytoskeletal interactions,” The Journal of Biological Chemistry, vol. 285, no. 12, pp. 9147–9160, 2010.

[120] B. A. Bao, C. P. Lai, C. C. Naus, and J. R. Morgan, “Pannexin1 drives multicellular aggregate compaction via a signaling cascade that remodels the actin cytoskeleton,” The Journal of Biological Chemistry, vol. 287, no. 11, pp. 8407–8416, 2012.

[121] I. Grimm, S. N. Ullsperger, and H. Zimmermann, “Nucleotides and epidermal growth factor induce parallel cytoskeletal rearrangements and migration in cultured adult murine neural stem cells,” Acta Physiologica, vol. 199, no. 2, pp. 181–189, 2010.

[122] V. Benfenati, G. P. Nicchia, M. Svelto, C. Rapisarda, A. Frigeri, and S. Ferroni, “Functional down-regulation of volume-regulated anion channels in AQP4 knockdown cultured rat cortical astrocytes,” Journal of Neurochemistry, vol. 100, no. 1, pp. 87–104, 2007.

[123] W. Ma, V. Compan, W. Zheng et al., “Pannexin 1 forms an anion-selective channel,” Pflügers Archiv, vol. 463, no. 4, pp. 585–592, 2012.

[124] R. Z. Sabirov and Y. Okada, “The maxi-anion channel: a classical channel playing novel roles through an unidentified molecular entity,” Journal of Physiological Sciences, vol. 59, no. 1, pp. 3–21, 2009.