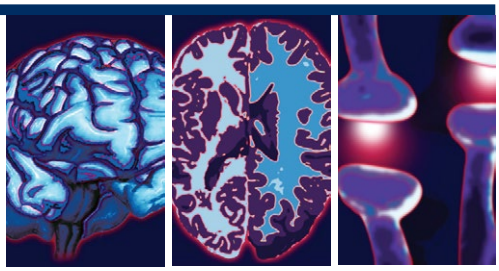
Review



Connexins in the Brain: Psychopharmaceutical Implications

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Abstract

Background

Connexins are the channel forming constituents of hemichannels that facilitate cell-extracellular communication, and gap junction (GJ) intercellular channels that directly connect the cytoplasm of interacting cells. The role of connexins in the brain is an area of growing interest. The purpose of this review is to highlight the current state of research and where future research might be headed, with particular attention to the role of connexins in psychology and psychiatric pharmaceuticals.

The primary connexin isoforms in the brain include connexin45 (Cx45), Cx43, Cx36, and Cx30. These connexins display differential expression in the major neuronal cell types, and have functions including synchronization of neuronal oscillations, metabolite homeostasis, and release of gliotransmitters. Importantly, these processes utilize both GJ intercellular communication, and hemichannel cell-extracellular communication pathways. At the behavioral level, connexins in the brain have been shown to be involved in memory, alcohol indulgence, motor coordination, and anxiety.

Conclusions

There are many compounds that broadly inhibit connexins, and a handful of targeted, isoform-specific, channel function agonists and antagonists. However, these compounds have primarily researched in areas other than the brain. Therefore, connexins potentially provide new pharmaceutical targets for psychiatric disorders.

Keywords

Gap junction, Hemichannel, Connexin, Astrocyte, Neuron, Therapeutic, Gliotransmitter

Introduction

Gap junctions are aggregates of intercellular channels that directly connect the cytoplasm of adjacent cells. Gap junction intercellular channels are composed of oligomers of a family of proteins called connexins. The pores of these channels allow for the passage of small molecules, less than approximately 1kDa in size, thereby creating a conduit for direct cell-cell communication [1]. Importantly, gap junction intercellular channels form from the docking of two half-channels, called hemichannels, which can also facilitate cell-extracellular communication [2].

Connexins are expressed in nearly every cell type with the exception of adult skeletal muscle, red blood cells, and sperm [3]. The role for connexins in the heart in conducting the electrical action potential is now well defined, but their function in many organs and tissues, including the brain, remain poorly defined. Here we summarize the current state of knowledge on the physiology and pathology of connexin expression and

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function in the brain, and discuss the potential for connexin-based therapeutics as psychotropic agents.

Connexin Expression in the Brain

A number of connexin isoforms are expressed in the brain, primarily including Cx30, Cx32, Cx36, and Cx43[4]. Neurons, astrocytes, microglia, and oligodendrocytes all express, and are coupled by connexins. In addition to facilitating cellular functions by direct cell-cell coupling, hemichannels also play a significant role in paracrine and autocrine signaling in the brain [4,5].

■ Neurons

Neurons have been shown to express primarily Cx36 and Cx45 [6]. Definitive GJ plaques have been identified between neurons in the in inferior olive, spinal cord, retina, olfactory bulb, visual cortex, suprachiasmatic nucleus, and locus coeruleus for Cx36 [7-11]. Cx45 GJs have only been identified in the retina and olfactory bulb [9].

■ Astrocytes

Astrocytes have been shown to express a number of different connexin isoforms. Isoforms that have been demonstrated to form astrocytic GJs include Cx26, Cx30, and Cx43 [11,12]. In particular, Cx43 is the isoform primarily expressed in the astrocytyes.

One of the most important functions of astrocytes is metabolite homeostasis. Specifically, astrocytes have been shown to regulate the extracellular milieu in the brain, maintaining an ideal concentration of ions and metabolites for neuronal function [13,14]. Cx43 facilitates the process of K^+, H^+, and glutamate/glutamine homeostasis through intercellular channels by connecting the network of astrocytes [15,16]. In addition, astrocytes communicate to one another through gliotransmitters, which as the name implies, are signaling molecules released from glial cells such as astrocytes. Astrocytic gliotransmitters include ATP and glutamate which are released through hemichannels [5,17,18], and play a role in Ca^{2+} wave propagation, synaptic plasticity, neurotransmitter release at synapses, memory consolidation, and inflammation and cell death under pathological conditions [17,19,20].

■ Microglia

Unactivated microglia can express low levels of Cx36, Cx43, and Cx45 [21,22]. However, upon activation, microglia form GJs composed of Cx43 [23]. In co-cultures, microglia has been found to form Cx36-based GJs with neurons [21]. This has been proposed to be a potential route for transmission of pro-death signals from microglia to neurons in disease and injury.

■ Oligodendtrocytes

Oligodendrocytes express Cx29, Cx32, Cx36, and Cx45 [6]. Although GJs have not been demonstrated between oligodendrocytes, heterotypic (i.e. each cell contributes a different connexin isoform) GJs have been observed between oligodendrocytes and astrocytes [24]. The function of these heterocellular couplings has not been elucidated to date.

Behavioral Effects of Altered Connexin Expression and Function

While there has been a significant amount of research into the role of connexins in the pathophysiology of CNS injury (stroke and spinal cord injury) and neurological diseases such as Alzheimer's (please see [4-6] for excellent reviews), there is little data on the role of connexins in behavior and psychological disorders. However, the studies that have been performed suggest diverse functions for connexins in behavior including addiction and depression.

Some of the data comes from Cx36 knockout mice. Cx36 knockout mice display a reduction in the amplitude of gamma oscillations [25]. The dampening of gamma oscillations suggested these animals could have a deficiency in perception or memory. Indeed, later work showed that Cx36 knockout mice displayed stimulus complexity dependent memory impairment [26].

Interestingly, Cx36 knockout mice also show altered responses to alcohol. Tests of motor function in Cx36 knockout mice given ethanol by injection gave mixed results, showing significantly more ataxia in an open-field test, but significantly improved coordination in a rotarod test of Cx36 knockout versus wild-type mice [27]. The authors also tested hedonic valence for ethanol using a "drink-in-the-dark" procedure, and found that Cx36 knockout mice consumed significantly less alcohol than wild-type mice. The authors attributed this difference to a hyper-dopaminergic state in the ventral tegmental area (VTA) of Cx36 knockout mice, as dopamine neurons in wild-type mice displayed a significant reduction in spontaneous inhibitory

postsynaptic currents in the VTA in response to ethanol, while Cx36 knockout mouse dopamine neurons were resistant to this effect [27].

Other data have come from pharmacological inhibition of GJs in the brain. In one recent study, application of the GJ inhibitors mefloquine and carbenoxolone in the ventral hippocampus and medial prefrontal cortex significantly reduced the peak frequency and total power of theta rhythms [28]. Using an elevated plus maze and open field tests, it was found that bilateral injection of GJ inhibitors into the ventral hippocampus reduced anxiety-like behaviors. This effect was recapitulated in mice subjected to unilateral ventral hippocampus GJ inhibition with contralateral prefrontal medial cortex inhibition, but not unilateral ventral hippocampus inhibition alone. Bilateral injections of GJ inhibitors in the dorsal hippocampus also failed to reduce anxiety, suggesting that GJ coordination of theta oscillations in the ventral hippocampus-medial prefrontal cortex pathway modulates anxiety [28].

Other studies have looked at the effects of induced disorders on the expression of GJs in the brain. Sun, *et al.* examined Cx43 expression and GJ communication in the brains of rats subjected to chronic unpredictable stress as a model of rat depression [29]. They found that GJ intercellular communication was suppressed in the prefrontal limbic cortex and Cx43 expression was reduced in rats subjected to chronic unpredictable stress, and that these effects were reduced by treatment with antidepressants. Interestingly, the authors also found that depression symptoms could be induced by application of the nonspecific GJ inhibitor carbenoxolone, and by the Cx43-specific inhibitors Gap26 and Gap27. Given that astrocytes are the primary CNS cells expressing Cx43, the results strongly indicate that cortical astrocytic intercellular communication plays a role in the neuro-circuitry of emotion.

Another recent study used a novel Cx43 hemichannel-specific inhibitory peptide called Gap19 to determine the role of Cx43 hemichannels in memory [30]. Mice were microinfused with Gap19 in the brain ventricle and subjected to Y maze testing. Gap19 did not affect locomotion or spatial working memory, but reduced short-term spatial working memory in the delayed spontaneous alternation Y maze model. Taken together, these results indicate that astrocytes in the hippocampus mediate spatial working memory through paracrine gliotransmitters released by Cx43 hemichannels.

Finally, there is some evidence from humans that connexins play a significant role in psychology. Mefloquine is a drug (trade name Lariam®) that was developed by the army in the 1970's in a large scale drug screen for compounds with antimalarial properties [31]. In the late 1980's, following FDA approval, Lariam® became the antimalarial drug of choice for people traveling from the United States to regions of the world harboring chloroquine-resistant malaria [32]. However, mefloquine is no longer widely used in the US due to a number of adverse psychiatric side effects including anxiety, panic attacks, paranoia, persecutory delusions, dissociative psychosis, and anterograde amnesia [32]. The severity of these adverse effects is apparent in the description provided by the author David S. MacLean in his book *The Answer to the Riddle Is Me* detailing his experience in India in which mefloquine induced extreme psychosis and amnesia [33]. Unexpectedly, these effects might stem from inhibition of GJs in the inhibitory neurons of the limbic system [32], because as any connexin researcher will know, mefloquine is a commonly used GJ inhibitor [34].

Potential for Connexin-Based Therapeutics as Psychiatric Medications

It seems clear from the preceding discussion that the broad-spectrum GJ inhibitors mefloquine, carbenoxolone, and other well researched GJ inhibitors such as flufenamic acid and 18-β-glycyrrhetinic, will likely not make good psychotherapeutics due to the potential for adverse side effects. However, targeted inhibitors could see more use. At this time, there are a number of GJ-based therapeutics in the pharmaceutical pipeline. We will not go into a detailed review of non-CNS-related applications of these drugs in this discussion (please see Grek, *et al.* for an excellent review of that topic [34]). Suffice it to say that these therapeutics have indications across the board in oncology, transplant, implants, wound healing (including reduced scarring in healthy patients and healing of ulcers in diabetic patients), inflammatory diseases, myocardial infarction, arrhythmia, stroke, migraine, neurodegenerative diseases, retinal disease, tissue engineering, and acute lung injury [2,6,34-36].

With respect to psychotherapeutic applications, there are no published studies to base expectations on. However, the reports discussed above suggest

that improved GJ communication could have anti-depressive effects. Although most connexin-based therapeutics have an inhibitory effect on GJ and/or hemichannel communication [37], a few of these compounds have been shown to improve intercellular communication. One such therapeutic is aCT1. aCT1 is a Cx43 mimetic peptide based on the last 9 amino acids of the Cx43 C-terminus [38]. We have previously shown that aCT1 increases GJ size [39], and enhances GJ communication [38]. aCT1 is currently undergoing clinical trials, and is the first connexin-based therapeutic to have a published clinical trial. Specifically, aCT1 was shown to improve healing of chronic venous leg ulcers [40], was efficacious in treating neuropathic diabetic foot ulcers in a multicenter, randomized trial [41], and reduced cutaneous scarring following laparoscopic surgery [42]. Another such therapeutic is PQ1, a small molecule that has been shown to increase GJ intercellular communication [43]. This drug has been tested in cancer models and shown to increase the efficacy of chemotherapeutics [44,45]. There is also budding research into the role that current mainstay psychiatric medications (clozapine, fluoxetine, haloperidol, and lithium) play in regulating connexins in the brain [46]. Cx43 was up-regulated in the prefontal cortex of rats when administered clozapine and fluoxetine, yet was down-regulated in rats administered haloperidol and lithium, thus suggesting that Cx43 may be a target of existing psychiatric pharmaceuticals.

Conclusions

Connexins are expressed throughout the brain and clearly play a role in the function of the CNS as it pertains to cognition, perception, and behavior. However, these roles have yet to be clearly delineated and future studies will be needed to further understand the complex and intertwining mechanisms that are played by intercellular communication within and between cell types of the CNS, and paracrine and autocrine signaling through small molecules and ions that are released through connexin hemichannels. Connexin-based therapeutics are an emerging line of drugs with broad indications, and we still have yet to understand the ways in which current drugs utilize connexins. We propose that many of these compounds can also have applications in the treatment of psychiatric disorders and diseases.

References

1. Laird DW. Life cycle of connexins in health and disease. *Biochem. J* 394(Pt 3), 527-543 (2006).
2. Rhett JM, Fann SA, Yost MJ. Purinergic signaling in early inflammatory events of the foreign body response: modulating extracellular ATP as an enabling technology for engineered implants and tissues. *Tissue. Eng. Part B Rev* 20(5), 392-402 (2014).
3. Evans WH, Martin PE. Gap junctions: structure and function (Review). *Mol. Membr. Biol* 19(2), 121-136 (2002).
4. Orellana JA, Martinez AD, Retamal MA. Gap junction channels and hemichannels in the CNS: Regulation by signaling molecules. *Neuropharmacology* 75(1), 567-582 (2013).
5. Saez JC, Contreras JE, Bukauskas FF, *et al.* Gap junction hemichannels in astrocytes of the CNS. *Acta. Physiol. Scand* 179(1), 9-22 (2003).
6. Orellana JA, Sáez PJ, Shoji KF, *et al.* Modulation of brain hemichannels and gap junction channels by pro-inflammatory agents and their possible role in neurodegeneration. *Antioxid. Redox. Signal* 11(2), 369-399 (2009).
7. Fukuda T, Kosaka T, Singer W, *et al.* Gap junctions among dendrites of cortical GABAergic neurons establish a dense and widespread intercolumnar network. *J. Neurosci* 26(13), 3434-3443 (2006).
8. Kamasawa N, Furman CS, Davidson KGV, *et al.* 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(4), 1093-1117 (2006).
9. Rash JE, Davidson KG, Kamasawa N, *et al.* Ultrastructural localization of connexins (Cx36, Cx43, Cx45), glutamate receptors and aquaporin-4 in rodent olfactory mucosa, olfactory nerve and olfactory bulb. *J. Neurocytol* 34(3-5), 307-341 (2005).
10. Rash JE, Olson CO, Davidson KG, *et al.* Identification of connexin36 in gap junctions between neurons in rodent locus coeruleus. *Neuroscience* 147(4), 938-956 (2007).
11. Rash JE, Staines WA, Yasumura T, *et al.* 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. U S A* 97(13), 7573-7578 (2000).
12. Nagy JI, Li X, Rempel J, *et al.* Connexin26 in adult rodent central nervous system: demonstration at astrocytic gap junctions and colocalization with connexin30 and connexin43. *J. Comp. Neurol* 441(4), 302-323 (2001).
13. Chen KC, Nicholson C. Spatial buffering of potassium ions in brain extracellular space. *Biophys. J* 78(6), 2776-2797 (2000).
14. Contreras JE, Sánchez HA, Eugenín EA, *et al.* 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. U S A* 99(1), 495-500 (2002).
15. Langer J, Stephan J, Theis M, *et al.* Gap junctions mediate intercellular spread of sodium between hippocampal astrocytes in situ. *Glia* 60(2), 239-252 (2011).
16. Giaume C, Theis M. Pharmacological and genetic approaches to study connexin-mediated channels in glial cells of the central nervous system. *Brain. Res. Rev* 63(1-2), 160-176 (2010).
17. Orellana JA, Froger N, Ezan P, *et al.* ATP and glutamate released via astroglial connexin 43 hemichannels mediate neuronal death through activation of pannexin 1 hemichannels. *J. Neurochem* 118(5), 826-40 (2011).
18. Ye ZC, Wyeth MS, Baltan-Tekkok S, *et al.* Functional hemichannels in astrocytes: a

novel mechanism of glutamate release. *J. Neurosci* 23(9), 3588-3596 (2003).

19. Abbracchio MP, Burnstock G, Verkhratsky A, *et al.* Purinergic signalling in the nervous system: an overview. *Trends. Neurosci* 32(1), 19-29 (2009).

20. Stehberg J, Moraga-Amaro R, Salazar C, *et al.* Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB. J* 26(9), 3649-3657 (2012).

21. Dobrenis K, Chang HY, Pina-Benabou MH, *et al.* Human and mouse microglia express connexin36, and functional gap junctions are formed between rodent microglia and neurons. *J. Neurosci. Res* 82(3), 306-315 (2005).

22. Eugenin EA, Eckardt D, Theis M, *et al.* Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions after treatment with interferon-gamma and tumor necrosis factor-alpha. *Proc. Natl. Acad. Sci. U S A* 98(7), 4190-4195 (2001).

23. Garg S, Md Syed M, Kielian T. Staphylococcus aureus-derived peptidoglycan induces Cx43 expression and functional gap junction intercellular communication in microglia. *J. Neurochem* 95(2), 475-483 (2005).

24. Orthmann-Murphy JL, Freidin M, Fischer E, *et al.* Two distinct heterotypic channels mediate gap junction coupling between astrocyte and oligodendrocyte connexins. *J. Neurosci* 27(51), 13949-13957 (2007).

25. Buhl DL, Harris KD, Hormuzdi SG, *et al.* Selective impairment of hippocampal gamma oscillations in connexin-36 knock-out mouse in vivo. *J. Neurosci* 23(3), 1013-1018 (2003).

26. Frisch C, De Souza-Silva MA, Söhl G, *et al.* 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(1), 177-185 (2005).

27. Steffensen SC, Bradley KD, Hansen DM, *et al.* The role of connexin-36 gap junctions in alcohol intoxication and consumption. *Synapse* 65(8), 95-707 (2011).

28. Schoenfeld TJ, Kloth AD, Hsueh B, *et al.* Gap junctions in the ventral hippocampal-medial prefrontal pathway are involved in anxiety regulation. *J. Neurosci* 34(47), 15679-15688 (2014).

29. Sun JD, Liu Y, Yuan YH, *et al.* Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology* 37(5), 1305-1320 (2012).

30. Walrave L, Vinken M, Albertini G, *et al.* Inhibition of Connexin43 Hemichannels Impairs Spatial Short-Term Memory without Affecting Spatial Working Memory. *Front. Cell. Neurosci* 10(1), 288 (2016).

31. Maugh TH 2nd. Malaria drugs: new ones are available, but little used. *Science* 196(4288), 415 (1977).

32. Ritchie EC, Block J, Nevin RL. Psychiatric side effects of mefloquine: applications to forensic psychiatry. *J. Am. Acad. Psychiatry. Law* 41(2), 224-235 (2013).

33. MacLean DS. The Answer to the Riddle Is Me: A Memoir of Amnesia. Houghton Mifflin Harcourt. 304 (2014).

34. Grek CL, Rhett JM Ghatnekar GS. Cardiac to cancer: connecting connexins to clinical opportunity. *FEBS. Lett* 588(8), 1349-1364 (2014).

35. Rhett JM, Wang H, Bainbridge H, *et al.* Connexin-based therapeutics and tissue engineering approaches to the amelioration of chronic pancreatitis and type I diabetes: Construction and characterization of a novel prevascularized bioartificial pancreas. *J. Diab. Res* 2016 (7262680) (2015).

36. Grek CL, Rhett JM, Bruce JS, *et al.* Targeting connexin 43 with alpha-connexin carboxyl-terminal (ACT1) peptide enhances the activity of the targeted inhibitors, tamoxifen and lapatinib, in breast cancer: clinical implication for ACT1. *BMC. Cancer* 15(1), 296 (2015).

37. Evans WH, Bultynck G, Leybaert L, Manipulating connexin communication channels: use of peptidomimetics and the translational outputs. *J. Membr. Biol* 245(8), 437-449 (2012).

38. Rhett JM, Jourdan J, Gourdie RG. Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol. Biol. Cell* 22(9), 1516-1528 (2011).

39. Hunter AW, Barker RJ, Zhu C, *et al.* Zonula occludens-1 alters connexin43 gap junction size and organization by influencing channel accretion. *Mol. Biol. Cell* 16(12), 5686-5698 (2005).

40. Ghatnekar GS, Grek CL, Armstrong DG, *et al.* The effect of a connexin43-based Peptide on the healing of chronic venous leg ulcers: a multicenter, randomized trial. *J. Invest. Dermatol* 135(1), 289-298 (2015).

41. Grek CL, Prasad GM, Viswanathan V, *et al.* Topical administration of a connexin43-based peptide augments healing of chronic neuropathic diabetic foot ulcers: A multicenter, randomized trial. *Wound. Repair. Regen* 23(2), 203-212 (2015).

42. Grek CL, Montgomery J, Sharma M, *et al.* A Multicenter Randomized Controlled Trial Evaluating a Cx43-Mimetic Peptide in Cutaneous Scarring. *J. Invest. Dermatol* 137(3), 620-630 (2017).

43. Bigelow K, Nguyen TA. Increase of gap junction activities in SW480 human colorectal cancer cells. *BMC. Cancer* 14(1), 502 (2014).

44. Ding Y, Nguyen TA. Gap Junction Enhancer Potentiates Cytotoxicity of Cisplatin in Breast Cancer Cells. *J. Cancer. Sci. Ther* 4(11), 371-378 (2012).

45. Ding Y, Nguyen TA. PQ1, a quinoline derivative, induces apoptosis in T47D breast cancer cells through activation of caspase-8 and caspase-9. *Apoptosis* 18(9), 1071-1082 (2013).

46. Fatemi SH, Folsom TD, Reutiman TJ, *et al.* Chronic psychotropic drug treatment causes differential expression of connexin 43 and GFAP in frontal cortex of rats. *Schizophr. Res* 104(1-3), 127-34 (2008).