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
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 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 astrocytes. 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 Ca²⁺ 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.

#### Oligodendrocytes

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
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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 microinjected 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 prefrontal 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


