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Novel model for the mechanisms of glutamate-dependent excitotoxicity: Role of neuronal gap junctions

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Abstract

In the mammalian central nervous system (CNS), coupling of neurons by gap junctions (electrical synapses) increases during early postnatal development, then decreases, but increases in the mature CNS following neuronal injury, such as ischemia, traumatic brain injury and epilepsy. Glutamate-dependent neuronal death also occurs in the CNS during development and neuronal injury, i.e., at the time when neuronal gap junction coupling is increased. Here, we review our recent studies on regulation of neuronal gap junction coupling by glutamate during development and injury and on the role of gap junctions in neuronal cell death. A novel model of the mechanisms of glutamate-dependent neuronal death is discussed, which includes neuronal gap junction coupling as a critical part of these mechanisms.

Keywords

Gap junctions; Connexin 36; Neuronal death; Glutamate; Excitotoxicity

1. Introduction

Gap junctions are formations between two neighboring cells that include intercellular channels and allow direct diffusion of ions and small molecules between the cells (Bennett and Zukin, 2004). The channels are made of two hemichannels and each hemichannel consists of six subunits, known as connexins, which are integral membrane proteins. Connexins are encoded by a large multigene family with at least 21 genes in mammals (Sohl et al., 2005). Among them, 11 are expressed in the nervous system, most of which are localized in glial cells (Condorelli et al., 2003; Nagy et al., 2004). Connexin 36 (Cx36) is the main neuronal connexin (Condorelli et al., 1998; Rash et al., 2000; Sohl et al., 1998), although some other connexins may be expressed by neurons too (e.g., connexin 45, and others) (Sohl et al., 2005). In addition to gap junctions, unopposed connexin-containing hemichannels and channels that consist of pannexins (i.e., the vertebrate proteins that are homologous to the invertebrate gap junction proteins, innexins) also are found in the nervous system (MacVicar and Thompson, 2010; Saez et al., 2010; Sosinsky et al., 2011).

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In the mammalian central nervous system (CNS), the coupling of neurons by gap junctions and the expression of Cx36 increase during early postnatal development, usually during the first two postnatal weeks (e.g., in the hypothalamus, cortex and hippocampus) (Arumugam et al., 2005; Belluardo et al., 2000; Park et al., 2011). In some areas of the brain and in the spinal cord, however, the increase presumably occurs during late embryonic development (Lee et al., 2010; Mentis et al., 2002; Pastor et al., 2003). It is believed that gap junctions between developing neurons play a role in a number of developmental events, including synaptogenesis (Personius et al., 2007), neuronal differentiation (Hartfield et al., 2011), migration (Cina et al., 2007; Elias et al., 2007) and neural circuit formation and maturation (Dupont et al., 2006; Maher et al., 2009; Yuste et al., 1995). The contributions of gap junctions to these developmental events presumably are via the passage of Ca^{2+} , IP_3 , cAMP and small molecules between the cells and coordination of metabolic and transcriptional activities in developing neurons (Kandler and Katz, 1998a). In addition, gap junctions contribute to generation of the highly synchronized excitatory electrical activity that is a hallmark of the developing brain (Ben-Ari, 2001; Garaschuk et al., 2000). This network-driven activity often involves cooperation between gap junctions and chemical neurotransmitter receptors, e.g., GABA (γ -aminobutyric acid) A receptors (GABA_AR) in the hippocampus (Strata et al., 1997) and cholinergic receptors and GABA_ARs in the cortex (Dupont et al., 2006; Hanganu et al., 2009).

The general level of neuronal gap junction coupling and Cx36 expression decreases during later stages of development: usually during postnatal weeks 3–4 (Arumugam et al., 2005; Belluardo et al., 2000; Park et al., 2011), in some CNS regions during weeks 1–2 (Lee et al., 2010; Mentis et al., 2002; Pastor et al., 2003); and this decrease overlaps with the major period of chemical synapse formation and increased synaptic activity (Kandler and Katz, 1998b). In the mature CNS, Cx36 gap junction coupling not only is lower than during early development, but also is restricted almost exclusively to homogeneous neuronal subpopulations, e.g., to subtypes of GABAergic interneurons (Deans et al., 2001; Galarreta and Hestrin, 2001); however, it is also possible that the developmental uncoupling simply does not occur in those neurons (e.g., see Meyer et al., 2002 and Parker et al., 2009). The coupling and Cx36 expression increase in the CNS following neuronal injuries, such as ischemia (de Pina-Benabou et al., 2005; Oguro et al., 2001; Wang et al., 2012), spinal cord and traumatic brain injury (TBI) (Chang et al., 2000; Frantseva et al., 2002; Ohsumi et al., 2006), retinal injury (Striedinger et al., 2005), epilepsy (Gajda et al., 2003; Perez Velazquez et al., 1994) and inflammation (Garrett and Durham, 2008).

Programmed cell death is a critical process in the developing CNS that helps to establish the final number of neurons and contributes to the distribution of various cell classes and neuronal circuit formation (Nijhawan et al., 2000). The activity of NMDARs also is the factor that plays a role in cell survival versus death decisions during development; NMDAR activity above or below a specific level results in neuronal cell death (Adams et al., 2004; Contestabile, 2000; Goodman and Shatz, 1993; Scheetz and Constantine-Paton, 1994). In addition, glutamate-dependent excitotoxicity (which is mostly caused by hyperactivation of NMDARs) plays critical role in neuronal death in the mature CNS following injury, including ischemic stroke, TBI and epilepsy (Arundine and Tymianski, 2004; Choi, 1988; Hazell, 2007). However, whether during development, NMDAR excitotoxicity or neuronal injury, gap junctions play a role in neurodegeneration or neuroprotection is controversial as their contribution to both neural cell death and survival has been reported (reviewed in Decrock et al., 2009 and Perez Velazquez et al., 2003). Further, until recently, the mechanisms controlling changes in neuronal gap junction coupling during development and neuronal injury were not understood. In this paper, I will review our recent studies that have addressed these critical issues.

2. Regulation and role of neuronal gap junctions in neuronal death in the developing CNS

In our recent work we studied the cellular and molecular mechanisms that are responsible for the sequential increase and then decrease in neuronal gap junction coupling during brain development. We also studied the role of these mechanisms in control of death/survival in developing neurons. Using dye coupling, electrotonic coupling and western blot analysis in the rat and wild-type (WT) mouse hypothalamus and neocortex, we showed that neuronal gap junction coupling and the expression of Cx36 increase between postnatal day 1 (P1)–P15 and then decrease between P15–30 (Arumugam et al., 2005; Park et al., 2011). Similar changes were found in neuronal cultures between, respectively, day *in vitro* 3 (DIV3)–DIV15 and DIV16–32 (Arumugam et al., 2005; Park et al., 2011; Wang et al., 2012).

We established that the developmental increase in neuronal gap junction coupling is regulated by an interplay between the activity of group II metabotropic glutamate receptors (mGluR) and GABA_ARs (schematically illustrated in Figure 1a) (Park et al., 2011). Specifically, we showed that chronic (2 week) activation of group II mGluRs augments, and inactivation prevents, the developmental increase in neuronal gap junction coupling and Cx36 expression. However, changes in GABA_AR activity have the exact opposite effects. We also showed that the regulation by group II mGluRs is via cAMP/protein kinase A-dependent signaling and the regulation by GABA_ARs is via depolarization of neurons (that commonly occurs during development; Stein and Nicoll, 2003) that results in influx of Ca²⁺ through voltage-gated Ca²⁺ channels and activation of Ca²⁺/protein kinase C-dependent signaling. Further, we found that other neurotransmitter receptors are not involved in these regulatory mechanisms, including acetylcholine, GABA_B and other glutamate receptors. Finally, we demonstrated that the prolonged, receptor-mediated up-regulation of Cx36 in developing neurons is associated with increase in Cx36 mRNA levels, requires a neuron-restrictive silencer element in the Cx36 gene promoter and, thus, involves transcriptional regulatory mechanisms. However, the receptor-mediated down-regulation of Cx36 is not associated with changes in Cx36 mRNA levels, but requires the 3'-untranslated region of the Cx36 mRNA and, thus, involves post-transcriptional mechanisms (Park et al., 2011).

Interestingly, we found that acute (60 min) activation of group II mGluRs in developing (DIV3) cortical neurons induces only transient increase (within 3 hours; with the following decrease) in Cx36 expression without change in the Cx36 mRNA levels (Song et al., 2012). This suggests that the initial response to activation of group II mGluRs in young neurons is posttranscriptional. However, it may convert into a transcriptionally-regulated modification, if the increased level of receptor activity sustains (Park et al., 2011; Song et al., 2012).

Further, in our studies we addressed the mechanisms for uncoupling of neuronal gap junctions during development. In the rat hypothalamus, the developmental uncoupling also is regulated by glutamate (Figure 1c) (Arumugam et al., 2005). However, the regulation occurs via activation of NMDARs and CREB (Ca²⁺/cAMP response element binding protein)-dependent down-regulation of Cx36, as inactivation of NMDARs or CREB prevents the developmental uncoupling and Cx36 down-regulation and CREB overexpression accelerates these developmental changes (Arumugam et al., 2005). These results are in agreement with the previous study (Mentis et al., 2002), that demonstrated a role for NMDARs in the developmental uncoupling of motoneurons in the rat spinal cord.

Based on the results obtained, we proposed the following model of the mechanisms for developmental regulation of neuronal gap junction coupling (Park et al., 2011). During early postnatal development, GABA_AR-dependent excitation maintains the expression of Cx36 in neurons at a low level (via Ca²⁺/protein kinase C-dependent signaling and 3'-untranslated

region of the Cx36 mRNA). The subsequent transition from GABA_AR excitation to inhibition, in combination with increased activity of the group II mGluRs, result in the developmental up-regulation of Cx36 (via the neuron-restrictive silencer element in the Cx36 gene) and increased neuronal gap junction coupling (Figure 1a). However, the developmental increase in the activity of NMDARs then causes down-regulation of Cx36 (via Ca²⁺-dependent signaling, including CREB) and neuronal uncoupling (Figure 1c). Because these developmental mechanisms were demonstrated in different brain regions (including the hypothalamus, cortex and spinal cord) and animal species (rats and mice), it is possible that such the mechanisms have universal character in the CNS. The variations among different CNS regions in the timing of neuronal gap junction coupling and uncoupling presumably can be explained by the interregional differences in the activity of these mechanisms (i.e., receptor and synaptic activity and timing of the excitation/inhibition switch for GABA_ARs). However, other additional, region-specific factors and/or neurotransmitter systems presumably may contribute too.

We also tested the role of neuronal gap junctions in death/survival in developing neurons (indicated in Figure 1b) (de Rivero Vaccari et al., 2007). In rat and mouse hypothalamic neuronal cultures, a 3 day inactivation or hyperactivation of NMDARs both induced neuronal death specifically during the peak of developmental gap junction coupling (i.e., on DIV14–17). In contrast, increasing or decreasing NMDAR activity for 3 days at the time when neuronal gap junction coupling was low, had no (on DIV1–4) or greatly reduced (on DIV28–31) impact on cell survival. Neuronal death caused by NMDAR hypofunction or hyperfunction, that was induced on DIV14–17, was prevented by pharmacological inactivation of gap junctions and genetic knockout of Cx36. We concluded that, during development, Cx36-containing gap junctions play critical role in neuronal death caused by increased or decreased NMDAR function (de Rivero Vaccari et al., 2007).

Finally, using developing hypothalamic and cortical neuronal cultures, we showed (Park et al., 2011) that chronic (2 week) activation of group II mGluR not only augments the developmental increase in neuronal gap junction coupling, but also makes neurons significantly more susceptible to NMDAR-mediated excitotoxicity. In addition, chronic inactivation of group II mGluRs not only prevents the developmental increase in neuronal gap junction coupling, but also completely prevents NMDAR-mediated death in developing neurons. Together, these observations led us to conclude that the mechanisms controlling developmental changes in neuronal gap junction coupling (Figure 1a) also control death/survival in developing neurons (Figure 1b). We suggest that via regulation of neuronal gap junctions these mechanisms contribute to the control of apoptotic death in developing neurons and formation of neuronal circuits (Park et al., 2011).

3. Regulation and role of neuronal gap junctions in neuronal death in the mature CNS

Recently, we studied the regulation and role of neuronal gap junctions during neuronal injury (Wang et al., 2012). Photothrombotic focal cerebral ischemia in adult mice and oxygen-glucose deprivation (OGD) in mature cortical cultures were used as, respectively, *in vivo* and *in vitro* models of ischemic stroke. In addition, three other *in vitro* injury models in mature cortical cultures were used: hyposmotic shock as a model of cytotoxic and osmotic edemas that occur during stroke and TBI (Unterberg et al., 2004); hydrostatic pressure injury that represents mechanical aspects of TBI (Morrison et al., 1998); and administration of 4-aminopyridine as a model of epileptic seizures (Wong and Yamada, 2001). In these models, neuronal gap junction coupling and/or Cx36 expression were studied and showed a significant increase 2 hours post-injury (schematically illustrated in Figure 1d) (Wang et al., 2012). Because there is excessive and rapid (within minutes) release of glutamate from

injured neurons (De Cristobal et al., 2001; Guyot et al., 2001; Lobner and Choi, 1994) and group II mGluRs regulate the developmental increase in neuronal gap junction coupling (Park et al., 2011), we tested whether group II mGluRs also regulate the increase in neuronal gap junction coupling following neuronal injury.

We showed that inactivation of group II mGluRs prevents the injury-mediated increases in neuronal gap junction coupling and expression of Cx36. In addition, an activation of group II mGluRs increased background levels of the coupling and Cx36 expression. Further, the results suggested that the regulation of neuronal gap junctions likely is via action potential-dependent synaptic release of glutamate, is via post-transcriptional control of Cx36 expression, is specific for neuronal (Cx36-containing), but not astrocytic (connexin 43-containing) gap junctions and that other neurotransmitter receptors are not involved directly in these regulatory mechanisms (including NMDARs, AMPA receptors, group I mGluRs, group III mGluRs, GABA_ARs and GABA_B receptors). Importantly, inactivation of group II mGluRs, genetic knockout of Cx36 and/or pharmacological blockade of gap junctions all substantially reduced the injury-mediated neuronal death (as demonstrated in all the five used *in vivo* and *in vitro* injury models). Based on these findings, we concluded that group II mGluRs control the injury-mediated increase in neuronal gap junction coupling (Figure 1d). Further, via regulation of neuronal gap junctions, group II mGluRs also control death/survival mechanisms in injured neurons (Figure 1e) (Wang et al., 2012).

A critical role for neuronal gap junctions in NMDAR-mediated excitotoxicity also was documented in adult mice (Wang et al., 2010b). A single intraperitoneal administration of NMDA to WT mice induced 24 hrs later a substantial neuronal death in some regions of the forebrain (see below). This neuronal death was dramatically reduced by co-administration of mefloquine, a relatively selective blocker for Cx36-containing gap junctions (Cruikshank et al., 2004), and was statistically insignificant in Cx36 knockout mice. The expression of NR1 subunit of the NMDAR and the amplitude of NMDAR-mediated neuronal Ca²⁺ responses were higher in Cx36 knockout than in WT mice, suggesting that the reduced level of NMDAR-mediated neuronal death in Cx36 knockout animals is not due to the reduced expression or activity of NMDARs. In addition, NMDA permeability of the blood-brain barrier (BBB) in the whole brain was not different between WT and Cx36 knockout mice, suggesting that the reduced neuronal death in Cx36 knockout mice is not due to the reduced permeability of the BBB to NMDA. Finally, a blockade of neuronal gap junction coupling with mefloquine and Cx36 knockout both dramatically reduced neuronal death that was caused in mice by photothrombotic focal cerebral ischemia. Together, the data supported a role for neuronal gap junctions in ischemic neuronal death. They also suggested that the coupling of neurons by gap junctions is required for NMDAR-mediated excitotoxicity in the mature CNS. Further, the neuroprotective mechanism of mefloquine very likely was solely based upon blockade of Cx36-containing neuronal gap junctions, since mefloquine did not have any additional neuroprotective effects in Cx36 knockout mice (Wang et al., 2010b).

An interesting aspect in that study (Wang et al., 2010b) was that systemic administration of NMDA induced neuronal death only in three regions of the forebrain: rostral dentate gyrus, hypothalamus and medial habenula. This was found in both WT (substantial neuronal death) and Cx36 knockout mice (statistically non-significant, but still detectable neuronal death). Although, permeability of the BBB in the whole brain to NMDA was not different between WT and Cx36 knockout mice, we believe that the only logical explanation for neuronal death only in three forebrain regions is that, somehow, permeability of the BBB to NMDA in those three regions is higher than in the rest of the forebrain.

4. Novel model for mechanisms of glutamate-dependent excitotoxicity

Brain injuries, such as those that occur following TBI and stroke, are characterized by two distinct areas: core and penumbra (Guyot et al., 2001; Stoffel et al., 2002). The core is the area subjected directly to a physical impact (TBI) or anoxia (stroke) and shows almost complete loss of neural cells due to necrosis (acute cell death). The penumbra, located immediately outside the core and not subjected to direct injury, suffers from the secondary injury and shows signs of apoptosis (delayed cell death). It has been suggested previously (Arundine and Tymianski, 2004; Choi, 1988; Hazell, 2007) that one of the most critical factors responsible for the secondary neuronal death is excessive release of glutamate from injured cells that causes glutamate-dependent excitotoxicity. The excitotoxic mechanisms of glutamate are well-characterized and include hyperactivation of glutamate receptors (primarily NMDARs), massive influx of Ca^{2+} ions and overactivation of Ca^{2+} -dependent signaling pathways that eventually causes death of neurons (Arundine and Tymianski, 2004; Choi, 1988; Hazell, 2007) (Figure 2A, a1). As such, the development of NMDAR antagonists for the purposes of neuroprotection is a rational approach for the treatment of neurological disorders. Indeed, a number of NMDAR antagonists were designed and tested in different models of neuronal injury to assess their neuroprotective effects. However, trials for the majority of NMDAR antagonists failed to provide clinical benefit (Ikonomidou and Turski, 2002).

Based on our studies, we proposed a novel model for the mechanisms of glutamate-dependent excitotoxicity (Figure 2B) (Wang et al., 2012). According to this model, during neuronal injury, the main reason for massive glutamate-dependent neuronal death is not an overactivation of NMDARs *per se*, but rather the expression of neuronal gap junctions. While NMDAR hyperactivity triggers neurodegenerative processes, in the absence of neuronal gap junctions this neurodegeneration is limited to a small group of neurons, which for various reasons may be especially sensitive to excitotoxicity (as we discussed in de Rivero Vaccari et al., 2007) (Figure 2, b1, which is the same as a1). However, in the presence of neuronal gap junctions, the amount of NMDAR-mediated neuronal death is greatly multiplied and death also occurs in nearly all (or all) coupled neurons (Figure 2B, b2). In this respect, the background level of neuronal gap junction coupling, normally found in many mature brain regions (Bennett and Zukin, 2004), is critical for NMDAR-mediated excitotoxicity to occur. In addition, an activation of group II mGluRs, caused by injury-mediated release of glutamate, induces synthesis of new neuronal gap junctions and these new gap junctions enhance the extent of neuronal death (Figure 2B, b3).

We believe that the presence of Cx36 gap junction coupling underlies a universal mechanism for neuronal death during different types of neuronal injuries, including stroke, TBI, epilepsy and presumably others. This mechanism is engaged as soon as extracellular glutamate rises to pathological levels, which triggers NMDAR excitotoxicity and initial gap junction-dependent neuronal death. The pathological mechanism then proceeds by means of group II mGluR-dependent induction of neuronal gap junctions. The induction occurs within 2–3 hours of the initial injury, i.e., during a critical time window, where therapeutic intervention is the most successful in limiting the extent of injury-mediated cell death and functional deficits.

Our proposed model may explain the phenomenon of enhanced susceptibility of the developing brain to NMDAR excitotoxicity. It has been shown previously (Zhou and Baudry, 2006) that administration of NMDA induces substantial neuronal death in acute hippocampal slices from juvenile (1–3 week-old) rats, but not adult (3 month-old) rats, though OGD causes neuronal death in slices from both animal groups. The developmental change in the NMDAR subunit composition perhaps is one possible explanation for this

phenomenon (Zhou and Baudry, 2006). However, we speculate that the presence and absence of NMDAR-mediated excitotoxicity in the brains of juvenile and adult animals, respectively, are determined by the age-related difference in the amount of neuronal gap junction coupling, i.e., high in the juvenile brain and low in the adult brain. In addition, the increased expression of neuronal gap junctions, mediated by the OGD-induced glutamate release and activation of group II mGluRs, contributes to the observed ischemic neuronal death in slices from adult animals.

As discussed above, one of the principles of neuronal gap junction coupling is that, in the mature CNS, coupling occurs mainly between neurons of the same type. For example, under non-pathological conditions in the brain of adult rodents, Cx36-containing gap junctions are restricted largely to subtypes of GABAergic interneurons, allowing formation of gap junction-coupled interneuronal networks (Deans et al., 2001; Galarreta and Hestrin, 2001). Therefore, based on our model, we predict that the networks of coupled interneurons (shown in red, Figure 2b, b2–b3) would be particularly susceptible to death signals propagated from the locus of initial death, i.e., from neurons dying as a result of NMDAR excitotoxicity (shown in blue, Figure 2b, b1–b3). The initial, primary death signals, i.e., those that induce NMDAR-mediated death, may or may not be gap junction-dependent. However, death in proximal, coupled interneurons would be expected to be gap junction-dependent. In addition, the second wave of gap junction-dependent cell death is mediated via newly synthesized Cx36-containing gap junctions and may potentially affect both interneurons and other neuronal types (shown in black, Figure 2b, b3).

Multiple lines of evidence also indicate participation of non-Cx36-containing gap junctions, hemichannels and pannexin-containing channels in injury-induced neuronal death (Davidson et al., 2012; Frantseva et al., 2002; Huang et al., 2012; Iglesias et al., 2008). Recent studies suggest the existence of gap junctions between pyramidal neurons in the adult rodent cortex and hippocampus (Mercer et al., 2006; Wang et al., 2010a), and these gap junctions presumably are made from a connexin(s) other than Cx36 (Connors and Long, 2004). Given that in the mature cortical regions, interneurons and pyramidal cells are not coupled to each other (Deans et al., 2001; Galarreta and Hestrin, 1999; Gibson et al., 1999), the possibility exists that these two neuronal types form separate gap junction-coupled networks that are differently affected by the injury. Therefore, the amount of contribution to neuronal death by gap junctions containing Cx36 or other neuronal connexins may vary. Nevertheless, given the substantial neuroprotection provided by blockade of Cx36 gap junctions or inactivation of the mechanisms controlling the injury-dependent increase in the coupling (de Rivero Vaccari et al., 2007; Wang et al., 2010b; Wang et al., 2012), we suspect that Cx36-containing gap junctions are the primary determinant of injury-mediated neuronal death.

The contribution of neuronal gap junctions to cell death presumably is through propagation of gap junction-permeable neurodegenerative signals between the coupled neurons; for example NMDAR-, AMPA receptor-, kainate receptor-, inflammation- and apoptosis-dependent signals such as Ca^{2+} , Na^{+} and IP_3 (Cusato et al., 2006; Decrock et al., 2009). This agrees with a model of the “bystander cell death”. The second possibility is the contribution via channel-independent mechanisms. This has not yet been demonstrated for Cx36. However, a number of studies showed that many non-neuronal connexins may control cell death via direct or indirect regulation of transcriptional programs and apoptotic pathways (reviewed in Decrock et al., 2009). It is also not excluded, that the two modes of gap junction-mediated cell death (i.e., channel-dependent and -independent) may coexist, that should be clarified in the future.

As indicated above, the role of gap junctions in cell death mechanisms still is controversial as their contribution to both neuronal death and survival has been reported. For example,

deteriorative effects of genetic Cx36 knockout and blockade of gap junctions on injury- and glutamate-induced neuronal death were documented (Ozog et al., 2002; Striedinger et al., 2005). Meanwhile, under our experimental conditions with the use of various *in vivo* and *in vitro* injury models and NMDAR-mediated excitotoxicity, we consistently observed a neuroprotective effect following blockade of neuronal gap junctions (de Rivero Vaccari et al., 2007; Park et al., 2011; Wang et al., 2010b; Wang et al., 2012). Two recent reviews discuss in detail the possible reasons on why both enhanced and reduced survival has been observed in different model systems, when gap junction coupling is blocked or eliminated (Decrock et al., 2009; Perez Velazquez et al., 2003). It is clear, however, that this controversy still exists and waits for its experimental resolution.

5. Conclusions

In conclusion, our studies provide new mechanisms for glutamate-mediated excitotoxicity beyond ionotropic glutamate receptors. They suggest that neuronal, Cx36-containing gap junctions are a crucial part of these mechanisms. They also identify neuronal gap junction coupling as a critically important therapeutic target for the development of new neuroprotective agents.

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Abbreviations

BBB	blood-brain barrier
CNS	central nervous system
CREB	Ca ²⁺ /cAMP response element binding protein
Cx36	connexin 36
DIV	day <i>in vitro</i>
GABA	γ -aminobutyric acid
GABA_AR	GABA _A receptor
mGluR	metabotropic glutamate receptor
NMDA	<i>N</i> -methyl-D-aspartate
NMDAR	NMDA receptor
OGD	oxygen-glucose deprivation
P	postnatal day
TBI	traumatic brain injury
WT	wild-type

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Figure 1. Neuronal gap junction coupling in the CNS

This figure schematically illustrates the summary of conclusions from our recent studies on the role of neurotransmitter receptors in changes in neuronal gap junction coupling (a, c, d) and the role of neuronal gap junctions in neuronal death (b, e) during development and neuronal injury (see text for details, abbreviations and references). Activation of group II mGluRs increases neuronal gap junction coupling and Cx36 expression during both development and neuronal injury (a, d: red arrows upward); however, note the difference in duration of developmental and injury-mediated increases. Activation of GABA_ARs counteracts to the developmental increase in coupling (a: red arrow downward). Activation of NMDARs causes the developmental uncoupling of neuronal gap junctions and down-regulation of Cx36 (c: red arrow downward). GJ, gap junctions; P1 and P30, postnatal days 1 and 30.

Figure 2. Glutamate-dependent excitotoxicity during neuronal injury

(A) Traditional model of the mechanisms for glutamate-dependent excitotoxicity. (B) Novel model of the mechanisms of glutamate-dependent excitotoxicity. a1 and b1: Neuronal death caused directly by overactivation of NMDARs. b2: Existing neuronal gap junctions contribute substantially to neuronal death caused by overactivation of NMDARs. b3: New neuronal gap junctions are induced by activation of group II mGluRs and also contribute to glutamate-dependent neuronal death. ⊕, this sign indicates the increase in receptor activity or expression of Cx36. In figures a1 and b1–b3 (lower row), lines between circles represent gap junctions and circles represent neurons: yellow, live neurons; blue, death caused directly by overactivation of NMDARs; red, death caused by propagation of presumptive gap

junction-permeable death signals via existing gap junctions; black, death caused by propagation of presumptive gap junction-permeable death signals via newly synthesized gap junctions. See text for details. This figure is adapted with permission from (Wang et al., 2012).

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