Connexin43 and Bergmann glial gap junctions in cerebellar function

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Connexin43 (Cx43), a major component of astrocytic gap junctions, is abundantly expressed in Bergmann glial cells (BGCs) in the cerebellum, but the function of Cx43 in BGCs is largely unknown. BGCs are specialized astrocytes closely associated with Purkinje cells. Here, we review our recent studies of the role of Cx43 in gap junctional coupling between BGCs and in cerebellar function. We generated Cx43 conditional knockout mice with an S100b-Cre transgenic line (Cx43fl/fl:S100b-Cre), in which there was a significant postnatal loss of Cx43 in BGCs and cerebellar astrocytes. Gap junctional coupling between BGCs measured by dye coupling was virtually abolished in Cx43fl/fl:S100b-Cre mice. Electrophysiologic and behavioral analyses suggested that Cx43-mediated gap junctions and Cx43 hemichannels in BGCs are not necessary for the neuron-glia interactions required for cerebellum-dependent motor coordination and motor learning. These findings raise questions regarding the regional differences in the impact of the loss of Cx43 in the brain.

Keywords: connexin43, gap junction, astrocyte, Bergmann glia, conditional knockout mouse

INTRODUCTION

Astrocytes, the most abundant cell type in the mammalian brain, are extensively coupled by gap junctions (Giaume and McCarthy, 1996) through which ionic and metabolic homeostasis (e.g., spatial buffering of K+ and glutamate) is maintained, and electrical coupling and intercellular signaling (e.g., Ca2+ wave) occur (Ransom and Ye, 2005). The gap junction channel is formed by two hemichannels, each of which comprises six protein subunits called connexins. Connexin43 (Cx43), the major constituent of astrocytic gap junctions, is abundantly expressed in astrocytes throughout the brain (Dermietzel et al., 1989; Giaume and McCarthy, 1996). Cx43 knockout in mice causes early postnatal lethality due to heart malfunction (Reaume et al., 1995), and therefore conditional knockout (CKO) of Cx43 in mice with Cre/loxP system has been used to study the function of Cx43 in the brain.

The first Cx43 CKO mice, exhibiting the loss of Cx43 essentially in all astrocytes in the brain, were generated using the Cx43 floxed allele (Cx43fl) in which the Cx43 coding sequence is flanked by two loxP sites (Theis et al., 2001), in combination with a Cre transgenic line under the control of the human glial fibrillary acidic protein promoter (hGFAP-Cre) (Theis et al., 2003). Cx43fl/+/hGFAP-Cre mice are viable and show no histologic abnormalities in the brain (Theis et al., 2003), but exhibit several features in situ and in vivo that are related to brain physiology and/or function, as follows: in situ accelerated hippocampal spreading depression (Theis et al., 2003) and impaired Ca2+ wave propagation in neocortex (Haas et al., 2006); in vivo enhanced locomotor activity (Theis et al., 2003); increased exploratory behavior, impaired motor capacity, and changes in brain acetylcholine level (Frisch et al., 2003); and increased apoptosis and inflammation after...
Astrocytic gap junction
Most of astrocytes are coupled by gap junction and form functional syncytium in the brain.

Conditional knockout (CKO) mouse
Tissue- or cell type-specific knock out of genes can be generated in mice by using Cre/loxp system, which requires genomic region flanked by two loxp sites (floxed allele) and tissue- or cell type-specific expression of Cre recombinase. Cre-mediated excision deletes the genomic region flanked by two loxp sites.

Bergmann glial cells (BGCs)
Specialized form of astrocytes in the cerebellum, with their somata located in the Purkinje cell layer and processes extending through the molecular layer of the cerebellum.

cerebral ischemia (Nakase et al., 2004). In addition, $\text{Cx43}^{\text{fl/fl}}:\text{hGFAP-Cre}$ mice in combination with a null mutation of Cx30, another astrocytic connexin, have impaired spatial K$^+$ buffering and a reduced threshold for the generation of epileptiform events in the hippocampus in situ (Wallraff et al., 2006). Figiel et al. (2007) showed that deletion of Cx43 in cortical astrocytes causes a loss of glutamate transporter GLT-1. Recently, Lin et al. (2008) reported that $\text{Cx43}^{\text{fl/fl}}:\text{hGFAP-Cre}$ mice are insensitive to hypoxic preconditioning, and in this case Cx43 functions as hemichannels that serve as a pathway for the efflux of ATP. Although these findings successfully demonstrated the involvement of astrocytic Cx43 in brain physiology and/or function, questions remain regarding the role of Cx43 in the cerebellum.

Bergmann glial cells (BGCs) are unipolar astrocytes that extend long processes across the molecular layer of the cerebellum (Figures 1A,B). BGC processes form intimate structural relationships with the dendrites of Purkinje cells (Figures 1B,C) (Grosche et al., 2002; Yamada and Watanabe, 2002), which are inhibitory neurons that act as the sole source of output from the cerebellar cortex. The processes of mature BGCs surround the synapses on Purkinje cells that are formed with glutamatergic excitatory axon parallel fibers and climbing fibers.

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fibers (Palay and Chan-Palay, 1974; Spacek, 1985). Extensive gap junctional coupling is observed in mature BGC processes (Clark and Barbour, 1997; Müller et al., 1996) where Cx43 is abundantly expressed (Figure 1D) (Nagy et al., 2001), but the role of Cx43 is largely unknown.

To investigate the role of Cx43 in cerebellar function, we constructed a new Cx43 CKO model with temporal and regional specificity of a Cre-mediated recombination directed to the cerebellum (Tanaka et al., 2008), as deficits in other brain areas, e.g., striatum, can also lead to impaired motor coordination (Blundell et al., 2008). Furthermore, Cx43<sup>fl/fl</sup>:hGFAP-Cre mice generated on a different genetic background exhibit cellular disorganization of the cortex, hippocampus, and cerebellum, accompanied by ataxia and motor deficits (Wiencken-Barger et al., 2007), suggesting the importance of controlling the onset and regional specificity of Cx43 CKO to study neuron-glia interactions in the adult cerebellum. Using a new Cx43 CKO model, we recently investigated the contribution of Cx43 to gap junctional coupling between BGCs, and examined whether Cx43 in BGCs, either as a gap junction channel or a hemichannel, plays an important role in cerebellar functions via Purkinje cell-BGC interactions.

**GENERATION OF A NEW Cx43 CKO MICE WITH EFFICIENT POSTNATAL RECOMBINATION IN BGCs AND CEREBELLAR ASTROCYTES MEDIATED BY S100b-Cre TRANSGENE**

First we generated a new Cre transgenic line that can be used to make Cx43 CKO mice suitable for studying cerebellar functions (Tanaka et al., 2008). S100B is an EF-hand-type protein expressed primarily in astrocytes in the mammalian central nervous system (Boyes et al., 1986; Haan et al., 1982; Van Eldik et al., 1984). In rodents, the pattern of S100b expression shows a rostral-caudal gradient during postnatal development with robust expression in the BGCs in the cerebellum (Landry et al., 1989). The generation of a transgenic mouse line using a 5.4-kb genomic sequence of S100b to drive Cre recombinase (S100b-Cre) led to efficient deletion of Cx43<sup>β</sup> in the cerebellum (Figure 2A), which can be monitored by β-galactosidase expression with a nuclear localization signal under control of the Cx43 promoter upon Cre-mediated recombination of the floxed Cx43 coding region (Theis et al., 2003). Since β-galactosidase is expressed instead of Cx30, reporter gene expression marks those cells which have lost Cx43 expression driven by the Cx43<sup>β</sup> allele. The recombination pattern of Cx43<sup>β</sup>:S100b-Cre mice with a rostral-caudal gradient as indicated by lacZ staining (Figure 2A, upper left), was very similar to that of the endogenous S100b pattern during postnatal development (Landry et al., 1989). Immunohistochemical analysis of the Cx43<sup>β</sup>:S100b-Cre mice revealed that nuclear β-galactosidase immunoreactivity was located in BGCs in the Purkinje cell layer, and in cerebellar astrocytes in the granule cell layer and white matter, all of which were S100B-positive and NeuN-negative (Figure 2B).

S100b-Cre-mediated recombination of Cx43<sup>β</sup> in the cerebellum began and progressed during postnatal development (Figure 2C). Excision in most of the BGCs in the Cx43<sup>β</sup>:S100b-Cre mice was completed by P28 (Figure 2C). The results obtained using different reporter strains consistently indicated that S100b-Cre-mediated recombination in the cerebellum was restricted to the BGCs and cerebellar astrocytes and did not occur in Purkinje cells or granule cells (Tanaka et al., 2008). It is therefore likely that the efficient and cell type-specific recombination in BGCs and cerebellar astrocytes in the adult cerebellum is due to the postnatal onset of S100b-Cre-mediated recombination in the precursors of those cells. The temporal and spatial Cre-mediated recombination pattern induced by S100b-Cre is markedly different from that of GFAP-Cre lines using either the human or mouse Gfap promoter, in which Cre-mediated recombination occurs in neural progenitors of prenatal embryos, resulting in widespread recombination in neurons and glial cells (Bajenaru et al., 2002; Casper and McCarthy, 2006; Garcia et al., 2004; Kwon et al., 2001; Zhuo et al., 2001). Thus, our transgenic S100b-Cre line appears to be an ideal tool for studying glial function in the cerebellar molecular layer and/or granule cell layer in postnatal late developmental stages and adulthood in mice.

**A CRUCIAL ROLE FOR Cx43 IN GAP JUNCTIONAL COUPLING BETWEEN BGCs**

The S100b-Cre transgenic line was then used to generate Cx43 CKO (Cx43<sup>β</sup>:S100b-Cre) mice, to examine the role of Cx43 in the postnatal cerebellum, particularly in the Purkinje cell-BGC interactions in the cerebellar molecular layer. In Cx43<sup>β</sup>:S100b-Cre mice, a loss of Cx43 was evident in most of the cerebellar astrocytes and in virtually all of the BGCs in the cerebellar molecular layer in the adult (Figure 3A). No compensatory changes were observed in the expression of Cx30, another connexin expressed in astrocytes and BGCs (Nagy et al., 1999, 2001), in the cerebellum of Cx43<sup>β</sup>:S100b-Cre mice (Tanaka et al., 2008). The cerebellum with Cx43<sup>β</sup>:S100b-Cre mice exhibited dendrite disorganization of the Purkinje cells in the molecular layer, particularly in the Purkinje cell-BGC interactions.
The failure of glutamate uptake at Purkinje cell synapses by the glutamate transporters GLAST and GLT-1 expressed in BGCs results in motor discoordination in mice (Rothstein et al., 1996; Watase et al., 1998), which most likely is caused by the multiple innervation of Purkinje cell by climbing fibers (Watase et al., 1998). Defects in Gfap KO mice (Shibuki et al., 1996), such as impaired cerebellar long-term depression (LTD) at the parallel fiber (PF)-Purkinje cell (PC) synapses and impaired motor learning “eyeblink conditioning”, may be caused by enhanced glutamate uptake through the upregulation of GLT-1 in the cerebellum (Hughes et al., 2004). Importantly, the deletion of Cx43 in cortical astrocytes is associated with a loss in GLT-1 expression (Figiel et al., 2007). In addition, gap junctions are permeable to

**Dispensable role of Cx43 in BGCs in cerebellar function**

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**Eyeblink conditioning**

A form of classical conditioning in which animals are trained to blink in response to a tone.

**Bell architecture of the Cx43<sup>fl/fl</sup>-S100b-Cre mice**

was normal, including normal morphology of both the BGCs and Purkinje cells (Tanaka et al., 2008), suggesting that Cx43 does not have a key role in the structural support of BGCs or cerebellar astrocytes in the late cerebellar developmental stages or adulthood.

We then investigated whether the loss of Cx43 in the BGCs affected gap junctional coupling *in situ*. Dye coupling experiments using Lucifer yellow demonstrated that Cx43 contributes significantly to gap junctional coupling between BGCs (Figures 3B,C). The magnitude of Lucifer yellow dye coupling was closely related with the amount of Cx43 protein, which was reduced in Cx43<sup>fl/fl</sup> mice to approximately 30% that of wild-type (WT) mice, and in Cx43<sup>fl/fl</sup>-S100b-Cre mice to approximately 10% that of WT mice (Tanaka et al., 2008).
Tanaka et al. (2008). Error bars represent SEM. *, in BGC in parasagittal cerebellar slices from 2.5-month-old control (Cx43fl/+), Cx43fl/+:S100b-Cre mice (n = 9) cells (Figure 4A). To induce LTD of PF-EPSCs, we administered PF-stimulation with simultaneous somatic depolarization of Purkinje cells (140 ms, −70 to +10 mV) at 1 Hz for 5 min (Koekkoek et al., 2005). The conjunctive stimuli induced a significant decrease in the amplitude of PF-EPSCs (10–90% rising time, 1.6 ± 0.2 and 1.3 ± 0.2 ms; p > 0.2; decay time constant, 15.5 ± 2.0 and 15.6 ± 1.3 ms; p > 0.9) between control (n = 8) and Cx43fl/+:S100b-Cre (n = 9) cells (Figure 4B). To study the motor behavior of Cx43fl/+ β: S100b-Cre mice, we first performed an open field test with 2.5-month-old control Cx43fl/+ (n = 13) and Cx43fl/+:S100b-Cre (n = 10) mice. The genotypes did not significantly differ in either horizontally directed locomotor activity or in time spent in the center of the open field (data not shown). We then performed a rotarod test to determine whether these mice had normal motor coordination. We trained 2.5-month-old control Cx43fl/+ (n = 14) and Cx43fl/+:S100b-Cre (n = 11) mice over 5 days to balance on an accelerating rotating rod (Figure 4C). Although the latency of Cx43fl/+ β: S100b-Cre mice to fall decreased slightly on the third day of training compared to that of control mice, their performance improved on the fourth and fifth days. There was no statistically significant difference in overall performance between the genotypes. There was no significant difference in the body weight of the mice used for the rotarod test (data not shown).
The impact of the loss of Cx43 in BGCs and the reduction of Cx43 in astrocytes in the deep cerebellar nuclei (data not shown) on delay eyeblink conditioning were then evaluated in 3- to 3.5-month-old control Cx43<sup>fl/+</sup> (n = 10) and Cx43<sup>fl/fl</sup>:S100b-Cre (n = 7) mice. Eyeblink conditioning is a task in which an animal learns to associate a conditioned stimulus (CS) with a noxious unconditioned stimulus (US) that elicits an eyeblink (Christian and Thompson, 2003). The memory trace for delay eyeblink conditioning, in which the preceding CS and the US terminate simultaneously, is considered to be formed in the cerebellar cortex and deep cerebellar nuclei (Attwell et al., 2002; Christian and Thompson, 2003; Mauk and Buonomano, 2004), and cerebellar LTD appears to be a neural correlate of delay eyeblink conditioning (Christian and Thompson, 2003). During eyeblink conditioning of control and Cx43<sup>fl/fl</sup>:S100b-Cre mice, a CS (tone 1 kHz, 352 ms, 83–85 dB) was paired with the US, a periodical shock (100 ms, 100 Hz pulses). Both genotypes exhibited an increased frequency of conditioned responses during the 7-day training period (Figure 4D). There were no statistically significant differences in acquisition, extinction, or relearning kinetics of the conditioned responses, however, between control and Cx43<sup>fl/fl</sup>:S100b-Cre mice (Tanaka et al., 2008).

**DISCUSSION AND FUTURE PERSPECTIVES**

A new model of Cx43 CKO (Cx43<sup>fl/fl</sup>:S100b-Cre) mice in which Cx43 ablation occurs preferentially in the postnatal cerebellum has been developed. In contrast to previous reports of Cx43<sup>fl/fl</sup>:hGFAP-Cre mice (Frisch et al., 2003; Theis et al., 2003), behav-
ioral analyses of the Cx43<sup>fl/fl</sup>:S100b-Cre mice in the present study did not reveal enhanced locomotor activity nor increased exploratory behavior in the open field test (data not shown), further suggesting limited S100b-Cre-mediated recombination of the Cx43<sup>fl</sup> allele in the forebrain of Cx43<sup>fl/fl</sup>:S100b-Cre mice (Figure 2A) (Tanaka et al., 2008). Cerebellum-dependent behaviors, such as motor coordination and eyelid blinking conditioning, were not significantly impaired in Cx43<sup>fl/fl</sup>:S100b-Cre mice (Figure 4C,D), in contrast to the previously reported impairment in rotator performance of Cx43<sup>fl/fl</sup>:hGFAP-Cre mice (Frisch et al., 2003). The restricted nature of the S100b-Cre-mediated deletion might explain the lack of rotator impairment in the Cx43<sup>fl/fl</sup>:S100b-Cre mice, because the functions of other brain regions can also affect motor coordination (Blundell et al., 2008).

Our results suggest that Cx43 expressed in BGCs, either as a gap junction channel or hemichannel, is not required for glutamate uptake, K<sup>+</sup> buffering, or other regulatory mechanisms that may be involved in the Purkinje cell synaptic plasticity related to cerebellum-dependent behaviors in adult mice. These results further support the idea that Cx43 in BGCs is a more broadly expressed component of cerebellar astrocytes, and the BGCs do not have an essential role in the eyelid conditioning motor learning circuit. It remains unclear, however, whether other connexins expressed in BGCs, such as Cx30 and Cx29 (Altevogt and Paul, 2004; Eiberger et al., 2006; Nagy et al., 2001), functionally compensate for the absence of Cx43. Cx30 forms functional gap junction channels that are not permeable to Lucifer yellow (Manthey et al., 2001) and is expressed in the BGCs of Cx43<sup>−/−</sup>:S100b-Cre mice (Tanaka et al., 2008). The role of these other connexins must be clarified by studying the consequences of their combined ablation in BGCs using [Cx30<sup>−/−</sup>, Cx43<sup>−/−</sup>:S100b-Cre] mice or [Cx29<sup>−/−</sup>, Cx30<sup>−/−</sup>, Cx43<sup>−/−</sup>:S100b-Cre] mice.

Although mutual interactions between Purkinje cells and BGCs have been described in several reviews (Bellamy, 2006; Lopez-Bayghen et al., 2007; Metea and Newman, 2006), our results suggest that these Purkinje cell-BGC interactions are less dependent on gap junctional coupling between BGCs. The morphology of the BGCs, which forms a perpendicular array, may facilitate the spatial buffering of molecules as in the case of astrocytes in the stratum radiatum in the hippocampus (Wallraff et al., 2006). In addition, the functional independence of Cx43 in BGCs compared to astrocytes in other brain regions where the loss of Cx43 results in physiologic and/or functional impairments (Frisch et al., 2003; Haas et al., 2006; Theis et al., 2003) may reflect a functional heterogeneity of these astrocytic subsets located in different brain regions. For example, Ca<sup>2+</sup> increases in BGCs restricted to microdomains (Kettenmann and Schipke, 2004) are different from the intrinsic calcium oscillations observed in hippocampal astrocytes (Fiacco and McCarthy, 2006).

Several lines of evidence indicate brain region-specific differences in response to the knockout of astrocyte-specific genes: (1) In Gfap KO mice (Shibuki et al., 1996), glutamate uptake is reduced in association with a failure in glial transporter GLT-1 trafficking in the cortex and hippocampus, whereas glutamate uptake is enhanced in the cerebellum (Hughes et al., 2004). (2) In S100b KO mice, microarray analysis revealed alterations of different groups of genes between the hippocampus and cerebellum (Ohshima, Kim, Konishi, and Itohara, unpublished data), which might be responsible for functional impairments detected in the hippocampus (Nishiyama et al., 2002; Sakatani et al., 2007, 2008) and for the preserved cerebellar functions (Ohshima, Kim, Konishi and Itohara, unpublished data). Based on these observations, the impact of Cx43 knockout may be different in the cerebellum compared to other brain areas. It should be also noted that large-scale global transcriptomic alterations that compensate for the functional consequences of Cx43 deletion in the cerebellum may occur in the Cx43 CKO, as reported for Cx43<sup>−/−</sup> astrocytes and newborn mouse brains (Iacobas et al., 2007, 2008). Our results, together with the results of others, suggest that transcriptomic alterations in Cx43 CKO may differ between the cerebellum and other brain areas. Further studies of Cx43 CKO mice using different Cre lines with region-specificity will help to elucidate the heterogeneity of astrocytic subsets in the context of their functional connections to each brain area.

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REFERENCES

Alvogt, B. M., and Paul, D. L. (2004). Four classes of intercellular channels between glial cells in the CNS. J. Neurosci. 24, 4313–4323.

Attwell, P. J., Ivarsson, M., Millar, L., and Yeo, C. H. (2002). Cerebellar mechanisms in eyelink conditioning. Ann. N.Y. Acad. Sci. 978, 79–92.

Bajenaru, M. L., Zhu, Y., Hedrick, N. M., Donahoe, J., Parada, L. F., and Gutmann, D. H. (2002). Astrocyte-specific inactivation of the neurofibromatosis I gene (NFI) is insufficient for astrocytoma formation. Mol. Cell Biol. 22, 5100–5113.

Bellamy, T. C. (2006). Interactions between Purkinje neurones and Bergmann glia. Cerebellum 5, 116–126.

Blundell, J., Hoang, C. V., Potts, B., Gold, S. J., and Powell, C. M. (2008). Motor coordination deficits in mice lacking RGS9. Brain Res. 1190, 78–85.

Boyes, B. E., Kim, S. U., Lee, V., and Sung, S. C. (1986). Immunohistochemical co-localization of S-100b and the glial fibrillary acidic protein in rat brain. Neuroscience 17, 857–865.

Casper, K. B., and McCarthy, K. D. (2006). GFAP-positive progenitor cells produce neurons and oligodendrocytes throughout the CNS. Mol. Cell Neurosci. 31, 676–684.

Christian, K. M., and Thompson, R. F. (1982). Production and characterization of monoclonal antibodies against the “brain-specific” proteins 14–3-2 and S-100. Proc. Natl. Acad. Sci. U.S.A. 79, 756–759.

Gold, S. J., and Powell, C. M. (2008). Motor coordination deficits in mice lacking RGS9. Brain Res. 1190, 78–85.

Grosche, J., Kettenmann, H., and Reichenspacher, A. (2002). Bergmann glial cells form distinct morphological structures to interact with cerebellar neurons. J. Neurosci. Res. 68, 138–149.

Haan, E. A., Boss, B. D., and Cowan, W. M. (1989a). The neural basis of temporal summation. Proc. Natl. Acad. Sci. U.S.A. 99, 4037–4042.

Iacobas, D. A., Iacobas, S., and Spray, D. C. (2003). The neural basis of temporal summation. Proc. Natl. Acad. Sci. U.S.A. 99, 4037–4042.

Jakos, M., Allritz, C., Lehmann, C., and Engele, I. (2007). Gap junctional control of glial glutamate transporter expression. Mol. Cell Neurosci. 35, 130–137.

Kang, J., and Nedergaard, M. (2008). Neural-activity-dependent ATP waves in the mouse neocortex are independent from astrocytic calcium waves. Cereb. Cortex 18, 237–246.

Langer, C. M., Wayne, C., and Yamasaki, Y. (2003). Electrical coupling among Bergmann glial cells and its modulation by glutamate receptor activation. Glia 17, 274–284.

Nagy, J. L., Patel, D., Ochalski, P. A., and Stelmack, G. L. (1999). Connexin30 in rodent, cat and human brain: selective expression in gray matter astrocytes, co-localization with connexin43 at gap junctions and late developmental appearance. Neuroscience 88, 447–468.

Nagy, J. L., Xi, L., Rempel, J., Stelmack, G., Patel, D., Staines, W. A., Yasumura, T., and Rash, J. E. (2001). Connexin26 in adult rodent central nervous system: demonstration at astrocytic gap junctions and colocalization with connexin30 and connexin43. J. Comp. Neurol. 441, 302–322.

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

Palay, S. L., and Chan-Palay, V. (1974) Cerebellar Cortex – Cytology and Organization. Berlin, Springer.

Ransom, B. R., and Ec, Z. (2005) Gap junctions and hemichannels. In Neuroglia, H. Kettenmann and B. R. Ransom, eds (New York, Oxford University Press), pp. 177–189.

Reaume, A. G., de Sousa, P. A., Kalksni, S., Langille, B. L., Zhu, D., Davies, T. C., Juneja, S. C., Kidder, G. M., and Rossant, J. (1995). Cardiac malformation in neonatal mice lacking connexin43. Science 267, 1831–1834.

Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kindl, R. W., Kanai, Y., Hediger, M. A., Wang, Y., Schiele, J. P., and Welty, D. F. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–686.

Sakatani, S., Seto-Ohshima, A., Itohara, S., and Hirase, H. (2007). Impact of S100B on local field potential patterns in anesthetized and kainic acid-induced seizure conditions in vivo. Eur. J. Neurosci. 25, 1144–1154.

Tanaka, S., Seto-Ohshima, A., Shinohara, Y., Yamamoto, Y., Yamamoto, H., Itohara, S., and Hirase, H. (2008). Neural-activity-
dependent release of S100B from astrocytes enhances kainate-induced gamma oscillations in vivo. J. Neurosci. 28, 10928–10936.

Shibuki, K., Gomi, H., Chen, L., Bao, S., Kim, J. J., Wakatsuki, H., Fujisaki, T., Fujimoto, K., Katoh, A., Ikeda, T., Chen, C., Thompson, R. F., and Itohara, S. (1996). Deficient cerebellar long-term depression, impaired eyblink conditioning, and normal motor coordination in GFAP mutant mice. Neuron 16, 587–599.

Spacek, J. (1985). Three-dimensional analysis of dendritic spines. III. Gial sheath. Anat. Embryol. (Berl.) 171, 245–252.

Tanaka, M., Yamaguchi, K., Tatsukawa, T., Chieko, N., Nishiyama, H., Theis, M., Willecke, K., and Itohara, S. (2008). Lack of connexin43-mediated Bergmann glial gap junctional coupling does not affect cerebellar long-term depression, motor coordination, or eyblink conditioning. Front. Behav. Neurosci. 2, 1.

Theis, M., de Wit, C., Schlaeger, T. M., Eckardt, D., Krüger, O., Döring, B., Risau, W., Deutsch, U., Pohl, U., and Willecke, K. (2001). Endothelium-specific replacement of the connexin43 coding region by a lacZ reporter gene. Genesis 29, 1–13.

Theis, M., Jauch, R., Zhuo, L., Speidel, D., Wallraff, A., Döring, B., Frisch, C., Sohl, G., Teubner, B., Euwens, C., Huston, J., Steinhaüser, C., Messing, A., Heinemann, U., and Willecke, K. (2003). Accelerated hippocampal spreading depression and enhanced locomotor activity in mice with astrocyte-directed inactivation of connexin43. J. Neurosci. 23, 766–776.

Van Eldik, L. J., Ehrenfried, B., and Jensen, R. A. (1984). Production and characterization of monoclonal antibodies with specificity for the S100 beta polypeptide of brain S100 fractions. Proc. Natl. Acad. Sci. U.S.A. 81, 6034–6038.

Wallraff, A., Köhling, R., Heinemann, U., Theis, M., Willecke, K., and Steinhaüser, C. (2006). The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. J. Neurosci. 26, 5438–5447.

Watase, K., Hashimoto, K., Kano, M., Yamada, K., Watanabe, M., Inoue, Y., Okuyama, S., Sakagawa, T., Ogawa, S., Kawashima, N., Horii, S., Jakimoto, M., Wada, K., and Tanaka, K. (1998). Motor discoordination and increased susceptibility to cerebellar injury in GLAST mutant mice. Eur. J. Neurosci. 10, 976–988.

Weber, P. A., Chang, H. C., Spaeth, K. E., and Nicholson, B. J. (2004). The permeability of gap junction channels to probes of different size is dependent on connexin composition and permeant-pore affinities. Biophys. J. 87, 958–973.

Wiencken-Barger, A. E., Djukic, B., Casper, K. B., and McCarthy, K. D. (2007). A role for Connexin43 during neurodevelopment. Glia 55, 675–686.

Yamada, K., and Watanabe, M. (2002). Cytodifferentiation of Bergmann glia and its relationship with Purkinje cells. Anat. Sci. Int. 77, 94–108.