

Journal Club

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Passing Potassium with and without Gap Junctions

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Review of Wallraff et al. (<http://www.jneurosci.org/cgi/content/full/26/20/5438>)

Glia, originally thought to play a passive role in the CNS, are now recognized as active regulators of CNS activity. For example, astrocytes are essential for the maintenance of extracellular ion concentrations, notably K^+ , at physiological levels (Orkand et al., 1966). Any deviation of extracellular K^+ concentration ($[K^+]_o$) from ~ 3 mM can affect neural activity. Elevated $[K^+]_o$ occurs during seizure activity, ischemia, and spreading depression, in which $[K^+]_o$ can reach 10–50 mM (Walz, 2000; Somjen, 2002), with consequent effects on neuronal excitability and ultimately on cell viability. To limit increased $[K^+]_o$, astrocytes are equipped with a variety of K^+ uptake mechanisms, including the $Na^+-K^+-ATPase$, $Na^+-K^+-2Cl^-$ cotransporters and voltage-activated K^+ channels. Furthermore, local elevations in $[K^+]_o$ shift the K^+ equilibrium potential (E_K) to more positive values relative to the membrane potential (V_m), thus driving K^+ into these cells along an electrochemical gradient. K^+ can also be spatially buffered via diffusion through the astrocytic cytoplasm to areas of lower $[K^+]_o$, and then K^+ is driven back out of the cell at distal sites at which E_K is still more negative than the V_m . Spatial redistribution of K^+ is be-

lieved to be enhanced by gap junction coupling between astrocytes, although the experimental evidence for this mechanism is minimal (Walz, 2000; Kofuji and Newman, 2004). In their recent paper in *The Journal of Neuroscience*, Wallraff et al. (2006) examined the role of gap junction coupling between astrocytes in K^+ buffering and its subsequent physiological effect.

Using hippocampal slices, the authors examined coupling in transgenic mice from which connexin43 (Cx43), the most abundant astrocytic Cx, had been conditionally deleted. Cx43/Cx30 double knock-out (dko) mice were also used because Cx30 is the other major connexin in astrocytes. Dye coupling of the gap junction-permeable tracer biocytin was reduced in the Cx43 knock-out and abolished in the dko [Wallraff et al. (2006), their Fig. 1*B,D* (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F1>)]. Importantly, the morphology and density of dko astrocytes labeled with GFAP were similar to wild-type (wt) [Wallraff et al. (2006), their Fig. 2*B,D* (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F2>)]. The dko astrocytes had a slightly more negative resting membrane potential and a greater membrane resistance than wt, likely attributable to the lack of gap junction coupling. To detect the current passing through gap junctions (I_{gj}), whole-cell currents (I_c) were recorded in wt and dko cells before and after coupling was ablated by a low HCO_3^- /low pH solution. Wt I_c was reduced by 50% when coupling was blocked, and dko I_c was

decreased by 20% [Wallraff et al. (2006), their Fig. 3*D* (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F3>)], a difference that the authors attributed to I_{gj} . Although low HCO_3^- /low pH abolished coupling, the 20% decrease in I_c in the dko preparation represents a modification in conductance that was independent of I_{gj} . It would be interesting to see whether gap junctional blockers such as carbenoxolone produce I_c profiles similar to the low HCO_3^- /low pH solution.

To test the involvement of gap junctions in limiting extracellular K^+ accumulation, the authors used antidromic stimulation of CA1 pyramidal neurons to increase $[K^+]_o$. The slices were treated with GABA and glutamate receptor blockers to inhibit synaptic activity, and $[K^+]_o$ was measured with a K^+ -sensitive electrode in the middle of the CA1 subfield. Compared with wt, a larger $[K^+]_o$ response was generated in dko slices after maximal stimulation (elicited by either paired-pulse or high-frequency trains) but not during low-to-moderate stimulation [Wallraff et al. (2006), their Fig. 4*D,E* (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F4>)]. During maximal stimulation, $[K^+]_o$ climbed as high as 17 mM in dko slices, whereas wt slices hit a “ceiling” at 12 mM. The rate of decay of $[K^+]_o$ after stimulation was also slower in the dko mice [Wallraff et al. (2006), their Fig. 5*B,D* (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F5>)]. Together, these data suggest a deficiency in K^+ clearance in the absence of gap junctions, but only when K^+ levels are high.

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To further examine alterations in K^+ clearance between wt and dko slices, rises in $[K^+]_o$ were generated in the stratum pyramidale in the same manner as before, and $[K^+]_o$ was recorded at various distances from the site of stimulation. Up to 300 μm away from the stratum pyramidale, corresponding to the stratum radiatum, normalized $[K^+]_o$ remained unchanged between wt and dko slices. Astrocytes in this region have long overlapping processes, and the authors reasoned that K^+ can be buffered to neighboring astrocytes independently of gap junctions via “indirect coupling” whereby K^+ released from one astrocyte is taken up by another (Fig. 1). However, at a distance of 400–500 μm away, corresponding to the stratum lacunosum moleculare, $[K^+]_o$ levels were significantly reduced in the dko [Wallraff et al., their Fig. 6B (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F6>)]. Astrocytes in this region have short non-overlapping processes and appear to depend on gap junctions to facilitate K^+ buffering (Fig. 1). Because the expression of other proteins (e.g., ion channels) may be altered by knocking out Cxs, testing whether gap junction blockers cause a similar impairment in K^+ spatial buffering in the wt would be an important control. Furthermore, the authors reported a steep decline in population spike potential as they moved away from the site of stimulation, but direct depolarization of the astrocytic syncytium cannot be ruled out as a potential confounding factor.

Last, extracellular field potentials were measured to investigate the potential pathological effect of reduced K^+ clearance on neuronal activity. The dko slices developed spontaneous epileptiform events [Wallraff et al., their Fig. 7A,C (<http://www.jneurosci.org/cgi/content/full/26/20/5438/F7>)] and experienced seizure-like discharges in the CA1 stratum

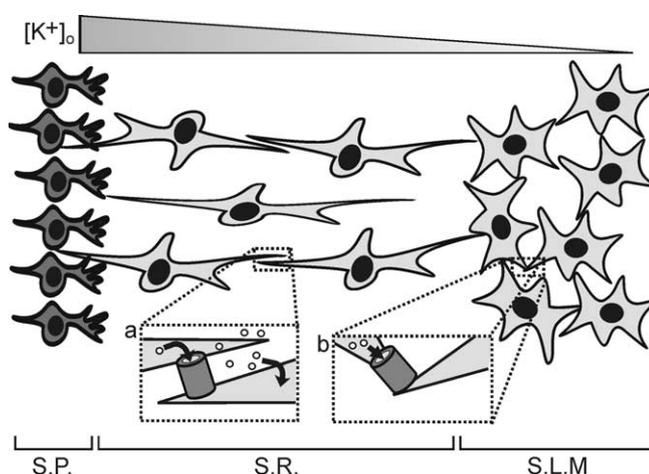


Figure 1. Overview of K^+ buffering of CA1 neurons in the hippocampus. Neuronal activity in the stratum pyramidale (S.P.) causes a rise in $[K^+]_o$, that is subsequently taken up by astrocytes in the stratum radiatum (S.R.). K^+ is distributed along the long processes of these cells to neighboring S.R. astrocytes by gap junctions and by indirect coupling (*a*) whereby K^+ released from one cell into the extracellular space is taken up by another neighboring astrocyte. In this case, dko astrocytes can still buffer K^+ because indirect coupling remains intact. K^+ is subsequently distributed via gap junctions (*b*) in stratum lacunosum moleculare (S.L.M.) astrocytes. In this case, K^+ buffering is impaired in dko because astrocytes lack preferential organization for indirect coupling.

pyramidale at a higher frequency than the wt slices when exposed to Mg^{2+} -free perfusion media. These findings are in contrast to previous studies that identified gap junction blockers as effective anticonvulsants. However, the pharmacological agents used in other studies were not able to selectively block astrocytic gap junctions, which could indicate that the anticonvulsant effect may be the result of blocked neuronal gap junctions or action on another unidentified target.

Wallraff et al. (2006) have demonstrated that K^+ redistribution occurs by both gap junction-dependent and -independent processes, thereby supplying evidence for a pathway that has been postulated for decades. Efficient handling of K^+ has relevance to the treatment of pathological conditions, and indeed the authors found an increased susceptibility to epileptiform events in the Cx43/Cx30 dko mice. These findings may extend to

other conditions, such as stroke, in which elevated $[K^+]_o$ occurs. More efficient K^+ buffering by astrocytes could curb excessive K^+ accumulation and in turn reduce the potential for neuronal damage.

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