Extended Methods

Analytical solution derivation

We wanted to find analytical solutions at steady state for the following variables in the pump-leak formulation for a single compartment including KCC2: intracellular concentrations of sodium, potassium, chloride and impermeant anions ($[Na^+]_i$; $[K^+]_i$; $[Cl^-]_i$; and $[X^z]_i$, with charge z); and membrane voltage (V). The steady state should occur in the presence of both a pump leak mechanism (sodium-potassium ATPase with pump rate modified by the sodium gradient, $J_p = p \cdot \left(\frac{[Na^+]_i}{[Na^+]_o} \right)^3$) and chloride-potassium extrusion (type 2 potassium-chloride co-transporter, KCC2, with conductance g_{KCC2} and driving force proportional to the difference in the Nernst reversal potentials of potassium and chloride — see Doyon et al. (2016)). The usual passive forces acting across the membrane on each ion are also included. To allow for an analytical solution inclusive of differences in osmolarity between the intracellular and extracellular environments (**Fig. 6A-E**), we derive the analytical solution with $\Pi_i = \Pi_o + N_{H_p}$, where Π_i is the intracellular osmolarity and Π_o the extracellular. Thus N_{H_p} is often but not always equal to 0.

The situation described above ought to satisfy the following five equations at steady state, in which the conductance of an ion is denoted g_{ion}, an ion's Nernst reversal potential as E_{ion}, F the Faraday constant and A_m the ratio of surface area to volume. This system is similar to that given in Keener and Sneyd (2009).

$$-\frac{d}{dt}\left(\frac{F}{A_m}[Na^+]_i \right) = g_{Na}(V - E_{Na}) + 3J_p \tag{S1}$$

$$-\frac{d}{dt}\left(\frac{F}{A_m}[K^+]_i \right) = g_K(V - E_K) - 2J_p - g_{KCC2}(E_K - E_{Cl}) \tag{S2}$$

$$\frac{d}{dt}\left(\frac{F}{A_m}[Cl^-]_i \right) = g_{Cl}(V - E_{Cl}) + g_{KCC2}(E_K - E_{Cl}) \tag{S3}$$

$$0 = [K^+]_i + [Na^+]_i - [Cl^-]_i + z[X^z]_i \tag{S4}$$

$$\Pi_o + N_{H_p} = \Pi_i = [K^+]_i + [Na^+]_i + [Cl^-]_i + [X^z]_i \tag{S5}$$

We first solve the system for constant J_p at steady state, i.e. equations S1, S2 and S3 set to 0, and then show that a parametric solution exists for p such that the function mapping J_p to p is bijective. Thus we begin by solving each of (S1), (S2) and (S3) for the reversal potential of the intracellular ion that they refer to, and then for the intracellular ions' concentration itself. By simple rearrangement,

$$[Na^+]_i = [Na^+]_o \cdot e^{-\frac{FV}{RT}} \cdot e^{-\frac{3J_pF}{RTg_{Na}}} \tag{S6}$$

and

$$E_{Cl} = \frac{g_{Cl}V + g_{KCC2}E_K}{g_{Cl} + g_{KCC2}} \tag{S7}$$

Let β be equal to $g_Kg_{Cl} - g_{KCC2}g_{Cl} + g_Kg_{KCC2}$. If we substitute (S7) into (S2) for E_{Cl}, we can solve for E_K and $[K^+]_i$, hence enabling us to substitute back into (S7) in order to solve for $[Cl^-]_i$.

Thus,

$$E_K = V - 2J_p \frac{g_{Cl} + g_{KCC2}}{\beta}$$

and hence

$$[K^+]_i = [K^+]_o \cdot e^{-\frac{FV}{RT}} \cdot e^{\frac{F}{RT} \cdot 2J_p \frac{g_{Cl} + g_{KCC2}}{\beta}} \tag{S8}$$

so then

$$[Cl^-]_i = [Cl^+]_e \cdot e^{\frac{FV}{RT}} \cdot e^{-\frac{F}{RT} \cdot \frac{2J_p \cdot g_{KCC2}}{\beta}} \tag{S9}$$

We have now found equations for all permeant intracellular ions in terms of constants and V. An extension of these results means that we can find an equation for X in terms of V by rearranging the osmotic equilibrium equation (S5).

$$[X^z]_i = (\Pi_o + N_{H_p}) - [Na^+]_i - [K^+]_i - [Cl^-]_i \tag{S10}$$

In order to solve for V, we substitute (S10) into (S4), the equation that ensures intracellular charge neutrality. Thus we obtain:

$$0 = z \cdot (\Pi_o + N_{H_p}) + (1 - z) \cdot ([K^+]_i + [Na^+]_i) - (1 + z) \cdot [Cl^-]_i \tag{S11}$$

Before substituting in for the permeable intracellular ions, let us denote $\theta = e^{-\frac{FV}{RT}}$. Then with substitution of (S9), (S8) and (S6) and multiplication through by θ equation S11 becomes:

$$0 = (1-z) \cdot \left([K^+]_o \cdot e^{\frac{2J_p F \cdot (g_{Cl} + g_{KCC2})}{RT \cdot \beta}} + [Na^+]_o \cdot e^{-\frac{3J_p F}{RT \cdot g_{Na}}} \right) \cdot \theta^2 + z \cdot (\Pi_o + N_{H_p}) \cdot \theta - (1+z) \cdot [Cl^-]_o \cdot e^{\frac{-2J_p F \cdot g_{KCC2}}{RT \cdot \beta}}$$

This quadratic equation can be solved in terms of θ using the quadratic formula.

$$\theta = \frac{-z \cdot (\Pi_o + N_{H_p}) + \sqrt{z^2 \cdot (\Pi_o + N_{H_p})^2 + 4(1 - z^2) \cdot [Cl^-]_o \cdot e^{-\frac{2J_p F \cdot g_{KCC2}}{RT \cdot \beta}} \cdot \left([Na^+]_o \cdot e^{-\frac{3J_p F}{RT \cdot g_{Na}}} + K_e \cdot e^{\frac{2J_p F \cdot (g_{Cl} + g_{KCC2})}{RT \cdot \beta}} \right)}}{2 \cdot (1 - z) \cdot \left([Na^+]_o \cdot e^{-\frac{3J_p F}{RT \cdot g_{Na}}} + [K^+]_o \cdot e^{\frac{2J_p F \cdot (g_{Cl} + g_{KCC2})}{RT \cdot \beta}} \right)} \tag{S12}$$

From this one can solve the system for any constants — at least those constants which give positive real solutions for θ — and then use $V = -\frac{RT}{F} \ln \theta$ to transform the solution into the corresponding membrane voltage. This implies that initial values of the intracellular ion concentrations do not affect the final steady state (this includes shifts in X that do not change the average intracellular charge z). Note that (S12) when $z = -1$, to avoid division by 0, the solution is found by substituting $z = -1$ into (S11) and simplifying.

Finally, we extend the solution from the constant pump rate assumed above to a pump rate modulated by the sodium concentration, as used in our model. The sodium-dependent pump rate updated by $J_p = p \cdot \left(\frac{[Na^+]_i}{[Na^+]_o} \right)^3$ cannot be solved purely analytically because one ends up attempting to find a solution for an expression unsolvable in the reals (*W-Lambert Function*). In this case, one might substitute different values of J_p into the solution above, and then use the function $f(J_p, [Na^+]_i)$ defined by $J_p = p \cdot \left(\frac{[Na^+]_i}{[Na^+]_o} \right)^3$ to solve for p.

f rearranged with p as the subject of the formula resembles a parametric function. Were p an independent variable determining the ionic solutions of the analytic

solution, each simulation beginning with constant p would have a unique steady state. To make this claim, the function mapping J_p and the steady state $[Na^+]_i$ to p needs to be injective (more strictly, $f : J_p \rightarrow p$ must be bijective). The reason for this constraint is that if ever a p is produced by more than one J_p and $[Na^+]_i$, we would have at least two possible steady states for the time series run with that pump rate constant, and thus a poor mapping between the constant pump rate solution and the cubic pump rate model.

Indeed, the mapping between p and J_p is found to be bijective over the range of J_ps for which we are concerned by numeric methods. This proves that the analytic solution derived here is sufficient for finding a parametric solution for the cubic pump rate pump leak model used in our manuscript.

Meta-analysis data and weighting

We have included here the data from studies in adult rodents of disease-mediated shifts in KCC2 expression and their effects on intracellular chloride concentration used in the KCC2 meta-analysis in Figure 3B (Table S1) as well as a description of the scoring system used to weight results for the weighted least squares regression technique employed in the meta-analysis (Table S2).

In addition, Figure S1 summarises the numbers of reports found using the search techniques described in Materials and Methods as well as the reasons for exclusions of studies considered for the meta-analysis.

Study	Region	$\Delta[Cl^-]_i$	ΔKCC2	ΔNKCC1	Weight
Lagostena et al. (2010)	hippocampus	↑ 118%	↓ 98%	↓ 16%	36
Lee et al. (2011)	hippocampus	↑ 72%	↓ 42%	↑ 7%	35
Campbell et al. (2015)	neocortex	↑ 59%	↓ 37%	↑ 27%	34
Tang et al. (2015)	spinal cord	↑ 56%	↓ 35%	↓ 11%	31
MacKenzie and Maguire (2015) A	hippocampus	↑ 37%	↓ 17%	no data	24
MacKenzie and Maguire (2015) B	hippocampus	↑ 2%	↓ 4%	no data	23
Mahadevan et al. (2015)	hippocampus	↑ 20%	↓ 20%	no data	20
Ferrini et al. (2013)	spinal cord	↑ 42%	↓ 45%	no data	19
Coull et al. (2003)	spinal cord	↑ 151%	↓ 75%	no data	18

Table S1: Changes in the activity of cation chloride co-transporters are associated with changes in intracellular chloride concentration. For each study, the shift in the chloride gradient, change in KCC2 expression, and change in NKCC1 expression was extracted. NKCC1 was assumed to be unlikely to alter chloride homeostasis as adult tissue only was considered (Ben-Ari, 2002), although studies reporting non-signficant changes in NKCC1 expression were weighted higher than those that did not test NKCC1 expression. However, when studies also reported significant expression changes in NKCC1 or functional inhibition confirming that NKCC1 was contributing towards the disease-mediated shift in chloride concentration, they were excluded.

Category		Score
Experimental preparation		
Region	*Cortical brain tissue*	2
	Spinal cord tissue	1
Technique used	*Acute tissue slice*	3
	Organotypic tissue slice culture	2
	Dissociated cell culture	1
KCC2 expression data		
Technique used to measure KCC2 expression	*RT-PCR*	3
	Immunoblotting	2
	Immunohistochemistry	1
Change in KCC2 expression	*Stated in-text*	2
	Extracted from graphic	1
Sample size	≥ 21	4
	$10 - 20$	3
	$5 - 9$	2
	≤ 4	1
NKCC1 expression data		
Technique used to measure NKCC1 expression	*RT-PCR*	3
	Immunoblotting	2
	Immunohistochemistry	1
Change in NKCC1 expression	*Stated in-text*	10
	Extracted from graphic	8
	None	0
Sample size	≥ 21	4
	$10 - 20$	3
	$5 - 9$	2
	≤ 4	1
E_{GABA} data		
Technique used to measure E_{GABA}	*Gramicidin perforated patch-clamp*	4

	Whole-cell patch-clamp	1
Change in E_{GABA} expression	*Stated in-text*	4
	Extracted from graphic	1
Sample size	≥ 21	4
	$10 - 20$	3
	$5 - 9$	2
	≤ 4	1

Table S2: Scoring system used to weight studies for the least squares regression meta-analysis in Figure 3C.
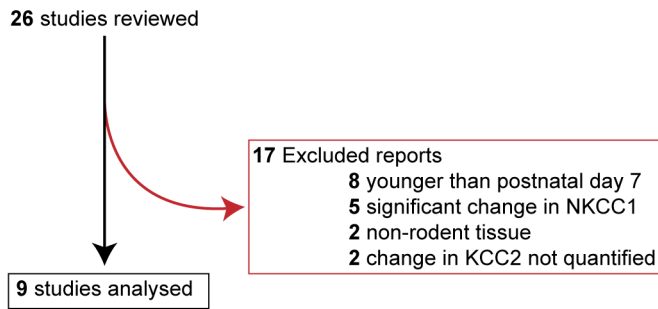

Figure S1: Study numbers resulting in 8 included studies in the meta-analysis as well as reasons for the exclusion of 17 other reports.

References

Ben-Ari Y (2002). Excitatory actions of gaba during development: the nature of the nurture. *Nat. Rev. Neurosci.* **3**, 728–39.

Campbell SL, Robel S, Cuddapah VA, Robert S, Buckingham SC, Kahle KT & Sontheimer H (2015). GABAergic disinhibition and impaired KCC2 cotransporter activity underlie tumor-associated epilepsy. *Glia* **63**, 23–36.

Coull JAM, Boudreau D, Bachand K, Koninck PD, Prescott SA, Nault F, Koninck YD & Sik A (2003). Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* **424**, 938–942.

Doyon N, Vinay L, Prescott SA & De Koninck Y (2016). Chloride Regulation: A Dynamic Equilibrium Crucial for Synaptic Inhibition. *Neuron* **89**, 1157–1172.

Ferrini F, Trang T, Mattioli TAM, Laffray S, Del'Guidice T, Lorenzo LE, Castonguay A, Doyon N, Zhang W, Godin AG, Mohr D, Beggs S, Vandal K, Beaulieu JM, Cahill CM, Salter MW & De Koninck Y (2013). Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl homeostasis. *Nature Neuroscience* **16**, 183–192.

Keener J & Sneyd J (2009). *Mathematical Physiology: Systems Physiology* Vol. 8/I Springer, New York, 2 edition.

Lagostena L, Rosato-Siri M, D'Onofrio M, Brandi R, Arisi I, Capsoni S, Franzot J, Cattaneo A & Cherubini E (2010). In the adult hippocampus, chronic nerve growth factor deprivation shifts GABAergic signaling from the hyperpolarizing to the depolarizing direction. *J. Neurosci.* **30**, 885–93.

Lee HHC, Deeb TZ, Walker JA, Davies PA & Moss SJ (2011). NMDA receptor activity downregulates KCC2 resulting in depolarizing GABAA receptormediated currents. *Nature Neuroscience* **14**, 736–743.

MacKenzie G & Maguire J (2015). Chronic stress shifts the GABA reversal potential in the hippocampus and increases seizure susceptibility. *Epilepsy Res.* **109**, 13–27.

Mahadevan V, Dargaei Z, Ivakine EA, Hartmann AM, Ng D, Chevrier J, Ormond J, Nothwang HG, McInnes RR & Woodin MA (2015). Neto2-null mice have impaired GABAergic inhibition and are susceptible to seizures. *Frontiers in Cellular Neuroscience* **9**, 368.

Tang D, Qian AH, Song DD, Ben QW, Yao WY, Sun J, Li WG, Xu TL & Yuan YZ (2015). Role of the potassium chloride cotransporter isoform 2-mediated spinal chloride homeostasis in a rat model of visceral hypersensitivity. *Am. J. Physiol. Gastrointest. Liver Physiol.* **308**, G767–78.