1. Kessaris, N., Pringle, N. & Richardson, W.D. *Phil. Trans. R. Soc. Lond.* B **363**, 71–85 (2008).
2. Copray, S., Huynh, J.L., Sher, F., Casaccia-Bonnefil, P., & Boddeke, E. *Glia* published online, doi:10.1002/glia.20881 (16 April 2009).
3. Ye, F. *et al. Nat. Neurosci.* **12**, 829–838 (2009).
4. Haberland, M., Montgomery, R.L. & Olson, E.*N. Nat. Rev. Genet.* **10**, 32–42 (2009).
5. Shen, S., Li, J. & Casaccia-Bonnefil, P. *J. Cell Biol.* **169**, 577–589 (2005).
6. Shen, S. *et al. Nat. Neurosci.* **11**, 1024–1034 (2008).
7. Popko, B. *Nat. Neurosci.* **11**, 987–988 (2008).
8. Kao, H.Y. *et al. Genes Dev.* **12**, 2269–2277 (1998).
9. Sekiya, T. & Zaret, K.S. *Mol. Cell* **28**, 291–303 (2007).
10. Shimizu, T. *et al. Dev. Biol.* **282**, 397–410 (2005).
11. Clevers, H. *Cell* **127**, 469–480 (2006).
12. Fancy, S.P.J. & Rowitch, D.H. *Genes Dev.* published online, doi:10.1101/gad.1806309 (1 July 2009).
13. Genoud, S. *et al. J. Cell Biol.* **158**, 709–718 (2002).
14. Liu, A. *et al. EMBO J.* **25**, 4833–4842 (2006).
15. John, G.R. *et al. Nat. Med.* **8**, 1115–1121 (2002).

Stop and go GABA

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A recent study shows that GABA switches from stimulating to inhibiting interneuron motility during neocortical development. This change in response is gated by the expression of the chloride transporter KCC2.

Trains, planes and automobiles all rely on a set of extrinsic signals and the ability to signal to each other so as to prevent traffic jams and crashes. Similarly, inhibitory interneurons manage to evenly distribute throughout the developing neocortex. Traffic jams, or disruptions in the distribution of interneurons, are suspected to be involved in a range of neural dysfunctions, including epilepsy and schizophrenia. Previous studies have shown that interneuron invasion and distribution throughout neocortical lamina is regulated by the transcription factors Dlx and Lhx6 (refs. 1,2), the repulsant CRX4 (ref. 3), and by the neurotransmitter GABA. A recent paper by Bortone and Polleux[4], published in *Neuron*, now shows that migrating GABAergic interneurons undergo an intrinsic change that fundamentally alters their response to GABA.

GABA has been implicated in many aspects of neurodevelopment, from the control of cell proliferation in embryonic progenitor cells to the regulation of migration of adult-generated neurons destined for the olfactory bulb[5]. In developing cerebral cortex, GABA in the extrasynaptic space provides a tonic activation of GABA receptors[6,7]. Activation of GABA receptors promotes the migration of both pyramidal neurons[8] and interneurons[4]. GABA also promotes the migration of neuroblasts in the developing hippocampus. In this case, GABA is released by mechanisms distinct from those necessary to release synaptic vesicles[9].

In mature neurons, activation of $GABA_A$ receptors typically results in chloride influx and cell hyperpolarization because the intracellular concentration of chloride in neurons is lower than the extracellular concentration. In neuronal progenitors, however, this gradient is reversed, and GABA therefore typically depolarizes migrating neurons. This is a result of both the expression of the co-transporter NKCC1, which causes intracellular chloride to accumulate, and the relatively low expression of the chloride extruder KCC2. As neurons mature, increasing KCC2 expression results in a net extrusion of intracellular chloride, shifting the chloride reversal potential to a more hyperpolarized potential[10].

In a recent study, Bortone and Polleux[4] used a BAC-transgenic mouse that expresses enhanced green fluorescent protein (eGFP) in a subset of migrating interneurons originating from the medial ganglionic eminence (MGE).

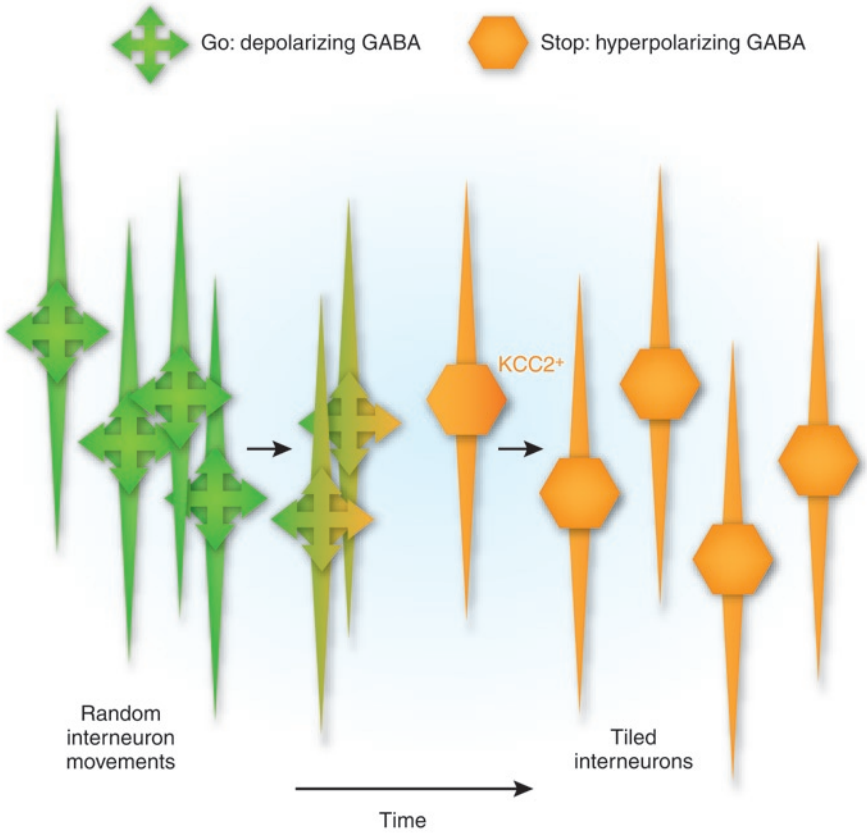

Figure 1 KCC2 expression acts as a molecular switch for interneuron migration. Depolarizing GABA promotes migration of immature interneurons (green cells). Developmentally increasing expression of KCC2 renders GABA hyperpolarizing and reduces interneuron motility (orange cells). This dual function of GABA may promote proper spacing of interneurons within the neocortex.

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Using time-lapse imaging of eGFP-positive interneurons, the authors quantified the dynamics of interneuron migration from embryonic to early postnatal time points. During this time, they observed a change in the dynamic motion of interneurons. The interneurons gradually increased the amount of time that they spent pausing as they approached their final destination in neocortical lamina.

After documenting the migration in slices, the authors turned to an *in vitro* coculture system that allowed them to more directly observe how GABA signaling regulates interneuron migration. By coculturing the MGE containing eGFP-positive interneurons on top of cortical pyramidal cells, the authors observed that application of GABA caused a small, but substantial, increase in pause time, similar to the age-related termination of migration that they observed in prior brain slice experiments. More interestingly, the addition of GABA created a substantial increase in the variability of motility amongst interneurons. Some appeared to move faster and more frequently, whereas others stopped altogether. This variability in response led the authors to hypothesize that there were intrinsic differences amongst the population of migrating interneurons.

Using immunohistochemistry, the authors found that expression of KCC2 was highly variable in immature interneurons. They then correlated the expression levels of KCC2 with the interneurons responsiveness to GABA. Cells with low expression levels showed no substantial changes in migration when GABA was applied, whereas cells with higher levels of KCC2 expression were more likely to pause when GABA was added. To show that this effect was mediated through $GABA_A$ receptors, the authors applied GABA with the $GABA_A$ receptor antagonist bicuculine methiodide (BMI). Interestingly, both GABA and BMI resulted in an increase in pause time for interneurons with below average KCC2 expression. These results suggested that GABA was working as either a motogenic signal or a stop signal, depending on whether GABA produced depolarization or hyperpolarization in the migrating interneuron.

These results suggest that KCC2 expression is acting as a molecular switch for migration (**Fig. 1**). To address this hypothesis, the authors used *ex vivo* electroporation of slices to either knockdown or prematurely overexpress KCC2. Knockdown of KCC2 was achieved using a short-hairpin RNA targeting mouse KCC2 mRNA (shKCC2) that did not target human KCC2. Premature overexpression of KCC2 was carried out using a plasmid containing human KCC2. If KCC2 acts as a migration switch, then preventing the expression of endogenous KCC2 should keep interneurons in an immature migratory state, and conversely, premature expression of KCC2 should hinder migration. The results from these experiments show that KCC2 expression is necessary and sufficient to reduce cortical interneuron migration. The authors also found that premature overexpression of KCC2 greatly reduced the number of interneurons that reached the cortex compared with control interneurons transfected with eGFP.

From these experiments, it is clear that KCC2 expression is working as a migration switch, but how is depolarization by GABA promoting migration and hyperpolarization by GABA terminating migration? Several studies have linked calcium signaling to migration[11,12]. Building on that work, Bortone and Polleux[4] found that calcium transients were present in cells that expressed shKCC2, and conversely, they observed very few calcium transients when interneurons prematurely expressed KCC2. In addition, application of the L-type calcium channel blocker nifedipine substantially increased the pausing time of interneurons. From these experiments it is clear that in this population of interneurons, activation of voltage-sensitive calcium channels are necessary for migration. However, voltage-sensitive calcium channels are promiscuous, as any source of depolarization could potentially activate these channels.

Is GABA the only source of depolarization capable of activating voltage-sensitive calcium channels? Another potential candidate is glutamate. AMPA and NMDA-type glutamate receptors are expressed by migrating interneurons. When they tested this idea, the authors found that blockade of glutamate in interneurons prematurely expressing KCC2 further enhanced the pausing time observed when $GABA_A$ receptors were activated. This suggests that migrating interneurons are under constant tonic AMPA/NMDA receptor–mediated depolarization that stimulates their motility. However, when KCC2 levels were knocked down with shKCC2, activation of $GABA_A$ receptors combined with blockade of glutamate receptors led to an increase in motility, suggesting that both GABA and glutamate provide a cumulative motogenic effect in immature interneurons. Interestingly, recent studies using overexpression of KCC2 in migrating pyramidal neurons found that migration control by GABA is different for interneurons and pyramidal neurons. In contrast to interneuron migration, expressing KCC2 prematurely in pyramidal neurons had no effect on radial migration, but negatively affected activity-dependent dendritic remodeling[13,14]. Thus, it appears that migration of excitatory principle neurons does not involve a GABA stop signal.

GABA is a versatile developmental signal in cell culture assays, but there has been very little direct genetic evidence for a fundamental role of GABA receptor activation in the proliferation or migration of neocortical neurons. It is possible that complex compensatory changes with multiple GABA receptors and chloride accumulation mechanisms obscure phenotypes seen *in vitro* and in slices. Nevertheless, if GABA-based mechanisms are critical for interneuron spacing and migration, then it will be important to confirm the results of Bortone and Polleux[4] with conditional genetic approaches *in vivo*.

The findings of Bortone and Polleux[4] provide a tantalizing explanation for how interneurons might evenly distribute in the cortex. Interneurons appear to undergo a process of tiling to ensure spacing throughout neocortical lamina. Indeed, the lack of interneuron clusters in normally developed neocortex argues against a purely random migration pattern and for the presence of tiling. Switching responses to GABA could contribute to proper interneuron spacing. Interneurons expressing low levels of KCC2 would continue to move when they approach other GABA-releasing interneurons. Once KCC2 expression increases, an interneuron would become anchored and would continue to stimulate the movement of any approaching less-mature interneurons. Because KCC2 expression gates how GABA will affect migrating interneurons, understanding how KCC2 expression is regulated will be important for deciphering how migration is ultimately controlled.

1. Cobos, I., Borello, U. & Rubenstein, J.L. *Neuron* **54**, 873–888 (2007).
2. Liodis, P. *et al. J. Neurosci.* **27**, 3078–3089 (2007).
3. Stumm, R.K. *et al. J. Neurosci.* **23**, 5123–5130 (2003).
4. Bortone, D. & Polleux, F. *Neuron* **62**, 53–71 (2009).
5. Bolteus, A.J. & Bordey, A. *J. Neurosci.* **24**, 7623–7631 (2004).
6. Cuzon, V.C., Yeh, P.W., Cheng, Q. & Yeh, H.H. *Cereb. Cortex* **16**, 1377–1388 (2006).
7. Farrant, M. & Nusser, Z. *Nat. Rev. Neurosci.* **6**, 215–229 (2005).
8. Behar, T.N., Schaffner, A.E., Scott, C.A., Greene, C.L. & Barker, J.L. *Cereb. Cortex* **10**, 899–909 (2000).
9. Manent, J.B. *et al. J. Neurosci.* **25**, 4755–4765 (2005).
10. Rivera, C. *et al. Nature* **397**, 251–255 (1999).
11. Komuro, H. & Rakic, P. *Neuron* **17**, 275–285 (1996).
12. Zheng, J.Q. & Poo, M.M. *Annu. Rev. Cell Dev. Biol.* **23**, 375–404 (2007).
13. Cancedda, L., Fiumelli, H., Chen, K. & Poo, M.M. *J. Neurosci.* **27**, 5224–5235 (2007).
14. Wang, D.D. & Kriegstein, A.R. *J. Neurosci.* **28**, 5547–5558 (2008).