Journal Club

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The Influence of GABA on Dendritic Development *In Vivo*

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Review of Cancedda et al. (<http://www.jneurosci.org/cgi/content/full/27/19/5224>)

Neurotransmitters play a key role in neuronal development. One particularly interesting neurotransmitter, GABA, has an excitatory function during nervous system development but becomes inhibitory in the mature brain (Ben-Ari, 2002). This transition in GABA signaling results from a shift in the chloride equilibrium potential, E_{Cl} , thus shifting the response of chloride-permeable GABA_A receptors. The intracellular chloride concentration is regulated by two transporters during neuronal development. The Na⁺/K⁺/Cl[−] cotransporter NKCC1 is mainly expressed in immature neurons and actively transports chloride ions into the cell, leading to a depolarized E_{Cl} and thus membrane depolarization after GABA_A receptor activation. In maturing neurons, expression of a second transporter, the K⁺/Cl[−] cotransporter KCC2, increases. KCC2 transports chloride ions out of the cell, leading to E_{Cl} that is hyperpolarized compared with the resting membrane potential.

A recent study by Cancedda et al. (2007) in *The Journal of Neuroscience* examined the significance of early depolarizing GABA responses by causing a premature hyperpolarizing shift in GABAergic signaling. The authors electroporated an expression vector

containing enhanced green fluorescent protein (EGFP) and KCC2 into the lateral ventricle of embryonic rats *in utero* at embryonic day 17 (E17) to E18. The authors then examined the migration and morphological development of neurons in the somatosensory cortex. Consistent with previous work (Chudotvorova et al., 2005; Akerman and Cline, 2006), a hyperpolarizing shift in E_{Cl} was present in KCC2/EGFP-transfected neurons in the ventricular zone (VZ) at postnatal day 0 (P0) to P1. Consequently, exogenous application of GABA caused membrane hyperpolarization in transfected cells rather than the depolarization observed in control cells [Cancedda et al. (2007), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F1>)]. By postnatal day 6, E_{Cl} was still hyperpolarized in KCC2-transfected neurons compared with cells transfected only with EGFP [Cancedda et al. (2007), their Fig. 4C (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F4>)].

By this age, neurons have migrated from the VZ to the cortical plate, but the authors reported no differences in the migration pattern of KCC2/EGFP and EGFP-only neurons or their cortical layering, as established at P14, when these patterns are fully developed [Cancedda et al. (2007), their Figs. 2 (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F2>), 3 (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F3>), respectively]. In contrast, early KCC2 expression caused a dramatic alteration in the morphology of the dendritic arbors. At both P6 and P14, the total length of the dendritic tree was decreased in KCC2 neurons, as was

the number of dendritic branches. These differences were more pronounced at P14, the time at which the E_{Cl} of control neurons reached the same hyperpolarized level as transfected neurons [Cancedda et al. (2007), their Figs. 4 (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F4>), 5 (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F5>)].

The authors then explored GABA_A receptor activation at nonsynaptic sites, which may be particularly important during early development, when synapses are less abundant. The GABA_A receptor blocker bicuculline made control neurons less excitable, as measured by the action potential frequency in response to depolarizing voltage steps. In contrast, KCC2-transfected neurons became more excitable after GABA_A blockade [Cancedda et al. (2007), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/27/19/5224/F6>)]. This result suggests that a steady tonic GABA_A receptor current is active in young cortex, which usually keeps immature neurons in a depolarized state. To investigate the general significance of a depolarized membrane potential during development, the authors examine the effects of overexpressing the inward rectifying potassium channel K_{ir}2.1. Consistent with previous work (Burrone et al., 2002), K_{ir}2.1 caused a hyperpolarizing shift in the resting membrane potential of transfected neurons and a reduction in intrinsic excitability. More interestingly, this manipulation also caused a decrease in total dendritic branch length and the number of branches at P14 [Cancedda et al. (2007), their Fig. 7

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(<http://www.jneurosci.org/cgi/content/full/27/19/5224/F7>).

In summary, this study shows that modulating the excitability of a developing neuron results in abnormal dendritic growth. Interestingly, these effects were more pronounced with KCC2 than $K_{ir}2.1$ overexpression. As the authors suggest, hyperpolarization caused by $K_{ir}2.1$ overexpression merely attenuates GABA-induced excitation, whereas KCC2 overexpression converts excitation into inhibition. Nevertheless, early KCC2 expression had a weaker hyperpolarizing effect on the resting membrane potential than $K_{ir}2.1$. This suggests that the influence of GABA is not restricted to tonic signaling. Indeed, *in vitro* studies suggest that phasic depolarizations at focal points determine growth events by controlling the fate of single developing dendritic processes (Korkotian and Segal, 2001;

Lohmann et al., 2005). Future work is perhaps required to dissect the precise mechanisms by which the different signaling modes influence dendritic development. Furthermore, synaptic circuit formation is dependent on neural activity (Zhang and Poo, 2001), which raises the question of the impact of manipulating excitatory GABA on the function of the neuronal network. Electroporation of neurons in the ventricular zone presumably affects only glutamatergic neurons and their progenitor cells. The question thus remains whether GABA is also involved in the morphological maturation of GABAergic neurons.

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