

# GABA EXCITES AND SCULPTS IMMATURE NEURONS WELL BEFORE DELIVERY: MODULATION BY GABA OF THE DEVELOPMENT OF VENTRICULAR PROGENITOR CELLS

**Excitatory GABA Action Is Essential for Morphological Maturation of Cortical Neurons *in Vivo*.** Cancedda L, Fiumelli H, Chen K, Poo MM. *J Neurosci* 2007;27(19):5224–5235. GABA exerts excitatory actions on embryonic and neonatal cortical neurons, but the *in vivo* function of this GABA excitation is essentially unknown. Using *in utero* electroporation, we eliminated the excitatory action of GABA in a subpopulation of rat ventricular progenitors and cortical neurons derived from these progenitors by premature expression of the Cl<sup>-</sup> transporter KCC2, as confirmed by the changes in the reversal potential of GABA-induced currents and the resting membrane potential after GABA<sub>A</sub> receptor blockade. We found that radial migration to layer II/III of the somatosensory cortex of neurons derived from the transfected progenitors was not significantly affected, but their morphological maturation was markedly impaired. Furthermore, reducing neuronal excitability of cortical neurons *in vivo* by overexpressing an inward-rectifying K<sup>+</sup> channel, which lowered the resting membrane potential, mimicked the effect of premature KCC2 expression. Thus, membrane depolarization caused by early GABA excitation is critical for morphological maturation of neonatal cortical neurons *in vivo*.

## COMMENTARY

The fact that GABA excites immature neurons is now almost dogma! There is no exception to the universal rule that developing neurons have a higher internal Cl<sup>-</sup> concentration, which roughly shifts from 25 to 30 mM in immature neurons to less than 7 mM in mature neurons. In immature

neurons, GABA depolarizes neurons and brings their membrane potential to levels that are sufficient to generate sodium and calcium action potentials. This effect is also sufficient to remove the voltage-dependent Mg<sup>2+</sup> block from NMDA channels, leading to a large calcium influx; thus, GABA in developing neurons acts in synergy with NMDA signaling (1). The obvious outcome is that GABA potentiating drugs, such as benzodiazepines, will exert opposite actions on the mother's brain from those that it exerts on the fetus.

The reasons underlying the differing actions of immature and mature neurons are thought to be due to an early expression

of the chloride cotransporter KCC2, which acts to import chloride, and a late expression of KCC2, which exports it (2). This finding and other observations indicate that GABA provides most of the excitatory drive in immature neurons by generating a large calcium influx, serving as a trophic factor, and at an appropriate stage following chloride removal, by leading to the excitatory to inhibitory (E to I) shift. Initially discovered with conventional intracellular recording techniques in immature hippocampal neurons almost 2 decades ago, these observations have been confirmed in a wide range of animal species and brain structures, including primate neurons in utero (1). It is not yet completely clear why these properties are needed for developing neurons and what advantages justified their evolutionary conservation. However, a variety of hypotheses can be put forward, with chloride control, water balance, and osmotic pressure all being likely factors.

Although these basic elements have been known for over 2 decades, the implications are just beginning to be understood. Indeed, if GABA excites immature neurons, the effect will be to excite a host of activity-dependent mechanisms, and a plethora of developmental mechanisms are activity dependent, including neuronal migration, differentiation, neuronal growth, synapse, and network formation. The hypothesis that GABA acts as a trophic factor has long been suggested based on *in vitro* neuronal culture experiments. The recent article by Cancedda and colleagues addresses these issues using a genetic manipulation that imposes an early removal of chloride by expressing KCC2 in immature neurons and thus, instigating an early shift of the actions of GABA and inhibiting these neurons sooner than normally would occur. The investigators used an *in utero* transfection technique (3) that enables embryos to be transfected with green fluorescent protein (GFP) in addition to the KCC2 construct and then performed assessments during various delays after delivery. To confirm the success of the operation, they measured the GABA reversal potential, which enables estimation of the chloride concentration and the polarity of the actions of GABA. As expected, neurons with the GFP label had more hyperpolarized actions of GABA, and transfected neurons had a lower ongoing activity, confirming the inhibitory actions of GABA.

The authors found, in essence, that migration of neurons, at least to layers 2 to 3 of the cortex, were not affected by the manipulation, suggesting that neurons will migrate to their normal target even with a hyperpolarizing GABA. Also, cortical layering was not affected, indicating that the polarity of the actions of GABA does not play a role in migration. However, the morphology and total length and arbor of dendrites differ significantly between neurons with Excitatory GABA (transfected with EGFP) and those with inhibitory GABA (transfected with KCC2/GFP construct). Interestingly, neurons transfected with an inward-rectifying  $K^+$  channel vector, which also leads to a

hyperpolarization, produce the same result. Therefore, imposing an early inhibitory GABA or hyperpolarizing neurons alters their growth.

A previous study, using a similar paradigm, found that an early transfection of KCC2 in neurons in cultures produced (in addition to the expected E to I shift in the actions of GABA) a massive increase of the density of GABAergic synapses (4). The number of GABA, but not glutamate synapses, was increased, as was the frequency of miniature synaptic currents. Therefore, the timely removal of chloride and the E to I shift is a pivotal signal for growth and formation of GABA synapses. This observation, which was not expected *a priori*, indicates an important biological function in the inhibitory actions of GABA as a major growth and formation signal of development. Interestingly, another recent study showed that shortly before delivery, there is a transient and abrupt E to I shift, which also is mediated by a dramatic reduction in the removal of internal  $Cl^-$  concentration such that internal levels drop transiently to values that are lower than those ever observed again in development or in the adult. This shift is triggered by the release of maternal oxytocin, which also induces labor (5). Both observations illustrate the strategic function of the excitatory to inhibitory shift of the actions of GABA.

In summary, the excitatory actions of GABA are now tied to a variety of major developmental issues. The clinical implications of this work are still uncertain. However, the impact of substances that act on GABA systems (including alcohol) and are consumed during gestation—a time when they produce opposite effects on GABAergic transmission in the mother and fetus—may have been underestimated. Recent studies in fact do suggest, somewhat in contrast to the study of Cancedda and colleagues, that migration and network formation are also affected by GABA-acting drugs. Thus, *in vitro* assays show that GABA receptor antagonists significantly retard neuronal migration and produce small ensembles of displaced neurons (6). The clinical implications of these observations are important particularly in relation to the epilepsies. Indeed, a type of reversed shift of the actions of GABA (i.e., from I to E) appears now to be a basic feature of epileptic networks, including human epileptic neurons (7). Concepts presented in these and similar studies suggest that: epileptogenesis recapitulates ontogenesis. Other studies suggest that GABA-acting drugs may exert deleterious actions on cortical construction. For instance, a recent study shows that some antiepileptic agents given during gestation may produce heterotopic masses in the fetus, most likely by affecting neuronal migration (8). Clearly, this domain of research will fuel a lot of renewed efforts to analyze the actions of GABA-acting drugs on fetal development.

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## References

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