Making Connections With GABA

De Novo Synaptogenesis Induced by GABA in the Developing Mouse Cortex.

Oh WC, Lutzu S, Castillo PE, Kwon HB. *Science* 2016;353:1037–1040.

Dendrites of cortical pyramidal neurons contain intermingled excitatory and inhibitory synapses. We studied the local mechanisms that regulate the formation and distribution of synapses. We found that local γ-aminobutyric acid (GABA) release on dendrites of mouse cortical layer 2/3 pyramidal neurons could induce gephyrin puncta and dendritic spine formation via GABA type A receptor activation and voltage-gated calcium channels during early postnatal development. Furthermore, the newly formed inhibitory and excitatory synaptic structures rapidly gained functions. Bidirectional manipulation of GABA release from somatostatin-positive interneurons increased and decreased the number of gephyrin puncta and dendritic spines, respectively. These results highlight a noncanonical function of GABA as a local synaptogenic element shaping the early establishment of neuronal circuitry in mouse cortex.

Commentary

The role of GABAergic signaling in the developing brain has been a subject of considerable debate—depolarizing and excitatory, depolarizing but inhibitory, hyperpolarizing, promoting of neonatal seizures, protective from neonatal seizures, dependent on intracellular chloride concentration, independent of intracellular chloride concentration, different in cortical versus subcortical neurons, variable based in vitro versus in vivo investigation, determined by relative activity of NKCC1 and KCC2 cation-chloride cotransporters, unrelated to cotransporter activity, and so on. Numerous commentaries have been written supporting or refuting these conflicting views (1–4). However, one role of GABA in the developing brain that has been consistently supported, and elegantly investigated by the recent work of Oh et al., is its role in synaptogenesis.

In the manuscript, the investigators use an armamentarium of imaging, electrophysiologic, genetic, and pharmacologic in vitro and in vivo approaches to convincingly demonstrate that $GABA_A$ receptor activation induces dendritic synapse formation in early postnatal mouse cortex. In the first set of experiments, organotypic slice cultures of somatosensory cortex were prepared from neonatal mice and transfected with a combination of tdTomato to visualize neuronal structure and dendritic spines (indicative of excitatory glutamatergic synapses) and Dio-Teal-gephyrin to visualize gephyrin puncta (indicative of inhibitory GABAergic synapses). Transfected neurons in cortical layer 2/3 were imaged by two-photon microscopy, and localized GABA release was achieved using caged GABA and high-frequency uncaging stimulation focally delivered to the edge of a pyramidal cell dendrite. The investigators were therefore able to monitor in real time GABA-induced dendritic spinogenesis and gephyrin cluster formation.

In a series of stunning images the rapid formation of new dendritic spines and gephyrin clusters are shown directly opposite the sites of focal GABA uncaging. These structures appeared within 1 to 5 minutes with success rates of over 50%. Similar uncaging of glutamate could elicit spinogenesis but not gephyrin clustering. The induction of synaptogenesis by GABA was primarily seen in cultured slices of less than equivalent postnatal day 14. Importantly, the investigators were able to replicate these results in anesthetized neonatal mice in vivo. Additional experiments established that GABA-induced synaptogenesis was associated with local elevations in intracellular calcium and was prevented by the $GABA_A$ receptor antagonist gabazine, calcium channel blockers, calcium-free solutions, and the NKCC1 sodium-potassium-chloride cotransporter inhibitor bumetanide. These new spines and gephyrin puncta were functional, as demonstrated by the appearance of currents elicited by glutamate (spines) or GABA (gephyrin puncta). The investigators further confirmed their findings through usage of optogenetic approaches to induce GABA release from somatostatin-containing interneurons onto pyramidal cell dendrites in cultured somatosensory cortical slices. Photostimulation of channelrhodopsin 2-expressing interneurons to induce GABA release from axonal boutons was demonstrated to result in local formation of dendritic spines and gephyrin clusters.

These studies establish that both focal GABA application and endogenous GABA release rapidly cause dendritic glutamatergic and GABAergic synapse formation in early postnatal mouse cortex. GABA-induced synaptogenesis is dependent on $GABA_A$ receptors, elevation of intracellular calcium, and NKCC1

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chloride cotransporter activity. These findings are consistent with a previous in vivo study, which revealed GABA-induced membrane depolarization in neonatal mouse cortical neurons (5). An important role of depolarizing GABA in neonatal synaptogenesis has been indicated for some time, as interfering with the normal developmental ontology of neuronal chloride gradients via either premature downregulation of NKCC1 or premature upregulation of KCC2 hinders dendritic branching and spinogenesis (6, 7). However, the study of Oh et al. is the first to directly observe de novo glutamatergic and GABAergic synapse formation in response to GABA.

Despite the thorough and rigorous approaches taken by the investigators, several unanswered questions remain regarding the relative roles of GABA and glutamate in activity-dependent developmental synaptogenesis. In this study, GABA was found to induce both glutamatergic and GABAergic synapse formation through $GABA_A$ receptor and calcium-dependent mechanisms. What is not addressed is how a dendrite "decides" to form a gephyrin puncta (GABAergic synapse) versus a spine (glutamatergic synapse) upon exposure to a GABA stimulus. Identification of factors underlying this "decision", which could potentially be mediated by activation of different subtypes of GABA receptors, local availability of structural synaptic components, or access to different second messenger molecules, kinases, and posttranslational modification machinery, would provide significant insight into how the balance is set between inhibitory and excitatory synapses. Similarly, the mechanisms that cause glutamatergic stimulation to result in only spinogenesis but not gephyrin clustering are not explored. Furthermore, the long term stability of these GABA-induced synapses and their influence on developing cortical networks is not examined. Finally, the question arises as to whether GABA-mediated synaptogenesis could play a role in aberrant synaptic plasticity and epileptogenesis in pathologic settings in the adult brain in which GABA is rendered locally depolarizing.

The potential implications of this study for the management of epilepsy in neonates are many. Irrespective of whether positive modulators of $GABA_A$ receptor activity (such as phenobarbital and benzodiazepines) are "inhibitory" or "excitatory" in the neonatal brain, the prolonged use of these agents in newborns could be reasonably hypothesized to interfere with normal postnatal synaptogenesis and contribute to later developmental and behavioral impairments (8, 9). Similarly, efforts to prematurely induce hyperpolarizing actions of GABA via administration of NKCC1 inhibitors (such as bumetanide) (10) to newborns may also have unanticipated developmental consequences. Important priorities for future investigations using the tools reported by Oh et al. include the replication of these studies in neonatal seizure models and in the presence of clinically used GABAergic antiseizure medications.

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