Tuning the neurogenesis channel

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Earlier work has implicated the neurotransmitter GABA in controlling forebrain progenitor proliferation. In this issue of Neuron, Everlien et al., 2022 demonstrate that diazepam binding inhibitor acts to keep the neurogenesis-promoting effect of GABA at bay.

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Neurotransmitters are of primordial importance for communication between neurons and may have played an even more primeval role in the evolution of nervous systems (Moroz et al., 2021). Thus, not without reason, researchers have been fascinated since long by the idea that beyond their prototypical function at chemical synapses, neurotransmitters could contribute to the shaping of the nervous system during development. What if neurotransmitters could act on neural progenitor cells even before they become secreted at synapses and could influence their decisions of whether to continue dividing or to differentiate? Such mechanisms could e.g., link the next round of neurons being generated to the number of cells already producing a particular neurotransmitter and contribute to the intricate matching of excitatory and inhibitory neurons. Indeed, a plethora of studies have uncovered highly heterogeneous effects of pharmacological stimulation with neurotransmitters or their receptor agonists on the proliferation and differentiation of neural progenitors, both in the developing and adult nervous system. In their classical study, LoTurco and colleagues, 1995, observed that glutamate and γ-aminobutyric acid (GABA) could depolarise progenitors located in the rat embryonic ventricular zone and inhibit their DNA synthesis. This data suggested that activation of ionotropic neurotransmitter receptors expressed on progenitors reduced their rate of proliferation. Follow-up studies confirmed and extended these initial findings by showing that distinct progenitors residing in the ventricular and subventricular zone, respectively, responded differentially to GABA or glutamate (Haydar et al., 2000). Likewise, regulation of neural stem and progenitor cell activity by GABA was observed in the postnatal and adult neurogenic niches of the subventricular zone of the lateral ventricle and the adult hippocampus (Liu et al., 2005; Song et al., 2012).

However, in most of these studies, the physiological relevance of these observations has remained elusive. Given that GABA exerted its effect apparently through ionotropic GABA-A receptors, one might expect that modulation of the channel’s activity via its diazepam binding site could tune the response of progenitors to this neurotransmitter. Indeed, in earlier work, the authors of the present study had identified diazepam binding inhibitor (DBI) and its cleavage product octadecaneuropeptide (ODN) as a positive cell cycle regulator of neural stem and progenitor cells in the postnatal subventricular zone and adult subgranular zone, thereby promoting olfactory bulb and hippocampal neurogenesis (Alfonso et al., 2012; Dumitru et al., 2017). In the present work, Everlien et al., 2022 now address the role of DBI and ODN in embryonic neurogenesis. They set out by noting a conspicuous DBI expression gradient
from the ventricular zone to the pial surface, both in the embryonic cortex as well as the ganglionic eminences, with highest expression in radial glia and second highest in intermediate progenitors, but much less in early neurons. This prompted them to undertake loss- and gain-of-function experiments to tease apart the effects of DBI on embryonic neurogenesis. By transducing radial glia and intermediate progenitors in utero with lentiviruses encoding short-hairpin RNAs against DBI, they found that cells targeted for DBI knockdown produced more cortical and striatal neurons than control transduced cells (Fig. 1). Conversely, progenitors overexpressing DBI generated less neurons. To find out whether DBI was primarily acting on radial glia or intermediate progenitors, Everlien et al., 2022 examined the expression of γ2 subunit of GABA-A receptors. DBI and ODN are endozepines that bind at interfaces between α and γ2 subunits, thereby reducing GABA-induced channel activity (Calcaterra and Barrow, 2014). Compared to radial glia, γ2 expression was enriched in intermediate progenitors, suggesting them as the main culprits for the DBI-mediated effects on neurogenesis. The authors succeeded in nailing this further down by directly recording from brain slices of the embryonic lateral ganglionic eminence and finding not only that – at this stage depolarising - responses to the GABA-A receptor agonists muscimol were by a magnitude larger in intermediate progenitor cells as compared to radial glia, but more importantly ODN-mediated suppression of channel activity was drastically increased. Finally, the physiological role of modulation of GABA-A receptor channel activity in tuning progenitor proliferation was corroborated by showing that DBI overexpression failed to reduce cell division in mutant mice lacking the diazepam binding site at the α/γ2 subunit interface. Likewise, progenitors lacking γ2 subunits altogether no longer responded to DBI and exhibited enhanced proliferative activity. Thus, by showing that allosteric GABA-A receptor modulation via DBI and ODN affects progenitor proliferation, Everlien et al., 2022 unequivocally identify the GABA-A receptor as a physiological knob for fine-tuning progenitor dynamics.

However, while providing long-sought definitive evidence for the physiological role of GABA signalling in forebrain development, many new questions arise. First, why do DBI and ODN exert so drastically opposing effects on cell proliferation in the embryonic (i.e., reduction) and adult brain (i.e., enhancement)? The authors discuss the possibility that such opposing effects of GABA-A receptor modulation may be caused by distinct downstream signalling events elicited in embryonic versus adult stem and progenitor cells, albeit triggered by an apparently identical in vivo manipulation. Such differential translation of the same incoming stimulus into distinct actions could depend on the functional state of stem and progenitor cells and their interactions with the microenvironment. For instance, in adult neurogenic niches, most neural stem cells are quiescent and GABA signalling appears to suppress their activation (Song et al., 2012). In contrast, embryonic neural stem cells are largely in cell cycle. However, this does not explain the distinct responses observed between cycling intermediate progenitors in the embryonic brain and transit-amplifying progenitors in the adult brain. Comparing transcriptome changes in response to DBI gain-or loss-of-function between these progenitor types may shed light on the apparent enigma.

Similarly enigmatic is the fact that the effect of DBI knockdown appears to be essentially cell autonomous. The authors co-injected control and DBI knockdown-mediating lentiviruses in utero, resulting in the transduction of nearby cells and yet only the knockdown cells underwent increased proliferation and neurogenesis. This is more surprising as DBI is secreted and thus DBI released from surrounding cells may have compensated for the loss of DBI in the sparse population of progenitors expressing the DBI short hairpin. Likewise, the effect of DBI gain-of-function appeared to be limited to those progenitors transduced with the overexpression virus. This would indicate that the working range of DBI is very short and essentially limited to the cells expressing it.

Another fascinating question concerns the source of GABA. Here ambient levels might drastically vary between the neurogenic zones of the dorsal and ventral telencephalon. While
the latter forebrain region is the site of origin of GABAergic inter- and projection neurons, and hence be exposed to higher GABA levels secreted from early neurons, young GABAergic interneurons enter the cortex first via tangential and subsequently radial migration (Fig. 1) and would be expected to be considerably sparser. And yet, Everlien et al., 2022, observe very similar effects of DBI loss-of-function in the two forebrain territories. It will be important to get accurate measures of GABA ambient levels surrounding progenitor cells. A provocative question is whether any genetic defect causing delay or failure of GABAergic interneuron migration would result in a reduction of cortical neurogenesis. Furthermore, given that different types of cortical and subcortical neurons are generated at sequential stages of embryonic development, it will be important to learn whether some neurons are more vulnerable to manipulating the GABA-DBI balance than others and thereby cause changes to cortical and subcortical microcircuits.

Finally, diazepam, e.g., as Valium, is being prescribed in anxiety disorders, to relief muscle spasms, and in convulsive disorders (Calcaterra and Barrow, 2014). When taken during pregnancy, based on the findings presented here by Everlien et al., 2022, one might expect that this could outcompete DBI leading to enhanced activation of GABA-A receptors, thereby promoting embryonic neurogenesis. A recent study reported so-called odd ratios of benzodiazepine medication and Dandy–Walker malformations (DWM) (Tinker et al., 2019) which are characterised among other defects by hypoplasia of the cerebellar vermis. While it is unclear whether DWM involves aberrations in neurogenesis, and if so, whether they can be caused by diazepam medication acting through mechanisms akin to the embryonic or rather the adult forebrain, the study by Everlien et al., 2022, warrants further interest in uncovering the full role of allosteric modulation of GABA-A receptor during brain development.

The authors declare no competing interests.


Figure 1. Diazepam-binding inhibitor keeps proliferation of forebrain progenitors at bay. The pro-proliferative effect of GABA (here hypothesised to be secreted by migrating GABAergic interneurons entering the developing cortex) is balanced by secreted DBI. Upon loss of DBI, GABA acting on GABA-A receptors enhances proliferation of intermediate progenitor cells which eventually generate a surplus of pyramidal neurons. CP Cortical plate; DBI Diazepam binding inhibitor; GABA_A GABA-A receptor; IN Interneuron; IP Intermediate progenitor cells; PYN Pyramidal neuron; RG radial glia; SVZ Subventricular zone; VZ Ventricular zone