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GABA Excitation in the Adult Brain: A Mechanism for Excitation-Neurogenesis Coupling

The production of new neurons in the adult hippocampus is exquisitely regulated, and alterations in this process may underlie both normal and pathological hippocampal function. In this issue of *Neuron*, Tozuka et al. describe electrophysiological recordings that target proliferating progenitor cells in adult mouse hippocampal slices. They report that GABAergic synaptic inputs directly depolarize the proliferating progenitors, thereby activating molecular players that favor neuronal differentiation and providing a mechanism for direct excitation-neurogenesis coupling in vivo.

Many of in vivo manipulations that influence electrical activity affect neuron production from neural stem/progenitor cells in the adult hippocampus (reviewed in Lehmann et al., 2005). Indeed, recent evidences suggests that hippocampal activity itself can act directly on proliferating progenitors (Deisseroth et al., 2004), giving rise to a computationally intriguing scenario in which network activity controls insertion of new elements into the network itself. Among other possible roles, this process could help tune memory storage and clearance capabilities to demands being placed upon the network. Such excitation-neurogenesis coupling appears to involve an activity-dependent, Ca^{2+} channel-driven proneural-gene-expression program within the proliferating progenitor cells themselves (Deisseroth et al., 2004). It has been unclear, however, which of many possible mechanisms triggers this excitation-neurogenesis coupling in vivo. That is, how do the proliferating cells directly sense activity signals in the intact circuit?

Transgenic mice expressing GFP under control of the nestin promoter and its second intron have been a useful tool to identify proliferating stem/progenitor cells in the living adult hippocampus. In these mice, GFP labels both radial glia-like progenitor (type-1) cells and actively proliferating neuronal progenitor (type-2) cells but not postmitotic neurons (Fukuda et al., 2003; Filippov et al., 2003; Seri et al., 2004). Recent work has shown that the type-2 cells, a major subpopulation of the progenitor pool in the adult hippocampus, express $GABA_A$ receptors that can be activated by synaptic stimuli (Wang et al., 2005). In a series of elegant experiments, Tozuka et al. take advantage of this system by independently confirming that type-2 cells receive direct GABAergic synaptic inputs from the hippocampal circuitry and going on to present morphological evidence suggesting that GABAergic terminals are present on or very near the type-2 cells. Both groups report synaptic event kinetics consistent with direct synaptic innervation of the patch-clamped type-2 cell. Importantly, Tozuka et al. present two additional lines of evidence suggesting that the proliferating progenitors are wired into the dentate gyrus networks and respond robustly to normal hippocampal rhythmic activity. First, although single stimuli to the dentate hilus suffice to drive GABAergic synaptic responses in progenitor cells, a specific physiological pattern of activation (theta-burst stimulation) is required to generate synaptic responses if the perforant path input is stimulated. Second, application of the muscarinic receptor agonist carbachol, which typically generates theta-range oscillatory-network activity in the hippocampus, potently increases the rate of spontaneous GABAergic synaptic events. Together, these results suggest that proliferating progenitors are wired up to the pre-existing hippocampal network in a precise way. Indeed, preferential wiring to the GABAergic system may be a common theme in the early stages of adult neurogenesis; even neuronal progeny of proliferating cells (dentate granule neurons $\sim$2 weeks after their last mitosis) express evoked and spontaneous synaptic currents that are exclusively mediated by GABA receptors (Overstreet Wadiche et al., 2005).

What effect does this GABAergic synaptic input have on membrane potential in the proliferating progenitor cells? Tozuka et al. show that like most GABAergic responses early in development (Ben-Ari, 2002; Owens and Kriegstein, 2002), the GABAergic inputs onto progenitors actually strongly depolarize type-2 cells from their resting potential of $\sim$–60 mV. (This depolarization occurs because the adult progenitors have elevated intracellular chloride levels, a phenomenon previously described in embryonic neuronal progenitor cells [Lo-Turco et al., 1995; Owens and Kriegstein, 2002].) This

depolarization in turn causes an increase in intracellular Ca^{2+} similar to that evoked in cultured adult neural progenitors by mild depolarization (Deisseroth et al., 2004), and similar downstream effects of Ca^{2+} influx also result. Specifically, like the response of adult progenitor cells in culture to direct mild depolarization, Tozuka et al. find that GABA application increases the expression of NeuroD, a transcription factor that is required for neuronal phenotype generation in hippocampal dentate granule neurons (Liu et al., 2000; Schwab et al., 2000). Taken together, these data suggest that Tozuka et al. have hit upon a highly plausible mechanism by which depolarization of proliferating progenitors can be generated in vivo by network activity to drive excitation-neurogenesis coupling.

Additional mechanisms in this process, of course, still may be involved. Other ligand-gated channels that could directly provide depolarization to the progenitors include AMPA- and NMDA-type glutamate receptors as well as the 5HT3 serotonin receptor. Although no effect of glutamate receptor agonists on type-2 cells was observed by Tozuka et al., other groups have shown that NMDA receptors can generate small currents in type-2 adult progenitors in exactly the same experimental system (Wang et al., 2005) and also can modulate neuronal phenotype in stem/progenitor cell progeny (Deisseroth et al., 2004). The variance in these results might be explained by a rapid rundown of NMDA receptor-mediated responses in these small cells after attaining the whole-cell configuration or by slightly different maturational states of the progenitors in different experiments since receptor expression may change as proliferating progenitors move along the neurogenic pathway. It also seems likely that some heterogeneity exists even within the type-2 cell population, such that distinct subpopulations of progenitors might express distinct ligand-activated channels and G-protein-coupled receptors that could give rise to elevations in intracellular Ca^{2+}.

Tozuka et al. conclude with important experiments to test for changes in neurogenesis in vivo in response to GABA receptor stimulation and inhibition. Since the GABA agonists that they administer systemically will act in opposing directions, to both directly excite the progenitors and overall to reduce hippocampal network activity, it might be expected that the results will be complicated, and indeed they are. First, administration of $GABA_A$ receptor antagonists over 4 days, expected to increase overall hippocampal activity, greatly increased the number of newborn type-2 cells observed in hippocampus, consistent with the general idea that elevated hippocampal activity drives increased neurogenesis. Second, administration of $GABA_A$ receptor agonists over 4 days significantly decreased the number of type-2 newborn cells observed in hippocampus. This is consistent with overall reduced network activity and the reduction in doublecortin-positive newborn cells previously observed after 7 days of diazepam treatment (Deisseroth et al., 2004). Finally, 28 days after a paradigm consisting of a single BrdU labeling of newborn neurons followed by 7 days of agonist administration, Tozuka et al. observed a modest (~40%) $GABA_A$ receptor agonist-induced increase in the number of newborn calbindin-positive neurons in the adult dentate gyrus. This finding is plausibly interpreted as being due to $GABA_A$ receptor-driven depolarization and Ca^{2+} influx leading to neuronal phenotype consolidation/stabilization in progeny of the proliferating progenitors. This, in turn, would lead to an increase in the number of mature neurons observed 1 month later. Again, this finding is consistent with the increased number of observed mature (NeuN-positive) new neurons in the dentate gyrus 1 month after a 7-day course of Ca^{2+} channel agonist treatment (Deisseroth et al., 2004).

This elegant series of experiments importantly extends and complements other work in the field (Wang et al., 2005; Deisseroth et al., 2004; Overstreet Wadiche et al., 2005) but also points to some of the challenges inherent in attempting to elucidate the in vivo mechanisms that couple network activity to cellular differentiation events. Although it is becoming increasingly clear that direct depolarization of proliferating adult progenitors favors signaling pathways that lead to increased neuronal phenotype expression, in vivo manipulations, such as pharmacological treatments, are inherently complex. Within any given network, some cells will be excited while others will be inhibited, and the effect on the network will depend on a host of factors including the detailed connectivity within the network, the time course of the treatment, and the adaptations that occur in the network in response to the treatment. For example, newborn cells could in turn modulate the progenitor pool to control proliferation, as recently observed in the subventricular zone (Liu et al., 2005). To some extent, these challenges underscore the value of using reduced experimental preparations in combination with in vivo systems. It also will be important to develop and employ methods to track and control circuit activity in vivo, to determine how progenitor cells proliferate, differentiate, and survive in response to different known levels of physiological network activity. The work of Tozuka et al. is a very important step in this direction.

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