1. Methods to identify adult-born neurons

* 3H-thymidine: Altman injected 3H-thymidine into rat brains. Radioactive labeling showed that both glia (Fig1) and neurons (Fig2) had taken up 3H-thymidine into their nuclei (Altman 1962). His finding contradicted previous observations that 3H-thymidine injected IP were not observed in the brain.
* BrdU: Rodents are nice, but what about evolved species? To test this, BrdU was injected into monkey brains. Kornack et al. found that neurons, oligodendrocytes, and astrocytes in the hippocampal dentate gyrus of adult macaque monkeys were double labeled with BrdU and cell-type specific markers, not merely inferred by morphology (Kornack and Rakic 1999). In yet another step up the evolutionary ladder, Eriksson et al. found new neurons (co-labeled with NeuN, calbindin or neuron specific enolase) generated in the dentate gyrus of adult humans (Eriksson, Perfilieva et al. 1998).
* Retrovirus: The prior two chemicals are incorporated into the DNA during S-phase thus restricting labeling to the nucleus. But what about a method that labels the morphology of the neuron in question? GFP expressing retrovirus can do this and was injected into the dentate gyrus of adult mice. Zhao et al. used this technique to characterize the dendritic development of adult born granule cells (GC) in a staged/time-dependent manner (Zhao, Teng et al. 2006). Their findings pointed to a 4 week spine-maturation period from birth, which instructed future work in the field.

2. Evidence for functional integration: so neurons are born in the adult brain, focus here on dentate gyrus, but do they actually contribute to the network?

* Do newborn neurons form functional synapses? Toni et al. combined retroviral expression with immuno-EM and found GFP-positive synapses on many types of cells in the hippocampus, such as on dendrites in the CA3 and thorny excrescence in the hilus (Fig2) (Toni, Laplagne et al. 2008). Next, Toni et al. combined patching with light-evoked neurotransmitter release from adult born neurons expressing ChR2. They found that adult born GCs can elicit postsynaptic currents in CA3 principal cells, hilar interneurons, and hilar mossy cells (Fig4). Additionally, they demonstrated that these functional synapses are of the glutamate type by pharmacology (Fig5, application of kyn, AMPA/NMDA receptor antagonist blocked light-evoked postsynaptic responses). These findings demonstrate that adult born GCs have the capability to transmit information onto postsynaptic target cells.
* But do they receive synaptic input? Using Ca-imaging and loose patch recordings of individual cells in acute hippocampal slices, Marin-Burgin et al. set out to address this. They found spiking probability for a given input strength was highest in 4 week old adult born GCs compared to mature ones (Fig2) (Marin-Burgin, Mongiat et al. 2012). It required lower stimulus strength to activate 50% of the immature rather than mature GC population (Fig2) and E/I balance was higher immature GCs (Fig3). These findings suggest that adult born GCs have a lower inhibitory influence than mature ones.

3. Danielson paper (Danielson, Kaifosh et al. 2016)

* Pattern separation: Used ablation of adult born GCs by focal x-ray irradiation (may have gotten olfactory neurons too), mice could not pattern separate as well. In the positive direction, removed Bax from nestin cells to make their hippocampi have more adult born GCs, mice became great pattern separators. In Danielson paper, used nestincreert2 mice to express Arch-eGFP in adult born GCs. Shined light in the DG during either context A or context B. When light was on in context A (recall, Fig6C), arch no different than control case, both groups froze more in A. However, found a “discrimination” deficit only when adult born GCs were inhibited in the context B (different context, Fig6D).
* Activity: Used 2P-microscopy in GCamp6 adult born GCs to measure activity from mice that don’t have a cortex... Found more frequent transients in abGCs than mGCs (Fig1).
* Spatial tuning: In mice that were walking on a circular treadmill, used same Ca-imaging of abGCs. Found that mGCs are more tuned than abGCs (Fig2B-E).

In conclusion, abGCs cause depression, autism, schizophrenia and diseases we don’t even have names for yet.

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