The effect of synaptogenesis on the survival of adult born hippocampal neurons

Key words: Neurogenesis, synaptogenesis, dentate granule cells, entorhinal cortex, glutamate pathway, enriched environment, NMDARs

Background: After embryonic development, neurogenesis ceases in all of the nervous system except the dentate gyrus and the olfactory bulb. Recent evidence has shown that adult neurogenesis in the dentate gyrus is not merely a remnant of development, but a tightly regulated process that equips mammals with the ability to adapt to various environmental changes. In the dentate gyrus of the hippocampus, an area involved in memory formation, newborn neurons differentiate into dentate granule cells (DGCs). About 50% of new DGCs successfully integrate into hippocampal circuits, a process enhanced by environmental enrichment, stress and the completion of tasks that require learning. Enriched environments are frequently employed in studies of adult-born DGC survival because the large number of novel environmental encounters provides a simple means to encourage learning and subsequently elevates neuronal firing of newborn cells. Neuronal activity and synaptogenesis are important in determining which cells survive. For synaptogenesis to ensue, all the following events need to occur: formation of dendritic spines, glutamate input coming from the entorhinal cortex and the formation of axonal projections. Newborn DGCs receive excitatory glutamate signaling predominantly via the N-methyl-D-aspartate receptors (NMDARs) from the entorhinal cortex, an area responsible for encoding familiarity, and they send excitatory glutamate signaling to the CA3 region, an area responsible for memory consolidation. Given these findings, it is important to understand the role of enriched environments in elevated neuronal activity and thereby in DGC survival.

Hypothesis: Since enriched environmental exposure increases neuronal firing in adult-born DGCs, I hypothesize that this elevated neuronal firing increases the likelihood of synaptogenesis, and hence survival. To determine if DGC survival is dependent on increased excitability, I will use a modified teg-tag transgenic mouse line that allows persistent labeling of newborn neurons, and I will track such neurons until a successful synaptic connection is made by implanting a cranial glass window for two-photon in vivo imaging. Such technique has already allowed scientists to obtain long-term in vivo imaging of dendritic spines in the CA1 hippocampal region. If environment-dependent neuronal activity increases neuronal survival, I will go on to study if the glutamate pathway plays a role in neuronal survival by blocking NMDARs in newborn DGCs.

Experimental Design:

Aim 1: Determine if DGC survival and hippocampal integration is dependent on increased neuronal excitability elicited by environmental factors: To label activated newborn neurons during an environmental exposure, I will use the doxycycline-dependent (dox) teg-tag transgenic mouse that I optimized during my internship at UCSD (Fig. 1). In these mice, neuronal firing activates the Fos promoter and induces the expression of tetracycline-controlled transactivator (tTA), which activates the expression of the tau-LacZ/YFP tag. The presence of a mutated tTA (tTA*) will allow persistent tau-LacZ expression in neurons irrespective of dox treatment. Animals will receive bromodeoxyuridine (BrdU), a proliferation marker, at the beginning of the experiment to label all the neurons that were born during that period. The enriched environment will include a running wheel, colorful house nest and toys. A chronic cranial glass window will
be implanted into the mouse brain before environmental exposure for in vivo two-photon imaging. The two-photon microscope offers long-term in vivo imaging in awake animals owing to the head plate that provides specific coordinates to continuously reimage the same neurons over time. Using the transgenic mouse model, I will label only the newborn neurons that were activated during the enriched environment exposure and follow them with two-photon imaging until they make a successful synaptic connection. The formation of synaptic connections will be tracked daily using real-time imaging. The formation of a synaptic connection with a CA3 region neuron indicates that the neuron has integrated into the hippocampal circuitry. In addition, control experiments will be conducted with no exposure to an enriched environment. Since proper synaptic connections is required for neuronal survival, with these experiments, I expect to estimate the proportion of surviving neurons among those activated during environmental exposure. If the enriched environment is influencing the survival of newborn neurons, the number of successful synaptic connections of activated neurons should be higher in the enriched-environment group than in the control group. Such results will suggest that elevated neuronal firing during exposure to the enriched environment improved newborn neuron survival. If the results are very similar to control animals, this will suggest that environmental exposure during development increases neuronal activity of newborn neurons but that it is not sufficient to ensure their survival.

**Aim 2:** Determine if neuronal survival is regulated by glutamate signaling between the entorhinal cortex-DG-CA3 region: NMDARs are predominantly responsible for the glutamate excitatory signaling that newborn neurons receive from the entorhinal cortex during their development. Therefore, blocking NMDARs in the DGCs will impair the signaling input they receive. Carboxyethyl ester (CGP 39551), a high-affinity NMDAR antagonist, will be administered intraperitonially (ip) to block NMDARs. By blocking the glutamate pathway, I will be able to determine the role of glutamate signaling in the survival of newborn neurons. I will use the same experimental setup described in Aim 1. Two control groups will be included to differentiate between the effects of the enriched environment plus NMDAR blockage and the effects of NMDAR blockage alone. I expect to obtain an estimate of the number of neurons that were activated and survived in the presence of the NMDAR antagonist. Such results will be compared to the number of neurons that were activated and survive in the absence of the NMDAR antagonist. If antagonist treatment decreases the number of activated neurons that made a successful synaptic connection and survived, it will suggest that enriched environmental exposure increases glutamatergic signaling, providing the newborn neurons with sufficient signaling input to ensure the formation of a proper synaptic connection. If the number of antagonist-treated neurons making proper synaptic connections is higher than or equal to that in control groups, I would conclude that environmental effects are not exerted via the glutamate pathway. If the glutamate pathway does not have an effect on neuronal survival, I will examine the GABAergic signaling pathway, which is predominantly activated during early development of newborn neurons.

**Broader impacts:** An understanding of the formation of synaptic connections and neuronal survival in the adult brain will shed light on the putative function of adult neurogenesis in hippocampal information processing and storage. In addition, these studies will provide us with a new method that can be used to modulate adult neurogenesis. Also, working with undergraduates in this project will allow me to continue mentoring and training young scientists.

**References:**
1. Deng et al., eLIFE, 2, (2013).