In vivo methods for observing and manipulating neural progenitor cell dynamics

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The tight spatiotemporal control of the proliferation of neural progenitor cells (NPCs) during development and in the adult is crucial for correct brain function. Disruption of these processes can lead to a wide spectrum of disorders ranging from subtle behavioral problems to grossly abnormal brain formation or cancers. Most of our understanding of NPCs comes from *in vitro* studies and histo-chemical analysis of fixed or cultured brain tissue. We have developed a model system for examining NPC proliferation in the intact brain. Using the developing tectum of Xenopus laevis we have established a series of *in vivo* cellular imaging techniques that enable us to observe and manipulate the dynamics of NPCs. First, using single cell electroporation of fluorescent lineage markers, we have demonstrated that the NPCs in the Xenopus tectum closely resemble radial glial cells, which are known to be NPCs in the mammalian cortex. Labeling individual NPCs results in the subsequent labeling of clones of related neurons, which are oriented radially within the tectum and contain cells of multiple phenotypes. Second, we have developed *in vivo* techniques using fluorescent lipid markers to observe NPC mitosis. This provides quantitative data on NPC proliferation in different parts of the brain, across development. Third, in order to characterize the cell-cell signaling mechanisms used by NPCs, we have combined these approaches with calcium imaging techniques. This enables us to quantify calcium dynamics within identified NPCs and to relate this to the activity of other cells within the network. We have observed that NPCs within the proliferative zones exhibit a variety of signaling events as they progress through their cell cycles. We are currently using pharmacological manipulations to explore whether these signals play distinct roles in coordinating NPC proliferation.