



# Cellular imaging and emerging technologies for adult neurogenesis research

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The first report on the generation of new neurons in the adult mammalian brain occurred in the early 1960s, however, nearly 40 years passed before the scientific community generally recognized the existence of adult mammalian neurogenesis. Development of new technologies that facilitate the identification of newborn neurons in the early 1990s has been central to expanding our understanding of adult neurogenesis as a process influencing mammalian brain plasticity. Subsequently, the field of adult neurogenesis progressed tremendously thanks to continuous technical advances allowing *in vivo* and *in vitro* manipulations of adult neural progenitors. Today, a core understanding of various aspects of adult neurogenesis has emerged, including neural progenitor proliferation and fate-specification, and the migration, maturation, and synaptic integration of newborn neurons into functional circuits. However, numerous questions remain open. This research topic issue gathers a series of articles dedicated to major methodological advancements that have significantly contributed to progress in the understanding of adult neurogenesis in the mammalian brain. It includes six review articles that give a critical update of current approaches, outlining their strengths as well as their limitations, and the need of further improvement in technological tools to address specific key issues. Four methods articles dealing with new *in vitro*, *in vivo*, or *ex vivo* approaches to study adult neurogenesis together with one original research article are included.

*In vitro* assays are key tools for deciphering the cellular and molecular mechanisms of adult neurogenesis. Generally, adult neural precursor cells can be expanded *in vitro* using two different culture methodologies: neurospheres, or adherent monolayer cultures. Babu et al. (2011) present a detailed protocol for isolation and enrichment of neural precursor cells from mouse adult hippocampal neurons. They highlight potential modifications to the protocol useful for isolating and expanding precursor cells from other brain regions.

Newly generated neurons *in vivo* can be identified and/or manipulated by various approaches, such as the incorporation of nucleotide analogs, retrovirus-mediated gene transfer, and genetic methods using transgenic mice. Among nucleotide analog methods, bromodeoxyuridine (BrdU) has been the marker of choice in recent years. BrdU labeling of dividing cells allows various types of analysis including birth-dating, cell cycle analysis, and evaluation of survival of newly generated cells following different experimental paradigms. Sultan et al. (2011) present original data obtained by coupling BrdU and activated caspase-3 labeling to cellular mapping. They analyzed the number, spatial distribution, and apoptosis of newborn cells in the granule cell layer of the olfactory bulb following olfactory conditioning paradigms. This revealed

a region-specific reduction in newborn cell death that correlates with the time point at which animals acquired the task. However, a key limitation of the BrdU method is its ability to recognize only a single pool of BrdU-positive cells at one time in the same animal. Llorens-Martín and Trejo (2011) describe the recent development of multiple birth-dating analyses involving the injection of different thymidine analogs (i.e., IdU and CldU) that can be unequivocally distinguished using specific antibodies. This method allows two to three cell subpopulations of different ages to be labeled in the same animal. Focusing on the hippocampus, the authors describe the main results obtained by this technique, outlining some of the key applications as well as the main concerns associated with multi-dating approaches.

Recent developments in genetic methods to identify and manipulate newborn neurons are invaluable tools to progress in the study of adult neurogenesis. Imayoshi et al. (2011) specifically discuss this topic describing the application of site-specific recombinases and the Tet inducible system in combination with transgenic or gene targeting strategy. They present several genetic models to suppress adult neurogenesis. An alternative method to deplete adult generated neurons is described in the article by Tan et al. (2011) that give technical details and results obtained by image-guided irradiation at different doses and survival time. Among the additional manipulation possibilities Imayoshi et al. (2011) also discuss the need to develop new genetic techniques to effectively increase neurogenesis in brain regions that are normally non-neurogenic. In a further refinement of genetic approaches, the review by Arenkiel (2011) describes approaches to reveal connectivity of newborn neurons in the adult brain, and to manipulate cell and circuit activity. He summarizes the current viral tracing methods, heterologous receptor expression systems, and optogenetic technologies that hold promise toward elucidating the wiring and circuit properties of adult-born neurons.

Two method papers detail new approaches to analyze integration of adult-born neurons relaying on immunohistochemical detection of endogenous markers. Rosenzweig and Wojtowicz (2011) describe the development of a method for *ex vivo* analysis of dendritic growth in immature adult-born hippocampal granule neurons. The method is based on laminar quantification of cell bodies, primary, secondary, and tertiary dendrites separately and independently from each other. The data demonstrate the suitability of this technique for analysis of dendritic growth and complexity comparing different experimental conditions. Luzzati et al. (2011) developed a new procedure suitable to analyze neurogenesis in parenchymal neurogenic niches, which represents a reliable alternative to the whole mount approaches to analyze cyto-architectural features of

adult germinative niche. The method, which is based on existing freeware software, combines confocal laser scanning microscopy and serial section reconstruction in order to span large volumes of brain tissue at cellular resolution. An example is described by investigating the morphology and spatial organization of a group of doublecortin-positive neuroblasts located in the lateral striatum of the late post-natal guinea pig.

The latest progress toward *in vivo* imaging of neurogenesis in animal models are discussed in the reviews by James et al. (2011) and Couillard-Despres et al. (2011). The first review centers on quantitative multi-photon microscopy applied to the dynamic study of SVZ-derived neuroblast migration in acute slices. In this paper James et al. (2011) present a brief overview of SVZ–neuroblast time-lapse imaging studies and the current knowledge of cellular patterns of SVZ–neuroblast migration, highlighting putative underlying regulatory mechanisms. They give technical useful suggestions for setting up a two-photon microscope imaging system and identifying several unsolved questions about SVZ–neuroblast migration. These questions might be addressed with current or emerging strategies to further harness the deep potential of two-photon microscopy.

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