Genetic fate-mapping of adult-generated hippocampal dentate granule cells to assess anesthesia-induced neurotoxicity

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Introduction

All commonly used anesthetics increase brain cell death in developing animals. The mechanisms underlying this toxicity, however, remain unknown. As an additional tool to examine anesthesia-induced neurotoxicity, we have developed a genetic fate-mapping approach to label newborn cells in the developing brain. This study will provide new insights into the long-term impact of anesthetics on developing neuronal populations.

Methods

- Gli1-CreER2T2 mice expressing a conditional, tamoxifen-inducible form of Cre-recombinase were crossed to a green fluorescent protein reporter line. (Figure 1)
- Bi-transgenic offspring were treated with 250mg/kg tamoxifen on P7 to activate cre-recombinase in Gli1-expressing neural progenitor cells, leading to the persistent expression of GFP in these cells and all their progeny.
- Mice were exposed to six hours of 1.5% isoflurane or room air two weeks later (P21). Brain structure was assessed immediately after anesthesia exposure, or following a 60-day recovery. (Figure 2)
- Brains were removed, post-fixed, cryoprotected in sucrose and snap frozen in 2-methyl-pentane at -25°C. Sagittal sections were cut on a crystal at 60μm, slides mounted and stored at ~80°C until use.
- Sections between 0.60 and 0.84 mm lateral to the midline were immunostained with 1:100 chicken anti-GFP and 1:100 anti-caspase-3 for the P21 groups; or 1:100 chicken anti-GFP, 1:200 mouse anti-calretinin and 1:200 rabbit anti-Ki67 for the P81 groups. Slides were rinsed in PBS, dehydrated in alcohol series, cleared in xylene, and mounted with Krytol.
- GFP-expressing cells were imaged using a Leica SP5 confocal microscope equipped with a 10X (0.3 NA) and 63X (1.4 NA) objectives. Confocal image stacks through the z-depth were collected from the midpoint of the upper and lower borders of the dentate cell body layer.
- Confocal image stacks were imported into Neurolucida software for quantification of GFP/Caspase-3 double labeling (P21 groups) and GFP/calretinin/Ki67 triple labeling (P81 groups). Counts were normalized to the volume of dentate present in each image stack.
- For all analyses, statistical significance was determined using Sigma Stat software (version 12.3), and appropriate statistical tests are noted in the results. All procedures conforms to National Institutes of Health and institutional guidelines for the care and use of animals. This work was supported by the Masimo-China-COHMC Pediatric Anesthesia Research Fellowship Program.

Figure 1. Cre-recombinase, under control of the Gli1 promoter, is initially inactive because it is fused to a modified estrogen receptor ligand binding domain, and cannot enter the nucleus of the cell. Tamoxifen treatment of these animals activates the CreER2T2 fusion protein, allowing it to enter the nucleus and excise the lox-P flanked stop codon. This leads to persistent GFP expression under the control of the β-actin promoter.

Figure 2. Bitransgenic offspring were treated with tamoxifen on P7, leading to Cre-mediated recombination in progenitor cells, and the persistent expression of GFP in these cells and all their progeny. On P21, animals were exposed to room air or 1.5% isoflurane for 6 hours. Either immediately after or 60 days after exposure, mice were sacrificed.

Figure 3. Anesthetic exposure increases caspase-3 expression in two-week-old dentate granule cells immediately following exposure. Sections are immunostained for green fluorescent protein (GFP) and caspase-3, a marker of apoptotic cell death. Scale bar = 250 μm

Figure 4. Images show representative cells after a six-hour exposure to isoflurane, which dramatically increased caspase-3 immunoreactivity. Arrows denote double-labeled cells. The two cells in the center of the image have short, aspiny dendrites projecting into the dentate molecular layer, morphological features of immature granule cells.

Conclusions

- Anesthetic exposure significantly increases cell death among two-week-old dentate granule cells.
- GFP expression reveals the morphology of caspase-3 immunoreactive cells.
- Fate-mapped dentate granule cell numbers recover 60 days after anesthesia exposure. The cohort of fate-mapped GFP-expressing granule cells exhibits similar rates of proliferation 60 days after anesthesia exposure.
- Mice exposed to anesthesia early in life show no evidence of granule cell migration defects in adulthood.

This study demonstrates the utility of the Gli1-CreER2T2::GFP (bitransgenic) fate-mapping approach.

These findings suggest that the dentate gyrus, a neurogenic niche region with life-long neurogenesis, can restore normal neuron numbers following a single, developmental exposure to isoflurane by modulating exposure rates of neurogenesis and/or natural apoptosis.