Majority of cells lining the walls of the 3rd ventricle in the adult rat brain are not neural progenitor cells

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Introduction
• The subventricular zone (SVZ) along the lateral wall of the lateral ventricles of the adult brain, as well as the subgranular zone (SGZ) of the dentate gyrus of the hippocampus, are sources of neurogenesis1.
• However, it remains unclear whether ependymal cells and tanyocytes, the cells lining the walls of the third ventricle, function as neural progenitor cells (NPCs)2-3.
• Tanyocytes and some ependymal cells express nestin, a class VI intermediate filament widely accepted as a marker for NPCs4.
• However, Hendrickson et al. found that few to none of the proliferating cells (BrdU+) in the third ventricle walls were cells that expressed nestin5.
• Therefore, we used a different cell proliferation marker, Ki67, to quantitatively measure the number of nestin-positive cells that proliferate in the third ventricle walls. We compared with BrdU results using fluorescence immunohistochemistry.

Methods
One adult male Sprague-Dawley rat brain was preserved in 4% paraformaldehyde and stored in 0.05% sodium azide until sectioning with a vibrating blade microtome (Leica VT1000 S). Chromogenic immunohistochemistry (IHC) was performed on 40 µm sections against the nuclear protein antigen Ki67. 10 mM sodium citrate buffer with heat was used for antigen retrieval. We used the primary antibody rabbit monoclonal anti-Ki67 (Thermo Scientific; clone SP6) at 1:500 dilution. The secondary antibody used was biotinylated donkey anti-rabbit IgG (Chemicon) at 1:250 dilution. Light cresyl staining was performed. Images were taken using a Nikon basic digital camera and widefield bright microscope.

Fluorescence IHC was performed on 40 µm sections to double stain for Ki67 and nestin. 10 mM sodium citrate buffer with heat was used for antigen retrieval. We used the primary antibody rabbit monoclonal anti-Ki67 (Thermo Scientific; clone SP6) at 1:500 dilution. The secondary antibody used was Alexa 488-conjugated goat anti-rabbit (Molecular Probes) at 1:500 dilution. For nestin, we used primary antibody mouse monoclonal anti-nestin at 1:1000 (Rat-401, Millipore). The secondary antibody we used was biotinylated donkey anti-mouse (Jackson Immunoresearch) at 1:250, followed by tyramide treatment using the TSA kit (PerkinElmer). Cell nuclei were stained with DAPI (Sigma). Images were produced using a Nikon A1Rl confocal fluorescence microscope coupled with a Bio-Rad MRC-1024 laser confocal scanning system. Every third section was stained through the entire ventricle, and Ki67+/nestin- and Ki67-/nestin+ cells were counted.

Results

Question: Do cells lining the third ventricle walls of the adult rat brain act as neural progenitor cells?

Conclusions
• The majority of nestin-positive cells in the walls of the 3rd ventricle do not proliferate under normal conditions6.
• Of the cells that did proliferate, only a small percentage may be producing neurons5.
• Tanyocytes may play a significant structural and/or secretory role in brain function7.
• Different results using the different proliferation markers (BrdU and Ki67).
• Next Step
To determine the exact function of nestin-positive cells lining the walls of the 3rd ventricle

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References
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