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# Studying oligodendrocyte precursor cell differentiation and migration using immunohistochemistry

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## Introduction

In the adult mammalian brain, multipotent progenitor cells involved in the reproduction of neurons and glial cells have been well investigated only in two germinal regions: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus. However, one class of glial cells, the oligodendrocyte precursor cells (OPCs) have been found to multiply and sometimes differentiate into neurons in vitro. OPCs in the developing and mature CNS were mainly thought to serve as the primary source of remyelinating cells called oligodendrocytes. However, recent reports on the electrophysiological properties of OPCs have challenged the notion that they are committed progenitor cells of the oligodendrocyte lineage with the sole function of generating these myelin-forming cells. Recent studies have shown that some OPCs may differentiate into functional neurons, that is, not all OPCs may be committed to becoming oligodendrocytes. These studies have provided evidence suggesting the existence of GABAergic cortical neurons issued from OPCs with spontaneous synaptic currents and an ability to fire action potentials—both indicators of a neuronal phenotype. These findings support the idea that GABAergic cortical neurons could be derived from the OPCs.

In this study, adult mice are injected with 5-Bromodeoxyuridine (BrdU), a uridine analog that is incorporated into DNA, which serves as a marker for cell division. Doublecortin (DCX) is a microtubule-associated protein strongly expressed by neuronal precursor cells during neuron migration from the subventricular space and the subgranular zone of the dentate gyrus. The alpha receptor for platelet-derived growth factor (PDGFR), are proteins expressed in OPCs and is one of the markers which specifically determines the presence of OPCs as this protein is only expressed by proliferating progenitor cells.

Immunohistochemistry is used to detect BrdU and DCX in order to investigate whether new neurons can be derived from OPCs. Specifically, we examined closely associated pairs of OPCs under fluorescent microscopy and determine if they have recently undergone division. Based on previous research, we hypothesized that the role of OPCs is not solely to differentiate into oligodendrocytes but also possibly become neurons.

## Results

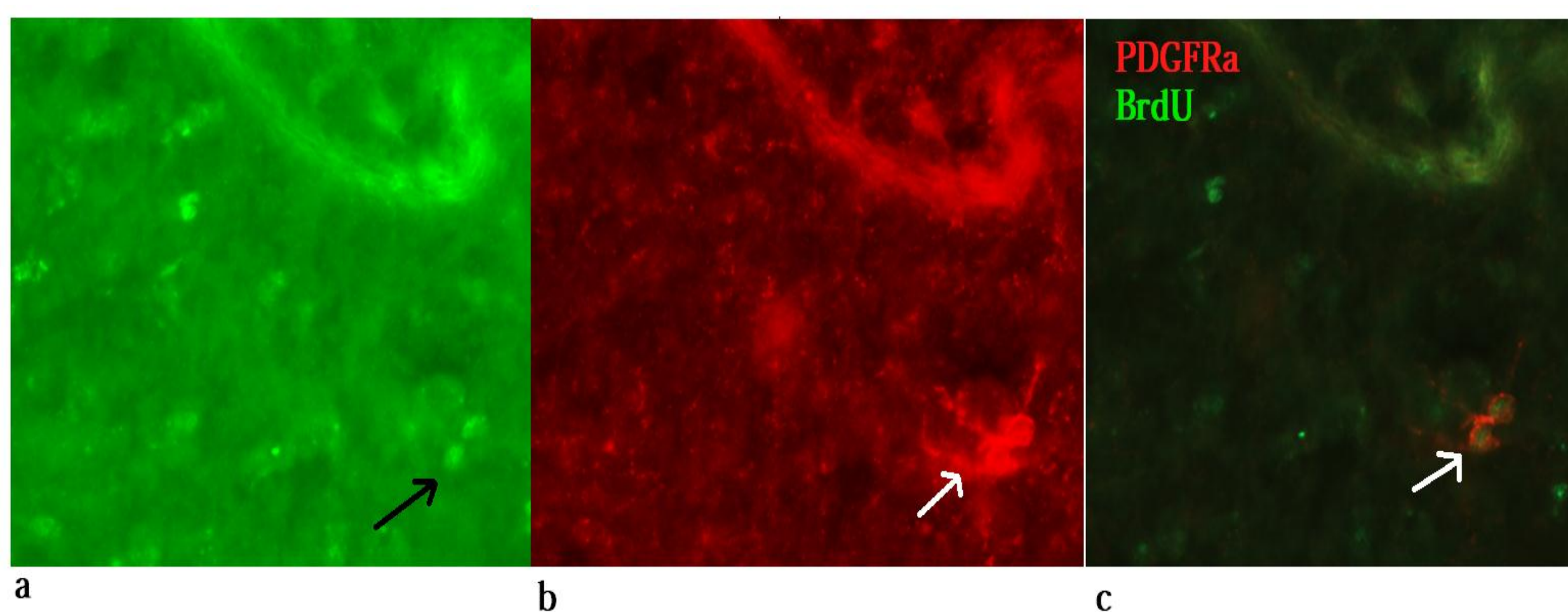
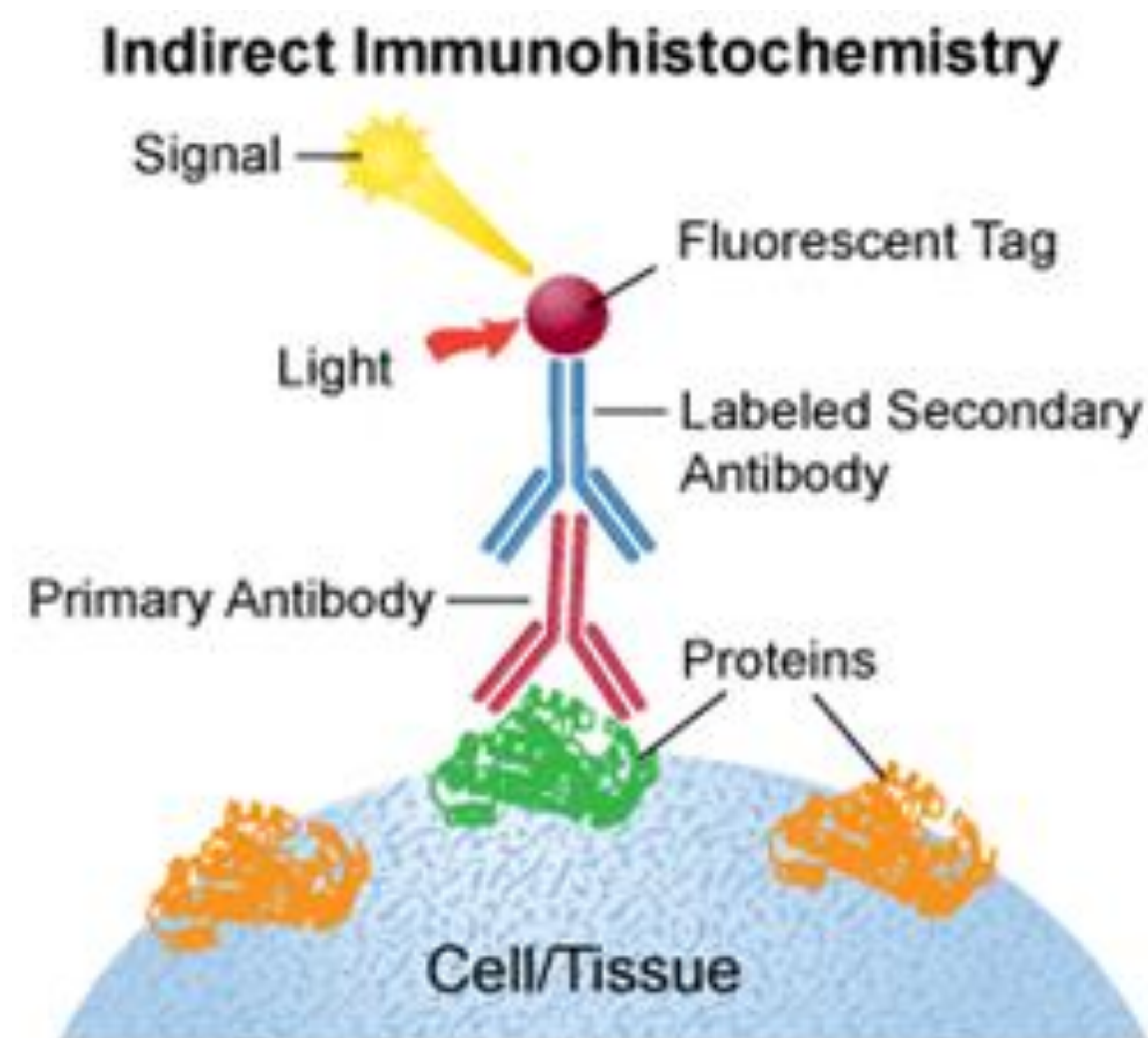


Figure 1. BrdU (a), PDGFR $\alpha$  (b), and double stained (c) sagittal brain slice showing a pair of oligodendrocyte precursor cells above the cerebellum at 20x magnification.

## Principle of Immunohistochemistry



## Discussion

Areas of the cortex, non-dentate gyrus hippocampal tissue, the cerebellum and the corpus callosum were examined to determine if any closely associated pairs of OPCs could be observed. A total of 6 pairs were found and their identity as OPCs was confirmed with both nuclei staining antibodies for PDGFR-alpha and the Hoescht stain under fluorescent microscopy first.

Figure 1 shows a single pair of OPCs after double labelling with BrdU and PDGFR $\alpha$  only, while DCX labelling was not observed. The expression of PDGFR proves this pair is OPC as presence of the PDGFR serves as a marker for OPCs, while BrdU incorporation into neural nuclei suggests the cells were actively dividing during the time they were injected with BrdU. Due to the absence of DCX expression, we cannot conclude whether the OPC's are migrating as the presence of this protein is a marker for migrating cells.

The BrdU labelling resulted in a very low intensity of fluorescence and may have been a result of some experimental errors. For example, before the BrdU epitope can be expressed, 2N HCL is added to denature the tissues and expose the epitope. Initially, the optimal concentration of hydrochloric acid needed was miscalculated and improper denaturation of the cells means that the BrdU epitope was not properly exposed to the BrdU antibody within the nuclei of the OPCs.

Additionally, the primary and secondary antibodies used for BrdU was generated in sheep and goat, which have very similar genomes. During antibody synthesis, these two were not incubated with each other. Some kind of interaction is occurring between the antibodies and may be a possible factor affecting the staining. It may have also caused the overall fluorescence intensity of the antibodies to decrease. If triple labelling was successful, OPCs would appear yellow when all three figures are merged together.

## Methods

### BrdU injection and perfusion

12-week old C57 mice (n=2) received i.p. injections of BrdU (Sigma) at a concentration of 200mg/kg for 7 consecutive days. The mice were transcardially perfused forty-eight hours following the last injection.

### Immunohistochemistry

First, 14um-thick brain sections were cut using a cryostat and treated with 2N HCl to reveal the BrdU epitope inside the cell nucleus. Then, sections were stained using an anti-BrdU antibody (abcam). The sections were triple labelled with an anti-doublecortin antibody (DCX; Santa Cruz) and an anti-alpha receptor for platelet-derived growth factor antibody (PDGFR $\alpha$ ; Millipore). The cell nuclei were coloured using Hoescht 33342 stain (Invitrogen).

Fluorescent microscopy was used to observe and document the staining.

## Conclusions

Out of the six pairs of recently divided OPCs, a single pair of OPCs was found to be double labelled with BrdU and PDGFR $\alpha$ , which confirms it was recently divided and was a pair of OPCs.

Due to the absence of DCX expression, we cannot conclude whether the OPC's are migrating as the presence of this protein is a marker for migrating cells.

## Future Studies

Future studies should repeat this process with a different antibody or perhaps a different marker for OPC migration. Although not tested in this study, one of our the hypotheses is that OPCs may serve functions other than become oligodendrocytes or neurons, so a different marker which may determine the presence of electrophysiological properties may further enhance the hypothesis that these OPCs serve other important functions and are not quiescent cells.

## References

- Belachew, S., Chittajallu, R., Aguirre, A.A., Yuan, X., Kirby, M., Anderson, S., Gallo, V.(2003). Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *Journal of Cell Biology*, 161, 169-186.
- Cameron, H.A., Dayer, A.G. (2008). New Interneurons in the Adult Neocortex: Small, Sparse, but Significant? *Biological Psychiatry*, 63, 650-655.
- Dayer, A.G., Cleaver, K.M., Abouantoun, T., Cameron, H.A. (2005). New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. *Journal of Cell Biology*, 168, 415-427.
- Nishiyama, A., Komitova, M., Suzuki, R., Zhu, X. (2009). Polydendrocytes (NG2 cells) multifunctional cells with lineage plasticity. *Nature Reviews Neuroscience*, 10, 9-22.

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