Neuron
Review

NG2-glia as Multipotent Neural Stem Cells: Fact or Fantasy?

William D. Richardson,[1,*] Kaylene M. Young,[2,3] Richa B. Tripathi,[1] and Ian McKenzie[1]
[1]Wolfson Institute for Biomedical Research and Research Department of Cell and Developmental Biology, University College London (UCL), Gower Street, London WC1E 6BT, UK
[2]Menzies Research Institute, The University of Tasmania, Hobart 7001, Australia
[3]Present address: Research Department of Neuroscience, Physiology and Pharmacology, UCL, Gower Street, London WC1E 6BT, UK
*Correspondence: w.richardson@ucl.ac.uk
DOI 10.1016/j.neuron.2011.05.013

not immediately obvious. Although it seemed likely that the adult cells were descended by lineage from their perinatal counterparts this was not formally demonstrated until later, with the advent of genetic fate-mapping approaches in transgenic mice (see below). In this article we refer to both the perinatal and adult cells as NG2-glia.

The sheer number of NG2-glia in the adult brain and their uniform distribution in both gray and white matter seemed counterintuitive. Given their presumed role as oligodendrocyte precursors, should they not be concentrated in white matter where they would presumably be in most demand for myelinating axons? Why should so many precursor cells persist in the mature adult brain in any case? Moreover, the complex process-bearing morphology of NG2-glia in vivo seemed more in keeping with differentiated cells than immature precursors. Perhaps NG2-glia served a dual purpose—as a source of oligo-

injury. It also raises a raft of intriguing new questions concerning the role of myelination during normal adulthood.

Cre-lox Fate Mapping: Potential Pitfalls

generation in the developing cortex, for example (Mission et al., 1991). The given scenario is consistent with a study using *Olig2-C eER**, in which some astrocytes as well as oligodendrocytes (and motor neurons) were found among the progeny of *Olig2*-expressing neuropithelial precursors in the embryonic ventral spinal cord (Masahira et al., 2006).

Whatever the precise sequence of events during prenatal gliogenesis, it now seems likely that NG2-glia do not generate astrocytes during normal healthy adulthood. Several Cre-lox studies—using *Pdgf a-C eER** (two independent lines: Rivers et al., 2008; Kang et al., 2010), *NG2-C eER** (Zhu et al., 2011; see above), and *Olig2-C eER** (Dimou et al., 2008) converge on that conclusion. While some reporter-positive astrocytes were observed in the cortical gray matter in the *Olig2-C eER** line, their number did not increase significantly between 8 and 65 days post-tamoxifen administration, indicating that they were not generated continuously from dividing NG2-glia (Dimou et al., 2008). It appears that the *Olig2-C eER** transgene is expressed in some protoplasmic astrocytes in the normal gray matter, resulting in labeling of some of these in addition to NG2-glia. A subsequent study from the same lab (Simon et al., 2011) marked NG2-glia in a different way, by long-term BrdU labeling of 2- to 3-month-old mice, and confirmed that no astrocytes were found among their pr-glia./Crgcs/T1scnR*n(al.,)Gd[(ge(were)-489(f28t)-211(NG2udieFacttheir)3(motra)Fasy?(2011)]TJ00*)Tj/T10

levels −2.3 mm to −1.1 mm), whereas ours were predominantly in the aPC.

generation but little or no astrocyte production. An accumulation of GFAP$^+$ BrdU$^+$ reactive astrocytes was found in the vicinity of the lesion, as expected, but these were mostly reporter-negative (i.e., not NG2-glia derived). Very similar results to these were reported following cortical stab wounds in *NG2-C eER*: R a26-YFP* mice (Komitova et al., 2011). A subsequent BrdU fate mapping study (Simon et al., 2011) failed to find evidence for any astrocyte production from dividing NG2-glia after cortical stab injury. The emerging consensus from these studies is that the reactive (hypertrophic, strongly GFAP$^+$) astrocytes that form the glial ''scar'' around sites of injury in the cortex are derived predominantly or exclusively from pre-existing astrocytes, not from NG2-glia. This conclusion has been supported by complementary experiments in which astrocytes were labeled specifically by injecting a *GLAST-C eER** lentiviral vector into the cortex of reporter mice, and their fates followed before and after cortical stab injury (Buffo et al., 2008). Before injury, the labeled astrocytes were quiescent (did not incorporate BrdU after a long label) but, after injury, they started dividing and generated many new astrocytes, but not other cell types, at the site of the wound.

This also seems to be what happens after spinal cord injury. Barnabé-Heider et al. (2010) made a transverse cut through the dorsal funiculus of the spinal cord, severing the ascending and descending axon tracts. They observed new oligogenesis but insignificant astrocyte production from NG2-glia (marked using *Olig2-C eER**), despite a robust astrocytic reaction/gliosis. Most interestingly, they identified two separate components of the astrocytic reaction—a localized accumulation of GFAP$^+$ astrocytes at the core of the lesion site in the dorsal funiculus and a more diffuse accumulation/gliosis around the lesion site and throughout the spinal cord at the level of the injury. These two components had different cellular origins; the ''core'' astrocytes were derived from multipotent stem cells in the ependymal zone surrounding the central canal of the cord (labeled with *F J1-C eER**), whereas the ''diffuse'' astrocytes were derived from pre-existing astrocytes in the parenchyma (labeled with *C e i 30-C eER**). It is curious that Barnabé-Heider et al. (2010) found that all protoplasmic astrocytes in the spinal cord were Olig2-immunoreactive in their experiments. Despite this, the *Olig2-C eER** transgene did not drive recombination in astrocytes, perhaps because the level of CreER* expression was below threshold (see above, under heading ''Cre-lox Fate Mapping: Potential Pitfalls''). While this worked out well for Barnabé-Heider et al. (2010), it does raise the possibility that *Olig2-C eER** might trigger recombination in astrocytes in addition to oligodendrocyte lineage cells in some circumstances.

In marked contrast to the above is a study by Tatsumi et al. (2008), who followed the fates of NG2-glia following freeze-thaw lesions in the cerebral cortex. They used *Olig2-C eER**

mice (the same line used by both Dimou et al. [2008] and Barnabé-Heider et al. [2010]) to mark presumptive NG2-glia prior to injury and reported a robust proliferative response followed by production of ''bushy'' protoplasmic astrocytes between one and 2 weeks postinjury. Astrocytes appeared to be the major differentiated product of NG2-glia in this injury model; oligodendrocytes were not observed. However, in this study, as in that of Dimou et al. (2008), *Olig2-C eER** triggered recombination in some protoplasmic astrocytes in addition to NG2-glia in the uninjured cortex (~20% of reporter-positive cells were astrocytes at short times after 4HT administration; Tatsumi et al., 2008). This leaves open the possibility that the reactive astrocytes formed after injury were derived from division of pre-existing astrocytes. Tatsumi et al. (2008) discounted this idea because they failed to find BrdU-labeled $GFAP^+$ astrocytes shortly after injury but it is possible that there could have been a delayed mitogenic response of astrocytes or slow upregulation of GFAP in previously GFAP-negative astrocytes, either of which might have obscured a transient population of $BrdU^+$ astrocytes. Nevertheless, the apparent absence of oligodendrocyte production in the experiments of Tatsumi et al. (2008) marks their study out from the others; perhaps the particular environment of the freeze-thaw injury, as compared to stab injury for example, inhibits NG2-glia from differentiating into oligodendrocytes. It is important to confirm or refute this observation through cryolesioning experiments in different *C eER** mouse lines, because it could perhaps provide a link to late-stage multiple sclerosis lesions, in which inhibition of oligodendrocyte differentiation is thought to contribute to remyelination failure.

Other researchers have examined the response of NG2-glia during experimentally induced demyelination. In a gliotoxin-induced focal demyelination model, Zawadzka et al. (2010) found robust remyelination from NG2-glia (labeled using *Pdgf a-C eER*: R a26-YFP*)—as expected from previous studies (Redwine and Armstrong, 1998; Watanabe et al., 2002; Dawson et al., 2003; reviewed by Franklin and ffrench-Constant, 2008; Figure 1F). A small proportion of YFP^+ cells were Aquaporin-4^+ astrocytes (~3%), but the great majority of reactive astrocytes were derived from *Fgf 3*-expressing cells (ependymal cells and/or preexisting astrocytes) (Young et al., 2010), because they were YFP-labeled in *Fgf 3-C eER*: R a26-YFP* mice (Zawadzka et al., 2010). Schwann cells, the myelinating cells of the peripheral nervous system (PNS), are commonly found in remyelinating CNS lesions including some human multiple sclerosis lesions. Often these remyelinating Schwann cells surround blood vessels, which in the past has been taken to suggest that they enter the CNS from the PNS, using the vessels as a migration route. However Zawadzka et al. (2010) found that most remyelinating Schwann cells ($Periaxin^+$) in their CNS lesions were YFP^+ in *Pdgf a-C eER*: R a26-YFP* mice, suggesting that they were derived from NG2-glia (Figure 1G). In strong support of this,-423(p7ee)-269(C-xprigodeat)3298(Schwann)-262(cells)-421(were)-26alsoat(labeled4-237(in)]TJ/T11TJT*Olig2ra-C:on Rosa26-YFP

cord, from ependymal cells around the central canal. The latter cells represent a relatively unexplored population that is a key target for future investigation. It will be important to discover whether these cells retain, or can be induced to recapitulate, some of the neuronogenic flavor of their forebears in the embryonic neuroepithelium.

Oligodendrocyte Generation and Myelin Dynamics in the Adult

Most newly formed oligodendrocytes in the postnatal forebrain survive long-term and myelinate axons. Myelin formation has been demonstrated by microinjecting (YFPNy)-308429rmed)-317(longodendrocytes)-317(It)weli46.3-9.734.299TD[ds[(linate)-

experience and that such memories are an intrinsic part of the circuits involved in the behavioral response, not something that is generated or stored remotely (Carew and Sahley, 1986). The general idea is that, during repetitive use, circuit connectivity becomes strengthened so that the neurons comprising the circuit acquire a lower threshold for firing and become more

et al., 2007) or by physical exercise (Simon et al., 2011). Therefore, a key research focus for the future is the potential role of adult myelination in learning and memory and how that might be affected by the environment.

ACKNOWLEDGMENTS

We thank David Attwell (UCL) and three anonymous reviewers for their constructive comments and suggestions for improvement. Work in the authors' laboratory was supported by the UK Medical Research Council (MRC), The Wellcome Trust, and the National Institutes of Health, USA. I.M. was the recipient of a Royal Society USA/Canada Exchange Fellowship. K.M.Y. is supported by the BUPA Foundation and the Alzheimer's Society, UK.

REFERENCES

Aguirre, A., and Gallo, V. (2004). Postnatal neurogenesis and gliogenesis in the olfactory bulb from NG2-expressing progenitors of the subventricular zone. J. Neurosci. *24*, 10530–10541.

Aguirre, A.A., Chittajallu, R., Belachew, S., and Gallo, V. (2004). NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the postnatal hippocampus. J. Cell Biol. *165*, 575–589.

Arisi, G.M., Foresti, M.L., Mukherjee, S., and Shapiro, L.A. (2011). The role of olfactory stimulus in adult mammalian neurogenesis. Behav. Brain Res., in press. Published online March 29, 2011. 10.1016/j.bbr.2011.03.05.

Bakiri, Y., Karadottir, R., Cossell, L., and Attwell, D. (2010). Morphological and electrical properties of oligodendrocytes in the white matter of the corpus callosum and cerebellum. J. Physiol. *589*, 559–573.

Barnabé-Heider, F., Göritz, C., Sabelström, H., Takebayashi, H., Pfrieger, F.W., Meletis, K., and Frisén, J. (2010). Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell *7*, 470–482.

Barres, B.A., and Raff, M.C. (1993). Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature *361*, 258–260.

Barres, B.A., Koroshetz, W.J., Swartz, K.J., Chun, L.L., and Corey, D.P. (1990). Ion channel expression by white matter glia: the O-2A glial progenitor cell. Neuron *4*, 507–524.

Bartzokis, G., Beckson, M., Lu, P.H., Nuechterlein, K.H., Edwards, N., and Mintz, J. (2001). Age-related changes in frontal and temporal lobe volumes in men: a magnetic resonance imaging study. Arch. Gen. Psychiatry *58*, 461–465.

Bartzokis, G., Lu, P.H., Tingus, K., Mendez, M.F., Richard, A., Peters, D.G., Oluwadara, B., Barrall, K.A., Finn, J.P., Villablanca, P., et al. (2010). Lifespan trajectory of myelin integrity and maximum motor speed. Neurobiol. Aging *31*, 1554–1562.

Belachew, S., Chittajallu, R., Aguirre, A.A., Yuan, X., Kirby, M., Anderson, S., and Gallo, V. (2003). Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J. Cell Biol. *161*, 169–186.

Bengtsson, S.L., Nagy, Z., Skare, S., Forsman, L., Forssberg, H., and Ullén, F. (2005). Extensive piano practicing has regionally specific effects on white matter development. Nat. Neurosci. *8*, 1148–1150.

Bergles, D.E., Roberts, J.D., Somogyi, P., and Jahr, C.E. (2000). Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature *405*, 187–191.

Bernier, P.J., Bedard, A., Vinet, J., Levesque, M., and Parent, A. (2002). Newly generated neurons in the amygdala and adjoining cortex of adult primates. Proc. Natl. Acad. Sci. USA *99*, 11464–11469.

Bliss, T.V., and Collingridge, G.L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. Nature *361*, 31–39.

Bostock, H., and Sears, T.A. (1976). Continuous conduction in demyelinated mammalian nerve fibers. Nature *263*, 786–787.

Buffo, A., Rite, I., Tripathi, P., Lepier, A., Colak, D., Horn, A.P., Mori, T., and Götz, M. (2008). Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. Proc. Natl. Acad. Sci. USA *105*, 3581–3586.

Bullmann, T., Härtig, W., Holzer, M., and Arendt, T. (2010). Expression of the embryonal isoform (0N/3R) of the microtubule-associated protein tau in the adult rat central nervous system. J. Comp. Neurol. *518*, 2538–2553.

Butt, A.M., Duncan, A., Hornby, M.F., Kirvell, S.L., Hunter, A., Levine, J.M., and Berry, M. (1999). Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. Glia *26*, 84–91.

Butt, A.M., Kiff, J., Hubbard, P., and Berry, M. (2002). Synantocytes: new functions for novel NG2 expressing glia. J. Neurocytol. *31*, 551–565.

Butt, A.M., Hamilton, N., Hubbard, P., Pugh, M., and Ibrahim, M. (2005). Synantocytes: The fifth element. J. Anat. *207*, 695–706.

Carew, T.J., and Sahley, C.L. (1986). Invertebrate learning and memory: From behavior to molecules. Annu. Rev. Neurosci. *9*, 435–487.

Chang, A., Nishiyama, A., Peterson, J., Prineas, J., and Trapp, B.D. (2000). NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. J. Neurosci. *20*, 6404–6412.

Chittajallu, R., Aguirre, A., and Gallo, V. (2004). NG2-positive cells in the mouse white and grey matter display distinct physiological properties. J. Physiol. *561*, 109–122.

Dawson, M.R., Polito, A., Levine, J.M., and Reynolds, R. (2003). NG2-expressing glial progenitor cells: An abundant and widespread population of cycling cells in the adult rat CNS. Mol. Cell. Neurosci. *24*, 476–488.

De Biase, L.M., Nishiyama, A., and Bergles, D.E. (2010). Excitability and synaptic communication within the oligodendrocyte lineage. J. Neurosci. *30*, 3600–3611.

Delaunay, D., Heydon, K., Miguez, A., Schwab, M., Nave, K.A., Thomas, J.L., Spassky, N., Martinez, S., and Zalc, B. (2009). Genetic tracing of subpopulation neurons in the prethalamus of mice (Mus musculus). J. Comp. Neurol. *512*, 74–83.

Diers-Fenger, M., Kirchhoff, F., Kettenmann, H., Levine, J.M., and Trotter, J. (2001). AN2/NG2 protein-expressing glial progenitor cells in the murine CNS: Isolation, differentiation, and association with radial glia. Glia *34*, 213–228.

Dimou, L., Simon, C., Kirchhoff, F., Takebayashi, H., and Götz, M. (2008). Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J. Neurosci. *28*, 10434–10442.

Doerflinger, N.H., Macklin, W.B., and Popko, B. (2003). Inducible site-specific recombination in myelinating cells. Genesis *35*, 63–72.

Doetsch, F., Caillé, I., Lim, D.A., García-Verdugo, J.M., and Alvarez-Buylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell *97*, 703–716.

Dore-Duffy, P., Katychev, A., Wang, X., and Van Buren, E. (2006). CNS microvascular pericytes exhibit multipotential stem cell activity. J. Cereb. Blood Flow Metab. *26*, 613–624.

ffrench-Constant, C., and Raff, M.C. (1986). Proliferating bipotential glial progenitor cells in adult rat optic nerve. Nature *319*, 499–502.

Fields, R.D. (2008). White matter in learning, cognition and psychiatric disorders. Trends Neurosci. *31*, 361–370.

Franklin, R.J., and ffrench-Constant, C. (2008). Remyelination in the CNS: From biology to therapy. Nat. Rev. Neurosci. *9*, 839–855.

Gallo, V., Zhou, J.M., McBain, C.J., Wright, P., Knutson, P.L., and Armstrong, R.C. (1996). Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K^+ channel block. J. Neurosci. *16*, 2659–2670.

Geha, S., Pallud, J., Junier, M.P., Devaux, B., Leonard, N., Chassoux, F., Chneiweiss, H., Daumas-Duport, C., and Varlet, P. (2010). $NG2^+$/$Olig2^+$ cells are the major cycle-related cell population of the adult human normal brain. Brain Pathol. *20*, 399–411.

Gregg, C., Shikar, V., Larsen, P., Mak, G., Chojnacki, A., Yong, V.W., and Weiss, S. (2007). White matter plasticity and enhanced remyelination in the maternal CNS. J. Neurosci. *27*, 1812–1823.

Guo, F., Ma, J., McCauley, E., Bannerman, P., and Pleasure, D. (2009). Early postnatal proteolipid promoter-expressing progenitors produce multilineage cells in vivo. J. Neurosci. *29*, 7256–7270.

Guo, F., Maeda, Y., Ma, J., Xu, J., Horiuchi, M., Miers, L., Vaccarino, F., and Pleasure, D. (2010). Pyramidal neurons are generated from oligodendroglial progenitor cells in adult piriform cortex. J. Neurosci. *30*, 12036–12049.

Hamilton, N., Hubbard, P.S., and Butt, A.M. (2009). Effects of glutamate receptor activation on NG2-glia in the rat optic nerve. J. Anat. *214*, 208–218.

Hasan, K.M., Kamali, A., Kramer, L.A., Papnicolaou, A.C., Fletcher, J.M., and Ewing-Cobbs, L. (2008). Diffusion tensor quantification of the human midsagittal corpus callosum subdivisions across the lifespan. Brain Res. *1227*, 52–67.

Hayashi, T., Seki, T., Sato, K., Iwai, M., Zhang, W.R., Manabe, Y., and Abe, K. (2001). Expression of polysialylated neural cell adhesion molecule in rat brain after transient middle cerebral artery occlusion. Brain Res. *907*, 130–133.

Honjin, R., Sakato, S., and Yamashita, T. (1977). Electron microscopy of the mouse optic nerve: A quantitative study of the total optic nerve fibers. Arch. Histol. Jpn. *40*, 321–332.

Horner, P.J., Power, A.E., Kempermann, G., Kuhn, H.G., Palmer, T.D., Winkler, J., Thal, L.J., and Gage, F.H. (2000). Proliferation and differente4v38(F.,201(333(ne9Ja.oe-(Arch.)]TJ0-1.146(W.R.,iDhe)-28656–7Nr7wtoughout22677324(the)-3int79(Dhe)-287(ad

Patneau, D.K., Wright, P.W., Winters, C., Mayer, M.L., and Gallo, V. (1994). Glial cells of the oligodendrocyte lineage express both kainate- and AMPA-preferring subtypes of glutamate receptor. Neuron *12*, 357–371.

Paukert, M., and Bergles, D.E. (2006). Synaptic communication between neurons and $NG2^+$ cells. Curr. Opin. Neurobiol. *16*, 515–521.

Pekcec, A., Löscher, W., and Potschka, H. (2006). Neurogenesis in the adult rat piriform cortex. Neuroreport *17*, 571–574.

Peters, A. (2004). A fourth type of neuroglial cell in the adult central nervous system. J. Neurocytol. *33*, 345–357.

Pringle, N.P., Mudhar, H.S., Collarini, E.J., and Richardson, W.D. (1992). PDGF receptors in the rat CNS: During late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. Development *115*, 535–551.

Psachoulia, K., Jamen, F., Young, K.M., and Richardson, W.D. (2009). Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. Neuron Glia Biol. *5*, 57–67.

Raff, M.C., Miller, R.H., and Noble, M. (1983). A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature *303*, 390–396.

Redwine, J.M., and Armstrong, R.C. (1998). In vivo proliferation of oligodendrocyte progenitors expressing PDGFalphaR during early remyelination. J. Neurobiol. *37*, 413–428.

Richardson, W.D., Kessaris, N., and Pringle, N.P. (2006). Oligodendrocyte wars. Nat. Rev. Neurosci. *7*, 11–18.

Rivers, L., Young, K., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., Kessaris, N., and Richardson, W.D. (2008). PDGFRA/NG2-positive glia generate myelinating oligodendrocytes and cortical projection neurons in the adult mouse CNS. Nat. Neurosci. *11*, 1392–1401.

Salter, M.G., and Fern, R. (2005). NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature *438*, 1167–1171.

Scholz, J., Klein, M.C., Behrens, T.E.J., and Johansen-Berg, H. (2009). Training induces changes in white matter architecture. Nat. Neurosci. *12*, 1370–1371.

Seki, T., and Arai, Y. (1991). Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. Anat. Embryol. (Berl.) *184*, 395–401.

Seki, T., and Arai, Y. (1993). Distribution and possible roles of the highly polysialylated neural cell adhesion molecule (NCAM-H) in the developing and adult central nervous system. Neurosci. Res. *17*, 265–290.

Shapiro, L.A., Ng, K.L., Zhou, Q.Y., and Ribak, C.E. (2007). Hd(43[(Raff,fers.)-309enrrds1.138TD[(sialylate(F.tsari59en77(chang249(develoi59sur(prervou38(he)-2)-2newoly)-42(bo