

1 **Diversity in the oligodendrocyte lineage: plasticity or heterogeneity**

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3 Sarah Foerster, Myfanwy FE Hill and Robin JM Franklin

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5 *Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, Clifford Allbutt*
6 *Building, Cambridge Biomedical Campus, University of Cambridge, Cambridge CB2 0AH, UK*

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24 **Abstract**

25

26 Heterogeneity is a widely recognised phenomenon within the majority of cell types in the
27 body including cells of the central nervous system (CNS). The heterogeneity of neurons based
28 on their distinct transmission modes and firing patterns has been recognised for decades, and
29 is necessary to coordinate the immense variety of functions of the CNS. More recently,
30 heterogeneity in glial cells has been described, including heterogeneity in oligodendrocyte
31 progenitor cells (OPCs) and oligodendrocytes. OPC subpopulations have been described
32 based on their developmental origin, anatomical location in the grey or white matter, and
33 expression of surface receptors. Oligodendrocytes are categorised according to differences in
34 gene expression, myelinogenic potential and axon specificity. Much of what is described as
35 heterogeneity in oligodendrocyte lineage cells (OLCs) is based on phenotypic differences.
36 However, without evidence for functional differences between putative subgroups of
37 oligodendrocyte lineage cells (OLCs), distinguishing heterogeneity from plasticity and lineage
38 state is difficult. Identifying functional differences between phenotypically distinct groups is
39 therefore necessary for a deeper understanding of the role of OLCs in health and disease.

40

41 **Key words**

42

43 oligodendrocyte, oligodendrocyte progenitor cell, heterogeneity, myelin, remyelination

44

45 **Main points**

46

- 47 1. Phenotypic differences have been described between subpopulations within the cells of
48 the oligodendrocyte lineage.
- 49 2. Heterogeneity cannot be distinguished from functional plasticity based solely on
50 phenotypic differences.
- 51 3. Distinct functional differences between subclasses of oligodendrocyte lineage cells need to
52 be demonstrated unambiguously to prove heterogeneity.

53

54

55 **Introduction**

56

57 The central nervous system (CNS) integrates information it receives from all parts of the body,
58 and in turn coordinates and influences their activity. To coordinate this immense variety of
59 functions, different neuronal subtypes with distinct transmission modes and firing patterns
60 are necessary. Similarly, region-specific astrocyte functions are required for the maintenance
61 of CNS homeostasis and neuronal survival (Tsai et al., 2012). These examples demonstrate a
62 functional heterogeneity of different cell types in the CNS, raising the question whether a
63 similar heterogeneity exists for oligodendrocyte lineage cells (OLCs, an umbrella term for
64 oligodendrocyte progenitor cells (OPCs) and their progeny oligodendrocytes). Evidence for
65 diversity within both the oligodendrocyte and OPC populations has accumulated over the last
66 decade. However, there is not yet a fully coherent perspective on the functional implications
67 of this diversity or the extent to which this diversity represents true heterogeneity as distinct
68 from functional plasticity. There are several different methods of categorising heterogeneity
69 of OPCs and oligodendrocytes, many of which are not mutually exclusive. Here we examine
70 the evidence in support of the OLCs being a heterogenous cell population and discuss what
71 the functional roles for these different sub populations might be.

72

73 **Definition of heterogeneity**

74

75 The term heterogeneity derives from the Greek for 'heteros' (ἕτερος), meaning two, other or
76 different, and 'genesis' from the Latin, originally borrowed from the Greek (γένεσις), meaning
77 origin or development (Oxford English Dictionary). Therefore, implicit in the term is the sense
78 that, for a population to exhibit heterogeneity, its components must have distinct
79 developmental origins. However, currently it is more commonly used to describe a situation
80 where, in addition to origin, a single cell type can show distinct morphological and/or
81 phenotypic profiles, including gene expression, and a distinctive range of functions including
82 proliferation potential, motility, and response to injury. The gold-standard to unambiguously
83 identify heterogeneous populations of a cell type is the proof of functional differences. A
84 critical point is that true heterogeneity should not be confused with identification of cells at
85 different cell states within a cell population (e.g. adult versus adult activated OPCs following

86 injury), which is better termed functional plasticity, or cells captured at different points along
87 a differentiation path (e.g. pre-myelinating versus mature oligodendrocytes).

88

89 **Defining OPCs and Oligodendrocytes**

90

91 In the adult CNS, OPCs are estimated to comprise at least 5% of all cells, residing in both white
92 and grey matter (Dawson, Polito, Levine, & Reynolds, 2003; Pringle, Mudhar, Collarini, &
93 Richardson, 1992). Typically, OPCs are identified by the presence of the proteoglycan NG2
94 (Stallcup & Beasley, 1987) or by platelet derived growth factor receptor A (PDGFRA) (Pringle
95 et al., 1992). *In vivo* lineage tracing studies show that the vast majority of OPCs express both
96 NG2 and PDGFRA (Figure 1) (Kang, Fukaya, Yang, Rothstein, & Bergles, 2010; Karram et al.,
97 2008; Rivers et al., 2008); hence, the two marker proteins can be used interchangeably,
98 rendering it possible to compare studies performed using either marker. Additionally, the
99 ganglioside antibody A2B5 is used for the identification of OPCs in *in vitro* studies (Raff, Miller,
100 & Noble, 1983). Immunostaining of OPCs isolated using A2B5 indicates that the vast majority
101 of these cells also express NG2 and PDGFRA (Figure 1) (unpublished data from our laboratory).
102 However, neither marker is exclusively restricted to OPCs: NG2 can label activated microglia
103 and pericytes, PDGFRA can also label pericytes while the A2B5 antibody can label neural stem
104 cells and neurons. Therefore, to unambiguously identify an OPC, a combination of the OPC
105 markers or co-localisation with an OLC marker, such as the transcription factors Olig2 (Zhou,
106 Wang, & Anderson, 2000) or Sox10 (Kuhlbrodt, Herbarth, Sock, Hermans-Borgmeyer, &
107 Wegner, 1998), should be used. However, as the OLC markers are also expressed by cells in
108 later stages of differentiation they cannot alone be used for the identification of OPCs.

109

110 As an OPC starts to differentiate, marker proteins such as the ectonucleotide
111 pyrophosphatase/phosphodiesterase 6 (ENPP6) (Xiao et al., 2016), O4 (Sommer & Schachner,
112 1981) and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) (Poduslo & Norton, 1972)
113 are expressed, identifying a differentiation state between a progenitor and a fully mature
114 oligodendrocyte. These pre-myelinating oligodendrocytes differentiate into cells with
115 progressively more complex process networks and eventually mature myelin sheaths, thus
116 becoming a mature oligodendrocyte. Mature, sheath forming, oligodendrocytes express
117 myelin sheath proteins including myelin basic protein (MBP) (Sternberger, Itoyama, Kies, &

118 Webster, 1978), myelin/oligodendrocyte glycoprotein (MOG) (Linnington, Webb, &
119 Woodhams, 1984), myelin-associated glycoprotein (MAG) (Sternberger, Quarles, Itoyama, &
120 Webster, 1979), myelin regulatory factor (MYRF) (Cahoy et al., 2008) and proteolipid protein
121 (PLP) (Sobel, Greer, Isaac, Fondren, & Lees, 1994).

122

123 Progression along a differentiation and maturation pathway is a continuous and seamless
124 process. Combinations of marker proteins, all of which appear and disappear within defined
125 phases of differentiation, can be used to define distinct stages of development, which are
126 useful as reference points but should not be taken to imply that differentiation necessarily
127 proceeds in quantal steps. Additionally, it should be noted that the expression of marker
128 proteins (so far only shown for OPCs) can change with the activation state (Moyon et al.,
129 2015), during development (Clarke et al., 2012; Karram et al., 2008; Ligon et al., 2006; Stallcup
130 & Beasley, 1987) (Figure 1) and/or ageing (unpublished data from our laboratory). Therefore,
131 proof of heterogeneity inferred from marker protein expression is difficult as it may only
132 represent lineage stage.

133

134 **Developmental OPC heterogeneity – does origin matter?**

135

136 During embryonic development of the CNS, OPCs are generated from radial glia cells in
137 multiple localised areas. The diversity of OPCs based on their origin is known as
138 developmental heterogeneity. In the murine spinal cord, most OPCs arise from the pMN
139 domain of the ventral ventricular zone, and subsequently populating the entire neural tube
140 (Fogarty, Richardson, & Kessaris, 2005; Pringle & Richardson, 1993). Additionally, a minority
141 of OPCs is generated from progenitors in the dorsal dP3, dP4, dP5 and dP6 domains beginning
142 at E16.5 (Cai et al., 2005; Fogarty et al., 2005; Vallstedt, Klos, & Ericson, 2005). In the adult
143 mouse, OPCs from ventral and dorsal regions are intermixed, with a heavy predominance of
144 pMN-derived (ventral) cells (85-90%). OPCs arising from dorsal progenitors mostly populate
145 the dorsal and lateral funiculus (Tripathi et al., 2011).

146

147 Developmental heterogeneity of OPCs also occurs in the telencephalon, where OPCs arise
148 from three distinct regions in a spatiotemporal manner. The earliest OPCs develop from the
149 medial ganglionic eminence (MGE) and the anterior entopeduncular (AEP) region in the

150 ventral developing telencephalon, starting from E11.5. Subsequently, at E16.5, a second
151 population of OPCs are formed from the ventral lateral and caudal ganglionic eminence (LGE,
152 CGE). Both OPC populations spread from ventral to dorsal, eventually populating the entire
153 telencephalon. After birth, a third population of OPCs arises in the developing cortex, which
154 populate the dorsal parts of the telencephalon (Kessaris et al., 2006). During postnatal
155 development, the majority of the first population of OPCs from the MGE-AEP region is
156 eliminated, leaving the adult brain with OPCs derived from the ventral LGE-CGE region and
157 the dorsal cortex (Kessaris et al., 2006). In the adult telencephalon, dorsally derived OLCs
158 mainly populate the cortex (~50% dorsal OLCs, ~35% ventral OLCs) and the corpus callosum
159 (CC) (~25% dorsal OLCs, ~15% ventral OLCs), whereas the anterior commissure (AC), the pre-
160 optic tract (POA) and the lateral olfactory tract (LOT) are almost exclusively populated by
161 ventral OLCs (Tripathi et al., 2011). The question arises, why should there be developmental
162 heterogeneity in the oligodendrocyte lineage? Do different OLC populations fulfil different
163 roles, or is developmental diversity simply an evolutionary ploy to accommodate for the rapid
164 growth of the CNS?

165

166 Different molecular cues are needed for ventral and dorsal OPC specification in development.
167 Shh-signalling is required to generate ventral OPCs but is redundant for dorsal OPC
168 specification (Cai et al., 2005; Fogarty et al., 2005). In contrast, the induction of FGF signalling
169 as well as the inhibition of WNT and BMP signalling pathways may play an important role in
170 the specification and timing of appearance of dorsal OPCs (Chandran et al., 2003; Fogarty et
171 al., 2005; Langseth et al., 2010; Vallstedt et al., 2005). In addition to differences in
172 specification factors, dorsally derived OPCs also exhibit a preference to myelinate dorsal areas
173 in the CNS (Kessaris et al., 2006; Tripathi et al., 2011). In the course of spinal cord
174 development, the dorsal funiculus is initially populated by ventrally-derived oligodendrocytes
175 but by adulthood comprises more than 80% of dorsally-derived oligodendrocytes. That
176 ventrally derived oligodendrocyte numbers decrease after postnatal day 13 (P13), whereas
177 dorsally derived oligodendrocyte numbers stay constant, argues strongly for a selective
178 advantage of dorsally derived oligodendrocytes in the dorsal funiculus of the spinal cord
179 (Tripathi et al., 2011). Similar competition between ventrally and dorsally derived
180 oligodendrocytes occurs in the cortex and CC in the murine forebrain (Kessaris et al., 2006).

181

182 Although OPCs respond to neuronal electrical stimulation (Gibson et al., 2014; Li, Brus-Ramer,
183 Martin, & McDonald, 2010; Makinodan, Rosen, Ito, & Corfas, 2012; Mensch et al., 2015), not
184 all OPCs necessarily respond in the same way (discussed below) (Chittajallu, Aguirre, & Gallo,
185 2004; Clarke et al., 2012; Káradóttir, Hamilton, Bakiri, & Attwell, 2008; Spitzer et al., 2019),
186 leading to the hypothesis that this diversity in function might be linked to developmental
187 origin. However, there is no evidence that this is the case (Tripathi et al., 2011).

188

189 To test whether ventral OPCs can functionally compensate for the absence of dorsal OPCs,
190 individual developmentally-distinct OPC populations in the telencephalon were ablated by
191 region-specific expression of diphtheria toxin A (DTA). The ablation of any one of the three
192 distinct OPC populations did not, however, cause a reduction in the total number of OLCs at
193 P12 or in myelination in adult mice (Kessaris et al., 2006), indicating that different OLCs can
194 functionally compensate for one another. RNA-sequencing data support these findings, as no
195 differences in the gene expression profile between the developmentally distinct OPC
196 populations has been detected (Marques et al., 2018). Whether ventrally and dorsally derived
197 oligodendrocytes show transcriptional differences remains to be investigated.

198

199 **Do OPCs show different propensities for self-renewal?**

200

201 Self-renewal prevents a stem cell pool becoming depleted (stem cell exhaustion), which, in
202 the context of OPCs, would result in an inability to generate new oligodendrocytes under
203 homeostatic conditions and following demyelinating injury. BrdU labelling experiments had
204 initially suggested that a non-dividing population of adult OPCs exists alongside a separate
205 dividing population (Psachoulia, Jamen, Young, & Richardson, 2009; Rivers et al., 2008; Simon,
206 Götz, & Dimou, 2011). However, a subsequent study indicated that the toxicity of BrdU in
207 these studies may have led to erroneous conclusions being drawn on the proliferative
208 capacity of adult OPCs (Young et al., 2013). The use of the non-toxic BrdU analogue EdU has
209 more reliably demonstrated that all OPCs proliferate in the adult CNS (Clarke et al., 2012;
210 Young et al., 2013). However, a difference exists between white matter (WM) and grey matter
211 (GM) OPCs, with the former proliferating more rapidly and having a shorter cell cycle time
212 (Dawson et al., 2003; Dimou, Simon, Kirchhoff, Takebayashi, & Götz, 2008; Rivers et al., 2008;
213 Young et al., 2013). This difference has been recapitulated *in vitro*, where WM OPCs have a

214 three to four fold greater proliferative response to PDGF-AA than GM OPCs (Hill, Patel,
215 Medved, Reiss, & Nishiyama, 2013). WM tissue transplanted into GM areas of brain slices
216 retain their greater proliferative response to PDGF-AA, suggesting that NG2⁺ cells in the WM
217 have an intrinsically higher proliferative capacity than those in GM (Hill et al., 2013). The
218 functional implication of a different proliferation, and therefore self-renewal rates, are not
219 yet fully understood.

220

221 **Do OPCs have distinct differentiation capacities?**

222

223 Similar to the differences in proliferation, WM OPCs have a higher propensity to differentiate
224 into mature oligodendrocytes than OPCs from GM regions (WM: 40.6%, GM: 11%) (Dimou et
225 al., 2008). To resolve whether this difference is due to extrinsic or intrinsic differences
226 between the two populations, OPCs derived from both GM and WM were transplanted into
227 the antithetical region. Here it was shown that WM derived cells were able to differentiate
228 more efficiently in both WM and GM than GM derived cells when transplanted into WM
229 (Viganò, Möbius, Götz, & Dimou, 2013). The authors posit that this demonstrates an intrinsic
230 difference, but could not definitively rule out a role for environmental priming of the cells
231 before transplantation.

232

233 A detailed *in vivo* characterisation of ion channels in neonatal OPCs identified different
234 profiles of Na⁺ and K⁺ channel expression in WM and GM OPCs (Chittajallu et al., 2004;
235 Káradóttir et al., 2008; Spitzer et al., 2019). With respect to voltage gated potassium channels,
236 there is a marked increase in the expression of KDR (slow-inactivating delayed-rectifier) and
237 Kir (inward-rectifier) potassium channels in GM OPCs, when compared to WM OPCs
238 (Chittajallu et al., 2004). However, the expression of KA (fast-inactivating A-type) potassium
239 channel is similar between the two OPC subpopulations (Chittajallu et al., 2004). The
240 difference in potassium channel expression levels is of particular interest since
241 oligodendrocyte specific knockout of Kir4.1 increases OPC differentiation (Schirmer et al.,
242 2018). Therefore, and consistent with the studies discussed above, this apparent difference
243 in the potassium channel expression between GM and WM may imply functional
244 heterogeneity. However, these data are collected during the early postnatal period (p5-10)
245 and do not necessarily represent the expression profiles of adulthood.

246

247 A difference in OPC expression in Na⁺ channels has also been reported (Chittajallu et al., 2004;
248 Clarke et al., 2012; Káradóttir et al., 2008). Several studies have identified a subpopulation of
249 OPCs in both WM and GM that exhibit a transient Na_v channel mediated inward current,
250 followed by a K⁺ channel mediated outward current, in response to depolarisation (Chittajallu
251 et al., 2004; Clarke et al., 2012; Káradóttir et al., 2008). The remaining OPCs did not show this
252 response (Chittajallu et al., 2004; Clarke et al., 2012; Káradóttir et al., 2008). However,
253 whether two OPC populations based on the responsiveness to depolarisation exist is still
254 unclear, as other studies have found that all OPCs exhibit similar Na_v density and Na_v
255 mediated inward currents (De Biase, Nishiyama, & Bergles, 2010; Spitzer et al., 2019). In
256 addition, whether the ability to spike in response to depolarisation is functionally relevant for
257 OPCs remains unknown. To date, only a positive correlation of the number of Na_v channels
258 and active cell cycle progression of OPCs has been reported (Spitzer et al., 2019).

259

260 In addition, Spitzer and colleagues have shown that there is a higher proportion of neonatal
261 OPCs with detectable NMDA-evoked currents in the WM, and that WM OPCs have an
262 increased NMDA receptor density than GM OPCs (Spitzer et al., 2019). The percentage of
263 OPCs expressing NMDA receptors decreases with age, although at different rates in WM and
264 GM (Spitzer et al., 2019). The presence of NMDA receptors is dispensable for OPC
265 proliferation and differentiation as the knockout of the NMDAR subunit NR1 does not show
266 any effect on myelination (De Biase et al., 2010; Saab et al., 2016). However, the
267 oligodendrocyte specific knockout of NMDA receptors leads to an axon pathology caused by
268 decreased oligodendroglial axonal support in aged animals (Saab et al., 2016). Whether
269 oligodendrocyte heterogeneity based on the capacity of metabolic support to neurons exists
270 also remains to be investigated.

271

272 In addition to the CNS region in which an OPC resides, the expression of G-protein receptor
273 17 (GPR17) confers OPC diversity with respect to their differentiation potential. GPR17
274 inhibits OPC differentiation by acting on the differentiation inhibitors ID2 and ID4 (Chen et al.,
275 2009). GPR17-driven lineage tracing has revealed that only a proportion of adult NG2⁺ cells
276 (75% in the GM and 60% in the WM) express GPR17 (Viganò et al., 2016). Using a BrdU label
277 retention approach, it was shown that 82.0% of GPR17⁺/BrdU⁺ but only 23.4% of the

278 GPR17⁻/BrdU⁺ populations retained NG2-immunoreactivity, suggesting that more of the
279 GPR17⁺ OPCs remain in cell cycle and do not undergo differentiation (Viganò et al., 2016). The
280 block of differentiation in GPR17⁺ OPCs in homeostasis is released after various types of
281 injuries (demyelination induced by cuprizone or EAE, and cerebral damage by acute injury or
282 ischemia)(Coppolino et al., 2018; Viganò et al., 2016): however, how the differentiation
283 capacity of GPR17⁺ OPCs compares to GPR17⁻ OPCs after injury is not known.

284

285 **Are some OPCs better at regeneration than others?**

286

287 Alongside providing new oligodendrocytes for myelination during development and
288 adulthood, OPCs have a central role in oligodendrocyte regeneration (a process known as
289 remyelination) (Franklin & Ffrench-Constant, 2017). In response to oligodendrocyte loss,
290 local OPCs migrate to the site of CNS damage, proliferate, and differentiate into
291 oligodendrocytes, or in the concomitant absence of astrocytes, into Schwann cells capable of
292 creating new myelin sheaths (Monteiro de Castro, Deja, Ma, Zhao, & Franklin, 2015; Zawadzka
293 et al., 2010).

294

295 By tracing the response of dorsal OPCs to demyelination in the ventral WM of the spinal cord,
296 it was shown that dorsal OPCs populated the lesion and differentiated in mature
297 oligodendrocytes (Zhu et al., 2011). A subsequent study demonstrated that dorsal OPCs
298 respond more vigorously than ventral OPCs to focal acute demyelination in the spinal cord,
299 with more of them undergoing proliferation. Thus, following demyelination of ventral WM,
300 where the majority of OLCs are of ventral origin, the subsequent remyelination involves a
301 disproportionately high contribution from dorsally derived cells (Crawford, Tripathi,
302 Richardson, & Franklin, 2016) (Figure 2). The genetic ablation of dorsally derived OPCs led to
303 a reduction in mature oligodendrocytes following demyelination (Crawford et al., 2016),
304 demonstrating that ventrally derived OLCs cannot fully compensate for the lack of dorsally
305 derived OLCs. However, the situation changes with ageing, where the majority of dorsal OLCs
306 remains undifferentiated (presumably in an OPC state) in the aged animals, while ventral
307 OPCs continue to differentiate into oligodendrocytes at the same rate as in young adults
308 (Crawford et al., 2016). This suggests that the age-associated decline in OPC function has a
309 greater impact on dorsal OPCs than on ventral OPCs. The underlying reason for this remains

310 unknown. In addition, in response to the toxin-induced demyelination, dorsal OPCs show an
311 increased propensity to differentiate into Schwann cells when compared to ventral OPCs
312 (Crawford et al., 2016). However, this propensity is lost with ageing, consistent with the
313 conclusion that dorsal and ventral OPCs age at different rates. Taken together, these data
314 indicate that the regenerative properties of adult OPCs are determined by their
315 developmental origin and is an example of true functional heterogeneity within the OLC
316 lineage.

317

318 **Are oligodendrocytes heterogeneous in the CNS?**

319

320 The notion of oligodendrocyte diversity was first introduced by del Río Hortega who identified
321 four different classes of oligodendrocytes based on their morphology (del Río Hortega, 1928).
322 Class 1 (CI) oligodendrocytes occur in both WM and GM and are characterised by a high
323 number of thin processes leading to thinly-myelinated small diameter axons. Class 2 (CII)
324 oligodendrocytes have fewer, but thicker processes and are exclusively found in WM.
325 Oligodendrocytes categorised in class 3 (CIII) and class 4 (CIV) are mostly found in the WM of
326 the brain stem and spinal cord, areas with an abundance of larger diameter axons. In
327 comparison to CI and CII oligodendrocytes, they are less abundant and extend fewer
328 processes (del Río Hortega, 1928). Following this early classification of oligodendrocyte
329 diversity, additional morphological subclasses have been identified (Murtie, Macklin, &
330 Corfas, 2007; Vinet et al., 2010).

331

332 The development of an MBP-GFP (membrane bound) reporter mouse line, only labelling
333 around 1% of oligodendrocytes in the brain, has enabled imaging of the myelin sheaths
334 formed by a single oligodendrocyte (Chong et al., 2012). 3D reconstruction revealed a
335 diversity within the oligodendrocyte population with respect to the number of myelin sheaths
336 formed per oligodendrocyte (between 10 and 60 myelin sheaths per oligodendrocyte) and
337 myelin sheath length (between 20µm and 200µm per myelin sheath) (Chong et al., 2012).
338 This diversity is not region-specific, and occurs along axons with similar functional properties
339 (Chong et al., 2012; Tomassy et al., 2014), suggesting that internode length might not be
340 determined by the regional diversity of oligodendrocytes (as proposed by del Rio Hortega),
341 but rather local environmental cues. Indeed, using an *in vitro* co-culture of cortical OPCs with

342 neurons, Chong and colleagues were able to demonstrate that the density of OPCs (not
343 oligodendrocytes) negatively regulates the myelinogenic potential of oligodendrocytes
344 through repulsive interaction (Chong et al., 2012). Whether there is a difference in OPC
345 density in different CNS regions and how the local density of OPCs would be regulated in the
346 CNS to explain the observed morphological subclasses of oligodendrocytes remains unknown.

347

348 To assess the intrinsic diversity in regional OLC populations without the influence of axon
349 properties, Bechler and colleagues have examined the compact myelin sheath formation of
350 cortical and spinal cord OPCs in an assay where artificial microfibres substitute for the role of
351 the axon in providing a substrate for myelination. Oligodendrocytes from the spinal cord
352 formed myelin sheaths which are twice as long as those formed by cortical oligodendrocytes,
353 even though the number of sheaths formed per oligodendrocyte was similar (Bechler, Byrne,
354 & Ffrench-Constant, 2015). This suggests that the origin of the OPCs determines the
355 myelinogenic potential of the oligodendrocytes. However, the difference in internode length
356 formed by cortical and spinal cord oligodendrocytes was less pronounced when the OPCs of
357 different origins were cultured on dorsal root ganglion neurons or brain slices, indicating that
358 neurons also influence the myelinogenic potential of the oligodendrocytes (Bechler et al.,
359 2015).

360

361 The optimisation of the single-cell RNA sequencing of CNS cells has allowed the analysis of
362 oligodendrocyte diversity to be explored in greater depth. OLCs in ten different CNS regions
363 of juvenile and adult mouse CNS revealed 12 distinct OLC populations spanning the
364 differentiation stages of OPCs to mature oligodendrocytes. In the juvenile mouse, all CNS
365 regions contain oligodendrocytes from at least 2 different oligodendrocyte populations.
366 Whereas one mature oligodendrocyte population was present in all CNS regions, the other
367 oligodendrocyte populations are prevalent in certain CNS regions. However, within the adult
368 brain regions examined (cortex and CC) the diversity of oligodendrocyte populations is
369 reduced, with only two oligodendrocyte populations being present (Marques et al., 2016).
370 Whether the transcriptionally different oligodendrocyte populations fulfil distinct functions
371 in the brain remains to be investigated. These findings raise several important questions
372 including, how can transcriptional diversity of oligodendrocytes arise from transcriptionally
373 homogenous OPCs (Marques et al., 2018)? Possible explanations include technical limitations

374 of the sequencing technique to study gene expression in OPC (limited amounts of RNA,
375 fragility of OPC population) or environmental influences exerted during, or after, the
376 oligodendrocyte differentiation process.

377

378 Strong evidence for functional heterogeneity of oligodendrocytes has been obtained using
379 three different viruses to label oligodendrocytes, together with neuronal axon projections of
380 motor and sensory neurons in the CC. The analysis revealed that colossal oligodendrocytes
381 can be classified into three categories: those that either preferentially myelinate axons from
382 1) the motor cortex, 2) the sensory cortex, and 3) from both brain regions without preference
383 (~75% of all oligodendrocytes assessed) (Osanai et al., 2017). It is conceivable that the 25% of
384 oligodendrocytes showing a preference towards specific axons are adult-born
385 oligodendrocytes, specifically myelinating an axon based on its activity.

386

387 **Concluding remarks**

388

389 An expanding body of evidence has been published describing phenotypical differences
390 within the OPC and oligodendrocyte populations (Table 1). However, only a minority of these
391 publications addresses the important question of whether the observed phenotypical
392 differences are intrinsically driven (indicating OLC heterogeneity) or dictated by
393 environmental cues (OLC functional plasticity). As intrinsic heterogeneity is often established
394 due to different extrinsic (developmental) signals, the definition of intrinsic and extrinsic
395 heterogeneity can be blurred. The definition implies that extrinsically heterogeneous cells
396 would show similar properties within an identical environment. In contrast, cells that are
397 intrinsically heterogeneous will still exhibit different functional behaviour even in the same
398 environment. While one study argues for a non-existence of oligodendrocyte diversity (Chong
399 et al., 2012), other studies showed intrinsic diversity of aspects of OPC, such as OPC
400 differentiation capacity, (Crawford et al., 2016; Viganò et al., 2013) and oligodendrocyte
401 biology (Bechler et al., 2015). However, whether these intrinsic differences have any
402 functional implications has only been addressed in one study (Table 1). Crawford and
403 colleagues showed that dorsal OPCs are the proportionally greater contributors to WM
404 remyelination, and that the deletion of dorsal OPCs leads to a reduced remyelination
405 efficiency (Crawford et al., 2016) (Table 1). Nevertheless, no evidence has been found for the

406 functional heterogeneity in the homeostatic adult CNS, leaving the field without the definitive
407 proof required to unambiguously assert heterogeneity. However, the discovery of new
408 functions of OLCs are likely to reveal other examples of functional heterogeneity, and allow
409 current phenotypic descriptions of diversity to be better mapped on to newly elucidated OLCs
410 functions.

411

412 In favour of the existence of functional OLCs heterogeneity is the notion that the cortex, an
413 area coordinating complex tasks, is mainly populated by dorsal OLCs, whereas other
414 evolutionarily conserved brain areas are populated by ventral OLCs, suggesting that a variety
415 of oligodendrocyte subtypes are needed for optimal CNS function. In addition, the most
416 heterogeneous set of myelination profiles of the murine cerebral cortex exists in the upper
417 layers which is due to neurons from different cortical layers having different longitudinal
418 myelination profiles along their axons (Tomassy et al., 2014). While this effect might be driven
419 by neuronal activity, it is possible that distinct oligodendrocyte subpopulations are needed to
420 create such a specific myelination pattern. To this end, oligodendrocytes are transcriptionally
421 distinct in the adult CNS, which is indicative of functional distinct oligodendrocyte
422 subpopulations (Marques et al., 2016). This would echo what is known about the other
423 principal macroglial cell type, the astrocyte, where it has been shown that functionally distinct
424 astrocyte populations are necessary to support optimal neuronal transmission (Tsai et al.,
425 2012). As oligodendrocytes are also critical for neuron circuit function, it is likely that distinct
426 oligodendrocytes exist to meet the special needs of different neuronal circuits. Furthermore,
427 OPCs and oligodendrocytes form intercellular connections with neurons (via synapses) and
428 astrocytes (via gap junctions), respectively. Neurons exhibit functional heterogeneity with
429 respect to their mode of transmission and firing patterns, and astrocytes were shown to
430 become specialised for interactions with their own particular neuronal neighbours (Tsai et al.,
431 2012). Therefore, the existence of OLCs heterogeneity to accommodate the specific
432 functional requirements of individual neuron-glia networks is likely.

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714 **Figure legends**

715 **Figure 1: Overlap of OPC markers**

716

717 Overlap of OPC markers (NG2, PDGFRA and A2B5) and oligodendrocyte lineage cell markers
718 (Olig2 and Sox10) in neonatal (left) and adult (right) OPCs based on published *in vivo* lineage
719 tracing experiments (Clarke et al., 2012; Kang et al., 2010; Karram et al., 2008; Ligon et al.,
720 2006; Rivers et al., 2008; Stallcup & Beasley, 1987). A2B5 data was generated from
721 immunostaining of with the A2B5 antibody (unpublished data). The overlap of OPC marker
722 expression changes during development: adult OPCs show a higher overlap of the OPC marker
723 proteins when compared to neonatal OPCs.

724

725 **Figure 2: Developmental origin of OPCs determines their remyelination response**

726

727 Following a focal toxin-induced demyelination injury dorsal OPCs make a disproportionately
728 high contribution to remyelination when compared to ventral OPCs. Detailed analysis of the
729 OPC response to the injury showed that a higher proliferative response of dorsal OPCs causes
730 their increased response to demyelination. MGE = medial ganglionic eminence, AEP = anterior
731 entopeduncular, LGE = lateral ganglionic eminence, CGE = caudal ganglionic eminence, CC =
732 corpus callosum, AC = Anterior commissure, p = progenitor domain, MN = motor neuron, dP =
733 dorsal progenitor domain, DF = dorsal funiculus, LF = lateral funiculus.

734

735 **Table legends**

736

737 **Table 1: Summary of current literature on OPC and oligodendrocyte diversity**

738

739 Several phenotypical differences have been described between subclasses of
740 oligodendrocyte lineage cells. However, the assessment of phenotypic differences does not
741 allow to distinguish between cell/lineage plasticity and heterogeneity. Therefore, functional
742 differences between subclasses of oligodendrocyte lineage cells need to be investigated to
743 unambiguously prove heterogeneity.