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Adult-born SVZ progenitors receive transient glutamatergic synapses during remyelination of the corpus callosum

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Abstract

We demonstrate that, after focal demyelination of adult mice corpus callosum, demyelinated axons form functional glutamatergic synapses onto adult-born NG2⁺ oligodendrocyte progenitor cells (OPCs) migrating from the subventricular zone (SVZ). One week after lesion, this glutamatergic input is significantly reduced compared to endogenous callosal OPCs, and is lost upon differentiation into oligodendrocytes. Therefore, axon-OP synapse formation is a transient and regulated step that occurs during remyelination of callosal axons.

Keywords

Oligodendrocyte progenitors; synapse; subventricular zone; cell migration; myelin

After demyelination of the corpus callosum, repopulation of the lesion and remyelination are not only ensured by local oligodendrocyte progenitor cells (OPCs)¹, but also by NG2-expressing OPCs generated *de novo* in the subventricular zone (SVZ) and migrating into the lesion^{2,3,4}. During development, unmyelinated axons can form glutamatergic synapses onto NG2⁺ cells present in the white matter after the first postnatal week^{5,6}. However, in the context of demyelinating disorders, it is unknown whether demyelinated axons in the adult brain retain the potential to form new synapses with OPCs generated in the SVZ and repopulating a lesion and how these synapses are regulated during the remyelination process.

In order to answer these questions, we performed whole-cell recordings on SVZ-derived progenitors selectively label with a GFP retrovirus at different time points after focal demyelination of the adult mouse corpus callosum (**Fig. 1a**). Virtually all infected cells were restricted to the SVZ (**Supplementary Fig. 1a**), as predicted by the low number of Ki67⁺

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AUTHOR CONTRIBUTIONS

A. Etxeberria and J.M. Mangin designed, performed and analyzed all experiments. A. Aguirre helped with the LPC injection experiments and with the preparation of the retrovirus. V. Gallo participated in the design of the experiments, supervised the whole project and wrote the manuscript with A. Etxeberria and J.M. Mangin.

cycling cells in the adult white matter compared to the SVZ⁴ (**Supplementary Fig. 1b**). Two days after viral injection, we induced a demyelinating injury in the anterior corpus callosum by the injection of lysolecithin (**Fig. 1a**). We confirmed that SVZ progenitors are indeed strongly recruited by a demyelinating lesion compared to a control saline injection (**Supplementary Fig. 2**).

At three days post lesion (3DPL), we observed GFP⁺ cells not only in the SVZ, but also migrating at the border between the SVZ and the corpus callosum, and inside the corpus callosum itself (**Fig. 1a** and **Supplementary Fig. 3**). At this stage, we could distinguish two main populations of GFP⁺ cells (**Supplementary Fig. 3**). One population of GFP⁺ cells expressed doublecortin (Dcx), a marker of migrating neuroblasts, and was mainly found in the SVZ (**Supplementary Fig. 3a,d**). The other population expressed Olig2 and the proteoglycan NG2, a marker of OPCs, and was mainly found within the corpus callosum (**Supplementary Fig. 3b,c,e,f**). NG2⁺ cells could be distinguished from Dcx⁺ cells based on their membrane properties and the presence of a large transient K⁺ current (K_a) (**Supplementary Fig. 3g,h**).

We then investigated whether these two progenitor populations received glutamatergic synapses from callosal axons. We found that, as early as 2-3 DPL, approximately 30% of GFP⁺NG2⁺ cells at the border (n = 3/9) and 48% of GFP⁺NG2⁺ cells in the corpus callosum (n = 12/25) exhibited evoked EPSCs (eEPSCs; **Fig. 1b**) and spontaneous EPSCs (sEPSC; **Fig. 1c,d**). The average amplitude of eEPSCs was -37 ± 7 pA, with an average decay time constant of 1.4 ± 0.1 ms. Average amplitude of sEPSCs was 15.9 ± 1 pA, with an average decay time constant of 1.2 ± 0.1 ms and an average frequency of 0.076 ± 0.017 Hz. Both evoked and spontaneous EPSCs were blocked by CNQX, a specific antagonist of AMPA/Kainate receptors (**Fig. 1b,c**). Moreover, sEPSC decay time constant was increased by 162 ± 10 % in presence of 100 μ M cyclothiazide, a specific modulator of AMPARs (n = 3) (**Fig. 1c,d**). Finally, the frequency of sEPSCs was increased by 263 ± 18 % in the presence of ruthenium red, a secretagogue known to increase vesicular neurotransmitter release (n = 7) (**Fig. 1d,e**). By contrast, we never observed any evoked or spontaneous EPSCs in GFP⁺Dcx⁺ cells (n = 16), even in the presence of ruthenium red (n = 3) (**Supplementary Fig. 4**). These results demonstrate that synapse formation specifically occurs onto GFP⁺NG2⁺ OPCs.

We next investigated whether synaptically-connected, SVZ-derived OPCs could give rise to myelinating oligodendrocytes. The percentage of GFP⁺NG2⁺ cells exhibiting sEPSCs significantly increased from 48% (n=12/25) to 91% (n=11/12) between 2-3 DPL and 6-7 DPL (**Fig. 2a**), indicating that virtually all GFP⁺NG2⁺ cells became synaptically connected by 6-7 DPL. We observed a significant decrease in the percentage of GFP⁺NG2⁺ OPCs in favor of GFP⁺CC1⁺ oligodendrocytes between 6-7 and 10 DPL (**Fig. 2b,c,d**; see also ref. 4), suggesting that synaptically connected GFP⁺NG2⁺ cells do convert into CC1⁺ cells. Moreover, since the percentage of GFP⁺CC1⁺ cells whose processes aligned with callosal axons remained stable between 6-7 and 10 DPL (82% vs. 79%), at least a fraction of CC1⁺ cells generated from synaptically connected NG2⁺ cells should be actively remyelinating.

We then investigated whether GFP⁺CC1⁺ oligodendrocytes would still exhibit functional glutamatergic synapses. As previously reported⁷, we found that differentiated GFP⁺CC1⁺ oligodendrocytes displayed a significantly higher membrane capacitance (77 ± 7 pF; n=14) compared to GFP⁺NG2⁺ cells (26 ± 3 pF; n=7, $p<0.001$) at 6-7 DPL. More importantly, we observed that approximately 28% of GFP⁺CC1⁺ oligodendrocytes (n = 4/14) exhibited spontaneous EPSCs (**Fig. 2e,f**). The low proportion of GFP⁺CC1⁺ cells exhibiting synaptic activity at 7 DPL, as compared to GFP⁺NG2⁺ OPCs at the same time point (91%, n=11/12), suggests that synaptic integration is a transient property of differentiating OPCs. While only a very small fraction of GFP⁺ cells co-expressed NG2 and CC1 around 7 DPL, we found that one such GFP⁺NG2⁺CC1⁺ cell clearly exhibited EPSCs in response to callosal stimulation (**Supplementary Fig. 5**), further confirming the ability of NG2⁺ cells to differentiate into CC1⁺ oligodendrocytes while receiving glutamatergic synapses.

Finally, we explored how axon-OPC glutamatergic synapses were affected during the demyelination/remyelination process. First, we confirmed the presence of VGLUT-1 puncta in the vicinity of SVZ-born GFP⁺ OPCs (**Supplementary Fig. 6a**), as previously reported for endogenous NG2-DsRed⁺ OPCs in the corpus callosum⁶. We also observed that both SVZ-born GFP⁺ OPCs and NG2-DsRed⁺ cells were immunoreactive for the postsynaptic AMPA receptor subunit GluR2/3 (**Supplementary Fig. 6b,c**). Western blot analysis demonstrated that VGLUT-1 and GluR2/3 expression were strongly reduced in corpus callosum at 7 DPL, compared both to 4 DPL or to control NaCl injection (**Fig. 3**). To confirm that decrease at the functional level, we recorded EPSCs evoked by callosal stimulation and observed a significantly lower eEPSC average amplitude in GFP⁺DsRed⁺ cells at 7 DPL (-20 ± 12 pA; n=16) than in either control callosal NG2-DsRed⁺ cells (-34 ± 13 pA, n=12, $p<0.05$) or in NG2⁺GFP⁺ cells at 2-3 DPL (-37 ± 7 pA, n=12, $p<0.05$) (**Fig. 3c**). While proteins involved in vesicular release, including VGLUT-1, are expressed in astrocytes⁸, the high glutamate concentration needed for the fast EPSCs recorded in NG2⁺ cells are unlikely to be due to glutamate released from astrocytes.

We demonstrate here that new axon-NG2⁺ cell synapses are actively formed during the early stages of the remyelination process. The finding that these synapses are selectively formed on OPCs present in the demyelinating lesion and not on Dcx⁺ neuronal precursors suggests a specific function of glutamatergic synapses in axon-OPC recognition. We also provide evidence that axonal glutamatergic synapses contact NG2⁺ cells that give rise to mature oligodendrocytes, although synaptic connectivity appears to be lost during the subsequent stages of differentiation. Finally, we found that while synapse formation occurs during early phases of axon-OPC recognition, the subsequent reduction in glutamatergic activity observed one week after the lesion may be a necessary condition to allow OPCs to differentiate into oligodendrocytes, as predicted by the inhibitory influence of glutamate on OPC differentiation^{9,10}. Future experiments would be needed to determine the exact function of transient glutamatergic synapses during the remyelination process.

Supplementary Material

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Figure 1. Adult-born SVZ NG2⁺ cells display synaptic currents after migrating into a demyelinated lesion of the corpus callosum

a) Confocal images showing an example of a GFP⁺ cell recorded inside the lesion filled with biocytin (red) during patch-clamp recording and subsequently stained for NG2 (blue). Scale bar = 200 μ m. The insert show the location of GFP retrovirus injection in the SVZ (green arrow, 0.5/1.25/2.5 mm) and lysolecithin injection in the corpus callosum (red arrow, 1.3/1.0/2.0 mm). **b)** Example of current evoked by callosal axon stimulation (arrowhead) in the GFP⁺NG2⁺ cell shown in (b) ($V_h = -80$ mV) under control conditions, in the presence of 100 μ M CTZ and after application of 10 μ M CNQX. **c)** Spontaneous synaptic glutamatergic activity recorded from the same cell under control conditions, in the presence of 100 μ M CTZ and after blockade by 10 μ M CNQX. **d)** Spontaneous excitatory postsynaptic currents recorded from a GFP⁺NG2⁺ cell in the corpus callosum under control conditions and in the presence of the secretagogue ruthenium red (100 μ M). **e)** Graph showing the amplitude distribution of the spontaneous events of the cell shown in (e). The insert in (f) shows the events in the histogram. All procedures were approved by the Institutional Animal Care and Use Committee of Children's National Medical Center.

Figure 2. Synaptically connected SVZ-derived OPCs give rise to oligodendrocytes
a) Histograms showing that the percentage of connected GFP⁺NG2⁺ cells in corpus callosum significantly increases from 48 % (12/25) at 2-3 DPL to 91 % (11/12) (*p<0.05; Fisher exact test) at 6-7 DPL. **b)** Histograms showing the percentage of GFP⁺NG2⁺ oligodendrocyte progenitors and mature GFP⁺CC1⁺ oligodendrocytes within the total GFP⁺ population at 3, 6 and 10 DPL. The remaining GFP⁺NG2^{neg} and GFP⁺CC1^{neg} cells are either GFAP⁺ or Dcx⁺ (see also ⁴). Data are shown as mean ± sem (n = 3). The percentage of GFP⁺NG2⁺ cells significantly decreased in favor of mature GFP⁺CC1⁺ cells between 3 and 10 DPL (*p<0.05). **c,d)** Immunostaining of GFP⁺ cells in corpus callosum with antibodies to NG2 and CC1 at 3 (c) and 10 DPL (d). Empty arrows point to GFP⁺NG2⁺ cells and white arrows point to GFP⁺CC1⁺ cells. Scale bars = 40µm. **e)** Immunostaining of a biocytin-filled CC1⁺NG2^{neg} oligodendrocyte displaying glutamatergic synaptic currents shown in (f). Scale bars = 20µm. **f)** Voltage clamp recording showing spontaneous glutamatergic currents in the presence of 100 µM CTZ and after blockade by 10µM CNQX.

Figure 3. Glutamatergic synaptic transmission between axons and NG2⁺ cells is reduced one week after demyelination

a) Representative western blots of VGLUT-1 and GluR2/3 in control and lysocleithin-injected white matter tissue at 4, 7 and 21 DPL. **b)** Histograms showing the ratio of VGLUT-1 (top) and GluR2/3 (bottom) protein expression normalized to actin levels at 4, 7 and 21 DPL (mean $\pm$ sem n = 3). Expression of both VGLUT-1 and GluR2/3 was significantly decreased at 7DPL (*p<0.05). **c)** Histograms comparing the average eEPSC amplitude in GFP⁺NG2-DsRed⁺ cells at 7 DPL (mean $\pm$ sd, n=16) and in NG2-DsRed⁺ cells in age-matched, uninjected control littermates (n=12). Insert shows a representative average eEPSC in lysocleithin-injected corpus callosum and uninjected controls (*p<0.05).