



HAL
open science

Generation of Oligodendrocytes and Oligodendrocyte-Conditioned Medium for Co-Culture Experiments

Elisa Mazuir, Anne-Laure Dubessy, Loane Wallon, Marie-Stéphane Aigrot,
Catherine Lubetzki, Nathalie Sol-Foulon

► **To cite this version:**

Elisa Mazuir, Anne-Laure Dubessy, Loane Wallon, Marie-Stéphane Aigrot, Catherine Lubetzki, et al.. Generation of Oligodendrocytes and Oligodendrocyte-Conditioned Medium for Co-Culture Experiments. *Journal of visualized experiments: JoVE*, 2020, 156, pp.e60912. 10.3791/60912. hal-03052042

HAL Id: hal-03052042

<https://hal.science/hal-03052042>

Submitted on 12 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title: Generation of oligodendrocytes and oligodendrocyte conditioned medium for co-culture experiments

Authors and affiliations :

Elisa Mazuir¹, Anne-Laure Dubessy^{1,2}, Loane Wallon^{1,3}, Marie-Stéphane Aigrot¹, Catherine Lubetzki^{1,2}, Nathalie Sol-Foulon^{1,4}.

¹Sorbonne Université, Inserm, CNRS, UMR7225, Institut du Cerveau et de la Moelle épinière, ICM, Paris, France

²Assistance Publique-Hôpitaux de Paris, GH Pitié Salpêtrière, Paris, France

³Ecole Supérieure des Techniques de Biologie Appliquée, Paris, France

⁴Corresponding author

Email addresses of co-authors:

Elisa Mazuir (elisa.mazuir@icm-institute.org)

Anne-Laure Dubessy (annelaure.dubessy@gmail.com)

Loane Wallon (loane.wallon@gmail.com)

Marie-Stéphane Aigrot (marie-stephane.aigrot@upmc.fr)

Catherine Lubetzki (catherine.lubetzki@aphp.fr)

Nathalie Sol-Foulon (nathalie.sol-foulon@upmc.fr)

Keywords:

Oligodendrocytes, conditioned medium, secreted factors, cell culture, neuro-glia interactions, central nervous system, rat.

Summary:

We herein display an efficient method for the purification of oligodendrocytes and production of oligodendrocyte conditioned medium that can be used for co-culture experiments.

Abstract:

In the central nervous system, oligodendrocytes are well-known for their role in axon myelination, that accelerate the propagation of action potentials through saltatory conduction. Moreover, an increasing number of reports suggest that oligodendrocytes interact with neurons beyond myelination, notably through the secretion of soluble factors. Here, we show a detailed protocol allowing purification of oligodendroglial lineage cells from glial cell cultures containing also astrocytes and microglial cells. The method relies on an overnight shaking at 37°C, allowing selective detachment of the overlying oligodendroglial cells and microglial cells, and then elimination of microglia by differential adhesion. We then describe the culture of oligodendrocytes and production of oligodendrocyte conditioned medium (OCM). We also provide the kinetics of OCM treatment or oligodendrocytes addition to purified hippocampal neurons in co-culture experiments, studying oligodendrocyte-neuron interactions.

Introduction:

Oligodendrocytes (OLs) are glial cells of the central nervous system (CNS) that generate myelin wrapping around axons. OLs originate from oligodendrocyte precursor cells (OPCs) which proliferate within the ventricular zones of the embryonic CNS and then migrate and differentiate into fully mature OLs (*i.e.* myelin-forming cells)¹. OPCs are abundant during early development, but also persist in the adult brain where they represent the major proliferative cell population². A single OL ensheathes multiple axons in non-excitabile sections (*i.e.* internodes), and the edge of each myelin loop attaches to the axon forming the paranodal domain which is crucial for the insulating properties of myelin^{1,3}. In between the paranodes

are small unmyelinated gaps called the nodes of Ranvier. These nodes are enriched in voltage-gated sodium channels (Nav), allowing the regeneration and rapid propagation of action potentials through saltatory conduction⁴. This tight interaction also enables axonal energy support through neuronal uptake of lactate from OLs^{5,6}.

The maturation of oligodendroglial lineage cells and the myelination process are tightly regulated by their interactions with neurons⁷. Indeed, OLs and OPCs, also named NG2 cells, express an array of receptors for neurotransmitters, and can receive input from excitatory and inhibitory neurons, allowing them to sense neuronal activity that can trigger their proliferation and/or differentiation into myelinating cells². In turn, OPCs/OLs secrete microvesicles and proteins into the extracellular space which alone or synergistically mediate neuromodulative and neuroprotective functions⁸⁻¹². However, the molecular mechanisms controlling the multiple modes of interactions between oligodendroglial lineage cells and neurons are yet to be fully deciphered.

Moreover, in several CNS pathological conditions, OLs are primarily affected, thus disturbing their interaction with neurons. For instance, in Multiple Sclerosis (MS) neurological dysfunction is caused by focal demyelination in the CNS, secondary to OLs loss, that can lead to axonal damage and related disability accumulation. Remyelination can take place, albeit insufficient in most cases¹³. Progress in the last decade, due to the development of immunotherapies, have reduce the relapse rate but promoting remyelination remains to date an unmet need. As such, a better understanding of OLs role, functions and influences is of particular interest to the development of new therapies for a wide spectrum of CNS conditions.

Here, we describe the methods of OLs purification and culture. This enables to precisely examine intrinsic mechanisms regulating their development and biology. In addition, such highly enriched OLs cultures allow the production of oligodendrocyte conditioned medium (OCM), which can be added to purified neuron cultures to gain insight into the impact of OLs secreted factors on neuronal physiology and connectivity. Furthermore, we describe how to implement an *in vitro* co-culture system where purified oligodendrocytes and neurons are combined together, allowing to address the mechanisms regulating (re)myelination.

Protocol:

The care and use of rats in this experiment is conformed to institutional policies and guidelines (UPMC, INSERM, and European Community Council Directive 86/609/EEC). The following protocol is established for a standard litter of 12 pups.

1. Preparation of the flasks (\approx 5 min) – in a laminar flow hood under sterile conditions

NOTE: perform the following steps the day before dissection

1. Coat the T150 cm² flasks with filter cap (1 flask for 2 pups) using 5 ml of polyethylenimine (PEI, 100mg/l, see protocol in supplemental information).
2. Store the flasks at 4°C overnight.
3. Rinse coated flasks 3 times with sterile distilled water the day of dissection.

2. Preparation of media (\approx 10 min) – in a laminar flow hood under sterile conditions

1. Prepare 500 ml of culture medium, consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of Fetal Calf Serum (FCS) and penicillin-streptomycin (100 IU/ml).
2. Prepare 20.6 ml of the enzyme digestion medium in a 50 ml tube, consisting of 20 ml of DMEM, 200 μ l of DNase (50 μ g/ml), 200 μ l of papain (30 U/ml) and 200 μ l of L-cysteine (0.24 mg/ml).
3. Filter-sterilize the media using a 0.22 μ m filter.
4. Let the media in the laminar flow hood at room temperature (RT) until dissection.

3. Preparation of dissection (\approx 10 min) – in a laminar flow hood under sterile conditions

1. Prepare 100 ml of Phosphate Buffered Saline without calcium and magnesium (PBS; 1X). Filter-sterilize using a 0.22 μ m filter.
2. Prepare 50 ml of ice-cold Phosphate Buffered Saline (PBS; 1X) solution supplemented with 750 μ l glucose 45%. Filter-sterilize using a 0.22 μ m filter.
3. Fill a 100mm-petri dish with PBS (1X) for instruments clearing and three 60 mm-petri

dishes with ice-cold PBS-glucose for tissue harvesting.

4. Put the petri dishes on ice until dissection.

4. Dissection

NOTE: dissection is performed from male and female Wistar rat pups at postnatal day (P)2.

1. In order to provide a sterile environment, make sure to clean the bench with 100% ethanol.
2. Sterilize all surgical tools with 100% ethanol.
3. Gently spray the neck of the pup with 70% ethanol.
4. Use large surgical scissors to decapitate the animal and place the head in a 100 mm-petri dish containing ice-cold PBS-glucose.
5. Use curved forceps to maintain the head of the animal at eye level.
6. Use small surgical scissors to make a small incision at the base of the skull and cut the skull following brain midline.
7. Use forceps to gently peel off the two parts of the skull from the midline.
8. Use a small surgical spoon to remove the brain from head cavity. Put the brain in a 60 mm-petri dish containing ice-cold PBS-glucose on ice.
9. Under a binocular microscope, use fine forceps to remove the cerebellum, the brainstem and olfactory bulbs from cerebral hemispheres.
10. Use fine forceps to separate the two cerebral hemispheres.
11. Use fine forceps to peel off the meninges.

NOTE: Ice-cold PBS is critical for correct meninges removal.

12. Put the cerebral cortices in a 60 mm-petri dish on ice.

5. Tissue dissociation - in a laminar flow hood under sterile conditions

1. Use a sharp scalpel to finely chop the cerebral cortices.
2. Transfer the minced tissue into a 50 ml tube containing enzyme digestion medium.

3. Incubate for 30 min in a humidified incubator at 37°C – 5% CO₂.
4. Use a p1000 micropipette to gently remove the enzyme digestion medium in making sure that the cortical tissue remains at the bottom of the 50 ml tube.
5. Use a p1000 micropipette to add 1 ml of DMEM-10%FCS and gently triturate the tissue.
6. Use a 70 µm filter and a piston of a 1 ml syringe to filter the cortical tissue into a 50 ml tube. Note: you can rinse residual tissue on the inner tube wall several times with DMEM-10%FCS.
7. Fill the 50 ml tube with DMEM-10%FCS.
8. Centrifuge at 423 g for 5 min at RT.
9. Carefully remove supernatant and resuspend cell pellet with 2 ml of DMEM-10%FCS.
10. Gently triturate cell pellet with a p1000 micropipette and then with a p200 micropipette.
11. Dilute cell suspension with the appropriate volume of DMEM-10%FCS. Note: 2 brains = 1 T150 cm² = 5 ml of DMEM-10%FCS.
12. Plate 5 ml of the cell suspension on a T150 at a density of 10⁵ cells/cm².
13. Add 20 ml of warm DMEM-10%FCS to each T150.
14. Incubate in a humidified incubator at 37°C – 5%CO₂.
15. Renew half of the culture medium after 6 days *in vitro* (DIV) with warm DMEM-10%FCS.

6. Shaking preparation- in a laminar flow hood under sterile conditions

NOTE: perform the following steps the day before shaking

Renew half of culture medium with new warm culture medium into the flask and incubate at 37°C – 5%CO₂.

7. Shaking

1. Coat three 100 mm-petri dishes with PEI.
2. Store petri dishes at 4°C overnight.

3. Cover the flasks cap with parafilm and put flasks in a plastic bag.
4. Shake flasks containing glial cells overnight at 250 rpm at 37°C.

NOTE: First shaking is performed at 8 DIV and you can perform up to 3 different shaking (see Figure 1 for timing)

8. OL lineage cells harvesting and culture- in a laminar flow hood under sterile conditions

1. The next day, prepare Bottenstein-Sato (BS) medium according to Table 1.
2. Rinse coated petri dishes 3 times with sterile distilled water.
3. Harvest flasks' supernatant containing mainly OL lineage cells but also some microglial cells and plate it on non-coated 100 mm-petri dishes. This step allows removal of microglial cells through differential fast adhesion on the dish surface.
4. Incubate the petri dishes for 15 min in a humidified incubator at 37°C – 5%CO₂.
5. Fill each T150 flask with 25 ml of warm and freshly made culture medium and incubate them in a humidified incubator at 37°C – 5%CO₂ until second shaking.
6. Transfer the supernatant of the petri dishes on new non-coated 100mm-petri dishes to allow adhesion of residual microglial cells.
7. Incubate the petri dishes for 15 min in a humidified incubator at 37°C – 5%CO₂.
8. Remove the supernatant, which contains non-adherent OL lineage cells, and put it into 50 ml tubes (supernatant from 2 petri dishes for a 50 ml tube). Discard petri dishes plated with microglia.
9. Centrifugate for 5 min at 423 g.
10. Carefully remove supernatant and resuspend cell pellet with 1 ml of BS.
11. Pool all pellets in a common 50 ml tube and adjust volume to 10 ml with BS.
12. Cell density is determined by counting cells under the microscope.

NOTE: A cell density between 3 and 5x10⁵/ml should be obtained.

13. Add 20 ml of BS if cell density is higher than or equal to 4 x10⁵/ml to obtain a final volume of 30ml, or add only 10 ml of BS if cell density is less than 4x10⁵/ml to obtain a final volume of 20 ml.
14. Plate two or three pre-coated 100 mm-petri dishes with 10 ml of cell suspension.

15. Incubate in a humidified incubator at 37°C – 5%CO₂.

16. 2 hours later, clear the debris from the petri dishes by renewing all BS medium.

NOTE: Examine the culture under the microscope before and after clearing to verify cell density and efficiency of debris removal.

17. Incubate for 2 days in BS medium in a humidified incubator at 37°C – 5%CO₂.

NOTE: Examine the culture under the microscope. The confluence should be 70 to 80%.

9. OCM production - in a laminar flow hood under sterile conditions

1. Prepare NB-B27low medium according to Table 2.
2. Renew culture medium with 10 ml of warm NB-B27low medium.
3. Incubate for 2 days in a humidified incubator at 37°C – 5%CO₂.
4. Harvest the OCM, *i.e.* supernatant containing OL secreted factors.
5. Filter-sterilize OCM using a 0.22 µm filter.

NOTE: Store OCM at 4°C for a maximum of 2 months

10. OCM addition - in a laminar flow hood under sterile conditions

NOTE: OCM can be added to purified hippocampal neuron cultures prepared according to the following protocol¹⁴, and obtained by adding, 24h after isolation, the anti-mitotic agents uridine and 5- fluorodeoxyuridine (5µM) for 36h.

1. At 3 DIV, remove all neuron culture medium containing anti-mitotic agents and add 500 µl of freshly made warm OCM.
2. Renew half of medium every 3 days with freshly made warm NB-B27.

NOTE: Such cultures can be maintained up to 21 DIV.

11. Addition of OL to purified hippocampal neuron culture- in a laminar flow hood under sterile conditions

NOTE: OLs can be added to purified hippocampal cultures obtained the same way as described above.

1. Prepare co-culture medium according to [Table 3](#).
2. Take the OL culture at 70 to 80% confluence.
3. Rinse with 2 ml of warm PBS 1X.
4. To detach cells, add 2 ml of trypsin 0.25% to a 100mm petri dish.
5. Incubate 5 min in a humidified incubator at 37°C – 5%CO₂.
6. Add 2 ml of DMEM-10%FCS to block enzymatic reaction.
7. Harvest the supernatant containing OL lineage cells.
8. Centrifuge at 423 g for 5 min at RT.
9. Carefully remove supernatant and resuspend cell pellet in warm co-culture medium to obtain a concentration of 1.25×10^5 cells/ml.
10. Add OL to purified hippocampal neurons culture by removing 200 μ l of neuron culture medium and adding 200 μ l of cell suspension per well (2.5×10^4 cells/well) of 24 well plate.
11. Renew half of co-culture medium every 2-3 days.

NOTE: Co-cultures can be maintained up to 24 DIV.

Representative results:

In this protocol, OL lineage cells are purified from glial cultures by shaking off astrocytes and microglia. Purity and phenotypic examination of OL cultures can be assessed by immunostaining with glial markers¹⁵. Analysis of different markers expression indicated that OL cultures were mostly pre-OLs with $90 \pm 4\%$ of O4⁺ cells, $85 \pm 7\%$ NG2⁺ cells, and $4.7 \pm 2.1\%$ of PLP⁺ cells, while $7.2 \pm 2.5\%$ of cells were GFAP⁺ astrocytes (mean \pm sd, n=3; Figure 2). [In addition, \$4.6 \pm 0.7\%\$ of cells were CD11b⁺ microglial cells \(mean \$\pm\$ sd, not shown\).](#)

OCM produced from such cultures can be added at 3 DIV to purified hippocampal neuron cultures. This treatment promotes the clustering of nodal proteins, consisting of Na_v channels associated with Neurofascin 186 and Ankyrin G along the axon of hippocampal GABAergic

neurons before myelination, at 17 DIV (Figure 3A and 3B). Of note, electrophysiological recordings revealed that these clusters are associated with an increased conduction of action potentials¹⁴. In addition, expression of phosphorylated intermediate filament protein H stained by Smi31 is increased in OCM-treated hippocampal neurons (Figure 3A). Oligodendroglial secreted factors are therefore implicated in neuronal maturation and physiology.

Myelination of hippocampal neurons can be studied through addition of OL at 14 DIV. From 20 DIV to 24 DIV, immunostaining of myelin markers, such as proteolipid protein (PLP) allows visualisation of myelin segments (Figure 4).

Figure 1: Protocol timeline of OL lineage cells isolation and OCM production. After dissecting out cerebral cortices from P2 Wistar rats (step 4), perform tissue dissociation to culture glial cells (step 5). At 8, 12 and 15 DIV (*i.e.* days before shaking), renew half medium with warm DMEM-10%FCS (step 6). The next day, shake glial cultures overnight at 250 rpm at 37°C (steps 7.3 and 7.4). Harvest supernatant containing OL lineage cells and few microglia cells and plate it for 15 min in a humidified incubator at 37°C-5%CO₂ (steps 8.3 to 8.7). Centrifugate supernatant for 5 min at 423 g, resuspend cell pellet with BS and incubate for 2 days in a humidified incubator at 37°C-5%CO₂ (steps 8.8 to 8.17). To produce OCM, incubate for 2 days in NB-B27low (step 9). To isolate OLs for co-culture experiments, detach cell using trypsin (step 11.4).

Figure 2: OL lineage cells phenotype in cultures. Images were acquired using an Olympus FV-1200 Upright Confocal Microscope. Maximum intensity projections are represented. (A) OL cultures contain mostly pre-OLs (*i.e.* expressing only NG2 (red), or both O4 (green) and NG2 (red); cells expressing both markers are indicated with yellow stars), but also some immature OLs (*i.e.* only expressing O4 and not NG2; white stars). (B) Few mature OLs (*i.e.* PLP+; green) and few astrocytes (GFAP+ cells; red) are found in OL lineage cell cultures. Scale bars 25 µm.

Figure 3: Representative applications. (A) and (B) Hippocampal neurons treated with OCM at 3 DIV and fixed at 17 DIV express phosphorylated intermediate filament protein H (Smi31; green; Figure 3A). GABAergic neurons, identified by glutamate decarboxylase isoform of 67kDa (GAD67) expression (white), display accumulation of Ankyrin G and Na_v sodium

channels (red; Figure 3A and 3B, respectively) at the axon initial segment and form Ankyrin G and Na_v clusters along their axon (Figure 3A and 3B, respectively). Scale bars 25 μm.

Figure 4: Representative applications. OL lineage cells added to hippocampal neuron culture at 14 DIV myelinate some hippocampal axons, here fixed at 23 DIV (PLP as a myelin marker; green). Nodes of Ranvier (Na_v; red) are observed in between myelin segments. Scale bar 25 μm.

Discussion

Here, we provide a detailed protocol to obtain highly enriched oligodendroglial lineage cell cultures from mixed glial cultures, adapted from a previously published method¹⁶, and the subsequent production of OL conditioned medium. This shaking technique is not expensive, can be repeated three times and is optimal to obtain high quantity of purified OLs, as cells cultured in Bottenstein-Sato (BS) medium containing PDGF α proliferate. Glial cells are prepared using cerebral cortices of Wistar rats at P2, a time point at which a vast majority of the oligodendroglial lineage cells are pre-oligodendrocytes expressing NG2 and O4¹⁵. Of note, OL lineage maturation is similar at P2 in mouse and rat, and this protocol can also be used to isolate mouse pre-oligodendrocytes¹⁷.

After mixed glial cell cultures shaking, detached cells consist mainly of oligodendroglial lineage cells, but also some microglial cells and few astrocytes. Microglial cells are removed through differential adhesion on uncoated petri dishes. Of note, removal efficiency can be improved by performing an additional adhesion step. However, about 5% of microglial cells are still found in enriched oligodendroglial cell cultures, as well as 5 to 9% of astrocytes. It is possible to decrease contamination from astrocytes to less than 5% by performing an additional immuno-panning step using O4 antibody coated petri dishes, for a detailed protocol see supplemental information in¹⁴. The removal of debris two hours after plating oligodendroglial cells is a critical step, which relies on the strength of the flow applied with the pipet. At this step, it is important to examine the culture under the microscope before and after clearing to verify the efficiency as the presence of too much debris may impair cell viability and growth.

Of note, it is also important to use freshly made BS medium, otherwise it could alter oligodendrocytes survival. In addition, purified cells survive only up to 6 days after plating. Indeed, it is known that other glial cells and neurons promote OPC survival and proliferation or differentiation through secreted factors or direct contacts^{2,18}.

Other methods allow OLs isolation immediately after brain dissociation, using immunolabelling with O4 antibody followed by fluorescent-activated cell sorting by flow cytometry (FACS) or magnetic-activated cell sorting (MACS). In addition, GFP-positive OPCs or GFP-positive oligodendrocytes can be purified by fluorescent-activated cell sorting from *PDGF α R:GFP* or *PLP:GFP* mice, respectively^{19,20}. These sorting methods are more relevant for studying physiological state of oligodendrocytes compared to cultures treated with growth factors which could altered their phenotype. Notably, fluorescent-activated cell sorting has been used for gene-profiling approaches in the normal physiological state and demyelinating conditions²¹. As cell survival could be altered by cell sorting, it is better to perform functional assays immediately after sorting.

We have shown that OL cultures can be detached and added to purified hippocampal neuron cultures at 14 DIV. Such OL-neuron co-culture allows the study of early steps of myelination which starts during the first week of co-culture (Dubessy AL, unpublished results). Other models of OL-hippocampal neuron myelinating co-culture have been achieved by adding oligodendrocytes immediately after sorting^{22,23}. Furthermore, we produced OCM to further dissect OL - neuron interactions and address the role of OL secreted factors on neuron cultures. By using this technique, we demonstrated that hippocampal GABAergic neuron subtypes (*i.e.* parvalbumin⁺ and/or somatostatin⁺) can form clusters of nodal proteins along their axon which are induced by OCM prior to myelination^{14,24}. Mass spectrometry analysis of OCM has unraveled several secreted proteins and led to identify oligodendroglial Contactin-1 that in synergy with extracellular matrix proteins mediates early steps of nodal clustering²⁴. Primary cultures are useful models that allow the assessment of oligodendroglial lineage cell differentiation and interactions with neurons. However, other approaches have also been developed to evaluate OL functions and myelination, demyelination and remyelination from *ex-vivo* cerebellar organotypic slice cultures^{25,26}, and *in vivo* studies, notably with zebrafish and tadpole models²⁷ which are needed in final steps of pre-clinical studies.

Acknowledgements:

The authors would like to thank Rémi Ronzano for his wise advices in manuscript editing. This work was funded by ICM, INSERM, ARSEP foundation grant to NSF, and Bouvet-Labruyère price.

Disclosures:

None of the authors have competing interests or conflicting interests.

References:

1. Zalc, B. The acquisition of myelin: a success story. *Novartis Foundation Symposium*. **276**, 15–21; discussion 21-25, 54–57, 275–281 (2006).
2. Habermacher, C., Angulo, M.C., Benamer, N. Glutamate versus GABA in neuron-oligodendroglia communication. *Glia*. **67** (11), 2092–2106, doi: 10.1002/glia.23618 (2019).
3. Sherman, D.L., Brophy, P.J. Mechanisms of axon ensheathment and myelin growth. *Nature Reviews. Neuroscience*. **6** (9), 683–690, doi: 10.1038/nrn1743 (2005).
4. Freeman, S.A., Desmazières, A., Fricker, D., Lubetzki, C., Sol-Foulon, N. Mechanisms of sodium channel clustering and its influence on axonal impulse conduction. *Cellular and molecular life sciences: CMLS*. **73** (4), 723–735, doi: 10.1007/s00018-015-2081-1 (2016).
5. Lee, Y. *et al.* Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature*. **487** (7408), 443–448, doi: 10.1038/nature11314 (2012).
6. Nave, K.-A. Myelination and the trophic support of long axons. *Nature Reviews. Neuroscience*. **11** (4), 275–283, doi: 10.1038/nrn2797 (2010).
7. Monje, M. Myelin Plasticity and Nervous System Function. *Annual Review of Neuroscience*. **41**, 61–76, doi: 10.1146/annurev-neuro-080317-061853 (2018).
8. Birey, F. *et al.* Genetic and Stress-Induced Loss of NG2 Glia Triggers Emergence of Depressive-like Behaviors through Reduced Secretion of FGF2. *Neuron*. **88** (5), 941–956, doi: 10.1016/j.neuron.2015.10.046 (2015).

9. Frühbeis, C. *et al.* Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. *PLoS biology*. **11** (7), e1001604, doi: 10.1371/journal.pbio.1001604 (2013).
10. Jang, M., Gould, E., Xu, J., Kim, E.J., Kim, J.H. Oligodendrocytes regulate presynaptic properties and neurotransmission through BDNF signaling in the mouse brainstem. *eLife*. **8**, doi: 10.7554/eLife.42156 (2019).
11. Sakry, D. *et al.* Oligodendrocyte precursor cells modulate the neuronal network by activity-dependent ectodomain cleavage of glial NG2. *PLoS biology*. **12** (11), e1001993, doi: 10.1371/journal.pbio.1001993 (2014).
12. Sakry, D., Yigit, H., Dimou, L., Trotter, J. Oligodendrocyte precursor cells synthesize neuromodulatory factors. *PLoS One*. **10** (5), e0127222, doi: 10.1371/journal.pone.0127222 (2015).
13. Stadelmann, C., Timmler, S., Barrantes-Freer, A., Simons, M. Myelin in the Central Nervous System: Structure, Function, and Pathology. *Physiological Reviews*. **99** (3), 1381–1431, doi: 10.1152/physrev.00031.2018 (2019).
14. Freeman, S.A. *et al.* Acceleration of conduction velocity linked to clustering of nodal components precedes myelination. *Proceedings of the National Academy of Sciences of the United States of America*. **112** (3), E321-328, doi: 10.1073/pnas.1419099112 (2015).
15. Baumann, N., Pham-Dinh, D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiological Reviews*. **81** (2), 871–927, doi: 10.1152/physrev.2001.81.2.871 (2001).
16. McCarthy, K.D., de Vellis, J. Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *The Journal of Cell Biology*. **85** (3), 890–902, doi: 10.1083/jcb.85.3.890 (1980).
17. Dean, J.M. *et al.* Strain-specific differences in perinatal rodent oligodendrocyte lineage progression and its correlation with human. *Developmental Neuroscience*. **33** (3–4), 251–260, doi: 10.1159/000327242 (2011).
18. Domingues, H.S., Portugal, C.C., Socodato, R., Relvas, J.B. Oligodendrocyte, Astrocyte, and Microglia Crosstalk in Myelin Development, Damage, and Repair. *Frontiers in Cell and Developmental Biology*. **4**, 71, doi: 10.3389/fcell.2016.00071 (2016).

19. Klinghoffer, R.A., Hamilton, T.G., Hoch, R., Soriano, P. An allelic series at the PDGFalphaR locus indicates unequal contributions of distinct signaling pathways during development. *Developmental Cell*. **2** (1), 103–113 (2002).
20. Spassky, N. *et al.* The early steps of oligodendrogenesis: insights from the study of the plp lineage in the brain of chicks and rodents. *Developmental Neuroscience*. **23** (4–5), 318–326 (2001).
21. Moyon, S. *et al.* Demyelination Causes Adult CNS Progenitors to Revert to an Immature State and Express Immune Cues That Support Their Migration. *Journal of Neuroscience*. **35** (1), 4–20, doi: 10.1523/JNEUROSCI.0849-14.2015 (2015).
22. Gardner, A., Jukkola, P., Gu, C. Myelination of rodent hippocampal neurons in culture. *Nature Protocols*. **7** (10), 1774–1782, doi: 10.1038/nprot.2012.100 (2012).
23. Thetiot, M. *et al.* An alternative mechanism of early nodal clustering and myelination onset in GABAergic neurons of the central nervous system. *bioRxiv*. 763573, doi: 10.1101/763573 (2019).
24. Dubessy, A.-L. *et al.* Role of a Contactin multi-molecular complex secreted by oligodendrocytes in nodal protein clustering in the CNS. *Glia*. **67** (12), 2248–2263, doi: 10.1002/glia.23681 (2019).
25. Barateiro, A., Fernandes, A. Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. *Biochimica Et Biophysica Acta*. **1843** (9), 1917–1929, doi: 10.1016/j.bbamcr.2014.04.018 (2014).
26. Thetiot, M., Ronzano, R., Aigrot, M.-S., Lubetzki, C., Desmazières, A. Preparation and Immunostaining of Myelinating Organotypic Cerebellar Slice Cultures. *Journal of Visualized Experiments: JoVE*. (145), doi: 10.3791/59163 (2019).
27. Mannioui, A., Zalc, B. Conditional Demyelination and Remyelination in a Transgenic *Xenopus laevis*. *Methods in Molecular Biology (Clifton, N.J.)*. **1936**, 239–248, doi: 10.1007/978-1-4939-9072-6_14 (2019).