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CHAPTER

6

Investigating demyelination, efficient remyelination and remyelination failure in organotypic cerebellar slice cultures: Workflow and practical tips

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Chapter outline

Abstract

Healthy myelin is essential for proper brain function. When the myelin sheath is damaged, fast saltatory impulse conduction is lost and neuronal axons become vulnerable to degeneration.

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Thus, regeneration of the myelin sheath by encouraging oligodendrocyte lineage cells to remyelinate the denuded axons is a promising therapeutic target for demyelinating diseases such as multiple sclerosis. *Ex vivo* organotypic cerebellar slice cultures are a useful model to study developmental myelination, demyelination, remyelination and remyelination failure. In these cultures, the cerebellum's three-dimensional architecture and various cell populations remain largely intact, providing a realistic and relatively cost-efficient model that can be easily manipulated by the addition of viral vectors, pharmaceuticals or (transgenic) cells to augment or replace resident cell populations. Moreover, slice cultures can be treated with lysolecithin or polyinosinic:polycytidylic acid to induce demyelination and mimic efficient as well as inefficient remyelination. It can be challenging to set up slice cultures for the first time, as in our experience, seemingly minor differences in technique and materials can make a great difference to the quality of the cultures. Therefore, this report provides an in-depth description for the generation and maintenance of *ex vivo* organotypic cerebellar cultures for demyelination-remyelination studies with a focus on practical tips for scientists that are new to this technique.

1 Introduction

During central nervous system (CNS) development, oligodendrocyte lineage cells wrap neuronal axons in lipid-rich membrane sheets interspersed with myelin proteins (Franklin & ffrench-Constant, 2008). This myelin sheath is essential for proper brain function, as it not only provides protection, metabolic and trophic support to the axon, but also serves as an insulating layer that facilitates the fast conduction of neuronal impulses (Nave & Werner, 2014). When damage to the myelin sheath occurs, swift saltatory impulse transmission is disturbed and the axons are left bare and vulnerable to degeneration (Franklin & ffrench-Constant, 2008; Nave & Werner, 2014). A healthy brain will respond to myelin injury with the local upregulation of factors that recruit oligodendrocyte progenitor cells (OPCs) and/or pre-existing mature oligodendrocytes (Duncan et al., 2018; Yeung et al., 2019), which become activated and ultimately remyelinate the denuded axons (Franklin & ffrench-Constant, 2008). However, in demyelinating diseases such as multiple sclerosis (MS), remyelination efficiency often fails over time, leading to permanent loss of conduction and secondary neurodegeneration (Franklin & ffrench-Constant, 2008; Gruchot et al., 2019). This results in progressive accumulation of sensory, motoric and cognitive deficits, depending on the area(s) of the CNS affected (Franklin & ffrench-Constant, 2008). In some clinical subtypes, *e.g.,* primary progressive MS, remyelination efficiency appears to be poor or non-existent from the start, leading to faster and more debilitating disease progression (Faissner, Plemel, Gold, & Yong, 2019). Although MS has been studied for over 150 years, many aspects of the disease remain elusive. Current therapeutics mainly target the peripheral immune system, and even though these drugs reduce the frequency and severity of damage-inducing relapses, they are unable to completely halt disease progression or restore lost function. In this context, the promotion of remyelination to prevent secondary neurodegeneration is a promising therapeutic target (Lubetzki, Zalc, Williams, Stadelmann, & Stankoff, 2020).

Ex vivo organotypic cerebellar slice cultures are an established and well-characterized model for studying developmental myelination (Daviaud, Garbayo, Schiller, Perez-Pinzon, & Montero-Menei, 2013; Haber, Vautrin, Fry, & Murai, 2009; Hill, Medved, Patel, & Nishiyama, 2014; Rakotomamonjy & Guemez-Gamboa, 2019; Sherafat, Hill, & Nishiyama, 2018) as well as demyelination-remyelination paradigms (Birgbauer, Rao, & Webb, 2004; Doussau et al., 2017; Miron et al., 2010; Shen & Yuen, 2020; Zhang, Jarjour, Boyd, & Williams, 2011). Investigating demyelination in organotypic brain slice cultures goes back as early as the 1950s, when serum from mice with experimental autoimmune encephalitis, a widely used experimental animal model for inflammatory demyelination, induced demyelination in primitive slices of rat cerebellum (Bornstein & Appel, 1959). Over the next 50 years, improvements in culturing technique greatly enhanced the viability of brain slices (Stoppini, Buchs, & Muller, 1991) and in 2004 it was discovered that the phospholipid lysophosphatidylcholine, named lysolecithin, can be used to demyelinate axons in rat cerebellum slices (Birgbauer et al., 2004). These advances enabled the implementation of brain slice cultures as a model for CNS demyelination, *e.g.*, in the context of MS. Slice cultures can be generated from various areas of the CNS, including the hippocampus, cortex and spinal cord. As we learn more about regional differences within the CNS, care should be taken to select the appropriate region for each research question. Traditionally, the cerebellum has been used for demyelination-remyelination studies and to this day, it is often selected because its structure is well-preserved and contains both gray and white matter. In contrast to traditional cell culture, in cerebellar slice cultures the three-dimensional brain architecture remains largely intact and neuronal axons as well as neuron, microglia, oligodendrocyte and astrocyte populations are preserved (Haber et al., 2009; Hill et al., 2014), providing a more representative context for investigating cell behavior in response to demyelination. The cultures can be generated from neonatal rats (Birgbauer et al., 2004; Sheridan & Dev, 2012; Wolters et al., 2021) as well as mice (Miron et al., 2010; Sherafat et al., 2018; Tian et al., 2020), including genetically modified strains, and once in culture can be manipulated with relative ease by the addition of drugs (de Almeida et al., 2021; Wolters et al., 2021), the use of viral vectors to alter gene expression (Gaudillière, Konishi, Ria de la Iglesia, Yao, & Bonni, 2004; Wiegert, Gee, & Oertner, 2017) and the depletion and/or addition of cells (Coleman, Zou, & Crews, 2020; Pritchard, Mir, & Dev, 2014). For example, microglia can be specifically depleted from slice cultures by incubating the slices with liposomes containing clodronate disodium salt (Ta et al., 2019; Van Weering et al., 2011), a bisphosphonate used in the treatment of osteoporosis, or by adding pharmaceutical inhibitors of the colony-stimulating factor 1 receptor (Coleman et al., 2020; Liu et al., 2019). Similarly, high concentrations of bromodeoxyuridine (BrdU), a chemical compound commonly used in cell proliferation assays, can be employed to deplete the OPC population (Baudouin et al., 2021; Bouslama-Oueghlani, Wehrlé, Sotelo, & Dusart, 2003; Younkin & Silberberg, 1973). One cerebellum yields multiple slices, and a typical experiment lasts under 6 weeks, all in all providing a time-efficient, cost-effective and versatile model.

Our lab has previously used cerebellar slice cultures from rats (Qin et al., 2017; Werkman et al., 2020) and mice to study remyelination failure in the context of MS. Besides the classic demyelination-remyelination paradigm with lysolecithin, we have established a double-hit design with lysolecithin and the TLR3 agonist polyinosinic:polycytidylic acid, or Poly(I:C), to model the chronic inflammation and inefficient remyelination observed in MS. Addition of Poly(I:C) to slice cultures leads to aggregation of the extracellular matrix (ECM) protein fibronectin and reduced remyelination, as fibronectin aggregates have been hypothesized to contribute to remyelination failure in MS (Stoffels et al., 2013; Stoffels, Hoekstra, Franklin, Baron, & Zhao, 2015; Stoffels, Zhao, & Baron, 2013a; Wang et al., 2018; Werkman et al., 2020). Culturing cerebellar slices is an established technique for which a number of excellent protocols have already been published (Birgbauer et al., 2004; Hill et al., 2014; Shen & Yuen, 2020; Thetiot, Ronzano, Aigrot, Lubetzki, & Desmazières, 2019). Previous protocols on culturing cerebellar slices have focused on clearly visualizing the dissection phase using video (Hill et al., 2014; Shen & Yuen, 2020; Thetiot et al., 2019), describing different experimental paradigms such as demyelination-remyelination (Birgbauer et al., 2004; Shen & Yuen, 2020) and hypoxia (Shen & Yuen, 2020), or establishing methods for (live) imaging of slice cultures (Hill et al., 2014; Thetiot et al., 2019). However, despite the available information, setting up slice cultures for the first time, or in a new lab, can still be a challenging endeavor. In our experience, seemingly minor differences in technique and materials can make a great difference to the quality of the cultures. Therefore, we aimed to complement the existing literature by providing a detailed description of our workflow with a focus on practical tips for the slice culture novice. Here, we describe our protocol for the generation and culture of *ex vivo* organotypic cerebellar slice cultures, including how lysolecithin and Poly(I:C) can be used to mimic efficient and inefficient remyelination, and provide in-depth discussion on how to identify and avoid potential caveats when first establishing *ex vivo* organotypic cerebellar slice cultures.

2 Materials

- Cell culture incubator
- Laminar flow cabinet
- Dissection microscope
- McIlwain tissue chopper
- 50 mL tube holder
- Small beaker
- Surgical scissors (Fine Science Tools, 14000)
- Spring scissors (Fine Science Tools, 15040)

- 2 × regular forceps
- 2 × Dumont Forceps #5 (Fine Science Tools, 11251) or #5SF (Fine Science Tools, 11252-00)
- 2 × fine double spatula of 120 mm length and 4 mm width
- Millicel cell culture inserts (0.4 µm pore size, Millipore, PICM03050)
- Sterile 6-well plates
- Sterile 50 mL tubes
- Sterile petri dishes (100 mm width)
- Kimwipes
- *Dissection medium (50 mL):* 48.1 mL Hank's balanced salt solution (HBSS; Mediatech; 21–023-CV), 650 µL 45% Glucose (Sigma; G8769), 750 µL 1 M HEPES (Gibco-Invitrogen; 15630080)
- *Culture medium (1000 mL):* 50 mL 10 × Minimum essential medium (MEM) + Earle's salts (Gibco-Invitrogen; 21430-020), 250 mL Basal Medium Eagle (BME) +Earle's salts (Gibco-Invitrogen; 41010), 250 mL Heat-inactivated horse serum (Life technologies; 26050088), 14.4 mL 45% Glucose (Sigma; G8769), 10 mL GlutaMax (Gibco-Invitrogen; 35050061), 25 mL 1 M HEPES (Gibco-Invitrogen; 15630080); sterile distilled water; Sodium bicarbonate 7.5% solution (Gibco-Invitrogen; 25080094) or (sterile-filtered) 1M sodium hydroxide (NaOH); 1M hydrochloric acid (HCl) (Notes 1–6)
- Sterile phosphate-buffered saline (PBS)
- 70% ethanol
- Lysolecithin (Sigma; L1381; stock solution 125 mg/mL in PBS; Note 7)
- Poly(I:C) (Sigma-Aldrich, P1530; stock solution 1mg/mL in PBS; Note 8)

3 Preparation

1. We recommend using either neonatal rats at postnatal day 1 (P1) to P3 or neonatal mice at P7 to P9 (Note 9). Naturally, all animal experiments must comply with local legislation and ethical guidelines, and need permission from the appropriate regulatory boards.
2. Put the appropriate number of Millicell cell culture inserts in a 6-well plate (Note 10) with 1.2 mL of culture medium per well and equilibrate in the incubator at 5% CO_2 and 35 °C (Note 11).
3. Fill a 50 mL tube with approximately 20 mL of dissection medium for collection of the dissected brains. Prepare one tube per litter, storing the tubes on ice. Also, fill 35 mm petri dishes with approximately 4 mL of collection medium. These will be used for further dissection of the cerebellum. Prepare two petri dishes per neonatal rat/mouse, storing the dishes at 4 °C.

4. *Materials for dissecting the brains:* cold 10 cm petri dish (Note 12), small beaker or similar container with tissue and 70% ethanol to hold tools, 2× regular forceps, surgical scissors, spring scissors, 50 mL tubes with dissection medium on ice.
5. *Materials for dissection of the cerebellum (in laminar flow cabinet):* dissection microscope, McIlwain tissue chopper, 50 mL tube holder, 50 mL tube with tissue and 70% ethanol to hold tools, 50 mL tube with sterile PBS to rinse tools, 2× fine double spatula, 2× Dumont Forceps #5 or #5SF (Fig. 1; Notes 13–14).

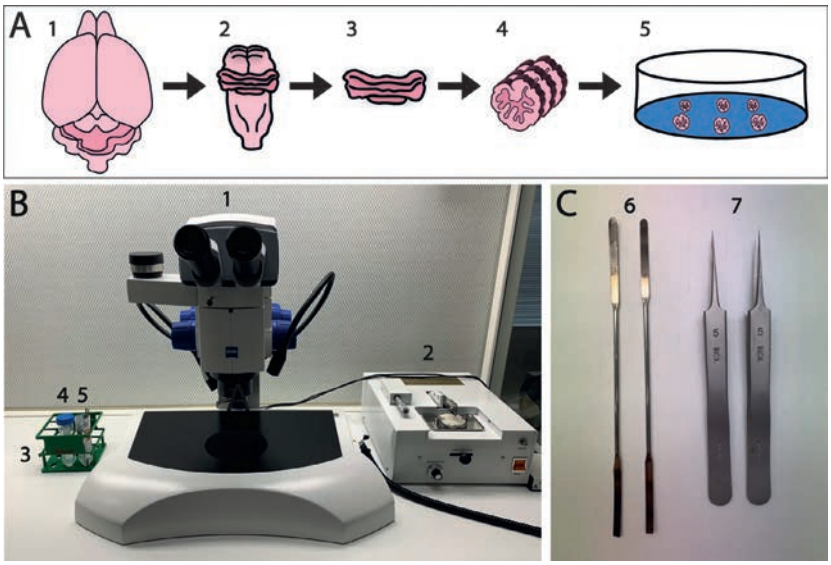

FIG. 1

Workflow and equipment. (A) Schematic depicting the steps to generate *ex vivo* organotypic cerebellar slice cultures, respectively (A,1) isolation of brain from neonatal rats or mice, (A,2) dissection of the hindbrain and removal of the meninges, (A,3) further dissection of the cerebellum, (A,4) cutting the cerebellum in slices of 375 μm thickness using the McIlwain Tissue Chopper and (A,5) culturing slices at an air-liquid-surface on semiporous Millipore inserts. (B) Overview of necessary equipment in laminar flow cabinet: (B,1) dissection microscope, (B,2) McIlwain tissue chopper, (B,3) 50 mL tube holder, (B,4) sterile 50 mL tube with sterile PBS to rinse tools and (B,5) 50 mL tube with a clean Kimwipe and 70% ethanol to hold tools. (C) Required tools: (C,1) Dumont Forceps #5 or #5SF (2×) and (C,2) fine double spatula of 120 mm length and 4 mm width (2×).

4 Dissection

4.1 Brain dissection (at the bench)

1. Decapitate the neonatal rat/mouse with sharp surgical scissors (do this as quickly as possible).
2. Put the head on the cold 10 cm petri dish, then hold the head by the nose with the forceps.
3. Cut the skin from back to nose using spring scissors, then lift the skin aside using forceps.
4. Carefully cut open the skull. First, cut a horizontal line between the eye sockets. Then, cut a vertical line starting from the middle of the horizontal line between the eyes to just before the beginning of the cerebellum. Next, use the forceps to gently move the skull pieces away to expose the brain and cerebellum. Work carefully to avoid damaging the cerebellum.
5. Gently take the brain out using forceps and store in a 50 mL tube with dissection medium on ice (Note 15).
6. Repeat step 1–5 with the rest of the neonatal rats/mice.

4.2 Cerebellum dissection (in the laminar flow hood)

7. Work in the laminar flow hood for the following steps. Take a 35 mm petri dish containing prechilled dissection medium out of the 4 °C fridge. Take the 50 mL tube in which you collected the brains and carefully transfer the first brain to the 35 mm dish using a fine spatula (Note 16). It is helpful to tilt the 50 mL tube until it is almost at a 90° angle.
8. Use the dissection microscope for the following steps. Place the 35 mm petri dish containing dissection medium and the brain under the microscope.
9. Separate forebrain and hindbrain by gently holding the forebrain down with the forceps in your non-dominant hand, while using the forceps in your dominant hand to pinch through the tissue just above the pons. Then, the forebrain can either be discarded or stored in dissection medium on ice to be used for primary cell culture.
10. Remove the meninges from the cerebellum (Fig. 2B–C; Note 17).
11. Carefully separate the cerebellum from the pons and medulla oblongata (Fig. 2A–D; Note 17). If necessary, remove any remaining loosely attached pieces of meninges.

4.3 Preparation of the slices (in the laminar flow hood)

12. Dip the spatulas in the medium (Note 18), then take the cerebellum out of the dissection medium using the spatulas. Place the cerebellum on the table of the McIlwain tissue chopper at a 90° angle with the blade (Fig. 2E), making sure the cerebellum is as straight as possible.

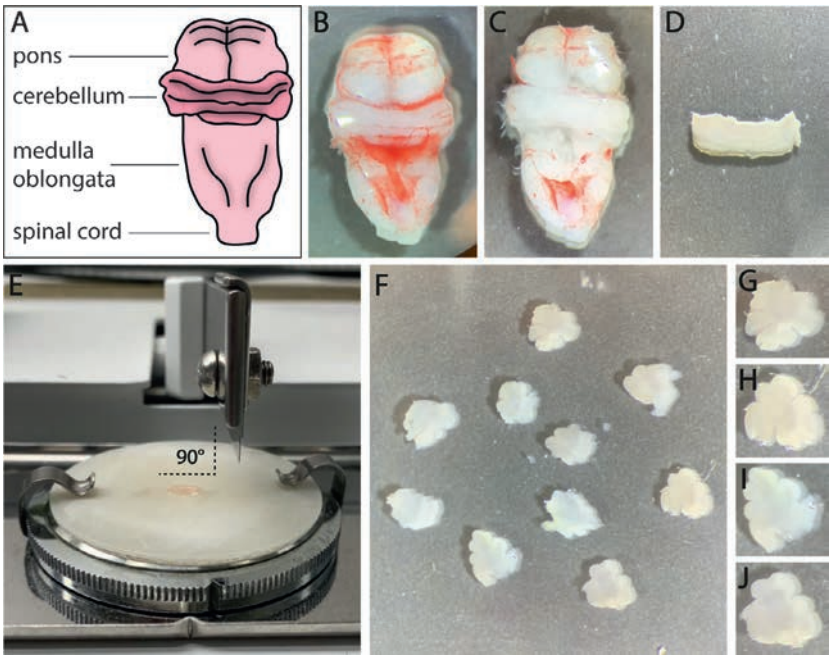

FIG. 2

Dissection of the cerebellum. (A) Anatomy of the rodent hindbrain. (B) Rat hindbrain before meninges removal as seen through the dissection microscope. (C) Rat hindbrain after meninges has been removed from the cerebellum as seen through the dissection microscope. (D) Dissected rat cerebellum as seen through the dissection microscope. (E) Cerebellum on McIlwain Tissue Chopper placed at a 90° angle with the blade. (F) Cerebellar slices after cutting as seen through the dissection microscope. (G–J) Representative images of cerebellar slices of good quality as seen through the dissection microscope.

13. Use a clean 200 µL pipette to remove excessive medium around the cerebellum (Note 19).
14. Cut slices of 375 µm thickness (Notes 20–21).
15. Use the spatulas dipped in dissection medium to collect the slices and put the slices in a clean 35 mm petri dish with ice cold dissection medium.
16. The slices will float in the dissection medium. Carefully push the slices to the bottom by tapping the slices with a spatula.
17. Use the dissection microscope to find slices of sufficient quality (Fig. 2G–J; Note 22). One cerebellum generally yields 8–10 slices of good quality.
18. Take the pre-equilibrated culture plate out of the incubator and transfer the slices to the inserts (4–6 slices per insert, one slice at a time) using the spatulas

(which have first been dipped in medium). Close the lid and put the culture plate back into the incubator. Any excess medium around the slices does not need to be removed, as it will soon evaporate and does not influence the viability of the slice cultures.

19. Carefully clean the blade with 70% ethanol and a clean Kimwipe to prevent sticking of the next cerebellum to the blade. Lastly, disinfect the blade by generously spraying with 70% ethanol and let air dry.
20. Repeat steps 7–18 for the next brain.

5 Demyelination-remyelination experiments

1. Culture the slice cultures at 5% CO_2 and 35 °C. First change the culture medium on the morning after the isolation (Note 23). Then, change the medium every 2–3 days. Use 1.2 mL medium per well. The medium does not contain antibiotics or antifungals, therefore you have to work very cleanly during the isolation as well as when changing the medium.
2. On day 10 (mice) or day 21 (rats; Notes 24–25), demyelination is induced by incubating the slices with pre-warmed medium containing 0.4 mg/mL (mice) or 0.5 mg/mL (rats) lysolecithin (diluted from a 125 mg/mL stock solution; Sigma; L1381; Notes 7, 26) for 17 h (Note 27).
3. After 17 h, the inserts are rinsed once with warm PBS, whereafter fresh medium is added.
4. OPTIONAL STEP: To mimic remyelination failure, on day 2 post-lysolecithin treatment slices are changed to fresh medium containing 25 µg/mL (mice) or 50 µg/mL (rats) Poly(I:C) (diluted from a 1mg/mL stock solution; Sigma-Aldrich; P1530; Note 8) to induce the formation of remyelination-inhibiting fibronectin aggregates. After 48 h of treatment with Poly(I:C), the slices are changed to fresh medium.
5. A typical collection scheme is as followed: slices are collected on post-lysolecithin day 0 (control), day 2 (demyelination), day 7 (early remyelination) and day 14 (late remyelination). Slices can be fixed with paraformaldehyde (PFA; Note 28) for immunostaining (*e.g.*, to detect the myelin marker myelin basic protein (MBP) and the axonal marker neurofilament H (NF-H; Notes 29–30) and the percentage of myelinated axons can be calculated using MATLAB software (Note 31). Alternatively, slices can be collected for protein or RNA analysis.

6 Concluding remarks

This report provides an in-depth description for the generation and culture of *ex vivo* cerebellar slices for demyelination-remyelination studies with a focus on practical tips aimed at scientists that are new to this technique. As outlined in this protocol,

the optimum neonatal age, slice thickness, culture time, medium components and lysolecithin incubation time may slightly differ between labs and can be adapted by the individual user to ensure high quality cultures. Since the first report of a demyelination experiment in cerebellar slice cultures in 1959, *ex vivo* cerebellar slice cultures have continued to develop and improve, becoming an indispensable tool in the study of demyelination and remyelination. In recent years, protocols have been published describing for the first time how viable cerebellar slice cultures can be generated from adult mouse cerebellum (Mewes, Franke, & Singer, 2012; Tan et al., 2018), enabling comparisons between (re)myelination in the developing and mature CNS as well as the study of age-related factors in remyelination failure. Moreover, the development of small lysolecithin-containing cryogel scaffolds (Eigel et al., 2019) provides a new tool for investigating local demyelination in slice cultures, which likely more accurately mimics the focal demyelinating lesions that are key to the pathology of MS, and will allow to better study local differences in de- and remyelination, *e.g.,* between white and grey matter. Lastly, in the 21st century, protocols have become available that describe how to reproducibly isolate and culture slices derived from surgically-resected and post-mortem human brain (Qi et al., 2019; Schwarz et al., 2019; Verwer et al., 2002, 2015). Although these experiments are limited by the scarce availability of human brain tissues, they will help to bridge the translational gap that exists between the development of therapeutics in animal models and their (lack of) effectiveness in human subjects. In conclusion, *ex vivo* organotypic cerebellar slice culture remains a useful and versatile model that will continue to provide new insights into myelin (re)generation in the context of health and disease.

Notes

1. Work in the flow cabinet to prepare the culture medium. For 1000 mL of medium, add the 10 × MEM (50 mL), BME (250 mL), horse serum (250 mL), 45% glucose (14.4 mL), GlutaMax (10 mL) and 1M HEPES (25 mL) to a sterile beaker and fill up with sterile distilled water to approximately 950 mL. Add a sterile stir bar to the beaker, cover the beaker with foil, put on stir plate and thoroughly mix contents. Then, while stirring, adjust the pH to 7.2 by adding either sterile sodium bicarbonate or (sterile-filtered) 1M NaOH and/or (sterile filtered)1M HCl. Let the medium stir for a few minutes after you have adjusted the pH to make sure the pH remains stable. Then, fill up with sterile distilled water to 1000 mL. Go back to the laminar flow cabinet and sterilize the medium using a bottle-top filter with 0.2 µm pore-size.
2. It may be necessary to work outside of the laminar flow cabinet during preparation of the medium to access the stir plate and pH meter. To reduce the bacterial load, disinfect the surfaces of your workbench, stir plate and pH meter with 70% ethanol. The pH meter should first be calibrated and then the tip of the pH meter, which will be immersed in the medium, should be thoroughly sprayed with 70% ethanol and then directly rinsed three times with sterile ddH2O. Furthermore, it is advisable to cover the beaker with sterilized aluminum foil

(spray with 70% ethanol and let it dry 'downside-up' in laminar flow cabinet under UV light for 10 min) whenever possible.

3. Aliquot the medium into labeled 50 mL tubes. Only use 40 mL per tube as the medium expands during freezing. Store tubes in −20 °C freezer for up to 3 months. When you need a new tube, preferably let it thaw overnight in at 4 °C. Then, before you use the medium for the first time, sterilize once more using a 0.2 µm syringe filter fitted onto a 30 mL syringe, directly into a fresh 50 mL tube.
4. A low pH of 7.2 is critical for the viability of the slice cultures (Stoppini et al., 1991). This low pH makes the medium an orange/brown shade, but it will turn slightly to a more yellow, when it has been equilibrated with CO_2 in the incubator. When defrosting the first tube of a new batch of medium, always check the pH of the medium before the start of your experiment. Correct pH control is critical to slice culture success.
5. In our experience, as well as the experience of others (Gee, Ohmert, Wiegert, & Oertner, 2017), different batches of horse serum can affect the quality of the slice cultures, similar to primary cell culture, where the quality of fetal bovine serum can strongly affect the extent of oligodendrocyte differentiation. We have had good experiences with horse serum from Life Technologies (heat-inactivated horse serum, 26050088, New Zealand origin) and PAA Cell Culture Company (horse serum, B15-021). When purchasing horse serum that is not yet heat-inactivated, it should be incubated in the water bath at 56 °C for 30 min to inactivate the complement system. To reduce variability, use the same batch of medium within an experiment whenever possible.
6. It is a topic of debate whether antibiotics and/or antifungals should be added to the slice culture medium; some protocols encourage their use (Shen & Yuen, 2020; Zhang et al., 2011), while others do not (Miron et al., 2010; Sherafat et al., 2018). We have chosen not to use antibiotics nor antifungals, as they have the potential to alter cellular properties (Gee et al., 2017; Llobet, Montoya, López-Gallardo, & Ruiz-Pesini, 2015). This of course then requires meticulous antiseptic handling of the slice cultures. In the unlucky event of bacterial contamination, the medium will appear bright yellow and hazy, and may emit a foul odor. Under the light microscope, small moving particles may be observed in the medium and/or around the slice cultures. Fungal contamination will present as white/yellowish patches in or around the slice cultures that slowly expand. Under the light microscope, the patches will appear slightly 'furry' and detached pieces of tissue may be observed in the medium. In case of infection, the entire contaminated plate should be discarded and the incubator disinfected to prevent further spread.
7. Commercially available lysolecithin derived from bovine brain is delivered as a powder (Sigma, L1381). To achieve a 125 mg/mL stock solution, the vial with powder is first spun down for 2 min at 1000 g to ensure that virtually all lysolecithin moves to the bottom of the vial. Next, 200 µL of sterile PBS is added and the vial is left on a float in the water bath at 50 °C for 1 h to completely dissolve the lysolecithin. Then, the solution is gently pipetted up and down, hereby rinsing the inner sides of the bottle to ensure any remaining lysolecithin

is dissolved. Lysolecithin should dissolve easily and use of a vortex should not be necessary. The lysolecithin can be aliquoted in 10 tubes of 20 µL and stored at −20 °C. When an aliquot is need for an experiment, the tubes should be thawed in the water bath at 50 °C for 1 h. Thawing and refreezing should be avoided as it may alter the properties of lysolecithin (Donsì, Wang, & Huang, 2011).

8. Poly(I:C) is a synthetic analogue of double-stranded RNA and a Toll-like receptor 3 agonist. We have previously used Poly(I:C) (Sigma-Aldrich, P1530) to induce formation of remyelination-inhibiting fibronectin aggregates by primary astrocytes as well as in slice cultures (Qin et al., 2017; Werkman et al., 2020). For preparation of the stock solution, 25 mg of Poly(I:C) should be dissolved in 10 mL of sterile PBS. For optimal solubilization, the solution should be heated at 50 °C for 60 min and cooled down to room temperature to allow re-annealing. As not all Poly(I:C) goes into solution, a Nanodrop (DNA-50 setting; OD260) should be used to determine the concentration (an OD260 of 1 equals 50 µg/mL). Next, the appropriate volume of PBS should be added to achieve a final concentration of 1 mg/mL. Poly(I:C) can be filter-sterilized and aliquoted in tubes and stored at −20 °C until use.
9. Neonatal rats and mice of ages P0-P10 are routinely used for cerebellar slice cultures (Birgbauer et al., 2004; Hussain et al., 2011; Meffre et al., 2015; Mi et al., 2009; Miron et al., 2010; Najm et al., 2015; O'Sullivan, Schubart, Mir, & Dev, 2016; Rutkowska, Sailer, & Dev, 2017; Shen & Yuen, 2020; Sherafat et al., 2018; Sun, Liu, Liu, Xiao, & Zhang, 2012; Ulc et al., 2019; Zhang et al., 2011). Slices tend to show poor survival when isolated from neonates beyond P12 (Birgbauer et al., 2004; Dusart, Airaksinen, & Sotelo, 1997; Sherafat et al., 2018), possibly because cutting slices from older, more extensively myelinated, brain will generate more myelin debris, which is known to inhibit remyelination *in vivo* (Clarner et al., 2012; Kotter, Li, Zhao, & Franklin, 2006). Most often researchers either use neonates from P0-P3 (Miron et al., 2010; Shen & Yuen, 2020; Ulc et al., 2019; Zhang et al., 2011) or slightly older animals from P7-P10 (Birgbauer et al., 2004; Hussain et al., 2011; Meffre et al., 2015; Mi et al., 2009; O'Sullivan et al., 2016; Rutkowska et al., 2017; Sherafat et al., 2018; Sun et al., 2012). Using neonatal brains allows for easier removal of the meninges, as these are not yet strongly attached to the brain parenchyma, and has the added advantage that the forebrain can also be collected and used for primary cell culture. However, especially when working with mice, using older animals provides the advantage that the brains are bigger, which allows for easier manipulation and a higher yield of slices.
10. Handle the inserts with a sterile forceps, first add the medium to the well and then the inserts, tilt the plate at a 45° angle when placing the inserts in the wells to avoid bubble formation.
11. In our experience, as well as the experience of others (Mewes et al., 2012), slice cultures should be incubated with 5% CO_2 and at 35 °C for optimal viability.

12. To prepare a cold sterile work surface on which to dissect the brains, tape a sterile 10 cm petri dish to the bottom half of a 10 cm petri dish containing crushed ice.
13. Dissection of the cerebellum and preparation of the slices will be done in the laminar flow hood suited for tissue culture. Disinfect with 70% ethanol and, if possible, 10 min of UV exposure, prior to use. Take extra care to disinfect the blade and surface of the tissue chopper, which should be sprayed generously with 70% ethanol and then be allowed to dry.
14. For the dissection of the cerebellum and the removal of the meninges it is helpful to use forceps with fine points, such as Dumont Forceps #5SF, which are in good condition. This is especially relevant when handling mouse brains, which are smaller and more fragile.
15. The colder the tissue stays during the isolation, the better the viability of the slices will be.
16. During the procedure, tools that are not in use can be stored in a tube containing 70% ethanol (Fig. 1B). Always rinse your tools with sterile PBS before manipulating the brain to avoid damaging the tissue.
17. Removing the meninges is important to prevent contamination of the slice cultures with fibroblasts. However, it can be challenging to remove the meninges completely without damaging the cerebellum, especially in older neonates (beyond P3), as there the meninges will be more strongly attached to the brain parenchyma, and in mice, where the meninges is less vascularized and therefore not as easily visible. Gently hold the forebrain down with the forceps in your non-dominant hand, while using the forceps in your dominant hand to carefully remove the meninges. Once you get a good grip, the meninges should come of relatively easily without exerting much force. Be very careful not to damage the cerebellum; it is preferable to leave a small bit of meninges than to damage the cerebellum. Some authors have reported increased viability of their cerebellar slice cultures when leaving the hindbrain attached to the cerebellum (Shen & Yuen, 2020; Zhang, Jarjour, Boyd, & Williams, 2011).
18. Always keep the spatulas wet with medium when handling the cerebellum to avoid having the tissue stick to the spatulas.
19. If there is too much medium, the cerebellum will shift around during the chopping, which will result in uneven slices.
20. In rare events, the tissue may stick to the knife after the first cut. In this case, it helps to play around with the velocity of tissue chopper. Generally, using the lowest velocity setting is sufficient to prevent sticking.
21. Slices of 350-400 µm are routinely used for cerebellar slice cultures. We cut slices of 375 µm, but as the slices are in culture for a longer period of time (up to 6 weeks), they will flatten out and lose much of their volume, until they are about 100 µm in thickness. For longer experiments, it is advisable to choose a slightly higher initial cutting thickness.

22. Only use slices that clearly retain the structure of the cerebellum and are not damaged. Do not use slices that appear to be too thick or too thin and/or that have been cut unevenly. Do not use the outermost slices, which are smaller in diameter.

23. It is recommended to first change the culture medium within 24 h of isolating the slices, preferably on the morning after the isolation. In our experience, waiting longer with the first medium change significantly reduces the viability of the slice cultures. After the first time, the medium should be changed every 2–3 days.

24. Directly after dissection, slices will appear transparent and slightly pinkish to the naked eye (Fig. 3A). With regular light microscopy, the cerebellar structure can clearly be seen (Fig. 3D). The day after isolation, slices will have turned white and non-transparent (Fig. 3B). Under the microscope, the cerebellar structure will be retained, but slices may appear slightly swollen and numerous brown rounded flecks may appear in the white matter tracts of the cerebellum, representing foamy macrophages that clear out (myelin) debris (Fig. 3E). After approximately 5–6 days in culture, healthy slices will flatten out and become transparent (Fig. 3C). Under the microscope, the slices will appear transparent, flatter, and the cerebellar structure may not be seen as clearly as before (Fig. 3F). Migration of cells may be seen around the edges of the slices and is a sign of healthy cultures. If slices become remain white and opaque after 1 week in culture or contain big white and opaque necrotic spots, they are not viable and should not be used for experiments.

25. Slices are routinely retained for anywhere between 4 and 21 days *in vitro* (Birgbauer et al., 2004; Hussain et al., 2011; Luo et al., 2018; Miron et al., 2010; Shen & Yuen, 2020) to allow for myelination and the clearance of debris before demyelination is induced with lysolecithin. Cerebellar slices derived from mice and/or from older neonates tend to myelinate faster. As a general rule, it is discouraged to induce demyelination with lysolecithin within the first 7 days *in vitro* (Birgbauer et al., 2004; Sherafat et al., 2018).

26. We recommend using lysolecithin from bovine brain (Sigma, L1381), as this gives more consistent results than the cheaper variant of lysolecithin derived from egg yolk (Sigma, L4129). Inquiries with different groups have confirmed our observations that some batches of egg yolk-derived lysolecithin are more difficult to dissolve and cause overt destruction of neurons and slices at conditions that should normally only cause demyelination, but should leave the axons unharmed.

27. Most groups incubate their cerebellar slice cultures with 0.4–0.5 mg/mL lysolecithin anywhere between 16 and 24 h. Interestingly, there is a narrow range in the concentration of lysolecithin, and especially, the duration of exposure to lysolecithin (Birgbauer et al., 2004). Exposing the slices to lysolecithin for too long will cause overt destruction of neuronal axons, while incubating for under 15 h, or for longer periods of time with concentrations 0.2 mg/mL or lower, will fail to induce demyelination (Birgbauer et al., 2004). The optimal incubation time may differ between type of tissue slice (*e.g.,* spinal cord cultures *vs.* cerebellum), the species, as well as between labs. We recommend to start out with 0.5 mg/mL for 17 h, which is most often used, and PFA fix the slices after 2 days. Then, an immunofluorescent staining for

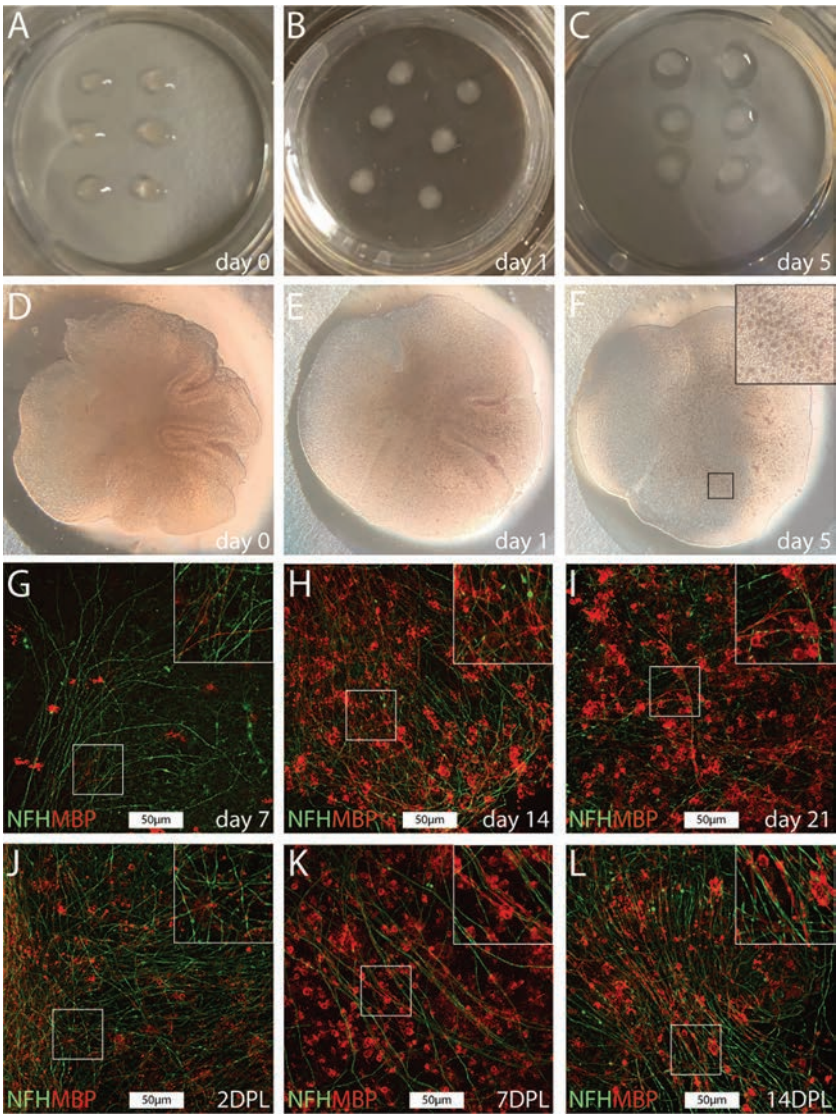

FIG. 3

Representative images of *ex vivo* organotypic cerebellar slice cultures as seen with the naked eye and through the microscope. (A–C) Macroscopic views of P1-P3 rat *ex vivo* organotypic cerebellar slice cultures on (A) day 0, *i.e.* the day of isolation, (B) day 1 and (C) day 5. (D–F) Rat *ex vivo* organotypic cerebellar slice cultures as seen with regular light microscopy on (D) day 0, (E) day 1 and (F) day 5. (G–L) Fluorescent staining for myelin basic protein (MBP; red) and neurofilament H (NFH; green) of rat *ex vivo* organotypic cerebellar slice cultures during developmental myelination after (G) 7 days, (H) 14 days and (I) 21 days *in vitro* as well as (J) 2 days, (K) 7 days and (L) 14 days after demyelination with lysolecithin (DPL, days post-lysolecithin), representing demyelination, early remyelination and late remyelination respectively. Scale bars are 50 µm. Inserts are digitally enlarged by 200%.

MBP & NFH (Note 31) can be performed to establish whether the slices show sufficient demyelination without axonal damage, or whether additional optimization of the lysolecithin concentration and/or incubation time is required.

28. **Fixing slice cultures with PFA:** Prewarm sterile PBS and 4% PFA to 37 °C. Aspirate the medium from underneath the inserts using the vacuum. Rinse the slices once in warm sterile PBS by carefully pipetting 2 mL of PBS in the well and 2 mL on top of the insert. Then, carefully aspirate the PBS using the vacuum. Next, pipette 2 mL of 4% PFA in the well and 2 mL on top of the insert. Wrap the lid of the 6-well plate with parafilm to prevent evaporation and incubate overnight on 4 °C. The next day, aspirate the 4% PFA and wash once with (cold) PBS, then change to PBS (For certain epitopes, shorter fixation periods, e.g., at room temperature for 1 h in 4% PFA, may be required). The slice cultures can be used directly for immunofluorescent staining or can be preserved in PBS at 4 °C for at least up to 3 months. For longer periods, it is advisable to add 0.01% sodium azide to prevent bacterial growth.
29. For immunostaining, slices can be cut out from the inserts using a scalpel with a small blade, spring scissors and Dumont #5 forceps. We recommend to use the scalpel to make 4 cuts in a square shape around the slice, leaving a little bit of membrane in each of the corners to provide enough tension for each cut. Lastly, use the spring scissors to cut through the corners and pick up the slice with the forceps placing it in a 24-well plate with PBS. Be careful to not let the slice dry out at any point.
30. **Immunostaining for MBP and NF-H:** Transfer slices of interest to a PBS-containing 24-well plate and rinse three times with PBS. Pre-incubate with blocking solution for 3 h (1 mM/L HEPES, 2% heat-inactivated horse serum, 10% heat-inactivated normal goat serum, 1% BSA, and 0.25% Triton X-100 in PBS). Next, incubate with the primary antibodies for MBP (Rat-anti- MBP; 1:250; Millipore; MAB386) and NF-H (Chicken-anti-NF-H; 1:3000; Encore Biotechnology; AB2149761 or Rabbit-anti-NF-H; 1:750; Abcam; AB8135) for 48 h in blocking buffer at 4 °C in a humidified chamber. Wash three times with PBS for 5 min, then incubate with the appropriate secondary antibodies in blocking buffer at 4 °C in a humidified chamber. Rinse twice with PBS, then wash in PBS with 0.05% Triton-X-100 for 1 h. Nuclei can be counterstained with DAPI (1 μg/mL DAPI in PBS with 0.05% Triton for 30 min). Lastly, after washing in PBS for 10 min, slices can be mounted, face-up, on glass slides with SlowFade Gold mounting medium.
31. We use MATLAB software to quantify the percentage of myelinated axons (Fig. 4) as previously described (Qin et al., 2017). Per condition, 2 pictures are taken from 2 different slices (total 4 pictures) using fluorescent or confocal microscopy at 10-20 × magnification. These pictures are entered into the MATLAB program, which is designed to calculate the area that is positive for both NFH and MBP, while only taking into account linear structures, *i.e.* excluding cell bodies of neurons and oligodendrocytes. The authors are happy to be contacted for more details about the software.

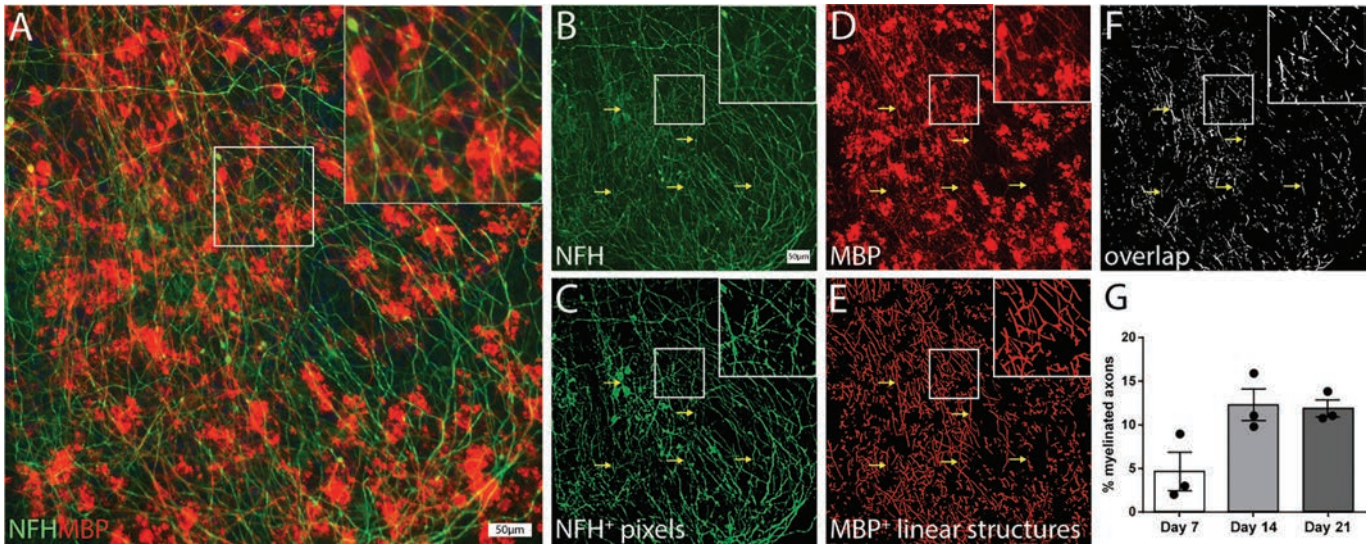

FIG. 4

Quantifying the percentage of myelinated axons using MATLAB. Rat *ex vivo* organotypic cerebellar slice cultures were stained for neurofilament H (NFH) and myelin basic protein (MBP) as described previously. Per condition, 2 pictures were taken from 2 different slices (total 4 pictures) using a Leica DMI 6000 B microscope at 10 × magnification. Then, MATLAB software was used to determine the number of myelinated axons. The software has been designed to calculate the area that is positive for both NFH and MBP, only taking into account linear structures in the MBP channel, *i.e.* excluding cell bodies of oligodendrocytes. (A) Fluorescent staining for neurofilament H (green) and myelin basic protein (red) of rat *ex vivo* organotypic cerebellar slice cultures at 14 days *in vitro*. (B) NFH channel. (C) Overview of NFH^+ pixels generated by MATLAB software. (D) MBP channel. (E) Overview of MBP^+ pixels generated by MATLAB software, only taking into account linear structures. (F) Overlap of NFH^+ and MBP^+ pixels generated by MATLAB software. (G) Quantification of the percentage of myelinated axons in rat *ex vivo* organotypic cerebellar slice cultures after 7 days, 14 days and 21 days *in vitro* ($n = 3$). Data are presented as the mean ± standard error of the mean. Scalebars are 50 µm. Inserts are digitally enlarged with 200%.

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References

Baudouin, L., et al. (2021). Co-culture of exogenous oligodendrocytes with unmyelinated cerebella: Revisiting ex vivo models and new tools to study myelination. *Glia*, *69*, 1916–1931.

Birgbauer, E., Rao, T. S., & Webb, M. (2004). Lysolecithin induces demyelination in vitro in a cerebellar slice culture system. *Journal of Neuroscience Research*, *78*, 157–166.

Bornstein, S., & Appel, M. (1959). Demyelination in cultures of rat cerebellum produced by experimental allergic encephalomyelitic serum. *Transactions of the American Neurological Association*, *84*, 6.

Bouslama-Oueghlani, L., Wehrlé, R., Sotelo, C., & Dusart, I. (2003). The developmental loss of the ability of Purkinje cells to regenerate their axons occurs in the absence of myelin: An in vitro model to prevent myelination. *The Journal of Neuroscience*, *23*, 8318–8329.

Clarner, T., et al. (2012). Myelin debris regulates inflammatory responses in an experimental demyelination animal model and multiple sclerosis lesions. *Glia*, *60*, 1468–1480.

Coleman, L. G., Zou, J., & Crews, F. T. (2020). Microglial depletion and repopulation in brain slice culture normalizes sensitized proinflammatory signaling. *Journal of Neuroinflammation*, *17*, 1–20.

Daviaud, N., Garbayo, E., Schiller, P. C., Perez-Pinzon, M., & Montero-Menei, C. N. (2013). Organotypic cultures as tools for optimizing central nervous system cell therapies. *Experimental Neurology*, *248*, 429–440.

de Almeida, M. M. A., et al. (2021). Agathisflavone modifies microglial activation state and myelination in Organotypic cerebellar slices culture. *Journal of Neuroimmune Pharmacology*. https://doi.org/10.1007/s11481-021-09991-6.

Donsì, F., Wang, Y., & Huang, Q. (2011). Freeze-thaw stability of lecithin and modified starch-based nanoemulsions. *Food Hydrocolloids*, *25*, 1327–1336.

Doussau, F., et al. (2017). Organotypic cultures of cerebellar slices as a model to investigate demyelinating disorders. *Expert Opinion on Drug Discovery*, *12*, 1011–1022.

Duncan, I. D., et al. (2018). The adult oligodendrocyte can participate in remyelination. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, E11807–E11816.

Dusart, I., Airaksinen, M. S., & Sotelo, C. (1997). Purkinje cell survival and axonal regeneration are age dependent: An in vitro study. *The Journal of Neuroscience*, *17*, 3710–3726.

Eigel, D., et al. (2019). Cryogel scaffolds for regionally constrained delivery of lysophosphatidylcholine to central nervous system slice cultures: A model of focal demyelination for multiple sclerosis research. *Acta Biomaterialia*, *97*, 216–229.

Faissner, S., Plemel, J. R., Gold, R., & Yong, V. W. (2019). Progressive multiple sclerosis: From pathophysiology to therapeutic strategies. *Nature Reviews Drug Discovery*, *18*, 905–922.

Franklin, R. J. M., & ffrench-Constant, C. (2008). Remyelination in the CNS: from biology to therapy. *Nature Reviews. Neuroscience*, *9*, 839–855.

Gaudillière, B., Konishi, Y., Ria de la Iglesia, N., Yao, G., & Bonni, A. (2004). A CaMKII-NeuroD signaling pathway specifies dendritic morphogenesis. *Neuron*, *41*, 229–241.

Gee, C. E., Ohmert, I., Wiegert, J. S., & Oertner, T. G. (2017). Preparation of slice cultures from rodent hippocampus. *Cold Spring Harbor Protocols*, *2017*, 126–130.

Gruchot, J., et al. (2019). The molecular basis for Remyelination failure in multiple sclerosis. *Cell*, *8*.

Haber, M., Vautrin, S., Fry, E. J., & Murai, K. K. (2009). Subtype-specific oligodendrocyte dynamics in organotypic culture. *Glia*, *57*, 1000–1013.

Hill, R. A., Medved, J., Patel, K. D., & Nishiyama, A. (2014). Organotypic slice cultures to study oligodendrocyte dynamics and myelination. *Journal of Visualized Experiments*, *51835*. https://doi.org/10.3791/51835.

Hussain, R., et al. (2011). Progesterone and nestorone facilitate axon remyelination: A role for progesterone receptors. *Endocrinology*, *152*, 3820–3831.

Kotter, M. R., Li, W.-W., Zhao, C., & Franklin, R. J. M. (2006). Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *The Journal of Neuroscience*, *26*, 328–332.

Liu, Y., et al. (2019). Concentration-dependent effects of CSF1R inhibitors on oligodendrocyte progenitor cells ex vivo and in vivo. *Experimental Neurology*, *318*, 32–41.

Llobet, L., Montoya, J., López-Gallardo, E., & Ruiz-Pesini, E. (2015). Side effects of culture media antibiotics on cell differentiation. *Tissue Engineering, Part C: Methods*, *21*, 1143–1147.

Lubetzki, C., Zalc, B., Williams, A., Stadelmann, C., & Stankoff, B. (2020). Remyelination in multiple sclerosis: From basic science to clinical translation. *The Lancet Neurology*, *19*, 678–688.

Luo, F., et al. (2018). Modulation of proteoglycan receptor PTPsigma enhances MMP-2 activity to promote recovery from multiple sclerosis. *Nature Communications*, *9*, 4126.

Meffre, D., et al. (2015). Liver X receptors alpha and beta promote myelination and remyelination in the cerebellum. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 7587–7592.

Mewes, A., Franke, H., & Singer, D. (2012). Organotypic brain slice cultures of adult transgenic P301S mice-A model for Tauopathy studies. *PLoS One*, *7*.

Mi, S., et al. (2009). Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. *Annals of Neurology*, *65*, 304–315.

Miron, V. E., et al. (2010). Fingolimod (FTY720) enhances remyelination following demyelination of organotypic cerebellar slices. *The American Journal of Pathology*, *176*, 2682–2694.

Najm, F. J., et al. (2015). Drug-based modulation of endogenous stem cells promotes functional remyelination in vivo. *Nature*, *522*, 216–220.

Nave, K.-A., & Werner, H. B. (2014). Myelination of the nervous system: Mechanisms and functions. *Annual Review of Cell and Developmental Biology*, *30*, 503–533.

O'Sullivan, C., Schubart, A., Mir, A. K., & Dev, K. K. (2016). The dual S1PR1/S1PR5 drug BAF312 (Siponimod) attenuates demyelination in organotypic slice cultures. *Journal of Neuroinflammation*, *13*, 1–14.

Pritchard, A. J., Mir, A. K., & Dev, K. K. (2014). Fingolimod attenuates splenocyte-induced demyelination in cerebellar slice cultures. *PLoS One*, *9*, 99444.

Qi, X. R., et al. (2019). Human brain slice culture: A useful tool to study brain disorders and potential therapeutic compounds. *Neuroscience Bulletin*, *35*, 244–252.

Qin, J., et al. (2017). GD1a overcomes inhibition of myelination by fibronectin via activation of protein kinase A: Implications for multiple sclerosis. *The Journal of Neuroscience*, *37*, 9925–9938.

Rakotomamonjy, J., & Guemez-Gamboa, A. (2019). Purkinje cell survival in organotypic cerebellar slice cultures. *Journal of Visualized Experiments*, *2019*, e60353.

Rutkowska, A., Sailer, A. W., & Dev, K. K. (2017). EBI2 receptor regulates myelin development and inhibits LPC-induced demyelination. *Journal of Neuroinflammation*, *14*, 250.

Schwarz, N., et al. (2019). Long-term adult human brain slice cultures as a model system to study human CNS circuitry and disease. *eLife*, *8*, e48417.

Shen, K., & Yuen, T. J. (2020). Ex vivo myelination and remyelination in cerebellar slice cultures as a quantitative model for developmental and disease-relevant manipulations. *Journal of Visualized Experiments*, *2020*, 1–12.

Sherafat, A., Hill, R. A., & Nishiyama, A. (2018). Organotypic slice cultures to study oligodendrocyte proliferation, fate, and myelination. *Methods in Molecular Biology*, *1791*, 145–156.

Sheridan, G. K., & Dev, K. K. (2012). S1P1 receptor subtype inhibits demyelination and regulates chemokine release in cerebellar slice cultures. *Glia*, *60*, 382–392.

Stoffels, J. M. J., Hoekstra, D., Franklin, R. J. M., Baron, W., & Zhao, C. (2015). The EIIIA domain from astrocyte-derived fibronectin mediates proliferation of oligodendrocyte progenitor cells following CNS demyelination. *Glia*, *63*, 242–256.

Stoffels, J. M. J., Zhao, C., & Baron, W. (2013). Fibronectin in tissue regeneration: Timely disassembly of the scaffold is necessary to complete the build. *Cellular and Molecular Life Sciences*, *70*, 4243–4253.

Stoffels, J. M. J., et al. (2013). Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. *Brain*, *136*, 116–131.

Stoppini, L., Buchs, P. A., & Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *Journal of Neuroscience Methods*, *37*, 173–182.

Sun, X., Liu, Y., Liu, B., Xiao, Z., & Zhang, L. (2012). Rolipram promotes remyelination possibly via MEK-ERK signal pathway in cuprizone-induced demyelination mouse. *Experimental Neurology*, *237*, 304–311.

Ta, T.-T. T., et al. (2019). Priming of microglia with IFN-γ slows neuronal gamma oscillations in situ. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 4637–4642.

Tan, G. A., et al. (2018). Organotypic cultures from the adult CNS: A novel model to study demyelination and Remyelination ex vivo. *Cellular and Molecular Neurobiology*, *38*, 317–328.

Thetiot, M., Ronzano, R., Aigrot, M. S., Lubetzki, C., & Desmazières, A. (2019). Preparation and immunostaining of myelinating Organotypic cerebellar slice cultures. *Journal of Visualized Experiments*. https://doi.org/10.3791/59163.

Tian, Z., et al. (2020). Platelet-activating factor deteriorates Lysophosphatidylcholine-induced demyelination via its receptor-dependent and -independent effects. *Molecular Neurobiology*, *57*, 4069–4081.

Ulc, A., et al. (2019). The guanine nucleotide exchange factor Vav3 modulates oligodendrocyte precursor differentiation and supports remyelination in white matter lesions. *Glia*, *67*, 376–392.

Van Weering, H. R. J., et al. (2011). CXCL10/CXCR3 signaling in glia cells differentially affects NMDA-induced cell death in CA and DG neurons of the mouse hippocampus. *Hippocampus*, *21*, 220–232.

Verwer, R. W. H., et al. (2002). Cells in human postmortem brain tissue slices remain alive for several weeks in culture. *The FASEB Journal*, *16*, 54–60.

Verwer, R. W. H., et al. (2015). Injury response of resected human brain tissue in vitro. *Brain Pathology*, *25*, 454–468.

Wang, P., et al. (2018). MMP7 cleaves remyelination-impairing fibronectin aggregates and its expression is reduced in chronic multiple sclerosis lesions. *Glia*. https://doi.org/10.1002/glia.23328.

Werkman, I., et al. (2020). TLR3 agonists induce fibronectin aggregation by activated astrocytes: A role of pro-inflammatory cytokines and fibronectin splice variants. *Scientific Reports*, *10*.

Wiegert, J. S., Gee, C. E., & Oertner, T. G. (2017). Viral vector-based transduction of slice cultures. *Cold Spring Harbor Protocols*, *2017*, 131–134.

Wolters, A., et al. (2021). Teriflunomide provides protective properties after oxygen-glucose-deprivation in hippocampal and cerebellar slice cultures. *Neural Regeneration Research*, *16*, 2243–2249.

Yeung, M. S. Y., et al. (2019). Dynamics of oligodendrocyte generation in multiple sclerosis. *Nature*, *566*, 538–542.

Younkin, L., & Silberberg, D. (1973). Myelination in developing cultured newborn rat cerebellum inhibited by 5-bromodeoxyuridine. *Experimental Cell Research*, *76*, 455–458.

Zhang, H., Jarjour, A. A., Boyd, A., & Williams, A. (2011). Central nervous system remyelination in culture - A tool for multiple sclerosis research. *Experimental Neurology*, *230*, 138–148.