Chapter 12

Electrophysiological Recordings of Oligodendroglia in Adult Mouse Brain Slices

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Abstract

Communication between neurons and oligodendrocyte lineage cells has attracted a great interest since multiple discoveries revealed its important roles in brain function under physiological and pathological conditions. Oligodendroglia responds to neuronal activity through the activation of a plethora of ion channels and receptors whose expression changes depending on the maturation state and whose characterization helps defining their interactions with neurons. Here, we describe in detail the methodology for carrying out electrophysiological patch-clamp recordings of oligodendroglial cells in acute brain slices of adult mice, with an emphasis on tailor-made solutions to make this experimental approach successfully. Additionally, we describe a protocol for combining photostimulation of neurons with patch-clamp recordings of oligodendroglia.

Key words Oligodendrocyte precursor cells, Oligodendrocytes, Whole-cell patch-clamp, Neuron-glia communication, Optogenetics, Electrophysiology

1 Introduction

Neuron-glia interactions in health and disease have been a central topic in neuroscience in the last two decades [1–3]. In particular, neuron-oligodendroglia chemical communication has been very intriguing since the discovery of true synaptic contacts between neurons and oligodendrocyte precursor cells (OPCs) more than 20 years ago [4]. Indeed, OPCs express different types of glutamatergic and GABAergic receptors that allow these cells to respond to the synaptic release of glutamate and GABA, the two major neurotransmitters of the CNS [5]. Although the characteristics of these synapses have been well described, less is known about their

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function [4, 6]. Furthermore, although electrophysiological analyses have allowed for the extensive characterization of oligodendroglia membrane properties in neonatal rodent animals (postnatal day (PN) 3 to PN15), less studies have been performed in the adult model (>PN45). Cumulative evidence has shown that neuronal activity can modulate oligodendroglia proliferation, differentiation, and survival, directly through synaptic receptors such as AMPARs, NMDARs, or GABARs [7, 8] or indirectly by, for instance, modulating surrounding astrocytes [9]. However, the mechanisms underlying OPC development, myelin formation, and repair as well as the roles of oligodendroglia in health and disease are still not totally understood. Therefore, oligodendroglia represents an important field of study that contributes to our understanding of brain physiology and pathophysiology. In line with this, state-of-the-art experimental technologies such as optogenetics, super-resolution microscopy, and optical techniques combined with electrophysiological recordings represent powerful tools to analyze oligodendrocyte (OL) lineage cells (i.e. oligodendroglia). In this chapter, we will describe in detail the methodology to carry out whole-cell patch-clamp recordings of oligodendroglial cells in acute brain slices of adult mice. In addition, we will add a short section dedicated to the description of a protocol to combine optogenetic stimulation of neurons with whole-cell patch-clamp recordings of oligodendroglia.

2 Materials

2.1 Animals

A suitable way to identify the different developmental stages of oligodendroglia in the adult (>PN45) is the use of transgenic mice expressing fluorescent reporters under the control of lineage stage-specific promoters. Among the existing mouse lines, CNPase-GFP or Sox10-Venus mouse lines, for instance, can be used for the identification of cells of the entire OL lineage [10, 11], PDGFRα-GFP for OPCs [12, 13] and the MBP-GFP mouse line [14] for mature OLs.

2.2 Buffer and Intracellular Solutions

It is recommended to prepare 10 times (10×) concentrated buffer solution stocks containing most of the inorganic salts. Store them at 4 °C and freshly prepare the final 1× solution the day of the experiment.

2.2.1 N-Methyl-D-Glucamine (NMDG)-Based Solution

Prepare the 10× stock considering the following composition of the final (1×) solution: 2.5 mM KCl, 1.2 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, and 1.2 mM thiourea. After dilution of this 10× stock to 1×, add 93 mM NMDG, 25 mM glucose, 5 mM Na-ascorbate, and 3 mM Na-pyruvate. This solution has a basic pH (around 10) that must be carefully adjusted to a pH 7.4 by

adding 10 M HCl (*drop by drop*). After bubbled the solution for at least 10 min with a 95% O_2/5% CO_2 gas mix, add 10 mM $MgCl_2$ and 0.5 mM $CaCl_2$ from the previously prepared 1 M stock solutions. This solution will be used for cutting and recovery of the slices. In the case of using old animals, viable brain slices from older-than-P120 mice are obtained by a step gradient change of extracellular sodium (please see details in [15]).

2.2.2 Artificial Cerebrospinal Fluid (aCSF) Solution

Prepare the 10× stock considering the following composition of the final (1×) solution: 126 mM NaCl, 2.5 mM KCl, 25.9 mM $NaHCO_3$, and 1.3 mM NaH_2PO_4. After diluting this stock to 1×, add 20 mM glucose and 5 mM Na-pyruvate. After bubbled the solution for at least 10 min with a 95%O_2/5%CO_2 gas mix, add 1 mM $MgCl_2$ and 2 mM $CaCl_2$ from previously prepared 1 M stock solutions. This solution will be used for the storage and recordings of brain slices.

2.2.3 Intracellular Solutions

To unmask the characteristic Na^+-inward current of OPCs, a Cs-gluconate intracellular based solution is recommended (*see* [16]). This solution contains 130 mM Cs-gluconate, 10 mM 4-aminopyridine, 5 mM tetraethylammonium chloride, 5 mM EGTA, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 2 mM Na_2-ATP, 0.2 mM Na-GTP, and 10 mM Na_2-phosphocreatine (pH ≈ 7.4), 296 mOsm/L. Alternatively, the following intracellular solution can be used: 130 mM K-gluconate, 0.1 mM EGTA, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM Na_2- ATP, 0.5 mM Na-GTP, 10 mM HEPES, 10 mM phosphocreatine. Dilute Cs- (or K-) gluconate and EGTA in 30 to 40 mL of milliQ water and adjust the pH to 7.3 with a solution of CsOH (or KOH, accordingly). Prepare this solution on ice. Add the required volumes of $CaCl_2$ and $MgCl_2$ solutions and dissolve the HEPES. Adjust the pH to 7.3 while mixing. Dissolve Na_2-ATP, Na-GTP, and phosphocreatine and adjust again the pH to 7.3. Complete the volume to 50 mL using a volumetric flask. It is recommended to store this solution at −20 °C in 1 mL aliquots.

2.3 Material for Brain Slice Storage and Recordings

Before starting the slice preparation, prepare a chamber for their storage. The "storage" chambers can be obtained commercially or manufactured in the laboratory (*see* **Note 1**). A conventional chamber is a table-shaped plastic structure with a nylon net on top, which is secured by a plastic hoop and must be tight to receive the slices (Fig. 1a). The chamber is placed inside a 250 mL glass beaker that contains either a NMDG-based or an aCSF solution (Fig. 1b). Although brain slices must be kept oxygenated inside the "storage" chamber, it is very important to avoid bringing the bubbling into direct contact of the tissue (*see* **Note 2**).

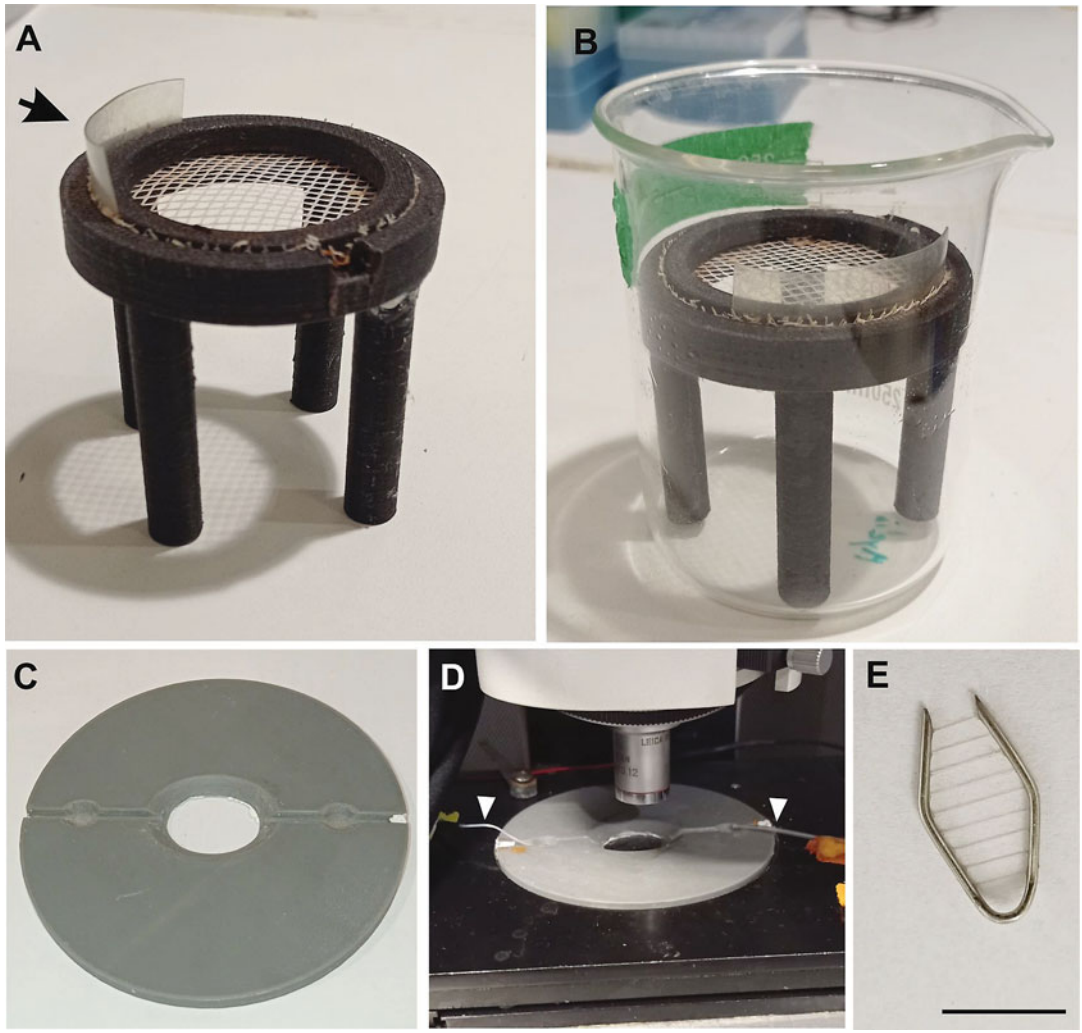

Fig. 1 Materials for brain slice preparation and recording. Picture of the *storage* chamber before (**a**) and after (**b**) placing it in a 250 mL beaker with either aCSF or NMDG-based solutions (**b**, *see* the text for details). Note the protective barrier to prevent direct bubbling the tissue into the storage chamber (arrow in **a**, *see* **Note 1** for details). A custom-made *recording* chamber (**c**) is shown. The recording chamber is mounted on an upright epifluorescence microscope (**d**) (note the needles of the perfusion system visible in the picture, white arrowheads). The slice-anchor avoids the movement of the slice during patch-clamp recordings (**e**). Scale bar: 1 cm

Once brain slices are obtained, one of them can be transferred to the recording chamber and placed under the microscope, where patch-clamp recordings are performed (Fig. 1c, d). Again, this chamber can be obtained commercially or custom-made in the laboratory. After placing the brain slice in this recording chamber, it is necessary to use a "slice-anchor" (or "harp") to immobilize it, preventing the slice from floating in the perfused extracellular

solution during patch-clamp recordings (Fig. 1e, *see* **Note 3**). Although fixing the slice with a harp could cause some tissue damage, it is by far the most common maneuver to immobilize the slice in the chamber without altering significantly the tissue properties.

2.4 Whole-Cell Patch-Clamp Recordings

Borosilicate glass capillaries containing an inner filament (1.5 mm outer diameter, 0.86 mm inner diameter; GC150F-10, Harvard Apparatus) are the most convenient choice to make the recording microelectrodes. Prepare your micropipettes (*see* **Note 4**) by pulling these capillaries in a conventional puller (for instance, a P-87, Sutter Instruments, or a P-1000 Microelectrode Puller, WPI). Use a silver wire coated with a thin layer of silver chloride (AgCl) (*see* **Note 5**) on your microelectrode holder to mount the micropipette containing an intracellular solution, and test for the pipette resistance using the membrane test pulse of your software (for instance, PClamp or AxoScope series, Axon Instruments) when the pipette is in the solution. A pipette resistance of 5 MΩ is appropriate for patching oligodendroglia.

2.5 Photostimulation During Whole-Cell Recordings

To combine electrophysiological recordings of oligodendroglia with photostimulation of neurons, a transgenic animal expressing a photosensitive ion channel in neurons (i.e., channelrodopsin-2) or, alternatively, the delivery of a genetic construct containing the photosensitive ion channel (i.e., via a viral-driven expression) [17, 18] is suitable to obtain the responsive cells in the tissue.

1. *A fully equipped patch-clamp setup.*
2. *A light source for photostimulation*: ultrahigh-power LED--based or laser-based system capable of delivering the desired wavelength (i.e., for channel rodhodpsin-2 λ ~470 nm, [17]).
3. *Current controller to power the light source.*
4. *Patch-fiber cord to stimulate the sample* (the characteristics are described in Subheading 3.4).
5. *Pulse generator device* to create programmable TTL pulses from a software.

3 Methods

3.1 Brain Slices

1. Before preparing the slices, the NMDG-based cutting solution—almost frozen—must be bubbled with 95% CO_2/5% O_2 mix for at least 10 min and maintained *on ice*. The solution must be at the freezing point during the slicing (*see* **Note 6**). For the brain extraction, it is recommended to have three beakers or wells with ice-cold NMDG-based solution (Fig. 2): (i) one beaker to clean the head, (ii) another to handle the head during brain extraction (a low well is recommended in

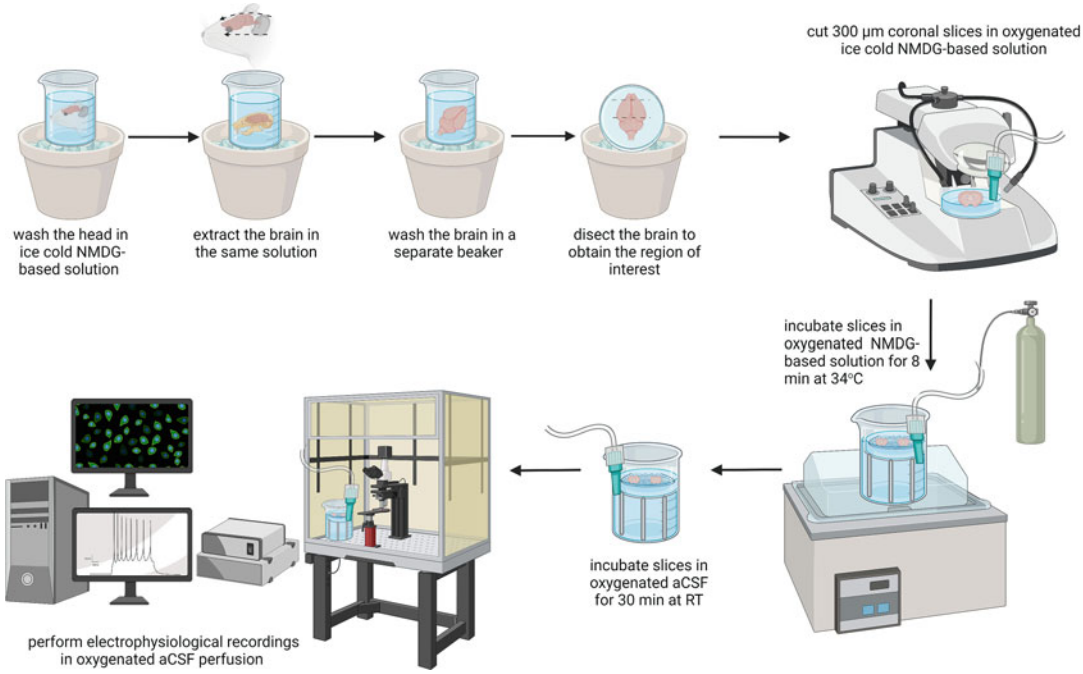

Fig. 2 Step-by-step summary of the procedures to perform electrophysiological recordings in brain slices (please refers to Subheadings 3.1 and 3.2 for a full detailed description). (This figure was made using BioRender)

this step), and (iii) a third one to clean the brain once it has been extracted from the skull.

2. Place the slicing chamber on the vibratome according to the manufacturer's instructions.
3. Fill a beaker containing a storage chamber with oxygenated NMDG-based solution (*see* **Note 6**). Fill another beaker (containing another storage chamber) with aCSF. In both cases, remove all bubbles adhering to the nylon net using a Pasteur pipette. Ensure that the slice is continuously oxygenated until the end of the experiment, and remember that bubbles *should not touch the slices*. Both solutions should be kept at 34 °C in a water bath during the slice cutting.
4. Anesthetize the adult mouse by inhalation of isoflurane 1% (we recommend volatile anesthetic since they are quick and painless and requires no particular technical skills; however, the selection of a particular anesthetic agent *must take into account possible effects upon the ionic currents of interest*). After checking lack of paw pinch reflex, perform the beheading with a sharp guillotine, a sharp-blunt or a blunt-blunt scissor (i.e., 14001-12 scissors, FST instruments). Please follow all guidelines of your local Animal Care Committee. Quickly immerse it in the first glass beaker containing ice-cold oxygenated NMDG-based solution (*see* **Note 7**).

5. Dissect the brain out by cutting the skull with a fine straight scissors (i.e., 14060-09 scissors, FST instruments) between the eyes. Continue with lateral cuts close to the base of the skull, in both sides of the head from posterior to anterior. It is recommended that all these steps be made inside the well to keep the tissue constantly wet and cold (Fig. 2).
6. Carefully remove the excised skull with fine forceps ideally angled (45 degrees, i.e., 00109-11 forceps, FST instruments) without damaging the brain, and immediately remove and place the brain in the third clean glass beaker containing ice-cold NMDG-based solution. This procedure removes the excess of blood (Fig. 2).
7. In a wet surface (for instance, a filter paper soaked in NMDG-based solution placed on a petri dish cover), dissect the brain area of interest, paying attention to the angle in order to obtain a flat surface.
8. Stick the brain with glue to the slicing platform, taking into consideration the correct brain orientation for the desired slices (i.e., sagittal, coronal, transverse). Fill the slicing platform with ice-cold oxygenated NMDG-based solution (Fig. 2). From beheading to **step 8** should take around 1 min.
9. Cut brain slices of 300 μm thick with the optimal vibration amplitude and frequency for your vibratome (*see* **Note 8**).
10. As a slice is made, transfer it to the storage chamber filled with oxygenated NMDG-based solution at 34 °C using a Pasteur pipette whose tip has been cut and smoothed by heating. Each slice *must remain in this solution for no more than 8 min*. Then, it must be transferred to the aCSF-containing storage chamber—kept also at 34 °C—resting there for 15 min. Before performing the electrophysiological recordings, the slices should rest for another 30 min in the same aCSF-containing solution this time at room temperature (RT) to allow them to restore the intracellular osmolarity and adapt to the new temperature.

3.2 Whole-Cell Recording

1. Unfreeze and filter one aliquot of the desired internal solution and store it on ice for the day.
2. Turn on all the electronic equipment on the electrophysiological rig and oxygenate the aCSF solution with the gas mix (95% O2/5% CO2) for a few minutes before use.
3. Before placing the brain slice on the recording chamber, verify that the perfusion system of the electrophysiological rig works properly. Continuously perfuse the recording chamber with the oxygenated aCSF solution by using either a gravity perfusion system for the influx coupled to a peristaltic pump connected to a suction tubing (outflow) or connect both influx

and suction tubing to the same peristaltic pump. Be careful in the setting of the inflow and outflow in order to keep the recording chamber volume constant. The optimal flow rate of the perfusion should be around 2.5–3.0 mL per minute. The recording chamber is mounted on the stage of an upright fluorescence microscope (Fig. 1c, d) carrying at least one low-magnification objective (i.e., 4×, 5×, or 10×) and one high-magnification water immersion objective (i.e., 40× or 63×).

4. Transfer a brain slice from the storage chamber to the recording chamber of the setup using the cut Pasteur pipette. To stabilize the slice inside the liquid, place the C-shaped slice-anchor on the top of the slice. *Make sure that the nylon threads of the slice-anchor do not interfere* with the region of interest.
5. Visualize the slice first with a low-magnification objective to identify the region under study. Once in the area of interest, switch to the 40× objective.
6. In order to identify the cell of interest and note its location on the screen, you can alternate fluorescence (for instance, to identify an OPC in case that you are using transgenic mice; Fig. 3a) and bright-field visualization. This helps to approach the target cell with the pipette (Fig. 3b).
7. Once the best cell to record has been identified, fill a glass micropipette with the internal solution and mount it on the microelectrode holder that is attached to the headstage. Check that the intracellular solution is in contact with the AgCl-coated silver wire and that there are no air bubbles at the tip of the pipette. Add some positive pressure into the micropipette before entering to the bath solution.
8. Lower the microelectrode until the surface of the slice *under visual control*. Be careful; do not touch the slice before checking the pipette resistance inside the bath solution. Use a pipette resistance around 4.5–5.5 MΩ. A higher resistance can be used but makes it difficult to achieve a whole-cell configuration, and a lower resistance makes it difficult to seal the cell.
9. After checking the resistance, adjust the pipette current offset to zero on the amplifier. This offset arises from the voltage difference between the operational amplifier and the recording electrode, normally originated from multiple sources: the intrinsic electrode properties such as resistance and capacitance, small drifts in the electrode position over time, and the small amount of current flowing into the input terminals of the amplifier. The latter can create a small current bias inside the amplifier, contributing to the initially detected offset that must be cancelled (*see* **Note 9**).

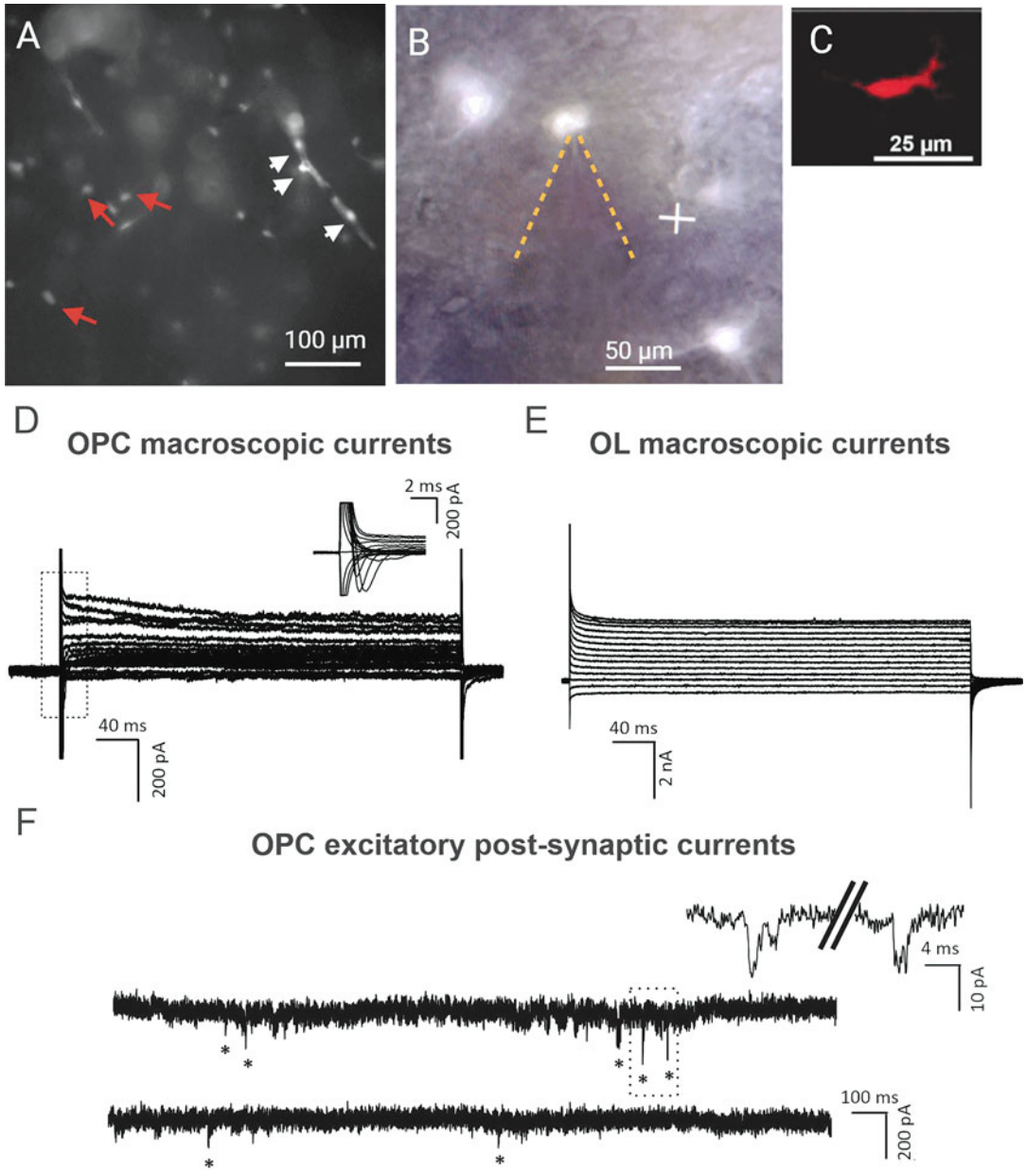

Fig. 3 OL lineage cell identification for whole-cell patch-clamp recordings. Identification of fluorescent oligodendroglial cells in transgenic mice (**a**). In this example, a coronal brain slice from an adult NG2-Cre$^{ERT2+/-}$; tdTomato$^{lox/lox}$ mouse. Note the presence of TdTomato+ pericytes clearly located in vessel walls (white arrowheads) compared with oligodendroglia presented as a rounded-shaped sparse cell population (red arrows). Images were acquired at λ emission = 580 nm by using an excitation laser beam source settled at λ 550 nm wavelength (**b**). After identification, we increased the transmitted light potency (at 40×) allowing for visualization of the pipette during the final approach to patch the target cell (note the pipette's shade above the cell indicated by a discontinuous yellow line). (**c**) To illustrate, here we show a representative example of an OPC, this time pseudo-colored in red after image treatment. Note the ovoid shape with almost no processes of an identified fluorescent OPC. (**d**) Currents elicited in an identified OPC held at −80 mV (Cs-gluconate intracellular based solution) by voltage steps from −120 to +40 mV. Note the presence of a

10. Once in the bath solution, continue applying a positive pressure to the micropipette by blowing in steadily and very gently before to enter to the tissue. This prevents the pipette from becoming clogged before reaching the cell of interest.
11. Change from positive to negative pressure (gently sucking) when the pipette is already on the surface of the cell membrane in order to get a gigaseal. A change in the holding potential from 0 mV to a negative potential of −60 mV (or up to −80 mV) while contacting the cell surface might also help to get the gigaseal. *The formation of a proper gigaseal is crucial to obtain a good recording* (*see* **Note 10**).
12. When the seal has been stabilized, cancel the pipette capacitance using the amplifier.
13. To obtain a whole-cell configuration, apply brief pulses of suction while monitoring the seal resistance. This pressure is applied to break the membrane at the tip of the pipette. It is recommended to wait a few seconds between each suction to avoid damaging the cell. The whole-cell configuration is reached when the value of the series resistance dramatically drops (from GΩ to hundreds of MΩ).
14. Since OPCs and immature and mature OLs have specific macroscopic current profiles (*see* ref. 19), it is recommended to apply a depolarizing voltage-step protocol (in whole-cell configuration, *see* details in Subheading 3.3.2) to obtain the cell macroscopic currents. This procedure will allow for a further confirmation and identification of the cell (for details, *see* Subheading 3.3.2).
15. When the OL cell type is identified and the recording is stable, apply your experimental protocols (i.e., electrical or pharmacological stimulation). In our hands, a signal acquisition filtered at 4 kHz and digitized at 20 kHz are recommended to obtain proper whole-cell recordings of OPCs and OLs [19].

3.3 OL Lineage Identification Hallmarks

3.3.1 Morphology

OL lineage cells are not easily visualized in a brain slice under brightfield or DIC microscopy due to their small size (compared to neurons), and the high refraction index observed in myelinated bundles (i.e. white matter tracts) [20]. Thus, as mentioned, the use of transgenic models provides the easiest method for OL lineage cell identification prior to patch-clamp recordings. For instance,

Fig. 3 (continued) characteristic fast transient inward sodium current that becomes evident at voltage step values of −40 mV (inset). (**e**). OL macroscopic currents elicited by the same voltage steps. Note the lack of the inward sodium current and the difference of current amplitudes (scale bars) compared to those obtained from OPC, as well as the classical linear phenotype of a mature oligodendroglia [19, 24]. (**f**) Spontaneous excitatory postsynaptic currents (EPSC, *) from the same OPC are shown (magnified examples in the inset)

OPCs can be identified by their small, hexagonal, or ellipsoidal cell body and the presence of two major branches (*see* Fig. 3c). A critical aspect to obtain a proper whole-cell recording is that the target cell has a well-preserved cellular membrane. Under DIC microscopy, healthy cells normally present a smooth and bright cellular outline; a swollen cell with a round and dark outline is not a recommended target.

3.3.2 Inward Sodium Currents

OPCs exhibit a characteristic transient inward voltage-dependent sodium current in response to depolarizing voltage steps (Fig. 3d, **Note 11**). Thus, once the whole-cell recording is stable, apply a voltage-step stimulation protocol starting from a holding potential of −80 mV to steps from +40 to −120 mV in 10 mV steps while recording the cell macroscopic currents (Fig. 3d). The functional expression of this current decreases in amplitude with oligodendroglia maturation, being smaller in pre-oligodendrocytes and completely absent in differentiated oligodendrocytes (Fig. 3e) [17, 20]. Therefore, studying this sodium current allows for further characterization of OL lineage cells. Additionally, OPCs express spontaneous excitatory postsynaptic currents (EPSCs) in different regions of gray and white matter [4, 5, 21, 22]. Then, after identification by their morphology and macroscopic sodium currents, these cells can also be characterized by checking the presence of EPSCs under voltage-clamp configuration (Fig. 3f).

3.4 Photostimulation of Neurons During OPC Whole-Cell Recordings

This procedure is suitable to study neuron-OPC communication properties. Before starting, ensure that the neurons of interest respond properly to photostimulation (please find a comprehensive detailed methodology in our previous publications on this subject [17, 18]).

1. A LED-based (or laser-based) light source and a patch-cord optic fiber(s) are needed to carry on these experiments (*see* **Note 12**). This equipment must be settled combined with the electrophysiology workstation in a way that it allows for the manipulation of all the elements necessary for the achievement of both patch-clamp recordings and light delivery pulses (i.e., let free room for maneuver micromanipulators, micropipettes, optic fibers, etc.).
2. Connect the patch-cord optic fiber to the light source. Minimize the length of the patch-cord to reduce the power loss. In order to photostimulate a broad area, it is recommended the use of an optic fiber with a high numerical aperture (i.e., 0.8 NA). Other parameters of the optic fiber such as its length and diameter should be adjusted according to the setup characteristics and experimental aims (Fig. 4).

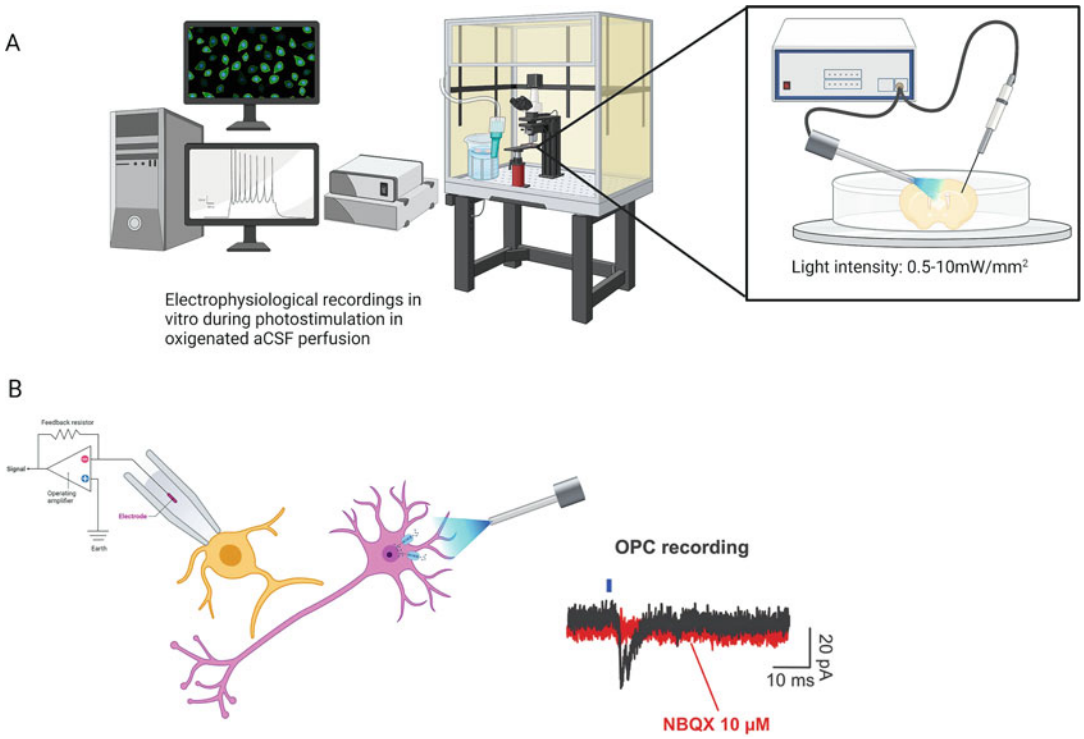

Fig. 4 OPC whole-cell patch-clamp recordings during photostimulation. Setting an experimental station for OL lineage cell recordings during photostimulation (**a**) requires an additional equipment able to deliver light pulses in a controlled manner. This equipment comprises a current controller, a light source, and a fiber patch cord. (**b**) In the example, light stimulation is delivered upon neurons expressing the channel-rhodopsin 2 (ChR2) during the recording of an OPC in whole-cell configuration. Note the light-evoked AMPAR-dependent excitatory postsynaptic current in response to photostimulation (blue line). Note that these evoked currents are blocked by the AMPAR antagonist NBQX (red) (**b**)

3. Measure the power at the tip of the optic fiber outside of the recording chamber. The current controller parameters should be adjusted to obtain the light power required for the stimulation (Fig. 4a, *see* **Note 13**).
4. Set the equipment to trigger your stimulation pattern. Usually, this step is achieved by connecting an external TTL-pulse generator to the light-delivery system, but it can also be incorporated as an asset of the delivery system (depending on the light-source distributor).
5. Trigger the stimulation pattern and check whether the light source is delivered the light properly (i.e., following the established pattern) outside of the recording chamber.
6. Before starting the experiment, carefully put the optic fiber into the recording chamber and check for photostimulation artifacts. One way to do it is by delivering light pulses over the tip of the microelectrode placed in the bath solution with *no patched* cell (*see* **Note 14**).

7. Repeat the procedure to patch a cell in the region of interest (*see* Subheading 3.2).
8. Bring the tip of the optic fiber just above the recorded cell and deliver the light pulses with the established parameters. The cell response can be recorded simultaneously (Fig. 4b) (For an extended and detailed methodology on optogenetics procedures, *see* ref. [17]).

4 Notes

1. A file containing the design of the chamber, to be printed in any conventional 3D-plastic printer, can be download in the online version of the chapter.
2. To prevent oxygen bubbles from contacting the tissue, you can build a diffuser. For this, you can cut the bottom of a 1 mL Eppendorf tube and attach it to the chamber inside the beaker; then, put the bubble tubing inside the cut Eppendorf tube. Alternatively, a protective barrier between the bubbling and the tissue can be made by cutting a small plastic sheet or the lid of a petri dish and sticking it to the edge of the chamber (Fig. 1a).
3. To make a slice-anchor, you can use a thick-platinum wire bended in a C-shape and then stick thin nylon threads to this structure crossing from one side to another (Fig. 1e). To make the nylon threads of the slice-anchor, you can use a pantyhose. Stretch one end of a few nylon threads and glue them to the C-shaped wire by using a strong liquid glue. Then, cut off the excess of nylon threads, and make sure you have enough rows to keep the tissue immobile and enough space between each thread to allow the entry of the recording micropipette into the tissue.
4. Before pulling the micropipettes, flame the two ends of the capillaries to make them smooth. This prevents damaging the AgCl-coated silver wire when placing the pipette into the microelectrode holder. Also, clean the center of the capillary with an alcohol solution (70% v/v) before placing it in the puller apparatus. This ensures no impurities on the tip of the micropipette.
5. The "ground" wire should be carefully chlorinated. It is recommended to check the condition of the ground wire (or pellet) the day before the experiment. When not properly chlorinated (i.e., it does not show a uniform grayish color), it can be a source of electrical noise or instability (i.e., a high junction potential). It is recommended to chlorinate the ground by electrochemical deposition of chloride, for instance, by using a 1 M HCl solution in an electrolysis device.

6. When possible, set the slicing chamber of the vibratome at –20 °C before starting the procedure. Similarly, the NMDG-based solution could be stored at −20 °C for 20 or 30 min before starting the procedure. Once it starts, you can maintain the NMDG-based solution on ice. You also should prepare a beaker with a storage chamber containing the NMDG-based solution at 34 °C for its use after obtaining the slices.
7. Oxygenated solutions are necessary to improve the longevity of the slices and maintain their suitable pH. Then, oxygenate the solution before starting the procedure but avoid direct contact of gas bubbles with the tissue during the dissection of the brain.
8. In our hands, a vibration amplitude/frequency of 1–1.2 mm/50–60 Hz are suitable parameters for cutting in a HM 650V Vibrating-Blade Microtome (Thermo Scientific).
9. There are different *voltage offsets* associated with patch-clamp recordings. Another common source arises from the generation of a liquid junction potential (LJP) originated when two electrolyte solutions with different ion concentrations get in contact. It occurs due to the unequal rate of diffusion of ions from one solution to the other, resulting in a potential difference between the two solutions. If the LJP is significantly high, it can interfere with the electrochemical-based measurements of the amplifier. Then, in addition to the initial current offset cancelation, a possible LJP must be considered when analyzing the final recordings. For instance, in our hands, by using intracellular solutions based on K-salts, the LJP was negligible; however, Cs-based solutions present a LJP of around −8 to −10 mV. For a further detailed discussion of this subject, please check ref. 23.
10. The corpus callosum (CC) is a common region to perform OPC whole-cell recordings in the neonatal mouse. However, in the adult, almost all oligodendroglia present in the CC correspond to mature OLs, and the tissue exhibits dense myelinated axon bundles, making difficult to perform patch-clamp recordings. A recommendation to work in this region is to enter to the tissue with a high positive pressure at the pipette tip while approaching the cell. A possible pitfall of this procedure is that the target cell can be displaced by this high positive pressure. To overcome this problem, once the target cell is identified, the micropipette tip should be quickly (few seconds) placed over its cell body just before releasing the pressure. This procedure generally produced a “fishing-like” effect where the cell is attracted to the tip of the pipette due to the sudden negative pressure generated by releasing the positive pressure (in occasions this is enough to produce the gigaseal).

11. In occasions the characteristic sodium current of OPCs might not be observed directly during the recording due to a low signal-to -noise ratio (i.e., cell culture [16]). Nevertheless, in these cases, it is recommended to perform an offline analysis of the amplitude of this conductance after applying a leak subtraction protocol [16].
12. Prizmatix , Artifex Engineering and Thorlabs are suitable companies to provide both light sources and path chords to perform photostimulation.
13. Most common optogenetic protocols that deliver light intensities are in a range of 0.5–10 mW/mm^2 at the tip of the fiber (*see* refs. 17, 18). Power at the tip can be tested by using fiber optic power meters with Internal Sensor (Thorlabs or Artifex companies).
14. Possible artifact sources originate in photoelectric or photothermic effects on the electrode. Commonly, they can be reduced or eliminated by simply reducing the power of the light source (i.e., light intensity).

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