

# Rodent Spinal Cord Demyelination Models



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**Abstract** Oligodendrocyte loss and subsequent demyelination is a significant component of the demyelinating diseases such as multiple sclerosis (MS) and traumatic CNS injury such as spinal cord (SCI) or traumatic brain (TBI) injury. Therefore, remyelination, either by enhancing endogenous myelination or engrafting exogenous myelinating cells, is a viable therapeutic target to restore function. To assess specific approaches to facilitate functional remyelination *in vivo*, appropriate injury

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models are needed. This chapter will discuss the strengths and weaknesses of a number of demyelinating lesions of the spinal cord and provide guidelines for choosing which model best suits which experimental condition. Step by step procedures for both creating and assessing the lesion will be provided.

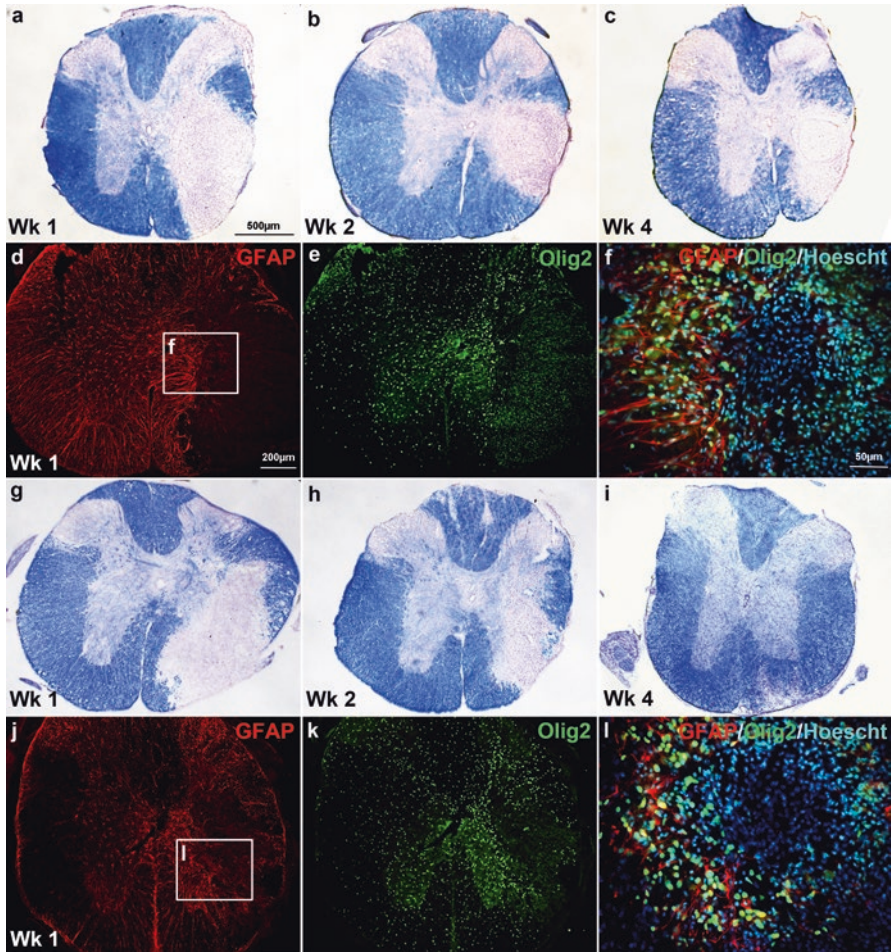
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## Introduction

There are a number of methods that can be used to demyelinate axons *in vivo*. These include injection of ethidium bromide (EB), lysolecithin, murine hepatitis virus (MHV), a cocktail of myelin-specific antibodies and complement, or by giving cuprizone in the diet [1, 2]. All of these lesions kill oligodendrocytes in the spinal cord, although cuprizone is much more effective in the corpus callosum than the spinal cord [3] and has the potential for remyelination (from endogenous or exogenously transplanted progenitors). However, depending on a number of factors (i.e. the mode of demyelination, targeted anatomical region, dose, length of administration, and species), other cell types (including axons) can also be lost [1]. Each of these factors must be considered to determine the extent/temporal progression of demyelination, severity of inflammation, and potential for remyelination. All of these lesions ultimately remyelinate by 3–6 months post-demyelination. However, the temporal progression and the extent of remyelination show differences which are discussed below [1, 2]. Importantly, while there is axonal loss in all of these models in rat studies, under optimal injection parameters, that damage is not different from saline injections [1]. Here, we briefly review each demyelinating lesion as well as provide detailed protocol for delivering and assessing focal spinal cord demyelinating lesion via injection of the gliotoxic agents EB and lysolecithin.

When injected into white matter, EB is a gliotoxic compound that intercalates into the small groove of helical DNA and kills both oligodendrocytes and astrocytes, which are highly sensitive to EB toxicity [4, 5] (Fig. 1a–f). While EB intercalates into both chromosomal and mitochondrial DNA, it only affects transcription of mitochondrial DNA [6–8]. At higher doses, neurons and endothelial cells are also killed. Importantly, there are differences as to how the rat and mouse spinal cord respond to EB toxicity. EB lesions in the mouse exhibit a much steeper dose-response curve as there is only a 0.1 mg/mL difference in concentration between little to no demyelination (0.15 mg/mL) and complete axon loss (0.25 mg/mL) [9] whereas the rat exhibits a much wider range of concentrations between little demyelination and axon loss [10–12]. Note that demyelination occurs rapidly and the lesion remains demyelinated at 4 weeks post-EB (Fig. 1a–c).

In contrast to mouse EB lesions where there is axon loss with chronic demyelination [9], there is no evidence in the rat of axon loss following EB lesions at concentrations appropriate for demyelination [13], even with repeated episodes of focal



**Fig. 1** Representative images of ethidium bromide- and lysolecithin-induced demyelination at the lesion epicenter. Eriochrome cyanine-stained spinal cord section at 1 (a, g), 2 (b, h), and 4 (c, i) weeks post-injection of ethidium bromide (a–f) or lysolecithin (g–l). Immunofluorescent staining of the astrocyte protein glial fibrillary acidic protein (GFAP; d, j) or the oligodendrocyte-specific transcription factor Olig2 (e, k) at 1 week post-injection of (d–f) ethidium bromide or (j–l) lysolecithin. (d, e) and (j, k) are the same field. The boxed regions in (d, e) are shown at higher magnification in (f, l), respectively. Magnification bars are indicated

demyelination [11]. However, electrophysiological evidence of spontaneous remyelination in the rat was not observed even up to 8 weeks post-EB [13], indicating the need for physiological evaluation if evidence of functional remyelination is to be concluded. Moreover, a substantial percentage of the chronic remyelination in this model is Schwann cell rather than oligodendrocyte myelination [1]. Importantly, this lesion also kills astrocytes (Fig. 1d) which are essential for oligodendrocyte remyelination [14]. The EB lesion model is best suited for acute remyelination by engrafted

exogenous myelinating cells rather than to examine mechanisms of spontaneous, endogenous remyelination. The third major difference between rat and mouse spinal cord EB lesions is the inflammatory response. In the rat, there is a transient inflammatory response that resolves by 1 month post-EB [13], while in the mouse there is a persistent inflammatory response that lasts at least 2 months post-EB [9]. Such chronic inflammatory responses can markedly affect remyelination [15].

At appropriate concentrations and doses, lysolecithin selectively kills oligodendrocytes in the injection site by solubilizing myelin sheaths and facilitating inflammatory responses [1], but spares most axons and astrocytes [16–18]. In contrast to spinal cord EB lesions, lysolecithin-induced demyelinating lesions completely remyelinate spontaneously by 4 weeks after initial treatment (Fig. 1g–i), with only a small fraction of Schwann cell remyelination [1, 19]. Astrocytes are not significantly effected by lysolecithin, but there is still loss (Fig. 1j–l). This is an excellent model to study spontaneous remyelination but is more limited for grafting studies due to its robust intrinsic repair.

Antibodies to galactocerebroside C (GalC), an oligodendrocyte glycolipid, specifically target oligodendrocytes for complement-mediated lysis [1, 20]. These lesions also spontaneously remyelinate, with the majority of oligodendrocyte remyelination, but the time course mirrors that of EB, e.g. 3–6 months post-demyelination. The disadvantage to this model is that complement lysis initiates a robust inflammatory response, which as indicated above, can compromise the interpretation of mechanistic data [15].

The cuprizone model is best suited to examine endogenous remyelination *in vivo*. Cuprizone is provided in the diet [21] and it kills oligodendrocytes preferentially by chelating  $\text{Cu}^{2+}$ , a necessary co-factor for many metalloenzymes [22]. The process of cuprizone remyelination is complex involving an initial OPC propagation phase and a subsequent maturation phase [23]. Demyelination is maximal after 6 weeks on a cuprizone diet and following cuprizone cessation, the peak of oligodendrocyte maturation occurs at 2–3 weeks post-cuprizone with remyelination resolving by 6 weeks of recovery [21, 24, 25]. Note that the corpus callosum is much more effectively demyelinated by cuprizone than is the spinal cord [3].

Experimental autoimmune encephalomyelitis (EAE) and viral-induced demyelination are demyelinating models which closely resemble human MS [26] as each involves an inflammatory component as the cause of demyelination ultimately resulting in neurological deficits [27–29]. EAE is induced via immunization with CNS myelin proteins (i.e. MBP, PLP, MAG, and MOG) along with co-administration of an adjuvant [30, 31]. Viral demyelination is induced by infecting susceptible strains of mice with one of a number of viruses; most commonly Theiler's murine encephalomyelitis virus (TMEV) or murine hepatitis virus (MHV) [32]. Depending on the species, strain, virus, and technical approach, both EAE and viral-induced demyelination can be tailored to model various forms of MS. Moreover, both models display heterogeneous patterns of demyelination which, despite mimicking the clinical pathology observed in MS [30–32], lack the anatomical specificity and reproducibility of the focal demyelination techniques we describe here (i.e. EB and lysolecithin injection).

## Materials

Name	Company	Catalog number
1 mL syringes	B-D	309628
10 mL syringes	B-D	309604
60 mL syringes	B-D	309653
15 mL conical tube	Fisher	14-959-70C
#15 blades	F.S.T.	10015-00
4-0 monofilament	Henry Schein	036-626, 4-0
26G <sup>5/8</sup>	B-D	305115
30 guage needle, bent 90°	Henry Schein	036-345
Alpha-dri Bedding	Cincinnati Labs	ADT
Bacitracin	Henry Schein	108-2912
Biosafety hood	Flow Sciences	FS3060m
Castroviejo needle holder	F.S.T.	12565-14
Clippers	Harvard Apparatus	64-0712
Cotton tip applicators	Henry Schein	003-263
Dural scissors	F.S.T.	15002-08
Friedman-Pearson micro	F.S.T.	16221-14
Ronguers, curved tips		
Gauze pads	Henry Schein	006-936
Gel foam	Henry Schein	031-551
Glass micropipette tips	Warner Instruments Corp	G120F-4
Graefe forceps	F.S.T.	11053-10
Heating pad	Henry Schein	263-5521
Jeweler's forceps	F.S.T.	11254-20
Lacrilube	Henry Schein	039-886
Micropipette beveller	Sutter Instrument	BV-10
Micropipette puller	Sutter Instrument	P-97
Mouse decapicone	Braintree Scientific	
Mouse vertebral stabilizer	Louisville Injury System	Handmade (equivalent)
Nitrogen compressed air tank	Scott-Gross	
Picoinjector or nanoinjector		PV800
Scalpel handle	F.S.T.	10003-12
Sponge spears	F.S.T.	18105-03
Spring scissors with fine blades	F.S.T.	15013-12
Staple applicator	F.S.T.	12031-09
Staples	F.S.T.	12032-07
Stereotaxis frame	Benchmark digital	
Straight iris scissors	Henry Schein	012-338
Surgical microscope	Zeiss	
Thoracotomy Scissors	FST	14002-14
Water heating pad	Harvard Apparatus	729715

Reagent	Company	Catalog number
1% lysolecithin	Sigma	L1381, 5 mg
0.9 % sodium chloride	Henry Schein	040-198
2,2,2 tribromoethanol	Sigma	T48402-25G (acute toxicity)
Buprenorphine (0.3 mg/mL)	Reckitt Benckiser	
Derma Chlor	Henry Schein	055-480
Ethidium Bromide (0.2 mg/mL)	Invitrogen	15585-011 (mutagen)
EtOH	Sigma	E7023
Gentamicin (0.6 mg/mL)	Henry Schein	006-913
Ketamine (100 mg/mL)	Hospira	
NaCL	Sigma	S7653
Xylazine (20 mg/mL)	LLOYD laboratories, Akorn Inc.	4811-20 mL

## Procedures

All animal procedures were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, “Guide for the Care and Use of Laboratory Animals, Eighth Edition” (Committee for the update of the Guide for the Care and Use of Laboratory Animals, National Research Council, 2010), and with the approval of the University of Louisville Institutional Animal Care and Use Committee. Adult C57Bl/6 mice 8–10 weeks of age weighing 15–25 g were used for this methods section (Envigo–Harlan; Indianapolis, IN). Rats weighing 180–220 g are also commonly used. Because of the similarities in procedures, details of the mouse surgery are presented below. This section has been divided into five sub-categories ((1) glass micropipette preparation, (2) preparing the micropipette for injection, (3) pre-surgical animal preparation, (4) performing the surgical procedure, (5) post-op animal care) to demonstrate pre-, intra-, and post-surgical preparations.

### *Glass Micropipette Preparation*

1. Prepare micropipette needles by pulling a glass capillary tube with an inner diameter (ID) = 0.53–2.0 mm, using a Sutter Instrument model P-97 micropipette puller (or equivalent). The size of the glass capillary depends on the volume of demyelinating solution. For this procedure, glass capillaries with an ID = 0.69 mm, OD = 1.2 mm, with a length = 10 cm (Warner Instruments, 64-0790) are commonly used. Note: Capillaries contain a filament that helps draw up and expel liquids.
2. Pull glass capillaries to very fine tips using a high temperature setting (600–800 °C) with small force (20 psi) to produce a long tapered tip for deep tissue injection. If using the Sutter Instrument, use program 21-1 (Heat—600, Pull—29, Velocity—37, Time 150).



3. Once pulled, trim the micropipette needles to an ID of 20–30  $\mu\text{m}$  for mice and 30–35  $\mu\text{m}$  for rats using #5 forceps with the aid of a micrometer. Each hash mark on a micrometer represents 5  $\mu\text{m}$ . Note: The ID is based on the viscosity of solution, cell diameter (if cellular therapies are used), and size of the animal being injected. The larger the ID, the less resistance for fluids.
4. Once an ID has been achieved, bevel pipettes using a Sutter Instrument model BV-10 micropipette beveler (or equivalent). Tips should be beveled at 35–45° to facilitate tissue penetration. After beveling, flush pipette needles with 100% ethanol to remove any debris.
5. Always prepare 5–10 pipettes for each operative day to replace broken or clogged needles.
6. Sterilize glass pipettes by laying them on adhesive tape inside a petri dish exposing them to UV light for one hour.

### ***Preparing the Micropipette for Injection***

1. Reconstitute lysolecithin to a 1% solution with sodium chloride (NaCl; pH 7.4) or prepare 0.2 mg/mL ethidium bromide solution [9]. Store lysolecithin in small aliquots (75  $\mu\text{L}$ ) at  $-20\text{ }^{\circ}\text{C}$ . Thaw aliquots to room temperature before use. Keep ethidium bromide at room temperature.
2. Handle the pre-pulled glass pipette with extreme caution to avoid damaging the fine tip. Label micropipette needles with hash marks to represent 0.25  $\mu\text{L}$  between each mark.
3. Attach the pipette needle directly to the arm of the stereotactic device.
4. Load the pipette needle by connecting a 60 mL syringe to the inlet of the vacuum compressed air tip with plastic tubing. Make sure seal is tight so that no air escapes.
5. Once connected, lower the pipette needle into your demyelinating solution.
6. Gently pull back on the syringe plunger to slowly fill the pipette needle with glial toxin until the required amount is achieved. Note: Avoid introducing air bubbles into the pipette needle. If air bubbles are present, the pipette preparation must be repeated. Pipettes will be able to inject 4–8 animals before needing to be refilled.
7. Remove the 60 mL syringe from the vacuum inlet and reconnect the pneumatic pico-pump (PV800) to the stereotaxic frame.

### ***Pre-Surgical Animal Preparation***

1. Anesthetize mice with an intraperitoneal injection (IP) of ketamine (50 mg/kg) and xylazine (5 mg/kg) or 2.0% avertin (2,2,2-tibromoethanol in 1.25% 2-methyl-2-butanol in sodium chloride solution) at 400 mg/kg using a decapi-cone or by grabbing the scruff of the animal at the shoulder blades. Make sure the animal is safely secured before inserting the needle. Insert the needle with the bevel up and gently pull back on the syringe to test for proper placement. Note: When the

needle is properly placed you will get negative pressure. If blood, feces or urine are pulled into the syringe then remove the needle without injecting, discard the syringe and repeat the procedure again. Avertin is not an FDA approved drug and needs IACUC approval before use.

2. Place the animal in a secured location until they are fully anesthetized. The animal will be under anesthesia for approximately 45 min–1 h.
3. Firmly pinch the toe of the animal to assure that adequate sedation has been achieved. A properly anesthetized animal will not respond to toe pinch. Anesthetic depth should be monitored every 5–10 min throughout the surgical procedure.
4. Shave a 2 in. square along the dorsal spinal column using clippers. Make sure the clipper blade lays flat against the animal's skin to prevent tissue damage.
5. Make sure all clipped hair has been removed from the surgical area.
6. Clean the surgical area with 100% chlorhexidine solution applied to a 2 × 2 gauze pad. Then repeat using a 10% chlorhexidine solution. Carefully apply a small amount of lacrilube to the eyes to prevent drying during the surgical procedure.
7. Administer 1 mL of pre-warmed subcutaneous fluids (SQ) to prevent dehydration.

### *Performing the Surgical Procedure*

To create the desired demyelinating lesion, stereotaxis is used to align the depth of the injection in the ventral-dorsal plane as well as the location of the injection in the medial-lateral plane. To generate demyelinating lesions of the venterolateral funiculus (VLF), the following parameters are used:

- VLF lesions in rats, inject at 1.6 and 1.3 mm depths and 0.7 mm lateral to midline.
- VLF lesions in mice, inject at 1.1 and 0.7 mm depths and 0.45 mm lateral of midline.
- Two unilateral injections spaced 1.75 mm apart were given at a depth of 1.1 and 0.7 mm and 0.45 mm lateral of midline for this demonstration.

*Additional Comments:* Aseptic technique should be used for all steps during this procedure (gloves, bonnet, mask). Surgical tools should be sterilized before coming in contact with the animal. Each surgical procedure may take 15–20 min. It is recommended to have at least 1–2 people assisting for each surgical procedure. If anesthetic boosting is required during the surgical procedure inject ketamine (50 mg/kg) IP or Avertin (20–40 mg/kg) IP. This surgical procedure may be modified for rats.

1. Move the animal to the surgical table dorsal side up. Surgical tape (optional) may be used to secure the animal to the operating station.
2. Under a surgical microscope with adequate lighting, use small surgical scissors to make a sagittal incision in the skin from T6 to T12.
3. Cut away the connective tissue overlaying the musculature of the dorsal column.



4. Using extra fine spring scissors, separate the muscles from the lamina, exposing the lateral aspects of the facets bilaterally. Adequate exposure is important to allow the vertebral column to be grasped when lifted onto the stabilizer using the facets. Note: The spine stabilizer used here was developed by the Louisville Injury System Apparatus (LISA). However other devices may work well provided the spine is securely immobilized.
5. Using forceps or closed spring scissors, feel for the hard surface of T11 (T11 will protrude slightly higher than the other vertebrae).
6. Once T11 has been located, use spring scissors to make shallow lateral cuts (2–3 mm deep) in the connective tissue between T8–T9 and T9–T10 repeat between T10 and T11. Note: If bleeding occurs hold sponge spears or cotton tipped applicators to area for 15–30 s or until bleeding subsides. Gel-foam may be applied to the area as aid for coagulation.
7. Using Graefe forceps pull the T9 and T10 vertebrae up and off of the column. Removal of the vertebrae will slightly expose the spinal cord so that a laminectomy may be performed.
8. Place the animal in the Louisville Injury System Apparatus or other equivalent device.
9. Use extra fine Graefe forceps to grasp the lateral facets and place the toothed blades of the stabilizer on either side of the facets, thereby securing the vertebral column in the stabilizer by tightening both sides of the stabilizer. Note: The vertebral column must be aligned parallel with the base of the stabilizer.
10. Very gently use #2 laminectomy forceps to remove the lamina from the vertebral column. Make sure the area does not contain any sharp edges which may interfere with pipette placement.
11. Clean the interlaminar soft tissue with a cotton tipped applicator. Open the dura using the tip of a bent (90°) 30 gauge needle. Once the dura has been penetrated expand the opening with laminectomy forceps. Remove any cerebrospinal fluid that may have leaked out when the dura was penetrated. Note: The dural opening should only be large enough to allow the micropipette tip to enter the spinal cord.
12. Transfer the animal in the spine stabilizer to the injection system (see accompanying movie). Place the animal perpendicular to the tip of the micropipette. Align the tip to the midline of the dural opening. Slowly lower the pipette tip until it is just 1–2 mm above the cord.
13. Using the graded measurements of the Y-axis, move the pipette needle 0.4 mm lateral of midline.
14. Slowly move the pipette needle in a downward direction until it just barely touches the spinal cord to obtain a surface reading of x-axis. From this reading, subtract 1.1 mm. Use a quick downward motion to pierce the spinal cord surface and slowly lower the pipette tip until the new measurement is reached.
15. Using the pico-pump, deliver half of the total volume per injection site of glial toxin into the spinal cord. Wait 2 min to allow the demyelinating agent to diffuse.

Note: Lesions depend not only on the target but also on the volume and concentration of the solution, each of which has a specific pattern of diffusion.

16. Using your x-axis reading, add 0.5 mm. Raise the tip to this new measurement. Inject a second bolus of glial toxin into the spinal cord. Wait 2 min. Note: If lesions are required at a different location, refer to a mouse [33] or rat [34] brain atlas for specific injection coordinates.
17. Remove the pipette needle from the spinal cord.
18. Calculate the z-axis to move 1.75 mm caudal to the first injection site. Note: This only applies if a second injection site is needed.
19. Repeat steps 14–17.
20. Loosen the pins of the facet blades and remove the animal from the spinal stabilizer apparatus.
21. Using 4-0 or 5-0 monofilament, tie a single suture through the muscle and adipose tissue overlaying the spinal column.
22. Close the skin incision by applying wound clips using a staple applicator. Apply bacitracin to the incision area. Administer 1 mL of pre-warmed SQ NaCl, and gentamicin (5 mg/kg) SQ. Place the animal in a recovery cage that has been placed halfway onto a warm water circulating heating pad. Note: Only place the cage half way on the heating pad so the animal may escape the warmed area if over-heating occurs.
23. Administer analgesics as directed by the institute's animal care and use program. Animals are fully ambulatory and capable of self-feeding and drinking as soon as they recover from anesthesia.
24. Repeat the procedure on any remaining animals. Note: Between each use the micropipette tip should be submerged in liquid to prevent clogging. The same pipette needle may be used throughout 4–8 surgeries before the needle dulls and has to be replaced. Separate needles should be used when multiple demyelinating agents are being considered. All used micropipette needles should be discarded at the end of surgery. It is not recommended to reuse needles.

### ***Post-op Animal Care***

1. Keep animals on a water-heating pad (cage half on half off) overnight in a recovery room. Cages should contain alpha-dri bedding with food pellets laid directly on the alpha-dri for ease of access.
2. Return animals to normal housing condition 24 h post-surgery.
3. Administer pain meds Buprenorphine (0.1 mg/kg) for 48 h post-surgery, or other IACUC approved pain medication.
4. Administer antibiotics Gentamicin (5 mg/kg) SQ once daily for 7–10 days, or other IACUC approved antibiotic. Fluids may also be given as need for dehydration.
5. Keep daily health logs to record each animal's health status for the remainder of the study.

## Applications, Complications, and Limitations

- (a) When injecting larger volumes into the spinal cord, the volume of fluid itself can compress the surrounding spinal cord or the force of injection can cause fluid to leak out through the needle trajectory. It is recommended two depths be injected so that the volume at each depth is half the total. This prevents undesirable fluid leakage. Each glial-toxin has different diffusion properties as well so the two injection sites also provide a more consistent concentration of toxin throughout the desired target area. The concentration, volume and stereotaxic coordinates for any desired lesion must be optimized empirically.
- (b) Micropipette needles may become clogged in between animal injections. To prevent this lower the pipette needle into the glial toxin in between animal injections. If the needle still becomes clogged use the pico-pump to displace the clog. If a needle becomes clogged during injection raise the needle up and out of the spinal cord, gently swab the tip with a cotton tipped applicator that has been moistened with saline and use the pico-pump to displace the clog. Avoid using high pressure settings while the needle is still in the spinal cord. High pressure may cause the needle to flood the cord with too much glial toxin compromising the procedure.
- (c) All of these lesions can be used to follow the spatiotemporal progression of remyelination by both endogenous or engrafted myelinating cells, e.g. oligodendrocyte precursor cells (OPCs). If one wants only to look at the contribution of engrafted myelinating cells, the lesion site can be X-irradiated [35, 36] either before or after SCI to prevent endogenous OPCs and Schwann cells from proliferating and migrating to the lesioned area and remyelinating. If endogenous remyelination is to be evaluated, the relative contributions of oligodendrocyte and Schwann remyelination must be quantified as both cells will migrate to the lesion site [11]. Lesions of the dorsal columns remyelinate more efficiently than do lesions of the lateral or ventral white matter.
- (d) One must be extremely careful to monitor axonal loss with these models. This can be done by immunostaining for neuron-specific antigens such as the neurofilament proteins. Silver staining can also be used. It is important that longitudinal sections be examined as it is difficult to assess axon continuity on cross sections. The extent and progression of the inflammatory response, which can affect both axonal pathology and remyelination, can be monitored by immunostaining for microglia/macrophage markers, OX42 or ED1 for the rat or Iba1 for the mouse or the leukocyte common antigen CD45 which identifies all rat and mouse leukocytes.
- (e) If axons are demyelinated chronically in the mouse, they can degenerate and/or become not patent for remyelination [9]. In all these lesion models, demyelination is complete within 7 days and it is recommended that therapeutic treatments are not delayed more than 1 month post-demyelination.

## Illustrative Results

The optimal approach to assess remyelination is to combine multiple outcome measures. We routinely use electrophysiological methods in combination with terminal histological assessment of remyelination. What electrophysiological approach one takes depends on where anatomically in the spinal cord the demyelination lesion was performed. Conduction is measured by somatosensory evoked potentials (SSEPs) for the ascending dorsal columns [37], transcranial magnetic motor evoked potentials (tcMMEPs) for the medial aspects of the VLF [36], and magnetic inter-enlargement reflexes for the mediolateral white matter [38]. A detailed description of these methods is beyond the scope of this chapter, but changes in conduction can be measured by electrophysiological assessment. Histologically, one must quantify both spared white matter and the extent of remyelination and by which cells remyelination occurs. It is imperative that the latter assessment utilize unbiased stereological methods. Figure 1 shows representative results of how EB lesions and lysolecithin lesions may appear after injection. For detailed description of tissue dissections, histological (EC staining) and immunohistochemical methods, see: <http://louisville.edu/kscirc/basic-research/laboratories/laboratory-of-molecular-neurobiology/protocols-1>.

## Conclusions

As outlined above, all of these models have strengths and weaknesses depending on the questions to be asked. None of them faithfully reproduce the clinical spectrum of demyelinating diseases. However, if one's experimental questions are carefully tailored and the inherent caveats are recognized, they provide model systems that enable physiologically relevant questions about CNS remyelination to be addressed.

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