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Title: Rat Model of Widespread Cerebral Cortical Demyelination Induced by an Intracerebral Injection of Pro-Inflammatory Cytokines

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Authors, please fill out the brief questionnaire below.

A. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **(Y/N)**____**N**___

Can you record movies/images using your own microscope camera? **(Y/N)**_____

If no, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope:

_____

B. Software Usage: Does your protocol include detailed, step-by-step, descriptions of software usage? **(Y/N)**___**N**____

C. Which steps of your protocol will viewers benefit most from having filmed? Please list 4-6 individual steps using the step numbers listed in this document. (Please do not list entire sections.) __2.7, 2.9, 2.10, 3.1 and 3.2_

Authors, please answer this question with the steps listed here in the protocol section for use by the videographer.

D. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1-2 individual steps using the step numbers listed in this document. (Please do not list entire sections.) __2.7: While drilling the holes in the skull, the dura mater should remain intact and a traumatic injury by penetration should be avoided. To ensure this, we 1) use a drill that can be installed on to the stereotactic frame, 2) inspect the hole frequently during drilling, 3) drill down in small steps

Authors, please answer this question with the steps listed here in the protocol section for use by the videographer.

E. Will the filming need to take place in multiple locations? (Y/N) ____**N**___ If yes, how far apart are the locations? _____

1. Introduction (Experimental Goal and Author Interviews) – As the beginning of your video, the introduction should clearly present the goal of your method to the viewer and its significance. Other information can be provided according to the various statements below, but the total introduction should not exceed 150 words.

A. Experimental Goal: (read by voice talent at JoVE)

The overall goal of this procedure is to generate a widespread demyelination of the cortex of both cerebral hemispheres in a rat model using subclinical immunization against myelin oligodendrocyte glycoprotein followed by intracerebral injection of cytokines through an implanted catheter. **(Intro)**

B. Required Interview Statements: (Said by you on camera. Don't forget to smile!)

1.1. Sonja Hochmeister: This method can help answer key questions in inflammatory demyelinating diseases of the brain, such as multiple sclerosis.

1.2. Muammer Üçal: The main advantage of this technique is that it can generate inflammation-induced grey matter demyelination in the cortex of both cerebral hemispheres without leading to white matter plaques.

C. Optional Interview Statements: (Said by you on camera. Don't forget to smile!)

1.3. Michaela Tanja Haindl: The implications of this technique extend toward a better understanding of mechanisms of cortical demyelination that is observed mostly in progressive types of multiple sclerosis.

E. Ethics title card: (for human subjects or animal work, does not count toward word length total)

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1.9. Procedures involving animal subjects have been approved by the Austrian Ministry of Science and Research.

Protocol: (read by voice talent at JoVE)

Authors: In order to ensure that your protocol can be filmed in a single day, the protocol text must be limited to 30 steps (marked with two-digit numbers, e.g. 2.1., 2.2.) and 60 "shots" (designated by a three-digit number, e.g. 2.1.1, 2.2.2). The scope of the scripted protocol text should include only those aspects of the procedure that require visualization in order to be well understood.

2. Catheter Implantation

2.1. To begin this procedure, assemble the catheter and the catheter cap with the inlet **[1-MED-over the shoulder]**. Next, cut the catheter to a length of 2 mm with a scalpel **[2-CU]**.

2.1.1. Talent assembles the catheter and the catheter cap with the inlet and tightens the screw

2.1.2. CU the catheter as it is cut

2.2. Then, shave the head of an anesthetized rat between the ears using the electric shaver **[1-MED-over the shoulder]**. Place the rat in the stereotactic frame and secure its head using the ear bars and bite plate **[2-CU]**. Ensure that the head is horizontal and check for its stability by applying pressure on it with finger or forceps **[3-CU]**.

2.2.1. Talent shaving the rat's head between the ears

2.2.2. CU the rat as its head is secured using the ear bars and bite plate

2.2.3. CU the head as pressure is applied on it

2.3. Afterward, apply lubricating eye drops to prevent cornea dryness during the surgery **[1-CU]**. Cover the eyes with an opaque material to prevent any surgical light exposure **[2-CU]**. Next, clean the shaven area by alternating the application of 70% ethanol and 10% povidone-iodine complex **[3-CU]**.

2.3.1. CU the head as lubricating eye drops are applied

2.3.2. CU the head as the yes are covered with an opaque material

2.3.3. CU the head as the shaven area is cleaned by alternating the application of ethanol and povidone-iodine complex

2.4. To implant the catheter, make a longitudinal incision of about 2 cm of length along the midline **[1-CU]**. Then, use bulldog clamps to hold the skin to the sides **[2-CU]**. Remove the blood using a cotton-tipped applicator **[3-CU]**.

2.4.1. CU the head as a longitudinal incision of about 2 cm of length is made along the midline

2.4.2. CU the head as the skin is held to the sides

2.4.3. CU the head as blood is removed

2.5. Afterward, remove the periosteum **[1-CU]**. Clean the tissue with the cotton-tip applicator **[2-CU]**. Expose the skull and allow it to dry for about 1 minute **[3-CU-TXT]**.

2.5.1. CU the head as the periosteum is removed

2.5.2. *Film as written

2.5.3. CU the head as it is exposed. Text: 1 min

2.6. Next, identify the anatomical landmarks, Lambda, Bregma, and medial suture **[1-SCOPE]**. With the drill installed on the stereotactic frame, position the tip of the drill bit at the Bregma as the starting point **[2-SCOPE]**. Move 2 mm posterior from the Bregma and 2.4 mm laterally to the medial suture **[3-SCOPE]**.

2.6.1. Show that the landmarks (Lambda, Bregma, and medial suture) are pointed at with forceps

2.6.2. Show that the tip of the drill bit is at Bregma

2.6.3. Show that drill bit is moved 2 mm posterior from the Bregma and 2.4 mm laterally to the medial suture

2.7. Then, drill a 0.5 mm diameter hole for the catheter at this position **[1-SCOPE]**. Drill 3 more holes of 1.3 mm diameter for the anchor screws a few millimeters away from the first hole **[2-SCOPE]**.

2.7.1. Show that a 0.5 mm diameter hole is drilled for the catheter

2.7.2. Show that 3 more holes of 1.3 mm diameter are drilled for the anchor screws a few millimeters away from the first hole NOTE: please cut the film before the blood comes up! This is important.

2.8. Remove the bone dust by irrigation with around 1-2 mL of sterile PBS and clean the skull **[1-SCOPE]**. Subsequently, tighten the anchor screws by 2 - 3 full turns **[2-SCOPE]**.

2.8.1. Show that the bone dust is removed from the skull

2.8.2. Show that the anchor screws are tightened by 2 - 3 full turns

2.9. Insert a 2-mm length catheter through the first hole, perpendicular to the skull surface **[1-SCOPE]**. While still holding the catheter, apply a little dental cement and let it polymerize with a brief exposure to the dental curing light in order to stabilize the catheter **[2-CU]**.

2.9.1. Show that a 2-mm length catheter is inserted through the first hole

2.9.2. CU the skull as dental cement is applied and dental curing light is used to solidify the dental cement

2.10. Then, apply more dental cement around the catheter, anchor the screws **[1-CU]**, and solidify the dental cement with the dental curing light for about 15 - 30 second **[2-CU]**. Confirm that the cement is hardened **[3-CU]**.

2.10.1. Show that more dental cement is applied around the catheter and the screws are anchored

2.10.2. Show that dental curing light is used to solidify the dental cement- NOTE: this is shown together with 2.10.1.

2.10.3. Show that the cemented is poked by the forceps tip

2.11. After implantation, close the skin with resorbable sutures anterior and posterior to the catheter **[1-CU]**. Then inject the antidote mixture subcutaneously **[2-MED-over the shoulder]**. Next, administer 2.5% enrofloxacin by subcutaneous injection for prophylactic antibiotic treatment **[3-MED-over the shoulder]**

2.11.1. CU the head as sutures are preformed

2.11.2. *Film as written

2.11.3. *Film as written

2.12. Return the animal to the modified cage and keep it under observation for 1-3 hours, with an application of infrared light to avoid hypothermia **[1-MED]**. Repeat the enrofloxacin treatment and administer carprofen at 1 mg/mL subcutaneously for pain relief by injection the day after the surgery **[2-CU]**.

2.12.1. Talent places the animal in the modified cage with infrared light

2.12.2. CU the animal as it is injected with carprofen subcutaneously

3. Immunization

3.1. To prepare the immunization mixture, connect two 10 mL Luer lock tip glass syringes to the short arms of a 3-way stopcock and close the third outlet with the long arm **[1-MED-over the shoulder]**. Next, pipette 1 mL of IFA and 50 μg of rMOG together **2-MED-TXT]** and adjust the mixture to a final volume of 2 mL with sterile PBS **[3-CU]**.

3.1.1. *Film as written

3.1.2. *Film as written. Text: IFA: incomplete Freund's adjuvant, rMOG: recombinant myelin oligodendrocyte glycoprotein

3.1.3. CU the mixture as it is adjusted to a final volume of 2 mL with sterile PBS- NOTE: 3.1.2. and 3.1.3. are filmed together

3.2. Place the diluted IFA and rMOG mixture in the open syringe **[1-MED]**. Then, insert the piston gently whilst maintaining a loose pressure on the opposite piston **[2-CU]**. Emulsify the inoculum by driving it from one syringe to the other via pushing the pistons back and forth until it is white and viscous **[3-MED-over the shoulder]**.

3.2.1. *Film as written

3.2.2. *Film as written

3.2.3. Talent pushing the pistons back and forth NOTE: 3.2.1, 3.2.2. and 3.2.3 are filmed together

3.3. Next, fix a 1 mL Luer lock syringe to the open short arm of the 3-way stopcock and fill it with inoculum **[1-MED-over the shoulder]**. Distribute all inoculum to 1 mL syringes and keep them on ice until the injection **[2-CU-TXT]**. Subsequently, inject 200 μL of the IFA and rMOG mixture subcutaneously at the tail base under a temporary isoflurane anesthesia using a 21 Gauge needle.

3.3.1. *Film as written

3.3.2. CU the syringes as they are placed on ice. Text: Administer the mixture on the day of the preparation. NOTE: 3.3.1. and 3.3.2. are filmed together

3.3.3. CU a rat as the IFA and rMOG mixture is injected subcutaneously at the tail base

4. Intracerebral Cytokine Injection

4.1. For intracerebral cytokine injection, adjust the length of the connector cannula to 2 mm **[1-MED]**. Fill a 1 mL syringe with the cytokine mixture **[2-CU-TXT]**. Then connect the syringe to the connector cannula **[3-MED]**.

4.1.1. *Film as written. Authors, how is the length of the connector cannula adjusted? A previously prepared 2 mm catheter is assembled to connector cannula (cannula is inserted into the catheter and the catheter is tightened to the screw present on connection hose – like it would be performed with the animal later). Then connector cannula is cut to the same length as the catheter. Then the template catheter is removed again.

4.1.2. CU a 1 mL syringe as it is filled with the cytokine mixture. Text: 500 ng/μL of TNF-alpha, 300 U/μL of recombinant rat IFN-gamma in sterile PBS

4.1.3. *Film as written

4.2. Next, fill the cannula with the cytokine mixture; avoid creating any bubbles **[1-CU]**. Then, mount the syringe onto the programmable syringe pump and program it to inject at a rate of 0.2 μL/min **[2-MED]**. Start the pump and keep it working in order to avoid an air bubble formation at the tip of the cannula **[3-CU]**.

4.2.1. CU the cannula as it is filled with the cytokine mixture

4.2.2. Talent mounts the syringe onto the programmable syringe pump and programs it

4.2.3. CU the pump as it starts working

4.3. Afterward, remove the catheter cap with the inlet **[1-MED-over the shoulder]**. Insert the connector cannula into the catheter, screw and tighten it **[2-MED-over the shoulder]**. Then, allow the injection to proceed for 10 minutes before stopping the pump **[3-CU]**.

4.3.1. *Film as written

4.3.2. *Film as written

4.3.3. CU the animal as it is being injected

4.4. Leave the cannula inside the catheter for 20 minutes to allow the injected volume to fully diffuse **[1-MED]**. Subsequently, unscrew the connector cannula and remove it slowly to avoid a vacuum effect **[2-MED]**. Reattach the catheter cap with the inlet and screw it **[3-MED]**. Allow the animal to recover from the anesthesia in a cage **[4-CU]**.

4.4.1. Talent stops the pump

4.4.2. *Film as written

4.4.3. *Film as written

4.4.4. CU the animal as it is placed in a cage

OPTIONAL: Author Protocol Interviews: In this section, please submit a brief statement stressing the required technique for the single most critical step of the procedure. This will be an interview style shot, interjected into the appropriate point of the protocol section of the video. **If there is no single critical step, then there is no need to fill this out.** If there are two critical steps, please fill out two statements, but this is the maximum. Each statement, should be 40 words or less. Please specify who will speak these parts and which step(s) in the protocol the statement pertains to. Use the step numbers given above.

Author name, Step _____: (write your 1st optional statement here)

Author name, Step _____: (write your 2nd optional statement here)

5. Results: PLP Immunoreactivity in Coronal Brain Sections

5.1. Cortical demyelination could be assessed at different time points after a cytokine injection by immunohistochemistry for PLP (Text: PLP: proteolipid protein). Figure 6A shows intact PLP immunoreactivity at day 15 in a MOG-immunized control animal that received only sterile PBS through the implanted catheter.
-LAB MEDIA: 57879_Hochmeister_Figure6A.tif

5.2. On day 1 after the cytokine injection, demyelination could already be detected in MOG-primed animals, albeit only in the close vicinity of the catheterized area (Figure 6B).
-LAB MEDIA: 57879_Hochmeister_Figure6B.tif

5.3. The PLP immunoreactivity stays intact in the contralateral cortex 1-day post-cytokine injection. On day 3, a gradual increase in the loss of the PLP immunoreactivity, which spreads in the ipsilateral cortex (Figure 6C), could be observed.
-LAB MEDIA: 57879_Hochmeister_Figure6C.tif

5.4. Contralateral cortical demyelination could also be detected at day 3 (Figure 6D), but it is rather restricted to the area beneath the anchor screws.
LAB MEDIA: 57879_Hochmeister_Figure6D.tif

5.5. Between days 9 - 15, demyelination affects large parts of the cortex of both hemispheres (Figures 6E, 6F, and 6G).
LAB MEDIA: 57879_Hochmeister_Figure6E-G.tif

5.6. The cortical demyelination is sustained for up to 30 days post-cytokine injection in both hemispheres (Figures 6H and 6I) with only a partial remyelination.
LAB MEDIA: 57879_Hochmeister_Figure6H-I.tif

5.7. Figure 6J shows a quantification of PLP loss in the cortical grey matter after the intracerebral cytokine injection.
LAB MEDIA: 57879_Hochmeister_Figure6J.tif

6. Conclusion (said by authors on camera)

6.1. Michaela Tanja Haindl: After watching this video, you should have a good understanding of how to generate demyelination of grey matter using subclinical

immunization against myelin proteins followed by intracerebral injection of cytokines through the implanted catheter.

6.2. Muammer Üçal: This animal model could help researchers in study of progressive types of multiple sclerosis, where grey matter demyelination is a hallmark.

6.3. Sonja Hochmeister: The implanted catheter enables multiple rounds of demyelination or intracerebral delivery of potential therapeutic drugs undergoing preclinical investigation without causing injection-induced trauma.

Provided Media

Insert your media filenames here.

5.1 – *57879_Hochmeister_Figure6A.tif-* *PLP immunoreactivity in control brain*

5.2 – *57879_Hochmeister_Figure6B.tif-* *PLP immunoreactivity at day 1, ipsilateral*

5.3 – *57879_Hochmeister_Figure6C.tif-* *PLP immunoreactivity at day 3, ipsilateral*

5.4 – *57879_Hochmeister_Figure6D.tif-* *PLP immunoreactivity at day 3, contralateral*

5.5 – *57879_Hochmeister_Figure6E-G.tif-* *PLP immunoreactivity at day 9-15; 6E: ipsilateral; 6F: contralateral; 6G: overview*

5.6 – *57879_Hochmeister_Figure6H-I.tif-* *PLP immunoreactivity at day 30; 6H: ipsilateral; 6I: contralateral*

5.7 – *57879_Hochmeister_Figure6J.tif-* *quantification of PLP loss*