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Title: Generating Acute and Chronic Experimental Models of Motor Tic Expression in Rats

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 25
Number of Shots: 53

Introduction

1. Introductory Interview Statements

NOTE to VO Talent: Please record the introduction and conclusion statements as well.

1.1. This protocol describes a unique experimental model of tic disorders by laying out the method for inducing motor tics in a freely behaving rat, either in a transient or chronic manner.

1.2. The main advantage if this method is that it describes the only available model of tic expression, which is widely used of study Tourette syndrome and other tic disorders.

1.3. Visual demonstration clarifies the complex process of custom-made device preparation and implantation surgery.

1.3.1. *Video editor is suggested to display Figure 1 & 2*

Introduction of Demonstrator on Camera

1.4. **Izhar Bar-Gad:** Demonstrating the procedure will be Esther Vinner, a graduate student from my lab and Katya Belelovsky, the lab manager.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrators look up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. All procedures were approved and supervised by the Institutional Animal Care and Use Committee and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Bar-Ilan University Guidelines for the Use and Care of Laboratory Animals in Research. This protocol was approved by the National Committee for Experiments in Laboratory Animals at the Ministry of Health.

Protocol

2. Generation of acute experimental model of motor tic expression in a rat

2.1. To begin, prepare an implant-cannula by cutting a 25-gauge stainless steel hypo-tube **[1-TXT]** and a 0.013-inch dummy removable internal wire **[2]**. Insert this dummy wire into the implant cannula until it reaches the end and bend the excess wire **[3]**.

2.1.1. Talent cutting the steel tube using a rotary tool. **TEXT: OD 0.02'', ID 0.015''**
2.1.2. Talent cutting wire using rotary tool. *Video editor: Show Figure 1 as an inset and emphasize Device #1 & 2*
2.1.3. Talent inserting the dummy wire in the implant-cannula.

2.2. Then, cut a 70-centimeter flexible polymer microbore tube to prepare the injector **[1-TXT]**.

2.2.1. Talent cutting the microbore tube. **TEXT: OD 0.06'', ID 0.02''** *Video editor: Show Figure 1 as an inset and emphasize Device #3*

2.3. Prepare an injection-cannula by cutting a 30-gauge stainless steel hypo-tube with a rotary tool **[1-TXT]**. Insert 3 millimeters of this injection-cannula into a flexible tube **[2]** and glue the joint between them to obtain an injector **[3].**

2.3.1. Talent cutting the 30-gauge stainless steel hypo-tube. **TEXT: OD 0.012'', ID 0.007''** *Video editor: Show Figure 1 as an inset and emphasize on Device #3.2*
2.3.2. Talent inserting the injection-cannula into the tube
2.3.3. Talent gluing the joints.

2.4. When performing the surgery, slide the implant-cannula onto the cannula holder and lower it up to the implantation target in the rats' brain **[1]**. Attach the implanted cannula to the skull of the rat by applying dental cement **[2]**. *Videographer: This step is important!*

2.4.1. Talent lowering the implant-cannula in the implantation target.
2.4.2. Talent applying dental cement to attach the implanted cannula at the skull.

2.5. Insert the prepared dummy into this implant-cannula **[1]** and cover all the implants by applying dental cement to the rest of the skull area **[2]**.

2.5.1. Talent inserting the dummy in the implanted cannula.
2.5.2. Talent applying dental cement on the skull to cover all other implants.

2.6. To perform the microinjection, attach the injector to the precision glass microsyringe filled with bicuculline **[1]** and configure the settings to a rate of 0.35 microliters per minute and a total volume of 0.35 microliters **[2]**. Place the rat in the experimental cage **[3]**. *Videographer: This step is important!*

2.6.1. Talent attaching the injector to the glass microsyringe
2.6.2. Talent configuring the rate of flow of the microsyringe
2.6.3. Talent putting the rat in the experimental cage.

2.7. Remove the dummy **[1]** and insert the injector into the implanted cannula through the end **[2].** *Videographer: This step is important!*

2.7.1. Talent removing the dummy out of the implanted-cannula.
2.7.2. Talent inserting the injector into the implanted cannula.

2.8. Start the infusion pump machine **[1]** and keep track of tic initiation and termination times using a stopwatch **[2]**.

2.8.1. Talent starting the infusion pump machine.
2.8.2. Talent starting the stopwatch for tic observation.
NOTE: 2.8.1 and 2.8.2 were filmed together as a single shot
Videographer: Film the rat as tics appear for result shot 5.1.1.

2.9. After one minute of injection, remove the injector **[1]** and slowly reinsert the dummy **[2]**.

2.9.1. Talent removing the injector.
2.9.2. Talent reinserting the dummy.

3. Generation of chronic experimental model of motor tic expression in a rat

3.1. Prepare a cannula-guide by cutting a 12-millimeter 25-gauge stainless steel hypo-tube **[1-TXT]** and an infusion-cannula by cutting a 30-gauge stainless steel hypo-tube **[2-TXT]**. Insert a 0.005-inch diameter wire into the infusion-cannula and bend it into an L shape in the intended location **[3]**.

3.1.1. Talent cutting 25 G stainless steel hypo-tube to prepare cannula-guide. **TEXT: OD 0.02", ID 0.015"** *Video editor: Show Figure 2 as an inset and emphasize Device #1* NOTE: use shot 2.1.1, as it describes the same procedure

3.1.2. Talent cutting 30 G stainless steel to prepare infusion-cannula. **TEXT: OD 0.012", ID 0.007"** *Video editor: Show Figure 2 as an inset and emphasize Device #2*

3.1.3. Talent inserting wire into the infusion-cannula and bending it.

3.2. Next, prepare flexible catheter-tubing by cutting an 8-centimeter polyethylene PE-10 tube **[1-TXT]**.

3.2.1. Talent cutting the PE-10 tube to prepare flexible catheter tubing. **TEXT: ID 0.011", OD 0.025** *Video editor: Show Figure 2 as an inset and emphasize on Device #3*

3.3. Remove the inner wire from the infusion-cannula **[1].** Glue the cannula-guide on the 3-millimeter overlap near the bent part of the infusion-cannula using Cyanoacrylate glue and accelerator **[2]**, then insert the horizontal part of the infusion-cannula into the catheter-tubing **[3]**.

3.3.1. Talent removing inner wire.

3.3.2. Talent gluing the cannula-guide to the infusion-cannula. *Video editor: Show Figure 2 as an inset and emphasize on Device #1 & 2*

3.3.3. Talent inserting the infusion-cannula in the catheter tube.

3.4. Eject the translucent cap of the pump flow-moderator **[1]**. Immerse the tubing adaptor in 70% alcohol **[2]** and attach it to the short cannula part of the flow-moderator until it touches the white flange **[3]**.

3.4.1. Talent removing the cap of the pump flow-moderator.

3.4.2. Talent immersing the tubing adaptor in 70% alcohol.

3.4.3. Talent attaching the swollen tubing adaptor to the short cannula of flow-moderator. *Video editor: Show Figure 2 as an inset and emphasize on Device #5.2*

3.5. Insert the flexible catheter-tubing into the tubing adapter **[1]** and hold its long cannula part using a clip-stand **[2]** while gluing all the connections **[3]**.

3.5.1. Talent inserting the catheter-tubing in the tubing adapter.
3.5.2. Talent using a clip stand to hold the long cannula.
3.5.3. Talent gluing all the connections using polyethylene compatible adhesive.

3.6. Wrap the mini osmotic pump with a paper wipe, and fix it vertically with the opening facing upwards, using a clip holder stand **[1]**.

3.6.1. Talent fixing the mini osmotic pump

3.7. Fill the pump with ACSF using a syringe with a 27-gauge blunt needle, taking care to avoid air entry **[1]**.

3.7.1. Talent filling the pump with ACSF.

3.8. Fill the long-cannula part of infusion-tube with ACSF using a syringe with a 27-gauge blunt needle **[1]** and insert it into the pump **[2]**. Place the pump in a saline beaker for at least 4 to 6 hours at 37 degrees Celsius **[3]**. *Videographer: This step is important!*

3.8.1. Talent injecting the long-cannula with ACSF.
3.8.2. Talent connecting the infusion-tube to the pump.
3.8.3. Talent placing the pump inside a saline beaker into a water bath.

4. Implantation of chronic model (osmotic pump) into the rat

4.1. Create a subcutaneous pocket in the rat's back by alternately opening and closing an alcohol sterilized hemostat under the skin through the midscapular line **[1]**. *Videographer: This step is difficult.*

4.1.1. Talent creating a subcutaneous pocket by opening and closing the hemostat under the skin.

4.2. Remove the pump from the water bath and place it on the rat's back, covered with a paper wipe **[1]**. Slide the cannula-guide of the infusion tube on the cannula holder **[2]**. Hold the pump with the hemostat and gently insert it into the subcutaneous pocket **[3]**. *Videographer: This step is difficult and important!*

4.2.1. Talent placing the pump on the rat's back.
4.2.2. Talent sliding the cannula guide on the cannula holder.

4.2.3. Talent placing the pump in the rat's back pocket.

4.3. Implant the infusion-cannula in the target and glue it to the skull using gel glue **[1]**. Fix the infusion-cannula, ensure that the catheter is kink-free to allow for neck movement, and cover all the implants with dental cement **[2]**.

4.3.1. Talent implanting infusion cannula in the target.
4.3.2. Talent applying dental cement along the skull. NOTE: Shot 4.3.2 includes 2 parts (was paused in the middle). Since the step is pretty long, part 2 shows how it should look at the end.

4.4. To perform pump replacement surgery, start injecting bicuculline into the pump using a syringe with a 27-gauge blunt needle. Continue to inject bicuculline while removing the syringe to prevent air from entering **[1]**.

4.4.1. Talent injecting bicuculline in the pump.

4.5. Insert the flow-moderator inside the pump **[1]** and place the pump in a saline beaker for at least 4 to 6 hours in a 37-degree Celsius water bath **[2]**.

4.5.1. Talent inserting the flow-moderator inside the pump.
4.5.2. Talent keeping the pump in saline beaker at water bath.

4.6. Make an incision on the skin above the implanted pump **[1]**. Wash the pocket with room temperature ACSF **[2]** and dry it with gauze pads **[3]**.

4.6.1. Talent making an incision for surgery.
4.6.2. Talent washing the rats' back pocket with ACSF.
4.6.3. Talent drying the skin with gauze pads.

4.7. Detach the ACSF-filled pump from the flow-moderator using a hemostat and discard it **[1]**. Similarly, detach and discard the flow-moderator from the bicuculline-filled pump **[2]**.

4.7.1. Talent detaching the pump from the flow moderator
4.7.2. Talent detaching the flow moderator from the pump.

4.8. Gently attach the bicuculline-filled pump to the implanted flow-moderator **[1]** and glue the incision line with a tissue adhesive **[2]**. *Videographer: This step is important!*

4.8.1. Talent attaching the pump to flow moderator
4.8.2. Talent gluing the incision with tissue adhesive

Results

5. The observation & quantification of tic expression in both the models

5.1. In the acute model, tics start to appear several minutes after the bicuculline microinjection and last for dozens of minutes, then eventually decay and cease **[1]**.

5.1.1. Tics appearing in the acute model. *Videographer: Please film this.*

NOTE: The best visible tics appear at the end of this shot.

5.2. In the chronic model, tics typically start to appear on the first day following the bicuculline-filled pump implantation which fluctuates during the day and is most clearly observable during the quiet-waking state **[1]**.

5.2.1. LAB MEDIA: ChronicModel.mp4.

5.3. Motor tics have a stereotypic kinematic signature that can be detected in the accelerometer and gyroscope signals **[1]**.

5.3.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the top two graphs*

5.4. Similarly, tic timing can also be assessed using the local field potential signal throughout the CBG pathway because of the appearance of large amplitude LFP transient spikes **[1]**.

5.4.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the LFP graph*

Conclusion

6. Conclusion Interview Statements

6.1. These models enabled the study of tic expression following different behavioral, environmental, and pharmacological interventions for prolonged periods of time.
6.1.1. *Video editor is suggested to display Figure 3.*