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Title: In Vivo Targeting of Neural Progenitor Cells in Ferret Neocortex by In Utero Electroporation

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Nereo Kalebic**: This protocol allows *in vivo* genetic manipulation to be performed in a key non-primate animal model to study the development of an expanded and folded brain **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Nereo Kalebic**: Using a high spatial and temporal specificity enables the in vivo targeting of the neural stem cells that are fundamental to brain development **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Author**: Demonstrating the procedure with Nereo Kalebic will be Barbara Langen, a veterinarian **[1][2]**.

1.3.1. INTERVIEW: Author saying the above

1.3.2. Named demonstrator(s) looks up from workbench or desk or microscope and acknowledges camera

Author NOTE: we had two veterinarians:
1. Barbara Langen is an external veterinarian who until recently worked with us and helped us establish this method (therefore she is also an author) and she will give the statement 2.4.
2. The current institutional veterinarian is Katrin Reppe and she was present at filming and she demonstrated the steps 2.3, 2.5, 2.6 and 2.7. Since she is our current institution veterinarian she has to be present to take care of the anesthesia and analgesia.

As to their introdcutions:
- Barbara Langen gives the statement 2.4 and we suggest that you write below: "Dr.Barbara Langen, veterinarian"

- We filmed a shot in the style of 1.3.2 for Katrin Reppe and we suggest to write below "Dr. Katrin Reppe, Veterinarian / Animal Welfare Officer
MPI-CBG" and put it before the first time K.Reppe appears which is 2.3

Ethics Title Card

1.4. All experimental procedures were conducted in agreement with the German Animal Welfare Legislation after approval by the Landesdirektion Sachsen.

Protocol

2. In Utero Electroporation and Surgical Preparation

2.1. Before beginning the procedure, pull glass capillaries on a micropipette puller **[1]** and use forceps to cut off the distal part of the capillary to adjust the diameter of the capillary tip **[2]**.

2.1.1. WIDE: Talent pulling glass pipette

2.1.2. Pipette tip being cut

2.2. On embryonic day 33, add the appropriate concentration of DNA in PBS supplemented with 0.1% Fast Green with gentle mixing **[1]** and place a 3-hour-fasted, anesthetized, pregnant, female ferret on an operation table with a heat pad **[2-TXT]**.

2.2.1. WIDE: Talent adding DNA to PBS, with DNA, PBS, and Fast Green containers visible in frame

2.2.2. Talent placing ferret onto table *Videographer: More Talent than ferret in shot*
TEXT: 3 ± 0.5 % isoflurane in O_2 -> 0.8 liters/min Author NOTE: Here we filmed the conditions of the anesthesia i.e. a shot of the anesthesia machine and a talent adjusting the level of isoflurane and O2. The actual placING of ferret was not shot, but we have a shot of ferret already placED. The room was simply to small to show the placing properly

2.3. Confirm the appropriate level of sedation by touching the periocular skin **[1]** and pinching the skin between the second and third or third and fourth toe of both hind limbs **[2]**.

2.3.1. Skin being touched

2.3.2. ECU: Skin between toes being pinched

2.4. **Barbara Langen**: As isoflurane can cause adverse effects, be sure to use protective equipment when handling the anesthetic in liquid form and to use absorption canisters to capture the gas during surgery **[1]**.

2.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

2.5. Inject the animal subcutaneously with analgesic, antibiotic, and glucose **[1-TXT]** and place ointment on the animal's eyes **[2]**.

2.5.1. Ferret being injected, with metamizole, amoxicillin, and glucose containers visible in frame **TEXT: Analgesia: metamizol 50 mg/kg; Antibiotic: 20 mg/kg amoxicillin; 5% glucose**

2.5.2. ECU: Ointment being applied

2.6. Use clippers to shave the abdomen **[1]** and clean the exposed skin with a water, soap, and iodine solution **[2]**.

2.6.1. Hair being shaved

2.6.2. Skin being washed, with soap container visible in frame NOTE: 2.6.2 - 2.7.2 in one shot (2.7.2 included)

2.7. Then dry the skin with gaze swabs **[1]** and disinfect with alcohol and iodine scrubs **[2]**.

2.7.1. Skin being dried

2.7.2. Skin being wiped, with alcohol and iodine containers visible in frame

3. In Utero Electroporation

3.1. For in utero electroporation, place a sterile drape over the animal **[1-TXT]** and use a scalpel **[1.2]** to make an approximately 5-centimeter skin incision at the linea alba **[2]**.

3.1.1. WIDE: Talent placing drape over ferret *Videographer: More Talent than ferret in shot* **TEXT: Use sterile technique and instruments**

3.1.1.2 Added shot: sterile instruments

3.1.2. Incision being made

3.2. Use scissors to cut through muscle layer **[1]** and place gauze swabs around the incision site **[2]**.

3.2.1. Muscle being cut

3.2.2. Swabs being placed NOTE: 3.2.2 till 3.3.2 in one shot

3.3. Wet the gauze with PBS **[1]** and place the uterus onto the swabs **[2]**.

3.3.1. Gauze being wet

3.3.2. Uterus being exposed

3.4. Using a pipette with a long tip, load one of the pulled glass capillaries **[1]** with 5 microliters of DNA solution per embryo to be injected **[2-added]**.

3.4.1. Capillary being injected

3.4.2. Added shot: Close up of the capillary

3.5. Attach the loaded capillary to a holder **[1]** and connect the other side of the holder to a tube and a mouthpiece **[2]**.

3.5.1. Talent attaching capillary to holder NOTE: from 3.5.1 till 3.5.2 in one shot

3.5.2. Talent connecting holder to tube and mouthpiece

3.6. Locate the head of the first embryo **[1]** and place a fiber optic light source next to the head **[2]**.

3.6.1. Head being located *Videographer: Important step*

3.6.2. Light source being placed *Videographer: Important step* NOTE: from 3.6.2 till 3.7.1 in one shot, but not 100% sure because below it says 3.7.1 – 3.8.1 in one shot

3.7. Using the pigmented iris as a reference point, penetrate the skin, skull, and cerebral tissue with the tip of the glass capillary **[1-TXT]** and use mouth pipetting to facilitate

the delivery of 3-5 microliters of the injection solution into the ventricle of one of the cerebral hemispheres **[2]**.

3.7.1. ECU: Shot of iris and hemisphere, NOTE: from 3.7.1 till 3.8.1 in one step

Author NOTE: We made several shots of this step and in one version we used tools to point first at the pigmented iris and then at the position of where the ventricle should be. Please use that version because I think it is the best. Again, please reach out if you have any questions during the editing.

3.7.2. then tissue being penetrated *Videographer: Important/difficult step; Video Editor: please emphasize iris when mentioned* **TEXT: Uninjected contralateral hemisphere as control**

3.7.3. ECU: Solution being delivered *Videographer: Important/difficult step*

3.8. Because the injected solution contains 0.1% Fast Green, a successful injection will result in a dark green staining of the injected ventricle, which will now be visible as a kidney-shaped structure **[1]**.

3.8.1. ECU: Shot of injected ventricle *Videographer: Important/difficult step*

3.9. After the injection, place the tweezer electrodes on the uterus above the head of the embryo **[1]** with the positive pole above the area to be targeted **[2]** and the negative pole below the injected area **[3]**.

3.9.1. Electrode(s) being placed *Videographer: Important step* NOTE: from 3.9.1 till 3.9.3 in one shot.

3.9.2. Shot of placed electrodes *Videographer: Important step; Video Editor: please emphasize positive pole*

3.9.3. Use 3.9.2. *Video Editor: please emphasize negative pole*

Author NOTE: For the positive pole (3.9.2) the shot is clear and I draw a little "+" sign on the electrode to make it clear. The negative pole is then by definition on the opposite side and not clearly visible on the shot

3.10. Set the pulse length of the electroporate to 50 milliseconds, the pulse voltage to 100 volts, the pulse interval to 1 second, and the number of pulses to 5 **[1]**.

3.10.1. Talent setting electroporator parameters

3.11. When all of the parameters have been set, press **Pulse [1]** and quickly drop several drops of warm PBS onto the electroporated embryo **[2-TEXT]**.

3.11.1. Talent pressing pulse NOTE: from 3.11.1 till 3.12.2 in one shot.

3.11.2. PBS being added to embryo **TEXT: Repeat injection and electroporation for each embryo**

3.12. When all of the embryos have been electroporated, return the uterus to the peritoneal cavity **[1]** and use a 4-0 suture to close the muscle layer with the peritoneum **[2]**.

3.12.1. Uterus being placed back into abdomen

3.12.2. Muscle and/or peritoneum being sutured

3.13. Close the skin in a similar manner **[1]** and cover the wound with aluminum spray **[2]**.

3.13.1. Skin being sutured

3.13.2. Wound being sprayed

3.14. Then return the animal to its cage with a heat source and monitoring until full recumbency **[1]**.

3.14.1. Talent placing ferret into cage Author NOTE: We filmed this but since the room in which we work was to small, the shot will not be very good so please decide if you want to include it or not. *Videographer: More Talent than ferret in shot*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.6.- 3.9.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.7., 3.8.

Results

4. Results: Representative Ferret Neocortex Imaging

4.1. Four days after electroporation, at embryonic day 37, most of the targeted cells and their progeny are still in the germinal zones **[1]** and the cells are seldom observed further basally within the cortical plate **[2]**.

4.1.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize green signal*
4.1.2. LAB MEDIA: Figure 2A

4.2. The progenitor identity can be examined by immunofluorescence for markers of cycling cells, such as PCNA (P-C-N-A) **[1-TXT]**, whereas a subset of progenitors undergoing mitosis can be shown by markers such as phosphohistone 3 **[2]**.

4.2.1. LAB MEDIA: Figures 2B-2C *Video Editor: please emphasize pink signal in Figures 2B and 2C* **TEXT: PCNA: proliferating cell nuclear antigen**
4.2.2. LAB MEDIA: Figures 2B-2C *Video Editor: please emphasize red signal in Figure 2D*

4.3. At postnatal day 0, 8 days after electroporation, the progeny of the targeted cells spread to all of the histological layers **[1]**.

4.3.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize green signal in Figure 3A*

4.4. Using a combination of transcription factor markers, different basal progenitor populations can be revealed **[1]**.

4.4.1. LAB MEDIA: Figure 3B

4.5. For example, Sox2 is a marker of proliferative progenitor cells, including basal radial glia **[1]**, and T-box brain protein 2 is a marker of neurogenic basal progenitors, which are mainly intermediate progenitors **[2]**.

4.5.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize blue signal in right/merged Figure 3B image*
4.5.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize pink signal in right/merged Figure 3B image*

4.6. By postnatal day 16, a majority of the targeted cells stop dividing and differentiate into neurons and glia **[1]**, with ferret postnatal day 16 neocortex exhibiting the characteristic folding pattern **[2]**.

4.6.1. LAB MEDIA: Figure 3C *Video Editor: please emphasize green signal*
4.6.2. LAB MEDIA: Figure 3C *Video Editor: please emphasize folding pattern in image*

Conclusion

5. Conclusion Interview Statements

5.1. **Wieland B. Huttner**: The in utero electroporation of various genes, including human-specific genes, in gyrencephalic ferrets has allowed us to model key processes that underlie the evolutionary expansion of the human neocortex **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera