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Title: Short-Duration Hypothermia Induction in Rats Using Models for Studies Examining Clinical Relevance and Mechanisms

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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? **NO**

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**.

    ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Current Protocol Length
Number of Steps: 13
Number of Shots: 26

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Daniel Omileke:** The gradual cooling protocol has a slower rate of cooling than current experimental models. This model is clinically relevant because the time to target temperature is closer to the human range **[1]**.

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

1.2. **Daniel Omileke:** The main advantage of these techniques is their simplicity and inexpensiveness. Additionally, these methods do not require specialist equipment **[1]**.

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

OPTIONAL:

1.3. **Daniel J. Beard:** The clinical rate of cooling achieved from the gradual model will allow a more informed pre-clinical investigation into the benefits of hypothermia as a treatment measure for stroke patients **[1]**.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Animal Care and Ethics Committee of the University of Newcastle.

Protocol

2. Datalogger Implantation

2.1. Begin by placing an anesthetized rat on a surgical heat mat in the supine position with the nose in a cone **[1]**. Secure the nose with surgical tape **[2]**.

2.1.1. Wide: Talent is placing animal onto mat. *Videographer: More Talent than rat in shot*

2.1.2. Nose being secured with tape.

2.2. Shave the fur from the lower right abdomen **[1]** and subcutaneously inject 200 microliters of 0.05% Bupivacaine as a local anesthetic **[2]**. Apply antiseptic solution to the freshly shaved region **[3]**.

2.2.1. Fur being shaved.

2.2.2. Rat being injected.

2.2.3. Antiseptic being applied.

2.3. Make a 2-centimeter longitudinal incision along the right abdominal region proximal to the right thigh deep enough to expose the space at the ventral thigh crease **[1-TXT]** and use hemostats and forceps to create a pocket under the skin large enough to hold a datalogger device **[2]**.

2.3.1. Incision being made. **TEXT: Sterilize all instruments before procedure**

2.3.2. Pocket being created.

2.4. Insert the temperature monitoring datalogger device into the pocket **[1-TXT]** and use 5-0 (five-oh) sutures to close the muscle and skin, making sure that the datalogger is not resting against the heating mat **[2]**.

2.4.1. Datalogger being inserted into pocket. **TEXT: See text for datalogger and rectal probe calibration details**

2.4.2. Sutures being placed.

3. Induction of Active (Fast) Hypothermia

3.1. To induce active hypothermia, first place one retort stand with a clamp 20 centimeters from the head of the rat and one 20 centimeters from the lower abdomen **[1]** and attach a 60-millilmeter, 12-volt fan with an at least 4000 rotations per minute setting to each stand with the fans aimed toward the lower back of the rat **[2]**.

3.1.1. Wide: Talent placing second stand, with first stand visible in frame

3.1.2. Talent attaching fan to stand.

3.2. Adjust the heat mat to the desired target temperature for initiating hypothermia **[1-TXT]** and turn on both fans **[2]**.

3.2.1. Talent adjusting mat to desired temperature. **TEXT: e.g., 32.5 °C**

3.2.2. Talent turning on fans.

3.3. In the prone position, apply 3-4 sprays of 70% ethanol every 5 minutes to the lower back of the rat while ruffling the fur for faster cooling induction **[1-TXT]** and while closely monitoring the rectal temperature **[2]**.

3.3.1. Talent applying ethanol spray to animal. **TEXT: Caution: Do not oversaturate** *Videographer: This step is important!*

3.3.2. Shot of rectal temperature.

3.4. Stop the ethanol application when the rectal temperature is within 1 degree of the target temperature **[1]**. When temperature is within 0.5 degree of the target, turn off the fans to allow the temperature to drop slowly to the target to without overcooling **[2]**.

3.4.1. Shot of monitor screen with temperature within 1 degree Celsius of target.

3.4.2. Shot of monitor screen with temperature within 1 degree Celsius of target and talent turning off both the fans. *Videographer: This step is difficult and important*

3.5. In the case of overcooling, replace one fan with an animal heat lamp **[1]** and use the heat lamp to mildly warm the animal back up to the target temperature **[2-TXT]**. Once the target temperature has been reached and stabilized, monitor the temperature for the remaining hypothermia period **[3]**.

3.5.1. Talent placing heat lamp onto stand.

3.5.2. Talent turning on heat lamp, with lamp on visible in frame. *Videographer: This step is difficult and important!* **TEXT: Optional: Use fan to prevent re-warming overshoot**

3.5.3. Talent monitoring the temperature.

3.6. At the end of hypothermic period, set the heat mat to 37 degrees Celsius and allow the animal to thermoregulate over a 30-minute period **[1-TXT].**

3.6.1. Talent adjusting the temperature to rewarm animal. *Videographer: This step is important.*

4. Induction of Clinically Achievable Gradual Onset Hypothermia

4.1. To induce clinically achievable gradual onset hypothermia, reduce the temperature of the core temperature regulated homeothermic heat mat in small increments to achieve hypothermia within a specific pre-determined time period **[1]**.

4.1.1. WIDE: Talent reducing the temperature of the heat mat.

4.2. When the target temperature has been reached, maintain the target temperature for the desired interval without any external cooling **[1]**. To rewarm the animal at the end of the hypothermic period, adjust the heat mat to allow the rat to rewarm to 37 degrees Celsius over the desired time interval **[2]**.

4.2.1. Talent removing fans/monitoring the body temperature of animal. *Videographer: This step is important*

4.2.2. Talent adjusting the temperature of heat mat. *Videographer: This step is important*

4.3. Upon the completion of either type of hypothermia experiment, remove the datalogger to allow analysis of the recorded body temperatures **[1-TXT]**.

4.3.1. Talent plugging datalogger into USB port **TEXT: See text for longer-term hypothermia study details**

Results

5. Results: Wistar Rat Responses to Induced Hypothermia

5.1. In this analysis **[1]**, hypothermia was achieved with rapid cooling to a target temperature of 32.5 degrees Celsius in 15 minutes as demonstrated **[2]**. A slight temperature overshoot, due to failing to stop the cooling from about 0.5 degrees Celsius above the target temperature, can be observed **[3]**.

5.1.1. LAB MEDIA: Figure 3A.

5.1.2. LAB MEDIA: Figure 3A. *Video Editor: Emphasize dashed data line from 0 time point to valley at about 30 °C.*

5.1.3. LAB MEDIA: Figure 3A. *Video Editor: Emphasize/encircle area of valley in dashed data line from about 32-30 °C.*

5.2. The target temperature was maintained for 30 minutes **[1]** and the rewarming was initiated at 1.5 hours **[2]**.

5.2.1. LAB MEDIA: Figure 3A *Video Editor: Emphasize grey shaded region*

5.2.2. LAB MEDIA: Figure 3A *Video Editor: Emphasize second vertical dotted line*

5.3. In this experiment, the gradual hypothermia induction approach **[1]** was used to allow the target temperature of 33 degrees Celsius to be reached in 2 hours **[2]**. The target temperature was maintained for 30 minutes **[3]** before rewarming at 2.5 hours **[4]**.

5.3.1. LAB MEDIA: Figure 3B.

5.3.2. LAB MEDIA: Figure 3B. *Video Editor: emphasize dashed data line from time 0 to time point right before grey region*

5.3.3. LAB MEDIA: Figure 3B. *Video Editor: Emphasize grey shaded region.*

5.3.4. LAB MEDIA: Figure 3B. *Video Editor: emphasize second dotted line*

Conclusion

6. Conclusion Interview Statements

6.1. **Daniel Omileke:** The gradual cooling technique led us to investigate whether a more clinically relevant cooling rate would prevent intracranial pressure elevation after ischemic stroke in rats **[1]**.

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