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Title: Multi-system Monitoring for Identification of Seizures, Arrhythmias, and Apnea in Conscious Restrained Rabbits

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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? **Yes**

- 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?

X 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: 29

Number of Shots: 52

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **David Auerbach:** Cardiac and respiratory dysfunction contributes to sudden death after a seizure. To study this phenomenon, a multi-system approach was applied to a rabbit model [1].
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.2. **Cameron Bosinski:** This technique enables the simultaneous monitoring and later analysis of neurologic, cardiac, and respiratory function of multiple rabbits surrounding seizures, arrhythmias and preceding death [1].
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

- 1.3. **David Auerbach:** While a disease may be primarily neuronal or cardiac, it may include electrical disturbances in both the brain and heart, such as seizures and arrhythmias, as well as respiratory dysfunction [1].
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.4. **Kyle Wagner:** It can be difficult to work with rabbits because of their hindleg strength. Take care to keep the rabbits calm when moving them and when attaching the monitors [1].
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: Can cut as necessary for time*

Ethics Title Card

- 1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Upstate Medical University.

Protocol

2. Implanting Electroencephalogram (EEG) and Electrocardiogram (ECG) Electrodes and Attaching Respiratory Monitors

- 2.1. Begin by connecting the computer to an amplifier with 64-pin headbox [1]. To make every 8th pin on the headbox a reference, set the **Reference Electrode** to Independent [2].
 - 2.1.1. WIDE: Establishing shot of talent connecting computer to amplifier
 - 2.1.2. SCREEN: 62256_screenshot_1. 0:00 – 0:16
- 2.2. For implantation of the ECG (**E-C-G**) electrodes, secure a rabbit in a supine position with the head at the investigator's knees and lower than the rest of its body [1]. With a second investigator spreading the fur to expose the underlying skin [2], subdermally insert 35-degree-angle bent electrodes into each axilla [3].
 - 2.2.1. Rabbit being secured
 - 2.2.2. Skin being exposed
 - 2.2.3. Electrode being inserted into axilla skin *Videographer: This step is difficult and important*
- 2.3. Place leads on the chest posterior to the right and left forelimbs [1] and on the abdomen anterior to the left hind limb [2]. Then place a ground pin electrode anterior to the right hind limb on the abdomen [3].
 - 2.3.1. Second lead being placed on forelimb, with first visible in frame as possible *Videographer: This step is difficult and important*
 - 2.3.2. Third lead being placed anterior to the left hind limb *Videographer: This step is difficult and important*
 - 2.3.3. **Ground** electrode being placed anterior to the right hind limb *Videographer: This step is difficult and important*
- 2.4. When all of the ECG leads have been properly placed, secure the rabbit in an appropriately sized restrainer with the hind limbs under the animal [1].
 - 2.4.1. Talent securing the rabbit in the restrainer *Videographer: More Talent than rabbit in shot*
- 2.5. Using a 45-degree angle approach, insert subdermal straight pin-electrodes into the scalp [1]. Place one EEG lead on the right anterior, left anterior, right occipital, and left occipital regions of the head [2] and place a central reference lead at the central point between the other four leads [3].
 - 2.5.1. Electrode(s) being inserted *Videographer: This step is difficult and important*

- 2.5.2. Shot of leads in place *Videographer: This step is difficult and important; Video Editor: please emphasize right anterior, left anterior, right occipital, and left occipital leads when mentioned*
- 2.5.3. Central lead being placed *Videographer: This step is difficult and important; Video Editor: please indicate this lead as "Cz"*
- 2.6. Run the wires between the ears and loosely tether them to the restrainer behind the head [1].
 - 2.6.1. Wires being run between ears and tethered to the restrainer
- 2.7. To monitor respiration during the experiment, attach a pulse oximeter to one ear over the marginal ear vein [1] and gently secure a facemask with capnography tubing over the mouth and nose of the animal [2]. Then attach the other end of the tubing to the vital signs monitor [3-TXT].
 - 2.7.1. Pulse oximeter being attached
 - 2.7.2. Talent placing mask over rabbit's face *Videographer: More Talent than rabbit in shot; This step is difficult and important* Authors say rabbit did not struggle during mask placement, but are aware JoVE won't include video if the animal appears to be in stress
 - 2.7.3. Talent attaching tubing to facemask and monitor **TEXT: Repeat for each rabbit**

3. Video EEG-ECG Recording

- 3.1. To record video-EEG-ECG, open an appropriate commercially available EEG software program [1] and adjust the video such that all of the rabbits can be observed within the field of view [2].
 - 3.1.1. Talent with EEG software, with monitor visible in screen
 - 3.1.2. SCREEN: screenshot_2 *Video editor: please emphasize the image of the rabbits*
- 3.2. Perform a baseline recording for the animal for a minimum of 10-20 minutes or until the heart rate stabilizes to 200-250 beats per minute for at least 5 minutes [1].
 - 3.2.1. SCREEN: screenshot_3: 00:00-00:10
- 3.3. Set the **low frequency filter** to 1 hertz and the **high frequency filter** to 59 hertz [1]. Add time-locked notes in real-time to indicate the timing of interventions, neuro-cardiac events, and motor or investigator artifacts [2].
 - 3.3.1. SCREEN: screenshot_4: 00:05-00:30 *Video editor: please speed up*
 - 3.3.2. SCREEN: screenshot_5: 00:17-00:23

4. Photic Stimulation

- 4.1. For photic stimulation, place a light source with a circular reflector 30 centimeters in front of the rabbit at eye level with the flash intensity set to the maximum [1] and place two mirrors on each side of the head and one mirror behind the rabbit so that the light enters the rabbit's eyes [2].
 - 4.1.1. WIDE: Talent placing a light source in front of the rabbit eye
 - 4.1.2. Mirrors being placed behind rabbit, with mirrors on either side visible in frame
- 4.2. Attach the light to a controller with an adjustable rate, intensity, and duration [1]. With the photic stimulator set to 1 hertz and the flash on, record the response for 30 seconds [2].
 - 4.2.1. Talent setting the photic stimulation parameters, with monitor visible in frame
 - 4.2.2. SCREEN: screenshot_6: 00:00-00:10
- 4.3. Cover the rabbit's face with a surgical mask to simulate or cause eye closure and stimulate for another 30 seconds [1].
 - 4.3.1. SCREEN: screenshot_6: 00:20-00:30
- 4.4. After recording the eyes open and eyes closed response at each frequency, turn off the photic stimulator for 30 second [1] and set the controller to the next frequency setting [2].
 - 4.4.1. Talent turning off stimulator
 - 4.4.2. Talent setting next frequency setting, with monitor visible in frame
- 4.5. After recording for 60 seconds at each frequency from 1 to 25 hertz in 2-hertz intervals, decrease the frequency from 60 to 25 hertz, in 5-hertz increments, and record for 30 seconds with the eyes open and 30 seconds with the eyes closed [2].
 - 4.5.1. SCREEN: screenshot_7: 00:00-00:20 *Video Editor: can speed up*
- 4.6. At the end of experiment, remove EEG and ECG leads from the rabbit [1] and return the animals to their home cages for routine care by husbandry staff [2].
 - 4.6.1. Talent removing EEG and ECG leads
 - 4.6.2. Talent placing rabbit into **transport carrier** *Videographer/Video Editor: shot will be used again*

5. Oral Medication Administration

- 5.1. Before administering the medication [1], collect 10-20 minutes of baseline EEG-ECG video from the untreated rabbit as demonstrated [2].
 - 5.1.1. WIDE: Talent at computer, recording baseline video, with monitor visible in frame
 - 5.1.2. Added shot: screenshot_3

- 5.2. For the oral administration of a medication of interest, mix 0.3 milligram/kilogram of the drug of interest in 3 milliliters of food-grade applesauce [1-TXT] and load the mixture into a 3-milliliter oral syringe [2].
 - 5.2.1. Talent preparing drug for oral administration **TEXT: e.g., E-4031 or moxifloxacin**
 - 5.2.2. Talent loading the drug in a syringe
- 5.3. Gently lifting the upper lip of rabbit, slide the tip of the syringe into the side of the mouth that is unobstructed by the rabbit's teeth [1] and inject the entire volume of drug-supplemented applesauce into the rabbit's mouth [2].
 - 5.3.1. Shot of **mask being removed, lip being lifted**, then syringe being inserted
 - 5.3.2. Applesauce being injected
- 5.4. Then collect 2 hours of EEG-ECG video data [1] before returning the rabbit to its home cage for routine care [2].
 - 5.4.1. SCREEN: screenshot_3: 00:00-00:42 *Video Editor: please speed up*
 - 5.4.2. Use 4.6.2. Rabbit being placed into cage

6. Intravenous Medication Administration

- 6.1. Before recording the EEG-ECG response to intravenous medication administration, shave the posterior surface of the rabbit ear [1] and use 70% ethanol to disinfect the site and to dilate the marginal ear vein [2].
 - 6.1.1. WIDE: Talent shaving ear *Videographer: More Talent than rabbit in shot*
 - 6.1.2. Skin being wiped
- 6.2. Carefully cannulate the marginal ear vein with a 25-gauge angiocatheter [1-TXT] and place an injection plug at the end of the catheter [2]. Tape a splint created from three rolled pieces of gauze to the ear to secure the catheter in place and to hold the ear upright [3]. **6.2.1.-6.2.3. are merged**
 - 6.2.1. Catheter being placed *Videographer: This step is difficult and important* **TEXT: Cover rabbit's face to reduce stress as necessary**
 - 6.2.2. Injection plug being placed *Videographer: This step is difficult and important*
 - 6.2.3. Splint being taped into place *Videographer: This step is difficult and important*
- 6.3. Inject 1 milliliter of 10 USP (U-S-P) units/milliliter of heparinized saline to keep the catheter patent [1]. When baseline recording has finished, inject saline supplemented with 1 to 10 milligrams/kilogram of the drug of interest into the catheter once every 10 minutes [2-TXT].
 - 6.3.1. Injection of heparinized saline
 - 6.3.2. Needle being inserted into catheter, then drug being delivered **TEXT: e.g., 1-10 mg/kg PTZ**

- 6.4. After each dose, carefully monitor the video-EEG-ECG-oximetry-capnography for any neuro-cardiac electrical and respiratory abnormalities or visual evidence of epileptiform activity. Note these changes in real-time, as well as during post-analysis [1].

6.4.1. SCREEN: screenshot_8: 00:00-00:42 OR screenshot_9: 00:00-00:33 *Video Editor: please select best video; can speed up*

7. Analysis of ECG and Video-EEG

- 7.1. To analyze the ECG video, use an appropriate commercially available software [1] to identify periods of tachycardia, bradycardia, ectopic beats, or other arrhythmias within the ECG data [2].

7.1.1. WIDE: Talent opening the ECG analysis software, with monitor visible in frame

7.1.2. SCREEN: screenshot_10: 00:00-00:33 *Video Editor: please speed up*

- 7.2. To reduce the amount of data to review, create a tachogram to increase the ease with which periods of tachycardia, bradycardia, or irregularities of the RR (R-R) interval can be identified [1].

7.2.1. SCREEN: screenshot_11: 00:00-00:22 *Video Editor: can speed up*

- 7.3. For video-EEG analysis after a drug administration experiment, visually scroll through the EEG tracing to distinguish epileptic versus non-epileptic movements for at least 1 minute after each dose of drug [1].

7.3.1. SCREEN: screenshot_8: 00:00-00:42 OR screenshot_12: 00:00-00:33 *Video Editor: please select best video; please speed up* Authors also offer supplemental videos 18-22 as options for this step

- 7.4. For video-EEG analysis after a photic stimulation experiment, create a spectral analysis plot in an appropriate EEG analysis software program [1].

7.4.1. Talent creating plot in EEG software analysis program, with monitor visible in frame

- 7.5. Then analyze the occipital leads of the EEG for the presence and absence of the occipital driving rhythm. The occipital driving rhythm will create a peak in the spectral analysis that corresponds to the frequency of the photic stimulator [1].

7.5.1. SCREEN: screenshot_13: 00:28-1:20 *Video Editor: please speed up and emphasize occipital driving rhythm peak when mentioned*

Results

8. Results: Identification of Seizures and Arrhythmias in Conscious Restrained Rabbits

- 8.1. Assessment of the ECG morphology [1] allows the detection of abnormal heart rates, conduction, and ECG waveforms [2].
 - 8.1.1. LAB MEDIA: Figure 6A
 - 8.1.2. LAB MEDIA: Figures 6B-6D *Video Editor: please sequentially emphasize Figure 6B, 6C, and 6D graphs*
- 8.2. The traces can also be used [1] to quantify the RR interval [2], heart rate [3], PR (P-R) interval [4], P duration [5], QRS (Q-R-S) interval [6], QT (Q-T) interval [7], QTc (Q-T-C) [8], JT (J-T) interval [9], and T-peak to T-end interval [10].
 - 8.2.1. LAB MEDIA: Figures 6E and 6F
 - 8.2.2. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize RR data row*
 - 8.2.3. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize heart rate data row*
 - 8.2.4. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize PR data row*
 - 8.2.5. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize RR data row*
 - 8.2.6. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize P duration data row and P Start, P Peak, and P End sections of data line*
 - 8.2.7. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize QRS data row and QRS Start and End section of graph*
 - 8.2.8. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize QT data row*
 - 8.2.9. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize QTc data row*
 - 8.2.10. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize JT data row*
 - 8.2.11. LAB MEDIA: Figures 6E and 6F *Video Editor: please emphasize Tpeak Tend data row and Tpeak Tend section of graph*
- 8.3. Recordings from the occipital EEG leads [1] typically exhibit a higher amplitude than that observed for frontal lead data [2] and the dominant frequency in all of the leads are commonly measured in the delta range [3].
 - 8.3.1. LAB MEDIA: Figure 7B
 - 8.3.2. LAB MEDIA: Figure 7B *Video Editor: please emphasize black and blue data lines*
 - 8.3.3. LAB MEDIA: Figure 7B *Video Editor: please emphasize delta section of data lines*
- 8.4. Here sleep spindle waves from a representative rabbit experiment are shown [1]. Multiple EEG montages of one period of sleep demonstrate that these waves arise from the center of the head, which is consistent with human findings [2].

- 8.4.1. LAB MEDIA: Figure 8A *Video Editor: please emphasize waves within dashed rectangle*
- 8.4.2. LAB MEDIA: Figure 8C *Video Editor: please emphasize yellow highlighted section of graph*
- 8.5. In addition to normal EEG changes, various, conscious, non-epileptic rabbit movements can also be observed during baseline recordings that can be used to distinguish these data from epileptiform discharges [1].
 - 8.5.1. LAB MEDIA: Figures 10 *Video Editor: please sequentially emphasize waves forms within dashed rectangles/emphasize dashed rectangles from top left to bottom right of graph*

Conclusion

9. Conclusion Interview Statements

9.1. **Kyle Wagner:** It is important to place the ECG leads securely into the skin to keep the wires away from the legs and to take care that the electrodes remain attached when transferring the rabbits into the restrainer [1].

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2-2.4.*

9.2. **David Auerbach:** This multi-system recording apparatus will enable future drug safety and efficacy studies and facilitate a comprehensive understanding of various acquired and inherited diseases [1].

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

9.3. **Cameron Bosinski:** This technique captures the course of potentially fatal multi-organ dysfunction after a seizure, **which will lead to** a better understanding of the mechanisms of SUDEP [1].

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